Investigation on the Influence of Ultra-violet Rays on the Physiological Activities of Azotobacter.

I. On the Lethal Action of Ultra-violet Rays on Azotobacter chroococcum.

By

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Since Downes and Blunt[1] reported on the lethal action of ultra-violet rays on bacteria, the numerous literature are found on the subject. However so far as the authors are aware, no report has been made on Azotobacter chroococcum which is one of the important soil bacteria and which may be exposed to the ultra-violet rays frequently on account of their habitat. Consequently the following investigation was undertaken.

Experimental:

Part I. Standardization of Methods and Apparatus Used.

Hanovia mercury lamp was used as the source of rays with 110 v. 3 A. electricity. The organism was exposed to the rays under various conditions at 35 cm. distance, on a black wooden desk which has a top of 36 sq. cm. surface.

1.) Determination of quantity of ultra-violet rays discharged from the lamp.

First the quantity of ultra-violet rays discharged from the lamp was determined since it is well known that the quantity varies by such factors as the age of lamp, the strength of electric current, temperature and others. The determination was made by acetone methylene blue[2] and also by molybdic acid[3] method, and was found that the discoloration in the former method was 2.0—3.0 per one hour, and aH (activated hydrogen) was 1.6—2.1 per 10 minutes by the latter.

2.) Difference of quantity of ultra-violet rays by the position on the table top:

Since it is well known that the strength of rays varies by the distance from the source, the intensity of rays was determined at three points A, B and C which

formed an equilateral triangle having the line A—B passes over a point O which was right under the source of light and situated at equal distance. The quantity of the rays was determined by the molybdic acid test in the following manner:

a.) Exposed the molybdic acid solution in Petri dish (8.5 cm. diam. and 1.7 cm. deep) of hard glass without cover at the different points noted above for 10 min., and obtained the following results:

Table I.

Quantity of Ultra-violet Rays at Different Positions.

Test No. / Position.	1	2	3	4	Average.	Ratio to O.
						(%)
O	2.17	2.12	2.11	2.13	2.1325	100.000
A	2.08	2.06	2.07	2.06	2.0825	97.655
B	2.10	2.09	2.06	2.06	2.0775	97.421
C	1.99	1.97	1.93	1.99	1.9575	91.794
Temp. (°C.)	23.5	18.0	17.0	17.5	19.0	

Table I indicates that no marked difference was obtained among the points A, B and O but at C the rays were much weaker so that for the experimental purpose, the points on line A—B and close to O were used.

b.) Next 50 cc. molybdic acid solution were exposed in Erlenmeyer flask (150 cc. volume) for 10 min. in the same manner as described in part a., and obtained the following results:

Table II.

Quantity of Ultra-violet Rays at Different Positions.

Test No. / Position.	1	2	3	4	Average.	Ratio to O.
						(%)
O	0.67	0.70	0.68	0.70	0.6875	100.000
A	0.66	0.69	0.68	0.70	0.6825	99.273
B	0.66	0.70	0.67	0.70	0.6825	99.273
C	0.66	0.69	0.67	0.70	0.6800	98.909
Temp. (°C.)	26.0	26.0	24.5	25.0	25.375	

The results in Table II indicates that very slight difference was obtained among these flasks although the flask at C was less effected.

From these experimental results, it may be stated that the quantity of rays is different by the different position, and the difference increases with the intensity of light source. Consequently it is advisable to carry out the experiment around the point O.

3.) The rate of transmission of ultra-violet rays through glass apparatus used.

The rate of transmission of ultra-violet rays through different glass apparatus, namely "Acme" Ultravit-glass, ordinary hard glass and quartz, was determined and found as follows:

a.) The transmission rate of Petri dish of ordinary cover and covered with Ultravit-glass was determined against an uncovered, exposing 25 cc. molybdic acid in each, and the following results were obtained:

Table III.

Rate of Transmission of Ultra-violet Rays for Different Glass.

