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Microbiology. — "*Metabolism of the nitrogen in Aspergillus niger.*"

By H. J. WATERMAN. (Communicated by Prof. M. W. BEIJERINCK).

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

In a previous-communication ¹⁾ I described the circulation of the carbon in *Aspergillus niger*.

The changes which the plastic aequivalent or assimilation quotient of the carbon and the respiration- or carbonic acid aequivalent underwent in the course of time gave a clear view into the metabolism. In the beginning of the development a great plastic aequivalent was constantly found, which, however, lowered quickly, whilst the carbonic acid aequivalent rose considerably in the course of time.

The curve indicating the change of the two aequivalents with time could not be explained by an adsorption of nutrient substance.

The existence of an adsorption, that is to say, a change of concentration caused by molecular attraction of the components at the surface of a liquid formed by these components, gas, etc. and theoretically foretold by W. GIBBS and J. J. THOMSON, has in many cases been experimentally confirmed. For such experiments it was desirable to artificially enlarge the surface, for example by formation of scum, in order to bring the phenomenon within the reach of the relatively rough methods of observation.

Animal and plant cells present a great surface in relation to their contents. So it might be possible experimentally to observe the adsorption by the disappearance of the food from the surrounding medium.

The above investigation, however, has proved that this is not the case and the following experiments confirmed this.

A living mould culture, some months old and washed out with distilled water, ca. 300 mgrs. dry and containing hardly any more glycogen, was during half an hour shaken with 50 cm³ solution of 2% glucose, 0,15% ammoniumnitrate, 0,15% KH₂PO₄, and 0,06% magnesiumsulfate in tapwater. The mould layer, which had absorbed hardly any glucose from the solution, was then repeatedly washed out with distilled water at room temperature, boiled for ten minutes with distilled water, then filtered. The filtration did not reduce "Fehling", consequently contained no glucose.

If the concentration of the glucose in the mould were likewise

¹⁾ Folia microbiologica, Holländische Beiträge zur gesamten Mikrobiologie. Bd. 1 p. 422.

2 %, at least 6 mgrs. should have been found, a quantity which can with certainty be indicated by "Fehling".

This proves that there is no question of a considerable permanent adsorption at the outer surface of the protoplasm, but that it behaves more like a semi-permeable wall towards the glucose.

The same experiment was once more repeated, but this time with a 2 % glucose solution without anorganic substances, with shaking for two hours. Now, too, the mould proved to contain no glucose. A duplo-experiment gave only traces of glucose.

Hence the adsorption in *Aspergillus niger* is of no significance for the accumulation of nutrient substances.

Now it is a matter of course that the first stage of the accumulation is an adsorption, but it evidently escapes observation. The high plastic aequivalent in the beginning pointing to an extensive fixation of carbon-containing material, relates to a further stage of assimilation.

The food has then already passed into other compounds, *e. g.* into glycogen.

If the observations have ascertained that physiologic processes may be represented by an adsorption curve, this cannot be explained by accepting an adsorption in the first part of the process but it may be a consequence of what happens in a later stage.

Such an adsorption curve does not in general represent a simple process; it is more a combination of a whole series of successive physical and chemical phenomena.

In the study of the nitrogen results have been obtained corresponding to those found with the carbon.

It has namely been observed that also the nitrogen compounds used for the nutrition, are accumulated in the organism in a way not yet explained. First I convinced myself that the plastic aequivalent of the nitrogen at the end of the experiment is subject to only slight changes, as is shown in table I.

Compare for this nrs. 1 with 2 and 3, 4 with 5, 9 with 10, 11 with 12, 13 with 14. Secondly the quantity is independent of the nature of the source of carbon provided the weights of the mould be alike. For the levulose we find the same numbers as for the glucose. Lowering of temperature does not (nrs. 9 and 10) influence the rate of nitrogen of the mould, nor is it changed by addition of boric acid (nrs. 11 and 12).

