THE BACTERIAL PURIFICATION OF GASWORKS' LIQUORS. THE ACTION OF THE LIQUORS ON THE BACTERIAL FLORA OF SEWAGE.

By FRANK CHARLES HAPPOLD and ARTHUR KEY.

(*From the Department of Pathology and Bacteriology, and the Department of Coal Gas and Fuel Industries, the University of Leeds.*)

For some time it has been a common practice to purify the spent liquor resulting from the recovery of ammonia from gasworks' ammonia liquor by methods which involve the principle of mixing the liquor with sewage and allowing the mixture to percolate through bacteria beds. In the majority of cases the purification takes place at the local sewage treatment works, the spent liquor being mixed with the whole of the town's sewage, amounting, on the average, to about 200 times the volume of the liquor. This is a satisfactory way of purifying the liquor, and provided the sewage works is of sufficient capacity, little difficulty is experienced. In some cases, however, the sewage works is not of a sufficient size to be able to deal with the spent liquor without prior purification. In a few cases, in order to effect this, percolating filters have been installed at the gasworks, as, for instance, at Coventry, where the present work had its origin. By the term "purify," gas engineers have largely envisaged the removal of phenolic and other bodies, which, when discharged into streams, might render them poisonous to fish life, either through the removal of dissolved oxygen, or because of a direct toxic action.

It is known that the phenolic bodies in spent gasworks' liquor are gradually destroyed when the liquor is mixed with sewage and passed through percolating filters. Fowler, Arden and Lockett (1910) isolated from bacteria beds so treated two organisms, one of which, *Bacillus helvolus*, they stated caused the oxidation of phenol. The organism was a Gram-negative bacillus which would not grow at temperatures above 30° C. They considered that the purification in the beds was due to a bacteriological oxidation. Harowitz Wlassowa (1930) suggested that this decomposition of phenol in waste waters is not a biological process, but takes place by a purely chemical process of oxidation if the liquid is aerated in the presence of wood carbon. Our own view based on previous experience (Happold and Raper, 1925) is that, while this might be so with phenolic substances having two hydroxy groups in the ortho position, it is unlikely with monohydric phenols unless the system contains ferrous salts and can produce hydrogen peroxide (Martinon, 1885).

In a previous communication (Happold, 1930) it was shown (*a*) that, amongst a large number of oxidase containing bacteria, *Vibrio tyrosinatica*

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(Beijerinck) was the only one which possessed tyrosinase, and which consequently catalysed the oxidation of monohydric phenols, with subsequent formation of more complex compounds; (*b*) that the similar oxidation of catechol was catalysed by all oxidase containing organisms, but that oxidation could also take place in sterile media if the reactions were sufficiently alkaline.

Since *V. tyrosinatica* was actually isolated from the sewage effluents of Delft[1], it was felt that this type of oxidation, followed as it is by some process of polymerisation, might account for the purification of spent liquor in bacteria beds, especially since the effluent from the Coventry beds was of the same reddish brown to black hue as is the product of the tyrosinase oxidation of phenolic bodies. If bacteria containing tyrosinase were found to be absent from the beds, it was felt that the destruction of phenols would have to be looked for in the catabolic activities of bacteria whose mode of action would be quite different from the well-recognised type of oxidation described by Raper and his colleagues. Accordingly an investigation was commenced of the bacteriology of the sewage, the liquor fed to the beds, and the effluent therefrom, at the Coventry gasworks. It was hoped to determine whether the removal of phenolic substances was a true biological process or not, by correlating the bacteriological findings with the efficiency of working of the beds.

The bacteria beds at the Coventry gasworks.

There are four beds of the revolving sprinkler type, the total volume of filtering material being 7700 cubic yards. The amount of spent liquor normally dealt with corresponds with about 5 gallons per cubic yard per day, but since recirculation is employed the actual rate of feed to the beds is much greater than this, approximating to 120 gallons per cubic yard per day. The method of working is illustrated from the following example. The effluent from the beds had an oxygen absorption value, given by the 4 hours' permanganate test, of 100 parts per 100,000. A small part of this was admitted to the sewers, and the remainder made up to its original volume with spent liquor from the still. This had an oxygen absorption value of 900, and that of the effluent was thereby increased from 100 to 115. This liquor was passed through the beds and emerged with its oxygen absorption value again reduced to 100. The process was repeated. Thus spent liquor of an oxygen absorption of 900 was continually added to the system, and purified liquor of oxygen absorption 100 was removed, the net purification being 89 per cent. A quantity of sewage was also continually added to the system in order to maintain the life in the beds, and the diluting effect of this sewage has been allowed for in all calculations given in this paper.

