corresponding to the stimulus 900 are rather too small, as well for the height of twitch as for the heat-production. Calculating from the observed lifting-height the corresponding magnitude of stimulus, we find 810 instead of 900. Now taking this number 810, to calculate the heat-production, we obtain 12, in perfect accordance with the observation. The supposition that the number 900 is an error and that 800 was meant is not very hazardous.

From the communicated series we may draw firstly this conclusion that the heat-production, considered as total effect, increases virtually with increased magnitude of stimulus in the manner indicated by the established formula.

In the three last series B_w, the increment-constant for the thermal effect, proved to be always smaller than the B_h corresponding to it, a fact predicted already in our deduction. We found for the number $n = \frac{B_h}{B_w}$ in series IV, V and VI the value 2.5, 4.23 and 2.31. Though of course even by this fact our deduction may not be deemed absolutely proven, it nevertheless affords a valuable support for considering the deduction proposed by me as a most useful working-hypothesis.

Bacteriology. — *"On a colourless bacterium, whose carbon food comes from the atmosphere."* By Prof. M. W. BEIJERINCK and A. VAN DELDEN.

We give the name of *Bacillus oligocarbophilus* [1]) to a colourless bacterium, whose carbon nutrition in the dark (and likewise in the light), takes place at the expense of a not yet well-known atmospheric

[1]) It is probable that W. HERAEUS (Ueber das Verhalten der Bacterien in Brunnenwasser sowie über reducirende und oxydirende Eigenschaften der Bacterien. Zeitschrift f. Hygiene, Bd. I, pag. 226) already in 1886, has had cultures of *B. oligocarbophilus* before him. He says the following: .... „Ausserordentlich auffallend war das Ergebniss dieser Versuche in der Hinsicht, dass eine Vermehrung der Bacterien in einer Flüssigkeit eingetreten war, welche keine organische Verbindungen sondern nur Salze enthielt. Ein unansehnliches, kaum sichtbares Pünktchen von Bacterienzoogloeën hatte sich im Verlaufe vom zehn Tagen so stark vermehrt, dass die ganze Oberfläche der Lösung von einer dicken Haut bedeckt war." Analytical results are not given, and the remark makes the impression of being accidental and is lost among insignificant observations. — WINOGRADSKY's statement, concerning the accumulation of organic carbon in nitrifying solutions, evidently refers likewise to this microbe, but his description suffers of indistinctness (Annales de l'Institut Pasteur, T. 4 pg. 270 et 462, 1891).— In the experiments of GODLESWKI (Bulletin international de l' Académie d. sc. d. Cracovie, Dec. 1892 pag. 408 et Juin 1895 pag. 178), the vanished CO^2 is not, as he thinks, absorbed by the ferments of nitrification but by the $MgO.MgCO^3$.

carbon compound (or compounds), from which the energy, wanted for the vital processes, is also derived [1]).

The culture of this bacterium on solid media or in nutrient solutions, containing soluble organic substances has not yet succeeded, which may, of course, have been caused by an erroneous choice of these substances. On the other hand, pure cultures on solid and in liquid substrata, without soluble carbon compounds, are easy to be made.

1. CRUDE CULTURES OF BACILLUS OLIGOCARBOPHILUS.

Bacillus oligocarbophilus is obtained by the following accumulation experiment, which, because of the purity of the thereby resulting vegetation, may be called a "perfect accumulation experiment."

Into a large ERLENMEYER-flask a thin layer is introduced of a nutrient liquid of the same composition as used for the water culture of higher and lower green plants, but with alkaline instead of acid reaction.

One takes for instance:

Distilled water	100
Kaliumnitrate	0.01 to 0.1
Dinatriumphosphate	0.02
"Mineral solution"	1 drop.

This "mineral solution" contains in one drop:

8	Mgrms	$MgSO_4 . 7 H_2O$
0.05	"	$MnSO_4 . 4 H_2O$
0.05	"	$FeCl_3 . 3 H_2O$

If from this liquid nitrogen, phosphor, kalium or magnesium is left out, special experiments have proved, that no, or but an insignificant growth is obtained. As to the necessity of the likewise added elements sulphur, manganese and iron, there still exists some doubt.

