International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 5, Issue 6 - 2018

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2018.05.06.017

Taxonomical Consideration, Biological and Biochemical Effect on Growth (Chlorophyll-a) and Nitrogenase activity(n mole C_2H_4/ µg chl-a / h) of *Scytonema bewsii* Fritsch Et.Rich under the Family Scytonemataceae (Cyanobacteria) Studied under Culture

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Abstract

The present research work of **Biological and Biochemical Effect on Growth(Chlorophyll-a)**, **Nitrogenase activity** (n mole C_2H_4/ µg chl-a / h) and mainly **different conditional** morphological development of ***Scytonema bewsii* Fritsch Et.Rich under the** family **Scytonemataceae (CYANOBACTERIA)** which are heterocystous, multicellular and filamentous Cyanobacteria deals with the observations of natural and isolated strains from various localities of diversed habitats. Their different characters observed in diffent conditions thoroughly studied in morphological, physiological and biochemical work under suitable laboratory cultural conditions. These strains showed maximum polybranches and geminate, minimum single with terminal heterocyte or heterocyst in nitrogen deficient medium and more lateral geminate branches in nitrogenous liquid as well as solid medium. Hormogonia are very less, mostly with intercalary heterocyte. All the terminal heterocyte bearing hormogones and filaments showed mucilaginous hollow papillae at the apical parts specially in liquid medium, apically broader and highly constricted, posses with 5-8 celled meristematic zones in nitrogen deficient medium, apically broader cell and yellowish in colour in nitrogen deficient medium whereas in nitrogenous medium apical cell oblong and blue-green in colour.

Physiological Characterization of Biological and Biochemical Effect on Growth and Nitrogenase activity of ***Scytonema bewsii*,** the growth(Chlorophyll-a) increased in all different days successively and maximum increased on 30th day. **Nitrogenase activity were** in terms of n mole C_2H_4/µg chl. a/h and in terms of n mole C_2H_4/vial/h showed maximum increase on 10^{th}days and decreased on 20^{th} and 30^{th} days successively.

Keywords: *Scytonema*, Heterocyst, Taxonomy, Nitrogenase activity, Falsebranch, Cyanobacteria, Scytonemataceae.

1. Introduction

The genus *Scytonema***(BGA)** first described by Agardh in1824 and the family described by Robenhorst in1865. ***Scytonema bewsii* Fritsch Et. Rich under the** family **Scytonemataceae (Cyanobacteria)** are very common in different types of rice growing fields. Geitler (1925, 1932) also supported the same concept, *Scytonema* plays an important role in ecology maintaining the soil fertility and increasing rice growth yield as a bio-fertilizer.

Fritsch (1945) and Desikachary (1959), also supported the same view as followed by Geitler (1932).The nitrogen fixing potential of this organism is of great significance for enriching of nitrogen level in soil. The ecological conditions *of* rice fields are more suitable environment for the growth of these organisms. De (1939) and Singh (1942) indicated that one of the probable causes for the ability of rice to grow year after year in the absence of manure, is due to fixation of atmospheric nitrogen by Cyanobacteria. Singh in his treatise 'Role of Blue-Green Algae in nitrogen economy of Indian agriculture' discussed in detail general characteristics of the Cyanobacteria, ecology of various communities and their nitrogen fixation in rice fields, reclamation of usarlands and conservation of other soils including sugar cane fields, maize fields, rabi fallows and grass lands.

Jeeji Bai (1977) *Scytonema stuposum* in culture medium showed various changesits morphological variations .Pandey (1974) observed in *Scytonemopsis ghazipurensis* and Anand & Gunaseeli (1978b) worked on *Scytonema* and *Tolypothix* in different culture media found different growth media could be identified.Komarek and Anagnostidis (1988) observed the germination of hormogonium either isopolar or heteropolar andreferred the International Association for Cyanophyte Research (IAC) and needed more intransitive cultural study under various conditions for proper identifications and suggested not merely on the basis of the natural material. The present study of **Biological and Biochemical Effect on Growth, Nitrogenase activity** and mainly **different conditional** morphological descriptions observed in details of ***Scytonema bewsii* Fritsch Et.Rich under the** family **Scytonemataceae.**

2. Materials and Methods

2.1. Isolation and Maintaine of strains

The present cultural study belonging to the ***Scytonema bewsii* Fritsch Et.Rich under the** family **Scytonemataceae** in total 22 strains, isolated from natural rice growing fields of some districts of Uttar Pradesh and West Bengal.

In the beginning different culture media have been used and among them BG11 medium (Stanier et. al., 1972) showed better growth. Therefore, during the study, all the experiments performed and maintained in BG11 liquid and solid medium.

