in favour of a view that the Todas are a people who have once had a culture higher than that they now possess. When the customs and institutions of the Todas are compared with those of other parts of India, it is found that there is most resemblance with the people of Malabar; and the view is advanced that the Todas migrated to the Nilgiri Hills from Malabar, and are possibly allied in race to the two chief castes at present existing in that district, the Nairs and Nambutiris.

In addition to the work on the Todas, observations were also made on members of other tribes. The vision of the Sholagas and Uralis, two wild jungle tribes, was investigated* from several points of view; and observations, chiefly on colour-blindness, were made on members of other castes or tribes.

A Study of the Process of Nitrification with reference to the Purification of Sewage.

By Harriette Chick, D.Sc.

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Introduction.—That nitrification is a biological process was first established with certainty, after long controversy, in 1888, by the decisive experiments of Plath† and Landolt,‡ who in this matter confirmed the previous researches of Schlösing and Muntz,§ Warington‖ and Soyka.¶

The discovery of the active living agents followed soon after, when Winogradsky** (1890 to 1892) isolated the two sets of organisms which, as he showed, co-operate to produce natural nitrification. These were (1) the nitrite-producer, *B. nitrosomonas*, which oxidises ammonia to the nitrite stage only and (2) the nitrite-producer, *B. nitrobacter*, which carries on the

* 'Bull. Madras Government Museum,' 1903, vol. 5, p. 3.

† Plath, 'Landw. Jahrbücher,' v. H. Thiel, vol. 16, hft. 6, and 'Centralbl. f. Agrikulturchem. v. Biedermann,' vol. 17, 1888.

‡ Landolt, 'Deutsch. Landw. Presse,' vol. 15, and 'Centralbl. f. Agrikulturchem.,' vol. 17, 1888.

§ Schlösing and Muntz, 'Comptes Rendus,' vols. 84 and 85, 1877, and vol. 89, 1879.

‖ Warington, 'Journ. Chem. Soc.,' vol. 33, 1878, and 'Landw. Versuchsst.,' vol. 24, 1880.

¶ Soyka, 'Zeitschr. f. Biologie,' vol. 14, 1878.

** Winogradsky, 'Ann. de l'Inst. Past.,' vol. 4, 1890, and vol. 5, 1891; also 'Archives des Sci. biol. de St. Petersb.,' vol. 1, 1892.

oxidation to nitrate but cannot act upon ammonia, being indeed inhibited in its development by minute traces of that substance. Winogradsky, by himself, and in conjunction with Omeliansky,* subjected these bacteria to a very exhaustive study. The most striking characteristic that they demonstrated was the marked repugnance of both forms to organic substances. Not only, in opposition to the rest of the plant world, do these organisms make no nutritive use of sugars, peptones, etc., but the presence of more than a trace of such organic substances was found to entirely inhibit their development, thus explaining the failure of all attempts to isolate these bacteria by using the ordinary nutrient culture-media. Winogradsky, on the other hand, had succeeded in cultivating them by employing a silica-jelly-medium impregnated with inorganic salts, and a total inability to grow on organic nutrient media was afterwards put forward by him as a definite practical criterion of the purity of cultures of nitrifying bacteria. This criterion has been challenged by Burri and Stutzer,† by Stutzer and Hartleb,‡ and later by Fremlin.§ It has been shown by Winogradsky,‖ and also by Gärtner, Fränkel, and Krüger¶ that the former workers were misled by an admixture of non-nitrifying organisms. The most recent work, that of Boulanger and Massol,** and of Wimmer†† confirms Winogradsky's criterion.

The special case of nitrification considered in this paper is that occurring during sewage purification, which aims at the complete oxidation and mineralisation of putrescible substances present. Nitrification is here of great importance, and the effluent of perfectly-treated sewage should contain all its nitrogen in the form of nitrates.

Although land-treatment of sewage is theoretically the most economical, yet artificial processes, by which space can be saved, have often to be employed. Two processes concern us here, both involving the use of "filter-beds" of coke or other porous material, in which the sewage, usually after having been treated in a "septic tank" is oxidised by bacteria. (1) *Contact Filters.*—In these the filter-bed is first entirely filled up with the liquor and then after a time allowed to empty slowly, and finally to remain empty for a period. This cycle usually occupies about eight hours, and often may have to be repeated before the effluent is sufficiently purified. (2) *Continuous*

* Winogradsky and Omeliansky, 'Centralbl. f. Bakt.,' 2 abt., 5, 1899.

† Burri and Stutzer, 'Centralbl. f. Bakt.,' 2 abt., 1 and 2, 1895 and 1896.

‡ Stutzer and Hartleb, 'Centralbl. f. Bakt.,' 2 abt., 2 and 3, 1896 and 1897.

§ Fremlin, 'Journal of Hygiene,' vol. 3, 1903.

‖ Winogradsky, 'Centralbl. f. Bakt.,' 2 abt., 2, 1896.

¶ Gärtner, Fränkel, Krüger, 'Centralbl. f. Bakt.,' 2 abt., 4, 1898.

** Boulanger and Massol, 'Ann. de l'Inst. Past.,' vol. 17, 1903.

†† Wimmer, 'Zeitschr. f. Hygiene,' vol. 48, 1904.

Filters.—In this procedure the liquor trickles continuously through the filter-bed, being uniformly distributed by sprinklers, while as perfect aëration as possible of the bed is maintained.

The objects of the present research were mainly the following:—

1. The detailed chemical study of the course of the nitrification occurring during the filtration of sewage, especially during the maturing period of the filter, and the comparison of the "contact" and "continuous" methods (Section I).

2. The isolation and study of the organisms concerned, and comparison with those isolated from the soil by Winogradsky. The amount of organic matter accumulated in a sewage filter is comparatively great, and it seemed most unlikely that nitrification should here also be the work of bacteria so extremely sensitive to the presence of organic matter. One seemed compelled to believe that other and different bacteria must be here engaged (Section II).

3. The study of the question of absorption of ammonia upon the surface of filtering material previous to nitrification (Section III).

These researches were begun in Vienna in 1901, and were resumed in Munich in 1903, after a break of two years. I am very happy to have this opportunity of thanking Professor Max Gruber for his kind hospitality extended to me in the hygienic institutes of both cities, as well as for the valuable advice and kind assistance he constantly gave me in the course of the work. My thanks are also due to the Royal Commission on Sewage Disposal for granting me leave of absence in 1903 to continue the research in Munich. I should also add that part of the expense of the work was defrayed by a grant from the Royal Society.

Section I.—*Chemical Study of Nitrification in Experimental Filters.*

Description of Apparatus and Methods of Analysis.—Small experimental filters were erected, consisting of glass cylinders 50 cm. high and 12 cm. in diameter; these were placed one above the other, fitted well together by means of specially ground rims, and covered on the outside with black glazed paper. There were altogether three filters, differing only in height—200 cm., 100 cm., and 50 cm. respectively. Fig. 1 is a diagram of the filter of medium height, showing the arrangements made to allow of samples being drawn off, and of the temperatures being measured at different depths; the tall filter, consisting of four cylinders, had the three upper ones similarly constructed. The filters were filled with small coke, carefully sifted and of a uniform size (mean diameter 3·5 mm.). By volumetric measurements with

water, it was found that when this coke is packed into a space, the volume of the interspaces between the pieces is 35 per cent.; the volume of the pores inside the pieces (amount of water retained on draining) is 20 per cent.; and the volume of solid coke is 45 per cent. of the whole space. The three filters were fixed to the wall, near together, and all treated in exactly the same way, *i.e.*, as continuous filters. Each received 4 litres of liquid daily, and the sewage employed was the liquid manure ("Jauche") from a neighbouring cowshed; this proved a very suitable material, after a rough filtration through glass wool, and dilution to 1 in 20 with tap-water.

