LOSSES OF AMMONIA FROM CULTURE SOLUTIONS.

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Received May 17, 1907.

The isolation of pure cultures of nitrous and nitric ferments baffled for a long time the efforts of many bacteriologists. It was reserved for Winogradski[1] to offer a brilliant solution of the difficult problem, and to demonstrate that the isolation of these organisms may be accomplished with comparative ease where the proper culture methods are employed. Our knowledge of such culture methods has been amplified further by Omelianski[2] who has proposed the following culture solution for the preparation of crude cultures of the nitrous ferments:

Ammonium sulphate	2 gms.
Sodium chloride	2 "
Potassium phosphate	1 "
Magnesium sulphate	0.5 "
Ferric sulphate	0.4 "
Distilled water	1000 cc.

To each 100 cc. of this solution there is to be added 1 gm. of magnesium carbonate, which is to serve as the base for neutralizing the nitrous and nitric acid formed in the oxidation of the ammonia.

The same solution has also been employed for the systematic study of the nitrifying power of different soils, or of the same soil under different conditions of treatment. Remy[3] who was the first to propose such systematic investigations of the various bacteriological functions of soils inoculated measured portions of this culture solution, and by means of qualitative tests noted the rate at which the ammonia reaction disappeared, and the nitrite and nitrate reactions became more intense. The same, or a similar procedure was followed by Wohltman, Fischer, and Schneider[4], and Ehrenberg[5]. Löhnis[6] found the addition of magnesium carbonate impracticable for the quantitative study of nitrification, because of the very considerable losses of ammonia from the culture solutions. He employed, instead, calcium carbonate with solutions containing only one gm. of ammonium sulphate per liter.

It was noted in our own studies on the nitrifying power of various soils that the ammonium sulphate culture solutions prepared according to the directions of Omelianski, lost large quantities of ammonia. In order to secure more exact data as to the extent of these losses we prepared one liter of the ammonium sulphate culture solution, and distrib-

[1] Ann. inst. Pasteur., Vol. 4, 1890, No. 4; Ibid. vol. 5, 1891, No. 2.

[2] Centr. Bakt. Parasitenk., II, 5, 539, (1899).

[3] Ibid, 8, 657, (1902).

[4] Ibid, 12, 304, (1904).

[5] Landw. J., 33, 1, (1904).

[6] Centr. Bakt. Parasitenk., II, 13, 709, (1904).

uted it in 100 cc. portions in round flat-bottomed Jena flasks. Eight of the ten flasks were sterilized in the autoclave at two atmospheres of pressure, while two were left unsterilized. Immediately after sterilization ammonia determinations were made in two of the sterilized portions of the solution, and likewise in the two unsterilized portions. The six remaining portions were placed in the incubator at 28° C., and two of these analysed for ammonia at the end of six days, twelve days and twenty-four days, respectively. The following table shows the amount of ammonia found in each case:

NITROGEN AS AMMONIA IN THE CULTURE SOLUTIONS.

Analysed Immediately	Unsterilized	43.45 mg.	Average 43.45 mg.
	"	43.45 "	
	Sterilized	36.82 "	" 36.99 "
	"	37.16 "	
At the end of six days.	"	27.26 "	" 27.17 "
	"	27.09 "	
At the end of twelve days.	"	24.23 "	" 24.40 "
	"	24.58 "	
At the end of twenty-four days.	"	17.57 "	" 17.44 "
	"	17.31 "	

The figures in this table show clearly that the losses of ammonia from the Omelianski culture solutions may be very large. It is evident that considerable amounts of it are lost during sterilization in the autoclave, for we note that the sterilized portions contained, on the average, 36.99 mg. of nitrogen as against 43.45 mg. in the unsterilized portions. Further losses of ammonia occurred in the sterilized portions, when they were allowed to stand for longer or shorter intervals. Thus at the end of six days the sterilized portions contained on the average 27.17 mg. of nitrogen; at the end of twelve days 24.40 mg., and at the end of twenty-four days only 17.44 mg. In other words, almost three-fifths of the entire amount of ammonia present in the fresh culture solution were lost in the course of twenty-four days.

