XXIII. THE USE OF THE BARCROFT DIFFERENTIAL MANOMETER IN THE ESTIMATION OF THE OXYGEN ABSORPTION OF SEWAGE.

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OUR earlier investigations on the purification of sewage by the activated sludge process [Wooldridge and Standfast, 1932; 1933; Wooldridge, 1933] led us to two main conclusions. Firstly, we concluded that probably the most important factor in sewage purification was a series of catalysed oxidation-reduction reactions determined by bacterial enzymes, present in either living or dead bacterial cells, or liberated by them into the fluid of the reacting system. Secondly we formed the opinion that neither of the two tests investigated was entirely satisfactory for use in a systematic study of the conditions influencing the purification of sewage by the activated sludge process; *e.g.* the technique for the determination of B.O.D. necessitates the incubation of many portions of the same sample for various periods of time. Considerable improvement could clearly be effected if the progress of oxygen absorption could be followed continuously on one sample. With this object in view experiments have been carried out on the measurement of the rate of oxidation of sewage in a Barcroft micro-respirometer.

Are gases other than carbon dioxide produced when sewage or activated sludge is shaken with excess of air?

Before it was possible to apply the manometric method to a study of sewage oxidations it was necessary to ascertain whether the quantity of gases other than oxygen and carbon dioxide which might be present changed during such experiments. It is well known that under anaerobic conditions such gases as hydrogen, hydrogen sulphide and methane are readily produced from sewage or sludge mixtures. It was thought probable however that, when these oxidations were effected in the presence of excess of air or oxygen, especially when the fluid was kept saturated with oxygen by continuous agitation, none of these gases would be produced. These conditions of oxygen saturation are secured in the Barcroft apparatus, wherein 3 ml. of the sewage are shaken in cups of a total capacity of 35–40 ml. In order to examine whether these gases are found in this type of experiment a larger proportion, *viz.* 150 ml. of sewage, sludge or sewage-sludge mixture was shaken with air in 500 ml. bottles for 6 hours, the gas above the mixtures being analysed after 20 min. shaking open to the air and after periods of shaking of 3 and 6 hours in sealed bottles subsequent to this. The gas samples were examined in a Haldane gas apparatus modified for the detection of combustible gases [Haldane, 1920], but in no case could any measurable quantity of combustible gas be detected. The results given in Table I represent one of a duplicate series in which all duplicates agreed.

Table I. *Examination of formation of combustible gas.*

The samples were shaken in 500 ml. bottles stoppered with rubber bungs containing inlet and outlet tubes to facilitate sampling the gas. Three bottles were employed in each series of experiments. The sewage or mixtures shaken consisted of (a) 150 ml. crude sewage, (b) 50 ml. thick suspension of sludge *plus* 100 ml. distilled water and (c) 50 ml. of sludge *plus* 100 ml. crude sewage. After 20 min. preliminary shaking with the tubes open to the air 75 ml. samples of gas were taken with all the usual precautions to avoid sampling errors. Similar samples were taken at the end of 3- and 6-hour shaking periods.

	Hours of shaking	Volume of gas analysed	Volume of gas after CO ₂ abs.	Volume of gas after ignition	Volume of gas after second CO ₂ abs.	Volume of combustible gas
Crude sewage	0	9.445	9.420	9.420	9.420	0
	3	9.420	9.375	9.375	9.375	0
	6	9.420	9.370	9.370	9.370	0
Sludge	0	9.290	9.283	9.283	9.283	0
	3	9.320	9.300	9.300	9.300	0
	6	9.408	9.389	9.389	9.389	0
Sludge + crude sewage	0	9.350	9.330	9.330	9.330	0
	3	9.470	9.288	9.288	9.288	0
	6	9.450	9.111	9.111	9.111	0

The production of gaseous hydrogen sulphide in these manometric sewage experiments was tested for by placing 0.3 ml. of lead acetate solution in small side arms of several cups containing sewage liquor, sludge or sewage-sludge mixtures. The respirometers were then closed and shaken at 24° for 21 hours. Even at the end of this time there was no sign of blackening of the acetate solution, indicating that the production of hydrogen sulphide under these aerobic conditions had been negligible.

