SOME FACTORS WHICH INFLUENCE OXYGEN CONSUMPTION BY BACTERIA IN LAKE WATER [1]

CLAUDE E. ZOBELL [2]

(From the Limnological Laboratory and the Department of Agricultural Bacteriology, University of Wisconsin)

The conditions which influence the distribution of oxygen in lake water are the subject of an extensive literature (Thienemann, 1928; Grote, 1934), but there is relatively little information on the rôle played by bacteria regardless of the alleged importance of these organisms as agencies which deplete the oxygen content of natural waters. Birge and Juday (1911) state that the respiration of the bacteria of decay is more important than the respiration of aquatic animals in the utilization of oxygen. According to Domogalla, Fred and Peterson (1926) the oxygen content of lake water below the photosynthetic zone is inversely proportional to the abundance of bacteria. Similarly, Kusnetzow and Karsinkin (1931) report that the zones of diminished oxygen tension in Lake Glubokoje are caused primarily by bacterial activity. Seiwell and Seiwell (1938) attribute the oxygen minimum layer in the sea to the increased decomposition of organic matter by bacteria in such zones. Pütter (1924) expresses the view that bacteria consume more oxygen in sea water than all other organisms combined.

Some workers have assumed erroneously that the amount of oxygen consumed by bacteria in water can be evaluated merely by storing samples of raw water in the dark in glass-stoppered bottles and determining the oxygen content after different periods of incubation at the *in situ* temperature. This procedure may serve to estimate the amount of materials in such water which can be oxidized by bacteria but it fails to indicate the rate or the amount of oxygen consumption *in situ* because the storage of water in the laboratory is always accompanied by increased and altered bacterial activity (ZoBell and Anderson, 1936). Therefore, in order to ascertain how much oxygen is consumed by bacteria in lakes, several factors which influence oxygen consumption must be considered.

[1] This investigation was supported by grants from the Brittingham Trust Fund and the Wisconsin Alumni Research Foundation. Part of the cost of technical assistance was defrayed by the Works Progress Administration.

[2] Visiting investigator on sabbatical leave from the Scripps Institution of Oceanography, University of California, La Jolla.

Influence of Temperature

Water temperatures ranging from 0° to 35° C. or higher occur in lakes. In order to evaluate the effect of temperature upon the rate at which bacteria consume oxygen, the following experiments were conducted. Water was collected from Lake Mendota in 5-gallon carboys and thoroughly shaken to insure uniformity of its bacterial and chemical composition. It was then siphoned into each of several dozen 145 ml. oxygen bottles. The latter were divided into four lots and transferred to water baths held at 8°, 18°, 25° and 37° C. respectively. Duplicate bottles from each lot were analyzed for the oxygen content of the water by the Winkler method at the beginning of the experiment and after different periods of incubation. In most cases duplicate determinations agreed to within 0.03 mgm./1. and additional bottles were analyzed if the divergence of duplicates exceeded 0.10 mgm./1. The bottles were incubated in the dark to prevent photosynthetic activity. Table I shows the amount of oxygen which was consumed by the bacteria after different periods of incubation.

TABLE I

Amount of oxygen consumed by bacteria in Lake Mendota water incubated at different temperatures. The water initially contained 9000 bacteria per ml. and 7.46 mgm. of oxygen per liter.

Incubation temperature	Milligrams of oxygen consumed per liter after				
	2 days	5 days	10 days	20 days	30 days
8° ± 0.3° C.	0.09	0.26	0.61	1.32	2.84
18° ± 0.3° C.	0.31	1.43	2.41	3.06	3.77
25° ± 0.1° C.	0.56	2.02	2.37	3.95	4.49
37° ± 0.2° C.	0.49	1.84	2.95	4.11	4.76

A multiplicity of correlative factors are involved in the interpretation of the results. In the first place it must be recognized that the rate of reproduction and the death rate of bacteria as well as their respiratory rate are influenced by the temperature of the water. During the first 24 hours the bacterial population of the water incubated at 8° C. had trebled while that incubated at 25° C. had increased nearly a hundredfold. The bacterial population of the water incubated at 25° C. reached its maximum on the third day after which it decreased. The bacterial population of the water incubated at 18° C. reached its maximum on the fourth day and then decreased. The bacterial population of the water incubated at 8° C. was still increasing after ten days at which time

it contained more bacteria than the water incubated at the higher temperatures.