Average compositions of the liquors treated during the three periods which have been considered are given in Table I. In the first period the liquor contained little ammonia, but excess of lime; in the second, fixed ammonia, mainly chloride, was present, but no lime; and from October 28th, 1931, on-

[1] This organism was kindly supplied by Professor Kluyver, of Delft.

wards, crude ammonia liquor, containing both free and fixed ammonia, also sulphides, was treated.

Table I. *Composition of liquors treated.*

	July 1930–Apr. 1931	Apr. 1931–Oct. 1931	Oct. 1931–Apr. 1932
	grm./100 c.c.		
Ammonia, free	—	—	1·40
,, fixed	—	0·400	0·40
,, total	0·009	0·400	1·80
Sulphide, as H_2S	Nil	Nil	0·22
Thiosulphate, as S	0·064	0·060	0·061
Thiocyanate, as CNS	0·128	0·170	0·193
Phenols, as C_6H_5OH	0·243	0·280	0·366
Higher tar acids, as catechol	0·100	0·110	0·12
Oxygen absorption	4hr., 27° C. (pts per 100,000)		
Due to phenols	433	500	652
thiosulphates	55	51	52
thiocyanates	106	140	160
sulphides	—	—	298
difference (mainly higher tar acids)	226	300	378
Totals	820	991	1540

In the first half of Table II the performance of the beds at the time of sampling is recorded. The first column gives the number and the second the date of the experiment; the third the amount of liquor being purified per hour; the fourth gives the oxygen absorption of the liquor; and the fifth the percentage purification obtained, allowing for the diluting effect of the sewage supplied to the beds. It is not the custom in gasworks to run the ammonia recovery plant continuously, but for periods of about a month, interspersed with intervals of a similar length. The beds therefore usually worked for a month or so, and were then rested, or treated with sewage only for a further period. The efficiency of the beds usually decreased slowly as the run progressed, and the duration of the run at the time of sampling is recorded in the remarks column of Table II. In the case of the samples taken during the third period the ammonia content of the liquor as fed to the beds is also given.

Bacteriological examination.

All samples received from Coventry have been immediately plated at different dilutions, incubated at 37° C. for 2 days, and then subjected to a direct count. After incubation a representative portion of the plate was treated with 1 per cent. dimethylphenylenediamine hydrochloride solution, and a selection of oxidase reacting and non-reacting colonies rapidly made. These were immediately sub-cultured on to fresh media and incubated. The plates were then momentarily flooded with the dimethyl reagent, the latter drained away, and a second count of oxidase reacting colonies now made. The isolated strains after sub-culture were examined further and typed, and their individual action on phenol and catechol studied.

Table II.

Sample no.	Date	Liquor purification per hour (gals.)	Oxygen absorption liquor	Purification %	Remarks	Bacterial counts (in thousands per c.c.) Sewage: Oxidase containing bacteria	Sewage: Non-oxidase	Effluent* from beds: Oxidase containing bacteria	Effluent* from beds: Non-oxidase	Notes
1	July 20th, 1930	1600	803	92	20 days after beginning of very good run	2	998	770	193	—
2	Oct. 14th, 1930	1600	734	82	2 days after start of run	2400	2420	11900	930	—
3	Oct. 23rd, 1930	1600	710	85	11 days after start of run	38	3162	795	341	—
4	Nov. 17th, 1930	1600	876	69	35 days after start of run	3700	11500	7650	1420	—
5	Jan. 13th, 1931	1600	741	54	34 days after start of run	380	4660	572	64	—
6	Feb. 10th, 1931	1600	761	70	7 days after start of run	8	3592	1780	1190	—
7	Mar. 20th, 1931	1600	753	64	7 days after start of run	12	5988	1520	19	—
8	Apr. 29th, 1931	1800	1109	49	45 days after start of run, unlimed spent liquor	340	4720	155	Nil	Effluent slightly acid
9	Sept. 23rd, 1931	1800	957	81	16 days after start of run, unlimed spent liquor	960	1700	6200	8700	
10	Nov. 2nd, 1931	500	1483	79	Crude liquor. Free NH_3 0·144 grm./100	180	940	880	270	Effluent pH = 8·5
11	Nov. 9th, 1931	800	1452	56	Crude liquor. Free NH_3 0·289 grm./100	132	648	sterile		Effluent over pH 9·0
12	Jan. 27th, 1932	800	1400	27	Crude liquor. Free NH_3 0·348 grm./100	180	700	sterile		Effluent over pH 9·0

* The counts given for the feed and effluent liquors are not the actual results obtained. These have been multiplied by a factor (which is not the same for all samples) in order to allow for the diluting effect of the spent liquor on the bacteria of the sewage. This enables the effluent counts to be compared directly with the sewage.

