EFFECT OF CONTINUOUS AERATION ON BACTERIAL OXIDATION OF ORGANIC MATTER

By L. A. ALLEN and G. E. EDEN
Water Pollution Research Laboratory, Langley Road, Watford

(With 3 Figures in the Text)

The changes produced by anaerobic growth of bacteria in a simple carbohydrate medium are those resulting from reactions of the hydrolytic oxidation-reduction type, the products of fermentation commonly including volatile and non-volatile acids, neutral volatile compounds, and gases. Thus Harden (1901) showed that fermentation of glucose, fructose, galactose, or arabinose by *Bacterium coli* yielded lactic, acetic, and succinic acids, alcohol, carbon dioxide and hydrogen. Organisms growing on a carbohydrate medium aerobically, on the other hand, derive their energy from direct oxidation of the substrate by molecular oxygen, accompanied by the output of carbon dioxide.

The differences between the changes produced by anaerobic and by aerobic growth of bacteria have important applications in the disposal of waste waters. The polluting strength of a liquid and the ease with which it may be treated are largely dependent on the content and character of the organic matter it contains. One method of reducing the content of organic matter is to allow the liquor to undergo extensive bacterial decomposition, the nature of the substrate largely deciding whether this should take place anaerobically or aerobically. Under anaerobic conditions this result may be achieved, for example, by the loss of CO_2 resulting from the fermentation of carbohydrates, or of methane from the decomposition of cellulose. The remaining products of anaerobic metabolism tend in some liquors, however, not only to be acidic and evil-smelling in character but to impose a definite limit to the course of decomposition. It was shown, for example, by Verzár, Nábrásky and Szányi (quoted by Stephenson & Whetham, 1924) that *Bact. coli* ceases to break down glucose at or below a *p*H value of 4·08, and this limit may be quickly reached in some media under anaerobic conditions by the accumulation of metabolic acids.

Under aerobic conditions reduction in the content of organic matter is accomplished by its oxidation to CO_2 by atmospheric oxygen. Stephenson & Whetham (1924), studying the effect of oxygen supply on the metabolism of *Bact. coli*, observed that with an increase in the oxygen supply the formation of CO_2 was greater, the appearance of the limiting *p*H value was deferred, and oxidation therefore continued for a longer time.

It is important to compare the extent to which aeration will purify a liquor by bacterial oxidation of the organic matter with the corresponding changes produced by anaerobic decomposition. If the degree of purification effected in the waste liquor from a given process were sufficient to enable the liquor to be re-used in the same process, the problem of disposal would be obviated and there would be considerable economy in the consumption of water.

The work described in this paper was prompted, when investigating possible methods of reducing the polluting strength of waste waters from the retting of flax, by the observation that, although an infusion of flax which was allowed to remain stagnant rapidly became acidic in character, a similar infusion through which a current of air was passed tended to become alkaline, and its polluting strength after 3 or 4 days was appreciably less than that of the stagnant liquor. This supported the claims of Carter (1919) and of Ruschmann (1922) that the liquor from the Rossi (1908) process of aerobic retting was less acidic and less offensive in character than that from anaerobic retting.

EXPERIMENTAL

The effect of aeration on bacterial growth and on the chemical changes produced in the medium was followed in a series of experiments in which an infusion of flax in one bottle received a continuous current of air, and that in a similar bottle remained quiescent. For experiments with pure cultures a sterilized infusion was used and aseptic precautions were observed. Changes induced in the liquor by bacterial action were assessed by a chemical examination of the contents of each bottle and by determination of the CO_2 evolved by respiration.

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Apparatus for following metabolic changes

The arrangement of the culture bottle and absorption tubes, both for the aerated and for the quiescent culture, are shown in Fig. 1. The two bottles *E* and *N*, each containing a measured quantity (usually 950 ml.) of flax infusion, were immersed in water in a thermostat at 30° C. A current of air, freed from carbon dioxide by passage through tube *A*, which contained soda-lime, was drawn through the liquid in *E* by means of a vacuum pump. The rate of aeration was controlled by means of the tap *M* and varied in different experiments from about 3 to about 5 bubbles a second. The air leaving the bottle was passed through an absorption tube *G* containing strong sulphuric acid, three absorption tubes *H*, *J*, *K*, each containing 25 ml. of 2*N* NaOH to absorb carbon dioxide evolved by respiration, and one tube *L* containing baryta. The last was a safeguard against overloading the absorption tubes, since turbidity indicated that absorption of CO_2 was not complete.

When aseptic conditions were required the air was sterilized by being passed through tube *B*, which was about 1 ft. in length and was packed with sterile cotton-wool, and through the absorption tube *C* containing 3% mercuric chloride. Empty tubes *D* and *F* were included, one on either side of the culture bottle, and cotton-wool plugs were inserted in the leading tubes at *a*, *b*, *c* and *d*. The portion of the train comprising *B*, *C*, *D* and *F*, together with their connexions and the bung and leading tubes from the bottle *E*, were sterilized in the autoclave before assembly. After assembly of the train the bungs and glass-rubber connexions were coated with molten paraffin wax as a precaution against leakage.