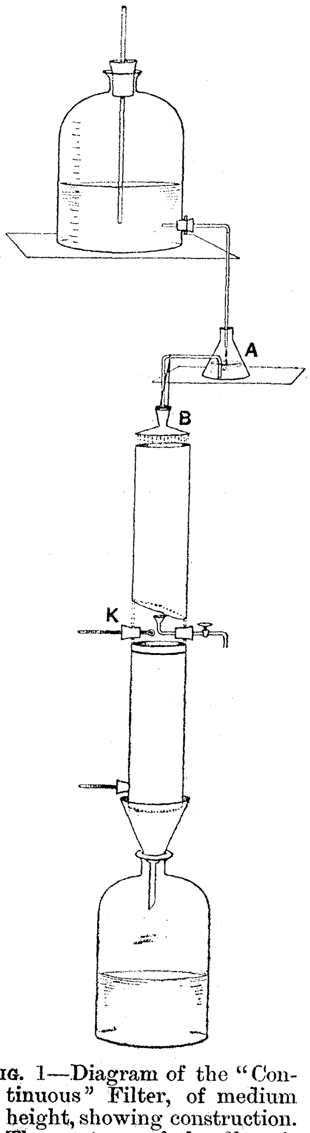

Fig. 1—Diagram of the "Continuous" Filter, of medium height, showing construction. The contour of the filter is represented as interrupted at K, to show the arrangement of the thermometer and the collecting funnel.

This liquid was contained in a 10-litre reservoir bottle of the Mariotte type, from which it dropped regularly into a small vessel containing a siphon arranged to empty when 100 c.c. had collected (A, fig. 1) into a sprinkler (B, fig. 1), the object of which was to distribute the liquid as evenly as possible over the surface of the filter.

The methods of analysis employed in following the course of the oxidation of nitrogen were those usually adopted in such work,* but the following details may be given:—

In the estimation of *free and saline ammonia*, 1 to 10 c.c. of the liquid was taken and diluted in a retort with about 500 c.c. NH_3-free water, and distilled, it being found unnecessary to add any alkali.† Three successive portions of 50 c.c. were distilled off and the ammonia they contained estimated by means of Nessler's reagent. *Albuminoid ammonia* was afterwards estimated by adding to the same retort a definite amount of "alkaline permanganate solution," distilling as long as ammonia came over in the distillate, and estimating these amounts in the same way.

Oxidised nitrogen in the filtrates was detected by means of the reaction with diphenylamine sulphuric acid. Nitrites were distinguished by reactions with acidified

* *Cf.* 'Report of Royal Commission on Sewage Disposal,' vol. 4, part 5.

† 'Sew. Com. Report,' vol. 4, part 5, appendices 3 and 4.

starch-zinc-iodide solution, and with metaphenylene-diamine, and estimated by the use of the latter. When both nitrites and nitrates were present, they were estimated together by the *indigo* method (*Tiemann-Gärtner's* "*Wasseranalyse*") and the figure for nitrates obtained by subtraction. This method was afterwards given up in favour of the *copper-zinc couple* method,* where, to allow for traces of ammonia originally present in the solution, or introduced during analysis, a control estimation was always made; this control was carried out in every way like the real analysis, except that no couple was introduced, and the ammonia obtained was subtracted from that found in the actual estimation.

Total nitrogen was estimated in the sewage by Kjeldahl's method, a small amount of Na_2SO_4 only being added during the preliminary heating.

The *oxidisability* ("Oxidirbarkeit," or measure of oxidisable substances present) was estimated by reduction of permanganate in alkaline solution on boiling for ten minutes, care being taken to keep the external conditions (such as concentration, size of flask, total amount of liquid present, temperature, and time of reaction) constant in all determinations. Periodic examination of sewage and filtrate in this way gave useful comparative results and a means of following the course of the general oxidation.

Course of General Oxidation During Maturation: First Appearance of Oxidised Nitrogen.—The filters were started on February 20, 1901, and their action carefully controlled by means of analysis from that time onwards. Especial attention was paid to the period of maturation from the time of first using to that of full efficiency, as it was thought this should throw light generally upon the manner of their working. The sewage employed contained on an average:—total nitrogen 10 parts, organic matter (by evaporation and ignition) 21 parts; oxidisability (expressed in terms of O) about 11 parts per 100,000 by weight. The course of the oxidation will be seen by reference to Tables IA, IB and II. On March 7 (see Table II, analyses 1 to 4) there was a marked amount of general oxidation taking place in all three filters, or at least a reduction of oxidisable substances, but there was no trace of oxidised nitrogen in the filtrates nor was there any diminution in ammonia. Oxidised nitrogen first appeared in the tall filter on March 18 (the filtrate being then clear, bright and without smell), three days later it was detected in the medium filter and seven days later in the short filter. During this first period (after starting and before the occurrence of nitrification) the amount of ammonia in the sewage was frequently compared with that in the filtrates and found to be always the same.† On March 7 this was proved to be so for all three filtrates, and even on March 28, when the first trace of oxidised nitrogen appeared in the filtrate from the short filter there was no diminution of the free and saline ammonia coming through (*cf.* analyses 10 and 13). So, generally, Tables II and III

* Sutton, 'Vol. Analysis,' 8th ed., p. 452.

† As the sewage varied considerably in composition from time to time, care was always taken that the sewage and filtrate analysed for comparison should correspond to one another as nearly as possible.

seem to show that loss of ammonia goes hand in hand with production of oxidised nitrogen.

Tables IA (Vienna) and IB (Munich) give a clear idea of the general progress of the oxidation of nitrogen. They are compiled from analyses made from time to time, and while the times given for the later stages are approximate, those for the first stage are exact, being based on almost daily tests.

The Nitrite Stage.—After the first appearance of oxidised nitrogen in the filtrates, nitrification went ahead, and, in the case of the tall filter, five days later there was nitrous nitrogen in the filtrate about equal in amount to the total nitrogen going on. The two stages in which nitrification occurs were well separated in time, and show very distinctly, first, the production of nitrites in quantity without nitrates, and finally the complete oxidation to nitrates, nitrites being absent. For example, in the case of the tall filter, five weeks after having been started (Table II, analyses 10 and 11) the sewage contained 17 parts ammoniacal and albuminoid nitrogen, which in the filtrate was reduced to 1·5 parts, while 10 parts nitrogen were present as nitrites, nitrates being altogether absent. Three days later a similar result was obtained (analyses 14 and 15), eight weeks later nitrates were being formed in small amount, and an analysis of the filtrate made four months after starting (analysis 21) showed a complete oxidation of the nitrogen, nitrates being present in quantity unaccompanied by nitrites. In the case of the short filter the process was much slower, for an analysis made after four months showed production in the filtrates of nitrites only (analysis 23). This comparative lack of efficiency may be referred to the lower temperature* of the short filter, for an analysis on the same date of the liquid from No. 1 tap of the tall filter, 50 cm. from the top (Table III, 6 and 7) showed the presence of nitrates in abundance, and only traces of nitrites.

* An exactly parallel fact was noticed with regard to the time of the first appearance of oxidised nitrogen in these two filters. Strictly speaking, the top section of the tall filter should have been exactly comparable with the short filter, and the only possible explanation is that, as the first division of the latter filter stood 150 cm. higher in the room, the discrepancy was due to a temperature difference. Regular observations of temperature had been made by means of the thermometers in the filters, and the higher position in the room was found to be constantly from 1·5° to 2·5° C. higher than the lower one, the temperature of the interior of the filters differing hardly at all from that of the surrounding air. This explanation is confirmed by the results of the Munich experiment, where all the six filters were arranged so that their tops, and not their bottoms, were on one level; hence the first divisions of all were at a similar temperature, and it was found that the first oxidation of nitrogen was observed after the same period in all the three filters of each set.

