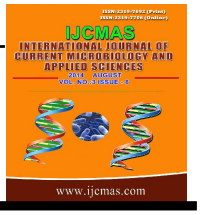
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 9 (2014) pp. 1028-1044**
http://www.ijcmas.com



Original Research Article

Induction of hydrogen evolution in *Nostoc* spp. by nitrogen and /or sulphur deficiency

Awatief F. Hifney[1]* and R. Abdel-Basset[1,2]

[1]Botany& Microbiology Department, Faculty of Science, Assiut University, 71516 Assiut, Egypt
[2]Biology Department, Faculty of Science, Taif University, Saudi Arabia, Po Box 888
**Corresponding author*

A B S T R A C T

In this study examined the filamentous cyanobacterium *Nostoc* sp PCC 7120 (wild type containing one nitrogenase, one uptake hydrogenase and one bidirectional hydrogenase), its hydrogen uptake deficient mutant Hup$^-$ 7120 and also *Nostoc* sp strain ATCC 29133 (strain lacking bidirectional hydrogenase) for their H_2 production capacities under nitrogen and/or sulphur deficiency growth condition compared with control culture condition. The hydrogen evolution under aerobic condition that was observed using a hydrogen electrode in all cultures indicates that oxygen did not totally arrest the hydrogen evolution process. In the present study nitrogen or sulphur deficiencies induced and stimulated hydrogen production in all *Nostoc* sp under investigation. Generally hydrogen production of all tested *Nostoc* Spp with nitrogen and/or sulphur deficiency exhibited dependence on culture age and presence or absence of light. All *Nostoc* spp. have similar growth rates and the same response mode of chlorophyll content, photosynthetic oxygen evolution (were reduced) to both element deficiency compared with regular growth condition

K e y w o r d s

Nostoc spp, nitrogen and /or sulphur deficiency, Photobiologic al hydrogen production

Introduction

World is facing energy crisis due to rapid depletion of limited fossil fuels. Among the various prompted prospecting non-conventional energy sources is hydrogen. Molecular hydrogen which is a clean, efficient and renewable energy resource is one such fuel which can overcome these problems. Photobiological hydrogen production by photosynthetic microorganisms involves generation of renewable energy from nature's most plentiful resources like solar energy and water (Ducat et al., 2011).

Among these microorganisms, cyanobacteria are of special interest which promises both oxygenic photosynthesis and hydrogen production (Ghirardi et al., 2007; McNeely et al., 2010). Nitrogen-fixing cyanobacteria are photosynthetic organisms with simple needs; they can live on air, water and minerals, using CO_2 and N_2 as sources of carbon and nitrogen and sunlight as their source of energy. The process of nitrogen fixation is carried out by the nitrogenase, an enzyme that reduces nitrogen from the atmosphere to ammonia, and produces molecular hydrogen

as a by-product. ATP is needed to drive the reaction, which can be summarized as: N_2 + $8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$

Apart from the nitrogenase, cyanobacteria may possess two types of hydrogenases, a bidirectional hydrogenase encoded by the hox gene and an uptake hydrogenase encoded by the hup gene, which is expressed in connection to nitrogen fixation (Lindberg et al., 2002)

S deprivation induces oxidative stress (for details see for example Agadeeswaran et al., 2014). Sulphur deprivation leads to installation of anaerobiosis, a fundamental prerequisite for H_2 evolution as oxygen is inhibitory to hydrogenases and nitrogenases since a long time ((Fedorov, 2001). Sulphur deprivation leads to inhibition of oxygen evolution due to degradation of PS11 and Rubisco (Melis et al., 2000).

Based on this background, the present investigation highlights the photobiological conversion of water to molecular hydrogen by some *Nostoc* spp. (cyanobacterial cultures) to optimize all the cultural conditions under *in vitro* conditions for pooling all the conditions to have maximum hydrogen production, theses condition were dark, light, separated and combined nitrogen and sulphur deficiency.

Materials and Methods

The nitrogen fixing cyanobacteria *Nostoc* sp ATCC 29133 (deposited in the Pasteur Culture Collection in 1973 as Nostoc sp. PCC 73102, Rippka et al., 1979), *Nostoc* sp. PCC 7120, *Nostoc* sp 7120 (hup^- Free mutant) cultures were grown in BG11 modified medium (Rippka and Herdman 1993) either in the presence of nitrogen and sulphur (control culture condition) or without addition of nitrogen ($BG11_0$ medium) or sulphur source (-S) or combined nitrogen and sulphur deficiency growth conditions (-NS). Cells in the late log phase were harvested by centrifugation (2700 g for 10 min at 25 °C),washed once in S free medium ($MgSO_4$ replaced by the same molarity of $MgCl_2$) and resuspended in S-depleted medium. Growth has been assessed by measurement of the optical density (Absorbance of cyanobacterial cells was monitored throughout the growth period at 750 nm). Chlorophyll content was determined according to the method described by Lefort-Tran et al., (1983). Photosynthetic activity and respiration of the control and treated sample was recorded using Clark type electrode computerized to an Oxygraph (Hansatech, Inc., donation from the Alexander von Humboldt foundation to R.

Abdel Basset). Samples (2ml) of cultures were used and photosynthesis was recorded at light conditions and then the light was switched off for determining respiration. Photosynthesis is expressed as (µmol $O_2\uparrow.mg^{-1}$ chlorophyll.h^{-1}) and respiration as (µmol $O_2\downarrow$/mg chlorophyll.h^{-1}). To study the effect of sulphur deficiency on hydrogen production, the isolates were grown initially under 3000 lux light intensity, at 23°C. Then, the isolates were centrifuged at 5000 rpm for 5 minutes and resuspended in BG-11 medium free of sulphur nutrients (i.e. without magnesium sulphate, zinc sulphate and copper sulphate). The isolates were incubated at selected optimum conditions obtained from previous experiments. The same protocol was used to study the effect of nitrogen deficiency on hydrogen production, (resuspended in BG-11 medium free of nitrogen). In the case of combined nitrogen and sulphur the isolates were resuspended in BG-11 medium free of nitrogen and sulphur. Hydrogen evolution was monitored using a Clark type oxygen electrode after being plated as recommended

by Hansatech Inc.; 2ml of culture with known chlorophyll a content was added to the electrode chamber and hydrogen evolution was registered in the dark and then in the light.

Results and Discussion

Hydrogen is starting to move from a fuel of the future to an energy carrier of the present, promising greatly reduced pollution and increased fuel efficiencies. A major goal is the production of renewable hydrogen at affordable costs. Since the growth conditions of algae can be optimized to find the best balance between growth rate and hydrogen production at lower cost, so that our goal in the present investigation to spot lights on the effect of mineral deficiency on hydrogen production of *Nostoc* species

It has been found that the growth (Absorbance at 750 nm) of all *Nostoc* spp. under investigation (*Nostoc* sp ATCC 29133, *Nostoc* sp. PCC 7120, *Nostoc* sp 7120 (hup^- mutant) were inhibited when grown without addition of nitrogen ($BG11_0$ medium), sulphur source (-S) or combined nitrogen and sulphur deficiency growth conditions (-NS) compared to control cultures, chlorophyll a content of all isolates have the same response to deficiency condition (-N,-S,-NS) as shown in Fig (1).

The effect of nitrogen (N) starvation on the abundance of pigment molecules in several cyanobacteria has been well documented, in *Anacystis nidulans* (Allen et al., 1969), *Synechococcus* sp. (Yamanaka and Glaser, 1980), *Anabaena* (Foulds and Carr 1977, Wood and Hasel Korn 1980) *Synechococcus* strain PCC 7002 (Stevens et al.,1981), and *Synechocystis* strain PCC 6803 (Elmorjani and Herdman 1987).