The bottle containing quiescent liquor was fitted similarly with a bung and leading tubes, the exit tube being attached to an absorption train to retain CO_2, with a guard tube of soda-lime at the end. The inlet tube to the bottle was attached to a glass tap *T*. At the end of the experiment this tap, which had remained closed throughout, was opened and was attached to the soda-lime, cotton-wool, and mercuric chloride tubes, which were disconnected for the purpose from the aerated bottle. A slow stream of CO_2-free air was then drawn for about an hour through the liquid in the bottle to carry over to the absorption tubes any CO_2 which had been formed and which had remained in solution or in the space above the liquor. When working with pure cultures precautions similar to those described for the aerated bottle were adopted, and cotton-wool plugs were inserted at *e*, *f* and *g*.

When samples of the liquor were required for bacteriological examination at intervals during the experiment the device *P*, which was described by Beesley (1914), was inserted into the bung of each bottle before sterilization and assembly. By manipulation of the tap *R*, a sample of the liquid could be drawn into the bulb *S* and withdrawn through *T* into a sterile test-tube under aseptic conditions.

Preparation of flax infusion

The flax infusion used in the experiments was prepared by immersing 450 g. of flax (with the stems upright) in 4½ l. of water in a 5 l. beaker for 4–5 hr. at 30° C., followed by 14–18 hr. in the ice-chest. The clear yellow liquid was then decanted from the flax and filtered through cotton-wool. For some experiments the undiluted infusion was used; for others it was diluted with an equal quantity of water. In some experiments the *p*H value of the infusion was adjusted to neutrality. The infusion was distributed in quantities of 950 ml. in bottles of a capacity of about 1150 ml. These were sterilized (when sterile infusion was required) in the autoclave at 15 lb. pressure for 20 min.

Chemical methods

At the close of an experiment the contents of each bottle were made up to 1 l. with CO_2-free water, and the results of chemical examination were compared with those of a similar examination of the unused liquid. The biochemical oxygen demand and the amount of oxygen absorbed from acid permanganate in 4 hr. at 26·7° C. were determined by methods outlined by the Ministry of Health (1942); *p*H values were determined either colorimetrically or electrometrically. CO_2 in the absorption tubes was determined by making the combined contents up to a measured volume and titrating an aliquot portion with standard hydrochloric acid to phenolphthalein in the presence of an excess of barium chloride. CO_2 in solution in the infusion was determined by addition of a slight excess of dilute sulphuric acid to a measured volume of the liquid in a flask (fitted with a reflux condenser) through which CO_2-free air was drawn and which was attached to a train of absorption tubes containing *N*/6 baryta. The liquid was raised just to the boiling-point, the current of air was passed for 30 min., and the baryta was then titrated with *N*/15 hydrochloric acid. A measure of the volatile acids in the infusion was obtained by distilling at constant volume an aliquot portion of the liquor which had been acidified with sulphuric acid, and titrating successive portions of 100 ml. of the distillate with standard baryta until a small constant value was obtained. The total titration value of the distillate was recorded.

The volatile neutral fraction of the metabolic products was obtained by the method used by

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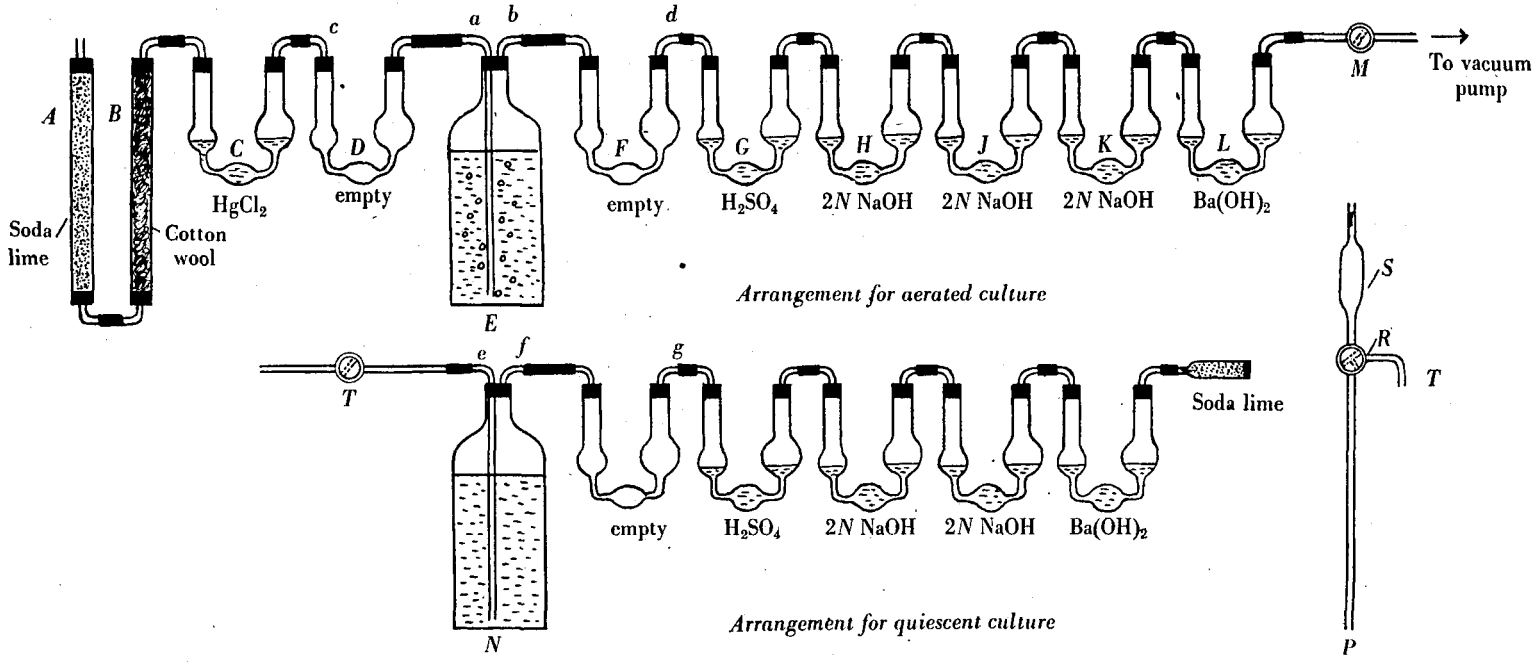