This complete separation of a nitrite from a nitrate stage is doubtless due to the comparatively strongly ammoniacal nature of the sewage employed. Previous observers*† have shown the inhibitive effect of ammonia upon nitrate-production, and it is probable that during the earlier stages of the maturing period the nitrate bacteria were unable to become established in the filter, and only later, when the ammonia of the sewage was being rapidly oxidised to nitrites, was the environment suited to their growth and development. The sewage employed frequently contained more than 15 parts free and saline ammonia per 100,000, a concentration which has been shown† to be sufficiently high to check completely the production of nitrates in pure culture. An interesting confirmation of this explanation was obtained in the maturing of the Munich filters, where nitrates appeared in the filtrates very soon after the first appearance of nitrites. There was here no such "nitrite stage," and the sewage was much less ammoniacal (2 to 4 parts ammoniacal nitrogen per 100,000).

Difference of Function in Different Strata of the Filters.—An attempt was made to study the course of the oxidation at different depths in the tall and medium filters (see Table III). In analysis 3 the "oxidisability" was taken as a criterion and the decrease in the first 50 cm. of the tall filter was found to be almost as great as in the whole length of the filter. One may therefore suppose that the mechanical deposition of suspended particles as well as the absorption of the more complicated organic matter in solution takes place principally in the upper layers of the filter. It is also apparent from analysis 4 that the formation of nitrites (these analyses were made during the nitrite stage) did not at that date take place in quantity in this upper layer but lower down for the most part; this same fact is also shown in analysis 2. In the latter case the free and saline ammonia was also estimated, and the decrease, which is so marked as the sewage passes through the filter, was found not to begin until after the first 50 cm. were passed. The same phenomenon appears in analysis 8 and is a striking instance of the principle, already alluded to, and discussed at length in the section devoted to absorption, that the disappearance of ammonia and the oxidation of nitrogen are closely associated both in time and space.

Comparison of Contact and Continuous Filters—Munich Experiments.—From the mature Vienna filters attempts were made to isolate the nitrifying organisms, but before much progress had been made the work was discontinued and was not again resumed until after two years. This second time, in Munich, in 1903, fresh filters had to be matured, and a second

* See footnote **, p. 242.

† Warington, 'Chem. Soc. Journ.,' vol. 35, 1879, and vol. 59, 1891.

opportunity was afforded for studying the maturing process, and nitrification generally.

Filters like those previously described were again erected, and three of them treated as formerly with a continuous trickle of diluted liquid manure. Another similar set of three filters was treated, for contrast, as contact filters by the procedure mentioned on p. 242. Eight litres of sewage were treated every 48 hours by each of the filters. The capacities of the tall,* medium and short filters were respectively, after wetting, 6, 4 and 2 litres, so that each filling remained in contact at least four hours in the two taller filters and two hours in the short one. For 38 out of the 48 hours of the cycle the filters were empty or emptying. These contact filters were cone-shaped below, with a narrow opening that could be closed with a cork for the purpose of filling them.

In the case of the continuous filters, oxidised nitrogen again made its first appearance in the filtrates four weeks after starting (compare Tables IA and IB). The fact that all three Munich filters behaved alike in this respect (forming a contrast to the Vienna ones) has already been explained as a temperature effect, see p. 246. It was noticed that during the maturing period the two different stages of nitrogen oxidation merged one into the other, and were not so clearly separated as was the case in the Vienna filters (Tables IA and IB); noticeably there was here no long period in which nitrites were formed in quantity without any accompanying nitrates. This difference has already been discussed (p. 247), and explanation is doubtless to be found in the much less ammoniacal nature of the sewage here employed.

The contact filters did not yield nearly such good results as the continuous filters (Table IB). The period which elapsed before nitrogen oxidation was apparent was, in the former, more than half as long again as in the latter. Again, when the short continuous filter showed complete oxidation of its nitrogen, the tall contact filter still showed presence of nitrites in its filtrate.

The Munich continuous filters had completely matured in about ten weeks from the time of starting, but they were yielding a very satisfactory effluent much earlier. After three months the sewage was changed for a much more strongly ammoniacal liquid (cows' urine, diluted 1 in 100, containing 14 to 17 parts ammoniacal nitrogen per 100,000), in order to test the capabilities of the filters as regards nitrogen oxidation. Most satisfactory results were obtained (Table II, analyses 33 to 36, and Table IV), the filtrates contained, as a rule, only traces of ammonia and nitrites, but abundance of nitrates. Attempt to further tax the capabilities of the filters

* In these Munich experiments the tall filters, both contact and continuous, were only 150 cm. high, instead of 200 cm., as in Vienna.

met with failure. Cows' urine diluted only 1 in 50, and containing about 30 parts ammoniacal nitrogen per 100,000, was put through the medium filter for about a fortnight, and also at a later date through all three filters, but it was found that they were incapable of oxidising so concentrated a liquid, and the quality of the filtrates deteriorated (*cf.* Table IV).

Throughout the history of these filters there was a considerable loss of total nitrogen from the sewage while filtering through, but it was specially noticeable during the period when the diluted urine was being treated, when in some cases not much more than half the original nitrogen was present in the filtrate (Table II, analyses 33 to 36). This loss is doubtless due to an escape of free nitrogen, set free possibly by decomposition of ammonium nitrite, a very probable intermediate product in the nitrification of ammonia ($NH_4NO_2 = 2H_2O + N_2$). This loss of nitrogen was not so marked in the case of the Vienna filters (Table II, analyses 1 to 23), though it occurred later to some extent. These differences are probably due to the absence or presence in quantity of the organisms involved.

The Munich continuous filters in their later history, and they were worked for about a year, possessed an efficiency rarely met with in large scale filters, showing that this type can give excellent results in the absence of much suspended matter.* The larger filters could not be considered to be heavily worked, but the short filter, which had a capacity of two litres and treated four litres of sewage daily, approximated more nearly to a practical installation. It oxidised daily about 0·5 gramme nitrogen, and this result must be considered extremely satisfactory when the high nitrogenous concentration of this special sewage (14 to 20 parts of ammoniacal nitrogen per 100,000 instead of the 3 to 8 parts usual in ordinary sewage) is kept in mind. The quantities of nitrate appearing in the filtrates from these filters have rarely, if ever, been obtained in practice on the large scale.

Section II.—*Bacteriological Investigations.*

Enumeration of Nitrifying Bacteria in the Filtrates.—It was thought worth while to attempt to count the numbers of nitrifying bacteria† present in the filtrates from the filters, as it was conceivable that such enumerations might furnish a bacteriological criterion of the quality of sewage effluents.

The practical utility of this procedure is, however, diminished by its

* This can be removed in practice by a preliminary screening or septic tank treatment.

† The recent work of C. C. Frye ('Report of Roy. Com. on Sewage Disposal,' vol. 2, 1902, p. 9) has experimentally verified the view which has been generally held, though doubted in some quarters, that all the nitrification taking place in sewage filters is the work of living organisms, and none of it purely chemical.

slowness, due to the sluggish growth of the organisms and to the extremely small numbers of them introduced in the higher dilutions. The method might, perhaps, give useful comparative results even without allowing the maximum time for development in the subcultures, and the rate of growth could be accelerated by a temperature of 28° to 30° C.

The enumerations were made as follows :—The filtrate was successively diluted with sterile water to a tenth degree six times ; of these six dilutions (viz., 1 in 10, 1 in 100, 1 in 1000, 1 in 10,000, 1 in 100,000, 1 in 1,000,000) 1 c.c. was used in every case for inoculation into bouillon and into Winogradsky's ammonia and nitrite-containing media respectively,* which were distributed in test-tubes, each containing about 5 c.c. The ammonia tubes were subsequently tested for production of nitrites with acidified starch-zinc-iodide solution, and the nitrite tubes for nitrates with diphenylamine sulphuric acid (after evaporation to dryness with NH_4Cl if any nitrite remained unoxidised).

The numbers in which the nitrifying bacteria are present are surprisingly large (see Table V), and it will be seen that there is no strict relation between the numbers present respectively of nitrite and nitrate producers ; but the latter would appear to be present generally in less amount even when the filtrate shows complete oxidation of its nitrogen to nitrates. The filtrates used were in all cases from the Munich continuous filters.

