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Microbiology. — *"Metabolism of the fosfor in Aspergillus niger"*. By Dr. H. J. WATERMAN. (Communicated by Prof. Dr. M. W. BEIJERINCK).

(Communicated in the meeting of December 28, 1912).

In an earlier communication[1]) I have shown that the metabolism of the nitrogen in this organism is analogous to that of the carbon[2]).

These two elements are accumulated in the organism and are later partly excreted, the carbon as carbonic acid, the nitrogen as ammonia.

We find besides that an excess of these elements retards the spore-formation. For the carbon compare tables II*a*, II*b* and III (p. 451, 452, and 464 Folia microbiologica); for the nitrogen see table VI (Preceding paper).

I have further found that the fosfor behaves in the same manner as the above elements.

In the first place I ascertained that the rate of fosfor of an old mature culture of *Aspergillus niger* is constant, independent of the way in which it is obtained.

The mould layer was before the analysis washed with distilled water and after drying destroyed by strongly concentrated nitric acid in a closed tube. In the thus obtained solution the fosfor was determined after FINKENER[3]) as ammonium fosfor molybdate $(NH_4)_3 PO_4\ 12\ MoO_3$. The results are found in Table I.

For shortness' sake I shall as for the nitrogen make use of the word "fosfornumber", which means the fosfor fixed in the mould per 100 parts of assimilated carbon. As in the experiments of table I all the glukose (1000 mgrs.) had been assimilated and this quantity corresponds with 400 mgrs. of carbon; the number of mgrs. of fosfor must thus be divided by 4 to find the fosfornumber.

As the table shows the fosfornumber is for an old mature mould-

1) See the preceding paper.

2) Folia microbiologica (1912) Bd I. p 442.

3) The liquid containing ammoniumnitrate and the nitric acid is heated till the first bubbles appear, then precipitated with ammoniummolybdate under continuous stirring. The precipitate is then washed out with a solution containing ammonium-nitrate and nitric acid and dissolved in dilute ammonia. To the thus obtained clear solution is added an excess of ammoniumnitrate and a small quantity of ammoniummolybdate, after which it is again heated until the first bubbles appear; finally hot nitric acid is added under continuous stirring. The precipitate is dried in an air current to constant weight at 160° C. in a GOOCH's crucible.

TABLE I.

Circumstances of cultivations: 50 cm^3. very pure distilled water, in which dissolved: 2% glucose and the anorganic substances mentioned below. Temp.: 33° C.

No.	Anorganic substances	Age	$(NH_4)_3PO_4 . 12MoO_3$ (mgrs.)	Fosfor in mould (mgrs)	Fosfor-number
	0,15 % ammoniumnitrate				
1	0,05 „ fosforic acid (crystallised)	90 dagen	33,3	0,55	0,15
	0,1 „ magnesiumsulfate („)				
2	0,1 „ calciumnitrate (free fr. water)	„ „	32,9	0,55	0,15
	0.1 „ rubidiumchlorid				
	0,15 „ ammoniumnitrate				
	0,1 „ potassiumchloride				
	0,1 „ magnesiumsulfate (crystall.)				
3	0,05 „ calciumnitrate (free fr. water)	50 „	25,2	0,4	0,1
	0,05 „ ammoniumfosfate				
	0,05 „ fosforic acid (crystallised)				
	0,00001 mgr. $MnCl_2 . 4Aq$				
4	As 3, but instead of 0,00001 mgr.: 0.0001 mgr. $MnCl_2 . 4Aq$	„ „	19,4	0,3	0,1
5	As 4, but instead of 0,0001 mgr.: 0,01 mgr. $MnCl_2 . 4Aq$	„ „	23,0	0,4	0,1
	0,4 % potassiumnitrate	30 „	66,8	1,1	0,25
6	0,15 „ KH_2PO_4				
	0,15 „ magnesiumsulfate (crystall.)				
	tapwater				
	0,15 % KH_2PO_4				
7	0,06 „ magn.sulf. (free from water)	„ „	37,2	0,6	0,15
	tapwater and				
	0,08 % NH_4Cl				
8	As 7, but instead of 0,08 % NH_4Cl: 0,12 % NH_4Cl	„ „	25,2	0,4	0,1
9		„ „	29	0,5	0,1
10	As 9, but instead of 0,12 %: 0,32 % NH_4Cl	„ „	28	0,45	0,1

layer rather constant so that in this respect, too, the fosfor corresponds quite with the carbon and nitrogen.