Table II gives a view of the quantity of nitrogen fixed in the mould layer at various periods of development.

After 3 days the accumulation of nitrogen is of importance. Per

TABLE I. FIXATION OF NITROGEN BY ASPERGILLUS NIGER.

50 cm³. tapwater ¹⁾, in which dissolved 0.15 % NH₄NO₃ ²⁾, 0.15 % KH₂PO₄, 0.06 % MgSO₄ (free from water) with the organic food given below. Temp. 33° C.

Nr.	Organ. food	Age of the mould	Carbon fixed in the mould (in mgrs.).	Particulars
1, 2, 3	2 gr. glucose	5 to 6 months	17.2; 17.3; 16.0	The culture liquid contained besides considerable quantities of ammonia
4, 5	2 " levulose	5 to 6 "	16.5; 18.6	
6	1 " glucose	5 "	9.8	
7	1 " "	4 to 5 "	9.7	
8	1 " "	4 "	10.1	
9, 10	1 " " ³⁾	4 "	9.1; 8.7	
11, 12	1 " " ⁴⁾	5 "	9.2; 9.2	
13, 14	1 " levulose	5 to 6 "	10.-; 10.6	

1000 mgrs. of assimilated glucose, i.e. per 400 mgrs. carbon $\frac{100}{80}$.

19, 3 = 24, 1 mgrs. N is fixed after that time, that is 6 % N on the weight of mould. This value I will call *nitrogennumber*.

After 4 days the accumulation was fairly the same. The nitrogen number was decreased to 5. Nearly all the ammoniumnitrogen (ca. 13 mgrs.) was taken up by the organism, for with NESSLER's test the liquid gave but an insignificant reaction. The remaining 7 mgrs. are furnished by the nitratennitrogen.

¹⁾ An analysis of this water made in October last gave:

solid substance	461,3 mgr. p. L.	SO ₄ "	60,5 mgr. p. L.
org. "	69,7 " " "	Cl'	97,7 " " "
reduction power	3,5 mgr. O ₂ p. L. (K Mn O ₄)	NO ₃ '	traces
dissolved O ₂	4,54 cM ³ p. L.	NO ₂ '	absent
free CO ₂	1,3 mgr. " "	NH ₄ '	"
fixed CO ₂	126,8 " " "	Silicic acid (SiO ₂)	2,0 mgr. p. L.
Total CO ₂	127,2 " " "	Al ₂ O ₃ + Fe ₂ O ₃	6,2 " " "
Temporary hardness (PFEIFER-WARTHA)	: 8,07°	Ca O	71,2 " " "
Total	" (")	Mg O	30,1 " " "
Permanent	" (")	Cu and Pb	absent
Mg	" (")	Na ₂ O	120,2 " " "
Total	" (CLARK)	" from rest of combustion	128,9 " " "

²⁾ With this corresponds somewhat more than 26 mgrs. nitrogen.

³⁾ The temperature in these experiments was 25° C.

⁴⁾ Addition of 5 mgrs. boric acid.

TABLE II. METABOLISM OF NITROGEN.

50 cm³ tapwater, in which dissolved 2 pCt. glucose (free from water), 0,15 pCt. ammoniumnitrate, 0,1 pCt. potassiumchloride, 0,05 pCt. crystallised fosforic acid, 0,1 pCt. crystallised magnesiumsulfate, 0,1 pCt. calciumnitrate (free from water). Temp. 34° C.

-Nr.	Days after inoculation	Quantity of nitrogen fixed in the mould in mgrs.	Growth and spore formation ¹⁾ .	Reaction of the culture liquid with	
				NESSLER.	Diphenylamin-sulfuric acid.
1	3	19.3	vigor., hardly any spores	slight	strong
2, 3, 4	4	20.3; 20.7; 19.6	" , very few "	"	"
5	5	17.5	" , " " "	rather strong	"
6	7	11.8	" , " " "	" "	"
7, 8	9	9.9; 10.4	" , rather many "	" "	"
9	15	8.8	" , " " "	" "	"
10	18	10.6	" , " " "	" "	"
11	19	10	" , " " "	" "	"

Excepting nr. 1 where only 80 % of the glucose was used, all the glucose in the other culture tubes had been assimilated.