The residual organic matter becomes more and more refractory to oxidation as the more oxidizable fractions are oxidized and finally a lack of oxidizable matter limits further oxygen consumption. Although six times as much oxygen was consumed by multiplying cultures in two days at 25° C. as at 8° C., there was virtually no difference after sixty days because at all temperatures most of the readily oxidizable organic matter had been oxidized. By applying the formula of Buchanan and Fulmer (1930):

$$m = \frac{2.303\, S \log b/B}{t(b - B)}$$

where m is the oxygen consumed per cell in time t; S the total amount of oxygen consumed in time t; B the number of bacteria at the beginning of the experiment and b the number after time t; it was found that during the first 24-hour period of incubation at 25° C. the bacteria in Lake Mendota water consumed 21 to 43×10^{-12} mgm. of oxygen per cell per hour. Although the bacterial population continued to increase throughout the second 24-hour period, the oxidizable organic matter content of the water was diminished to such an extent that the bacteria used only 5.4×10^{-12} mgm. of oxygen per cell per hour. After 5 days the bacteria incubated in water at 25° C. were using only 0.5×10^{-12} mgm. of oxygen per cell per hour. During the first 24-hour period of incubation at 8°, 18°, 25° and 37° C., the bacteria consumed an average of 9×10^{-12}, 20×10^{-12}, 32×10^{-12} and 61×10^{-12} mgm. of oxygen per cell per hour respectively. Bacteria from Lake Glubokoje were found by Liagina and Kusnetzow (1937) to consume an average of 18.8×10^{-12} mgm. of oxygen per cell per hour at 10° C. and 30.3×10^{-12} mgm. at 15° C.

The influence of temperature upon the rate of bacterial respiration independent of its effect on bacterial multiplication and other variables mentioned above was investigated by inoculating lake water enriched with 0.01 per cent each of glucose and asparagine with about a hundred million bacteria per ml. The inoculant was prepared by washing the actively multiplying colonies from several bottles of nutrient agar previously inoculated with the mixed microflora from Lake Mendota and incubated at 18° C. Under these conditions the rate of oxygen consumption was found to increase exponentially with the temperature with one exception as shown by Fig. 1. The Q_{10} from 8° to 25° C. was found to be 2.1. The decelerating rate of respiration in the water incubated at 37° C. is attributed to the inactivation of some of the

thermo-sensitive bacteria or their enzymes and is not due to a lack of either oxidant or oxygen. According to Edwards and Rettger (1937) the respiratory enzymes of bacteria are especially thermo-sensitive, many being destroyed at relatively low temperatures. Similarly ZoBell and Conn (1940) have shown that prolonged exposure to temperatures exceeding 25° C. is injurious to many water bacteria and that the respiratory enzymes of some are destroyed in 10 minutes at 30° C.

Influence of Oxygen Tension

According to Amberson (1928) the rate of oxygen consumption by unicellular organisms is independent of the oxygen tension over a wide

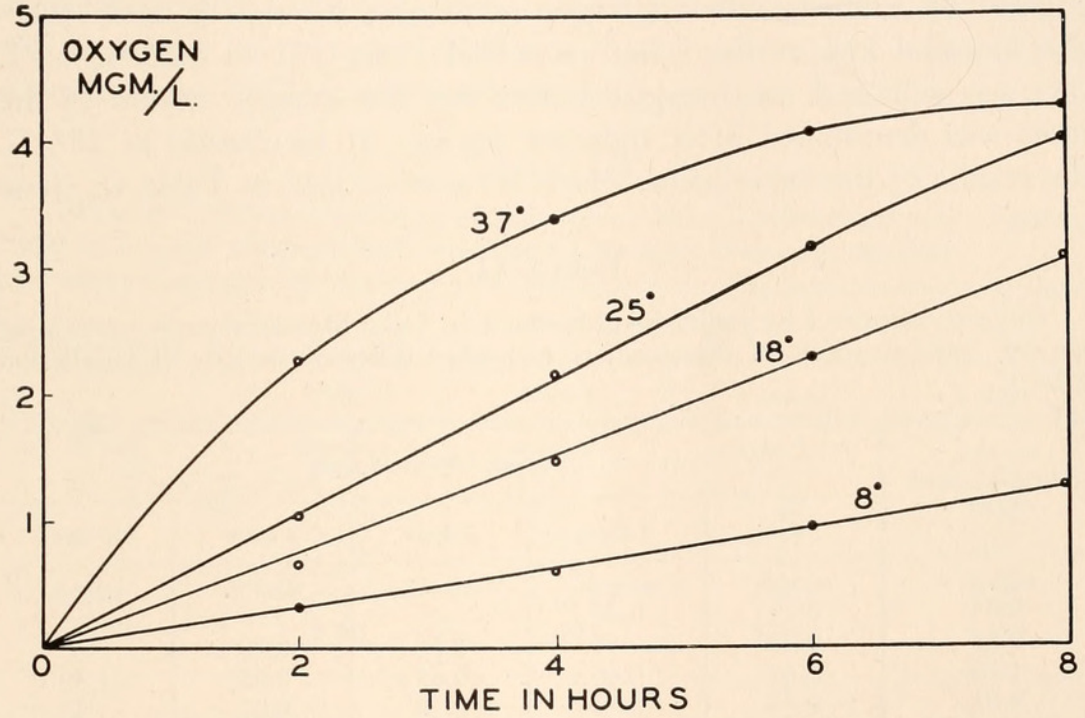

FIG. 1. Oxygen consumed after different periods of time at 8°, 18°, 25° and 37° C. by lake bacteria (resting cells) in lake water enriched with 0.01 per cent each of glucose and asparagine.