Hifney et al., 2013 found that the growth of *Spirulina* sp. expressed as daily change in Chl. a content (Xg/ml) were generally inhibited by studied nutrient deficiency (nitrogen, phosphorus and sulphur). The resulting decrease in chlorophyll and phycobilisome (PBS) content leads to a dramatic change in cell color from the normal blue-green to yellow-green, which is known as bleaching or chlorosis (Richaud, 2001) Nitrogen (N) limitation in cyanobacteria is well documented: a reduced growth rate is observed, accompanied by a cessation of phycobiliprotein synthesis and an ordered degradation of phycobilisomes (Hifney et al., 2013). Richaud et al., (2001) found that in N or S deprived cultures of wt *Synechocystis* strain PCC 6803, chlorophyll accumulation stopped almost immediately; this behavior has already been observed in *Synechococcus* strain PCC 7942 (Collier and Grossman 1992).

With exception of *Nostoc* sp 29133, photosynthetic oxygen evolution was highly reduced under deficiency growth condition compared to control culture in all isolates under testing especially in combined nitrogen and sulphur deficiency condition while enhancement of respiration rate was recorded in all treated and tested cultures (Fig 2). Enhanced respiration rates leads to higher rates of H_2 production as enhanced respiration induces a faster onset of anaerobiosis. The absence of oxygen switches off irreversible photoinhibition and D1- protein degradation, which is oxygen dependent Vass, et al. (1992). This leaves more PSII centers. Under anaerobic conditions these remaining PSII centers exist in the “reversibly inhibited state” that was described (Vass, et al. (1992).

Influencing hydrogen production under *in vitro* conditions was studied with the selected cyanobacterial isolates under nitrogen deficiency growth conditions and depicted in

Fig (3,4,5). In the present investigation nitrogen deficiency induced high level of hydrogen production in all studied isolates. In addition, hydrogen production in treated *Nostoc* culture depended on the presence or absence of light and on culture age. However the presence of nitrogen in the growth medium is essential for long-term hydrogen production since, it is necessary for nitrogen fixation and thus ultimately for cell metabolism. (Bothe and Kentemich, 1990). Kondratieva (1983) observed increased hydrogen production in *Anabaena cylindrica* at up to 1 per cent N_2 and complete inhibition with 15 per cent N_2. In *Mastigocladus laminosus* maximum hydrogen production was observed with 0.7 per cent N_2 (Miyamoto *et al*., 1979).

When a culture of *Nostoc* PCC 73102 is grown under nitrogen-fixation conditions, the nitrogenase will produce hydrogen, which will be rapidly consumed by the uptake hydrogenase. If the cells are incubated under an argon atmosphere, however, hydrogen will be evolved by the culture. In the absence of N_2, hydrogen will be produced instead of fixation N_2 as the nitrogenase will use all available electrons to generate H_2 (Tamagnini et al., 2002). It may be possible to genetically engineer the nitrogenase itself resulting in a decreased nitrogen fixation and an increased hydrogen production, even in the presence of N_2. Finally, limiting factors for hydrogen production by the nitrogenase could also be the efficiency of photosynthesis and of electron transport to the enzyme, two areas of possible improvement by genetic engineering. Again, gas phase nitrogen level for maximum hydrogen production varies with cultures (Tamagnini et al., 2002).

Hydrogen production of the three tested isolates under sulphur deficiency growth condition was illustrated in Fig. (6, 7, 8).

From the results it has been found that sulphur deficiency induced hydrogen production in all studied *Nostoc* spp either in presence or absence of the light (Fig. 6, 7, 8). Sulphur is critical for the completion of normal photosynthesis and in the absence of the element the algae cease emitting oxygen and stopp storing energy as carbohydrates, protein and fats. Instead, the algal cells use an alternative metabolic pathway to exploit stored energy reserved anaerobically in the absence of oxygen. The hydrogenase is activated; splitting large amounts of hydrogen gas from water and release it as a byproduct (Melis and Happe, 2001). Imbalance in the photosynthesis - respiration relationship by sulphur deprivation results in net consumption of oxygen by the cells, causing anaerobiosis in the growth medium; a condition that automatically elicited hydrogen production by the algae (Melis *et al*., 2000). The results in this investigation indicate that the hydrogen production under studied condition depends on the age of the culture and the presence or absence of the light.

Jeberlin Prabina and Kumar (2010) stated that compared to sulphur grown samples, marked increase in hydrogen production was noticed in *Anabaena* - TE1, *Nostoc*-TE1, *Fischerella*-TE1 and the green alga *Chlorella*-TE1 under sulphur deprived condition. Also, higher hydrogen production by sulphur deprived culture was noticed in isolates incubated under longer light duration whereas, sulphur grown isolates showed higher production with longer dark period. Jeberlin Prabina and Kumar (2010) found that sulphur stress had a pronounced effect on the production of hydrogen that it sustained higher production rate under 8:16 h dark: light cycle and highest production was recorded in *Anabaena* - TE1 (26.28 $mL.g^{-1}$ dry wt. L^{-1}).

Fig.1 Growth response (absorbance at 750 nm, chlorophyll a content) of *Nostoc* spp. to nitrogen (-N), sulphur (-S) and combined nitrogen and sulphur (-NS) deficiency growth conditions, compared with regular growth conditions (control)