The inoculation is made with a not too small quantity of garden-soil, the flasks are closed with a cotton plug, or with filter paper, without impeding the entrance of air by diffusion, and the culture is left in the dark at 23—25° C. After two or three weeks, the fluid, which itself remains perfectly clear, is seen to cover with a thin, white, or feebly rose-coloured, very dry film, difficult to moisten, and macroscopically resembling a *Mycoderma*-film, but consisting of minute bacteria, microscopically often invisible without staining, and sticking together by a slimy substance. This is *Bacillus oligocarbophilus*.

[1]) We also found another, rarer species, belonging to the genus *Streptothrix* COHN, with corresponding properties. It will not, however, be further discussed here.

The growth of the film continues for months, whereby a considerable accumulation of organic carbon may be observed, which is not only visible to the naked eye by the vigorous bacterial growth, but can also be proved by direct weighing, and by a comparison of the permanganate numbers found before and after the experiment, of which some instances are given below.

As there is reason to admit that our bacterium is generally distributed in garden-soil, and was without doubt always present in the crude material used for the inoculation, the failing of the film-formation in some of the flasks must necessarily result from the chosen culture fluid being less favorable to the feebler germs and not allowing their growth. So we observed that water, distilled in a copper apparatus, caused many more failures than when distilled in glass; we therefore afterwards always used the latter. In other cases monads, which immediately devoured the bacteria, were cause of the failure; by transfers and by the use of pure cultures, these voracious organisms could be rendered harmless or removed. When the distilled water is replaced by tap-water, the number of flasks remaining without growth after inoculation with the same quantity of garden-soil is much smaller.

If once a pellicle has formed, transfers into the said culture liquid, prepared either with distilled or with tap-water, come easily and without exception to development.

2. SOURCE OF NITROGEN REQUIRED.

In the above mentioned nutrient liquid we have chosen kaliumnitrate as source of nitrogen. As well, however, kaliumnitrite or some anorganic ammonium salt may be used. Very good results were obtained with:

Distilled water	100
Ammonium sulphate (or NH_4Cl)	0.01—0.1
Dikaliumphosphate	0.02
"Mineral solution"	1 drop

and with:

Distilled water	100
Kaliumnitrite	0.01—0.1
Dikaliumphosphate	0.02
"Mineral solution"	1 drop.

As both these liquids answer to the conditions of life of the microbes of nitrification, the formation of nitrite or nitrate is actually to be observed when using them, and when inoculating with garden-soil or with crude cultures. With the easily produced pure cultures

of *B. oligocarbophilus*, of which more below, a good development of the film is possible, by which experiment it can at the same time be proved, that this microbe itself does not nitrify. Hence, ammonium salts or nitrites, added to excess can, even for a year or longer, continue unchanged under the luxuriantly growing pellicle of *B. oligocarbophilus*, whereas, in the presence of nitrifying ferments, they completely disappear in a few weeks, being then found back as nitrates. If the ferments of nitrification alone are present, there is no question of film-formation and the nutrient solutions remain perfectly clear.

Not only the nature of the nitrogen-furnishing substances, but also their quantity can in these experiments, as already inferred in the recipes, vary between fairly wide limits, and the same may be said concerning the conditions for the water culture of higher and lower green plants. The limits allowable for *B. oligocarbophilus*, have not yet been precisely fixed, but they certainly have a broader range for this organism (circa 0.1—10 pro mille) than for the higher plants (0.5—5 pro mille).

By many experiments it was established, that in absence of kalium, phosphor, and magnesium, a still slighter growth occurs, than when no nitrogen compounds are given. Evidently *B. oligocarbophilus* finds in the almosphere, in a condition fit for nutrition, a quantity of nitrogen, which, although insufficient, should not be overlooked.

