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Introduction.

In the sewage treatment methods most widely used at present, the suspended organic solids are separated from the liquid and each disposed of by different methods. The highly putrescible solids are commonly fed to covered tanks in which anaerobic bacteria become established, and by the bacterial activities they are broken down into inoffensive products. This digestion or biolytic process is little understood, though the control of temperature and hydrogen-ion concentration is known to favor the rapid evolution of the gaseous end-products, methane and carbon-dioxide, and the conversion of the residual solids into a humus-like residue. Better control and increased efficiency of the digestion process should result from detailed studies of the biochemical reactions involved. The present study deals with biochemical transformations in the predominant nitrogenous compounds and in one class of carbonaceous materials, the soaps and fats, of sewage sludge. The aim has been to represent chemically the degradations occuring in a normally operating, sludge-digestion tank, rather than to formulate more favorable operating conditions for such tanks; the latter aim can be realized when all the sludge components have been studied.

The summarized results reported here will be developed more fully in a forthcoming bulletin, together with the details of the experimental method used in each part of the study and the analytical technique employed.

HISTORICAL.

Controlled digestion of sewage dates from the introduction of the septic tank in 1896[2], but not until 1903 were the suspended solids removed to a separate chamber of the tank for the digestion. Three years later, Dr. Imhoff in Germany built the forerunner of the widely-used tanks bearing his name[7]. Various modifications of the Imhoff design have played a prominent part in European and American sewage treatment practice, but in recent years the collection of the evolved gas for power production has led to the development of separate digestion units which can be heated by a portion of the gases to stimulate digestion.

The potential value of the methane has aroused interest in the nature of the biochemical processes producing it. Bach and Sierp[1] obtained high methane yields by inoculating some common foods with digested sludge, though the highest yield was obtained from digesting feces. Starchy materials gave hydrogen and carbon dioxide. Meat protein

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digested slowly and incompletely, but 30 to 50 per cent of the associated fat was decomposed. O'Shaughnessy[9] observed also a digestion of one-third of the grease of sewage solids. Good treatment-plant performance has been correlated with a low grease content of the sludge[10]. On the other hand, some workers in the field have denied grease digestion[11]. Buswell and Neave[3] examined a large number of sludges from a separate sludge-digestion unit and found no change in the percentage of grease during digestion; but, since 30 per cent of the organic matter digested, an equivalent reduction in grease was indicated by this constant percentage. Further investigation[4] of sewage solids undergoing the acid type of digestion showed a rapid destruction of grease with the production of lower fatty acids, some of which fermented further to give methane. Later work[5] showed a similar degradation under alkaline conditions and confirmed the methane production from lower fatty acids. Since these lower acids arise in the decomposition of all three major classes of organic matter,—carbohydrates, proteins and fats,—the studies have been continued to show how the acids arise, why they sometimes accumulate in inhibitory concentrations and the mechanism by which they yield methane.

Extensive studies[6] on an experimental sludge-digestion unit in this community have shown that 27 per cent of the sludge solids consists of grease; also "that 90 per cent of the grease is digested and that the weight of grease digested corresponds to more than 58 per cent of the gas." These figures show the importance of grease degradation in the sludge-digestion process.

Although the economic aim is high methane yield, the primary purpose of sludge digestion is the production of an inoffensive product of suitable physical properties. A high methane yield was obtained in the above mentioned acid digestion studies, but the resultant sludge had an offensive odor and a strongly hydrophilic nature. These properties were attributed to inhibition of the proteolytic reactions by the acid. Accordingly, the nitrogen transformation during both normal and acid digestion have been included in the present study.

The investigation, then, had three purposes: (a) to chemically define the changes in forms of nitrogen; (b) to study conditions favoring rapid grease digestion on account of its potential methane values, and (c) to formulate a mechanism for the methanic degradation of fatty acids with a view to stimulating methane yields.

EXPERIMENTAL.