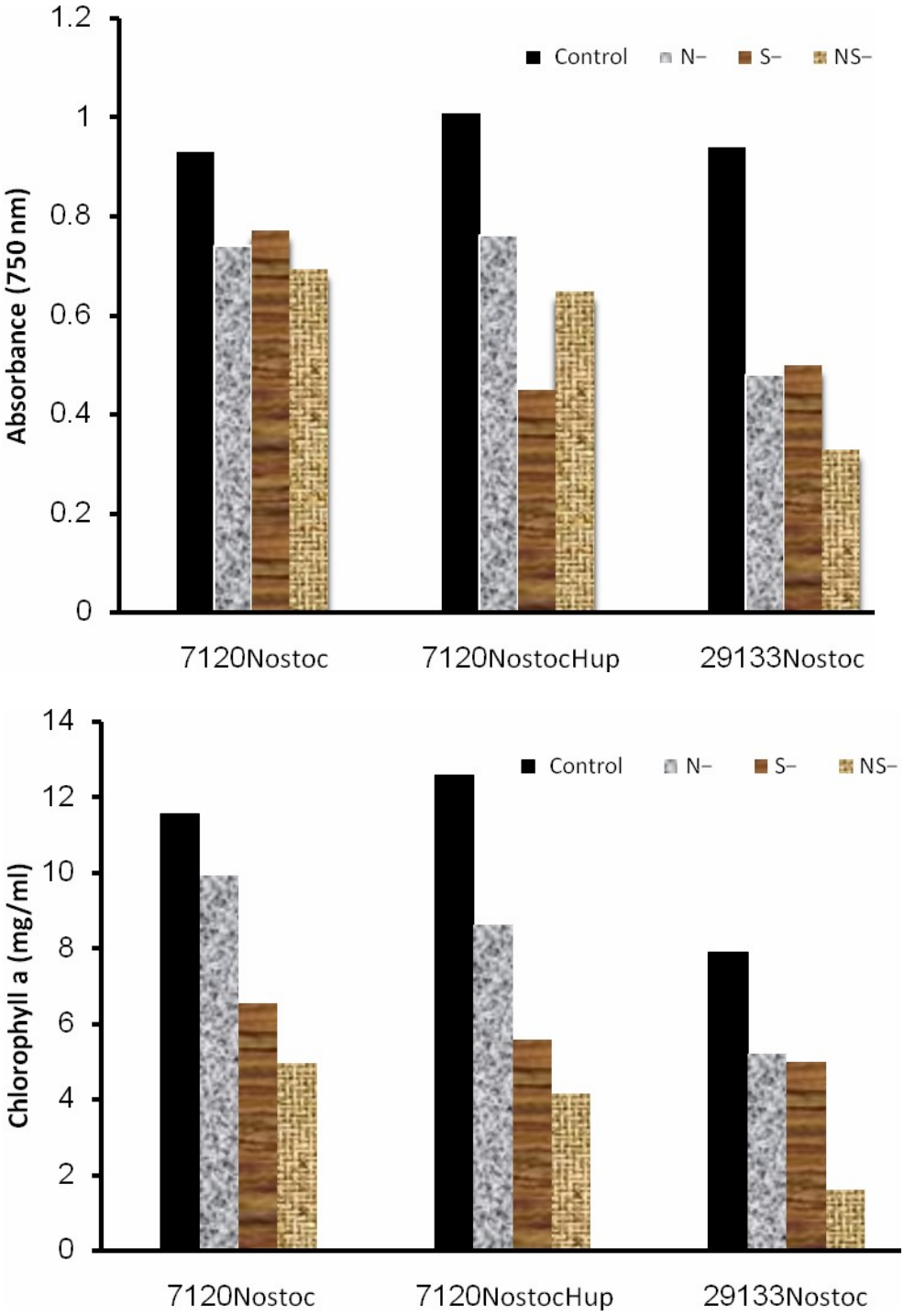
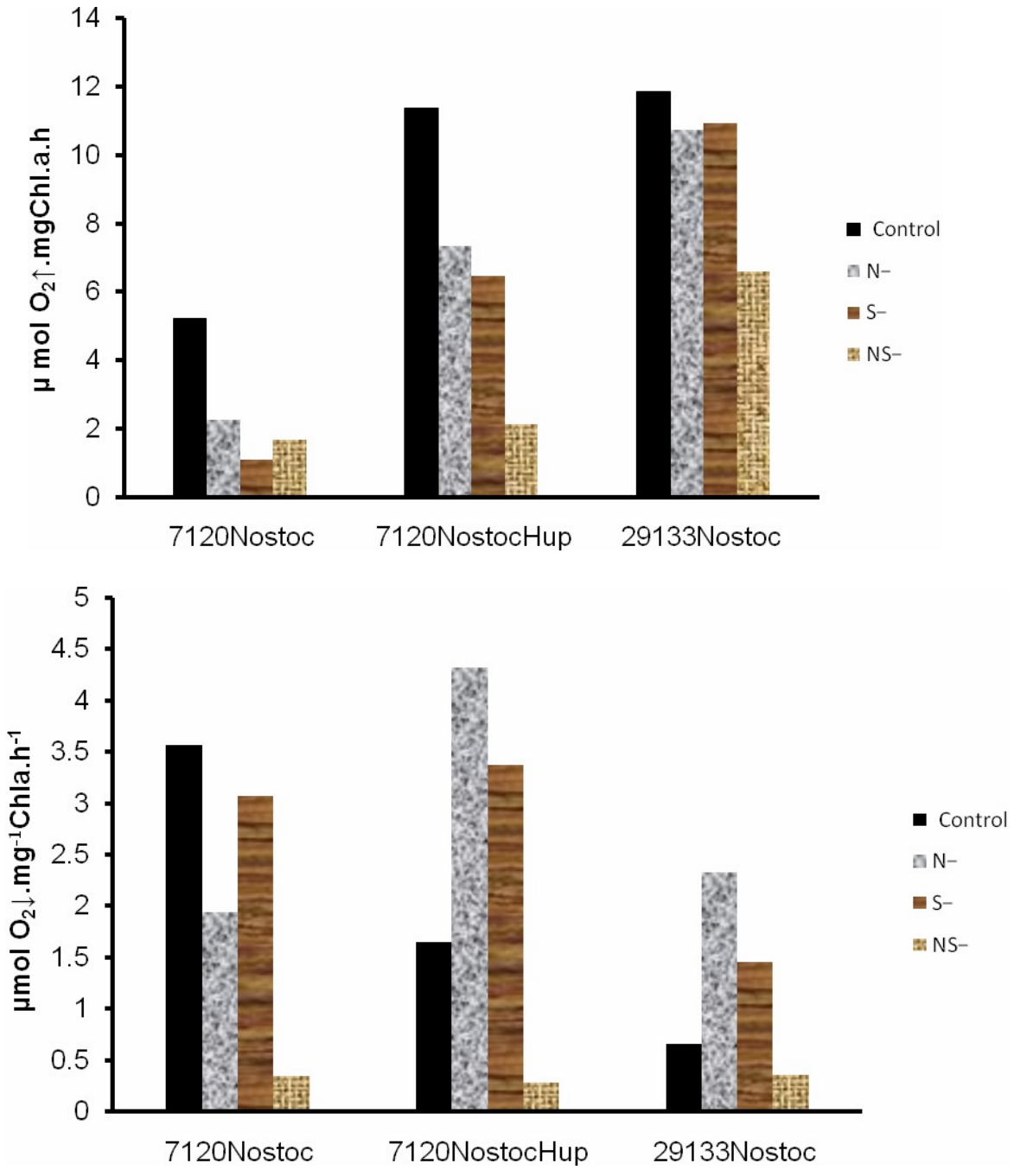


Fig.2 Effect of nitrogen and/or sulphur deficiency growth conditions on photosynthetic oxygen evolution and oxygen uptake in *Nostoc* spp. under testing as discussed in materials and methods



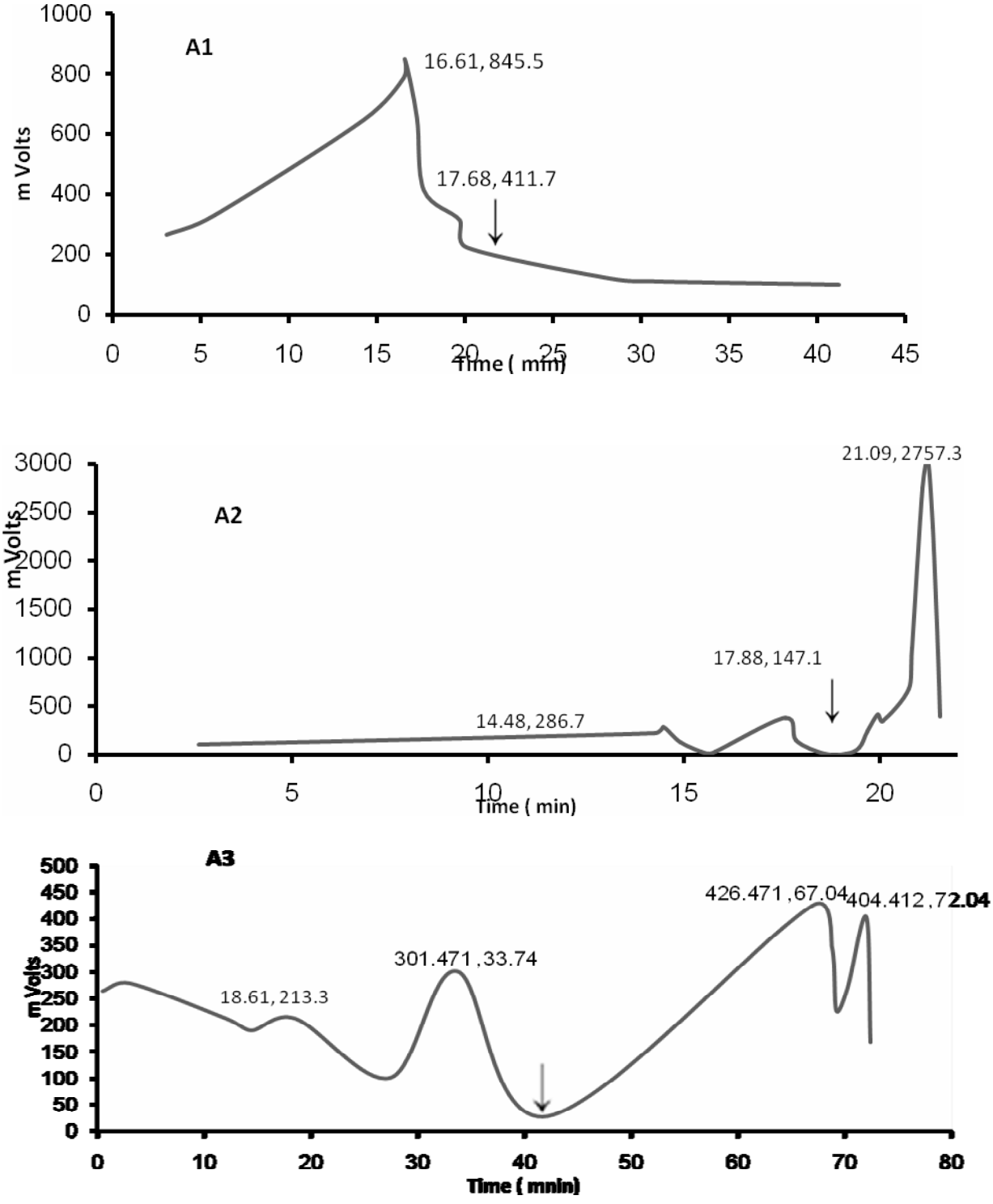


Fig.3 Recordings from the H_2- electrode of *Nostoc* sp 29133, under nitrogen deficiency growth conditions: after 5-7-14 days (A1- A 2- A 3 respectively); arrows indicate when the light was turned on.