Another series of culture solutions was prepared partly to check the foregoing results and partly to furnish information as to the losses in sterilized and inoculated solutions. Twenty 100 cc. portions of the Omelianski culture solution were prepared in the usual manner with the single difference that calcium carbonate was used instead of magnesium carbonate in ten of these portions. All but four of the twenty portions were sterilized in the autoclave, and ammonia determined in four of the sterilized portions, as well as in the four unsterilized portions. The following amounts of ammonia nitrogen were found:

Unsterilized		Sterilized	
$MgCO_3$	No. 1—40.03 mg.	$MgCO_3$	No. 5—34.84 mg.
	No. 3—40.20 "		No. 7—34.92 "
$CaCO_3$	No. 2—41.07 "	$CaCO_3$	No. 6—40.55 "
	No. 4—40.72 "		No. 8—40.37 "

We note here that in the portions supplied with magnesium carbonate

there was a distinct loss in the sterilized solutions as compared with the unsterilized portions. It seems, furthermore, that there was a slight loss even in the unsterilized portions provided with magnesium carbonate: for the yields in culture solutions 1 and 3 were 40.03 mg. and 40.20 mg., respectively, as against 41.07 mg. and 40.72 mg. for the corresponding solutions 2 and 4, where calcium carbonate was employed. Moreover, the losses of ammonia from the sterilized portions supplied with calcium carbonate were quite slight when compared with those in the corresponding solutions supplied with magnesium carbonate.

The remaining 12 sterilized portions of the culture solution were placed in the incubator and four of them analysed at the end of six days, four at the end of 12 days, and the last four at the end of 25 days. Six of these solutions were each inoculated after sterilization with 10 cc. of soil infusion equivalent to 5 gms. of soil. The following amounts of ammonia nitrogen, and, in the inoculated portions, also of nitrite and nitrate nitrogen, were found in the several solutions.

IN SIX DAYS.

	Ammonia Nitrogen.	Average	Nitrite Nitrogen	Nitrate Nitrogen
$MgCO_3$	Sterile— No. 9—31.63 mg. Inoculated— No. 11—29.64 mg.	30.63 mg.	0.19 mg.	0.062 mg.
$CaCO_3$	Sterile— No. 10—39.60 mg. Inoculated— No. 12—39.86 mg.	39.73 mg.	0.15 mg.	0.089 mg.

Comparing the amounts of nitrogen found at the end of six days with those found in the corresponding fresh solutions, we note further losses. The latter are greater in the portions containing magnesium carbonate, and are slight, but none the less distinct, in the portions containing calcium carbonate. In the inoculated culture solutions 11 and 12, small quantities of nitrite nitrogen were already present at the end of six days.

IN TWELVE DAYS.

		Ammonia Nitrogen.	Average	Nitrite Nitrogen.	Nitrate Nitrogen.
$MgCO_3$	Sterile— No. 13—	22.96 mg.	23.30 mg.	0.06 mg.	0.078 mg.
	Inoculated 15—	23.65 mg.			
$CaCO_3$	Sterile— 14—	38.83 mg.	37.23 mg.	0.30 mg.	0.250 mg.
	Inoculated— 16—	35.64 mg.			

At the end of twelve days the losses in the magnesium carbonate solutions had become much greater. Only 23.30 mg. of ammonia nitrogen remained, on the average, in these solutions. The inoculated portion 15 showed scarcely any nitrite and nitrate, indicating thus that the process of nitrification had not yet become active. In culture solutions 14 and 16, where calcium carbonate was used, small but distinct losses occurred. In the inoculated solution 16, the loss was greater than that in the corresponding uninoculated solution 14, a loss which was but partly accounted for by the nitrite and nitrate nitrogen found. It should be noted here, also, that in the inoculated solution 16 the amounts of nitrite and

nitrate nitrogen were 0.30 mg. and 0.25 mg., respectively, as against 0.15 mg. and 0.089 mg. in the corresponding solution 12 at the end of twelve days. The nitrification process had evidently made some headway in the intervening six days, where calcium carbonate was used, but not in the solutions where magnesium carbonate was used.

IN TWENTY-FIVE DAYS.