2.2 Preparation and maintenance ofculture media (Plate-4&5) :

To critical study the morphological features of the selected strain ***Scytonemabewsii* Fritsch Et.Rich** under the family Scytonemataceae. The media have been used of ammonical nitrogen and Nitrate nitrogen. The quantity of $NH_4Cl$and $NaNO_3$ for different concentrations have taken as (i) without nitrogen means Nitrogen deficient medium (-N;) (ii)Normal concentration of ammonical nitrogen and nitrate nitrogen (+N; 20 mM). (iii)More concentration of ammonical nitrogen and nitrate nitrogen (+1.5N; 30 mM). (iv)Rich (+2N; 40 mm) and high (+4N; 80 mM) concentrations of ammonical nitrogenand nitrate nitrogen respectively. The final pH range was adjusted at 7.5. This medium wasone for solidified agar slant and other for liquid medium and also with in sterile soils in a petridish and incubated for 25-30 days at 32 ± 2º C.and 4000-5000 Lux light intensity under 14/10 LD cycle .

2.3. Morphological observation and identifications

Morphological observations were studied with the help of Nikon and Motic microscopes with attaching photosystem.

2.4. Identification of the strains :

The taxonomic identification of the strains for morphological studies have been made after comparing with description of the standard monograph Geitler(1932); Desikachary(1959); Starmach, (1966); Komarek and Anagnostidis(1988) and according to our present studies.

2.5. Determination of growth Chlorophyll-a (µg/ml)

The replicates containing 10 ml of liquid medium were in the test tubes. The assessment of the growth i.e., Chllorophyll-a(De Marser and Hawmard, 1988) after the experiments of different conditions after the definit period of time i.e. 10^{th} day, 20^{th} day and 30^{th} day respectfully.

Three replicates in all experiments were performed.

2.6. Estimation of Nitrogenase activity (Acetylene Reduction Assay)

In nitrogen-deficient cultures medium, Nitrogenase activity was analysed into the tastetubegrown.at the exponential stage of growth. The activity of Acetylene Reduction Assay (ARA) (Kaushik and Venkataraman-1983) was measured in terms of using Gas Chromatograph (Amil- Nucon model-5700) with para pack N and T columns (Stewart et al., 1967). 10% of Acetylene equivalent of the total air space was injected into a glass vial of 15 ml capacity.

The replicates allvials at 28 ± 2 ^{0} C under 4000 – 5000 lux light intensity.were stopped with subseals and incubated for 120 minutes.The reaction was stopped by injecting 0.1 ml of 50 % Trichloro acetic acid (TCA) and the gas phase was analyzed for ethylene and the activity was expressed as n mole C_2H_4/ µg chl / h.. All the Experiments in three replicates were performed .

3. Results

3.1. Habits and Habitat

It is a cushion forming terrestrial alga which is growing on moist soil of rice fields and collected different districts of Uttarpradesh and WestBengal during harvesting period in the form of spreaded membranaceous thallus.

3.2. Thallus and Growth (Plates-4&5)

The young thallus looks greenish in colour by the naked eyes but under microscope it appear light blue green at the young and mature stage. However old filaments or growth appears brown in liquid medium and brownish blue-green in dried conditions. It forms cushion like large thallus (7-13 mm in length) which attain a height of 1-2 mm on 30th day.

Large clusters of growth appear on agar slant and their filaments do not penetrate into agar medium. Whereas, it usually grows in patches free floating and their branches grow in all directions in liquid medium. Under experimental conditions, the generation time varies between 3 and 4 days in nitrogen deficient medium. The growth in nitrogenous medium is 2.7 times more on 15th day as compared to nitrogen deficient condition. Maximum growth appears in two times nitrate nitrogen medium and two times ammonical nitrogen suppressed the growth.

3.3. Filaments and Sheath

The filaments are thick and vary 14-22 (-28) µm diameter in different cultural compositions. It is 14-18 µm broad (narrower) in nitrogen deficient medium, 18-20 µm in normal nitrogen medium and upto 22 µm (-28) (broader) in rich (2N) nitrogen medium. There are longer filaments in nitrogen deficient medium than nitrogenous medium.

Sheath is thick, hyaline and unlamellated in young stage and become yellowish and lamellated at mature and old growth. Maximum thick medium sheath and coloured appears in solid growth than the growth present in liquid medium.

