東北大学機関リポジトリ
TOUR
Tohoku University Repository
東北大学

CULTURAL AND PHYSIOLOGICAL STUDIES OF THE NITROGEN FIXING BLUE-GREEN ALGA, NOSTOC SPONGIAEFORME AG. II. THE INFLUENCE OF THE CHANGES OF HYDROGEN ION CONCENTRATION. ILLUMINATION AND AERATION ON THE GROWTH OF THE ALGA

著者	FUJIWARA Akio, OKUTSU Masahiko
journal or publication title	Tohoku journal of agricultural research
volume	10
number	3
page range	321-325
year	1959-11-25
URL	http://hdl.handle.net/10097/29288

CULTURAL AND PHYSIOLOGICAL STUDIES OF THE NITROGEN FIXING BLUE-GREEN ALGA, *NOSTOC SPONGIAEFORME AG.*

II. THE INFLUENCE OF THE CHANGES OF HYDROGEN ION CONCENTRATION, ILLUMINATION AND AERATION ON THE GROWTH OF THE ALGA

By

Akio FUJIWARA and Masahiko OKUTSU

Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, Sendai, Japan

(Received July 14, 1959)

Introduction

Blue-green algae are important organisms which support the maintenance of soil fertility in the paddy field and have the ability to carry out both photosynthesis and fixation of elementary nitrogen at the same time. For this reason nutritoinal and physiological studies are in progress in our laboratory. Some of the results of the experiments carried out to find tne conditions of the mass culture and to clarify their physiology are reported in this paper.

In the preceding paper (5), the composition of the cultural solution was examined in detail, then the modification of the cultural solution was performed. In the present paper, experiments were carried out to make clear the influence of the changes of hydrogen ion concentration, illumination and aeration on the growth under the cultural conditions.

Materials and Methods

As the algal strain inoculated to the cultural solution, *Nostoc spongiaeforme AG.* was applied as before. The cultural conditions except hydrogen ion concentration, illumination and aeration were the same as in the preceding paper (5). The new cultural solution fixed in the preceding report was used and the composition of the medium is illustrated in Table 1.

Table 1. The cultural solution

$Ca(NO_3)_2 \cdot 4H_2O$	100.00 g/l
K_2HPO_4	25.00 〃
$MgSO_4 \cdot 7H_2O$	62.50 〃
Na_2CO_3	20.00 〃
Fe(EDTA-Fe)	2.25 〃
H_3BO_3	7.15 mg/l
$MnSO_4 \cdot 4H_2O$	10.50 〃
$ZnCl_2$	1.00 〃
$CuSO_4 \cdot 5H_2O$	0.20 〃
$H_2MoO_4 \cdot H_2O$	0.10 〃
$CoSO_4 \cdot 7H_2O$	0.02 〃

The dry weight of the algal cells were adopted as the measure of the growth rate. The dry weight and the nitrogen content were respectively estimated through drying and the Kjeldahl method on an aliquot of the algal suspension at the end of the cultures. PH was measured by the glass electrode method.

Results and Discussion

1) Hydrogen ion concentration

The preceding paper made clear that the final pH appears to be between 7.0 and 7.5 in overwhelming numbers especially in the cultures of heavy growth, but, at that time, pH was adjusted to about 8.5 before the autoclaving after Allison's optimum (1) within which the alga make growth in all cultural stages. The present cultural experiment was planned to obtain the optimum pH range of *Nostoc spongiaeforme*, through adjusting pH of the cultural solution respectively to 7.00, 7.80, 8.30, 9.00, 9.70 ane 10.40 before the autoclaving. The cultural term was 12 days. The results are summarized in Table 2.

Table 2. PH experiment result.