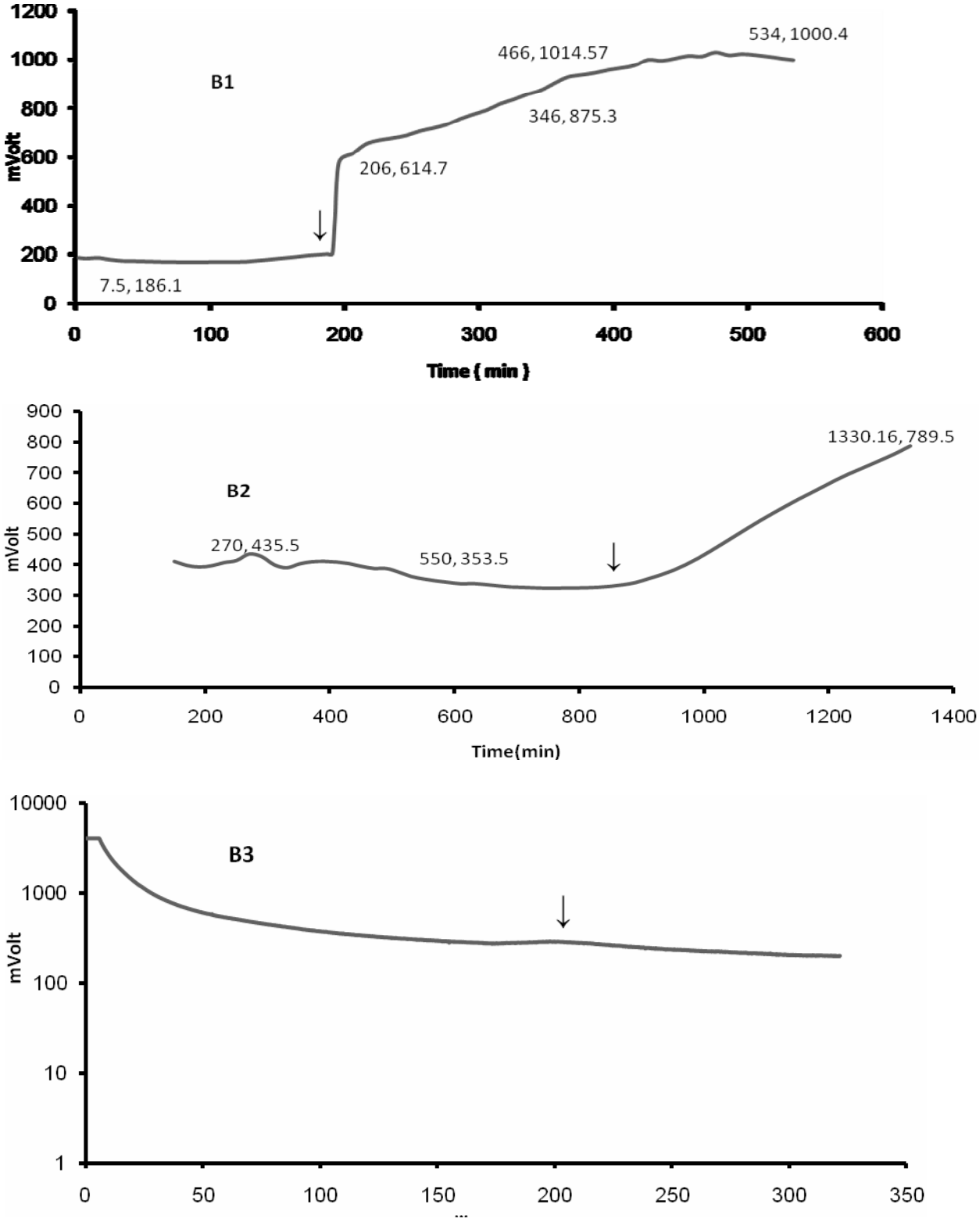


Fig.4 Recording from the H_2- electrode of *Nostoc* sp Hup$^-$7120, under nitrogen deficiency growth conditions: after 1--10-15 days (B1-B2-B3 respectively); arrows indicate when the light was turned on

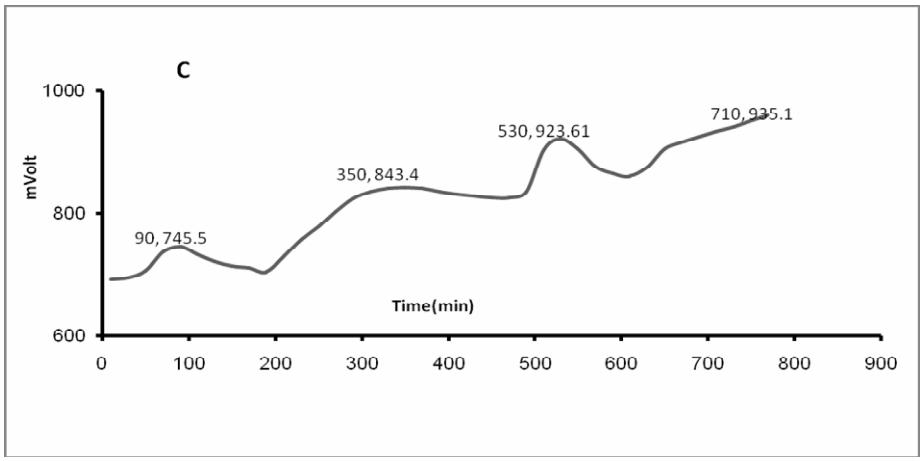
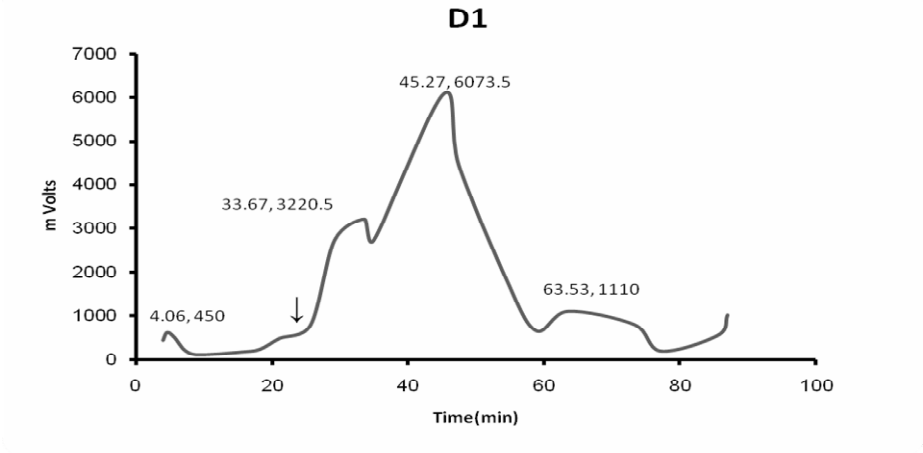


Fig.5 Recording from the H_2- electrode of Nostoc sp 7120, under nitrogen deficiency growth conditions: after 8 days; arrows indicate when the light was turned on.