Fig. 1. Apparatus for studying the effect of aeration on changes produced by bacteria in flax infusion and other liquors.

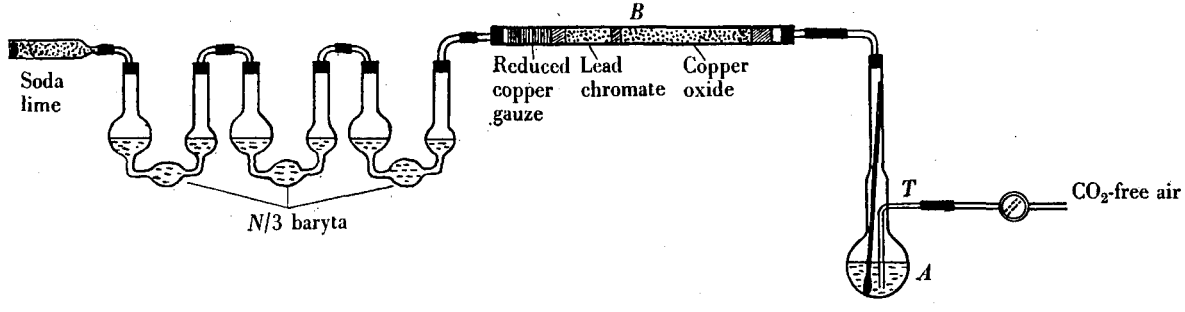

Fig. 2. Apparatus for determination of organic carbon.

Raistrick and others (1931). The distillate from 250 ml. of culture, which had been first neutralized to *p*H 7·0, was collected in a cylinder cooled in ice, the distillation was stopped when two-thirds of the liquid had passed over, the volume of the distillate was made up to 250 ml., and the carbon content was determined.

Organic carbon was determined by means of the apparatus shown in Fig. 2, that used by Mills for sewage (1931) being modified by the inclusion of a combustion tube *B*, containing copper oxide, lead chromate, and reduced copper gauze, which was heated to a dull red heat by a burner placed under the copper oxide. This was rendered necessary by the presence of volatile compounds and of oxalates in the samples of liquor. Gases issuing from the combustion tube were passed through three absorption tubes each containing 25 ml. of *N*/3 baryta. An aliquot portion of flax liquor, usually 40 ml., was transferred to flask *A*, and 150 ml. of strong sulphuric acid were run carefully underneath so as not to mix the two liquids; 10 ml. of a saturated solution of chromium trioxide were then added, a thermometer was placed inside the flask, and the stopper was immediately inserted. The combustion flask was heated by a micro-burner until a brisk evolution of gas occurred, and the flame was adjusted so that the current of gas did not overload the absorption tubes. The temperature of the liquid in *A* was then maintained steadily at 145–155° C. for 2 hr., when the heating was stopped and a slow current of CO_2-free air was admitted for half an hour through the tube *T* to carry over to the absorption tubes any residual CO_2 in the flask and combustion tube. CO_2 absorbed in the baryta tubes was estimated by titration with *N*/6 HCl, correction being applied for the CO_2 evolved in a blank experiment with all the reagents except the flax liquor. The value for dissolved CO_2, determined separately on another portion of the liquor, was subtracted from the total value found after combustion, thus giving the amount of CO_2 derived by oxidation of the organic carbon. The method was shown to be accurate in preliminary tests on solutions containing known amounts of sucrose, acetic acid, potassium tetroxalate, and sulphanilic acid.

Sources and characters of cultures

The pure cultures used were isolated in the laboratory from samples of retting liquor, with the exception of the strain of *Bact. coli* type I, used in the experiment recorded in Table 1, which was isolated from faeces. Coliform bacteria were classified by their reactions to the indol, methyl red, Voges-Proskauer, and Koser's citrate tests, and by the tests for liquefaction of gelatin and for ability to grow in MacConkey broth at 44° C. The streptococci were strains which produced no change in litmus milk and the growth of which was stimulated by yeast extract. Strains I and III slowly formed acid in milk containing yeast extract and rapidly clotted milk, to which both glucose and yeast extract were added. Strain II produced no change in the former medium, but rapidly clotted the latter. The strain of *Bacillus subtilis* was one which had been found to cause soft rot of potato and the retting of flax (Allen, 1944). The *Achromobacterium* species was typical of the group of Gram-negative, non-sporing rods commonly found in large numbers in retting liquor and characterized by their biochemical inactivity in carbohydrate media. This strain produced little or no change in glucose broth, did not form indol or reduce nitrates, and only slowly liquefied gelatin.

