Influence of Iodine on Physiological Activities of Microorganisms.

By

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The influence of iodine on the physiological activities of animals and plants has been investigated quite extensively in recent years. However its effect aside the bactericidal has not been investigated to any extent in regard to the micro-organisms. Consequently it was investigated here to ascertain the influence of iodine on Azotobacter chroococcum, B. subtilis and Saccharomyces cerevisiae.

Experimental.

I. *Influence of Iodine on Azotobacter chroococcum.*

A stock culture of Azotobacter chroococcum in this laboratory was used, and the tests were conducted in ASHBY's liquid medium, as follows :

100 cc. of the medium were placed in 300 cc. Erlenmeyer flask to which a known amount of iodine, as potassium iodide, was added to each, as noted in Table I. The flask was inoculated with 1 cc. of Azotobacter suspension which was prepared by taking a rejuvenated, two days old culture. The flask was incubated at 28°C., and the cells were counted at 24 hours intervals up to 120 hours, and finally the nitrogen was determined. The results are given in Table I.

(See Table I on next page.)

Table I indicates that the number of organisms increased with the concentration of iodine up to 0.007 per cent until the end of 96 hours, and after 120 hours 0.006 per cent was the best. Since it is generally accepted that the observation made within 48 and 72 hours may be taken as an index of such an investigation, the optimum concentration of iodine for the growth of Azotobacter may be considered as 0.007 per cent. The amount of nitrogen fixed was in parallel with the growth namely the largest amount of nitrogen was found in 0.007 per cent concentration.

Table I.

Influence of Iodine on Azotobacter chroococcum.

Hours. / % I.	Number of Azotobacter in 1 cc.						T. N. in 100 cc.
	Initial.*	24	48	72	96	120	
Control.	269	526	1549	2432	2845	2901	(mg.) 1.692
0.001	„	498	1831	2291	3493	2742	1.692
0.003	„	601	1859	2676	3089	3080	1.904
0.005	„	873	1990	3343	4113	2948	1.904
0.006	„	939	1975	2995	4089	4751	2.045
0.007	„	948	2329	3023	4526	3812	2.256
0.008	„	892	2225	2967	4056	3521	2.115
0.009	„	892	2541	3211	3277	3099	2.115
0.010	„	601	1981	2657	2761	3305	1.904
0.015	„	545	1512	2563	2498	2948	1.904
0.020	„	451	1868	2263	2592	3343	1.904
0.030	„	507	1324	2789	3080	2920	—
0.050	„	479	1155	2085	2545	3014	—
0.060	„	516	1099	1915	2357	2357	—
0.070	„	375	873	2206	2160	2770	—
0.080	„	272	808	2188	2817	2329	—
0.090	„	282	488	1474	2019	2620	—
0.100	„	366	507	1615	2103	1746	—
0.200	„	347	620	1324	1963	1822	—

Note:

* Number in ten thousands, counted by direct microscopic method.

The following experiment was also carried out to ascertain the lethal dose of iodine which was not found in the previous experiment and also to find out the change in hydrogen ion concentration in calcium carbonate free medium, by means of the quinhydrone electrode. The number of organism was determined by the plate method so that the viability can be clearly ascertained at the same-time. The results are noted in Table II.

Table II.

Lethal Influence of Iodine on Azotobacter chroococcum.

Hours. / % I.	Number of Azotobacter in 1 cc.							T. N.**	Lethal.***
	Initial.*	24	48	72	96	120	P_H		
								(mg.)	
Control.	223	563	1042	1390	2085	2366	6.82	1.410	+
0.003	„	685	1286	1728	2394	2648	6.95	1.410	+
0.005	„	770	1390	1455	2122	2742	7.01	1.410	+
0.007	„	864	1718	1794	2695	2779	7.05	1.692	+
0.010	„	798	1239	1512	1699	2657	6.81	1.269	+
0.050	„	592	1052	1408	1709	2066	6.80	1.128	+
0.100	„	404	958	1427	1577	2319	6.89	1.128	+
0.200	„	573	1005	1455	1559	2254	6.85	1.128	+
0.300	„	413	920	1305	1380	2075	6.81	1.128	+
0.400	„	535	948	948	1361	1991	6.44	1.128	+
0.500	„	338	817	1174	1493	1596	6.37	1.128	+
0.750	„	385	836	958	1187	1568	6.39	0.987	+
1.000	„	382	779	883	1127	1540	6.46	0.846	+
2.000	„	23	9	5	12	18	7.14	0.423	−
3.000	„	19	9	9	7	7	7.10	0.423	−