If the distilled water in the artificial solution is replaced by tap-water, a somewhat higher rate of organic substance is produced. As in tap-water a small quantity of nitrogen compounds occur, — here, at Delft, about 0.4 milligrams of combined nitrogen per litre, — whilst it contains the other necessary elements (phosphor and kalium, of course, excepted) in an obviously favorable form for the nutrition of our mikrobe, one can simply use for its culture:

Tap-water	100
Dikaliumphosphate	0.02.

It should, however, be kept in view, that the productivity in bacterial substance, in consequence of the film formation, is not determined by the volume, but chiefly by the extent of the surface of the medium, which is in free contact with the air. Hence, in a very thin layer of tap-water, the nitrogen may soon be consumed, whereas, with the same amount of nutrient liquid, but with a smaller surface, consequently in a thicker layer, the provision of nitrogen will suffice for a longer time. Therefore, in order to obtain from a flask of determined size, the maximum production of *B. oligocarbophilus*, a

nitrogen compound should be added when a small quantity of tap-water is used, which addition is not necessary when cultivating in a greater quantity in a flask of the same size.

3. PURE CULTURE.

Our bacterium does not grow at all or only to a slight extent on the commonly used bacteriological media, these containing too much organic food. But it is easy to produce pure cultures on solid media, when observing the same precautions which I described in the Meeting of the Academy of 27 June 1892 for the pure culture of the ferments of nitrification on agar-plates [1]), and to which I referred in the Meetings of 30 March 1901 (Proceedings p. 586) and 25 May 1901 (Proceedings p. 5) when discussing the culture conditions of the oligonitrophilous Cyanophyceae.

In all these cases it is necessary as completely as possible to remove all soluble organic substances from the solid medium, which is to be effected by a prolonged washing with distilled water. The agar thus prepared, with the required nutrient salts, for instance in the proportion:

Distilled water	100
Agar	1.5
K_2HPO_4	0.01
KNO_3 (of NH_4Cl)	0.01

is boiled and plated, and used for strew-or streakcultures originating from a film of *B. oligocarbophilus*. Very soon the common saprophytic bacteria which never lack in the film, are seen to develop on the plate and when these by their growth and respiration have consumed the soluble carbon compounds, which were not yet removed from the agar by the extraction with water, *B. oligocarbophilus* itself begins to grow. This is usually the case after 14 days. Then, however, the colonies become easily recognisable, our bacterium being the only species which in the given circumstances can feed on the atmospheric carbon, and so go on growing, whilst the growth of all other species soon comes to a stop.

Even the colonies of the nitrifying ferments, which, as I have demonstrated before (l. c.), can grow fairly well on this medium, when instead of nitrate an ammonium salt is used, remain very small, never exceeding 1 mM. or less. On the other hand, the colonies of *B. oligocarbophilus* attain dimensions of 1 cM. and more and may then easily be transferred in a pure condition into test-tubes

[1]) Nature, Vol. 46, pag. 264, 1892.

on the said medium. They grow on the agar as thin, snow-white or rosy-tinted, very dry, flatly extended layers, which strongly remind of the pellicle floating on the liquid.

Also on silica plates, prepared in glass dishes, which, after extraction of the chlorides are soaked with a nutrient solution, *B. oligocarbophilus* can produce very fine cultures, appearing after some weeks, as snowwhite colonies with indented margin, and which by a right selection of the salts, can finally spread over the whole plate. Then the remarkable phenomenon is observed, that the silica liquefies a little in the centre of the colonies and sinks in by evaporation.

The silica plates are made as follows. A commercial solution of potassium silicate, diluted with a known quantity of water, is titrated with normal hydrochloric acid. As the solidification is much favoured by an alkaline reaction, a complete neutralisation at the preparation of the plate should not occur, and as a plate, with a high percentage of silica, contracts strongly after coagulation, and expresses much water, the dilution must be sufficient for this contraction to be delayed. Into a small beaker-glass was introduced, in a certain case, 5 cM^3 of potassium silicate diluted with 25 cM^3 of water, and into a second glass the required quantity of hydrochloric acid, amounting to 10 cM^3 of normal acid. The acid is mixed with the diluted silicate and the mixture poured into a glass dish. The solidification delays the longer as the mass is more diluted, but it is easy, after some practice, to make very solid plates. The plate is first freed from the chlorides by streaming tap-water, then washed out with boiled water, and afterwards treated with the solution of nutrient salts. When these have sufficiently diffused into the plate, the glass dish is gently warmed at the underside, until the adhering water has evaporated and the plate shows a "dry", glossy surface. The surface is flamed in the BUNSEN-burner, by which only a partly but sufficient sterilisation is to be attained.