Effect of aeration on bacterial growth and on change in pH value

In the first series of experiments changes in bacterial counts and in *p*H values of the cultures were followed by withdrawing samples under aseptic conditions at intervals during the period of incubation. Tests were made in sterile flax infusion with pure cultures of *Bact. aerogenes*, *Bact. coli*, and two strains of streptococci, and in unsterilized infusion with the natural mixed flora of the flax. Results are shown in Table 1.

The *p*H value of the quiescent cultures decreased during the first 18–24 hr. from 6·8–7·0 to a value varying between 4·9 and 5·5 in different experiments. It then rose slightly and after 3–5 days the liquor was still distinctly acidic with a *p*H value of about 5·6. With the natural flora, with *Bact. aerogenes*, and with *Bact. coli*, aeration resulted in the development of marked alkalinity, which in the first two cases was preceded by the development of some acidity. This initial drop in the *p*H value of aerated cultures recalls the observation of Stephenson & Whetham (1924) that even under strongly aerobic conditions the initial stage in the breakdown of glucose by *Bact. coli* appeared to be anaerobic. With the cultures of streptococci the aerated liquor remained less acidic than the quiescent liquor, but did not become alkaline.

The plate counts suggest that aeration stimulated the growth of the mixed flora and of the two coliform strains. A similar result was recorded (Allen, 1940) for the effect of aeration on the growth of a non-sporing proteolytic bacterium in a casein medium, the plate count in the aerated medium increasing from 4280 to a maximum of 970 millions, while that in the quiescent medium increased only to a maximum of 24·5 millions per ml. It is possible that differences in the size of bacterial clumps may have been partly responsible. It is, however, well known that aeration greatly stimulates the growth of

yeast, and Kluyver, Donker & Hooft (1925) have suggested that this is due to the large amount of energy liberated when oxygen acts as the hydrogen acceptor.

Effect of aeration on chemical changes produced by bacterial growth

Two series of experiments were carried out to find the effect of aeration on the chemical changes resulting from bacterial growth. The first series was designed to compare the changes produced by different organisms under similar conditions and by the same organisms in infusions of different strengths. These results are shown in Tables 2 and 3. In the second series of similar experiments the organic carbon content of the infusion was determined before and after bacterial growth. In this way the percentage loss of organic carbon could be calculated and, by also determining the amount of carbon dioxide formed, a balance sheet for carbon could be made. Results of these experiments are shown in Table 4.

In general, aeration tended to produce an alkaline liquor in which the content of volatile acids, the value for oxygen absorbed from acid permanganate, and the biochemical oxygen demand, were appreciably less, and from which the amount of CO_2 evolved was appreciably greater, than the corresponding values for the quiescent liquor. The magnitude of these differences varied, however, according to the nature of the flora and with the strength of the liquor. Thus with the mixed flora, with *Bact. coli*, and with *Bact. aerogenes* in the two experiments with single-strength infusion (Tables 2 and 3), the *p*H values of the aerated cultures were 2·8–3·5 units higher than those of the quiescent cultures, and the amounts of volatile acids in the latter were appreciable and in the former very small. The loss of CO_2 (as mg. C per 100 ml.) from the

Table 1. *Effect of aeration on bacterial growth and on changes in pH value*

Natural flora of flax in unsterilized infusion					
Period of incubation (hr.)			0	24	47	72
Plate count on carrot agar (millions per ml.):						
Aerated culture			0·18	—	423	121
Quiescent culture			0·18	—	249	87
*p*H value:						
Aerated culture			7·0	6·2	7·1	8·4
Quiescent culture			7·0	4·9	5·5	5·8
Bact. aerogenes, type I, in sterilized infusion						
Period of incubation (hr.)	0	24	46	74	95	
Plate count on nutrient agar (millions per ml.):						
Aerated culture	0·82	163	1160	980	1150	
Quiescent culture	0·82	180	202	219	312	
*p*H value:						
Aerated culture	6·8	5·3	7·3	8·8	9·1	
Quiescent culture	6·8	5·2	5·4	5·6	5·6	
Bact. coli, type I, in sterilized infusion						
Period of incubation (hr.)			0	24	53	72
Plate count on nutrient agar (millions per ml.):						
Aerated culture			0·23	545	343	372
Quiescent culture			0·23	303	215	238
*p*H value:						
Aerated culture			6·8	7·8	8·5	8·8
Quiescent culture			6·8	5·3	5·4	5·6
Streptococcus, strain 1, in sterilized infusion						
Period of incubation (hr.)			0	24	72	120
*p*H value:						
Aerated culture			7·0	6·2	6·6	6·4
Quiescent culture			7·0	5·5	5·4	5·6
Streptococcus, strain 2, in sterilized infusion						
Period of incubation (hr.)	0	18	42	68	90	119
*p*H value:						
Aerated culture	6·8	5·4	5·6	5·8	5·8	5·8
Quiescent culture	6·8	5·0	5·0	5·3	5·4	5·7

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Table 2. *Effect of aeration on the changes produced in flax infusion by different organisms*