It is known that under certain conditions free nitrogen is liberated from soil and hence its possible evolution during the process of aerobic oxidation of sewages cannot be neglected in an investigation dependent upon changes in the volume of the gas in the apparatus. In order to test this possibility 30 ml. quantities of sewage, washed sludge or a mixture of sewage and sludge were shaken for 5 hours in sealed bottles of 400 ml. capacity at 20° with excess air. The concentrations of sewage, sludge and air in the bottles were thus practically identical with those generally employed in the Barcroft cups. The total nitrogen contents of the mixtures were estimated by the Kjeldahl process both before and after the aerobic shaking. The experiments were made in triplicate and, as will be seen from Table II, no change in the nitrogen content of the fluid was observed

Table II. *Examination of the production of nitrogen.*

Experiment	Composition of solution in bottles				% of nitrogen in solution	
	Sludge ml.	Crude sewage ml.	Distilled water ml.	Buffer ml.	Initial g.	Final g.
1	10	0	15	5	0.028	0.028
2	0	15	10	5	0.005	0.005
3	10	15	0	5	0.033	0.033

and therefore it appears that gaseous nitrogen was not liberated. It may be concluded that under the ordinary conditions of a few hours' shaking with excess air in a Barcroft manometer sewage systems do not normally liberate free nitrogen.

Nevertheless we are not justified in concluding from this experiment that free nitrogen can never be liberated from oxidising sewage systems, *e.g.* one containing a very high content of nitrate.

The oxygen absorption by sewage measured manometrically.

If equal volumes of a sewage and water are put respectively into the right- and left-hand cups of a Barcroft differential manometer and the apparatus is shaken in a water-bath at constant temperature then the difference that develops in the levels of the menisci of the manometer fluid will enable us continuously to determine the oxygen absorption of the sewage at the temperature of the experiment. In order to absorb CO_2 0.3 ml. of 10 % KOH solution is placed in the inner tube of all cups. This has been a routine procedure unless otherwise stated. In the majority of our experiments it has been found that 1 ml. of a crude sewage or 3 ml. of a sewage effluent are generally suitable, but the total volume of liquid in each cup is always made up to 3 ml. with buffer or water. The results given in the tables or figures indicate the oxygen absorption per ml. of sewage or mixture. The temperature of the experiments has not always been the same but it has generally been 24° , the lowest temperature at which the baths could be kept constant throughout the year. All manometers are shaken in the bath for 15–20 min. with the manometers open to the air to enable the system to reach temperature equilibrium before closing the taps. The factor for each manometer has to be worked out for each temperature, but once the factor is known for any particular temperature the actual change in volume of the gas in the right-hand cup can be readily obtained by multiplying the difference in levels of the manometer fluid by this factor. The results are most readily expressed in $\mu\text{l.}$ of oxygen absorbed by 1 ml. of sewage. If it is required to conform to current practice in sewage analysis and express results in terms of parts of oxygen absorbed by 100,000 parts of sewage then the result expressed in $\mu\text{l.}$ of oxygen absorbed per ml. of sewage must be multiplied by the factor 0.143.

For strong sewages 1 ml. is sufficient to give very definite results, but for weak sewages 2 or 3 ml. should be used. The results given in Table III illustrate the oxygen absorptions per ml. of several different sewages over a period of 5 days;

Table III. *Oxygen absorption of various sewages.*

No.	Hours											Parts per 10 ⁶ calc. 120 hrs.
	3	6	9	12	21	27	45	69	78	102	120	
1	45.6	71	89	98	133	155	220	313	339	400	443	63
2	26.4	71	156	—	262	295	405	484	498	534	560	80
3	43.9	—	—	—	204	235	296	398	—	—	586	84
4	18.4	67	—	—	160	190	248	298	—	355	387	55
5	23.6	52	100	—	194	220	270	306	323	368	401	57
6	10	27	36	—	82	92	121	149	165	—	177	25
7	—	62	—	98	120	133	165	192	202	220	240	34

the oxygen absorption was measured in $\mu\text{l.}$ but the last column contains the calculated 5-day figures representing parts of oxygen absorbed per 100,000 parts of sewage. Thus the sewages examined absorbed oxygen in 5 days equivalent to 25 to 85 parts per 100,000. Although there is some variation in rates of oxidation of the different samples they all show the general characteristic of a more rapid oxidation in the earlier (10–20) than in the later hours. In none of the experiments, however, had the absorption of oxygen completely ceased at the end of the 5 days.