Notes:

* in ten thousands, counted by plate method to find the vitality at the sometime;

** in 100 cc. after 120 hours;

*** + living, − dead.

Table II indicates that up to the concentration of 0.007 per cent, the same results were obtained as in the previous experiment, and 2.00 per cent had the lethal effect while in the less concentrations, higher was worse in their growth as well as the nitrogen fixation. As a whole, up to 0.01 per cent showed a stimulating effect. The hydrogen ion concentration decreased as the growth progressed and the nitrogen fixed.

II. *Influence of Iodine on B. subtilis.*

The same procedure was used as in case of Azotobacter except the standard nutrient broth was used instead of Ashby's solution. At 24 hours intervals, ammonia was determined by Pregle's micro method and finally the total ammonia was determined by magnesium oxide method. The growth was determined

macroscopically by turbidity and finally by plating. The results are given in Table III.

Table III.

Influence of Iodine on B. subtilis.

Hours. / % I.	NH_3-N in 100 cc. medium.					Growth.			Lethal.
	Initial.	24	48	72	120	48	72	120	
	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)				
Control.	0.966	1.600	2.248	2.488	5.640	###	####	####	+
0.001	„	1.591	2.272	2.542	5.640	###	####	####	+
0.003	„	1.580	2.228	2.468	5.640	###	####	####	+
0.005	„	1.732	2.340	2.744	5.640	###	####	####	+
0.007	„	1.696	2.300	2.804	5.640	###	####	####	+
0.010	„	1.652	2.420	2.708	4.512	###	###	####	+
0.020	„	1.692	2.180	2.692	4.230	###	###	####	+
0.050	„	1.600	2.208	2.488	3.948	##	##	###	+
0.100	„	1.564	2.124	2.128	2.840	+	+	###	+
0.250	„	1.528	1.580	1.652	1.974	+	+	##	+
0.500	„	1.572	1.591	1.600	1.833	+	+	##	+
0.750	„	1.440	1.512	1.540	1.551	+	+	##	+
1.000	„	1.408	1.509	1.532	1.551	+	+	+	+
2.000	„	1.382	1.395	1.410	1.410	−	−	−	−
3.000	„	1.397	1.402	1.408	1.410	−	−	−	−
4.000	„	1.406	1.397	1.402	1.410	−	−	−	−
5.000	„	1.372	1.388	1.400	1.410	−	−	−	−

Notes:

− no growth; + growth; ## good growth; ### better growth; #### best growth.

Table III indicates that a certain small amount of iodine stimulates the growth and 0.007 per cent seems to be the optimum where the ammonia production was greatest and at 1.00 per cent concentration practically no change was observed, and 2.00 per cent was lethal.

III. *Influence of Iodine on Yeasts.*

Saccharomyces cerevisiae was investigated in the same manner as in the previous experiments but using LAURENT's medium of the following composition:

Ammonium sulfate	4.71 g.
Magnesium sulfate	0.10 g.
Monobasic potassium phosphate	0.75 g.
Maltose	50.00 g.
Distilled water	1,000.00 cc.

The influence of iodine was determined by: (1) the growth, (2) the amount of sugar consumed and (3) the gas production.

(1) *Influence on the growth of Sacch. cerevisiae:*

100 cc. medium in 300 cc. Erlenmeyer flask was inoculated with 1 cc. of 24 hours old culture and incubated at 28°C. The count was made at 24 hours intervals by the direct microscopical method using the MEISSNER's solution as the diluent. The results are given in Table IV.