Period of incubation (hr.) ...	Natural flora of flax in unsterilized infusion 138			*Bact. coli*, type I, in sterilized infusion 94			*Achromobacterium* sp. in sterilized infusion 114		
	Fresh liquor	Aerated culture	Quiescent culture	Fresh liquor	Aerated culture	Quiescent culture	Fresh liquor	Aerated culture	Quiescent culture
CO_2 formed (as mg. C per 100 ml.)	—	46·7*	12·2*	—	39·0	9·6	—	4·2	0·4
*p*H value	—	8·7	5·6	8·4	9·9	6·6	6·4	6·7	5·3
Volatile acids (ml. *N*/10 per litre)	—	2·8	101·0	—	3·0	37·0	—	—	—
O_2 absorbed from acid $KMnO_4$ in 4 hr. (parts per 100,000)	84	40	55	101	55	63	118	115	111
Percentage reduction in O_2 absorbed	—	52	35	—	46	37	—	—	—
Biochemical oxygen demand (parts per 100,000)	73	6	80	80	47	58	—	61	64
Percentage reduction in Biochemical oxygen demand	—	92	+10	—	41	28	—	—	—

* Includes only CO_2 in absorption tubes. Small amount remaining in solution not determined.

Table 3. *Effect of aeration on the changes produced by* Bact. aerogenes, *type I, in sterilized flax infusion of different strengths, after different periods of aeration*

	Exp. I (91 hr.) Double-strength infusion			Exp. II (117 hr.) Single-strength infusion			Exp. III (139 hr.) Single-strength infusion		
	Fresh liquor	Aerated culture	Quiescent culture	Fresh liquor	Aerated culture	Quiescent culture	Fresh liquor	Aerated culture	Quiescent culture
CO_2 formed (as mg. C per 100 ml.)	—	—	—	—	33·1	13·1	—	32·6	10·9
*p*H value	7·0	7·6	6·4	6·8	9·1	6·3	6·8	9·3	5·8
Volatile acids (ml. *N*/10 per litre)	—	93·8	112·3	—	5·0	72·5	—	6·7	40·5
O_2 absorbed from acid $KMnO_4$ in 4 hr. (parts per 100,000)	317	168	195	146	77	89	146	84	88
Percentage reduction in O_2 absorbed	—	47	7	—	47	39	—	43	40
Biochemical oxygen demand (parts per 100,000)	188	140	233	104	45	91	104	47	97
Percentage reduction in Biochemical oxygen demand	—	26	+24	—	57	13	—	55	7

Table 4. *Effect of aeration on the changes produced by different bacteria in flax infusion*

Duration of exp.	Sterile infusion, no inoculum 66 hr.		*Streptococcus*—Strain 3 in sterilized infusion 93 hr.			Natural flora of flax in unsterilized infusion 113 hr.		
	Fresh liquor	Aerated culture	Fresh liquor	Aerated culture	Quiescent culture	Fresh liquor	Aerated culture	Quiescent culture
Organic carbon (mg. C per 100 ml.)	82·6	82·9	121·3	118·5	120·3	207·8	143·4	198·0
CO_2 as carbonate (as mg. C per 100 ml.)	0·4	0·3	1·3	0·4	0·9	0·8	3·3	3·4
CO_2 in absorption tubes (as mg. C per 100 ml.)	—	Nil	—	2·4	2·1	—	58·0	9·1
Total carbon	83·0	83·2	122·6	121·3	123·3	208·6	204·7	210·5
Percentage reduction in organic carbon	—	Nil	—	2·3	0·8	—	31·0	4·7
*p*H value	6·6	6·7	7·7	7·2	6·6	5·1	5·7	3·9
Volatile acids (ml. *N*/10 per litre)	—	—	—	—	—	—	69·0	63·5

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Table 4 (*cont.*)

Duration of exp.	*Bact. aerogenes*, type I, in sterilized infusion 114 hr.			*B. subtilis* in sterilized infusion 100 hr.		
	Fresh liquor	Aerated culture	Quiescent culture	Fresh liquor	Aerated culture	Quiescent culture
Organic carbon (mg. C per 100 ml.)	121·3	84·9	107·6	117·4	81·5	105·0
CO_2 as carbonate (as mg. C per 100 ml.)	1·3	6·2	4·5	1·5	3·4	4·3
CO_2 in absorption tubes (as mg. C per 100 ml.)	—	33·3	8·4	—	33·5	6·8
Total carbon	122·6	124·4	120·5	118·9	118·4	116·1
Percentage reduction in organic carbon	—	30·0	11·3	—	30·6	10·6
*p*H value	7·7	9·2	5·8	6·8	7·3	5·8
Volatile acids (ml. *N*/10 per litre)	—	12·7	45·2	—	—	—
Neutral volatile compounds (as mg. C per 100 ml.)	—	0·9	11·8	—	—	—
O_2 absorbed from acid $KMnO_4$ in 4 hr. (parts per 100,000)	151·3	87·0	104·3	147·5	77·0	110·8
Biochemical oxygen demand (parts per 100,000)	91·4	60·7	90·4	—	—	—

aerated cultures was 20–35·5 mg. greater than from the quiescent cultures, and aeration reduced the biochemical oxygen demand and the amount of oxygen absorbed from permanganate by a much larger percentage than did growth under quiescent conditions. On the other hand, the changes induced by the growth of the *Achromobacterium* species were very slight, whether the cultures were aerated or quiescent. With *Bact. aerogenes* in double strength infusion, although aeration considerably reduced the values for oxygen absorbed from permanganate and for biochemical oxygen demand, these values were still high at the close of the experiment, there were considerable amounts of volatile acids in the aerated liquor, and the difference between the *p*H values of the aerated and the quiescent cultures was comparatively small.