Experimental condition.	Number of tests.						Average.	
	1		2		3			
	QUv.	TR.	QUv.	TR.	QUv.	TR.	QUv.	TR.
Control, uncovered.	2.16	100.000	2.12	100.000	2.14	100.000	2.14	100.000
Petri dish.	0.62	28.704	0.63	29.717	0.62	28.972	0.623	29.126
Ultravit-glass.	0.95	43.981	0.94	44.340	0.97	45.327	0.953	44.547

Notes: QUv. = Quantity of ultra-violet rays; TR. = Transmission rate.

As Table III indicates, the transmission rate of Ultravit-glass was about 45% although it is claimed by the manufacturer to be over 50%. This difference may be due to the age of glass since it was partly used out-of-doors previously. The transmission rate of ordinary glass cover was about 30% against the control and 65.383% against Ultravit-glass.

b.) The transmission rate of Erlenmeyer flask was determined against a quartz flask (Florence) which was the only kind available in the laboratory, and the comparative figure was calculated on a unit area of which the results are shown in Table IV:

Table IV.

Rate of Transmission of Ultra-violet Rays for Different Flasks.

Experimental condition.	Number of tests.			Average transmission.
	1	2	3	
Quartz flask, (Florence).	1.61	1.52	1.63	1.587
Hard glass flask, (Erlenmeyer).	0.77	0.70	0.78	0.750
Transmission rate.	47.826	46.053	47.853	47.244
Transmission rate per unit area.	58.601	56.429	58.635	57.888

As Table IV indicates, the transmission rate of Erlenmeyer flask of ordinary hard glass was 57.888 per unit against the quartz flask.

Part II. Exposure of Azotobacter chroococcum to Ultra-violet Rays.

Azotobacter chroococcum was exposed to the rays in the following manner :

1.) Tests in Petri dish :

A loopful of the suspension of 2 days old culture of Azotobacter chroococcum which was grown on an agar slant, was transplanted in the middle of Petri dish in which 15 cc. ASHBY's agar has been poured and cooled. The inoculum was spread evenly within 2 cm. circle and exposed to the rays. Three of such Petri dishes were prepared and exposed one of them with the ordinary cover, the second one without cover and the third with Ultravit-glass cover. The following results were obtained as given in Table V.

Table V.

Exposure of Azotobacter chroococcum in Petri Dish.

No. Exp't. / Exposure.	Covered, hard glass.			Uncovered.			Ultravit-glass cover.		
	1	2	3	1	2	3	1	2	3
Control.	++	++	++	++	++	++	++	++	++
5 Sec.	++	++	++	++	+++	++	++	++	++
10 Sec.	++	++	++	++	++	++	+++	+++	++
15 Sec.	++	++	++	+	−	+	+++	++	+++
30 Sec.	++	++	++	−	±	−	++	++	++
1 Min.	++	++	++	−	−	−	++	++	++
3 Min.	++	++	++	−	−	−	++	++	++
5 Min.	++	++	++	−	−	−	++	++	++
10 Min.	++	++	++	−	−	−	++	++	++
15 Min.	++	++	++	−	−	−	+	+	+
30 Min.	+	++	+	−	−	−	+	+	+
1 Hrs.	+	+	+	−	−	−	+	±	±
2 Hrs.	+	+	+	−	−	−	−	−	−

Notes: (+) Rate of growth; (−) Killed; (±) Doubtful.

The data in Table V indicate that in case with the cover of ordinary glass, no appreciable difference in growth was noted up to 30 minutes exposure and after that a slight depression was obtained ; without cover or the direct exposure gave a good growth up to 5 seconds exposure, but on 15 seconds exposure, very feeble growth was obtained and no growth was observed after 30 seconds exposure ; through Ultravit-glass, 10—15 seconds exposure gave good growth and even after one hour exposure, fairly good growth was observed ; even two hours exposure did not destroy the organisms completely and a feeble growth was noted.