The dry weight obtained from them I have not determined in these experiments. For this my earlier investigations may be compared ²⁾.

Notwithstanding the quantity of fixed nitrogen had decreased on the 4th day from 6 to 5 % of the assimilated carbon, the absolute quantity was not lower. On the contrary, a slight increase was observed caused by the glucose still present after 3 days. The nitrogen excreted by the growing mould is assimilated again during the formation of new cells. When all the glucose had been used, which was already the case on the 4th day, nitrogen only was excreted. The consequence was a considerable lessening of the quantity fixed in the organism. After 5 days (nr. 5) only 17.5 mgrs. remained fixed. This was accompanied by a decrease of the nitrogen number as graphically demonstrated (fig. I). (see p. 1051).

After 5 days it was already decreased to 4.4, after 7 days to 3, after 9 days to 2.5. Then it undergoes but insignificant changes.

¹⁾ The use of very pure chemicals free from manganese causes the bad spore formation

²⁾ Folia Microbiologica, Bd. 1 p. 422 and these Proceedings XV p. 753.

The metabolism of the nitrogen corresponds thus in both cases with that of the carbon, namely a considerable accumulation at first,

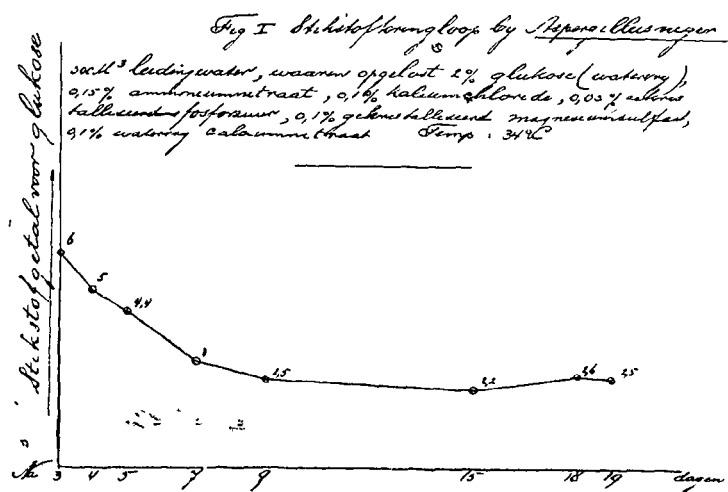


Fig. I. Metabolism of the nitrogen; 50 cm³ tapwater in which dissolved 2% glucose (free from water), 0,15% ammoniumnitrate, 0,1% potassiumchloride, 0,05% crystallised fosforic acid, 0,1% magnesiumsulphate, 0,1% calciumnitrate (free from water). Temp. 34° C.

which decreases very much in the course of time, finally to remain nearly unchanged.

Whereas carbonic acid is the form in which the carbon can leave the organism, the experiments in table II prove that the nitrogen is excreted as ammonium. The lowering of the nitrogennumber is parallel with a return of ammonium into the medium so that there is cause to consider, as before with the carbon, the course of the plastic aequivalent of the nitrogen and of the ammonium aequivalent in relation to time.

The decrease of the plastic aequivalent of the nitrogen is combined with an increase of the ammonium aequivalent. This view may, however, give rise to error as to the nitrogen, the ammonium being here a product of excretion, which likewise is mostly the form in which the nitrogen is given to the organism. By introducing the nitrogennumber this error is avoided. When excess of ammonium-nitrate is used it is chiefly the ammonium nitrogen which is assimilated, as proved before.

In a previous paper¹⁾ was shown that manganese does not change

¹⁾ These Proceedings, XV p 753.

T A B L E III. The conditions of the cultivation were the same as in table II.