It should be noted that for fermented liquors containing organic acids, the biochemical oxygen demand is probably a better index of their polluting strength than the amount of oxygen absorbed from permanganate. It has been pointed out by Richards & Cutler (1933), for example, that acetic acid is not oxidized by acid permanganate and that lactic acid takes up only about one-third of the oxygen absorbed by the sucrose from which it is formed. It is for this reason that the figures for biochemical oxygen demand in Tables 2 and 3 reveal much greater differences between the aerated and the quiescent cultures than are shown by the figures for oxygen absorbed from permanganate.

The conclusions to be drawn from the results in Table 4 may be summarized as follows:

No change in the composition of the liquor occurred as the result of aeration for 66 hr. in the absence of bacterial growth. With pure cultures of *Bact. aerogenes* or *Bacillus subtilis*, and with the mixed natural flora of the flax, aeration reduced the organic carbon content of the infusion by much greater amounts than did growth of the same organisms under quiescent conditions. The balance sheets for carbon show that, within the limits of experimental error, the loss of organic carbon from each of the cultures was accounted for by the CO_2 produced by bacterial action. After periods of aeration varying from 100 to 114 hr., 30–31 % of the total organic carbon had been converted to CO_2 by cultures of *Bact. aerogenes*, of *Bacillus subtilis*, and of the mixed flora of the flax. Results in Tables 2 and 3 suggest that *Bact. coli* is comparable with *Bact. aerogenes* in this respect. The experiment with *Bact. aerogenes* (Table 4) shows that neutral volatile compounds represented in the quiescent culture nearly 10 %, and in the aerated culture only 0·7 % of the total carbon of the original liquor. When double-strength infusion was used (in the experiment with the natural flora of the flax) the aerated culture again contained appreciable quantities of volatile acids, which in this case were exceptional in being rather larger in amount than those in the quiescent culture.

Rate of oxidation of organic carbon and rate of disappearance of sugar during aeration

The rate at which organic carbon was oxidized and sugar disappeared during aeration was followed in experiments on a somewhat larger scale, an infusion of flax being used in the first, and an infusion of beetroot in the second experiment. Eight litres of the liquor were poured into a small aeration tank of about 10 l. capacity, at the bottom of which a porous diffusion plate of 3 in. diameter was fitted. Air at the rate of 5 cu.ft. per hour was supplied to

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Table 5. *Effect of aeration with diffused air on content of organic carbon and of sugar in infusions of flax, and of red beetroot*

Period of aeration (hr.)	*p*H value	Sugar content (as dextrose)		Organic carbon content	
		mg. per 100 ml.	% of initial content	mg. per 100 ml.	% of initial content
		Flax infusion			
0	6·47	81	100	219	100
24	7·54	9	11·2	169	77·2
50	8·04	4	5·1	134	63·0
72	8·14	2	2·6	114	52·5
96	8·50	0	0·0	98	45·0
144	8·77	—	—	86	39·3
192	8·92	—	—	78	35·8
240	9·00	—	—	75	34·4
		Beetroot infusion			
0	7·61	120	100	117	100
20	7·46	105	87·5	100	85·1
44	7·61	60	50·0	74	63·2
68	7·65	5	4·5	51	43·7
104	8·32	0	0·0	43	36·5
140	8·38	—	—	36	31·0
168	8·49	—	—	33	28·2
216	8·52	—	—	30	25·5
284	8·64	—	—	28	24·0

the diffuser by means of a small rotary blower and the liquor was aerated continuously for several days at room temperature. At intervals samples were abstracted for determination of *p*H value, of organic carbon, and of sugar. For determination of sugar the sample was first treated with dilute hydrochloric acid on the boiling water-bath for 15 min., and reducing sugars were then estimated by the method of Stiles, Peterson & Fred (1926). Results are shown in Table 5.

It is evident that, with both infusions, the sugar had disappeared after aeration for 3–4 days, the rate being very rapid with the flax infusion in the early stages. The rate at which organic carbon in each infusion was oxidized is shown graphically in Fig. 3. Each curve shows initially a steep decline, which persists for some 70–90 hr. and is then followed by a more gradual decline. It appears, therefore, that for the first 3–4 days the more easily oxidizable substances, which included sugars, were rapidly converted to CO_2 by bacterial oxidation. During this phase about 55 % of the total organic carbon of the liquor was oxidized. The remaining organic matter appeared to be more resistant, and the rate of oxidation decreased until, after a further 2 or 3 days, it became very slow.

