

Microbiological Process Report

Factors Affecting the Survival of Bacteria in Sea Water¹

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During recent years, the use of marine outfalls for the disposal of sanitary and industrial wastes has increased greatly and information regarding the fate of bacteria in sea water has assumed public health significance. Unpolluted sea water does not contain large numbers of bacteria, and bacteria which enter the oceans by way of land drainage and sewage outfalls die quickly. The numbers of bacteria in sea water are determined by those factors which influence growth and reproduction as well as by those that control survival and cause death. General aspects of the survival of bacteria in sea water were discussed by Ketchum (1953), Harvey (1957), and Wood (1958). The subject was considered in detail by ZoBell (1946), Greenberg (1956), and Orlob, (1956).

Waksman and Hotchkiss (1937) suggested that the paucity of bacteria in sea water may be the result of any one or combination of various inter-related physico-chemical and biological factors including: (a) the adsorption of bacteria and their sedimentation; (b) the destructive action of sunlight; (c) the lack of required nutrients; (d) the presence in sea water of toxic substances; (e) the presence of bacteriophages; (f) the utilization of bacteria as food by protozoa and other predators; and (g) the competitive and antagonistic effects of other microorganisms. These possibilities provide a convenient framework within which knowledge of the influence of various factors on the survival of bacteria in sea water can be reviewed.

I. ADSORPTION AND SEDIMENTATION

In addition to living organisms, clear sea water contains 0.2 to 2.0 ppm of both inorganic and organic particles in suspension. Since approximately half of this particulate material is inorganic and claylike (Harvey, 1957) it may be expected that bacteria will be adsorbed and that the particles will eventually settle and be deposited on the ocean floor. Attached organisms thus become removed from surface waters and

accumulate in sediments. Marine sediments contain as much as 18 per cent organic matter (ZoBell, 1946) and bacteria in bottom deposits may grow and multiply.

Russell (1892) demonstrated that there were greater numbers of bacteria in marine sediments than in overlying waters, and Rubentschik *et al.* (1936) reported that coliform bacteria were adsorbed by muds from salt lakes. The adsorption capacity of muds varied and there were differences in the susceptibility of different bacteria to adsorption. Clays adsorbed bacteria to a greater extent than did larger particles. Similar results were obtained by Waksman and Vartiovaara (1938) who demonstrated that the clay fraction of marine sediments adsorbed more bacteria than did silt or sand. Weiss (1951) reported that sea water flocculated and decreased the capacity of silt to adsorb bacteria, but great numbers of bacteria were adsorbed when there was sufficient silt to cause turbidity. Diénert and Guillard (1940) and Nusbaum and Garver (1955) attributed reduced bacterial counts to adsorption and sedimentation of coliform bacteria in sea water. Orlob (1956) added settled sewage to each of two samples of sea water and periodically determined the number of surviving coliform organisms. One sample was agitated prior to testing. The other sample was disturbed as little as possible. Bacterial numbers were invariably higher in the agitated sample, suggesting that sedimentation was a factor of significance. Large numbers of coliform bacteria were detected in marine sediments underlying the paths of two outfalls that discharged unchlorinated sewage (Rittenberg *et al.*, 1958), and it appeared that sedimentation was responsible for the distribution observed. The importance of adsorption and sedimentation in the self-purification process of streams was discussed by Phelps (1944) and Gainey and Lord (1952).

II. DESTRUCTIVE ACTION OF SUNLIGHT

There is a great deal of information concerning the lethal action of ultraviolet light on bacteria but few studies have been made of the influence of sunlight on the survival of bacteria in sea water. Gaarder and Spärck (1931) attributed low numbers of bacteria in an oyster pool to the unfavorable effect of sunlight, but

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studies by Reuszer (1933) showed no relation between the numbers of bacteria in samples of surface water and the amount of sunlight during summer months. Vaccaro and Ryther (1954) found that sunlight had no effect on the respiration of marine bacteria suspended in bottles 10 in. below the surface. Although ZoBell and McEwen (1935) failed to find any evidence that sunlight influenced the daily or seasonal occurrence of bacteria, they observed that bacteria died rapidly in shallow layers of sea water exposed to midsummer sunlight. The lethal effect of sunlight did not extend to depths exceeding 20 cm.