2.) Tests in liquid culture:

The tests in liquid culture were carried out in two following manners:

a.) In quartz test tubes:— Quartz test tube of 1.7 cm. diameter and 30 cc. volume was filled with 10 cc. ASHBY's solution without calcium carbonate, and inoculated with 0.1 cc. of 24 hours old Azotobacter culture; exposed to the rays, and at different intervals a sample was taken out and the cells were counted directly under microscope after stained with MEISSNER's solution. The following results were obtained:

Table VI.

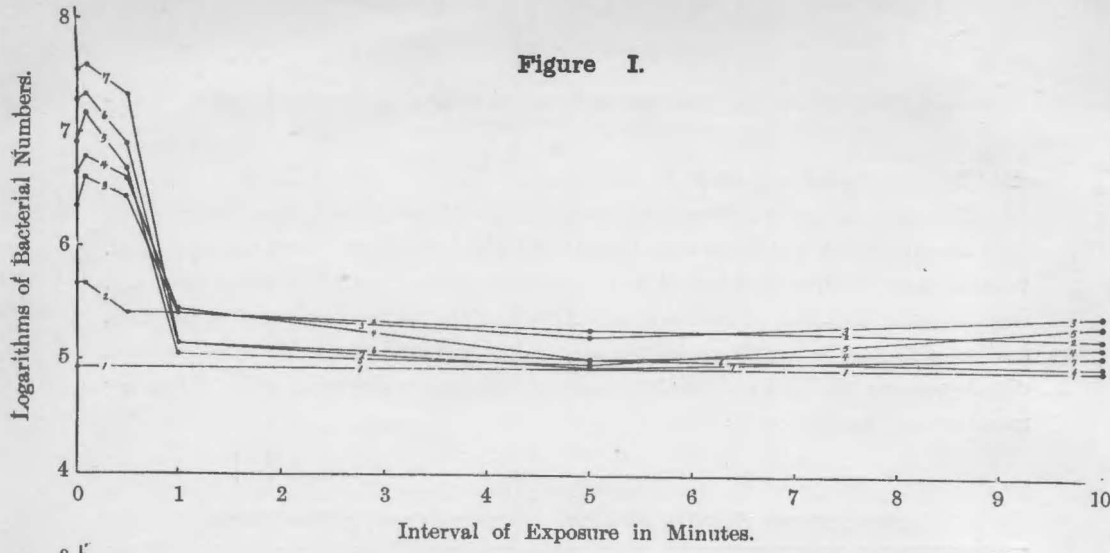
Experimental Results obtained by using Quartz Test Tube.