Not only *B. oligocarbophilus*, but also the ferments of nitrification grow on this medium very well. By mixing of the diluted solution of the silicate with chalk, magnesium carbonate, or ammonium-magnesium phosphate, snow-white plates may be obtained, which are particularly fit for the culture as well of all these microbes as of several lower algae. Even earth-diatoms, of the genus *Nitzschia* will grow thereon.

Once more it must be observed, that in the silica plates organic substances must be absent, even fragments of cork, fallen into the silicate solution, may disturb the experiment.

The pure cultures, obtained on agar or silica plates, are as well fit

for the further experiments on liquid media as the crude cultures, of which many experiments, continued for years, have convinced us. Every thought of symbiotic relations on which the carbon assimilation by our bacterium might repose is thereby excluded, so that at least the biological side of this part of our problem is clear.

Concerning the further properties of our bacterium in pure cultures, we can be brief. In the films, as well as on in the colonies on the solid media, it consists of minute, thin and short rodlets, probably always immobile. They are ca. 0.5 μ wide and 0.5—4 μ long. The length however is very variable and frequently particles are seen 0.5 μ wide and 0.7—1 μ long. Often, when not using reagents, such as dyeing substances or acids, no structure at all is to be observed, neither in the colonies nor in the flowing pellicle, but the bacteria at once become visible by staining the preparations. The thick cell-walls form the chief constituent of the colonies; albuminous matter is only present in a slight quantity in this bacterium.

4. THE NUTRITION WITH ATMOSPHERIC CARBON.

A good appreciation of the carbon accumulation may be had as well by a direct weighing as by the permanganate method.

For both determinations it is possible, to suck off the fluid, which is practically free from bacteria, wholly or partly from beneath the film, so that the quantity of the culture material, destined for the filtration or the determination of the permanganate number, is not too voluminous.

In our experiments there only resulted a precipitate of calciumphosphate or calciumcarbonate, when we had used our tap-water, which is rich in lime, and when kaliumphosphate to excess had been added. These precipitates can, however, be dissolved beneath as well as in the film by dilute acid, and then the acid can be expelled by further washing. The film is so dry and wetted with so much difficulty, that all these manipulations may be effected without much loss of material.

The permanganate number was determined after KUBEL's [1]) method.

In relation to the quantity of organic matter found by direct weighing or by the permanganate method and formed from the atmospheric carbon, the following should be well observed.

As *B. oligocarbophilus* grows only on the free surface of the

[1]) TIEMANN-GÄRTNER's Handbuch der Untersuchung der Wässer, 4e Aufl. pag. 255 1895.

medium, and not in the depth, the thickness of the layer of the nutrient solution and consequently its volume, is, as already observed, actually indifferent. That is to say, by enlarging the surface of the solution, a bacterial film of any dimensions is to be obtained, which circumstance is of importance for appreciating the productivity of a certain quantity of a nutrient solution, the more so as the thickness of the bacterial film is usually only one cell-layer. How very thin the required thickness of this layer can be, growth being still possible, may be derived from the fact, that, especially when using distilled water with nutrient salts, the film can mount at the apparently dry glass-wall from 1 to 1.5 decimeter high, and not seldom extends on it nearly to the cotton plug. Only in certain vinegar bacteria I observed the same.

As it seems that our bacterium forms no compounds prejudicial to its growth, so the only circumstance, which governs its increase relatively to a given volume of liquid, provided its surface be of a sufficient extent, is the lack of one or more elements necessary for the nutrition. Carbon cannot be among the number, our experiments being made with free entrance of air.