		Ammonia Nitrogen.	Nitrite Nitrogen.	Nitrate Nitrogen.
$MgCO_3$	No. 17 Sterile —	18.64 mg.	0.15 mg.	0.025 mg.
	19 Inoculated —	18.04 mg.		
$CaCO_3$	18 Sterile—	35.56 mg.	1.74 mg.	1.000 mg.
	20 Inoculated —	22.52 mg.		

A still further loss of ammonia occurred in solutions 17 and 19, containing magnesium carbonate. The second of these, which was inoculated with soil infusion, showed the greater loss without corresponding increase in the amount of nitrite and nitrate nitrogen. On the other hand, the solutions containing calcium carbonate not only suffered smaller losses of ammonia, but showed active nitrification in the inoculated portion. We note, thus, that at the end of twenty-five days the magnesium carbonate solutions had lost considerably more than one half of the ammonia nitrogen which they originally contained. whereas the calcium carbonate solutions lost scarcely more than one-eighth of the initial amount. In the inoculated portion, supplied with calcium carbonate, the amount of ammonia nitrogen found at the end of twenty-five days was only 22.52 mg., as compared with the 35.56 mg. found in the corresponding sterile portion. We find, at the same time, that a portion of the ammonia nitrogen which had disappeared from the inoculated portion had been converted into nitrite and nitrate nitrogen to the amount of 1.74 mg. and 1.00 mg. respectively. The quantity of nitrite and nitrate nitrogen thus recovered is not sufficient to account for the greater loss of ammonia from the inoculated solution. The discrepancy observed here would tend to support the claim made by Godlewski[1] and others that gaseous nitrogen may be liberated in the nitrification of ammonia. Godlewski attributes this loss not to the respiration processes of the bacteria themselves, but to the reaction between nitrous acid and ammonia.

$$NH_3 + HNO_2 = 2H_2O + N_2.$$

It is to be added here that the formation of nitrite and nitrate may proceed simultaneously even where large quantities of ammonia are still present in the culture solution. Winogradski and Omelianski point out[2] that the nitrate ferments are very susceptible to ammonia and do not show any appreciable activity until nearly all of it had been converted into nitrite. Their results were secured with solutions containing mag-

[1] Centr. Bakt. Parasitenk., II, 2, 458, (1896).

[2] Ibid, 5, 439, (1899).

nesium carbonate, and find strong support in observations made elsewhere. Where calcium carbonate instead of magnesium carbonate is present in the culture solutions, the process of nitrification is modified, and the presence of unoxidized ammonia does not seem to inhibit the development of the nitric ferments. Under actual soil conditions the formation of nitrites and nitrates may not only occur at the same time, but the nitrites may be oxidized as fast as they are formed, and the activity of the nitrous ferments may thus be concealed. It should be noted here, however, that organisms may exist in the soil, which have the power of converting ammonia directly into nitrate without the intermediate formation of nitrites. Such, at least, is the claim made by Kaserer,[1] and there are observations made by others, which would lend support to this claim.

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THE FLUORINE CONTENT OF MALT LIQUORS.

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Received July 3d, 1907.

In a previous paper[2] the authors outlined a method for the approximate estimation of fluorides when present in minute quantities, such as might occur in food products. At that time the hope was expressed that it might be possible to examine by means of the method, various classes of food products with a view to determining the limiting values for their fluorine content. For obvious reasons, malt liquors seemed excellent material for beginning such an investigation, and it is the purpose of the present paper to summarize the results obtained in the examination of a number of the brands of malt liquor on the market, as well as the materials used in brewing.

Method.—The method used was similar to that already described, except that on account of the large amounts of carbonic acid in the beers, it was found necessary in every case to add a somewhat greater quantity of barium acetate, enough being used to ensure an excess in the filtrate from the barium sulphate and fluoride. For the same reason more sulphuric acid (3-4 cubic centimeters instead of 2-3 as previously directed) is necessary during the etching. An insufficient amount of either of these would occasion negative results, even if an appreciable amount of fluoride were present.

In extracting the fluoride from malts and barleys it was found inadvisable to grind the sample and extract with hot water, as would be done in the brewery operation of mashing, because the large amount of sugary and starchy matters extracted interfere with the subsequent precipitation of the fluoride. Satisfactory results were obtained in the case of malt by

[1] Centr. Bakt., Parasitenk., II, 16, 681 and 769 (1906).

[2] This Journal, 28, 1437, (1906).