In the second place the action of various increasing fosfate concentrations on the metabolism of *Aspergillus niger* was studied. The results are found in table II. The fosfor was added as kaliumbifosfate to the nutrient liquid, whilst I ascertained by analysis that the rate of fosfor of this compound was indeed in accordance with the formula KH_2PO_4.

After one day already, growth was observed in all numbers, except in Nrs. 1 and 2. After two days it had considerably increased in Nrs 4—18, Nrs. 1 and 2 also showing a beginning of growth. After three days the growth of Nrs. 1 and 2 had not increased, as little in Nr. 3 where, however, more mycelium had been formed. The growth increased in the following Nrs. and was very strong in Nr. 8. This continued also after 7 and 14 days.

The retarding of the spore formation after 2 and 3 days is convincing in those experiments where much fosfor is added. After two days 3—6 had rather many spores. In 7 and 8 few had appeared whereas in the following Nrs. hardly any spores were seen. After 3 days 3—6 had many spores, 8 few, and the Nrs. with much fosfor very few. Only in Nrs 17 and 18 the spore-formation was considerable and about alike to that of Nr. 8. The same I have observed for the action of potassium, as before for the carbon and nitrogen, so that it seems of general significance. This may be explained thus: If an excess of the referring element, in this case fosfor, is present, the cells are continually overloaded with new food and with the therefrom arising intermediary products, by which the spore-formation is retarded. When the excess becomes very great it is possible that the process of the metabolism is so much accelerated that also the spore-formation is quickened. Probably such is the case in Nrs 17 and 18, where three days after inoculation more spores were produced than in Nrs. 9—16. After 7 days the differences in spore-formation are no more observable.

However, there are elements which in feeble concentrations counteract the spore-formation [1]) and then the limits will be quite different.

The quantity of mould is very small in Nrs. 1 and 2 where no fosfor was added, and amounts with increase of the fosfor; herewith the assimilation of glucose is parallel. After 4 days the solution in Nrs, 9, 10 and 11, no more contained fosfate, which after the same

[1]) These Proceedings, November 1912.

TABLE II.

50 cm³. very pure distilled water, in which dissolved: 0,15% ammonium nitrate, 0,1% kaliumchlorid, 0,1% magnesiumsulfate (crystall.) 0.05% calciumnitrate (free from water), 0,00025% $MnCl_2 . 4Aq.$, 2% glucose (free from water).

Nr.	Added fosfor in mgrs.	Fosfor in mgrs. in liquid as fosfate (after four days)	Fosfor in mgrs. in mould-material (after four days)	Assimilated glucose (after four days)	Fosfor-number (after four days)	Fosfor in mgrs. in liquid as fosfate (after five days)	Fosfor in mgrs. in mould-material (after five days)	Assimilated glucose (after five days)	Fosfor-number (after five days)	Fosfor in mgrs. in liquid as fosfate (after seven days)	Fosfor in mgrs. in mould-material (after seven days)	Assimilated glucose (after seven days)	Fosfor-number (after seven days)	Assimilated glucose (after sixteen days)	Dry weight in mgrs. (after sixteen days)
1,2	0													± 10%	
3	0,02													± 30 „	30
4, 5	0,22													more than 40 „	100; 106,5
6	0,44													55	150
7	0,66													83	259
8	0,88														
9	1,1	0	1,0	83%	0,3										
10	1,55	0		79 „	0,5										
11	2,1	0		87 „	0,6										
12	3,2														
13, 14	4,2	fosfate still present	2,9	96 „	0,75										
15	10,5									8,7	1,6	100%	0,4		
16	20,7						1,5	100%	0,4						
17	104,—														
18	207,—														

