OBSERVATIONS ON NITROGEN FIXATION AND ORGANIC MATTER PRODUCED BY *ANABAENA CIRCINALIS* RABH. AND THEIR SIGNIFICANCE IN RICE CULTURE

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Introduction

In the following account the nitrogen content, the nitrogen-fixing capacity, extra-cellular nitrogen liberated and the total organic matter produced by the blue-green alga, *Anabaena circinalis* Rabh., are reported. This species does not appear to have been dealt with in this regard earlier.

Methods

The material was isolated and cultured by one of us (R. S.) as reported earlier (Subrahmanyan and Sahay, 1964). Details of methods employed are described in that account. In the present instance, two series of experiments were carried out simultaneously: (i) in Kjeldahl flasks under sterile conditions and (ii) in petri dishes. The latter was meant more for determining total organic matter produced by this alga.

After a definite period, the total organic nitrogen was determined in one set of flasks and the second set was used for assessing extra-cellular nitrogen.

The petri-dish cultures were used for estimating total matter produced by growth, extra-cellular nitrogen as well as total nitrogen. Organic matter produced was estimated, after the period of incubation, by centrifuging and drying at 60° C. The dried alga was also used for estimating cellular nitrogen. The left-over medium was employed for assessing the extra-cellular nitrogen, and the total nitrogen was calculated from the sum of the two. The ratio of N fixed to dry weight of the alga was also calculated.

The period of incubation of 61 days and the quantity of culture medium used, 50 ml., were same in all the experiments.

Results and Discussion

Table I, A and B, shows the results from the petri-dish experiments. It is seen that organic matter produced amounts to 80 to 92 times the original weight of the inoculated alga, total organic nitrogen fixed between 4·7–6·5 mg., about five times the original weight, and extra-cellular nitrogen produced 0·604 mg. (average of 4 replications).

The results of experiments with Kjeldahl flasks are shown in Table II, A and B. It is seen that the amount of N fixed is 1·2–2·5 times the original dry weight of alga inoculated. The extra-cellular nitrogen amounted to 0·26 mg. (average of 4 replications).

From a comparison of the data shown in Tables I and II, it is evident that more N is fixed in the petri-dish cultures than in the flasks, just over twice the quantity of that in the flasks; the same applies to extra-cellular nitrogen also. It would appear that when the growing alga has a better access to the atmosphere as in the petri dishes, the growth is more as well as N fixed as this is proportional to growth. It is, therefore, permissible to infer that under the natural conditions of the field, the growth of the alga (applicable to other species as well) will be more efficient than in the laboratory cultures. In other words, N fixation and addition of organic matter by the blue-green algae are more pronounced under field conditions. In field experiments with inoculation of N-fixing blue-green algae at the rate of 2 gm./ha. estimations of uptake of N by the crop indicates that the algae are very efficient indeed under field conditions, their contribution alone amounting to 19 kg./N/ha. in a single crop season (Subrahmanyan and Sahay, 1964; Table III).

Hence, the dual capacity of the blue-green alga to fix atmospheric nitrogen and production of organic matter could be best exploited for enriching the rice field soils by conditioning the soil for their proper manifestation. This is borne out by the field and pot experiments conducted at the CRRI (unpublished data and published: Relwani, 1963; Relwani and Subrahmanyan, 1963; Subrahmanyan *et al.*, 1964 *a*, 1964 *b*, 1964 *c*; Relwani and Manna, 1964).