Although it is thus established, that only the number of bacteria, produced in a certain time per surface-unit, indicates the rate at which the atmospheric carbon is assimilated, we will yet give the quantities in relation to the volume of the solution, because then a comparison can be better made with the numbers found by other authors for polluted waters.

5. HOW MUCH CARBON IS ASSIMILATED.

First we determined by an experiment, in which, after vigorous shaking, a culture was divided into two equal portions, how much one half contained at direct weighing, of bacterial substance, whereas the other half was titrated with kaliumpermanganate. We used for this a three months old culture on:

Tap-water	100
Na_2HPO_4	0.02
KCl	0.02
KNO_3	0.02

The film from the part, destined for the weighing, was separated from the liquid by filtration, washed out on the filter with strongly diluted hydrochloric acid, and subsequently with distilled water, to remove the chlorids. Subsequently the filter with the film was

dried, first at 40°—50° C. and then at 100° C., until the weight remained constant. So we found that per litre 180 milligrams of bacterial matter were produced, and that, after deduction of 14 milligrams, used by a litre of our tap-water itself, the corresponding permanganate number was 94. We can thus, with an accuracy sufficient for our purpose, accept that the relation between the two figures is as 2 : 1, that is to say, that the doubling of the permanganate number gives the weight of the dry bacterial substance, and, as this latter number is much more quickly to be found than the weight, we have contented ourselves with it in most of our further determinations.

We shall now give some more figures. Like the preceding they all relate to bacterial films produced in ERLENMEYER-flasks on 100 cM^3. liquid with a free liquid-surface of about 80 cM^2.

By weighing we found in one case on:

Tap-water	100
KCl	0.02
KNO_2	0.02
K_2HPO_4	0.04

after 5 months' culture 235 milligrams per litre. On:

Distilled water	100
KCl	0.02
KNO_3	0.1
K_2HPO_4	0.02
"Mineral solution"	1 drop

after 5 months 220 milligrams per litre.

Some numbers, found by the permanganate method follow, and in the first place some relating to tap-water.

The greatest production which we had, was obtained with tap-water 0.02 K_2HPO_4 and 0.02 KNO_3, after a year's culture and amounted to 250 mgrs. of permanganate per litre, nearly corresponding with $250 \times 2 = 500$ milligrams of dry bacterial substance.

After a shorter time the production is likewise smaller; so we found in a culture on:

Tap-water	100
Na_2HPO_4	0.02
KCl	0.02
KNO_3	0.02

after 5 months' culture (January to May) 202 mgrs. of permanganate, corresponding with 404 mgrs. of bacterial matter per litre.

If the tap-water was replaced by distilled water, the production of dry organic substance was commonly smaller, which cannot, however, result from the nutrition by substances in the tap-water, oxidisable by kaliumpermanganate, for the 14 mgrs. of permanganate, which our tap-water consumed per litre, we found quantitatively back, at the end of the cultivation period, in the clear liquid beneath the pellicle of *B. oligocarbophilus*, which liquid can easily be sucked off with a pipette, without any considerable bacterial contamination. Moreover the experiments with distilled water have likewise exhibited great divergency in production, and though the cause has not been established with perfect certainty, we still think it probable, that these differences result from the greater or smaller density of the cotton plugs, by which the speed of air entrance is greatly influenced. We base this supposition on results obtained with flasks, only differing in the width of the mouths, and to which we shall refer later. It is furthermore certain that we have not to do here with the infection of other bacteria, or with monads, for the pure cultures displayed as considerable divergency as the crude ones. Neither can the chief cause be attributed to a change in percentage of the air in gaseous carbon compounds, the differences being observed simultaneously in cultures placed side by side in the same locality.

But we now give some further numbers. In an experiment with:

Distilled water	100
K_2HPO_4	0.02
KNO_2	0.1
KCl	0.01
"Mineral solution"	1 drop

sterilised and inoculated with a pure culture of *B. oligocarbophilus*, were found, after 37 days' cultivation (2 Jan.—19 Febr.) at 23° C., 66.6 mgrs. of permanganate, corresponding with circa 133 mgrs. of dry bacterial substance per litre.