Table 5 shows that with each infusion aeration was accompanied by an appreciable rise in *p*H value. With flax infusion the *p*H value rose steeply in the early stages and more gradually in the later stages

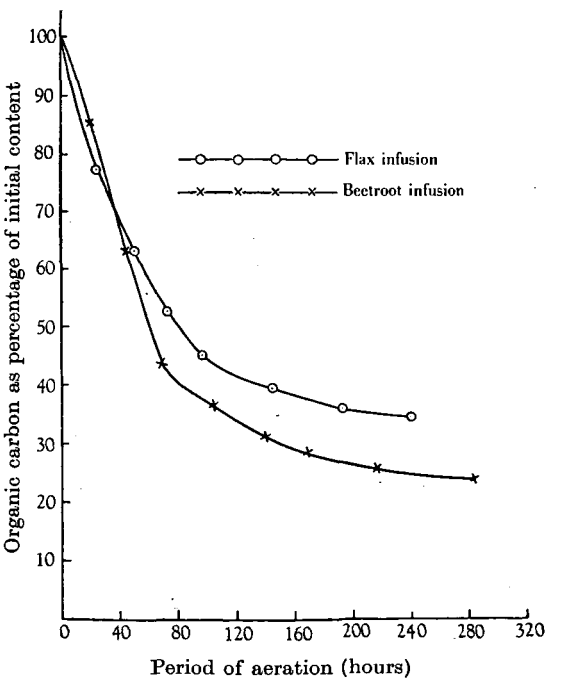

Fig. 3. Rate of oxidation of organic carbon during aeration of (*a*) flax infusion, (*b*) beetroot infusion, with diffused air.

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of aeration, but with beetroot infusion there was no appreciable rise for the first 68 hr., although by that time nearly 60 % of the organic carbon had been oxidized.

DISCUSSION

It is evident from these experiments that the general effect of aeration is to alter the metabolism of the bacteria in the direction of more complete oxidation of the substrate. Cook & Stephenson (1928), by measuring the oxygen uptake of cell suspensions in the presence of different substrates, showed that *Bact. coli* was capable of oxidizing, by means of molecular oxygen, glucose and certain of its products of fermentation, including lactate and acetate. Oxidation of glucose and of lactate was carried to two-thirds, and of acetate to three-quarters of completion. The rate of oxidation was most rapid at *p*H 5·6, but between 5·0 and 9·0 the rate was not greatly affected by change in *p*H value. Below *p*H 5·0 and above 9·0 a rapid fall in the rate of oxidation occurred, and below *p*H 4·2 or above 11·0 no oxidation took place. The rate of oxidation was not dependent on living cells but was proportional to the total concentration of cells, both living and dead.

The growth of coliform bacteria in flax infusion under quiescent conditions resulted in the formation of organic acids, neutral volatile compounds, and CO_2, but in the presence of a continuous current of air the amount of CO_2 formed was greatly increased and the amounts of other metabolic products were greatly reduced, often to a very small value. It seems, therefore, that the carbohydrates, which were fermented anaerobically to organic acids and other metabolic products, tended under sufficiently aerobic conditions to be oxidized to CO_2 and water. If at any stage the rate at which air was supplied were insufficient to cope with the demand for oxidation of this character, some of the carbohydrate would be fermented with the formation of the usual end-products and a consequent fall in *p*H value. Provided the *p*H value were not below 5·0 it would be expected from Cook & Stephenson's results that the acid metabolic products would gradually become oxidized to CO_2 and water on continued aeration. This no doubt accounts for the initial fall, followed by a rise, in *p*H value in some of the experiments recorded in Table 1. The rise in *p*H value of aerated cultures to a point considerably above that of the initial infusion may be due to the oxidation to carbonate of some alkali salts of organic acids extracted from the flax. With single-strength infusion bacterial oxidation usually prevented accumulation of volatile acids, but with double-strength infusion, when the amount of aeration supplied was insufficient for the complete oxidation of the increased concentration of carbohydrates, volatile acids accumulated. With *Bact. aerogenes* (Table 4) it is evident that, under conditions of aeration, either the oxidation of carbohydrates prevented the formation of volatile neutral compounds or, if these were formed, they were subsequently oxidized to CO_2 and water.

Bacillus subtilis differs from the coliform bacteria in being an aerobe. Less is known of its metabolism, but the work of Desmots (1904) and of Lemoigne (1911, 1912, 1923) (quoted by Thaysen & Galloway, 1930) indicates that the products of aerobic dehydrogenation of hexoses by aerobic spore-forming bacilli are mainly 2 : 3-butylene glycol, acetylmethylcarbinol, acetic acid, and β-hydroxybutyric acid, and that lactic acid is not formed. The fact that the aerated culture of *B. subtilis* (Table 4) had an appreciably higher *p*H value than the quiescent culture suggests that, if sufficient oxygen is supplied, this organism oxidizes the carbohydrate to CO_2 and water instead of converting it to the acidic products mentioned above. The increased amount of CO_2 yielded by the aerated culture is no doubt partly due to this, but partly also to the limited amount of air available to the quiescent culture, in which growth would cease when the supply of air had been exhausted.

The small change effected by aeration of a *Streptococcus* culture is explained by the microaerophilic nature of this group of organisms, since aeration markedly retards growth. This, however, does not explain the comparative inactivity of the strain of *Achromobacterium*, which grew under aerobic conditions. This organism was apparently unable to oxidize the carbohydrates present, being similar in this respect to *Bact. alkaligenes* (Cook & Stephenson, 1928).

The natural flora of the flax was invariably found to include coliform bacteria, which rapidly reached large numbers on incubation of the infusion, together with streptococci, comparatively inert Gram-negative rods, and spore-forming bacilli. The changes produced by the growth of the natural flora (Tables 2, 4 and 5) would therefore be due to the combined action of these types of bacteria.