It may be mentioned here that, besides making available to the current crop the N fixed in the form of extra-cellular product, bulk of the organic matter produced by the algal growth remains in the soil and becomes available to the next crop as organic enrichment, the spores in them continuing the next generation of growth. Thus there is bound to be a gradual build-up of the fertility by blue-green algal activity. This has been substantiated in pot

TABLE I

Analytical data relaling to nitrogen fixation and growth in Anabaena circinalis *Rabh. in petri-dish*

A. Data relating to growth

No. of replications	Fresh wt. of alga inoculated mg.	Fresh wt. of alga after incubation mg.	Dry wt. of alga after incubation mg	Dry wt./Fresh wt.	(mean)	Calculated dry wt. of alga inoculated mg.	Dry wt. after growth/ Dry wt. inoculated	(mean)
(i)	34·0	2722	72	0·02645	0·02656	0·8993	80·06	84·72
(ii)	38·0	3256	86	0·02641		1·0036	85·69	
(iii)	42·0	3864	103	0·02666		1·1197	91·99	
(iv)	48·0	3895	104	0·02671		1·2821	81·12	

B. Data relating to nitrogen fixation

No. of replications	Dry wt. of after growth mg.	Intra-cellular nitrogen in algae after growth mg.	% N	(mean)	Calculated dry wt. of alga inoculated mg.	Nitrogen initially present mg.	Extra-cellular nitrogen mg.	Combined nitrogen mg.	Net nitrogen fixed mg.	N fixed/ dry weight inoculated	(mean)
(i)	72·0	4·248	5·9	5·6	0·8993	0·0531	0·531	4·780	4·7269	5·27	5·22
(ii)	86·0	4·799	5·2		1·0036	0·0522	0·574	5·373	5·3208	5·30	
(iii)	103·0	5·320	5·6		1·1197	0·0627	0·630	5·950	5·8873	5·26	
(iv)	104·0	5·880	5·7		1·2821	0·0731	0·686	6·566	6·4929	5·06	

TABLE II

nalytical data relating to nitrogen fixation in Anabaena circinalis *Rabh. in Kjeldahl flask*

A. Data relating to nitrogen fixation

Replications	Fresh weight of algae inoculated mg.	Calculated dry wt. of algae inoculated		% N	N initially present mg.	Total N after fixation mg.	Net amount of nitrogen fixed mg.	N-fixed/dry wt. inoculated	
		Facter	Weight in mg.						
(i)	29·5	0·02656	0·7835	5·6	0·0439	1·2516	1·2077	1·54	2·53
(ii)	10·0		0·2656		0·0149	1·2320	1·2171	4·58	
(iii)	16·0		0·4250		0·0238	1·1900	1·1662	2·74	
(iv)	39·0		1·0359		0·0580	1·3384	1·2804	1·24	

B. Data relating to extra-cellular N liberated

Replications	Fresh wt. of algae inoculated mg.	Calculated dry wt. of algae inoculated		Extra-cellular nitrogen in mg.	
		Factor	Weight in mg.		
(i)	38·0	0·02656	1·0093	0·294	0·26
(ii)	19·0		0·5046	0·252	
(iii)	29·0		0·7702	0·266	
(iv)	28·0		0·7437	0·238	

Date of inoculation: 24–3–1964; Date of estimation: 24–5–1964; Period of incubation: 61 days; Volume of N free media used: 50 ml.

experiments (Subrahmanyan *et al.*, 1964; De and Sulaiman, 1950); field trials conducted at CRRI are encouraging and the results will form the subject of another communication.

For the present it may be stated that the work carried out so far envisage the prospects of a schedule of rice cultivation by succession in certain regions: manuring to promote growth of blue-green algae for about three seasons and growing rice crops for two seasons following *without* any manuring at all. As the higher yield is consistently maintained, this method should prove very economical.

SUMMARY

An account of the nitrogen-fixing capacity, extra-cellular N liberated and total organic matter produced by the blue-green alga *Anabaena circinalis* Rabh. is given. Studies indicate that N fixed as well as organic matter produced are more under conditions as near to nature as possible. It is suggested that the blue-green algae could be best exploited for enrichment of the soil by conditioning the soil for the manifestation of *the indigenous flora* and/or for known *introduced forms*. Owing to the action of these algae in the building up of the fertility of the soil, the possibility of an economic schedule of growing crops in succession is indicated leading to beneficial results.

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