In another experiment with:

Distilled water	100
Na_2HPO_4	0.02
KNO_2	0.01
"Mineral solution"	1 drop

likewise sterilised and after a culture of 40 days, at 23° C. the permanganate number amounted to 60 mgs., corresponding with 120 mgrs. of dry bacterial matter per litre.

In a third case in:

Distilled water	100
K_2HPO_4	0.02
$(NH_4)_2SO_4$	0.02
Na_2CO_3	0.01
"Mineral solution"	2 drops

after cultivating from 5 May to 1 Dec., 155 mgrs. of permanganate per litre were found.

In a culture in:

Distilled water	100
Na_2HPO_4	0.02
KCl	0.02
KNO_3	0.02
"Mineral solution"	1 drop

from 1 June to 1 Dec. we found 165.5 mgrs. of dry bacterial substance, corresponding with ca. 83 mgrs. of permanganate per litre. As we see, the differences are considerable.

When a little natrium acetate was added to the anorganic solution, and when using a pure culture for inoculation, we could neither state an augmenting nor a diminishing of growth.

Thus we obtained in:

Distilled water	100
KCl	0.02
KNO_2	0.1
Natriumacetate	0.02
K_2HPO_4	0.02
"Mineral solution"	1 drop

by means of weighing, 220 mgrs. of dry bacterial substance per litre, corresponding with 110 mgrs. of permanganate, which figures are not exceedingly high and might likewise have been producedi n the same time (4 months) from the air alone, without acetate.

In all these experiments with distilled water, the free surface of the liquid was also 80 cM^2, and the air had to pass through a dense cotton plug, with which the ERLENMEYER-flasks were closed. Already before we drew attention to the importance of the way in which the flasks are closed; be here still mentioned that we made some special experiments, which proved that a very narrow opening of the flasks, slackens the growth of *B. oligocarbophilus*, so that years may go by before the film has vigorously developed. We could not, however, expected anything else, for the considerable volume of air, required for the growth of the said quantities of bacteria, can only very slowly diffuse inward and outward through the narrow canal.

6. CARBONIC ACID CANNOT SERVE AS FOOD.

Various experiments were made to establish what may be the volatile atmospheric carbon compound which renders the growth of *B. oligocarbophilus* possible. That it cannot be carbonic acid, whether free or combined, resulted from the following experiments. In closed culture-flasks with the best nutrient solutions, and arranged in such a way, that at times a little free carbonic acid mixed with pure air, could artificially be introduced, it was not possible to get any growth. This experiment, which seemed of particular interest, has been so frequently repeated, and so long continued under different conditions, that we consider it as quite certain, that free carbonic acid cannot serve for the nutrition of *B. oligocarbophilus*.

For testing the influence of combined carbonic acid, cultures were made, firstly in the following solution:

Tap-water	100
Dikaliumphosphate	0.01
Kaliumnitrate	0.01
Natriumbicarbonate	0.1

When cultivating at the free air surely a luxurious growth was obtained, but it was by no means more vigorous than when the bicarbonate was left out.

If in this liquid the nitrate was replaced by an ammonium salt, the result was quite the same.

Secondly, the bicarbonate was replaced by common natrium carbonate, the same quantities of the different salts being used. But in this case the action proved rather injurious than favorable. It is true that the film had become considerable after a few months, but it was directly to be seen that the growth was so much inferior to that of cultures obtained in the same circumstances but in absence of carbonate, that the determination of the permanganate number seemed superfluous. Here, too, the replacing of nitrate by an ammonium salt or by a nitrite caused no change.

As a remarkable fact it may be mentioned, that in these experiments, in our large flasks, containing a litre of air, the thin bacterial film mounted very high up the dry glass-wall, which is likewise often observed in the solutions made with distilled water, and may repose on the absence of dissolved lime salts.