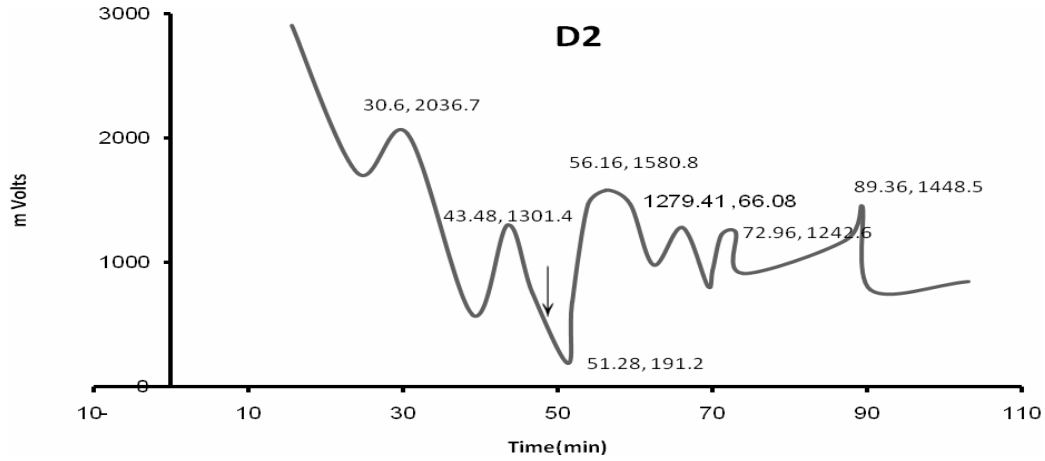
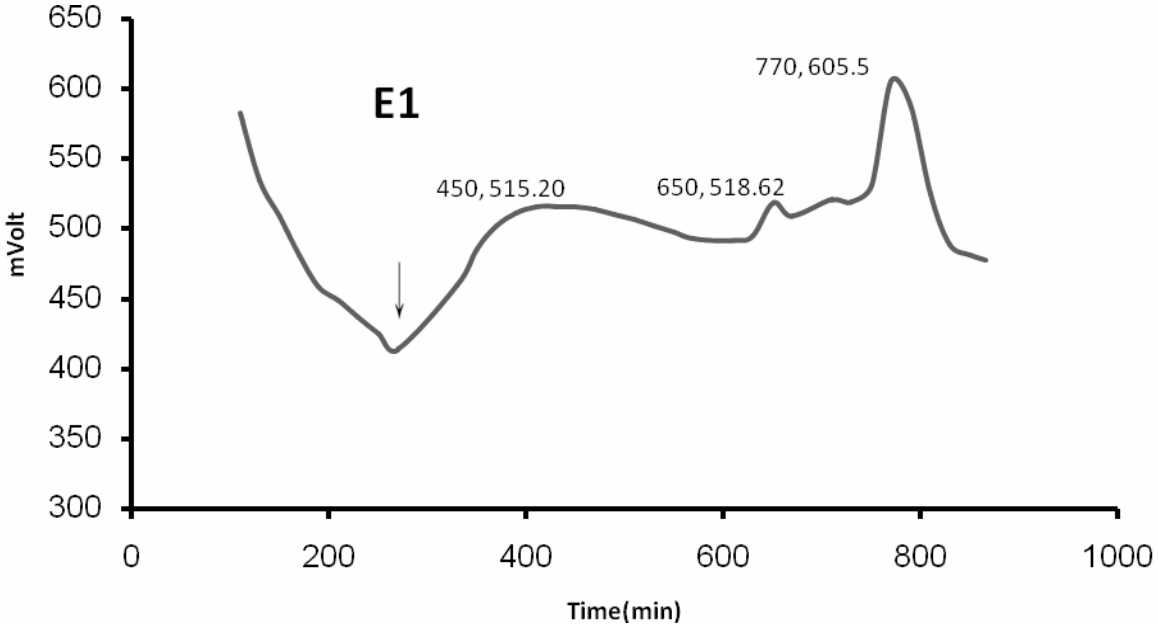


Fig.6 Recording from the H_2-electrode of *Nostoc* sp 29133, under sulphur deficiency growth conditions: after 1and 10 days (D1-D2, respectively); arrows indicate when the light was turned on



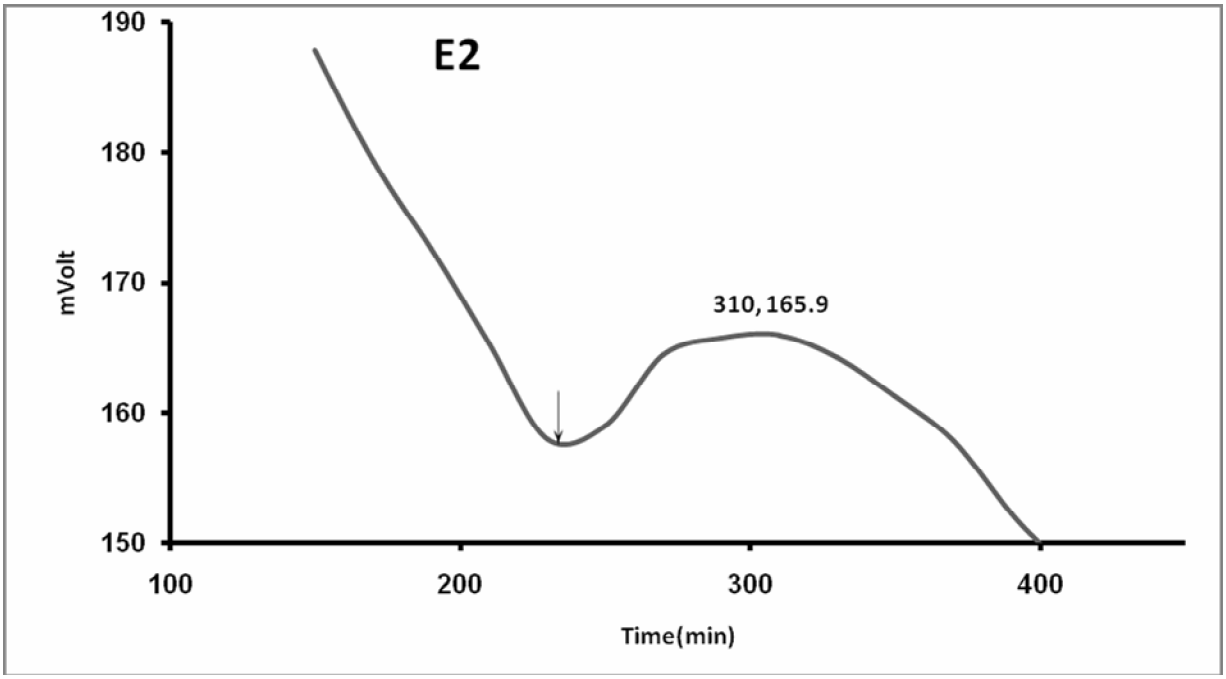


Fig.7 Recording from the H_2-electrode of *Nostoc* sp 7120 hup, under sulphur deficiency growth conditions: after 3 and 15 days (E1-E2 respectively); arrows indicate when the light was turned on.