range. Similarly Pütter (1924), Harvey (1928), Pomeroy (1938) and others report that the rate of oxygen uptake by bacteria is not influenced by the oxygen tension. However, Waksman and Carey (1935), Heukelekian (1936), Schlayer (1936) and others find that the rate of oxygen consumption by certain bacteria is proportional to the oxygen tension. These antithetical observations may be due to variations in experimental procedure. For example, Kempner (1937) finds that at relatively low temperatures or with old cells the respiration of *Escherichia coli* is independent of the oxygen tension, but when using

heavy suspensions of young cells at their optimum temperature, the oxygen uptake of *E. coli* is a function of the oxygen tension. These observations suggest that all of the oxygen may be consumed in the immediate vicinity of groups or clumps of young cells more rapidly than it can reach them by diffusion. Clifton and Logan (1939) and others have shown that young cells consume oxygen much faster than old ones. According to Waksman and Renn (1936) the effect of oxygen tension is most pronounced when resistant materials are being oxidized.

The following experiments were performed to ascertain what effect the oxygen tension has upon the respiration of bacteria in lake water. The oxygen content of water recently collected from Lake Mendota was adjusted by bubbling either nitrogen or oxygen through it until otherwise identical lots of the water contained from 0.31 to 8.26 mgm./l. This was siphoned into oxygen bottles and the oxygen tension of the water was determined after different periods of incubation at 25° C. The results of the experiment, which are summarized in Table II, show

TABLE II

Oxygen consumed by multiplying bacteria in Lake Mendota water containing different concentrations of dissolved oxygen after different periods of incubation at 25° C.

Initial dissolved oxygen	Oxygen consumed after				
	1 day	2 days	3 days	5 days	10 days
mgm./l.	*mgm./l.*	*mgm./l.*	*mgm./l.*	*mgm./l.*	*mgm./l.*
0.31	0.21	0.29	—	—	—
0.85	0.23	0.41	0.54	0.79	—
1.78	0.28	0.46	0.61	0.95	1.39
4.02	0.27	0.39	0.50	0.92	1.32
8.26	0.28	0.42	0.56	1.06	1.52

that within the limits of experimental error the oxygen tension of the water does not influence the rate at which oxygen is consumed by respiring bacteria.

In several other experiments which have been reported in detail by ZoBell and Stadler (1940*a*), it was similarly found that the respiration of "resting" cells in enriched lake water as well as cultures of bacteria is independent of the oxygen tension over a wide range. Although there is a slight tendency for the oxygen tension of lake water to influence the multiplication of bacteria, there is no evidence to indicate that the oxygen uptake of bacteria is influenced by the oxygen tension until

the oxygen content is as low as 0.3 mgm./1. Even when the water was supersaturated with oxygen (as much as 26.48 mgm./1.), bacteria consumed oxygen no more rapidly than when the water contained only 0.3 mgm./1. As the oxygen tension is reduced below 0.3 mgm./1. the rate of bacterial respiration decreases rapidly.

Influence of Organic Matter

According to Friedlein (1928) 100 mgm. of utilizable organic matter per liter approximates the minimum concentration in which certain bacteria can grow. This is greatly in excess of the concentration of total organic matter commonly found in lakes; Lake Mendota water containing an average of only 12 mgm./1. (Birge and Juday, 1934); and, as will be shown below, much of this is highly refractory to bacterial decomposition. While many bacteria indigenous to natural waters can

TABLE III

Effect of the concentration of glucose upon the rate of respiration of "resting" bacteria in water which initially contained 7.28 mgm. of oxygen per liter.

Concentration of glucose	Oxygen consumed after			
	1 hour	2 hours	4 hours	8 hours
mgm./l.	*mgm./l.*	*mgm./l.*	*mgm./l.*	*mgm./l.*
0	0.06	0.10	0.14	0.23
2	0.12	0.19	0.38	0.53
5	0.16	0.26	0.52	0.91
10	0.17	0.34	0.71	1.18
100	0.29	0.63	1.43	1.69
1000	0.32	0.69	1.38	1.75

maintain themselves indefinitely when sub-cultured in lake water as well as in sea water which contains less than 10 mgm. of total organic matter per liter, this concentration seems to be in the neighborhood of their threshold for multiplication.

The effect of concentration of organic matter upon the rate of oxygen consumption was investigated by adding from 2 to 1000 mgm. of glucose per liter of synthetic lake water inoculated with enough washed bacterial cells (mixed microflora from Lake Mendota) to give around fifty million per ml. After different periods of incubation in oxygen bottles at 25° C. the amount of oxygen consumed was determined. The results are summarized in Table III. Although the "resting" cells were washed twice by centrifuging and re-suspending in synthetic lake water to free them of oxidizable materials, they consumed about 1.0×10^{-12} mgm. of oxygen per cell per hour without the addition of any

organic matter. However, the rate of respiration was increased by the addition of glucose until a concentration of 100 to 1000 mgm./1. was reached. Similar results were obtained with glycerol, asparagine and lactic acid. The rate of respiration was not influenced by the addition of 2 mgm./1. of either ammonium sulphate or potassium nitrate thereby proving that the lack of available nitrogen was not a limiting factor. Further experiments are planned to determine the effect of concentration of other organic compounds upon the respiration and other vital activities of bacteria.