Degradation of Nitrogenous Matter.—The principal nitrogenous constituents of sewage have been shown by Neave and Buswell[8] to be urea, ammonia and protein. The soluble nitrogen compounds enter the digestion chamber or tank only in the liquor associated with the settled solids; hence, urea is the only soluble compound discussed here. In the present studies, 10 to 20 milligram amounts of urea have been shown to hydrolyze to ammonium carbonate in 5 days in sewage and in 24 to 48 hours in liquor from digesting sludge. This is in accordance with the assumption usually made regarding the fate of urea in the sewage tank.

The insoluble nitrogenous material has been given most attention. Dried sewage solids contained 2.5 to 3.5 per cent of nitrogen (equivalent to 15 to 22 per cent protein). Since these proteins are combined and denatured, they are not readily procured in a soluble form; accordingly, they were hydrolyzed with acid and the nitrogen distribution after hydrolysis used to characterize them. The average distribution for fresh and digested solids, respectively, was found to be: Humin-N 11.2, 15.2; Ammonia-N 8.5, 15.0; amino-N 53.0, 67.1; non-amino-N 27.3, 3.7, these values being percentages of the total nitrogen present in the dried solids.- The non-amino nitrogen fraction was shown by precipitation as the copper salts to be mainly purine bases, derived probably from nucleoproteins. About 25 per cent of the nitrogen of the fresh solids represents purines, but these bases undergo almost complete destruction. during the digestion process; otherwise the character of the sludge nitrogen shows little change.

A more complete picture of the nitrogen transformations was obtained on two series of one-liter bottles of sewage solids, digesting at room temperature, the one series undergoing the acid type of digestion and the other receiving enough lime to keep the pH above 6.4. At intervals one bottle was removed from each series for analysis. The results showed a rapid loss of urea and purine nitrogen, and a marked liquefaction of solid 'nitrogenous matter in the more alkaline series. The acid digestion favored the production of amines with only a very gradual peptization of solid proteins after 4 or 5 weeks. The alkaline digestion, on the other hand, showed no such accumulation of amines, and a pronounced proteolysis to soluble combined and free amino-N, and no excessive ammonia production; the trend of these values indicated resynthesis of peptized and split proteins into a new bacterial flora.

No marked change was observed in the total of all forms of nitrogen in the system, though later more precise balances were carried out specifically to test the current belief in the loss of gaseous nitrogen during the digestion process. Thus, 8 balances on fatty acid salts fed to digested sewage sludge indicated an average loss as gas of 1.0 per cent of the total nitrogen; feeding glycocoll gave 3.7 per cent loss; sewage solids showed 2.0 per cent average loss for 4 balances, and a single test with sodium propionate gave a loss of 6.8 per cent. Under the conditions of these anaerobic digestions, then, not more than 5 per cent of the total nitrogen is ordinarily lost as gas.

Accordingly, the nitrogen transformations may be characterized as a remobilization of the forms of nitrogen in which solid coagulated proteins and nucleoproteins of bacterial bodies, plant and animal debris, etc., are peptized and hydrolyzed' to free amino acids and ammonia; some of the free amino acid is resynthesized into bacterial protoplasm. A low pH inhibits this remobilization, and favors the decarboxylation of amino acids to amines of disagreeable odor.

Degradation of Carbonaceous Matter.—Average analyses of sludges from Imhoff and separate sludge-digestion units in this community show the following percentage composition before and after digestion, respectively: nitrogen as protein 19.4, 12.5; grease 25.2, 8.6; crude fiber

10.8, 9.8; humic acids 4.0, 6.9. Grease suffers the greatest loss in weight; consequently attention has been focussed on it as a source of methane.

Buswell and Neave[4] have reported acid digestion studies in which a 75 per cent destruction of fats and soaps occurred in 4 weeks, 25 per cent being recovered as lower fatty acids and 50 per cent as methane and carbon dioxide. They have shown[5] similar results for the alkaline degradation of soaps. In the present studies, attempts to isolate specific grease-digesting organisms were unsuccessful; only in actively fermenting carbohydrate or peptone cultures of a mixed flora were long carbon chains fragmented, and under these conditions the products of grease degradation could not be determined with certainty.