3.4. Vegetative cells and Hormogonia (Plates-4&5)

Generally, the shape of the vegetative cells is quadrate to rectangular which have variability in different composition of medium and different age conditions e.g. usually more cylindrical shape of cells is in nitrogen deficient grown growth. They vary in size from 7.5-14 µm long; 10-14µm long in nitrogen deficient medium, 9-11 µm in nitrogenous medium and shorter upto 7.5 µm in rich (2N) nitrogenous medium. They have also variation in their broadness under different ingradients of medium i.e. narrower 11.5-13 µm broad in nitrogen deficient medium. Normal 13-14.5 µm broad in nitrogenous medium and 13.5- 16 µm broad in rich (2N) nitrogen medium. Therefore average range of their diameter is 11.5-16 µm.

In comparision to other strains, the frequency of hormogonia in the parent alga is maximum hormogonia (long 10-14 celled) released in nitrogen deficient medium and minimum sized (short 5-9 celled) hormogonia are produced in rich (2N) nitrogenous medium.

3.5. Hormogonia and Heterocytes (Plates-4&5)

Usually, early released hormogonia are 10-14 celled longer in nitrogen deficient medium which have straight and irregular shape. In these hormogones, the intercalary heterocyte develop at first where they become up to 30 celled long. Whereas, terminal heterocytes develop in very short hormogones (upto 15 celled long). The frequency of intercalary heterocyte in hormogones varies 50-60% and remains show always with terminal and terminal with a intercalary hetreocytes with in hormogones which develops after sometime of terminal heterocyte. It is interesting that terminal heterocytes are also appears very Less in number in normal nitrogenous medium in liquid and solid condition but frequency is more in liquid medium. It is observed that occasionally a few hormogones with intercalary heterocyte posses with a hallow mucilaginous papilla at the apical ends in nitrogendeficient liquid medium. The apical parts of these hormogones are gradually broder and highly constricted in nitrogen deficient medium in place of slightly constricted with similar thickned hormogones present in nitrogenous medium.

Maximum heterocytes frequency is observed in nitrogen deficient liquid medium and they are also present in rich (2N) nitrogen medium. Their shape vary from rectangular sometimes, they also appeared in pairs at the base of single branches. But between the two heterocytes may be occupied by 2-81 vegetative cells. The average ratio of vegetative cells and heterocytes is 14:1. Usually intercalary and basal heterocytes are present at the base single branching and also in germinating hormogones. Hormogones with terminal heterocyte or detached single branches from polybranches sometimes confuse with the genera of *Tolypothrix*. At several places, long cylindrical heterocytes devide and a common polar nodules appears in medium cross walls of these heterocyte. They have distinct yellow in colour and marked polar nodules under both nitrogenous and nitrogen deficient medium. During the growth phase one pored basal heterocyte and two pored intercalary heterocyte appear usually single or in chain (upto 7) in nitrogen deficient medium and possess straight or roundish cell wall .

3.6. False Branches (Plates-4&5)

The false branches are common in all medium but their frequency varies according to variations in ingradients of medium. However, 25% more branches are observed in liquid than solid condition in nitrogen deficient medium. The branching present in nitrogen deficient and more in normal nitrogenous and maximum in rich (2N) nitrogenous media which are 4% and 20% more branching in liquid and 3% and 7% more branching in solid conditions respectively. In nitrogen deficient liquid medium, filaments develop usually polybranches, geminate and single branhes. The more polybranch formation takes place in liquid than in solid, and in nitrogen deficient than other media. The percentage of all types of branches are 32% polybranches, 19% lateral geminate branches, 15% cross geminate 20% single with terminal heterocytes and 14% single without terminal heterocytes in nitrogen deficient liquid medium. It is observed that formation of polybranches and geminate branches are restricted only in central part of the filament. In solid nitrogen deficient medium the percentage are 54%, 6%, 24% and 16% of geminate, polybranches, single with terminal heterocyte and single without terminal heterocytous branching respectively. Single or many terminal heterocytes are very common at the basal part of polybranches and single branches in nitrogen deficient liquid medium. Mostly a hyaline hollow mucilaginous Papilla appears at the apical part of the single heterocytous branches **(Plates-4&5).**

3.7. Apical Part andMeristematic zone and Apical cell(Plates-4&5):

A distinct part of 5-8 celled meristematic zone differentiated in terminal and subterminal position at terminal parts of each filaments. In the present alga, it is gradually broader and highly constricted than other parts of the filaments. It is colourless due to less reserve food materials. The meristematic zone differentiation is absent in normal (N) and rich (2N) nitrogenous medium grown filaments.

