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STUDIES ON THE CHEMICAL PRESERVATION OF WATER SAMPLES*

By

CLAUDE E. ZOBELL and BARBARA FAY BROWN[1]

Scripps Institution of Oceanography
University of California
La Jolla, California

During the storage of water samples collected from the sea, rivers or lakes, increased bacterial activity causes changes in the organic, oxygen, carbon dioxide, ammonia, nitrate or phosphate content or the H-ion concentration of the water (Waksman and Carey, 1935; ZoBell and Anderson, 1936). These changes occur even near 0° C, although refrigeration tends to retard bacterial activity. Consequently, chemical preservatives are frequently added to water samples which are to be analyzed for any of the aforementioned constituents or properties unless the water is to be analyzed soon after collection. Likewise plankton samples are often preserved for later observation.

While the choice of a preservative depends upon the constituent to be determined, in general it should be colorless, miscible with water, chemically inert, preferably volatile or readily neutralized, and of such a nature that it will not interfere with the analytical procedures to be used for the examination of the water. This report is concerned with a study of the relative efficiency of certain preservatives which are widely used or recommended for preventing bacterial activity in samples of sea or fresh water.

EXPERIMENTAL

In the first series of experiments oxygen consumption was used as a criterion of bacterial activity in samples of sea water treated with different preservatives. The work of Bronfenbrenner, Hershey and Doubly (1939) has established that oxygen consumption is a satisfactory method of evaluating the toxic or preservative action of disinfectants. The amount of oxygen consumed is more or less directly proportional to the number of bacteria present and their rate of

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respiration under given conditions (Johnson, 1936). The respiration of bacteria and allied micro-organisms is not influenced by the oxygen tension of the water until it is reduced to less than 0.3 mgm of oxygen per liter (ZoBell, 1940). If the bacteria are not respiring, it is doubtful if they are activating any other chemical changes.

The water samples were collected in 5-gallon carboys, thoroughly shaken to insure uniformity in composition and siphoned into 145 ml glass-stoppered bottles. Preservatives were added to some of the bottles and some were left untreated as controls. Sterile controls were prepared either by heating the water to 124° C for 15 minutes, or by passing it through a fine Mandler filter, after which it was reoxygenated prior to siphoning into glass-stoppered bottles. The dissolved oxygen content of the water was determined by the Winkler method at the beginning of the experiment, and other bottles were analyzed for oxygen after different periods of incubation in the water bath at 22° C. Representative results obtained with some reagents commonly used as preservatives are summarized in Table I.

TABLE I—Oxygen Consumed by Bacteria in 20 Days at 22° C in Sea Water Treated with Different Preservatives

Treatment	*Initial oxygen content*	*Oxygen content after 20 days*	*Oxygen consumed in 20 days*
	mgm/l.	*mgm/l.*	*mgm/l.*
None (control)	7.56	4.41	3.15
0.1% Toluol	7.62	5.16	2.46
0.1% Phenol	7.04	6.30	0.74
0.1% Tricresol	7.59	6.92	0.67
0.1% Formaldehyde	6.69	6.28	0.41
0.5% Chloroform	7.50	7.37	0.13
0.2% Carbon disulfide	7.01	4.34	2.67
1.0% Ether	6.76	3.92	2.83
Heat sterilized	7.36	7.36	0.00
Mandler filtered	7.48	7.45	0.03

The data indicate that none of the reagents in the concentrations used, with the possible exception of chloroform, could be relied upon to preserve samples of sea water for 20 days. However, further experiments have demonstrated that some of the reagents are effective in higher concentrations or for shorter periods of time. This is shown by the data in Table II. Similar results have been obtained with various samples of sea water, as well as with samples of fresh water. The results have been substantiated by experiments in which

bacterial multiplication, nitrate reduction and ammonia production have been employed as criteria of bacterial activity.

DISCUSSION OF RESULTS

Although toluol is commonly used as a preservative for biological materials (Tauber, 1937), it is not satisfactory for the preservation of water samples. Toluol is only slightly soluble in water, but even when its solubility and dispersion have been increased with alcohol or ether it does not prevent the growth of all water bacteria. In fact, certain micro-organisms oxidize toluol. Störmer (1908), Gray and Thornton (1928) and Tausson (1929) have isolated organisms from soil which utilize toluol, and Grant and ZoBell (1942) have reported the occurrence of bacteria in the sea which obtain their carbon and energy requirements from toluol. There was no bacterial activity in water saturated with a mixture of toluol dissolved in chloroform, but the mixture was little more efficacious as a preservative than chloroform alone.