Table IV.

Influence of Iodine on Saccharomyces cerevisiae.

Hours. / % I.	Number of Sacch. cerevisiae in 1 cc. medium.				
	Initial.*	24	48	72	120
Control.	12	139	270	336	718
0.001	„	218	359	451	742
0.003	„	150	361	472	887
0.005	„	178	392	453	692
0.007	„	275	387	479	852
0.010	„	188	359	380	606
0.020	„	162	336	338	535
0.050	„	136	268	305	434
0.100	„	94	209	311	495
0.250	„	61	263	286	554
0.500	„	33	23	19	14
0.750	„	7	12	$\bar{5}$	16
1.000	„	9	$\bar{5}$	$\bar{5}$	2
2.000	„	7	$\bar{5}$	9	1
5.000	„	4	$\bar{5}$	$\bar{5}$	2
10.000	„	9	2	2	7

Note: * in ten thousands.

The above results indicate that 0.007 per cent was the optimum and 0.5 per cent, the lethal. Accordingly yeasts seem to be less resistant against iodine than those two organisms investigated previously.

(2) *Consumption of sugar by Sacch. cerevisiae :*

In the course of investigation, the sugar in the medium was determined by STILE's method[1] to ascertain the amount of sugar consumed by the organism. The results are given in Table V.

Table V.

Determination of Residual Sugar in Medium.

Hours. / % I.	Residual maltose in medium.					
	Initial.	24	48	72	120	240
Control.	(%) 3.844	(%) 3.752	(%) 3.211	(%) 2.809	(%) 2.630	(%) 1.615
0.001	„	3.709	3.230	2.698	2.569	1.336
0·003	„	3.662	2.764	2.689	2.424	1.382
0.005	„	3.159	2.853	2.501	2.306	1.276
0.007	„	3.107	2.553	2.246	2.100	1.471
0.010	„	3.569	3.158	2.630	2.474	1.631
0.020	„	3.519	3.050	2.809	2.608	2.168
0.050	„	3.569	3.229	2.887	2.649	2.271
0.100	„	3.569	3.107	2.828	2.828	2.877
0.250	„	3.616	3.158	3.043	2.952	2.963
0.500	„	3.662	3.519	3.118	3.043	3.020
0.750	„	3.662	3.519	3.093	3.118	3.066
1.000	„	3.752	3.419	3.349	3.297	3.245
2.000	„	3.798	3.519	3.310	3.258	3.234
5.000	„	3.798	3.419	3.374	3.270	3.297
10.000	„	3.798	3.519	3.297	3.322	3.297

The percentage of decrease of sugar due to the consumption by the organism is noted in Table VI and also that of consumption in ratio to the original sugar added is given in Table VII.

Table V indicates that even right after the sterilization, the amount of sugar decreased from 5.00 to 3.844 per cent owing to the hydrolysis. This is in accord with those results reported by SMITH.[2] Up to 120th hour, the amount of residual sugar was the smallest in 0.007 per cent while 0.005 per cent became the least after that. From Table VI and VII, the change in the amount of sugar in the medium can be clearly seen.

Table VI.

Quantity of Maltose consumed by Saccharomyces cerevisiae.

Hours. / % I.	Quantity of maltose consumd.				
	24	48	72	120	240
	(%)	(%)	(%)	(%)	(%)
Control.	0.092	0.633	1.035	1.214	2.229
0.001	0.135	0.614	1.146	1.275	2.508
0.003	0.182	1.080	1.155	1.420	2.462
0.005	0.685	0.991	1.343	1.538	2.568
0.007	0.737	1.291	1.598	1.744	2.373
0.010	0.275	0.686	1.214	1.370	2.213
0.020	0.325	0.794	1.035	1.236	1.676
0.050	0.275	0.615	0.957	1.195	1.573
0.100	0.275	0.737	1.016	1.016	0.967
0.250	0.228	0.686	0.801	0.892	0.881
0.500	0.182	0.325	0.726	0.801	0.824
0.750	0.182	0.325	0.751	0.726	0.778
1.000	0.092	0.425	0.495	0.547	0.599
2.000	0.046	0.325	0.534	0.586	0.570
5.000	0.046	0.425	0.470	0.574	0.547
10.000	0.046	0.325	0.547	0.522	0.547

Table VII.