One-gram quantities of fatty acid salts were then fed to digested sludge and analyses made of the evolved gas, the initial and final carbon dioxide in solution and changes in the weight of organic inoculum. The total CO_2:CH_4 ratios resulting from fatty acid metabolism were sought as indications of the mechanism of the reaction. After deducting gases evolved by the unfed control, sodium salts of the acids gave the following CO_2:CH_4 ratios: formic 1:0.44, acetic 1:0.9, propionic 1:1.5, n-butyric 1:1.4, n-valeric 1:1.7, oleic 1:2. Glycerol gave a 1:0.9 ratio. But, while the loss of organic solids in the control agreed with the weight of gas given by the control, in other cases the loss of organic solids exceeded that shown by the control, this being particularly marked in the glycerol fermentation; in other words, the added nutritive stimulated the digestion of the sewage solids used as inoculum, and the control no longer represented a true correction for the fermentation gases. The above ratios are thus merely first approximations.

Either less inoculum or more nutritive was required to obtain precise ratios. Decreasing the inoculum below about 5 grams of total solids per liter resulted in an inconveniently large percentage of failures among the cultures, so the amount of salt fed was increased to 10 grams. Propionic and butyric acids gave the mean CO, :CH_4 ratios of 1:1.42 and 1:1.77, respectively, for a series of cultures.

For quantities of salt exceeding 10 grams per liter, either the inoculation failed or lethal concentrations of bicarbonate developed as the acid digested. Accordingly, an 8-liter reaction vessel was designed to permit repeated feeding of the same inoculum. This vessel was a cylindrical, galvanized-iron tank provided with a conical bottom terminating in a gate-valve, and a cover sloping up to a central, water-sealed, gas-collecting dome from which the gases were led to a brine-filled gasometer for measurement and analysis. Appropriate lateral connections on the tank permitted withdrawal of liquor or introduction of liquid food without admitting air into the system. Digested sludge, equivalent to about 50 grams of dry solids, in the tank full of sewage established the methane flora, and the fatty acid salt was fed once or twice daily as a 2 per cent solution, an equivalent quantity of liquor being displaced. Thus 20 to 30 grams of the fatty acid per week could be digested, and the experiment could be continued until the weight of sludge inoculum became a negligible percentage of the total metabolism.

To date, two runs on calcium acetate have been completed, and the experimental method has proved so satisfactory that the investigation is being extended with this apparatus to the higher members of the homologous series. The essential data on the calcium acetate runs are:

	Organic solids in inoculum		Acetic acid	Gas yield as per cent of theory		CO, :CH_4
	Initial	Final	metabolized	CO.	CH_4	ratio
Run 1	37.4 gm.	48.9 gm.	75.08 gm.	92.1	89.7	1:0.974
Run 2	37.8 gm.	38.4 gm.	114.18 gm.	94.7	92.9	1:0.975

The gases from the first run contained a total of 0.053 gram of hydrogen, and those from the second 0.106 gram. The theoretical gas yield was based on simple decarboxylation of the acetic acid according to the equation:

$$CH_3COOH = CO_2 + CH_4$$

Discussion of Results.—A review of the experimental data on all of the acids studied brings out the following points.

For acids higher than acetic, the total carbon dioxide produced (not merely that evolved) is more than that contained in the carboxyl group of the acid metabolized; therefore, some of the carbon atoms of the chain are oxidized, and the only source of oxygen for this reaction is shown analytically to be water.