It is evident from these results that the differential manometer can be used to follow the oxygen uptake of crude sewage but it remains to be seen whether differences such as those existing between sewages or effluents taken at different stages in the plant of a sewage disposal works can be readily demonstrated. The results given in Table IV show that this is possible and that the oxygen absorbed

Table IV. *Oxygen absorption of sewage at different stages of purification.*

The figures show the oxygen absorption in $\mu\text{l.}$ at N.T.P. per ml. of sewage preparation. The right-hand cups contained the sewage preparations unbuffered and diluted to 3 ml., and the left cups 3 ml. water. KOH paper was present to absorb CO_2 . The amounts used were: crude sewage (unfiltered) 1 ml.; effluent from sedimentation tank 2 ml.; effluent from filter 3 ml.; final effluent after land treatment 3 ml.

Hours shaken in Barcroft app. ...	1	3	5	10	25	45	70	91	120
Crude sewage (unfiltered)	10.9	23.6	43.5	112	212	270	308	346	400
Sedimentation tank effluent	4.9	12.5	22.9	48.8	78	101	118	135	153
Filter bed effluent	2.4	4.0	4.5	4.9	8.1	11.7	15.4	17.8	23.4
Final effluent after land treatment	0.6	1.0	1.3	2.0	3.7	6.0	8.8	10.7	16.3

in any given time by the four samples is always in the expected order, *viz.* crude sewage, sedimentation tank effluent, effluent from filters, final effluent. Even within a few hours an approximate idea of the relative strengths of the sewages and sewage effluents is clearly demonstrable. It should be noted that the final effluent shows a definitely measurable, although small, uptake of oxygen when 3 ml. of the effluent are used.

In view of this small absorption of oxygen the absorption values with a number of different effluents were measured. Table V represents the results obtained

Table V. *The oxygen absorption of various sewage effluents in the Barcroft manometer.*

Except for those in the last column the figures show the oxygen absorption in $\mu\text{l.}$ at N.T.P. per ml. of effluent. The right-hand cups contained 3 ml. of effluent and the left-hand cups water. CO_2 was absorbed by potash.

Effluent	Temp. ° C.	Hours									Parts per 10 ⁵ calc. 120 hrs.
		3	5	20	30	48	54	72	96	120	
I	22	—	2.3	3.0	4.5	6.0	8.4	9.0	—	22.0	3.1
II	22	2.2	3.0	6.5	8.3	10.4	11.4	13.6	21.2	24.3	3.5
III	21	2.4	—	5.3	5.9	7.7	8.9	10.8	15.4	22.0	3.1
IV	21	0.7	—	4.3	5.8	6.8	7.2	8.6	10.9	13.1	1.9
V	21	1.6	—	4.5	5.0	5.3	5.4	7.3	11.7	14.8	2.1
VI	21	1.9	—	5.9	7.2	9.3	9.5	13.2	19.7	27.4	3.9
VII	21	1.2	—	3.9	4.7	6.3	6.4	6.9	7.9	8.2	1.2
IX	24	4.0	4.5	6.0	10.8	11.8	—	15.6	18.9	23.0	3.3
XIII	24	—	—	6.6	—	13.2	14.8	19.9	29.1	36.0	5.1
VIII	21	0.6	1.0	3.3	4.0	6.6	7.1	8.5	12.8	16.3	2.3

with ten effluents (not all "satisfactory" final effluents) and it will be seen that after 5 days the absorption of oxygen per ml. of effluent varied between 8.2 and 36.0 $\mu\text{l.}$

By this method it is easy to distinguish the oxygen absorbing capacity of a strong sewage from that of an effluent after examination over a period of 5 days, but it is worth considering whether it is possible to form a conclusion from measurements at an earlier stage. Tables III and V show that with both sewages and effluents the rate of absorption of oxygen under these conditions is greatest in the first few hours. In spite of this, however, the values for the two groups are well separated almost from the beginning. In 20 hours all the sewages have absorbed more than ten times the amount absorbed by even the strongest treated effluent, whilst in 6 hours the same ratio generally holds although at this point the weakest sewage had absorbed six times as much oxygen as the strongest effluent. It is possible then that the careful following of the rate of oxygen absorption over a period as short as 6 hours might give something more than an approximate idea of the condition of the sewage, especially when a number of manometers are used.