Percentage of Maltose consumed by Sacch. cerevisiae against the Quantity of Maltose added.

Hours. / % I.	Percentage of maltose consumed against the initial amount added.				
	24	48	72	120	240
Control.	1.84	12.66	20.70	24.28	44.58
0.001	2.70	12.28	22.92	25.50	50.16
0.003	3.64	21.60	23.10	28.40	49.24
0.005	13.70	19.82	16.86	30.76	51.36
0.007	14.74	25.82	31.98	34.88	47.46
0.010	5.50	13.72	24.28	27.40	44.26
0.020	6.50	15.88	20.70	24.72	33.52
0.050	5.50	12.30	19.14	23.90	31.46
0.100	5.50	14.74	20.32	20.32	19.34
0.250	4.56	13.72	16.02	17.84	17.62
0.500	3.64	6.50	14.52	16.02	16.48
0.750	3.64	6.50	15.02	14.52	15.56
1.000	1.84	8.50	9.90	10.94	11.98
2.000	0.92	6.50	10.68	11.72	11.40
5.000	0.92	8.50	9.40	11.48	10.94
10.000	0.92	6.50	10.94	10.44	10.94

From these results, it is indicated that the change in the amount of sugar is in parallel with the number of yeasts. The growth was best in 0.007 per cent where more than half of the sugar added was consumed after ten days. Also it is noted that the sugar decreased where a considerable amount of iodine is present although no growth of organism took place, which may be due to the oxidation by iodine as pointed out by KAPPANNA[3] and others.

(3) *Carbon dioxide production by Sacch. cerevisiae :*

Further the influence of iodine on yeasts was investigated by the rate of carbon dioxide production by inoculating the LAURENT's medium (5 cc.) in SMITH fermentation tubes. The results are given in Table VIII and also shown photographically in Plate VII.

Table VIII.

Quantity of Carbon Dioxide produced by Sacch. cerevisiae.

Hours. / % I.	Quantity of CO_2 produced.					
	72	80	96	104	120	128
	(cc.)	(cc.)	(cc.)	(cc.)	(cc.)	(cc.)
Control.	0.2	0.9	3.0	3.9	6.0	6.8
0.003	0.1	0.5	1.9	2.7	5.6	5.5
0.005	1.1	2.3	4.9	5.9	11.6	12.6
0.007	2.1	4.1	8.6	10.3	14.4	Inabilitiy.
0.010	Trace.	0.4	3.1	4.6	9.6	9.7
0.020	0.7	1.9	4.5	5.6	8.5	9.5
0.050	1.0	2.4	4.85	5.5	6.0	8.0
0.100	0.1	1.1	4.9	5.0	5.2	6.0
0.250	—	0.2	1.3	2.0	2.7	4.5
0.500	—	0.1	2.0	2.9	3.1	4.1
5.750	—	—	—	0.3	2.3	3.0
1.000	—	—	—	0.7	3.5	4.3
3.000	—	—	0.9	1.6	2.7	3.7
4.000	—	—	—	—	—	—
5.000	—	—	—	—	—	—

These results indicate that the maximum gas production took place in 0.007 per cent which agrees with those results observed in the previous experiments. At the end of 120 hours, 6.0 cc. gas was produced in the control while 14.4 cc. was produced in 0.007 per cent. A slight gas production was observed

even in 3.0 per cent which is stronger than the lethal dose determined previously. This seems to be an experimental error owing to the difficulty of mixing iodine thoroughly in the fermentation tube.

Summary.

From the results obtained in this investigation, the following summary may be made :

1.) A small amount of iodine stimulates the physiological activities of microorganisms while beyond a certain quantity is harmful.