Isolation of the Nitrite Producer.—The isolation of a nitrite-producing bacterium in pure culture was found to present considerable difficulty and many unsuccessful trials were made. Attempts were first made to isolate it directly from the coke of the filters by the method of dilutions. This method, originally invented by Lister, was formerly employed by Warington† and P. and G. Frankland‡ for the same purpose, but with only partial success. The method here employed was similar to that used by the Franklands, except that much higher dilutions were made, and a larger number of tubes (about 200) containing appropriate culture media§ were sown with small amounts of liquid from the higher dilutions, bouillon tubes being also similarly inoculated as controls. Repeated attempts to isolate from the coke of the filter were only partially successful.|| A culture was, however, obtained which was comparatively, but not absolutely, pure ; this was used for further isolation experiments, and will be referred to as culture "*a*."

* Omeliansky, 'Centralbl. f. Bakt.,' 2 abt., 5, 1899.

† See footnote, p. 247.

‡ P. and G. Frankland, 'Phil. Trans.,' B, vol. 181, 1890.

§ Culture solutions used were diluted urine and Winogradsky's solutions.

|| Starting from such very impure material, the dilution method does not give an adequate return for the great labour it entails. The filtrates might have proved better original material, as in them the nitrifiers sometimes predominated (see Table V).

Culture "*a*," and all cultures showing production of nitrites* invariably contained in quantity a small oval bacillus or coccus, which was recognised as the nitrite-producing organism.

Attempts to obtain a pure culture were further made with the use of ammonium agar as medium,† but without success. The plate cultures showed vigorous formation of nitrites,* but all nitrifying subcultures were found to be impure. The employment of a similar medium composed of agar and diluted cows' urine was equally unsuccessful.

Ordinary gelatine plate cultures were made and bouillon was inoculated from the impure cultures; none of the colonies separated from the former were able to nitrify, although 40 were investigated. From the growths in bouillon, plate cultures were also made on nutrient gelatine and agar, and 70 of the organisms separated were further investigated, but in no case did nitrification occur. This seemed to show that the nitrifying organisms in filters resembled those of Winogradsky very closely. Therefore, in order to decide if the nitrifying organism was or was not able to live in the bouillon, an ammoniacal medium was directly inoculated from the growths in bouillon. Usually there was no nitrite-production (*e.g.*, Table VI, culture "*a*"), and indeed the oval bacillus could in no case be traced in the bouillon growths. In one instance, however (Table VI, culture "*b*"), inoculation from a bouillon growth led to nitrification, but this property was lost after a second generation in bouillon; it therefore seemed probable that, if the nitrifier had not been killed in the bouillon, it certainly had not been able to multiply there.

It thus was evident that, contrary to expectation, the nitrite-producing organisms of sewage filters were also unable to grow upon media containing organic matter; recourse was then had to silica plate cultures, which were made and inoculated according to the directions given by Omeliansky.‡ This operation was accomplished with comparative ease if the original sodium silicate was quite pure; the study and isolation of the separate colonies was, however, found to be exceedingly difficult. The sub-cultures

* The test for production of nitrites was usually made by allowing a little of the culture fluid, withdrawn with a sterile pipette, to drop into a small quantity of acidified starch-zinc-iodide in a porcelain dish. This was preferred to the similar test with diphenylamine, partly because of its specific nature, and partly because the ferric salt present in the sediment of the culture-tubes also yielded a slight blue colour with diphenylamine.

† $(NH_4)_2SO_4$, 2·0 gr.; NaCl, 2·0 gr.; K_2HPO_4, 1·0 gr.; $MgSO_4$, 0·5 gr.; $MgCO_3$, in excess; agar-agar (purified by washing, Beyerinck's method, 'Centralbl. f. Bakt.,' vol. 19, 1896), 20 gr.; distilled water, 1 litre.

‡ See footnote, p. 250.

obtained were to all intents and purposes pure cultures, showing pure pictures of an oval, almost spherical organism, resembling the nitrosomonas of Winogradsky, except that it seemed to be somewhat smaller in size. It appeared constantly in the form of zooglœa embedded in the particles of magnesium carbonate at the bottom of the culture tubes, and it stained easily and well. The individual bacteria were often found to be well separated in a culture, but an actively motile stage was not observed.

These cultures, however, still gave a growth, though extremely slow, in bouillon, and this consisted of the other quite inconspicuous organisms present. By means of the dilution method, pure cultures were obtained which yielded absolutely no growth in bouillon when preserved indefinitely either at 37° or at the room temperature. These pure cultures were not, however, robust, and they nitrified very feebly; attempts are now being made to obtain vigorous pure cultures.

Isolation of the Nitrate-producer.—The dilution method was also employed for the isolation of the nitrate organism, the original material being a culture obtained during the enumeration experiments (Table V), which showed active oxidation of nitrites. A culture was separated which consisted of the nitrate bacterium mixed with one other species, and the combination, referred to in future as culture "*d*," formed a very interesting symbiosis. Pure cultures were obtained from culture "*d*" by making surface plate cultures, in great dilution, on nitrite agar.* These pure cultures showed a small non-motile bacterium, agreeing in essentials with Winogradsky's organism, though somewhat larger in size. It was a bacterium very thick in comparison with its length, so that it often appeared to be almost a coccus; stains were badly taken up, and it frequently appeared imperfectly and irregularly stained. These pure cultures rapidly changed nitrite to nitrate, when growing in nitrite-containing medium,† the nitrite present being sometimes completely oxidised in less than two weeks. Bouillon on the other hand remained indefinitely sterile; the tubes were kept under observation for seven weeks without there being any sign of growth.

* Omeliansky, 'Centralbl. f. Bakt.,' 2 abt. 5, 1899.

† Winogradsky's nitrite culture solution was invariably employed, and the cultures were tested from time to time for the production of nitrates. When time enough had elapsed and all nitrite had disappeared, then, on testing the culture liquid, a negative reaction with starch-zinc-iodide, and a positive with diphenylamine proved the presence of nitrates. But if all nitrite were not oxidised, the remainder was decomposed by evaporating to dryness with a little NH_4Cl, and the residue dissolved in water and tested for nitrates with diphenylamine. This method has been shown to be quantitative when such substances as sugar and peptone are present (Frankland, 'Journ. Chem. Soc.,' 1888), and it is possible it might also prove a useful method of estimating nitrates in presence of nitrites in sewage effluents.

Pure cultures were also obtained with more difficulty directly from less pure material, by means of nitrite agar plates, but the organism isolated was in every case the same.

All attempts to isolate a nitrate organism by means of ordinary nutrient agar and gelatine were unsuccessful. In no instance was nitrite oxidised to nitrate by any organism separated on such plate cultures, though over 40 such organisms were investigated.

Experiments with "Symbiotic" Cultures of the Nitrate-producer.—Although nitrobacter, when alone, is incapable of growing in bouillon, it would appear to be capable of surviving an inoculation into bouillon if not alone, but growing with certain other bacteria. A very instructive set of experiments was made with culture "*d*" (Table VII), in which this strain was inoculated into bouillon through four generations. From each set of tubes nitrite medium was inoculated, and it was found that the change to nitrate occurred invariably in the tubes sown from the earliest bouillon generation, and in two instances also from those sown from bouillon of the fourth generation (Table VII, d_3 and d_4). The quantities inoculated were large, one or two drops, but it is impossible to believe that nitrobacter would still be present in a fourth generation if no multiplication had taken place in the bouillon. Pure cultures of the nitrate-producer showed no such effects; inoculated bouillon remained quite clear; examined under the microscope it showed complete absence of bacteria, and nitrite tubes inoculated from the bouillon in no case showed any oxidation to nitrate. In Table VIII are shown the results of further experiments in which three pure and four mixed cultures were compared in this respect, and one is compelled to conclude, in explanation, that the presence of the accompanying organism in some way protects the nitrate bacterium from adverse influences present in the bouillon, which it is unable to withstand if alone. Without further experiment, any attempt to explain in what this action really consists must be pure conjecture, but it is possible that the harmful organic substances present are in some way altered by the accompanying organism, and it would be interesting to see whether the nitrate organism in pure culture could thrive in bouillon previously exhausted by its companion.*

Phenomena which present an interesting analogy with these observations are found in the case of certain anaerobic organisms, one instance of which has been precisely investigated by Winogradsky, viz., that of *Clostridium Pasteurianum.*† This strictly anaerobic species was found to be capable of

* The experiment was not made in this instance because the cultures had then been isolated a considerable time and their properties were enfeebled.