Table IV

Relative rates of oxidation of 2.0 mgm./l. of organic compounds by cultures of lake bacteria as indicated by the amount of oxygen consumed after different periods of incubation at 25° C. The "oxygen demand" is the amount of oxygen required for the complete (100 per cent) oxidation of 2.0 mgm. of the compound.

Compound	Oxygen demand	Milligrams of oxygen consumed and per cent of each compound oxidized after					
		5 days		10 days		20 days	
	mgm.	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*
Succinic acid	0.88	0.42	47.8	0.63	71.6	0.83	94.4
Glycine	1.28	0.74	57.8	0.96	75.0	1.16	90.7
Asparagine	1.46	0.59	40.4	0.95	65.1	1.24	85.0
Lactic acid	2.12	1.29	60.9	1.76	83.0	2.10	99.1
Glucose	2.14	1.56	72.9	2.02	94.5	2.07	96.8
Starch	2.36	0.44	18.7	1.19	50.4	2.20	93.3
Cellulose	2.36	0.17	7.2	0.42	17.8	0.96	40.7
Glycerol	2.44	1.63	66.8	1.97	80.8	2.36	97.8
Propionic acid	3.04	0.72	23.7	1.38	45.4	2.24	73.7
Butyric acid	3.64	0.68	18.7	1.49	40.9	2.35	64.6
Lignin	3.70	0	0	0	0	0.23	6.2
Ethanol	4.16	2.06	49.6	3.12	75.0	4.01	96.4

The susceptibility to bacterial attack of different organic compounds which may occur in lacustrine materials was tested by adding 2.0 mgm./1. of the compounds to synthetic lake water (a mineral solution simulating lake water in composition but devoid of oxidizable substrates). The latter was inoculated with mixed microflora from Lake Mendota and distributed in 145 ml. glass-stoppered bottles. In the course of the experiment the bacterial population increased from an initial 26,000 per ml. to several million per ml. after 5 to 20 days incubation at 25° C. Dissolved oxygen was determined at the beginning of the experiment and after different periods of incubation. Some of the data are summarized in Table IV which shows the rapidity with which various com-

pounds are attacked by lake bacteria. Corrections have been made for the 0.07, 0.12 and 0.16 mgm./1. of oxygen which was consumed after 5, 10 and 20 days respectively in the inoculated controls to which no organic matter was added. The "oxygen demand" is the theoretical amount of oxygen required for the complete oxidation of 2.0 mgm. of the compound to carbon dioxide and water. The oxygen demand of the nitrogenous compounds would be somewhat higher if the ammonium resulting from their decomposition were oxidized to nitrite or nitrate.

From Table IV it will be observed that there is considerable difference in the amount of oxygen required for the complete oxidation of similar quantities of different compounds and that there is even a greater difference in the susceptibility of the compounds to bacterial decomposition. Part of the difference in the rate at which oxygen is consumed by bacteria in the presence of different compounds is attributable to differences in the number of bacteria in the mixed culture which are capable of attacking the given compound as well as to differences in the growth-promoting properties of the compounds. Moreover, the fact that the oxidation of some compounds requires four or five times as much oxygen as equal concentrations of other compounds might influence the results. An evaluation of these and other factors which are involved in the full interpretation of the experiment awaits further investigations but the experiment does indicate in a general way how small concentrations of certain organic compounds might influence oxygen consumption by bacteria in lake water. It seems especially noteworthy that 2.0 mgm./1. of some of the compounds are almost quantitively oxidized in 10 to 20 days and the more resistant compounds including cellulose and lignin are slowly oxidized by certain lake bacteria. Working with eleven samples of purified lignin differing either in source or method of preparation ZoBell and Stadler (1940*b*) found that 4.4 to 14.7 per cent of the lignin was oxidized by lake bacteria in 30 days at 28° C. Kinkel (1936) has reported on the decomposition of cellulose, chitin and pectin by aerobic bacteria found in Wisconsin lakes.

Lacustrine Organic Matter

Most of the organic matter which occurs in lake water is quite refractory to bacterial oxidation. This was demonstrated by noting the rate and amount of oxygen consumption in samples collected from various Wisconsin lakes at different times during the year. The water was collected in 5-gallon carboys, filtered through cotton gauze to remove gross particles, thoroughly shaken to insure uniformity in composition and after it was warmed to 25° C., the water was siphoned into 145 ml.

oxygen bottles. Duplicate bottles were analyzed for dissolved oxygen after different periods of incubation in the dark in a water bath at 25° C. Table V shows the oxygen-consuming capacity of Lake Mendota water collected near the surface at Station S 7 from October 11, 1938, to May 6, 1939. In February the samples were collected through twelve to twenty inches of ice.