2.) For Azotobacter chroococcum, B. subtilis and Saccharomyces cerevisiae, 0.007 per cent seems to be the optimum although there is a slight difference among them.

3.) About 2.00 per cent is the lethal dose for Azotobacter chroococcum and B. subtilis while 0.50 per cent is lethal to Sacch. cerevisiae.

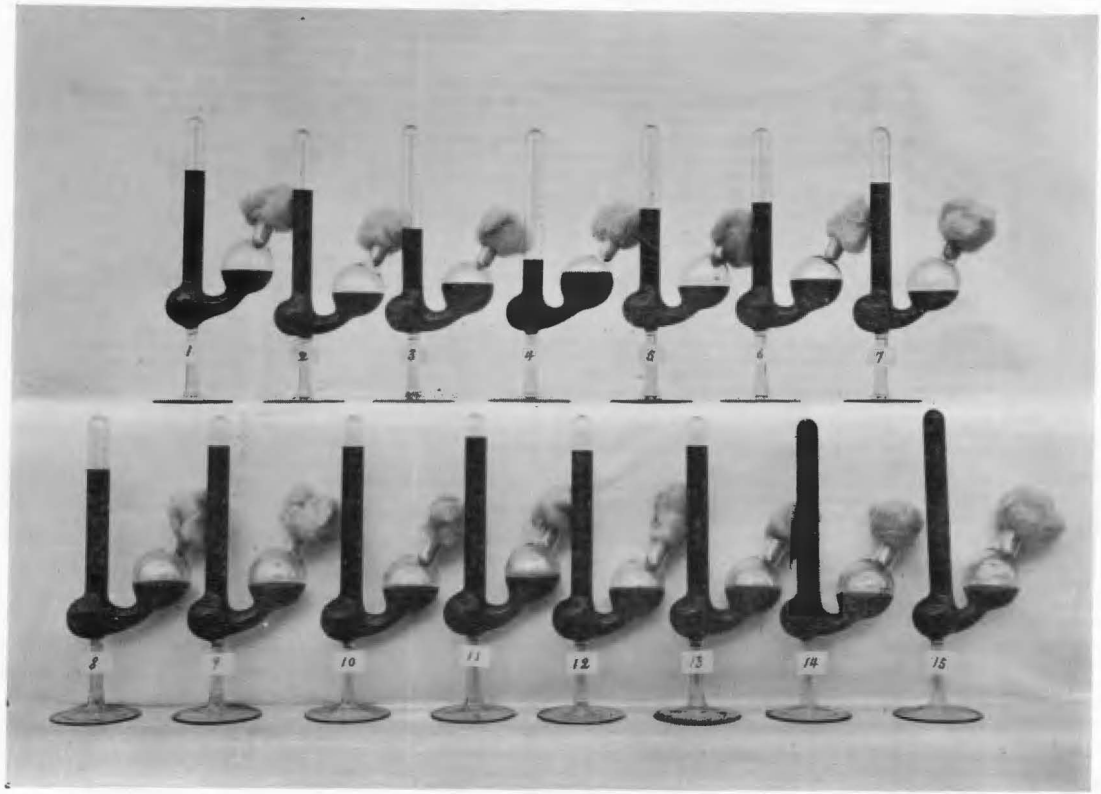
Literature.

1.) Stiles, H. R., Peterson, W. H. and Fred, E. B., Jour. Bact., 12 : 427, 1926.

2.) Smith, M. I., Biochem. Jour., 26 : 1467, 1932.

3.) Kappanna, A. N., Jour. Indian Chem. Soc., 5 : 387, 1927.

PLATE VII.



Notes: Percentage of iodine added to each fermentation tube:

(1) none; (2) 0.003; (3) 0.005; (4) 0.007; (5) 0.010; (6) 0.020; (7) 0.050; (8) 0.100; (9) 0.250; (10) 0.500; (11) 0.750; (12) 1.000; (13) 3.000; (14) 4.000; (15) 5.000.