† 'Archives des Sciences biolog. de St. Petersb.,' vol. 3, 1895.

growing aerobically when, and only when, associated symbiotically with a certain aerobic organism which removed the surrounding oxygen and created an oxygen-free environment for it. Such symbioses of various grades must be frequent in Nature where the "pure culture" is almost unknown. The part played by the artificial pure culture in the progress of bacteriology has, of course, been enormous, yet its possibilities are limited, and one must look to the investigation of regulated simple symbioses for a nearer approach, in the laboratory, to the workings of Nature.

A break of two years occurred during the course of these investigations. After they were again resumed, Dr. Schultz-Schultzenstein* published the results of bacteriological investigations, having the same aim as the present work. He isolated two kinds of nitrifying organisms from the material of coke sewage filters at Karolinenhöhe, near Charlottenburg, which corresponded exactly to those isolated from the soil by Winogradsky, and no other nitrifying organisms were found. His researches must be regarded as the first published successful attempt to investigate the organisms concerned with nitrification during the artificial purification of sewage, and the results are entirely confirmed by the present investigation. In spite of this anticipation of my identification of these bacteria, I have thought it worth while to describe my isolation experiments in detail, because in a subject of such technical difficulty the experience of an independent worker may be of use to others.

Section III.—*Absorption of Ammonia and Ammoniacal Compounds during Sewage Purification.*

It has been held that a most important preliminary to nitrification, both in the soil and in sewage filters, is to be found in an absorption of ammonia and ammonium compounds upon the surface of the particles of soil or of filtering material respectively. In the case of the soil, a long controversy has taken place as to whether a physical or a chemical process was here in question,† and the former view, maintained notably by Liebig‡ and his school, has on the whole prevailed. This "adsorption" of ammonia plays an important part in the current doctrine of the action of sewage filters, which considers that nitrification could not take place in the short time taken by

* Schultz-Schultzenstein, 'Mitt. a. d. Kön.-Prüfungsanst. f. Wasservers. u. Abwässerbeseit,' 1903.

† Way, 'Agric. Soc. England Journ.,' series 1, vols. 11—13, 1850—1852, and Mayer, 'Lehrbuch der Agrikulturchemie,' 1871. Lemberg, 'Zeitschr. d. deutsch. geol. Gesellsch.,' vol. 28, 1876.

‡ 'Liebig, 'Ann. Chem. Pharm.,' vol. 94, 1855, and vols. 105 and 106, 1858.

the liquid to pass through the filter, and that the nitrites and nitrates appearing at any particular time in the filtrates are the result of a slower change which has been effected by the nitrifying bacteria upon ammonia previously absorbed in some physical manner upon the surface of the filtering material. The procedure for the purification of sewage used in "contact beds" has been held to assist successively the processes of adsorption of ammonia and nitrification. Dunbar and Thumm* consider that, in the "filling" and "full" stages, putrescible and oxidisable substances are retained upon the surface of the filtering material, and are subsequently oxidised at times when the bed is full of air, the oxidation being the work of bacteria in the bed, among which the nitrifying bacteria rank high in order of importance.

As regards the complex oxidisable putrescible substances of high molecular weight, the solid suspended matter will be retained, of course, by mechanical filtration, while the soluble constituents may, doubtless, be supposed to undergo some physical adsorption.†‡ But the greater part of the nitrogen present in sewage is there in the form of free and saline ammonia, and these are the compounds most markedly retained as the sewage passes through the filter; yet for such simple compounds as these, adsorption by solids has been shown to take place only to a small degree or not at all.§ To attempt to explain removal of ammonia by adsorption then, would appear inadequate.

Special experiments were therefore made to investigate the behaviour of filtering materials with ammonia and its salts; also during the investigations in Section I, careful note was also made of any facts which should tend to confirm or refute the theory of nitrification quoted above.

If the theory of a previous ammonia absorption and a subsequent oxidation were true, then contact beds should be much more efficient nitrifiers than continuous filters, but the contrary proved to be the case, the latter doubtless owing their greater efficiency to their more perfect aëration. Again, while complicated organic substances appeared to be absorbed in the top layer of the filter (Table III, 3), the disappearance of free and saline ammonia was shown usually to take place lower down in the filter (Table III, 1), and to be always associated with the appearance of oxidised nitrogen (Table III, 2, etc.). Moreover, during the maturing of the filters, before oxidation of nitrogen had occurred, no absorption of ammonia could be detected, although this was

* Dunbar and Thumm, 'Beit. zur Abwasserreinigungsfrage,' 1902.

† Soyka, 'Archiv f. Hygiene,' vol. 2, 1884.

‡ Kattein and Lübbert, 'Gesundheitsingenieur,' vol. 25, 1903.

§ Weppen, 'Ann. d. Chem. u. Pharm.,' vol. 55, 1845, and A. Mayer, 'Lehrbuch d. Agrikulturchemie,' 1871.

frequently looked for. It was possible, however, that, at the very first, ammonia had been taken up by the filtering material, to saturation point, and that afterwards no more absorption was possible until nitrification had begun. Unfortunately no analyses were made at the very beginning, but this gap was afterwards filled by special experiments with clean sterile coke.

The tendency of these observations was thus in opposition to any theory of ammonia-absorption by the filtering material, and this opposition was confirmed by the following experiments, made to test the power of various solids to absorb ammonium salts. The solids employed were barium sulphate, sand, and ground-up "clinker," and the experiments were carried out as follows:—

A small quantity of a solid (1 gramme or 2 grammes), previously carefully purified, was weighed out into a small flask, which was then exhausted to remove air films, which might cause imperfect contact of solid and liquid.* A measured quantity (50 c.c. to 100 c.c.) of ammonium chloride solution was added through a tap-funnel, and the whole left standing for 24 hours. The clear liquid was then drawn off by a pipette, and the ammonia estimated in a small portion (1 to 5 c.c.); this was first diluted to about 500 c.c. with NH_3-free water, and then distilled and nesslerised. The remaining liquid was well shaken up and the muddy residue analysed similarly: a correction had to be made for the volume of the solid, which was measured, after centrifugalisation, in a graduated tube. In every case, as a control, a blank experiment was also made, similar in every detail except that NH_3-free water replaced the ammonium chloride solution. The solids had previously undergone a careful purification by washing, and often, by ignition also. The ammonium chloride solutions were exceedingly dilute, so as to approximate to the concentration of ammonia in ordinary sewage.

In Experiments 1 to 3 (Table IX) the ammonia yielded, both by the clear and the muddy portions of the liquid, was found to have diminished. It was therefore supposed that boiling was insufficient to drive off any ammonia which might have been absorbed by the solid; accordingly, in Experiments 4 to 7, a small amount (10 c.c. N/1 KOH) of alkali was added before distillation. In this case the analysis of the muddy portion of the liquid showed a small amount of the ammonium salt to have been absorbed by the solid, but nothing comparable to the effect required in a sewage filter. Moreover, the slight removal of ammonia demonstrated would appear to be a chemical rather than a physical phenomenon, alkali being necessary to free the absorbed ammonia from the solid.

In none of these experiments, however, was coke itself employed, and the surface of solid was very small in comparison with the amount of liquid taken;

* In Experiments 6 and 7 (Table IX) the flask was not thus exhausted, and the agreement of their results with those of previous experiments indicates that this precaution is unnecessary.

the following further experiments were therefore made, and confirmed the preceding ones.