time was still present in Nrs. 13 and 14. From these observations may be concluded that the mould had drawn to it all the fosfor in the three first experiments. As these quantities, especially that of Nr. 11, are considerably greater than those present in the old, mature mould layer (table I), it was indirectly proved that also the fosfor in the metabolism is accumulated in the organism in considerable quantity at the beginning of the development. This result was then in a direct way confirmed by analysis of the mould. So it was found for Nr. 9 that all the fosfor vanished from the solution was present in the organism (ca. 1 mgr.). For Nrs. 13 and 14 2.9 mgr were found in the mould, a quantity 7 tot 8 times as great as that in the old mould layer. The corresponding fosfornumber is **0.75**. This number may even be higher, as is seen from an experiment which may be mentioned here for comparison. In a five days old mould layer (culture liquid: 50 cm^3 distilled water, 0.15°/₀ am. nitrate, 0.1°/₀ KCl, 0.1°/₀ $MgSO_4$ (crystall), 0.05°/₀ Ca nitrate (free from water), 0.05°/₀ fosforic acid (crystall), 2°/₀ glucose), 3.9 mgrs. P was present. As all the glucose was then assimilated the fosfornumber was $= \mathbf{1.0}$.

In opposition to what is found for the carbon and nitrogen this quantity of fosfor is loosely fixed in the organism. Ten minutes' boiling with water will do to dissolve considerable quantities. Of a mature mould layer, treated in the same way, no, or hardly any fosfor is dissolved; the same is the case with lecithine or phytine. As the mould grows older the superfluous fosfor, accumulated in the organism, returns into the solution as fosforic acid. This was already indicated by the fact that the fosfornumbers of mature cultures were very small (table I).

It was ascertained by direct analysis both of the mould and the culture liquid of N°. 15 after 7 days, and of the mould of N°. 16 after 5 days. For N°. 15 the sum of the fosfor in the mould and in the liquid present after 7 days is $8{,}7 + 1{,}6 = 10{,}3$ mgrs. The totally added quantity was 10,5 mgrs., so that no loss of fosfor, in the form of hydrogenfosfid takes place.

By this study of the metabolism of the elements we obtain a better view of their signification than was hitherto obtained. We see that the quantities of the elements present in the mature mould, do not correspond with the quantities really active during the development. In the case of carbon the plastic aequivalent could in the course of time decrease to the half. For the nitrogen there was a threefold, for the fosfor I could point out a tenfold decrease. The quantities of the latter element required for the normal assimilation are much greater than is generally accepted, also as to the nitrogen, although in a less degree. When comparing the accumulation of the elements it thus seems, that during the metabolism this accumulation is greatest for those, which form a small permanent percentage of the constituents of the organism. So we see, that in the course of an experiment the same quantity of an element may be many times active in the metabolism, one cell taking up the products excreted by another cell.

Starting from this view the study of elements, such as manganese, which are already active in very dilute solutions, are interesting.

Meteorology. — *"A long range weather forecast for the East-monsoon in Java."* By Dr. C. BRAAK.. (Communicated by Dr. J. P. VAN DER STOK.)

(Communicated in the meeting of November 30, 1912).

In a preceding communication [1]) it was deduced from a study of factors of correlation that in the Indian Archipelago, with the exception of the western part north of the equator, a connection is clearly perceptible between barometric pressure and rainfall. The nature of this connection appeared to depend upon the geographical position as well as upon the different seasons.

In the following an attempt will be made to show that by means of this connection it is possible to make a long range weather forecast.

For this purpose Java has been chosen, because a forecast is of greater value for this island than for any other part of the Archipelago on account of its intense cultivation. Moreover this research will be limited to the east monsoon, as the connection is less distinct in the west monsoon, and because a forecast for this season of abundant rainfall is of secondary importance.

It will be necessary to prove, that the changes of the barometer-readings from year to year succeed each other according to definite rules, so that they may be determined in advance. Further we must also prove that it is possible to ascertain with sufficient accuracy how the rainfall depends upon the barometric deviations.

With regard to the deviations of airpressure Java has an advantage over any other part of the world, because the variations of climate are determined by the variations of the barometric pressure in North Australia, which are characterised by an extraordinary regularity. No station outside North Australia can vie with it in this respect, not even Bombay of Cordova (Argentina) which stations

[1]) These Proceedings 1912 p. 454.