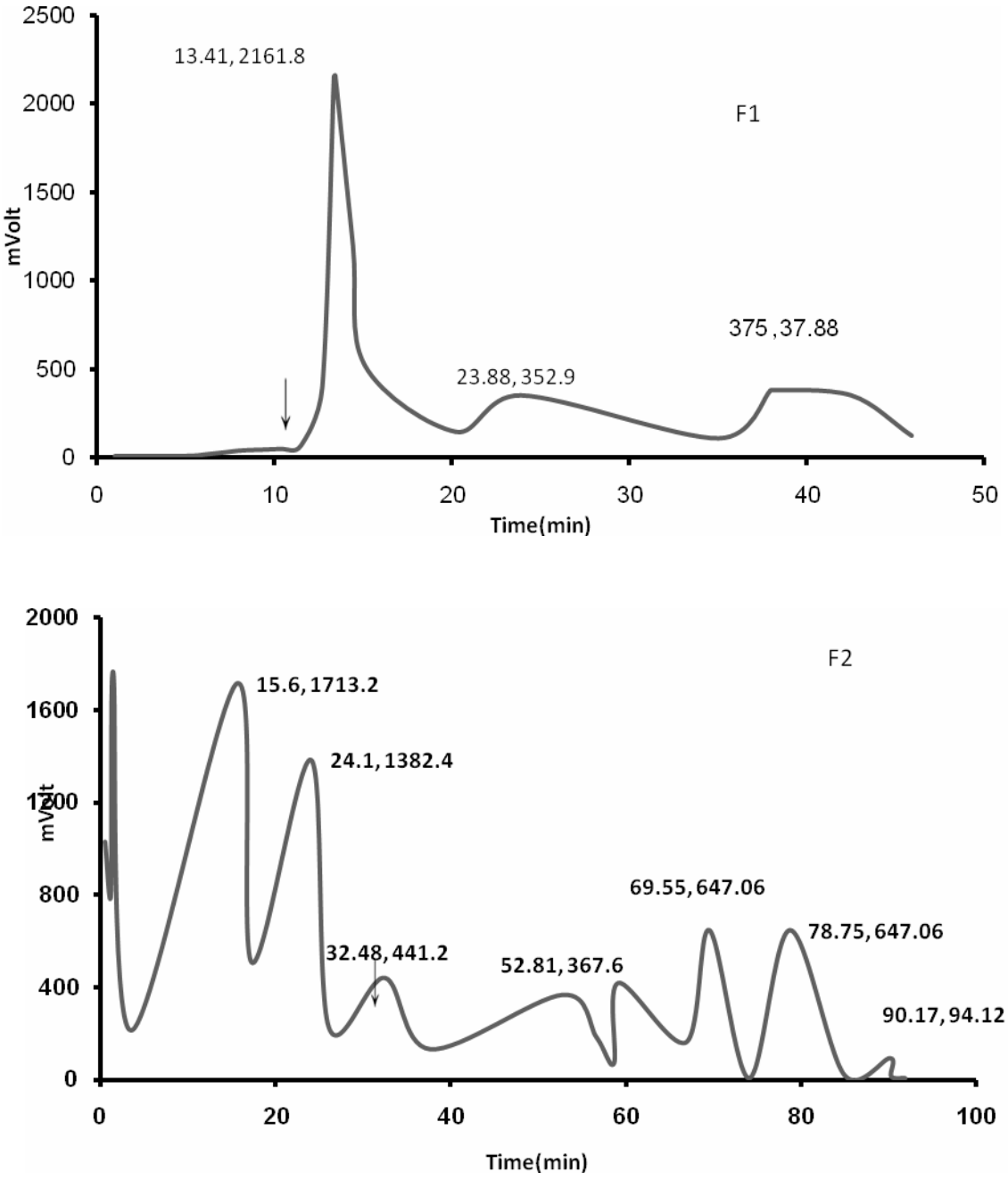


Fig.8 Recording from the H_2-electrode of *Nostoc* sp 29133, under sulphur deficiency growth conditions: after 1 and 11 days (F1-F2 respectively); arrows indicate when the light was turned on

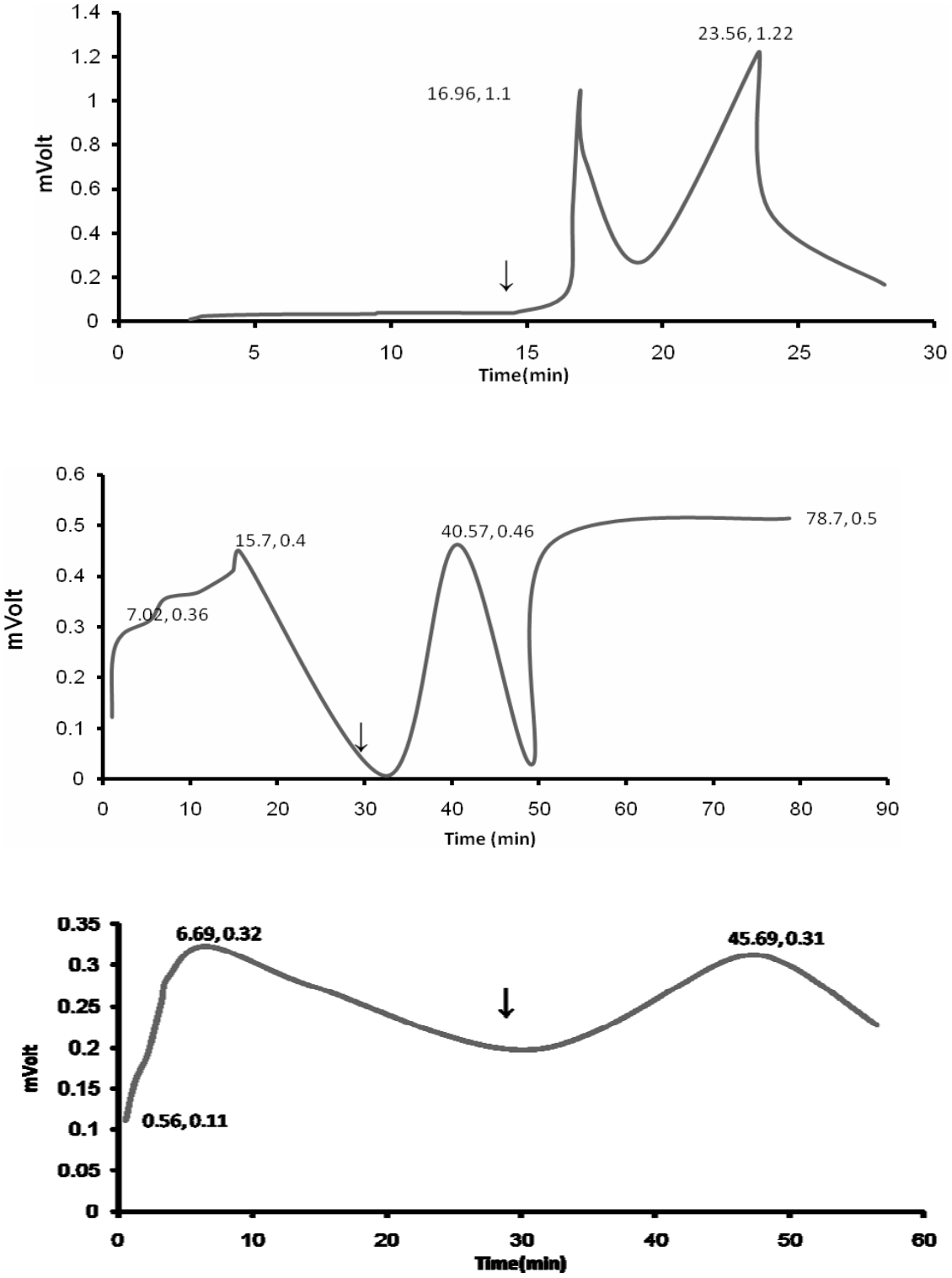


Fig.9 Recording from the H_2- electrode of Nostoc sp 29133 under sulphur and nitrogen deficiency growth conditions: after 1,6,11 days (G1-G2-G3) respectively; arrows indicate when the light was turned on