Nr.	Particular.	Number of days after inoculation.	Nitrogen in the mould-layers in mgr.	Nitrogen-number.	Growth and spore-forming.
1	No manganese	After 3 days ¹⁾	19.3	6.0	vigorous, <i>hardly any spores</i>
2	0.001 mgr. MnCl ₂ 4 Aq.	" 3 " ²⁾	17.5	5.0	" few spores
3, 4, 5, 6	No manganese	" 4 " ³⁾	19.6; 19.6; 20.7; 20.3	5.0	" very few spores
7	0.001 mgr. MnCl ₂ 4 Aq.	" 4 " ³⁾	18.5	4.6	" rather many spores
8	0.01 " " "	" 4 " ³⁾	17.3	4.3	" " " "
9	0.1 " " "	" 4 " ³⁾	17.0	4.2	" " " "
10	0.001 Zincsulphate 7 Aq.	" 4 " ³⁾	20.2	5.0	" very few spores
11, 12	No manganese and instead { of KCl, RbCl	" 4 " ³⁾	18.6; 19.5	4.8	" <i>hardly any spores</i>
13, 14	"	" 6 " ³⁾	18.4; 17.8	4.5	" " " "
15, 16	"	" 9 " ³⁾	12.7; 12.2	3.1	" rather many spores
17	"	" 12 " ³⁾	9.-; 9.4	2.2; 2.3	" " " "
18	No manganese	" 18 " ³⁾	10.6	2.6	" " " "
19	No manganese, but instead { of KCl, RbCl	" 18 " ³⁾	9.3	2.3	" few spores
20	With manganese	" 30 " ³⁾	8.-	2.0	" many spores
21	" "	" 35 " ³⁾	8.2	2.0	" " "
22, 23, 24, 25	" "	" 40 " ³⁾	8.3; 8.3; 8.4; 9.-; 9.-	2.2	" " "

¹⁾ 80 pCt. of the glucose is assimilated.²⁾ 88 pCt. of the glucose is assimilated.³⁾ No glucose is remaining in the solution.

the nature of the metabolism of the carbon, but does modify its velocity and that substitution of rubidium to potassium neither changes that nature. This I have also found true for the nitrogen under the influence of the said metal, as is shown in table III.

Nr. 2, where manganese is added, has a lower nitrogen number than 1, which is owing to the manganese. The nitrogen numbers of 7, 8, and 9, are lower than those of 3, 4, 5, and 6, where no manganese is added. The nitrogen number of 10 is like that of 3, 4, 5, and 6. The addition of 0,001 mgr. zinc sulfate ($\text{ZnSO}_4 \cdot 7 \text{ Aq.}$) changes neither the metabolism of the nitrogen nor that of the carbon.¹⁾

That the replacing of potassium by rubidium has little influence on the metabolism of the nitrogen is proved by comparing nrs. 11 and 12 with 3, 4, 5, and 6, and 19 with 18, whose nitrogen numbers are nearly equal.

In the above described experiments the nitrogen in the liquid was of different nature, both in the form of ammonium and of nitrate. For that reason I repeated the experiment and used ammonium-chlorid as only source of nitrogen.

Various concentrations were also studied. The results are found in table IV.

From these experiments we may conclude that the nature of the metabolism with ammonium-chlorid is the same as with ammonium-nitrate. The nitrogen number, high at first (6,1), descends rapidly; after 7 days it is already decreased to $\pm 2,5$, then to remain nearly constant. Furthermore we see that excess of nitrogen does not change the metabolism. All the nitrogen excreted is found exclusively as ammonium, the sum of the nitrogen in the mould and of that present in the solution being constant. The losses of nitrogen which may partly be ascribed to errors in the analysis, are, as seen in the table, of little import, and partly repose on the evaporation of ammonia. Thus we see that in the till now examined cases *ammonia is a normal excretion product in the metabolism of Aspergillus niger*.