Insufficient experimental data are available to evaluate the significance of the destructive action of sunlight. Bacteria in surface waters may be exposed to ultraviolet light but this need not result in their destruction. Kelner (1949) demonstrated that bacteria apparently killed by ultraviolet radiation can be reactivated by exposure to visible light. It is possible that bacteria inactivated by ultraviolet radiation in surface waters may be reactivated at depths penetrated by visible but not by ultraviolet light. The effect of sunlight is eliminated in laboratory studies where water samples are incubated in the dark.

III. LACK OF NUTRIENTS

Sea water may be considered a dilute culture medium in which the concentration of dissolved organic matter does not exceed 10 ppm (ZoBell, 1946). Sea water contains most of the known elements as inorganic ions and dissolved gases but some important nutrients such as iron, nitrogen, and phosphorous are present in very small amounts. Furthermore, the organic matter content of sea water is low except in the vicinity of land and in some bottom deposits.

Burke and Baird (1931) showed that many fresh water bacteria grow in sea water supplemented with organic matter. Similar studies by Krassilnikov (1938) and Vaccaro *et al.* (1950) demonstrated that the addition to sea water of organic nutrients, such as glucose and peptone, decreased the rate of death of *Escherichia coli*. Orlob (1956) added lactose broth to samples of sea water and found that the time preceding logarithmic disappearance of coliform bacteria was extended. The lag period varied directly with the concentration of broth added but, as soon as a significant decrease in the numbers of viable cells occurred, a death rate similar to that in unamended water was observed.

Information concerning the influence of oxygen on survival of bacteria in sea water is conflicting. Buttiaux and Leurs (1953) reported that oxygenation of sea water did not affect the survival of *Salmonella typhimurium*, whereas Waksman and Carey (1935a) showed that oxygen concentration was of importance for bacterial multiplication in stored sea water. ZoBell and Anderson (1936) attributed the results of Waksman

and Carey to surface phenomena rather than to oxygen concentration.

Waksman and Carey (1935a) showed that sea water contains sufficient organic matter to support a more extensive bacterial population than is usually observed, and demonstrated that the organic matter in sea water is not completely resistant to bacterial decomposition (Waksman and Carey, 1935b). When sea water was incubated in glass containers, 25 to 50 per cent of the organic matter in the water was decomposed within 10 to 12 days. The decomposition of organic carbon was limited by the amount of available nitrogen present in the water. Steininger (1951) found that the concentration of organic matter in water of tidal pools was adequate for multiplication of *Salmonella paratyphi* type B.

IV. TOXIC SUBSTANCES

A. Inorganic salts. The salinity of surface sea water is approximately 3.5 per cent (ZoBell, 1946) and, on the basis of concentration, inorganic salts are the most potentially toxic substances present in the sea. Sea salts may influence the survival of bacteria adversely by a general osmotic effect or by specific ion toxicity.

Kórfinek (1926) found that bacteria isolated from fresh water grew in media containing various concentrations of sea water. He observed no autolysis of the cells when they were suspended in sea water. Burke and Baird (1931) reported that the survival of fresh water bacteria in sea water was comparable to that in tap water. Persistence was influenced by temperature and the presence of organic matter. Growth and extended survival of fresh water bacteria was obtained in nutrient broth prepared to contain NaCl at 2 to 4 times the concentration at which it occurs in natural sea water. ZoBell and Feltham (1933) concluded that the rapid death of bacteria in sea water was not due primarily to its high salt concentration, and Nusbaum and Garver (1955) stated, "it is believed that the normal saline constituents of sea water are not antagonistic to enteric organisms."

Low salt concentrations have been shown to stimulate the growth of certain bacteria (Ware *et al.*, 1955). ZoBell (1936) reported that coliform bacteria survive longer in 6 per cent NaCl solutions than in natural sea water, indicating that salt per se, at levels common to marine environments, is not particularly detrimental to bacteria.