It is broadly rounded, hemispherical and yellowish in colour and possesses a hollow mucilaginous Papilla with circumferential constrictions specially in nitrogen deficient liquid medium whereas in normal(N) nitrogenous and rich (2N) nitrogen medium (liquid and solid both) apical cells are oblong and without distinction of colour from other vegetative cells and without mucilaginous papillae.

3.8. Perennating Bodies and Germination (Plates-4&5)

In nitrogen deficient medium formation of perennating bodies take place at many parts of filaments in old

growth by the granulation and development of deep greenish colour and these parts segregate in several parts due to formation of dead cells. However, in case of nitrogenous media perennatdting structures differentiate from parts of the filaments by the development of more granulated and spirally irregular broader trachomas with sheath.

In the present alga, formation of maximum branching takes place in nitrogen deficient medium which have moltly terminal heterocytes in chain (upto 7) are present mostly in all single branching while in nitrogenous medium branches become irregular and they are mostly geminate to polybranches. In some places, branches are irregular geminate due to presence of continuous growth.

3.9. Life Cycle Pattern(Platess-4&5)

In nitrogen deficient liquid medium, maximum hormogones appeared with intercalary heterocyte and some with terminal heterocyte which have mucilaginous hollow papillae at their apical parts also. Whereas, in solid medium maximum branches are polybranches and geminate; and minimum are single (with terminal heterocytes) branches. The hormogone frequency is very less in nitrogenous medium. Those hormogones present in such medium always produce maximum geminate and minimum single branching. Irregularly compressed and granulated parts of the trichomes form perennating structures after separation with help of dead cells through the remaining part of the trichomes. After germination, these perennating bodies produce maximum single branches with terminal heterocyte in nitrogen deficient liquid medium while in nitrogenous medium, they develop irregular branches (geminate, single and polybranches).

Table.1. In nitrogen deficient medium ***Scytonema bewsii*** showed growth (Chl.a µg/ml) in different days.

	10th Day	20th Day	30th Day
Cont.	0.097	0.403	0.569

(---) Growth absent.

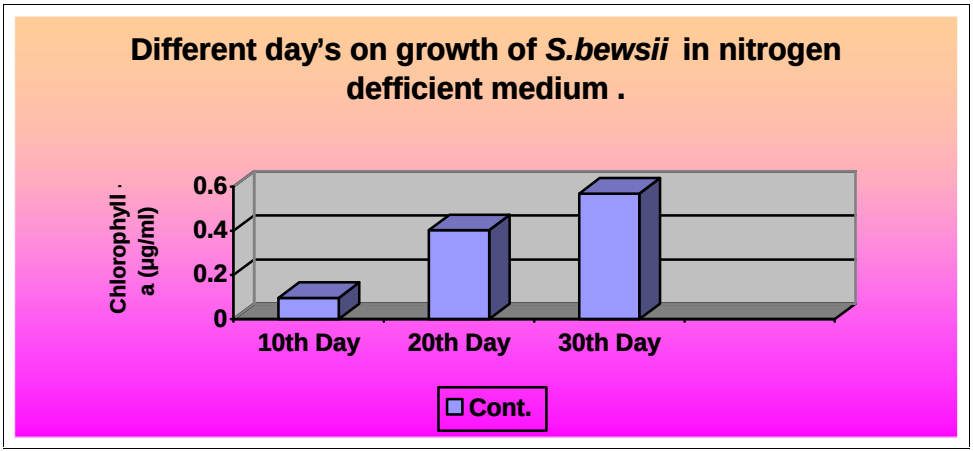


Figure-1. Different day's on Growth (Chl.a µg/ml) of ***Scytonema bewsii***

Table 2. In nitrogen deficient medium *Scytonema bewsii* **showed Nitrogenase activity (n mole C_2H_2/µg chl. a/h) in different days.**

	10th Day	20th Day	30th Day
Cont.	1.73	1.29	0.61

(---) nitrogenase activity absent.