If the tap-water was substituted by distilled water, the addition of natrium carbonate did not cause an increase of bacterial growth either. We found, for instance, in:

Distilled water	100
K_2HPO_4	0.02
$(NH_4)_2 SO_4$	0.02
Na_2CO_3	0.1
"Mineral solution"	1 drop

after 7 months (5 May—1 Dec.) 155 mgrs. of permanganate, corresponding with ca. 300 mgrs. of dry bacterial substance per litre, which production is less than that, obtained in other cases under the same circumstances but without carbonate, so that here also, the action of the carbonate, the long time of cultivation being taken into consideration, was not favorable. Quantities of carbonate, smaller than 0.1 %, were neither successful.

The results of this examination can be thus summarised, that for the growth of *B. oligocarbophilus* an atmospheric carbon compound is actually consumed, but that this cannot possibly be free carbonic acid. Furthermore, that also combined carbonic acid cannot serve for its nutrition.

7. Nature of the assimilated atmospheric carbon compound.

If the carbonic acid of the air cannot be the food of *B. oligocarbophilus*, what other atmospheric carbon source might then come into consideration?

It is clear, that we should think here of the carbon-containing component of the air, discovered in 1862 by the botanist Hermann Karsten [1]), and recently discovered anew by French experimenters, especially by Mr. Henriet [2]). It is true that the chemical nature of this substance has been hitherto unknown [3]), but yet it is certain that we have here to do with an easily oxidisable compound (or compounds), for a prolonged contact with alkali and air will already suffice to split off carbonic acid from it. Furthermore, according to the statement of the French investigator, the substance probably contains nitrogen.

This latter circumstance gives rise to the question whether this

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[1]) H. Karsten. Zur Kenntniss des Verwesungsprocesses. Poggendorff's Annalen Bd. 191, pag. 343. 1862. To this place, as also to the not unimportant older literature on the carbon compound of the air, my attention was drawn by Mr. G. van Iterson.

[2]) Comptes Rendus T. 135, pag. 89 et 101. 1902.

[3]) Henriet thinks that the substance must be a monosubstituted formamid with the formula HCO.NHR, where R represents a still unknown alkylrest. But then it is not easy to understand, why the production of carbonic acid takes place so readily. It might then rather be expected that, with an alkali a formiate would result and no carbonate.

nitrogen, like the carbon, is fit for assimilation by our microbe. Though this question has already partly been answered in the negative by the preceding experiments, it should still be remarked here that in nutrient liquids, without an expressly added nitrogen compound, for instance in:

Distilled water	100
K_2HPO_4	0.02
Mg, S, Mn, Fe	traces.

Or still better in:

Tap-water	100
K_2HPO_4	0.02

without any further addition, a not inconsiderable growth of *B. oligocarbophilus* may occur, so that at least traces of an assimilable nitrogen compound may be drawn from the air by this bacterium, whereas, for the possibility of assimilation of the free atmospheric nitrogen no indications were found.

We now turn to another question, which the assimilation of the atmospheric carbon gives rise to, namely: How great is the quantity of the volatile substance wanted for the formation of the bacterial film produced in our cultures? This question is closely connected with the following: How much of the compound is moreover consumed by the respiration of our bacterium, escaping as free carbonic acid? For answering these questions we have to measure the quantity of the carbonic acid corresponding with a determined weight of dry bacterial substance, granted that the carbon percentage of this substance be known.

Our experiments relating to the measurement of the quantity of carbonic acid produced, are not yet closed, but as to the first part of the question, we give the following calculation to fix the volume of air wanted for the production of the carbon, actually accumulated in the bacterial films. We hereby make two chemical suppositions which, to be sure, are fairly well in accordance with truth. First, we admit that the carbon, freed from the unkown compound, as carbonic acid by a prolonged contact with alkali, is consumed quantitatively by our bacterium and, secondly, that the bulk of the bacterial cells consists of a substance possessing nearly the composition of cellulose [1]).

[1]) If accepting that the composition of the bacterial cells corresponds with that of albuminous substances, then, instead of 44% C., 52 to 55% C. should be brought into account, and in this proportion the volume of the air should be augmented.