Experiment 8.—Exactly the same coke as had been used for the filters was taken and thoroughly washed and dried. An amount occupying a volume of 30 c.c. was placed in a flask with 50 c.c. NH_4Cl solution of concentration equal to about 5 parts ammonia per 100,000. In a control flask 50 c.c. of the liquid was placed alone. After 24 hours and after 48 hours the liquids in the two cases were examined, a small quantity (0·5 c.c. to 1 c.c.) being removed, diluted to 50 c.c. with NH_3-free water and tested with Nessler's reagent. In no case was the reaction fainter where the liquid had been in contact with the coke. (It was shown that the coke did not of itself yield ammonia by a control experiment in which NH_3-free water replaced the NH_4Cl solution.)

Experiment 9.—Coke, which had been thoroughly washed, dried, and sterilised, was placed in a cylinder to form a small filter, and a solution of NH_4Cl (10 parts NH_3 per 100,000) allowed to drop slowly through.* The filter occupied a volume of about 1 litre and during the first hour 50 c.c. came through, while in 15 hours a total of 500 c.c. was filtered. The first filtrate of 50 c.c. was tested for ammonia and compared with the original liquid. The tint given by the filtrate (after suitable dilution and addition of Nessler's reagent) was, if at all, only a shade paler, indicating only a negligible difference. After a second hour the filtrate was again compared with the control, and a similar result was obtained. The filtrate coming through in the next 13 hours was similarly tested, but no absorption of ammonia was detected.†

It may be objected that experiments with raw, cleansed, filtering material are not applicable to the occurrences in the mature filter, where the surface of the coke is probably coated, in some manner not yet investigated, and might possess the faculty of absorbing ammonia in a manner similar to that already demonstrated in the case of certain colloidal substances.‡ Therefore it is hoped, in the future, to make experiments with matured coke, eliminating, if possible, the action of bacteria. The available evidence is, however, opposed to such absorption, for it is in the uppermost layers of the filter that such a coating would be greatest, and yet disappearance of ammonia, at any rate during the maturing period, has been shown to take place lower down, and in any case coinciding, both as regards time and place, with nitrification.

Upon consideration of the experimental data at present available, one is therefore inclined to reject the current theory of nitrification and to consider

* The concentration of ammonia was greater than in the preceding experiments, where it approximated to that in ordinary sewage; here a more concentrated liquid was employed, in order to be comparable with the diluted urine which was then being treated on the filters.

† These last two experiments are in perfect accord with some of A. Mayer ('Lehrbuch d. Agrikulturchem.,' 1871), who showed that pure carbon in a porous condition was unable to effect any significant absorption with many salts long known to be absorbed by the soil.

‡ Van Bemmeden, 'Landw. Versuchsst.,' vol. 35, 1888, 'Zeitschr. f. physikal. Chem., vol. 18.

the disappearance and oxidation of the ammonia to be parts of one process, which is carried out by the nitrifying bacteria in the time taken by the sewage to pass through the filters.

For these experimental filters the time taken for the passage was measured directly.* For the Vienna continuous filters on July 1, 1901, the time was approximately $3\frac{1}{4}$ hours for the tall and medium filters and only 5 minutes for the short filter (nitrogen oxidation in this filter had not then progressed beyond formation of nitrites). For the Munich continuous filters on March 17, 1903, the time was 2 hours for the tall filter, and $\frac{1}{2}$ hour for the short one. At this date, a too concentrated sewage was being employed, and the filters were not at their best, but still four-fifths of the ammonia in the sewage was oxidised while passing through the tall filter.

Section IV.—*General Conclusions.*

1. Nitrification of ammonia during sewage purification occurs in two stages which may be referred to the activity of two classes of bacteria, one producing nitrites, and the second oxidising the nitrites to nitrates. These bacteria exist not only in the substance of the filter, but are also carried away in large quantities in the filtrates.

2. These organisms belong to the same group as those concerned with nitrification in the soil, isolated by Winogradsky. It is, at first, difficult to understand how organisms so susceptible to the presence of organic matter are able to live and do their work in sewage filters. The following, one or all, form possible explanations.

(*a*) The nitrifying bacteria may be, to a certain extent, protected by the presence of other organisms, and this view is strengthened by the results of certain experiments with the nitrate-producer, in symbiosis with such organisms, made in the course of the present investigation.

(*b*) It has been shown that porous materials, such as coke, are able to retain upon their surface complicated organic substances of high molecular weight, when these are presented in solution. We may suppose this absorption (together with the mechanical separation of the suspended

* 100 c.c. of a 2-per-cent. solution of sodium chloride were sprinkled over the top of the filter (that being the volume of liquid usually delivered at each discharge of the siphon). The filtrates were then continuously tested with silver nitrate until a copious precipitation was obtained; the ordinary sewage filtrate yielded only a slight reaction with silver nitrate. The passage of liquids through such filters is a very complicated process and one not yet thoroughly investigated, and though, doubtless, the times thus determined may be considered to apply to the *majority* of the liquid going on at any particular time, they must still, strictly speaking, be regarded as approximate and indeed minimal values.

materials in sewage, also largely of organic origin) to take place principally in the upper layers of the filter. The nitrifying organisms will then be able to live and multiply lower down in the filter where the amount of organic matter present will be comparatively small, and this view has been experimentally confirmed in the present work.

(*c*) It has been lately shown by *Wimmer*,* in the case of the nitrate organism, that a porous medium has a markedly mitigating effect when organic matter is present, and the coke and other materials of which sewage filters are made, are selected mainly on account of their porosity. It is only fair, however, to state that Wimmer's experiments were not made with absolutely pure cultures, and part of the beneficial effect observed may have been due to a symbiosis, though, from the nature of his experiments, it would seem unlikely.

(*d*) The nitrifying bacteria are doubtless present in very great numbers in the filters, and this may assist them in withstanding the effect of organic matter. This view is based upon certain observations of *Winogradsky* and *Omeliansky*,* in which nitrifying organisms, if present in sufficient quantity, were shown to withstand amounts of organic matter otherwise inhibiting them.

3. In the maturing of sewage filters, the two stages of nitrification may be markedly separate in time (Vienna experiments), or may be both developed together (Munich experiments). This difference is correlated with the greater or less ammoniacal content of the sewage. In the stronger sewage used for the Vienna filters, the well known inhibitory action of abundance of ammoniacal compounds (especially of free ammonia and carbonate of ammonia,† which are so largely represented in the sewage), presumably retarded the development of the nitrate-producer, until the nitrite-producer was sufficiently well established to be converting most of the ammonia into nitrites.

4. As a result of special experiments with coke, and of analyses of the filtrates at different depths of the filters, and at different stages during the maturing period, it would appear that there is no evidence of absorption of free and saline ammonia without contemporaneous nitrification. Further research is necessary, but the theory of a previous physical "adsorption" of ammonia and subsequent slower nitrification would appear, at present, to be without experimental foundation.

5. One is therefore inclined, in the present state of our knowledge, to consider the process of nitrification, during the filtration of sewage through

* See footnote, p. 242.

† Löhnis, 'Centralbl. f. Bakt.,' 2 Abt., 13, 1904, and Boulanger and Massol, 'Comptes Rendus,' vol. 140, 1905.

such filters, to be an extremely rapid biological process, requiring for its completion only the time taken for the liquid to pass through the filter (approximately 2 to 3 hours, possibly a little more). The rapidity of the process is probably to be explained by the very great number of nitrifying bacteria present and the very efficient aëration which obtains. In such filters also, the general conditions are ideal for quick action, as the continuous trickle secures rapidity of diffusion, and forms a great contrast to the much slower effect in stationary fluids.