TABLE II—Amount of Oxygen Consumed by Bacteria in Sea Water Enriched with 10 p.p.m. of Peptone after Different Periods of Incubation at 22° C

Treatment	*Oxygen consumed after:*			
	1 day	*2 days*	*5 days*	*10 days*
	mgm/l.	*mgm/l.*	*mgm/l.*	*mgm/l.*
None (control)	2.13	5.70	8.04*	—
0.10% Phenol	0.03	0.38	1.26	5.41
0.25% Phenol	0.00	0.08	0.94	1.60
0.50% Phenol	0.00	0.00	0.00	0.00
0.10% Formaldehyde	0.04	0.36	1.89	4.67
0.25% Formaldehyde	0.00	0.00	0.00	0.00
1.00% Ether	1.72	4.91	7.82*	—
5.00% Ether	0.26	1.07	2.21	4.39
0.50% Chloroform	0.00	0.00	0.07	0.08
0.10% Toluol	0.89	5.27	7.84*	—
0.20% Carbon disulfide	2.29	3.92	5.40	7.86*
0.10% Thymol	1.41	4.38	7.97*	—
Heat sterilized	0.00	0.00	0.00	0.00

* All of the dissolved oxygen was consumed.

One per cent phenol kills most bacteria in a few minutes at room temperature, and 0.1 per cent phenol usually inhibits growth. However, some soil and water bacteria are very resistant to phenol. According to ZoBell, Grant and Haas (1943), a few marine bacteria

have been observed which multiply in the presence of 1 per cent phenol. Some of them multiply in mineral media containing 0.1 per cent phenol as the only energy source. Phenol-resistant or phenol-oxidizing bacteria have been found in soil by Matthews (1924), Gray and Thornton (1928), Evans and Happold (1939), Vigier (1941) and others. Erikson (1941) studied *Micromonospora* species from lake mud which utilize phenol, cresol, toluol, α-naphthol, naphthalene and paradichlorobenzene. We have also encountered phenol-tolerant micro-organisms in sea water, but in about 95 per cent of the 140 ml samples of raw sea water treated with 0.5 per cent phenol there was no evidence of bacterial activity after 20 days at 22° C. Concentrations of phenol as low as 0.05 per cent are definitely inhibitory but are not dependable preservatives. It is doubtful if there are any micro-organisms which can tolerate 5 per cent phenol, but it is hardly practical to add this much to water samples.

None of the cresols used either alone or in combination have proved to be as good as phenol for the preservation of water samples. Pure cresol dissolves in water only with difficulty, and when emulsified with soaps or alkalis, certain salts are precipitated from sea water, thereby rendering the sample useless for many analyses. Certain micro-organisms tolerate a saturated solution of cresol in water. The oxidation of o-cresol, m-cresol and p-cresol by soil micro-organisms has been reported by Gray and Thornton (1928). Cresol-oxidizing bacteria were found in lakes by Erikson (1941). Repeatedly we have found water samples treated with an excess of tricresol becoming soupy with the profuse growth of micro-organisms, although cresol-tolerant organisms do not occur in all water samples.

Some bacteria multiply and are otherwise physiologically active in water treated with 0.1 per cent formaldehyde (Table II). However, in all of the 34 water samples tried, 0.25 per cent formaldehyde has proved to be a dependable preservative. This concentration reduces the solubility of oxygen in water, and formaldehyde may react with nitrogenous organic matter or interfere with chemical analyses which involve the use of oxidizing agents.

Diethyl ether, chloroform and carbon disulfide usually inhibit bacterial activity in water samples, but there are soil and water bacteria which grow in saturated solutions. Chloroform is the most effective of the three. Various combinations of ether, chloroform and carbon disulfide have proved to be no better than when these reagents have been used alone. However, we have found no bacteria which would grow in a combination of 0.25 per cent phenol and 0.5 per cent chloroform. Apparently the phenol-tolerant organisms which are

encountered occasionally cannot tolerate 0.5 per cent chloroform. As much as 0.8 per cent chloroform can be dissolved in water, but it requires considerable shaking of the sample to get 0.5 per cent chloroform in solution. If not completely dissolved, certain colorimetric indicators are preferentially dissolved in the droplets of chloroform, thereby complicating analytical procedures.