After Prof. BÖSEKEN's advice I investigated if this is always the case; if also by nitrogen nutrition with KNO_3 ammonia is excreted.

The results of these experiments are found in table V. We see from them that also with KNO_3 as exclusive nitrogen food the nitrogen is accumulated in the organism, albeit less quickly than NH_4Cl or NH_4NO_3 . The nitrogen number lowers also here whilst ammonia comes into the culture liquid. There are hardly

¹⁾ These Proceedings, XV p. 760.

TABLE IV. AMMONIUMCHLORID AS SOURCE OF NITROGEN.

A. 50 cm ³ tapwater, in which dissolved: 0.2 pCt. NH ₄ Cl, corresponding with 26.2 mgr N. 0.15 pCt. KH ₂ PO ₄ 0.15 pCt. Mg SO ₄ , crystallised 2 pCt. glucose (free from water)						B. 50 cm ³ tapwater, in which dissolved: 0.5 pCt. NH ₄ Cl, corresponding with 65.2 mgr. N. 0.15 pCt. KH ₂ PO ₄ 0.15 pCt. Mg SO ₄ , crystallised 2 pCt. glukose (free from water)					
Nr.	Mgr N in the mould	Nitrogennumber	Mgr. N. as ammonia in solution.	Sum.	Assimilated glucose in pCt.	Number of days after inoculation	Mgr. N. in the mould	Nitrogennumber	Mgr. N. as ammonia in solution.	Sum	Assimilated -glucose in pCt.
1	20.3		3.6	23.9	not determin.	3 days	19.9	—	44.5	64.3	not determin.
2	19.6	6.1	not determin.	—	79	3 "	23.5	6.2	not determin.	—	95
3	22.1		2.1	24.2	not determin.	4 "	21.4	—	38.9	60.3	not determin.
4	21.6	5.4	not determin.	—	98—99	4 "	21.7	5.6	—	—	97
5	16.2		5.6	21.8	not determin.	7 "	17.6	—	44.—	61.6	not determin.
6	17.2	4.3	not determin.	—	100	7 "	17.4	4.3	not determin.	—	100
7	9.25	2.3	10.5	19.75	100	10 "	10.9	2.7	49.4	60.3	100
8	8.7	2.2	12.2	20.9	100	10 "	10.—	2.5	49.8	59.8	100
9	8.7	2.2	13.4	22.1	100	21 "	8.1	2.—	52.8	60.9	100

any losses of nitrogen, so, *Aspergillus niger* is able to reduce nitrate to ammonia.

Furthermore it is proved that the nitrogennumber of a mature mould layer with glucose or levulose as exclusive organic food for *Aspergillus niger*, independent of the source of nitrogen, amounts about to 20.

Notwithstanding a young mould culture sometimes contains 2 to 2½ times as much nitrogen as a corresponding old one, such a young, duly washed layer, when boiled with distilled water gave no trace of the above named anorganic salts which had been added to the medium as exclusive source of nitrogen. Here thus, too, the

TABLE V. KNO_3 AS NITROGEN NUTRITION.

50 cm³ tapwater, in which dissolved 0,4 pCt. KNO_3 (28 mgr. N), 0,15 pCt. KH_2PO_4 ; 0,15 pCt. Mg SO_4 (crystallised) and 2 pCt. glucose. Temp. = 34° C.

Nr.	Quantity of nitrogen in the mould	Quantity of nitrogen in solution as ammonia, in mgrs.	Number of days after inoculation	Growth and spore-forming.	Assimilated glucose in pCt.	Nitrogen-number
1	8.4	Solution gives no reaction with Nessler	3 days	vigorous, very few spores	47 pCt.	4.5
2	14	Solution gives no reaction with Nessler	4 "	vigorous, beginning spore-forming	80 "	4.4
3	12.7	Solution gives no reaction with Nessler	4 "	vigorous, rather many spores	75 "	4.2
4	14.8	Only extremely slight ammonia reaction	5 "	vigorous, rather many spores	97 "	
5	14.2	Only extremely slight ammonia reaction	5 "	vigorous, rather many spores	92 "	
6	9.9	Already rather much ammonia in the liquid	7 "	vigorous, many spores	100 "	2.5
7	10.2		7 "	" " "	" "	2.6
8	8.1	4.9	10 "	" " "	" "	2.0
9	11.8	1.3	10 "	" " "	" "	2.9
10	9.4	3.8	22 "	" " "	" "	2.3
11	9.2	3.5	22 "	" " "	" "	2.3
12	9.5	not determined	22 "	" " "	" "	2.4