6. Temperature has a marked influence upon the oxidation of sewage, a higher temperature being noticeably more favourable. This indicates that the efficiency of sewage filters in practice would be much increased if at a reasonable cost they could be artificially maintained at a warm temperature during the winter.*

7. The previous conclusions are chiefly drawn from experiments with continuous filters, but filters working as contact beds were also investigated and the two methods compared. On the "ammonia adsorption theory," the contact method should have proved the most efficient. This, however, was not found to be the case. The advantages of the continuous method would seem to lie in the much more complete aëration and efficient diffusion, and also in the stratified distribution in the filter of the different stages of the sewage purification. Some of the present experiments were quite comparable with practical installations as regards quantity of liquid treated, concentration of nitrogen, etc., and the results were much more satisfactory than those usually obtained in practice. The obvious difficulty in practical employment of continuous filters is with regard to the solids in suspension, which can only be permitted upon the filter to a small extent without risk of clogging. The present experiments were all made with roughly filtered solutions, but the difficulty could be met in practice by a previous screening of the sewage or by passing it through a septic tank. Should clogging occur, it will probably take place in the superficial layers and could be remedied by simple mechanical treatment. In the case of contact beds, however, clogging necessitates the cleansing of the whole bed, an exceedingly costly process. From these considerations, and as a result of the present experimental study, the method of continuous filtration would appear to be a most advantageous method of purifying sewage.

* Ducat filters, which are artificially warmed in cold weather, perform an amount of nitrification which is well above the average.

Tables I—IX.

In these tables the estimations of "free and saline ammonia" are expressed as *ammoniacal nitrogen*, those of "albuminoid ammonia" as *albuminoid nitrogen*, those of nitrites as *nitrous nitrogen*, and those of nitrates as *nitric nitrogen*, all in parts of nitrogen per 100,000 by weight.

Nitrites were estimated by the metaphenylenediamine colorimetric method unless otherwise stated, the sum of nitrites and nitrates by the indigo method (Vienna analyses), or by the copper-zinc couple method (Munich analyses).

Throughout these tables the grades of reactions are represented as follows: reaction absent by 0, a trace, or faint reaction, by *f*, definite reaction by +, and intense reaction by + +.

Oxidisability is expressed in parts oxygen absorbed per 100,000 by weight, less that absorbed by any nitrites present. In figures marked with an asterisk the oxidisability was approximately gauged by the difference between the oxygen absorbed in the cold and on boiling.

Table IA.—Vienna Continuous Filters, showing the Progress of Nitrogen Oxidation during Maturing. Filters all started on February 20, 1901. Figures in brackets are weeks elapsed since the start.

Course of oxidation.	Tall filter.	Medium filter.	Short filter.
Oxidised N. first detected in filtrate	March 18 (4 w.)	March 21 (4 w.)	March 28 (5 w.)
Nitrites present in quantity, no nitrates	March 28* (5 w.)	March 28† (5 w.)	July 1‡ (19 w.)
Nitrates also present in quantity	May 24 (11 w.)	May 13 (9½ w.)	
Nitrates alone present	July 1 (19 w.)	July 1 (19 w.)	

* 10 parts per 100,000 N. as N_2O_3. † 1·3 parts ditto.
‡ 4·1 parts ditto, nitrates also present in small amount.

Table IB.—Munich Experiments. Filters all started April 1, 1903.

Course of oxidation.	Continuous filters.			Contact filters.		
	Tall.	Medium.	Short.	Tall.	Medium.	Short.
Oxidised N. first detected in filtrate	May 2 (4 w.)	May 2 (4 w.)	May 2 (4 w.)	May 16 (6½ w.)	May 16 (6½ w.)	May 16 (6½ w.)
Nitrites present in quantity, nitrates present	May 18 (7 w.)	May 23 (7½ w.)				
Majority of oxidised N. present as nitrates	May 27 (8 w.)	—	—	June 25 (12 w.)	June 25 (12 w.)	June 25 (12 w.)
Nitrates alone present...	June 8 (10 w.)	June 13 (10½ w.)	June 13 (10½ w.)	July 9 (14½ w.)	July 9 (14½ w.)	July 9 (14½ w.)

Table II.—Analyses of Sewage Affluent and Filtrates. Analyses 1—23 deal with the Vienna continuous filters, all started 20.2.01; Analyses 24—36 deal with the Munich continuous filters, all started 1.4.03. The results are expressed in parts per 100,000.

No. of Analysis.	Date.	Material analysed. Height of Filter.	Ammoniacal nitrogen.	Albuminoid nitrogen.	Nitrous nitrogen.	Nitric nitrogen.	Total nitrogen, Kjeldahl.	Total nitrogen calculated.	Oxidisability, in terms of O.
1	7.3.01	Raw sewage	Equal to sewage. (1–4)	—	0	0	—	—	10·3
2	7.3.01	Filtrate—tall		—	0	0	—	—	5·9
3	7.3.01	„ —medium ...		—	0	0	—	—	5·4
4	7.3.01	„ —short........		—	0	0	—	—	6·4
5	13.3.01	Raw sewage	—	—	0	0	—	—	15·3
6	13.3.01	Filtrate—tall	—	—	0	0	—	—	4·2
7	13.3.01	„ —short........	—	—	0	0	—	—	6·6
8	18.3.01	Raw sewage	—	—	0	0	—	—	15·3
9	18.3.01	Filtrate—short........	—	—	0	0	—	—	5·4
10	28.3.01	Raw sewage	15·2	2·0	0	0	17·2	17·2	42·8
11	28.3.01	Filtrate—tall	1·2	0·38	10·1	0	—	11·6	5·1
12	28.3.01	„ —medium ...	15·1	—	1·6	0	—	—	—
13	28.3.01	„ —short........	15·4	—	*f*	—	—	—	4·6
14	1.4.01	Raw sewage	14·7	2·0	0	0	17·2	16·7	—
15	1.4.01	Filtrate—tall	3·8	2·3	10·4	0	—	16·5	—
16	13–15.5.01 (16–18)	Raw sewage	14·7	2·5	0	0	—	17·2	13·5
17		Filtrate—medium ...	0·3	0·4	5·7	3·1	—	9·5	4·1*
18		„ —short........	10·8	1·3	*f*	—	—	12·1	5·2
19	24.5.01	„ —tall	0·3	—	6·0	1·1	—	—	—
20	1.7.01	Raw sewage	11·9	—	0	0	—	—	—
21	1.7.01	Filtrate—tall	0·1	—	0	7·8	—	—	—
22	1.7.01	„ —medium ...	4·1	—	*f*	5·0	—	—	—
23	2.7.01	„ —short........	3·7	—	4·1	*f*	—	—	—
24	19.5.03	Raw sewage	3·5	1·6	0	0	—	5·1	11·6
25	19.5.03	Filtrate—tall	0·2	0·2	2·5	0·4	—	3·3	2·4
26	23.5.03	Raw sewage	3·6	1·4	0	0	—	5·0	—
27	23.5.03	Filtrate—medium ...	0·1	0·2	1·2	1·5	—	3·0	—
28	26–27.5.03 (28–29)	Raw sewage	4·0	1·6	0	0	—	5·6	14·3
29		Filtrate—tall	0·07	0·2	0·6	2·0	—	2·9	—
30	15–17.6.03 (30–32)	Raw sewage	2·0	1·6	0	0	—	3·6	10·9
31		Filtrate—tall	0·04	0·2	0	2·1	—	2·3	3·6
32		„ —medium ...	0·07	0·24	0	1·7	—	2·0	4·1
33	24.6.03	Cows' urine, 1 p. c. ...	13·2	3·8	0	0	—	17·0	10·1
34	24.6.03	Filtrate—tall	0·08	0·15	0	8·3	—	8·5	1·61
35	13.7.03	Cows' urine, 1 p. c. ...	10·0	4·1	0	0	—	14·1	—
36	13.7.03	Filtrate—tall	0·05	0·17	0	8·1	—	8·3	—

Table III.—Showing the Course of Oxidation at Successive Depths in Tall and Medium Filters (Vienna).