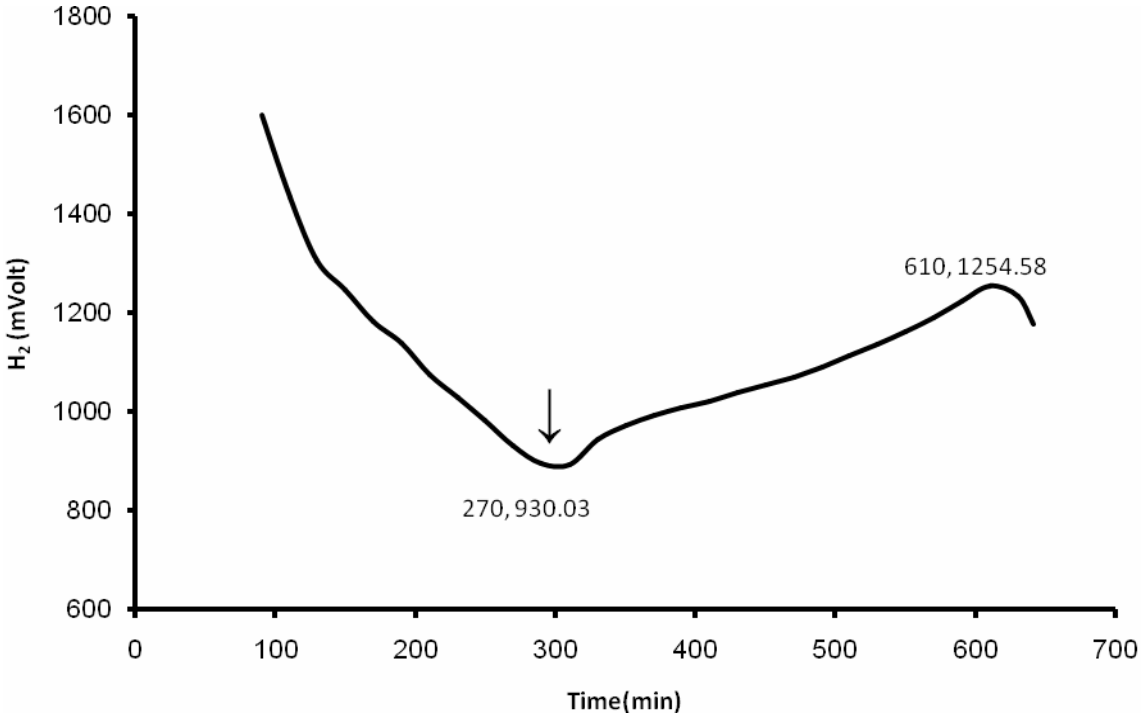


Fig.10 Recording from the H_2 electrode of Nostoc sp Hup$^-$ under sulphur and nitrogen deficiency growth conditions: after 3 days.
Arrows indicate when the light was turned on.

Melis (2007) found that during his work on hydrogen metabolism in *Chlamydomonas reinhardhtii*, the H_2 evolution process was induced upon sulfate nutrient deprivation of the cells, which reversibly inhibits photosystem-II and O_2-evolution in their chloroplast. In the absence of O2, and in order to generate ATP, green algae resorted to anaerobic photosynthetic metabolism, evolved H_2 in the light and consumed endogenous substrate. Melis and coworkers reported a two-stage process based on sulfur (S) deprivation in *Chlamydomonas reinhardtii*, which allowed the separation of the photosynthetic reactions from H2 formation (Melis et al., 2000). Cell cultivation in sealed conditions in media deprived of sulfur allowed light-dependent H2 evolution for several days. The efficiency was the highest for photobiological systems reported so far (2%) (Rupprecht et al., 2006).

Under these conditions photosynthesis is down-regulated and O2 consumption overtakes O_2 evolution, creating anaerobic conditions in the cell. This in turn activates hydrogenase expression and activity (Ghirardi et al., 1997; Happe et al., 2002). Cells of *C. reinhardtii* undergo morphological. Volgusheva et al. (2013) analyzed the activity of PSII during S deprivation in the WT and the Stm6 mutant of *Chlamydomonas reinhardtii*, quantified and analyzed the function of the remaining, reactivated PSII centers. They found that competent in charge separation and S-state advancement and are able to provide electrons to the PQ pool. Estimation of the activity of these remaining PSII centers indicates that the water splitting by PSII

supplies the majority of electrons forH2 synthesis. This estimation is corroborated by their experiments in which H2 production was inhibited by the addition of DCMU. The hydrogenase uses electrons from the PQ pool during the H2 production phase. This partly removes the block in PSII electron transport from QA to plastoquinone, thereby permitting electron flow from water oxidation in PSII to hydrogen Volgusheva et al., 2013.

This results showed that deficiency of nitrogen and /or sulphur induce higher respiration rate in all *Nostoc* sp under testing in comparison to control. Volgusheva et al. (2013) explained how enhanced respiration rates lead to higher rates of H_2 production. Enhanced respiration induces a faster onset of anaerobiosis. The absence of oxygen switches off irreversible photoinhibition and D1- protein degradation, which is oxygen dependent (Vass et al., 1992). Under anaerobic conditions these remaining PSII centers exist in the "reversibly inhibited state" that was described in Vass et al. (1992).

It has been found that wild type of *Nostoc* sp 7120 not able to produce hydrogen under combined nitrogen and sulphur deficiency growth condition, while both of the mutant and *Nostoc* sp. 29133 were able to produce hydrogen under the same condition, amount of nitrogen production depend on the age of culture and presence or absence of light (Fig 9 and 10).

All experiments in this investigation was recorded at aerobic condition without flushing with argon and hydrogen production were detectable i.e oxygen did not totally arrest the hydrogen evolution process even at indicating a certain degree of protection. These results are in agreement with Jeberlin Prabina and Kumar (2010). Lindberg et al., 2002, observed a hydrogen evolution of a hupl- mutant of *Nostoc punctiformae* under aerobic condition. In *Fischerella* - T E1 with 10 per cent oxygen, hydrogen evolution completely ceased. Prabaharan and Subramanian (1996) reported that in *Phormidium valderianum* 20 per cent oxygen did not totally arrest the process of hydrogen evolution. In *Anabaena* - TE1 and *Nostoc* - TE1 oxygen at one and two per cent did not significantly inhibit hydrogen evolution and in contrast, oxygen at and above concentrations of one per cent drastically reduced hydrogen evolution in the non-heterocystous *Fischerella* - T E1.

Low oxygen tensions of one and two per cent did not inhibit hydrogen evolution in the heterocystous *Anabaena cylindrica* and oxygen tensions of five and ten per cent inhibited hydrogen formation (reference). In non-heterocystous *Oscillatoria* sp oxygen concentrations above one per cent inhibited hydrogen production (Philips and Mitsui, 1983). Potential of hydrogen production of *Nostoc linckia* IAM M-30 was found to be 0.17 mol.mg Chl $a^{-1}.h^{-1}$ in the presence of air **(**Masukawa et al., 2001). However, hydrogen production by *Anabaena variabilis* PK84 was observed as 0.11 mol. mg Chl $a^{-1}h^{-1}$ in aerobic conditions with 2% CO_2 in outdoor conditions **(**Fedorov et al., 2001). Again, the oxygen tolerance for hydrogen production varied with organisms.

Acknowledgments

The authors sincerely thank the STDF (Science and Technology Development Fund, Egypt) for financing the project and its administration for excellent follow up and guidance.

References

Allen, M. M., and A. J. Smith. 1969. Nitrogen chlorosis in blue-green algae.Arch. Mikrobiol. 69:114–120.

Bothe H and T. Kentemich. 1990. Potentialities of H_2 production by cyanobacteria for solar energy conversion programs. In: Hydrogen Energy Progress VIII. Proc. 8th World Hydrogen Energy Conference, (eds.) T.N. Veziroglu and P.K. Takahash, USA. p. 729.

Canto de loura,I ,Dubacq J.P., and Thomas ,J.C.,. The Effects of Nitrogen Deficiency on Pigments and Lipids of Cyanobacteria, Plant Physiol. (1987) 83, 838-843

Ducat D.C., Way J.C. and Pamela A., Silver Engineering cyanobacteria to generate high-value product, *Trends Biotechnol.*, 29(2), 95-103, (2011).