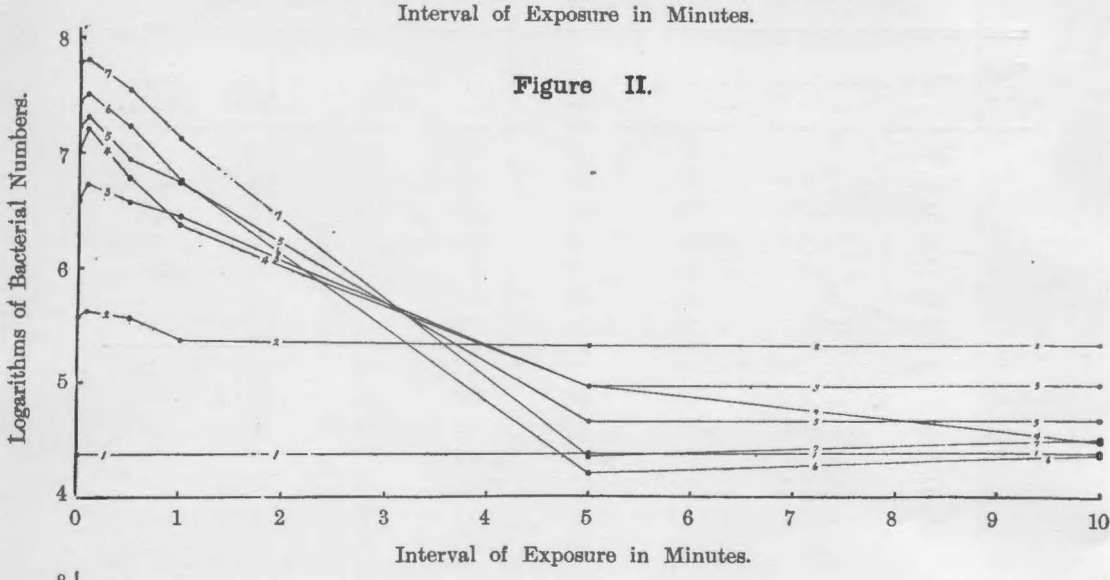
Test No.	Hours.	Time of exposure.						
		Control.	5 Sec.	30 Sec.	1 Min.	5 Min.	10 Min.	30 Min.
1	Initial.	85	85	85	85	58	85	85
	5	469	469	258	258	188	164	235
	24	2,254	4,038	2,723	258	163	258	164
	48	4,413	6,009	4,038	282	103	141	94
	72	8,075	14,648	4,883	113	94	211	211
	120	19,155	21,502	7,981	141	103	94	0
	168	35,305	38,873	21,502	141	88	117	164
2	Initial.	24	24	24	24	24	24	24
	5	376	423	364	235	211	211	164
	24	3,944	5,446	3,756	282	94	94	94
	48	10,423	16,338	6,103	2,347	94	70	70
	72	16,714	20,470	8,826	5,540	33	47	59
	120	28,169	32,019	16,995	5,728	16	23	33
	168	62,535	64,507	35,024	13,146	23	33	23
3	Initial.	65	65	65	65	65	65	65
	5	446	446	423	305	188	164	164
	24	3,850	4,319	1,596	352	141	94	103
	48	14,366	15,211	4,038	3,850	141	94	117
	72	19,718	31,455	15,962	4,695	103	117	94
	120	37,277	45,822	21,502	11,549	94	70	59
	168	38,028	60,845	33,991	18,310	88	61	33

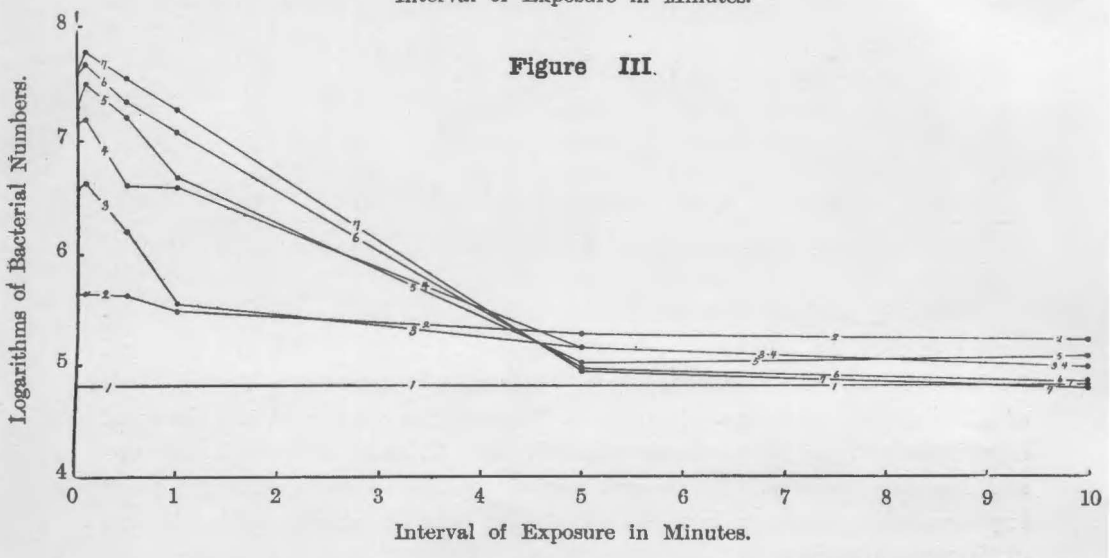
Note: The data show the number of organisms in 1 cc. expressed by thousand.