Group		PH of before the autoclaving	PH of after the autoclaving	Final pH	Dry Wt. mg	Total N mg	Total N %	Fixed N mg
1.	Complete	7.00	6.60	6.40	122.4	10.86	8.88	9.32
2.	Complete	7.80	7.20	6.65	164.1	9.73	5.93	8.20
3.	Complete	8.30	7.30	6.70	171.2	11.34	6.62	9.81
4.	Complete	9.00	7.70	7.10	311.4	24.94	8.01	23.41
5.	Complete	9.70	8.20	6.90	294.0	21.76	7.40	20.23
6.	Complete	10.40	9.00	7.50	269.1	18.18	6.74	16.65
7.	-N	7.00	6.85	6.20	142.9	10.38	7.27	10.38
8.	-N	7.80	7.10	6.30	191.6	14.08	7.35	14.08
9.	-N	8.30	7.25	6.40	181.3	13.43	7.39	13.43
10.	-N	9.00	7.60	6.85	283.0	19.01	6.75	19.01
11.	-N	9.70	7.90	7.10	253.5	18.85	7.43	18.85
12.	-N	10.40	8.45	7.45	231.3	13.79	5.96	13.79

PH value decreased through the autoclaving of the cultural solution and the value descended with the increase of the pH one. The best result was obtained at the one adjusted to pH 9.00 before the autoclaving and the growth in pH 9.70, 10.40, 8.30, 7.80 and 7.00 were in turn insufficient. It seemed that the optimum range was a little lower than that of Allison's range (7.00—8.50), because the best growths were obtained at the one preadjusted to pH 9.00—9.70 before the autoclaving, pH 7.70—8.20 at the commence of the culture and pH 6.85—7.30 at the end of culture. Therefore, in the following experiments, the pH value of the cultural solution is preadjusted between 9.00 and 9.50 before the autoclaving.

2) The duration of the illumination

It is said that the alternation of the light and dark periods is necessary for sufficient growth of the higher plants. However, in our cultural methods for algae, perfect continuous illumination viz. 24 hr illumination per day was used, but it is also reported that the presence of the dark period brought about better growth. Then applying 4000 lux light intensity as before, the illumination hr per day are respectively designed to 0, 4, 8, 12, 16, 20 and 24 hr and 10 days culture was carried out. Tne dark period treatment was performed by use of the black vinyl cloth. The results are given in Table 3.

Table 3. Illumination experiment result.

Group		The duration of the illumination hr	Dry wt. mg	Total N mg	Total N %	Fixed N mg	Final pH
1.	Complete	24	313.8	26.83	8.55	25.30	6.90
2.		20	305.4	26.39	8.64	24.86	7.30
3.		16	269.8	21.48	7.96	19.95	7.40
4.		12	251.8	17.79	7.07	16.26	7.15
5.		8	168.4	14.20	8.43	12.67	6.95
6.		4	100.6	7.16	7.12	5.63	7.35
7.		0					7.45
8.	-N	24	301.2	25.64	8.51	25.64	7.45
9.		20	257.0	20.65	8.34	20.65	7.40
10.		16	276.0	18.85	6.83	18.85	7.35
11.		12	219.4	20.16	9.19	20.16	7.15
12.		8	166.8	11.10	6.65	11.10	7.00
13.		4	120.4	10.26	8.52	10.26	7.30
14.		0					7.45

The growth rate of the cultures illuminated over 12 hr were increased with the increase of illumination time and the best growth was obtained with 24 hr viz. perfect continuous illumination. Whereas, the decrease of the illumination time brought about an abrupt fall of the growth rate as to the cultures of illumination under 12 hr, and the algae were unable to grow with no illumination viz. only continuous dark. The fact that the best growth was obtained with perfect continuous illumination to the lower algae especially the blue-green algae, might depend upon the nature of the algae which have no peculiar photoperiodism. Moreover, the blue-greens are able to grow in the dark if supplied with a suitable amount of carbohydrate as in Chlorella and they can fix nitrogen readily in the above-mentioned conditions (1, 2).

It is the most important problem to clarify the algal behaviour to the light in relation to the special pigments for the solution of blue-greens physiology, therefore photosynthesis together with nitrogen fixation have received much study.

If blue-greens could be produced as the source of food or feed containing much ninerals in the future, the results of this experiment should be expected to be a basis of the economical research. In the following experiments, 24 hr

per day or continuous illumination was used as before.