Elmorjani,K and Herdman,M (1987):Metabolic Control of Phycocyanin Degradation in the Cyanobacterium *Synechocystis* PCC 6803: a Glucose Effect, *Journal of General Microbiology* 133, 1685-1694

Fedorov A.S., Tsygankov A.A., Rao K.K.and Hall D.O., Production of hydrogen by an *Anabaena variabilis* mutant in photobioreactor

Foulds, I. J., and N. G. Carr. 1977. A proteolytic enzyme degrading phycocyanin in the cyanobacterium *Anabaena cylindrica*. FEMS Microbiol. Lett. 2:117–119.

Ghirardi M.L., Posewitz M.C., Maness P.C., Dubini A., Yu J. and Seibert M.,Hydrogenases and hydrogen photoproduction in oxygenic photosynthetic organisms, *Annu.Rev. Plant Biol.*, 58 : 71-91, (2007).

Ghirardi ML, Togasaki RK, SeibertM (1997) Oxygen sensitivity of algal H2- production. Appl Biochem Biotechnol 63–65:141–151.

Happe T, Kaminski A (2002) Differential regulation of the Fe-hydrogenase during anaerobic adaptation in the green alga Chlamydomonas reinhardtii. Eur J Biochem 269(3):1022–1032

Jeberlin Prabina,B and Kumar,K (2010): Studies on the optimization of cultural condition for maximum hydrogen production by selscted cyanobacteria.ARBN Journal of agricultural and Biological science ,vol5,No 5 ,22-31

Kondratieva E.N. 1983. Production of molecular hydrogen in microorganisms. In: Advances in Biochemical Engineering Biotechnology (ed.) A. Fiechter, Springer-Verlag, New York. pp. 139-180.

Kumazawa, S. and A. Mitsui, 1981. Characterization and optimization of hydrogen photoproduction by a saltwater blue-green alga, *Oscillatoria* sp. Miami BG7. I. Enhancement through limiting the supply of nitrogen nutrients. Int. J. Hydrogen Energy, 6: 339-348

Lefort-Tran M, Pouphile M, Spath S, Packer L. Cytoplasmic membrane changes during adaptation of the freshwater cyanobacterium *Synechococcus* 631 1 to salinity. Plant Physiol. 1 988; 7: 767-775.

Lindberg, P., Schutz, K., Happe, T., Lindblad, P., (2002).A hydrogen-producing,hydrogenase-free mutant strain of Nostoc punctiforme ATCC 29133. Int. J. Hydrogen Energy 27, 1291–1296.

Markov SA, Bazin MJ, Hall DO: Hydrogen photoproduction and carbon dioxide uptake by immobilized Anabaena variabilis in a hollow-fiber photobioreactor. *Enzyme Microbial Technol* 1995,17:306-310.

Masukawa H., Nakamura K., Mochimaru M. and Sakurai H., Photobiological hydrogen production and nitrogenase activity in some heterocystous cyanobacteria, *BioHydrogen II. Elsevier*, 63-66, (2001).

McNeely K., Xu Y., Bennette N., Bryant D.A. and Dismukes G.C., Redirecting reductant flux into hydrogen production viametabolic engineering of fermentative carbon metabolism in a cyanobacterium, *Appl. Environ. Microbiol.*, 76 (15) 5032-5038, (2010).

Melis, A., 2007. Photosynthetic H2 metabolism in *Chlamydomonas reinhardtii* (unicellular green algae). Planta 226, 1075–1086.

Melis, A., Happe, T., 2001. Hydrogen production. Green algae as a source of energy.Plant Physiol. 127, 740–748.

Melis, A., Zhang, L.P., Forestier, M., Ghirardi, M.L., Seibert, M., 2000. Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga Chlamydomonas reinhardtii. Plant Physiol.122, 127–135.

Miyamoto, K., P.C. Hallenbeck and J.R. Benemann, 1979. Hydrogen production by the thermophilic alga *Mastigocladius laminosus*: Effects of nitrogen, temperature and inhibition of pliotosynthesis. Applied Environ. Microbiol., 38: 440-446.

Oxelfelt F, Tamagnini P, Salema R, Lindblad P. Hydrogen uptake in Nostoc strain PCC 73102: effects of nickel, hydrogen, carbon and nitrogen. Plant Physiol Biochem 1995;33:617–23.

Philips E.J and A. Mitsui. 1983. Photoproduction of hydrogen by *Oscillatoria* sp. strain Miami BG 7. Appl. Environ. Microbiol. 45: 1212.

Philips, E.J. and A. Mitsui, 1986. Characterization and optimization of hydrogen production by a salt water blue-green alga *Oscillatoria* sp. Miami BG 7. II. Use of immobilization for enhancement of hydrogen production. Int. J. Hydrogen Energy, 11: 83-89

Prabaharan D and G. Subramanian. 1996. Oxygen-free hydrogen production by the marine cyanobacterium *Phormidium valderianum* BDU 20041. Biores. Technol. 57: 111-116.

Richaud,C. Zabulon,G. Joder,G. and Thomas,J.C. Nitrogen or Sulfur Starvation Differentially Affects Phycobilisome Degradation and Expression of the *nblA* Gene in *Synechocystis* Strain PCC 6803, Journal of bacteriology, May 2001, p. 2989–2994

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 1979;111:1–61.

Rippka R, Herdmann M. 1 993. Pasteur culture collection of cyanobacterial strains in axenic culture.Vol. 1 Catalogue of Strains 1 03 P. Institut Pasteur, Paris, France.

Rupprecht J, Hankamer B, Mussgnug JH, Ananyev G, Dismukes C,

Kruse O. (2006) Perspectives and advances of biological H2 production in microorganisms. Appl Microbiol Biotechnol 72(3):442–449.

Stevens,S.E.,Balkwill D. L, and Paone D. A. M:1981 The effects of nitrogen limitation on the ultrastructure of the cyanobacterium *Agmenellum quadruplicatum*. Arch. Microbiol. 130:204–212.

Tamagnini P, Axelsson R, Lindberg P, Oxelfelt F, W0unschiers R, Lindblad P. Hydrogenases and hydrogen metabolism of cyanobacteria. Microbiol Mol Biol Rev 2002;66:1–20.

Tsygankov AA, Borodin VB, Rao KK, Hall DO. H2 photoproduction by batch culture of Anabaena variabilis ATCC 29143 and its mutant PK84 in a photobioreactor. Biotechnol Bioeng 1999;64:709–15. under aerobic outdoor conditions, *BioHydrogen II. Elsevier*, 223-228 (2001).

Vass.I,Styring.S,Hundel.T.,Koivuniemit A,Arot,E-M,and Andersson , B: Reversible and irreversible intermediates during photoinhibition of photosystem II: Stable reduced QA species promote chlorophyll triplet formation. Proc Natl Acad Sci USA 89(4):1408–1412.

Wood,N.B., and Haselkorn, R. H. 1980. Control of phycobiliprotein proteolysis and heterocyst differentiation in *Anabaena*. J. Bacteriol. 141:1375–1385.

Yamanaka, G., and A. N. Glazer. 1980. Dynamic aspects of phycobilisome structure. Phycobilisome turnover during nitrogen starvation in *Synechococcus* sp. Arch. Microbiol. 124:39–47.

Volgusheva ,A, Styring,S., and Mamedov,F.(2013): Increased photosystem II stability promotes H2 production in sulfur-deprived *Chlamydomonas reinhardtii*.PNAS ,vol110,no18,7225

Jagadeeswaran,G, Li, Y-F and Sunkar,R.: (2014):Redox signaling mediates the expression of a sulfate-deprivation-inducible microRNA395 in Arabidopsis .The Plant Journal , 77, 85–96

Collier, J. L., and A. R. Grossman. 1992. Chlorosis induced by nutrient deprivation in Synechococcus sp. strain PCC 7942: not all bleaching is the same. J. Bacteriol. 174:4718–4726.

Hifney A. F, Issa A.A,. Fawzy M.A: Abiotic stress induced production of ß-carotene,allophycocyanin and total lipids in *Spirulina* sp. J. Biol Earth Sci 201 3; 3(1): 54-64