The survival of *E. coli* in water from the Great Salt Lake in Utah (salinity of approximately 32 per cent) was investigated by ZoBell *et al.* (1937) who observed a kill of over 95 per cent in 1 min, but conflicting results were obtained by Fraser and Argall (1954). The latter investigators reported that survival time increased with a decrease in temperature and that the test organism was recoverable after 24 hr.

Johannesson (1957) found that the survival of *E. coli*

in sea water was favored greatly by very low concentrations of cysteine and suggested that the toxic agent in sea water was iodate. The presence of iodate in sea water was demonstrated by chemical procedures and it was postulated that the favorable effect of cysteine on the survival of *E. coli* in sea water resulted from reaction of the sulfhydryl group of the amino acid with the toxic ion. Other workers (Shaw and Cooper, 1957) reviewed the mechanism of iodine oxidation in sea water and concluded that iodate would not be formed. They suggested that the oxidized form of iodine present in the sea is an equilibrium mixture of iodide and hypiodous acid (hypiodite). This view was repudiated by Sugawara and Terada (1958) and remains to be clarified. Perchlorate was recently identified in sea water (Bass-Becking *et al.*, 1958) and may be considered as a potentially toxic ion capable of influencing the survival of bacteria.

B. Microbial antagonism and antibiotic substances. In 1889 de Giaxa demonstrated that some marine bacteria were antagonistic to *Vibrio comma*, (de Giaxa, 1889) and when grown in association with isolated antagonists, *V. comma* was unable to produce experimental infections. Kiribayashi and Aida (1934) believed that the saprophytic organisms present in sea water influence the survival of enteric bacteria, and Kórínek (1927) noted that fresh water bacteria were influenced adversely by marine organisms.

Rosenfeld and ZoBell (1947) pointed out that the death of bacteria in sea water was greater than accounted for by salinity or osmotic pressure and suggested that antibiotic substances produced by microorganisms had toxic effects. They tested 58 species of marine bacteria and demonstrated that 9 produced antibiotics active against various test organisms. The most actively antagonistic marine bacteria were species of the genera *Bacillus* and *Micrococcus*. The antimicrobial action of sea water and culture broths of the antagonists was similar in that each was reduced by filtration. It was concluded that the rapid death of bacteria in sea water was due to some extent to an autochthonous microflora of antibiotic-producing organisms.

Ketchum *et al.* (1949) observed that a second inoculum of *E. coli* in sea water died more rapidly than the first, and Sherwood (1952) found that sea water which had supported growth of *E. coli* did not favor prolonged survival of *Salmonella typhosa* or *S. paratyphi*. These results were interpreted in terms of microbial antagonism and the production of antibiotic substances. Ketchum *et al.* (1949) suggested that antibiotic substances produced by marine organisms were responsible for the death of coliform bacteria in sea water, and Vaccaro *et al.* (1950) concluded that "the most probable cause of death of *E. coli* populations is an antibiotic action which requires the presence of the normal marine flora." Ketchum *et al.* (1952) observed a 99 per cent

decrease in the number of coliform bacteria in a tidal estuary. The contribution of dilution and predation was minor compared to that of unidentified factors.

V. BACTERIOPHAGES AND PREDATORS

Arloing *et al.* (1925) and d'Herelle (1926) believed that bacteriophages were a significant factor in the rapid death of bacteria in sea water, whereas Gilde-meister and Watanabe (1931) and de Balsac *et al.* (1952) expressed an opposite opinion. Guélin (1948) demonstrated that phages active against bacteria of the colityphoid group were present in sea water and that their numbers varied directly with the degree of sewage pollution. In more recent studies, Kriss and Rukina (1947) and Spencer (1955) isolated phages from sea water that were active against marine bacteria. Although bacteriophages are detected in sea water, they do not occur in great numbers. Recourse to enrichment procedures has been necessary to demonstrate their presence. There are no studies in which the lytic activity of bacteriophages in sea water has been evaluated.