Comparison of manometric and dissolved oxygen (B.O.D.) methods.

Before using the manometric method to any great extent either in research or in routine analytical work it is advisable to compare the oxygen absorption values obtained by the manometric and by the dissolved oxygen (B.O.D.) methods. For this purpose a number of sewages and effluents have been examined simultaneously by the two methods, the incubation being at the same temperature, viz. 22°. In the tests for B.O.D. care was taken to ensure that a satisfactory dilution was used, the sewage usually being incubated at two different dilutions. Estimations by both methods were made in duplicate, the illustrative examples being taken from experiments where duplicates were in close agreement. The results are shown in Table VI.

Table VI. *Comparison of manometric and B.O.D. values for various sewages.*

Manometric method (M.) as in previous table but expressed in parts per 10⁵. B.O.D. values obtained by usual method. Temperature 22°.

Sample no.	1		2		3		4		5 days	
	M.	B.O.D.	M.	B.O.D.	M.	B.O.D.	M.	B.O.D.	M.	B.O.D.
1	31.2	28.2	44.6	35.5	59.8	44.6	—	—	84	65
2	16.2	14.5	25.8	19.0	32.9	22.3	—	—	41	29
3	12.7	3.9	16.5	9.9	20.9	13.4	28.4	18.9	35	21
4	17.3	6.2	20.9	10.7	24.8	12.0	27.0	15.6	29	18
5	7.6	2.6	10.6	4.9	12.6	5.9	14.0	7.0	16	8
6	2.4	1.4	3.7	2.3	4.6	2.7	5.3	3.1	5.7	3.4
7	1.5	0.5	2.1	1.4	3.0	1.8	3.7	2.1	4.6	3.4
8	1.0	0.9	1.5	1.6	1.9	1.7	—	—	3.5	3
9	1.0	0.4	1.3	1.0	1.9	1.4	2.8	1.9	4.8	2.6
10	1.2	0.8	2.4	1.4	2.8	1.5	3.1	1.9	3.6	2.1
11	0.5	0.7	0.9	0.8	1.3	0.8	—	—	3.1	2.0
12	0.6	0.3	0.9	0.6	1.7	1.5	2.0	1.7	2.6	1.9
13	0.7	0	0.8	0.7	1.1	0.7	1.6	0.8	2.5	1.0
14	0.5	0.2	1.0	0.5	1.3	0.5	1.7	0.6	2.8	0.8
15	0.7	0.1	1.0	0.2	1.3	0.3	1.5	0.7	2.2	0.8

It will be noticed that in every instance the oxygen absorption values obtained by the manometric method are greater than the corresponding results obtained by the B.O.D. method; in many instances the B.O.D. value is about a third less than the manometric figure but comparison of the values for many sewages shows that no definite ratio between the two values appears to exist. A result such as this is to be expected since the conditions of the two methods are by no

means identical. In the manometric method the fluid is kept saturated with oxygen and so a more rapid oxidation might be expected than with a method wherein the tension of dissolved oxygen falls continuously as the period of examination extends. Furthermore the actual concentrations of the sewage are usually quite different in the two methods and carbon dioxide is continuously absorbed in the one method and not in the other. With such differences it is possible that dissimilar flora would develop in a period of 5 days.

Table VII. *Oxygen absorption by a sewage at different dilutions.*

Results expressed in $\mu\text{l.}$ at N.T.P. per ml. C.S. Conditions as in previous tables.

Sewage dilution %	Hours									
	1	2	5½	16	24	44	64	92	112	120
100	15	27.5	60	98	124	163	187	213	236	240
50	7.1	13.9	22	45.4	59	77	90	104	120	122
20	1.5	4.1	8.3	14.5	22.3	32.1	36	42.4	46	47
10	0.2	1.0	2.7	6.5	9.0	15	18.3	22	22	23

DISCUSSION.