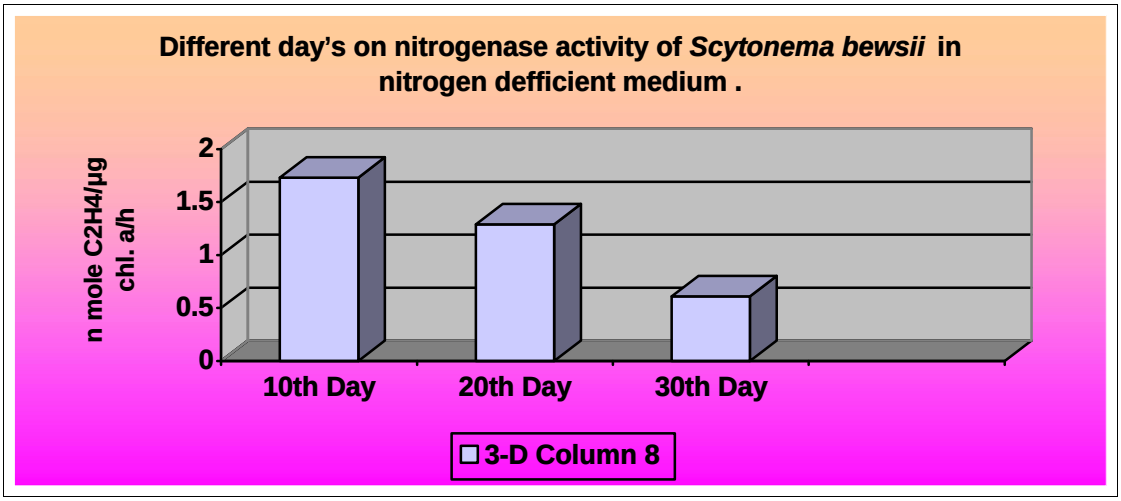
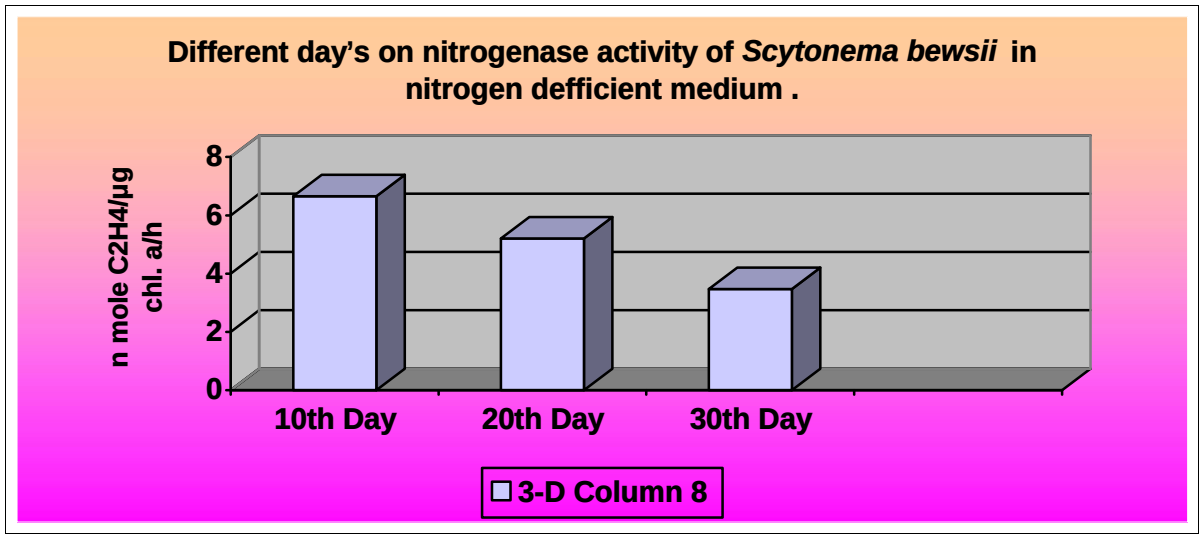


Figure-2. In nitrogen deficient medium ***Scytonema bewsii*** showed Nitrogenase activity (nmole C_2H_2/µg chl. a/h) in different days.

Table 3. In nitrogen deficient medium *Scytonema bewsii* showed Nitrogenase activity in term of n mole C_2H_4/vial /h) in different days.			
	10^{th} Day	20^{th} Day	30^{th} Day
Cont.	6.65	5.20	3.47
(---) nitrogenase activity absent.			