No.	Date.	Constituents.	Sewage.	Filtrate at successive depths.			
				Tap No. 1, 50 cm.	Tap No. 2, 100 cm.	Tap No. 3, 150 cm.	Tap No. 4, 200 cm.
		Tall filter.					
1	24.5.01	Ammoniacal N.	12·78	11·91	2·27	0·39	0·29
2	24.5.01	Oxidised N.......	0	*f*	+	+ +	+ + †
3	27.5.01	Oxidisability* ...	9·2*	3·4*	2·6*	3·0*	2·3*
4	27.5.01	Nitrites............	—	*f*	*f*	No. 2 × 5	No. 2 × 7
5	1.7.01	Ammoniacal N.	11·9	2·5	0·2	0·04	0·06
6	1.7.01	Nitrites............	0	*f*	0	0	0
7	1.7.01	Nitrates	0	+	+	+	7·8
		Medium filter.					
8	1.7.01	Ammoniacal N.	11·9	8·1	4·12		
9	1.7.01	Nitrites............	0	*f*	*f*		
10	1.7.01	Nitrates	0	*f*	4·96		

In 2 the test was made with diphenylamine, in 3 the estimations are approximate.

The high numbers in filtrate of 8 are due to deterioration in efficiency of the filter following partial clogging.

† Nitrous N = 6·0, nitric N = 1·1.

Table IV.—Munich Continuous Filters from June 24, 1903, to November 27, 1903, using Cows' Urine diluted 1 in 100; this contained 14—17 parts NH_3 per 100,000 and no oxidised nitrogen. In cases marked with an asterisk the urine was only diluted to 1 in 50.

Date.	Tall filter.			Medium filter.			Short filter.		
	NH_3 per 100,000.	Nitrite re-action.	Nitrate re-action.	NH_3 per 100,000.	Nitrite re-action.	Nitrate re-action.	NH_3 per 100,000.	Nitrite re-action.	Nitrate re-action.
26.6.03	*f*	0	+ +	*f*	0	+ +	1	*f*	+ +
30.6.03	0·1	0	+ +	0·1	0	+ +	2—3	*f*	+ +
9.7.03	0·05	0	+ +	2—3*	+	+ +	1—2	+	+ +
13.7.03 } 15.7.03 }	0·2	0	+ +	6—7*	*f*	+ +	2—3	*f*	+ +
23.7.03	0·05	0	+ +	4—5*	*f*	+ +	1—2	+	+ +
24.10.03	0·05	0	+ +	0·05	0	+ +	0·1	0	+ +
27.11.03	0·02	0	+ +	0·04	0	+ +	0·04	0	+ +
20.1.04	5·0*	*f*	+ +	8—10*	+ +	+ +	15*	+	+ +

The ammonia estimations by direct Nesslerisation are only approximate; nitrites were tested for by acidified starch-zinc-iodide; nitrates by diphenylamine (only specific if nitrites are absent or inconsiderable).

Table V.—Enumeration of Organisms in Filtrates.

Date.	Munich continuous filters.	No. of organisms per cubic centimetre.			Time sub-cultures were kept.
		Growing in bouillon.	Nitrite-producer.	Nitrate-producer.	
6.6.03	Tall	10,000	10,000	100*	6 weeks
6.6.03	Medium	1,000	10,000	100	6 "
6.6.03	Short...............	100,000	10,000	100	6 "
12.6.03	Tall	10,000	10,000	10,000	4 months
12.6.03	Medium	100,000	100,000	1,000	4 "
12.6.03	Short...............	100,000	10,000	100	4 "
18.6.03	Tall	10,000	10,000	100	Former 4 m.
18.6.03	Short...............	1,000	1,000	10	Latter 6 w.
24.6.03	Tall	10,000	1,000	10,000	4 months
24.6.03	Medium	10,000	1,000,000	10,000	4 "
27.6.03	Tall	1,000	—	10,000	4 "
27.6.03	Medium	10,000	—	10,000	4 "

* Culture containing 0·01 c.c. filtrate, used for dilution experiment on p. 252.

Table VI.—Results of Inoculating two different Impure Nitrite-producer Cultures, "*a*" and "*b*," into Ammoniacal Media, both directly and after passing through one or two generations in bouillon. + + indicates an intense nitrite reaction.

Series.	Sequence of inoculation.	"*a*," three weeks old.	"*a*," twelve days old.		"*b*," three weeks old.		"*b*," four weeks old.
1	From original material	+ +	—	—	+ +	+ +	+ +
4	From Series 1	—	+ +	+ +	+ +	+ +	—
3	From original through bouillon (Series 2)	0	0	0	+ +	+ +	+ +
6	From Series 2 again, through bouillon (Series 5)	—	—	—	0	0	—

Table VII.—Results of Inoculating into Nitrite Medium of Five Strains of a "Symbiotic" Culture of Nitrate-Producer, "*d*," after Successive Generations in Bouillon. + indicates a positive, + + an intense reaction.‡

Dates of successive inoculation into bouillon.	Date of inoculation from bouillon into nitrite medium.	Date of testing preceding cultures.	"*d* 1"		"*d* 2"		"*d* 3"		"*d* 4"		"*d* 5"	
			Nitrites.	Nitrates.	Nitrites.	Nitrates.	Nitrites.	Nitrates.	Nitrites.	Nitrates.	Nitrites.	Nitrates.
17.12.03 →	23.12.03	12.1.04	0	+ +	0	+ +	0	+ +				
↓ 12.1.04 →	18.1.04	3.2.04	+ +	0	0	+ +	0	+ +	0	+ +	0	+ +
↓ 18.1.04 →	26.1.04	10.3.04	+ +	0	0	+ +	0	+ +	0	+ +	+ +	0
↓ 26.1.04 →	3.2.04	10.3.04	+ +	0	+ +	0	+	+	0	+ +	+ +	0

Table VIII.—Showing the Different Behaviour of Pure and of "Symbiotic" Bouillon-Cultures of the Nitrate-Producer when Inoculated into Nitrite Medium, the latter tested for nitrites and nitrates 31 days after inoculation; + indicates a definite, + + an intense reaction.‡

Reactions of the nitrite medium.	Pure cultures, bouillon clear.						"Symbiotic" cultures, bouillon showing growth.					
	No. 25.		No. 39.		No. 67.		No. 49.	No. 29.		No. 12.	No. 21.	
Nitrite	+ +	+ +	+ +	+ +	+ +	+ +	0	0	0	0	+	+
Nitrate	0	0	0	0	0	0	+ +	+ +	+ +	+ +	+	+

Cultures No. 49 and No. 29 were strains of culture "*d*"; cultures No. 12 and No. 21 were of different origin.

‡ With cultures still containing nitrites, evaporation with ammonium chloride preceded the diphenylamine test for nitrates.

Table IX.—Experiments upon the Absorption of Ammonium Chloride from Dilute Solution by Various Finely Divided Solids.

No.	Solid tested; weight taken.	Original solution of NH_4Cl added.		Liquid after standing, ammonia content per 100,000 parts.		Ammonia in blank control in thousandths of a milligramme.	
		Volume of liquid in c.c.	Ammonia content per 100,000 parts.	Upper clear liquid.	Lower turbid liquid.	Clear liquid.	Turbid liquid.
1	$BaSO_4$, 1 gramme ...	100	1 ·00	1 ·02	0 ·91	0	0
2	Sand, 2 grammes	100	5 ·00	4 ·30	4 ·04	2	0
3	Sand, 2 „	50	5 ·00	4 ·14	3 ·78	3	0
4	Clinker, 2 grammes ...	50	1 ·00	0 ·82	0 ·89	2	0
5	Sand, 2 grammes	50	1 ·00	0 ·75	1 ·03	5	0
6	Sand, 2 „	50	1 ·00	0 ·83	1 ·05	3	0
7	Sand	—	1 ·00	0 ·85	0 ·99		

In Experiments 4—7, 10 c.c. N/1 KHO was added before distilling off the ammonia.