Assuming the total organic matter content of Lake Mendota water to be 12 mgm./l., the average found by Birge and Juday (1926) over

TABLE V

Biochemical oxygen demand (B.O.D.) of Lake Mendota water from Station S 7 after different periods of incubation at 25° C. Upon equilibration with air at 25° C. the oxygen concentration of the water was around 8.5 mgm./l. at the beginning of each experiment.

Description of samples			Oxygen consumed after				Ratio 5:20 day B.O.D.
Date of collection	Oxygen content	Temperature of water	5 days	10 days	20 days	30 days	
	mgm./l.	*° C.*	*mgm./l.*	*mgm./l.*	*mgm./l.*	*mgm./l.*	*per cent*
10/11/38	8.96	16.4	2.06	—	3.84	5.14	53.6
10/25/38	9.08	15.1	2.30	3.82	4.51	5.58	50.9
11/ 2/38	9.37	12.8	2.17	3.73	4.17	—	52.1
11/ 9/38	10.62	8.5	1.54	2.22	3.32	3.96	46.4
11/21/38	11.54	6.6	1.60	2.40	3.41	4.09	46.8
11/30/38	11.93	5.2	0.97	2.07	2.42	3.15	40.2
12/ 8/38	12.45	3.4	0.86	1.65	2.00	2.23	43.0
12/16/38	13.86	1.6	0.96	—	2.14	—	44.4
1/ 4/39	13.08	0	1.36	2.42	3.02	4.21	45.1
1/11/39	14.34	0	1.50	2.31	3.31	4.18	45.4
1/23/39	13.72	0	1.29	—	3.22	3.86	40.2
2/ 6/39	12.90	0	0.91	1.37	2.30	2.60	39.4
2/20/39	12.74	0	0.84	1.21	1.92	—	43.8
3/ 7/39	12.31	0	1.24	1.95	2.99	3.99	41.5
3/23/39	14.18	0.3	3.11	4.66	6.20	7.13	50.2
4/10/39	11.80	7.4	3.56	5.22	7.25	—	49.1
5/ 6/39	10.37	11.6	3.92	5.38	7.46	—	52.5

a period of years, and since the complete oxidation of 1.0 mgm. of lacustrine organic matter requires an average of 1.2 mgm. of oxygen, it is estimated that only 21 to 30 per cent of the total organic matter is oxidized in 20 days, and 31 to 37 per cent in 30 days at 25° C. The prolonged incubation of a few samples showed that oxygen continued to be used at a decreasing rate, but after 94 days less than 50 per cent of the organic matter was oxidized. Using the evolution of carbon dioxide as the criterion of decomposition, Allgeier, Peterson and Juday (1934) found that from 18 to 42 per cent of the organic carbon in lake water

was oxidized in 31 days at 25° C. According to Waksman and Renn (1936), about 50 per cent of the organic matter in sea water is decomposed by bacteria under similar conditions; and of this decomposed organic matter, about 60 per cent is completely oxidized and about 40 per cent is converted into bacterial cell substance.

Upon the addition of 10 mgm./l. of glucose to the lake water all of the oxygen (about 8.5 mgm./l. at 25° C.) was rapidly depleted from the lake water thereby indicating that available nitrogen is not a factor which limits the oxidation of the organic matter in lake water. Moreover, neither the rate nor the amount of oxygen consumption was influenced by the addition of 2 mgm./l. of ammonium sulphate.

The respirable or oxidizable organic content of Lake Mendota water as indicated by its oxygen-consuming capacity was found to decrease

TABLE VI

Oxygen consumed by bacteria (B.O.D.) in samples of water from Northeastern Wisconsin lakes after different periods of incubation at 25° C.

Lake	Date of collection	Oxygen consumed after			Ratio of 5:20 day B.O.D.
		5 days	10 days	20 days	
		mgm./l.	*mgm./l.*	*mgm./l.*	*per cent*
Crystal Lake	1/12/39	0.52	1.34	1.86	28.7
Crystal Lake	2/25/39	0.44	0.95	1.57	28.1
Trout Lake	2/23/39	2.27	3.41	5.33	42.6
Silver Lake	3/29/39	0.83	1.55	2.07	40.2
Little Star Lake	3/29/39	2.12	3.31	5.04	42.1

from the time of the fall overturn in October until the ice started to melt late in March (see Table V). The melting of the ice was accompanied by much terrigenous contamination which might account in part for the sudden increase of oxidizable material at this time although other factors, including an increase of phytoplankton, are involved. According to Birge and Juday (1934) the organic matter content of Lake Mendota varies very little throughout the year, centrifuge plankton constituting less than 10 per cent of the total. However, the plankton forms appear to be decomposed much more rapidly and more completely than the resistant residual dissolved organic matter in lake water.