Let us now consider the case when, in $^1/_2$ litre-flask with 100 cM³. of fluid and a free surface of 80 cM², after a month's culture a quantity of 20 mgrs. of dry bacterial substance is formed, which, calculated as cellulose, contains 44 % C.; we then find in the 20 mgrs. of dry matter 8.8 mgrs. of carbon. According to HENRIET the atmospheric carbon compound, present in a certain quantity of air, under prolonged action of alkali, gives out as much carbonic acid as occurs already in a free state in the same volume of air, that is per litre 0.3 cM³. = 0.6 mgrs., in which 0.163 mgrs. of carbon are present. Thus, for 8.8 mgrs. are wanted 55 litres of air. Consequently, in the course of a month these 55 litres of air must have diffused through the cotton plug inward and outward of our $^1/_2$ litre-flasks, in order to produce the found quantity of carbon, that is 76 cM³. hourly.

Though this figure should not be considered a priori as impossible, it still appears to be very high, and the difficulty of accepting it increases, if still the addition has to be made of a yet unknown, but apparently considerable amount consumed for the bacterial respiration, which, as remarked above, seems necessary. We therefore think that it must be admitted that the quantity of the atmospheric compound (or compounds) assimilable by *B. oligocarbophilus*, is much larger in our laboratory atmosphere, than in that of the Paris boulevard, analysed by HENRIET, and that we have here to do with an extremely variable factor. The circumstance, too, that we have not as yet been able in our greenhouse, where the air, in the common sense of the word, is surely much purer than in the laboratory, to obtain a vigorous growth of *B. oligocarbophilus* pleads for this view. But here we could not always keep the temperature high enough, so that we consider our experiments in this direction not yet closed. Besides, we should observe, that in an empty, isolated room of the laboratory, the quantities of combined carbon drawn from the air, were as great, or only little less than in the laboratory itself, where the air was certainly impurer.

We are accordingly conscious that further experiments, with fresh atmospheric air are wanted to decide, whether the carbon compound occurs in the atmosphere in a constant or in a varying percentage. Only thereby it will be possible to ascertain the distribution of this compound, by which, at the same time, the signification of *B. oligocarbophilus* in nature will become clearer.

As to this signification, the question arises whether our microbe in substrata containing sufficient mineral nutrients (N, P, K, Mg, S, Fe, Mn), but being poor in organic substances, is able to build up the latter in the dark from the volatile carbon compounds occurring in the

atmosphere of the surrounding medium. And furthermore, whether carbon nutrition takes place exclusively in the floating dry films, — hence, in the earth, only on the relatively dry surface of the earth particles, — or that also in the depth of fluids growth and carbon assimilation be possible. The hitherto gathered experience about the self-purification of rivers and the biological purification of water in general, seems to exclude the latter hypothesis, and our own experiments too, render it not probable. The result of these experiments consists, in our opinion, in the very discovery of a microbe, which, in consequence of the film-formation, has the specific faculty, to absorb for its nutrition and multiplication, from a gas, namely the air, traces of volatile carbon compounds, by which the struggle for existence with the rest of the microbic world can be successfully sustained. The biological purification of water would, according to this view, find a counterpart in the biological purification of the air by *Bacillus oligocarbophilus*

Physics. — "*The calculation of* $\frac{e}{m}$ *from the magnetic rotation of the plane of polarisation, for substances without an absorption band in the visible spectrum.*" By Dr. L. H. SIERTSEMA. (Communication No. 82 from the Physical Laboratory at Leiden by Prof. H. KAMERLINGH ONNES).

Starting from FITZGERALD's [1]) simple explanation of the magnetic rotation of the plane of polarisation derived from the ZEEMAN effect, and also from the supposition that the result of the magnetic force is only shown by the displacement of the dispersion curve of the medium ($n = f(\lambda)$) over a distance δ, HALLO [2]) finds for the magnetic rotation ω

$$\omega = \frac{2\pi}{\lambda} z \delta \frac{dn}{d\lambda},$$

where z represents the thickness of the medium. HALLO's investigations are concerned with the parts of the spectrum in the neighbourhood of an absorption band and for these we are justified in making the above supposition, as appears from a formula derived by VOIGT

[1]) FITZGERALD. Proc. Roy. Soc. 63 p. 31.

[2]) HALLO. Diss. Amsterdam 1902, p. 7.