Whilst it has been shown that oxygen absorption by sewage and sewage effluents can be followed manometrically, there remains to be considered the advisability of employing this method when so much previous work has been done with the test for biochemical oxygen demand in 5 days as the standard of reference. It is perhaps unfortunate that the manometric method furnishes results somewhat higher than those obtained by the older method and it is certain that before the new method can be generally applied, *e.g.* in testing whether an effluent is satisfactory, it will be necessary to make an exhaustive comparison of the values obtained by the two methods. Such a comparison might show that contrary to the deduction we have made from the few results given in this paper, a sufficiently satisfactory conversion of one set of results into the other can in fact be secured by employing some simple factor. In any case it is probable that a much shorter period of examination than five days may allow a satisfactory judgment of a sewage or an effluent to be formed using the manometric method. Although we have not carried out a sufficiently extensive examination, we would nevertheless suggest tentatively that a good effluent might be classified as one which does not give an oxygen absorption value as great as one part per 10^5 parts of effluent after shaking for a period of 24 hours in a Barcroft manometer at 22° . This conclusion is suggested by the results given in Table VI, where it will be noticed that effluents that show values by the ordinary B.O.D. test of less than 2 parts per 10^5 fall into this category. We are inclined to think, however, that were this method further explored a more definite standard of reference for judging effluents would be arrived at and one which might require a considerably shorter period, say 6 hours. The manometric technique however suffers from certain drawbacks when the examination is extended over very long periods. The most troublesome of these is that after several days drops of moisture may be deposited in the manometric tubes, especially if the latter are at a temperature considerably below that of the cups. In any case experiments should always be made at least in duplicate and when quantitative comparisons are desired a larger number of determinations is necessary. If after further experiment the method should prove of use in analytical work then the manometric scales could be calibrated and marked to read directly in parts of oxygen absorbed per 10^5

parts of the sewage provided that the temperature and the amount of sewage placed in the cups are standardised for all determinations. For the examination of effluents it might prove an advantage to use quantities larger than 3 ml. with or without a modification of the size of the cups. In any event the technique of the method and the determination of the constants for the apparatus, as described by Dixon [1934], are generally applicable. One advantage which the manometric method has over the ordinary B.O.D. test is that the sewages and effluents can be examined without the great dilution which is often otherwise necessary when examining a strong sewage by the B.O.D. test. Further with the B.O.D. test the sewage is often made up in two dilutions and then it is frequently found, even when each bottle has excess of dissolved oxygen at the end of 5 days that the final B.O.D. values are considerably different. With the manometric methods this dilution error, which may be dependent on the oxygen tension, is largely avoided since the fluid is fully saturated with oxygen throughout the experiment and the sewage is never diluted more than 1 in 3. Even if samples of the same sewage are used at widely different dilutions experiment has shown that the oxygen absorptions measured manometrically are near the values expected (see Table VII).

For the purposes of research into the mechanism of sewage purification processes however the manometric method has several important advantages over the B.O.D. test. An obvious advantage is that much smaller quantities of sewage can be examined. Further by this method the process of oxygen absorption can be followed continuously and the effect of an alteration in experimental conditions, such as change in temperature, the addition of some reagent *etc.*, can be readily ascertained. In this way considerable saving in time is effected. Thus substances may be added to the sewage that could not be used before since they interfered with the reagents employed in the subsequent determination of the dissolved oxygen. The fact that each manometer possesses two cups enables a condition to be varied on one side and not on the other, when its effect, if any, will be shown by the manometric reading. In this way, for example, we may investigate amongst other things, the effect of the addition of activated sludge to sewage. In fact when we have, say, 1 ml. of a sludge suspension and 2 ml. of sewage in the cup of a manometer, we have under fairly well controlled conditions, a system that is akin to the "activated sludge process" in miniature, for the presence of excess air and the shaking of the apparatus together form ideal conditions for such purification to take place. For this reason, among the others we have discussed, we have adopted this technique as a serviceable method for investigating sewage purification.

SUMMARY.

1. It is shown that the oxygen absorption from air by sewage and sewage effluents can be followed manometrically using the Barcroft differential manometer.

2. The oxygen absorption for any particular sewage is usually greater when measured manometrically than when it is estimated by the usual test for biochemical oxygen demand.

3. Under the aerobic conditions of the Barcroft apparatus such gases as hydrogen, methane, hydrogen sulphide and nitrogen do not appear to be liberated during oxidation of ordinary sewage systems.

4. The reasons for adopting the manometric method are discussed, and tentative suggestions are made for its use in routine sewage analysis.

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