From 65 to 75 per cent of the organic matter in the net plankton concentrate added to synthetic lake water was found to be oxidized in 20 days at 25° C. Filtering Lake Mendota water collected during the spring months through No. 25 bolting silk which removes the larger plankton forms reduced its oxygen-consuming capacity 4 to 13 per cent.

The Foerst supercentrifuge (Juday, 1926) removed enough particulate organic matter to reduce the oxygen-consuming capacity of lake water 9 to 21 per cent.

The lower the concentration of oxidizable material in lake water the more refractory it is to bacterial attack. For example, from the last of November (11/30/38) to the first of March (3/7/39) when the 20-day B.O.D. of Lake Mendota water was lowest, the ratio of the 5-day B.O.D. to the 20-day B.O.D. was also lowest; or proportionately less oxygen was consumed in 5 days when the total oxidizable content was lowest (see Table V). Similar observations were made on water samples from other Wisconsin lakes as shown in Table VI. Less oxidizable organic matter was found and the ratio of the 5-day to the 20-day B.O.D. was lower in oligotrophic Crystal Lake than in eutrophic Trout, Little Star and Silver Lakes.

TABLE VII

Oxygen consumed per 100 mgm. (dry basis) of Lake Mendota mud dredged from Station 1 in January 1939 after different periods of incubation at 25° C.

Sample No.	Mgm. oxygen consumed after				Ratio of 5:20 day B.O.D.
	5 days	10 days	20 days	30 days	
1380	1.44	2.93	3.67	4.26	39.2
1390	1.25	3.03	4.34	4.63	28.8
1406	1.73	2.78	4.01	4.56	43.1
1409	1.09	1.97	3.58	3.96	30.2
Average	1.38	2.68	3.90	4.35	35.4

The amount of biochemically oxidizable material in Lake Mendota mud was estimated by diluting dredged samples a thousandfold or more with synthetic lake water. The diluted material was equilibrated with air to satisfy any chemical oxygen deficit (Miyadi, 1934), transferred to oxygen bottles with continued shaking to insure uniformity of composition and incubated in the water bath at 25° C. Duplicate bottles were analyzed for oxygen after different periods of incubation. Table VII summarizes the results. The relative slowness with which oxygen is consumed (5:20-day B.O.D. equals 35.4 per cent) shows that the material in the mud is more resistant to bacterial attack than that in Lake Mendota water. Similarly Waksman and Hotchkiss (1938) found that the organic matter in marine bottom deposits is oxidized less readily by bacteria than that in sea water.

According to Black (1929) mud from Station 1 in Lake Mendota contains an average of 13 per cent organic matter (dry basis). As-

suming that 1.2 mgm. of oxygen is required for the complete oxidation of 1.0 mgm. of the organic matter, it is calculated that 24 per cent of the organic matter in the lake deposits is oxidized aerobically in 20 days and around 29 per cent is oxidized in 30 days at 25° C. Decomposition continues slowly thereafter at a constantly decreasing rate. The organic matter in the mud is decomposed much more readily in the presence of oxygen than anaerobically because Allgeier et al. (1932) found that only 1 per cent of the total organic carbon was destroyed in 7 months under anaerobic conditions. Steiner and Meloche (1935) have shown that from 30 to 48 per cent of the organic matter in lake deposits consists of ligneous materials which are very resistant.

Little relationship was noted between the oxidizability of the lacustrine materials by permanganate (Wereščagin et al., 1931) and its oxidizability as indicated by oxygen consumption by bacteria. The latter is believed to be much more representative of the capacity of the organic matter to utilize oxygen *in situ*.

Discussion

If the heterotrophic bacteria in Lake Mendota consume oxygen at the rate of 9 to 20 $\times$ 10^{-12} mgm. per cell per hour (values found in the laboratory at 8° and 18° C. respectively), a hundred thousand bacteria per ml. would consume 4 to 9 mgm. of oxygen per liter of water during the six summer months when the temperature of the water ranges from 8° to 18° C. At this rate the activities of heterotrophic bacteria could account for the depletion of the oxygen from stagnant waters particularly in the lake bottom where bacterial populations exceeding a million per ml. have been demonstrated (Henrici and McCoy, 1938). While these estimates based upon laboratory observations are highly speculative, they substantiate the conclusions of field workers that bacteria probably play a very important rôle in the utilization of oxygen in lakes. When more complete data become available it should be possible to evaluate with greater precision the rôle of bacteria. Combining field and laboratory observations Liagina and Kusnetzow (1937) have calculated that "the decrease of the oxygen content of the waters of Lake Glubokoje can be covered with excess by the respiration of the water bacteria."