A simple relation has been found between the number of water molecules required by each molecule of fatty acid decomposing and the number of carbon atoms in the acid; if n = the number of carbon atoms, then $\frac{n}{2} - 1$ molecules of water are required, the general equation for the digestion of saturated monocarboxylic acids being: $C_nH_{2n}O_2 + \frac{(n-2)}{2}$ $H_2O = \frac{(n+2)}{4} CO_2 + \frac{(3n-2)}{4} CH_4$

Thus acetic acid, with 2, carbon atoms, requires no water; each propionic acid molecule requires one-half a molecule of water; each butyric, one water molecule, etc.

The experimental verification of the general equation may be summarized thus:

Acid	Theoretical equation	Observed CO_2:CH_4	No. of runs
Formic	$4CH_2O_2 - 2H_2O = 3CO_2 + CH_4$	(2.5 : 1)	1
Acetic	$C_2H_4O_2 = CO_2 + CH_4$	1.03 : 1	2
Propionic	$4C_3H_6O_2 + 2H_2O = 5CO_2 + 7CH_4$	5.04 : 7	8
n—Butyric	$2C_4H_8O_2 + 2H_2O = 3CO_2 + 5CH_4$	(2.7 : 5)	3
n—Valeric	$4C_5H_{10}O_2 + 6H_2O = 7CO_2 + 13CH_4$	(6.7 :13)	1
Lactic	$2C_3H_6O_3 = 3CO_2 + 3CH_4$	1.06 : 1	1

Ratios in parentheses are from preliminary tests which require repetition with the tank apparatus. Lactic acid, while not a member of this fatty acid series, is included for comparison with propionic acid, because the difference in gas ratio between propionic and its hydroxy-derivative (lactic) supports the anaerobic oxidation mechanism proposed for these fermentations.

A consequence of this oxidation by water is that, for acids above acetic, the weight of carbon dioxide plus methane produced exceeds the weight of acid metabolized; thus, while 100 grams of metabolized acetic would yield the same weight of gas, a like quantity of propionic would yield 112 grams of gas; of butyric, 120 grams, and of stearic, 151 grams of gas. The mathematical nature of this relationship is such that the weight of gas approaches a theoretical limit of 164 per cent of the acid metabolized for an infinite number of carbon atoms, but the greatest increase, up to 151 per cent, occurs with acids up to 18 carbon atoms (stearic).

From the above general equation, it follows also that the percentage of methane in the gaseous products increases with increasing length of the carbon chain undergoing digestion, and attains a theoretical limit of 75 per cent by volume, or a CO_2 :CH_4 ratio of 1:3, for an acid chain of infinite length. In practice, of course, much of the carbon dioxide is not evolved as gas, but remains dissolved and chemically combined in the medium, and with highly buffered digestion mixtures even acetic acid can give evolved gases containing 80 to 90 per cent of methane.

A review of biological oxidation theories has revealed no mechanism applicable to the methanic degradation of fatty acids. The experimental results require an oxidation of some carbon- atoms in the fatty acid chain to carbon dioxide by water molecules, while other carbon atoms act as hydrogen acceptors and yield methane.

SUMMARY.

1. The anaerobic degradation of some of the dominant constituents of sewage sludge has been studied with reference to the fate of these substances in the sludge-digestion process.

2. The major nitrogenous constituents were shown to be urea, nucleoproteins and simple proteins, and their degradation products. Eapid hydrolysis of urea to ammonium carbonate was demonstrated. Proteins hydrolyzed and peptized rapidly at a pH of 6.4 but slowly at a pH of 5.0. The more alkaline digestion yielded fatty acids, ammonia and free amino acids as products. The purine fraction of the nucleoproteins suffered complete destruction. In acid sludges, proteolysis was arrested and odorous amines produced from the amino acids; also the solids retained their hydrophilic properties.

3. Grease (soaps and fats) has been shown to be an important sludge component in that it digests to lower fatty acids, methane and carbon dioxide. The lower acids also have been shown to yield methane and carbon dioxide. Other studies by this Division[6] show that 58 per cent of the total gas can be attributed to grease digestion.

4. For the fatty acid degradation, an anaerobic oxidative mechanism has been demonstrated in which water acts as the oxidizing agent.

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