The count of oxidase containing and oxidase non-containing bacteria obtained with the samples of sewage and the effluent from the beds are shown in the second half of Table II. Since the bacterial flora of the effluent from the beds had its origin in the sewage added, a comparison of the two gives a long-term picture of the resulting action of gasworks liquors, under the relative aerobic conditions of the beds, upon the original bacterial flora.

It should be mentioned that a series of cultures was made at 22° C., and that the results differed in no appreciable particular from those obtained at 37° C.

In comparing the counts of oxidase deficient bacteria in the sewage and the effluent, it will be noticed that that of the effluent was much less than that of the sewage, with one exception, no. 9, which was the only representative of a run conducted entirely with unlimed spent liquor. Evidently, for many such bacteria, gasworks liquor, spent and crude, exerts a distinct toxic action.

A different state of affairs was revealed by a study of the count of oxidase containing bacteria. In every case but one when spent liquor was treated, the effluent gave a larger oxidase positive count than the sewage. The exception was no. 8, which was the only sample to show a slightly acid reaction. This generalisation suggests either that some of the bacteria containing an oxidase system are playing a definite rôle in the purification process or that they develop most rapidly under conditions favourable to purification. A similar large development of oxidase positive types was observed when sewage plus 1/25th of its volume of spent liquor containing lime was incubated in the laboratory.

A further connection can be seen on comparing the counts of oxidase containing bacteria with the efficiency of the beds as expressed by the purification obtained. Samples taken when the purification was high showed either a large count of oxidase containing bacteria, or a large increase in this over the content in the sewage. Conversely, it will be seen that cases 8, 11 and 12, where the purification effected by the beds is at a minimum, were the only ones where the counts of oxidase containing bacteria in the effluents from the beds were less than the corresponding counts in the sewage.

Since 6 hours elapsed between the taking of the samples and the commencement of their examination, and since the samples were sent in tightly corked bottles, it was felt that considerable change might occur in the bacterial flora during transit. Consequently, on arrival, samples of sewage were mixed with 4 per cent. of their volume of spent liquor. These mixtures, and also samples of sewage alone, were sub-divided, one portion of each being sealed under vaseline seal for 24 hours at laboratory temperatures, and the other exposed in a shallow layer to the atmosphere. The following results, for instance, were obtained with sample 9:

Mixture	Total count (thousands per c.c.)	Oxidase positive (thousands per c.c.)
Sewage under seal	18,000	9,000
„ aerated	8,000	4,000
Sewage + spent liquor under seal	4,400	1,500
„ + „ „ aerated	20,000	15,000

It will be seen that while the sewage gave the greater count under anaerobic conditions (this is not invariably the case), the mixture with spent liquor gave the greater total and oxidase counts under aerobic conditions. These experiments can reassure us in the belief that the preponderance of oxidase positive colonies in the effluent from the beds was not caused by any change in transit.

Experiments in which sewage and effluent from the beds were treated with phenol and catechol solutions in the presence and absence of lime, and under aerobic and anaerobic conditions, showed that these two substances were more toxic to the bacterial population under anaerobic than under aerobic conditions. Table III gives an example.

Table III.

Mixture	Total count (thousands per c.c.)	Oxidase positive (thousands per c.c.)
Sewage, aerobic	12,000	8,000
,, anaerobic	12,000	6,000
Sewage + 0·02 % phenol, aerobic	6,500	5,200
,, ,, ,, anaerobic	340	68
Sewage + phenol + lime, aerobic	12,000	4,000
,, ,, ,, anaerobic	180	1
Sewage + 0·02 % catechol, aerobic	2,360	90
,, ,, ,, anaerobic	240	11
Sewage + catechol + lime, aerobic	3,900	572
,, ,, ,, anaerobic	2,960	2

An effluent from the beds gave similar results with corresponding mixtures.

The action of gasworks' ammonia liquor on the bacterial flora of sewage.

From 28th October, 1931 onwards, crude gasworks' liquor containing ammonia was substituted for the spent liquor. This liquor is strongly alkaline, and has doubtless accounted for the highly alkaline reaction of the effluent from the beds during this period. After November, 1931, the *p*H of the effluent was in excess of 9·5. The bacterial counts (Exps. 10, 11 and 12, Table II) showed that the liquor in such concentrations exerted a sterilising action, and this was also demonstrated in the laboratory with fresh sewage under both aerobic and anaerobic conditions. The sterilising action might be accounted for by the marked alkalinity of the liquor, due to the relatively high concentration of free ammonia, as indicated in Table II. The very much smaller purification effected by the beds under these more nearly sterile conditions is noteworthy.