Table VI indicates that in these three tests, the similar tendency was observed in that the number of cells decreased in 1 minute in the first test, and 5 minutes in the second and third tests, which may be taken as a sign of death of a majority of organisms. In order to ascertain the vitality of a majority of organisms, an agar slant culture was made after 72 hours, from each test tube and found no growth. The largest number of cells was found in 5 seconds exposure which seems to have the stimulating influence similar to that in case of the plate culture.

The results of these three tests are presented graphically as follows:







Explanation of graphs: Graph I, II and III represent the results of three expriments in a series respectively, andt he numerical figure noted in the curves designate the number of hours when the samples were taken viz.: 1=initial; 2=5; 3=24; 4=48; 5=72; 6=124; 7=186 hours.

b.) In Erlenmeyer flask of ordinary hard glass :— Fifty cc. ASHBY's solution without calcium carbonate were put in an Erlenmeyer flask of 150 cc. volume and inoculated with 1 cc. Azotobacter suspension, and treated in the same manner as in case of the test tube series. In addition, the amount of nitrogen fixed was determined by PREGEL's micro-analytical method together with the determination of hydrogen ion concentration by the quinhydrone method. The results are shown in Tables VII, VIII and IX.

Table VII.

Number of Azotobacter and Length of Exposure.

Test No.	Hours.	Time of exposure.							
		Control.	5 Sec.	30 Sec.	1 Min.	10 Min.	30 Min.	1 Hrs.	2 Hrs.
1	Initial.	171	171	171	171	171	171	171	171
	5	446	446	540	358	423	245	282	211
	24	11,549	12,394	9,484	9,104	9,484	6,479	5,164	7,981
	48	38,310	29,390	38,357	40,657	38,028	26,855	25,446	27,136
	72	45,728	42,160	44,038	48,826	64,132	65,540	35,962	32,770
	120	99,155	80,936	84,601	88,169	87,700	94,554	90,141	57,089
2	Initial.	244	244	244	244	244	244	244	244
	5	824	829	886	826	793	657	648	701
	24	13,146	13,897	20,187	13,427	9,953	8,291	9,014	10,141
	48	32,301	28,639	39,155	38,592	30,704	16,150	26,855	25,446
	72	50,141	53,146	66,479	72,113	58,310	36,714	40,329	62,629
	120	82,441	79,343	82,441	101,784	80,094	70,141	92,629	58,310
3	Initial.	130	130	130	130	130	130	130	130
	5	329	563	563	587	352	305	282	423
	24	4,225	4,789	6,103	4,413	4,601	5,446	4,131	5,014
	48	22,629	18,122	23,005	22,254	23,380	10,141	13,427	12,394
	72	35,305	54,648	61,878	65,728	53,239	34,930	43,662	36,714
	120	106,169	125,258	114,836	123,662	102,911	88,545	82,911	82,441

Note: The data show the number of organisms in 1 cc. expressed by thousand.

Table VIII.

Length of Exposure and Nitrogen fixed.

Test No.	Hours.	Time of exposure.							
		Control.	5 Sec.	30 Sec.	1 Min.	10 Min.	30 Min.	1 Hrs.	2 Hrs.
1	48	3.36	3.36	3.36	2.80	3.36	2.80	2.80	2.80
	72	6.24	6.32	6.40	6.40	6.32	6.24	5.82	5.60
	120	14.56	14.56	15.12	15.12	14.10	12.69	12.32	11.76
	168	26.80	26.32	26.80	28.40	25.42	24.68	25.12	24.80
2	48	3.64	3.92	3.92	3.64	3.64	3.36	3.36	2.80
	72	6.62	6.96	6.96	7.24	6.84	6.62	5.82	5.82
	120	16.92	16.92	17.45	18.23	15.12	14.56	12.69	12.32
	168	28.40	29.94	32.18	28.33	28.40	27.44	28.04	26.79
3	48	2.80	2.80	2.80	2.80	2.80	2.80	1.68	1.68
	72	5.60	6.16	6.32	6.16	6.16	6.16	5.04	5.04
	120	12.32	12.69	12.69	12.69	12.32	12.32	11.76	11.28
	168	26.32	26.32	26.80	26.92	25.44	24.00	24.88	23.52

Note: The data indicate the amount of nitrogen fixed in p.p.m.