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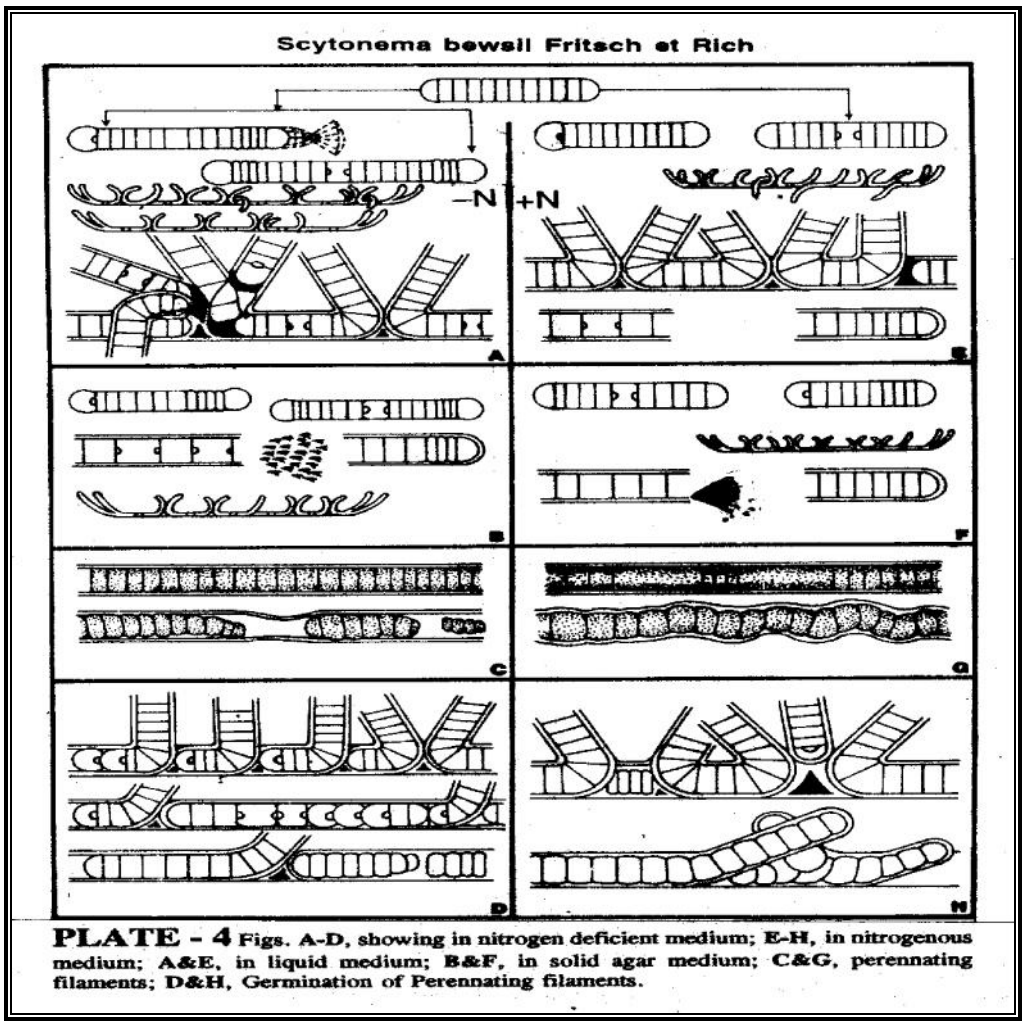

PLATE – 4 Figs. A-D, showing in nitrogen deficient medium; E-H, in nitrogenous medium; A&E, in liquid medium; B&F, in solid agar medium; C&G, perennating filaments; D&H, Germination of Perennating filaments.

4. Discussion

In India, during last few years a lot morework has been done on the development of Cyanobacterial biofertilizer technology. More efficient region specific strains with known capabilities of biomass production, nitrogenase activity and pesticide tolerance are being isolated and used for algalization. **Fogg (1949)** suggested that the substance, which prevents heterocyst formation, is ammonia itself or some substances readily derived from ammonia. He postulated that their exist concentration gradients of the inhibitory substances along the length of the filament and heterocyst are formed at the points of lowest concentration of the substance. The view was further substantiated by **Pandey and Mitra (1962)** that heterocysts are differentiated only when the level of combined nitrogen is below a critical concentration.

Scytonema bewsii **Fritsch Et. Rich under the** family **Scytonemataceae (Cyanobacteria)** are very common in different types of rice fields.**Rao, C.B (1973b)** reported it for the first time from India which was growing on mud settled down on rocks near dam in Latif dam near Banaras (U.P.).Further, **Khan and Mathur (1974)** reported from paddy fields, Majra village, near Element town, Dehradun (U.P.). It was also recorded from Mansar Lake, among other algae on rock surface, Jammu (J&K). However, from south India **Srinivasan (1963)** collected from moist soil near Melkote, Mysore (Karnataka).

The young thallus looks greenish in colour by the naked eyes but under microscope it appear light blue green at the young and mature stage. However old filaments or growth appears brown in liquid medium and brownish blue-green in dried conditions. It forms cushion like large thallus (7-13 mm in length) which attain a height of 1-2 mm on 30th day.