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excess of anorganic nitrogen compound attracted by the organism is very quickly converted into an unknown, nitrogen-containing intermediary product. Thus, here again the adsorption has no practical influence on the distribution of the nutrient substances between the medium and the mould.

These results which, as experiments have proved, hold also good for a few other examined organisms, show that in the literature the influence of the adsorption is often overrated ¹⁾.

In order to test the obtained views about the metabolism of the nitrogen the following experiments were made (table VI).

The quantities of added ammoniumnitrogen differ very much in the five series of experiments. In D there is just sufficient to satisfy the first wants (20.9 mgrs), in E was an excess of nitrogen, whilst in C, B and A there was a deficit of nitrogen relatively to the assimilated glucose. Still in B and C the additions were sufficient to satisfy the requirements of a mature mould layer.

In A this was not, however, the case and the quantity of nitrogen was even smaller than that fixed in an old mould culture containing little glycogen and obtained at the expense of 1000 mgrs. glucose. To this it must be ascribed that the assimilation of glucose is slackened. After 9 days 20 % is still unused. Fixation of nitrogen from the air could not be observed in this experiment, neither for A, nor for B or C, whilst yet these series of experiments might in particular come into consideration for an eventual fixation of atmospheric nitrogen in relation to the mentioned deficit in the nutrient solution. In the referring literature, however, are many statements tending to prove the contrary.

We further see that also the velocity of glucose assimilation in B is diminished although the general course of the process of nitrogen fixation remained the same, a high nitrogennumber at first which for all the series decreased with the time to 2 to 3.

The nitrogennumber of A, B, and C, and in slight degree also of D, was in the beginning bound to a certain limit determined by the added nitrogen and the mould.

Series A has a deficit of nitrogen with regard to the quantity of assimilable carbon; series E is characterised by a deficiency of carbon as to the quantity of fixed nitrogen.

We should still point to the association of the plastic equivalent of the carbon and the nitrogennumber. If the former is high this is also the case with the latter and the reverse.

Summary.

1. The nitrogen fixed in the mature mould is proportional to the plastic equivalent of the carbon independently of the nature of the carbon as well as of that of the nitrogen.

¹⁾ See also W. REINDERS and D. LELY, These Proceedings, 1912, p. 482.

TABLE VI. METABOLISM OF THE NITROGEN.
Ammoniumchlorid in different concentrations as only source of nitrogen.