Although little is known concerning the abundance and activity of chemosynthetic autotrophic bacteria in lakes, those which oxidize ammonium, nitrite, hydrogen sulphide and methane may utilize appreciable quantities of oxygen. The possible significance of such autotrophs is apparent from the fact that the oxidation of 1.0 mgm. of ammonium to nitrate requires 5.15 mgm. of oxygen, the oxidation of 1.0 mgm. of

hydrogen sulphide to sulphate requires 4.23 mgm. of oxygen and the oxidation of 1.0 mgm. of methane to carbon dioxide and water requires 4.0 mgm. of oxygen. Hydrographic data (Welch, 1935) indicate the presence of from a trace to a few milligrams per liter of ammonium, hydrogen sulphide and methane in lake water from various places and there is evidence that these substances are oxidized. Kusnetzow (1934) believes that methane-oxidizing bacteria consume more oxygen in certain lakes than all other bacteria and animals combined. According to Ravich-Sherbo (1930) there is an extensive layer of sulphur bacteria in the Black Sea where they oxidize the hydrogen sulphide from the hypolimnion at the expense of the oxygen from the epilimnion. A similar localization of sulphur bacteria in the marginal zone of Lake Ritom has been reported by Düggeli (1924).

Summary

The rate of respiration of lake bacteria increases with temperature, the Q_{10} from 8° to 25° C. being 2.1. Mixed microflora from Lake Mendota were found to consume from 21 to 43 $\times$ 10^{-12} mgm. of oxygen per cell per hour at 25° C. Prolonged exposure at 37° C. is injurious to some of the bacteria.

The rate of respiration is independent of the oxygen concentration of the water within the examined range of 0.31 to 26.48 mgm./1. As the oxygen tension is reduced below 0.3 mgm./1. the rate of bacterial respiration decreases rapidly.

Both the character and the concentration of organic matter influence the rate of oxygen consumption by lake bacteria. The rate of respiration of " resting " cells increases as the concentration of glucose, asparagine and lactic acid is increased up to 100 to 1000 mgm./1.

About one-third of the organic matter in Lake Mendota water is readily oxidizable, the remainder being quite resistant to bacterial attack. The organic content of bottom deposits is less oxidizable than that of water, and the remains of plankton organisms are more readily oxidized by bacteria than the dissolved organic matter occurring in lake water.

Acknowledgment

The author is indebted to Miss Janice Stadler and Dr. F. Cho for technical assistance and to Dr. Elizabeth McCoy, Dr. E. B. Fred, Dr. E. A. Birge and Dr. Chancey Juday for their encouragement and constructive criticisms.

REFERENCES

ALLGEIER, R. J., W. H. PETERSON, AND C. JUDAY, 1934. Availability of carbon in certain aquatic materials under aerobic conditions of fermentation. *Int. Rev. ges. Hydrobiol.*, **30**: 371–378.

ALLGEIER, R. J., W. H. PETERSON, C. JUDAY, AND E. A. BIRGE, 1932. The anaerobic fermentation of lake deposits. *Int. Rev. ges. Hydrobiol.*, **26**: 444–461.

AMBERSON, W. R., 1928. The influence of oxygen tension upon the respiration of unicellular organisms. *Biol. Bull.*, **55**: 79–91.

BAIER, C. R., 1935. Studien zur Hydrobakteriologie stehender Binnengewässer. *Arch. f. Hydrobiol.*, **29**: 183–264.

BIRGE, E. A., AND C. JUDAY, 1911. The inland lakes of Wisconsin. I. The dissolved gases of the water and their biological significance. *Bull. Wis. Geol. and Nat. Hist. Survey*, **22**: 1–259.

BIRGE, E. A., AND C. JUDAY, 1926. Organic content of lake water. *Bull. U. S. Bur. Fish.*, **42**: 185–205.

BIRGE, E. A., AND C. JUDAY, 1934. Particulate and dissolved organic matter in inland lakes. *Ecol. Monographs*, **4**: 440–474.

BLACK, C. S., 1929. Chemical analyses of lake deposits. *Trans. Wis. Acad. Sci.*, **24**: 127–133.

BUCHANAN, R. E., AND E. I. FULMER, 1930. Physiology and Biochemistry of Bacteria. Williams and Wilkins Co., Baltimore, vol. 3, p. 154.

CLIFTON, C. E., AND W. A. LOGAN, 1939. On the relation between assimilation and respiration in suspensions and in cultures of *Escherichia coli*. *Jour. Bact.*, **37**: 523–540.

DOMOGALLA, B. P., E. B. FRED, AND W. H. PETERSON, 1926. Seasonal variations in the ammonia and nitrate content of lake waters. *Jour. Am. Water Works Assoc.*, **15**: 369–385.

DÜGGELI, M., 1924. Bakteriologische Untersuchungen am Ritom-See. *Rev. d'Hydrolog.*, **2**: 65–205.

EDWARDS, O. F., AND L. F. RETTGER, 1937. The relation of certain respiratory enzymes to the maximum growth temperatures of bacteria. *Jour. Bact.*, **34**: 489–515.

FRIEDLEIN, F., 1928. Der quantitative verwendungstoffwechsel des Paratyphus B–Bazillus, des Bacterium coli und des Bacillus pyocyaneus. *Biochem. Zeitschr.*, **194**: 273–291.

GROTE, A., 1934. Der Sauerstoffhaushalt der Seen. *Die Binnengewässer*, **14**: 1–217.