The filaments are thick and vary 14-22 (-28) µm diameter in different cultural compositions. It is 14-18 µm broad (narrower) in nitrogen deficient medium, 18-20 µm in normal nitrogen medium and upto 22µm

(-28) (broader) in rich (2N) nitrogen medium. Generally, the shape of the vegetative cells is quadrate to rectangular which have variability in different composition of medium and different age conditions e.g. usually more cylindrical shape of cells is in nitrogen deficient grown growth.

Usually, early released hormogonia are 10-14 celled longer in nitrogen deficient medium which have straight and irregular shape. In these hormogones, the intercalary heterocyte develop at first where they become up to 30 celled long. Whereas, terminal heterocytes develop in very short hormogones (upto 15 celled long). The frequency of intercalary heterocyte in hormogones varies 50-60% and remains show always with terminal and terminal with a intercalary hetreocytes with in hormogones which develops after sometime of terminal heterocyte.

The false branches are common in all medium but their frequency varies according to variations in ingradients of medium. However, 25% more branches are observed in liquid than solid condition in nitrogen deficient medium. The branching present in nitrogen deficient and more in normal nitrogenous and maximum in rich (2N) nitrogenous media which are 4% and 20% more branching in liquid and 3% and 7% more branching in solid conditions respectively. In nitrogen deficient liquid medium, filaments develop usually polybranches, geminate and single branhes.

A distinct part of 5-8 celled meristematic zone differentiated in terminal and subterminal position at terminal parts of each filaments. In the present alga, it is gradually broader and highly constricted than other parts of the filaments. It is colourless due to less reserve food materials. The meristematic zone differentiation is absent in normal (N) and rich (2N) nitrogenous medium grown filaments.

In nitrogen deficient medium formation of perennating bodies take place at many parts of filaments in old growth by the granulation and development of deep greenish colour and these parts segregate in several parts due to formation of dead cells. However, in case of nitrogenous media perennatdting structures differentiate from parts of the filaments by the development of more granulated and spirally irregular broader trachomas with sheath.

Physiological Characterization the growth of alga increased in all different days successively and maximum increased was on 30th day. ***Scytonema bewsii*** showed the growth appeared in nitrogen deficient culture medium in three different days,i.e., chlorophyll-a 0.097 µg /ml, 0.403 µg /ml and 0.569 µg /ml in 10^{th} and 20^{th} and 30th day respectively.

Nitrogenase activity were in terms of n mole C_2H_4/µg chl. a/h increased maximum the nitrogenase activity on 10th days and decreased on 20^{th} and 30^{th} days successively. Nitrogenase activity in terms of per n mole C_2H_4/ vial/ h also shows same trends as it was in 10 ppm concentration cause maximum increase (53.08%) as compared to control on 10th day.

In nitrogen deficient liquid medium ***Scytonema bewsii*** showed Nitrogenase activity (n mole C_2H_4/µg chl. a/h) in different days. Nitrogenase activity in terms of n mole C_2H_4/µg chl. a/h showed 1..73 µg ,1.29 µg and 0.61 µg in three different 10^{th} ,20^{th} and 30^{th} day respectively.

In nitrogen deficient liquid medium Nitrogenase activity in terms of n mole C_2H_4/vial /h showed i.e., 6.65 µg, 5.20 µg and 3.47 µg in three different i.e., 10^{th} ,20^{th} and 30^{th} day respectively.

5. Conclusion

Scytonema bewsii **Fritsch Et. Rich** Its branches maximum polybranches and geminate, minimum single (with terminal heterocyte) in nitrogen deficient medium; The range of length is 7-13 mm and breadth is 14-28 µm and length is 7-13 mm; more lateral geminate branches in nitrogenous liquid as well as solid medium; hormogonia less, mostly with intercalary heterocyte, a few with terminal heterocytes; all the terminal heterocyte bearing hormogones and filaments and a few otherfilaments show mucilaginous hollow papillae at the apical parts specially in liquid medium; apically broader and highly constricted, posses with 5-8 celled meristematic zones in nitrogen deficient medium, apically broader cell or hemispherical and yellowish in colour in nitrogen deficient medium whereas in nitrogenous medium apical cell oblong and blue-green in colour as the other vegetative cells; germinating filaments produce mostly single (with terminal heterocytes single or in chain upto 7) branching in nitrogen deficient condition whereas irregular geminate branches in nitrogenous condition.

Physiological Characterization the growth of alga increased in all different days successively and maximum increased was on 30th day. **Nitrogenase activity were** in terms of n mole C_2H_4/µg chl. a/h and in terms of n mole C_2H_4/vial /h showed increased maximum on 10th days and decreased on 20th and 30th days successively.