3) **Aeration volume of air**

Although the aeration is the absolute important factor for the cultures of blue-greens as the source of CO_2, N_2, and O_2, there are no reports about the aerating volume except CO_2 percent in the air (3). To make clear the influence on the growth caused by the variation of the aerating volume, 11 days cultures were carried out with about 0, 1/5, 1 and 5 fold volume of the present aerating volume namely the continuous aeration of 0.25 *l*/*l* media/min. by air containing 3 percent CO_2 as the standard. The results are shown in Table 4.

Table 4. Aeration experiment result.

Group		Aerating volume *l*/*l* media/min	Dry wt. mg	Total N mg	Total N %	Fixed N mg	Final pH
1.	Complete	1.4	302.4	21.54	7.12	20.01	7.05
2.	Complete	0.25	300.1	22.88	7.61	21.35	7.25
3.	Complete	0.05	160.0	9.34	5.84	7.81	7.50
4.	Complete	0					7.70
5.	-N	1.4	268 8	17.10	6.36	17.10	7.10
6.	-N	0.25	264.0	19.16	7.26	19.16	6.80
7.	-N	0.05	194.4	17.30	8.90	17.30	7.30
8.	-N	0					7.70

Five fold aerating volume compared with the standard brought in heavy volatilization of media to about half volume of the initial, but the growth of this condition is almost equal to that of the standard. The growth of 1/5 fold aerating volume is considerably worse than the standard and the alga hardly grew in the no aerating condition (0 fold).

Aeration of 0.5 *l*/*l* media/min. which is 2 fold of the standard volume is adopted after the sterilization and the wetting of air in the following experiments because the good condition seems to lie between 1 and 5 fold aerating volume as used before.

In all 1), 2) and 3) experiments, our cultures were carried out with complete nutrition which contained combined nitrogen and without combined nitrogen for finding the influence of combined nitrogen for the growth and as the results blue-greens grew in the complete group slightly better than without the combined nitrogen group in all experiments. The combined nitrogen seems to be utilized in the early stage of culture, for nitrogen added per unit of cultures is only 1.53 mg. Although it is said that blue-greens prefer to utilize ammonia and nitrates, nitrogen fixing ability is lost if they were supplied (4).

In the following report will be described the experiment related to the influence on the growth and fixed nitrogen content through varying the

quantity and the quality of nitrogen sources.

Summary

The cultural experiments were performed to clarify the influence on the growth of *Nostoc spongiaeforme AG.* through controlling hydrogen ion concentration, illumination and aeration using the cultural solution modified in the preceding paper.

1) **Hydrogen ion concentration**

The best growth was obtained between pH 9.00—9.70 and the growth at pH 10.40, 8.30, 7.80 and 7.00 preadjusted before the sterilization were unfavorable.

2) **Illumination**

The growth experiments were performed with the illumination hr per day respectively designed as 0, 4, 8, 12, 16, 20 and 24. The growth with the illumination over 12 hr increased with the prolongation of the time and the growth with illumination below 12 hr abruptly descended with the decrease of the illumination time. The alga failed to grow in the dark.

3) **Aeration**

The aeration of 0.25 *l*/*l* media per min adopted as the standard, but to find the optimum of aeration, the cultural experiment was carried out at about 0, 1/5, 1 and 5 fold of the standard aeration.

The 5 fold aerating volume brought in severe volatilization of the media but the growths were almost equal to the standard one. The growth on 1/5 fold aeration is considerably worse than the standard one and the alga did not grow in no aerating condition.

References

1). Allison, F. E., S. R. Hover, and H. J. Movis (1937). Bot. Gaz., **98**, 432.
2). Kratz, W. A. and J. Myers (1955). Am. I. Bot., **42**, 282.
3). Tamiya, H (1955). J. Japanese Biochem. Soc., **27**, 1.
4). Russel, E. J. and E. W. Russel (1950). Soil conditions and plant growth (8th edition), 163.
5). Fujiwara, A. and M. Okutsu (1958). J. Sci. Soil and Manure, Japan, **29**, 35.