Table IX.

Length of Exposure and Change of pH.

Test No.	Hours.	Time of exposure.							
		Control.	5 Sec.	30 Sec.	1 Min.	10 Min.	30 Min.	1 Hrs.	2 Hrs.
1	Initial.	7.10	7.09	7.12	7.14	7.12	7.18	7.14	7.19
	48	6.76	6.72	6.74	6.73	6.75	6.72	6.72	6.70
	72	6.64	6.65	6.66	6.66	6.67	6.69	6.70	6.71
	120	6.62	6.62	6.58	6.58	6.52	6.59	6.62	6.58
	168	6.61	6.56	6.55	6.44	6.48	6.51	6.55	6.54
2	Initial.	6.92	7.08	7.06	7.04	7.14	7.14	7.12	7.14
	48	6.76	6.72	6.74	6.76	6.75	6.74	6.77	6.73
	72	6.66	6.65	6.64	6.64	6.67	6.69	6.70	6.69
	120	6.62	6.62	6.58	6.58	6.59	6.59	6.62	6.65
	168	6.57	6.56	6.55	6.44	6.50	6.51	6.59	6.60
3	Initial.	6.91	7.13	7.14	7.15	7.17	7.17	7.19	7.29
	48	6.58	6.59	6.58	6.58	6.60	6.61	6.61	6.60
	72	6.56	6.55	6.55	6.57	6.57	6.59	6.59	6.58
	120	6.57	6.52	6.51	6.56	6.57	6.58	6.59	6.58
	168	6.55	6.50	6.51	6.51	6.54	6.57	6.57	6.56

The results of these three tests agree closely and in case of Erlenmeyer flask of ordinary hard glass showed the similar tendency as in a previous tests. That is even after two hours of exposure, no death of organism was noted but the number decreased somewhat and also no appreciable change in both quantity of nitrogen fixed and P_H. When the exposure was short viz. 30 seconds to 1 minute, the number of organism increased more than that in the control, and a longer exposure caused a sudden decrease. It was true also in regard to the quantity of nitrogen fixed and the change of P_H. From these results, it seems to be true that a short exposure has stimulating influence and it is clear that the rate of transmission of ultra-violet rays through the glass has a great influence on the growth of organism but the lethal action is not proportional to the rate although they show the general tendency. The intencity of lethal action decreases much faster than the decrease of rate of transmission.

Summary.

The lethal action of ultra-violet rays on Azotobacter chroococcum under various conditions was investigated by using Hanovia mercury lamp, and the following summary may be given:

1. First the quantity of ultra-violet rays, discharged from Hanovia lamp, was determined by both acetone methylene blue and molybdic acid methods, and found to be 2.5—3.0 discoloration in one hour and aH 1.6—2.1 in ten minutes respectively.

2. Azotobacter chrooccocum was exposed to the rays under various conditions, namely in Petri dish, uncovered and covered by either with ordinary hard glass of 29.126 transmission rate or with "Acme" ultravit-glass of 44.57 transmission rate; and also in Erlenmeyer flask of hard glass of 57.888 transmission rate, and the following results were obtained:

Treatments.	Time of exposure.	Results.
Petri dish	2 hrs.	Not killed.
"Acme" ultravit glass	1 hr.	Majority killed.
	2 hrs.	Totally killed.
Direct exposure	30 seconds.	" "
Quartz flask	5 minutes.	" "
	5 seconds.	Stimulated the growth.
Erlenmyer flask, hard glass	2 hrs.	Not killed.
	30 - 60 seconds.	Stimulated the growth.

3. Besides the vitality of Azotobacter, the amount of nitrogen fixed and the change in the concentration of hydrogen ions under various treatments were determined.

4. The lethal action of ultra-violet rays decreases in greater proportion than the rate of transmission of the apparatus.

5. In all the cases, a short exposure stimulated the physiological activities of Azotobacter chroococcum.

Literature.

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