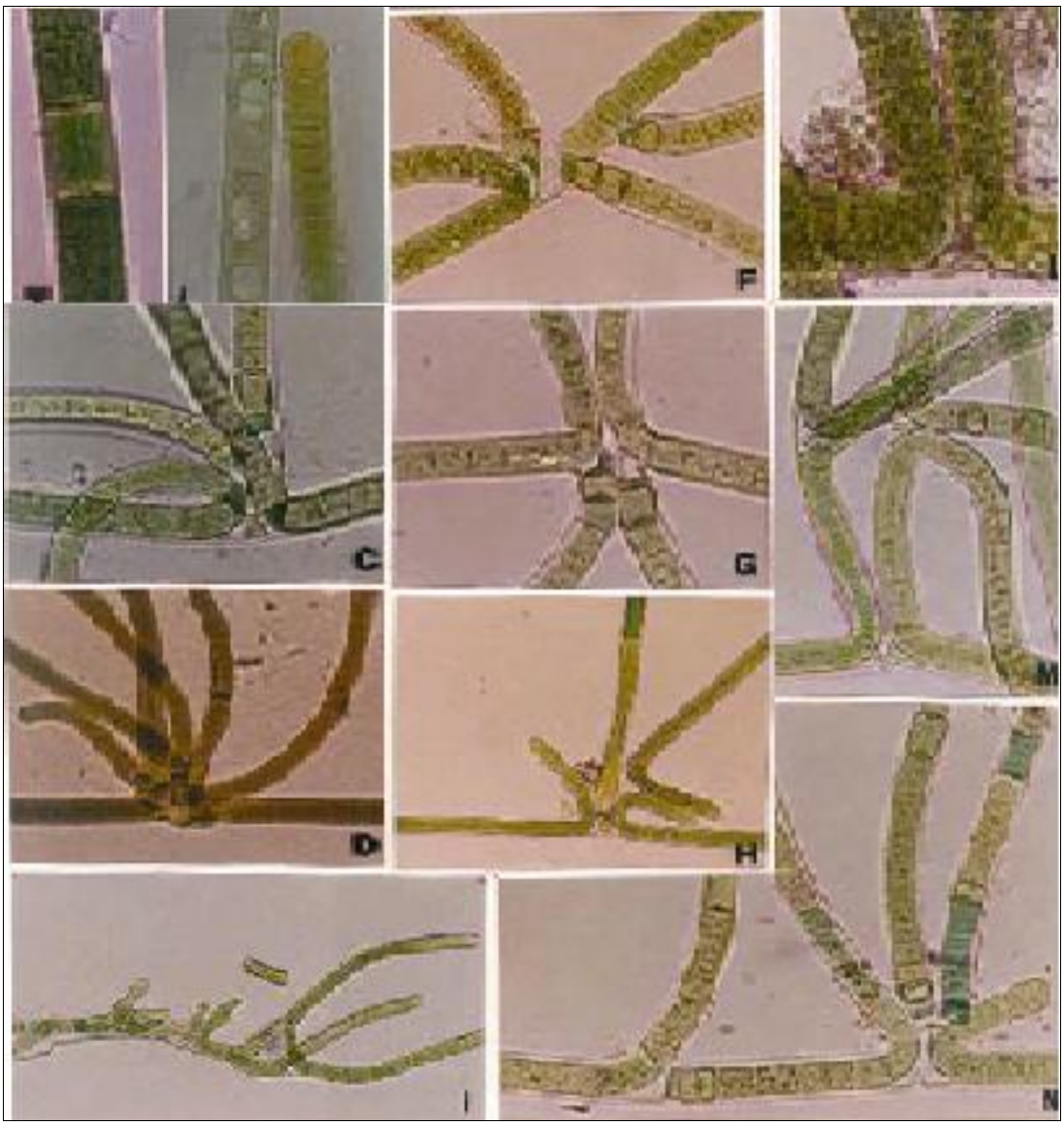

PLATE -5: ***Scytonema bewsii*** showing **polybranching, Single (Terminal or basal heterocytous)** branching and **repeated geminate branching.**

Conflict of Interest

The author of this paper have no conflict of interest

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How to cite this article:
Nikhil Chandra Halder and Giridhari Lal Tiwari. (2018). Taxonomical Consideration, Biological and Biochemical Effect on Growth(Chlorophyll-a) and Nitrogenase activity(n mole C_2H_4/ µg chl-a / h) of *Scytonema bewsii* Fritsch Et.Rich under the Family Scytonemataceae(Cyanobacteria)Studied under Culture
. Int. J. Adv. Res. Biol. Sci. 5(6): 153-162.
DOI: http://dx.doi.org/10.22192/ijarbs.2018.05.06.017