	A 50 cM ³ tapwater 0.15 pCt. KH ₂ PO ₄ 0.06 pCt. MgSO ₄ (free from water) 0.06 pCt. NH ₄ Cl (6.5 mgr. N.) <i>deficiency of nitrogen.</i>					B As in A, but instead of 0.05 pCt. 0.08 pCt. NH ₄ Cl (10.5 mgr. N.) <i>Temporary deficiency of nitrogen.</i>					C As in B, but instead of 0.08 pCt. 0.12 pCt. NH ₄ Cl (15.7 mgr. N.) <i>Temporary deficiency of nitrogen.</i>					D As in C, but instead of 0.12 pCt. 0.15 pCt. NH ₄ Cl (20.9 mgr. N.) <i>Equilibrium of nitrogen.</i>					E As in D, but instead of 0.16 pCt. 0.32 pCt. NH ₄ Cl (41.9 mgr. N.) <i>Excess of nitrogen.</i>									
Number of days after inoculation	Assimilated glucose in pCt.	Milligrams nitrogen		Nitrogen-number	Growth and spore-forming	Assimilated glucose in pCt.	Milligrams N		Nitrogen-number	Growth and spore-forming	Assimilated glucose in pCt.	Milligrams N		Nitrogen-number	Growth and spore-forming	Assimilated glucose in pCt.	Milligrams N		Nitrogen-number	Growth and spore-forming	Assimilated glucose in pCt.	Milligrams N		Nitrogen-number	Growth and spore-forming					
		In the mould	As NH ₃ in solution				In the mould	As NH ₃ in solution				In the mould	As NH ₃ in solution				In the mould	As NH ₃ in solution				In the mould	As NH ₃ in solution			In the mould	As NH ₃ in solution			
3	59	—	0	—	Vigorous, already many spores especially at the borders	76	10.1	0	3.3	Vigorous already many spores especially at the borders	87	15.0	0	4.3	Vigorous, beginning spore-form. especially at the borders	93	>18.4	Slight reaction with Nessler	—	Vigorous, beginning spore-form. especially at the borders	79	17.5	Strong re-act. with Nessler	5.5	Vigorous, beginning spore-form. especially at the borders					
4	66	7.1	0	2.7	Vigorous, many spores	93	10.6	0	2.8	Vigorous, rather many spores	100	14.7	0	3.7	Vigorous, rather many spores < B	100	19.6	0	4.9	Vigorous, beginning spore-form. especially at the borders	75	18.1		6.0	Vigorous, beginning spore-form. especially at the borders					
						93	—	0	—	100	—	0	—	100	—	0	—													
5		6.2	0	—								14.6	0	3.6																
																										6.7	—	—		
6	82	6.9	0	2.1		100	10.2	0	2.5							100	15.3	—	3.8		100	13.5	—	3.4						
	77	6.3	0	2.0	Vigorous, many spores	100	9.1	0	2.3	Vigorous, many spores		11.1	2.9	2.8	Vigorous, many spores	100	12.1	7.6	3.0	Vigorous, many spores	100	—	—	—	Vigorous, rather many spores					
Formed mould (mgrs dry)	A					B					C					D					E									
	after 4 days					—					403					468.5					497; 477.5					—				
	" 7 "					—					—					—					346					354				
	" 9 "					279					343					—					324					326				

2. The nitrogennumber, by which is meant the nitrogen per 100 parts of weight of assimilated carbon lowers with time; for a mature mould it is ca. 2 (glucose or levulose as source of carbon).

3. The metabolism of the nitrogen has much resemblance to that of the carbon.

a. An accumulation of carbon is combined with a high nitrogennumber; inversely the mature mould has a low nitrogennumber.

b. The nature of the metabolism of the nitrogen does not change under the influence of many factors; neither is this the case with the carbon.

c. The velocity of the metabolism is subject to great changes.

d. The same factors that accelerate the metabolism of the carbon also further that of the nitrogen.

e. Substitution of rubidium for potassium is of little influence on the metabolism of the nitrogen.

4. The nature of the metabolism of the nitrogen is independent of the source of nitrogen. At first the nitrogennumber is high, then it decreases whilst the freed nitrogen returns into the nutrient solution as ammonia. This is proved for the cases when ammoniumnitrate, ammoniumchlorid, or potassiumnitrate is given as nitrogenfood. *Aspergillus niger*, thus, reduces nitrates to ammonia but not to free nitrogen. Only in the culture tubes with a deficiency of nitrogen as to the quantity of carbon, no ammonia can return into the solution as it is directly used for the production of new cells.

6. In the cases of a deficiency of nitrogen no fixation of atmospheric nitrogen could be observed.

Finally my hearty thanks to Professor Dr. J. BÖRSEKEN and Professor Dr. M. W. BEIJERINCK for their valuable help in this investigation.

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Delft, November 1912.