HARVEY, E. N., 1928. The oxygen consumption of luminous bacteria. *Jour. Gen. Physiol.*, **11**: 469–475.

HENRICI, A. T., AND ELIZABETH MCCOY, 1938. The distribution of heterotrophic bacteria in the bottom deposits of some lakes. *Trans. Wis. Acad. Sci.*, **31**: 323–361.

HEUKELEKIAN, H., 1936. Oxygen tension and bacterial numbers. *Sewage Works Jour.*, **8**: 415–421.

JUDAY, C., 1926. A third report on limnological apparatus. *Trans. Wisconsin Acad. Sci.*, **22**: 299–314.

JUDAY, C., E. B. FRED, AND F. C. WILSON, 1924. The hydrogen ion concentration of certain Wisconsin lake waters. *Trans. Am. Micros. Soc.*, **43**: 177–190.

KEMPNER, W., 1937. Effect of oxygen tension on cellular metabolism. *Jour. Cell. and Comp. Physiol.*, **10**: 339–363.

KINKEL, DOROTHY, 1936. A study of chitin-, pectin- and cellulose-destroying bacteria from lake mud. Dissertation, University of Wisconsin, 35 pp.

KUSNETZOW, S. I., 1934. Microbiological investigations in the study of the oxygen regime of lakes. *Microbiol.*, **3**: 486–505 (Russian).

KUSNETZOW, S. I., AND G. S. KARSINKIN, 1931. Direct method for the quantitative study of bacteria in water and some considerations on the causes which produce a zone of oxygen-minimum in Lake Glubokoje. *Centralbl. f. Bakt.*, II Abt., **83**: 169–174.

LIAGINA, N. M., AND S. I. KUSNETZOW, 1937. The determination of the intensity of respiration of some species of water bacteria at various temperatures under laboratory conditions. *Microbiol.*, **6**: 21–27 (Russian with English summary).

MIYADI, D., 1934. Oxygen absorption of the lake deposit. *Proc. Imperial Acad.*, **10**: 236–239.

POMEROY, R., 1938. Influence of oxygen concentration on biochemical oxygen demand. *Sewage Works Jour.*, **10**: 465–472.

PÜTTER, A., 1924. Die Atmung der Planktonbakterien. *Pflügers Arch. f. Ges. Physiol.*, **204**: 94–126.

RAVICH-SHERBO, J., 1930. On the question of the bacterial thin layer in the Black Sea according to the hypothesis of Prof. Egounoff. *Travaux de la Station Biologique de Sebastopol.*, **2**: 127–141.

SCHLAYER, C., 1936. The influence of oxygen tension on the respiration of Pneumococci (Type I). *Jour. Bact.*, **31**: 181–189.

SEIWELL, H. R., AND GLADYS SEIWELL, 1938. The sinking of decomposing plankton in sea water and its relationship to oxygen consumption and phosphorus liberation. *Proc. Amer. Phil. Soc.*, **78**: 465–481.

STEINER, J. F., AND V. W. MELOCHE, 1935. A study of ligneous substances in lacustrine materials. *Trans. Wis. Acad. Sci.*, **29**: 389–402.

THIENEMANN, A., 1928. Der Saurstoff im eutrophen und oligotrophen See. *Die Binnengewässer*, **4**: 1–175.

WAKSMAN, S. A., AND CORNELIA L. CAREY, 1935. Decomposition of organic matter in sea water by bacteria. I. Bacterial multiplication in stored sea water. *Jour. Bact.*, **29**: 531–543.

WAKSMAN, S. A., AND MARGARET HOTCHKISS, 1938. On the oxidation of organic matter in marine sediments by bacteria. *Jour. Mar. Res.*, **1**: 101–118.

WAKSMAN, S. A., AND C. E. RENN, 1936. Decomposition of organic matter in sea water by bacteria. III. Factors influencing the rate of decomposition. *Biol. Bull.*, **70**: 472–483.

WELCH, P. S., 1935. Limnology. McGraw-Hill, New York, 471 pp.

WEREŠČAGIN, G. J., N. J. ANIČKOVA, AND T. B. FORSCH, 1931. Methoden der hydrochemischen Analyse in der limnologischen Praxis. *Arch. f. Hydrobiol.*, **23**: 167–230.

ZOBELL, C. E., AND D. Q. ANDERSON, 1936. Observations on the multiplication of bacteria in different volumes of stored sea water and the influence of oxygen tension and solid surfaces. *Biol. Bull.*, **71**: 324–342.

ZOBELL, C. E., AND JEAN E. CONN, 1940. Studies on the thermal sensitivity of marine bacteria. *Jour. Bact.*, **40**: (In press).

ZOBELL, C. E., AND JANICE STADLER, 1940*a*. The effect of oxygen tension on the oxygen uptake of lake bacteria. *Jour. Bact.*, **39**: 307–322.

ZOBELL, C. E., AND JANICE STADLER, 1940*b*. The oxidation of lignin by lake bacteria. *Arch. f. Hydrobiol.* (In press).