Part of the CO_2 yielded by aerated cultures is no doubt formed by oxidation of carbon compounds other than carbohydrates. This is suggested by the results in Table 5. Thus, during the first 24 hr. of aeration of the flax infusion 71·6 mg. of sugar, representing approximately 28 mg. of carbon per litre of infusion, were oxidized. During this period the total amount of organic carbon oxidized was 50 mg. per litre. After aeration for 96 hr., when all the sugar—80·6 mg., containing approximately 32 mg. of carbon—had been oxidized, the total amount of organic carbon which had been oxidized was 120·8 mg.

SUMMARY

Growth of bacteria in an infusion of flax under quiescent conditions resulted in the formation of an acidic liquor with an appreciable content of volatile acids. When a similar infusion was aerated by a continuous current of air, the liquid tended to become alkaline and the content of volatile acids was much smaller and in some cases negligible. Aeration reduced the polluting strength of the liquor, as measured by oxygen absorbed from permanganate and by the biochemical oxygen demand, by much larger amounts than did growth under quiescent conditions.

Experiments with infusion in which the natural flora was allowed to grow, and with sterile infusion inoculated with pure strains of different types of bacteria, showed that the general effect of aeration was to alter the metabolism of the bacteria in the direction of more complete oxidation of the substrate. Balance sheets for carbon showed that the organic carbon lost from the culture was accounted for by the CO_2 evolved. Thus carbohydrates, which under anaerobic conditions were fermented to organic acids, neutral volatile compounds, and gases, were oxidized, when the conditions were sufficiently aerobic; to CO_2 and water.

The magnitude of the effects observed depended largely on the nature of the organisms present, and partly on the strength of the infusion in which they grew. With pure cultures of *Bact. coli*, *Bact. aerogenes* and *Bacillus subtilis*, and with the natural mixed flora of the flax, aeration at moderate rates in bottles for 3–5 days reduced the value for oxygen absorbed from permanganate by 43–52 %, and the biochemical oxygen demand by 26–92 %, in different experiments. The organic carbon content of the infusion was reduced by 30–31 % by *Bact. aerogenes*, by *Bacillus subtilis* and by the mixed flora. With streptococci and with a strain of *Achromobacterium* the effects observed were very small. Aeration at higher rates with diffused air in small open tanks reduced the organic carbon content of a flax infusion by 50 % in about 80 hr., and of a beetroot infusion by 50 % in about 60 hr. Sugar was destroyed during the aeration and disappeared rapidly from the flax infusion in the early stages.

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REFERENCES

ALLEN, L. A. (1940). The influence of aeration on bacterial growth and on associated chemical changes. *Proc. Soc. Agric. Bact.* (Abstracts), p. 14.

ALLEN, L. A. (1944). Spore-forming bacteria causing soft rot of potatoes and the retting of flax. *Nature, Lond.*, **153**, 224.

BEESLEY, R. M. (1914). Experiments on the rate of nitrification. *J. Chem. Soc.* **105**, 1014.

CARTER, H. A. (1919). The microbiological retting of hemp, flax, and ramie. *Text. Rec.* **37**, 127.

COOK, R. P. & STEPHENSON, M. (1928). Bacterial oxidations by molecular oxygen. I. The aerobic oxidation of glucose and its fermentation products in relation to the viability of the organism. *Biochem. J.* **22**, 1368.

HARDEN, A. (1901). The chemical action of *B. coli communis* and similar organisms on carbohydrates and allied compounds. *J. Chem. Soc.* **79**, 610.

KLUYVER, A. J., DONKER, H. J. L. & HOOFT, F. V. t' (1925). Über die Bildung von Acetylmethylcarbinol und 2 : 3-Butylenglykol im Stoffwechsel der Hefe. *Biochem. Z.* **161**, 361.

MILLS, E. V. (1931). The determination of organic carbon in sewage. *J. Soc. Chem. Ind., Lond.*, **50**, 375T.

Ministry of Health (1942). *Methods of Chemical Analysis as applied to Sewage and Sewage Effluents.* London, H.M. Stationery Office.

RAISTRICK, H., and others (1931). Studies in the biochemistry of micro-organisms. *Philos. Trans.* B, **220**.

RICHARDS, E. H. & CUTLER, D. W. (1933). Purification of waste waters from Beet Sugar Factories. *Tech. Pap. Wat. Pollut. Res., Lond.*, no. 3.

ROSSI, G. (1908). Industrial microbiological vegetal retting process by pectic aerobic microbes in a gas current. Brit. Pat. no. 6532.

RUSCHMANN, G. (1922). Vergleich von Röstverfahren in Fabrikbetrieb. I. Warmwasserbassin-, aërobe Röste und Peufaillitverfahren. *Faserforschung*, **2**, 184.

STEPHENSON, M. & WHETHAM, M. D. (1924). The effect of oxygen supply on the metabolism of *Bacillus coli communis*. *Biochem. J.* **18**, 498.

STILES, H. R., PETERSON, W. H. & FRED, E. B. (1926). A rapid method for the determination of sugar in bacterial cultures. *J. Bact.* **12**, 427.

THAYSEN, A. C. & GALLOWAY, L. D. (1930). *The Microbiology of Starch and Sugars.* London: Oxford University Press.

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