It seems safe to conclude from the foregoing experiments that not only is the removal of phenolic and other bodies from gasworks' liquors due to the action of bacteria, but that the bacteria concerned are more likely to be found amongst those which possess an oxidase system.

The catabolic action of individual organisms on phenols.

Throughout the course of this investigation a wide sampling of the bacterial flora of the sewage and the effluent liquors has been made, and the biochemical properties of the individual strains studied. No organism analogous to *Vibrio*

tyrosinatica has been isolated, and whilst we do not feel justified in stating that none of the strains possess tyrosinase, we have not succeeded in demonstrating the presence of this enzyme in any of the strains up to the present. Of a large number of organisms isolated from the effluent liquor only one has been proved capable of utilising monophenols as a source of carbon. This organism contains an oxidase system, it is without action on any of the common sugars, and it fails to liquefy gelatine. It appears to be a gram negative vibrio, not unlike *V. tyrosinatica* in its morphological appearance, and it was actually the first organism containing an oxidase system isolated from the effluent liquor (strain O 1). At least 80 per cent. of the oxidase positive group isolated from the effluent liquor examined in the first experiment was of this type, and it has been the most constantly isolated strain ever since.

This strain (O 1) will grow and multiply in a medium containing 0·1 grm. $(NH_4)_2SO_4$, 0·1 grm. K_3PO_4, 0·5 grm. $MgCO_3$, and 0·4 grm. phenol per litre. 95 per cent. of the phenol is utilised in 10 days, and a viable count showed that growth runs parallel to phenol utilisation. If the phenol is omitted from the medium, demonstrable growth is absent. No other strain isolated was found to be capable of multiplying in this medium, nor was *V. tyrosinatica*. Ordinary bouillon containing 0·04 per cent. phenol allows many of the isolated strains to multiply, but the phenol is not attacked save with strain O 1.

It appears probable that at Coventry at least the removal of monohydric phenols in the bacteria beds depends on the presence of this vibrio. Another vibrio has been found containing an oxidase system, which can only be differentiated from strain O 1 by its inability to utilise phenols. It is important that a method for distinguishing these two strains should be discovered.

SUMMARY.

A study has been made of the bacterial flora in the sewage supplied to, and the effluent from, the bacteria beds which deal with the liquors produced at the Coventry gasworks. A high count in the effluent from the beds of bacteria containing an oxidase system, or a relatively large increase in the viability of such organisms when compared with the original sewage, has been found to be concurrent with good purification in the beds, and *vice versa*.

The bacterial flora undergoes considerable modification in the beds, and also when mixed with 4 per cent. of its volume of spent liquor in the laboratory. The total viable count of the treated sewage is reduced under these conditions, but the count of those bacteria which give the direct oxidase reaction and therefore catalyse the oxidation of catechol is relatively increased.

A gram negative vibrio (strain O 1) is the only bacterium isolated which has proved capable of breaking down monohydric phenols. It has been constantly isolated from the effluent liquors.

Gasworks' ammonia liquor in the relatively high concentrations used exerts a markedly toxic action on all types of sewage bacteria, and the purification

of this liquor by the beds is not so satisfactory. The toxicity is probably caused by the highly alkaline reaction of the liquor.

This investigation was commenced by one of us (F. C. H.) in conjunction with Dr Allan C. Monkhouse, who was at the time Research Chemist to the Liquor Effluents Sub-Committee of the Institution of Gas Engineers. Dr Monkhouse accepted another appointment shortly after the commencement of the work, and was therefore unable to assist further, but we should like to express our indebtedness to him during the initial period. We are also indebted to Prof. J. W. McLeod and Prof. J. W. Cobb for their kindly interest and encouragement, and to Mr P. N. Langford, Engineer and Manager of the Coventry Gas Department, for the facilities he so generously placed at our disposal at Coventry.

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REFERENCES.

FOWLER, ARDEN and LOCKETT (1910–11). *Proc. Roy. Soc.* B, **83**, 149.
HAPPOLD (1930). *Biochem. J.* **24**, 1737.
HAPPOLD and RAPER (1925). *Biochem. J.* **19**, 92.
HAROWITZ WLASSOWA (1930). *Gas u. Wasserfach*, **73**, 275.
MARTINON (1885). *Bull. Soc. Chim.* (2), **43**, 155.

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