Adding enough hydrochloric or sulfuric acid to water to render it more acid in reaction than pH 1.5 provides for its prolonged preservation, as shown by the data in Table III. This requires approximately 5 ml of concentrated HCl or 2.5 ml of H_2SO_4 per liter of sea water. Considerably less acid is required to preserve fresh water, the amount depending primarily upon the buffer capacity. Acidu-

TABLE III—Oxygen Consumed by Bacteria at 22° C in Sea Water Enriched with 10 p.p.m. of Peptone and Treated with Different Concentrations of Hydrochloric or Sulfuric Acid

Acid added	*Amount added*	*pH of water*	*Oxygen consumed after:*		
			5 days	*10 days*	*20 days*
	ml/l.		*mgm/l.*	*mgm/l.*	*mgm/l.*
None	—	8.23	7.81	—	—
Hydrochloric	2.5	1.73	0.95	2.46	5.16
Hydrochloric	5.0	1.38	0.00	0.00	0.04
Hydrochloric	10.0	1.11	0.00	0.00	0.00
Sulfuric	2.5	1.37	0.00	0.08	0.26
Sulfuric	5.0	1.13	0.00	0.02	0.03
Sulfuric	10.0	0.93	0.00	0.00	0.00

lating the water reduces the solubility of oxygen, but the water samples preserved with acid are suitable for most other analyses. Water samples to be analyzed for oxygen content should be treated with the Winkler reagents shortly after the collection of the water. There are a few micro-organisms occurring in nature, such as *Thiobacillus thiooxidans*, for example, which can grow at pH 1.5, but we have never found any evidence of their activities in the numerous samples of sea water, fresh water and sewage which have been acidulated. Phillips and Hatfield (1941) recommend preserving sewage samples for B. O. D. determinations by lowering the pH and then holding the acidulated samples at a low temperature.

Heavy metals such as mercury, copper and silver salts are generally good disinfectants. While low concentrations usually prevent bacterial activity in water samples, salts of heavy metals render the water unsuitable for most kinds of analyses.

Similarly oxidizing agents such as chlorine or iodine compounds,

permanganate or peroxides materially alter the composition of the water and interfere with many analytical procedures. Substances which are effective only in high concentrations dilute the sample too much or may be too expensive to be practical water preservatives.

The use of detergents including 0.1 per cent Aerosol OT, Dreft, Santomerse or Tergitol 4T failed to enhance the preservative action of any of the reagents listed in Table I sufficiently to recommend the addition of detergents. Some of the detergents produced undesirable precipitates in sea water. Petroff and Schain (1940) and others have shown that the killing power of some antiseptics is enhanced by detergents or by surface tension depressants.

Chloroform, formaldehyde or acid are just about as effective in the presence of organic matter as in its absence, so these reagents can be used to preserve plankton samples. There was no bacterial activity in sea water treated with 5 per cent peptone in the presence of 0.25 per cent formaldehyde, 0.5 per cent chloroform or enough acid to reduce the pH to less than 1.5. The acidulation of water does result in the destruction of certain calcareous structures of plankton organisms.

CONCLUSIONS

Toluol, tricresol, thymol, carbon disulfide and diethyl ether are not dependable preservatives for water samples to be examined for constituents or properties which may be altered by bacterial activity.

If they are not objectionable for other reasons, either 0.5 per cent chloroform or 0.25 per cent formaldehyde can be used to preserve water samples.

Nearly all water samples can be preserved indefinitely by the addition of 0.5 per cent phenol, but occasionally micro-organisms are encountered which live in the presence of 1 per cent phenol. When supplemented by 0.5 per cent chloroform, 0.5 per cent phenol is a dependable preservative.

Acidulating water to pH 1.5 which requires about 2.5 ml of sulfuric or 5.0 ml of hydrochloric acid per liter of sea water prevents the activity of micro-organisms ordinarily found in water.

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