The influence of predators on the survival of bacteria in sea water has not been subject to direct investigation. Waksman and Carey (1935b) and Waksman and Hotchkiss (1937) reported that the numbers of copepods and protozoa in sea water increased as the numbers of bacteria decreased. Since the reduction in bacterial numbers was not accompanied by decreased oxygen consumption, it appeared that organisms such as protozoa were developing at the expense of the bacteria. Stryszak (1949) believed that the increased survival of bacteria at low temperatures was due to reduced activity of predatory protozoa rather than to any direct effect of temperature on the bacteria. Ketchum *et al.* (1952) inferred that the contribution of protozoan predators to the death of bacteria in sea water was of minor importance. Predation by higher forms is difficult to evaluate.

VI. STERILIZATION

The earliest studies of the influence of sterilization on the survival of bacteria in sea water were published in 1885 by Nicati and Reitsch. In a series of laboratory experiments they showed that the survival of *V. comma* varied in different waters, and that survival time was consistently extended by sterilizing sea water prior to inoculation. Similar results were reported in 1889 by de Giaxa. He observed that the typhoid bacillus and the cholera vibrio survived 25 and 36 days respectively in heat sterilized sea water, whereas in untreated sea water they persisted for only 9 and 4 days. These early studies have been repeated by various investigators located throughout the world (Kiribayashi and Aida, 1934; ZoBell, 1936; Krassilnikov, 1938; Ketchum *et al.*, 1949; Vaccaro *et al.*, 1950; Nusbaum and Garver, 1955;

Richou *et al.*, 1955) and in each case it was reported that bacteria survived longer in heat sterilized sea water than in untreated sea water. The beneficial effect of heat sterilization on the survival of bacteria in sea water varies with the season of the year, being most marked in summer months (Vaccaro, 1950).

Heat is not the only sterilization procedure which renders sea water more favorable for the survival of bacteria. Beard and Meadowcroft (1935) showed that the rate of death of *E. coli* and *Salmonella typhosa* was greater in untreated than in filter sterilized water. Similar results were reported by ZoBell (1936) and Krassilnikov (1938) using waters sterilized by passage through Berkfeld and Seitz filters, respectively. In contrast to the foregoing observations, de Balsac *et al.* (1952) reported that the survival of bacteria in water that had been sterilized by passage through Chamberland filters was no greater than in untreated sea water.

Pasteurization (Vaccaro *et al.*, 1950) and boiling (Krassilnikov, 1938; Ketchum *et al.*, 1949; Vaccaro, *et al.*, 1950; Williams, 1950) also rendered sea water more favorable for bacterial survival. In one study (Vaccaro *et al.*, 1950) sea water was treated with 10 ppm chlorine and dechlorinated after 1 hr by adding thiosulfate. The survival of *E. coli* in water sterilized by chlorination was found to be greater than that in untreated water and identical with that in heat sterilized sea water.

Various types of semipermeable membranes have been employed in studies of the viability of bacteria in sea water. Beard and Meadowcroft (1935) suspended cells of *S. typhosa* and *E. coli* in filtered and unfiltered portions of samples of sea water. The suspensions were placed in dialyzing flasks and immersed in San Francisco Bay. The rate of death of the bacteria was greater in untreated water than in water which had been sterilized by passage through Chamberland filters. Similar results were obtained by ZoBell (1936) who exposed suspended coliform bacteria contained in semipermeable tubes (porcelain filter candles impregnated with collodion) in untreated sea water, filter sterilized sea water, and autoclaved sea water. Most rapid death was observed in untreated sea water and survival was greatest in autoclaved sea water. Williams (1950) attempted to simulate natural conditions by placing mixtures of sewage and sea water in Cellophane dialyzing tubing and suspending the tubes in Puget Sound. Seven of 11 samples showed more than a 95 per cent reduction in coliform counts in 24 hr, but the results were erratic. In some instances increases were noted, whereas in other experiments little or no change was observed. The survival of coliform bacteria contained in Cellophane dialyzing tubing placed in San Francisco Bay was also examined by Nusbaum and Garver (1955). In one case they noted a large initial increase in numbers (400 per cent increase in 2 days) and a subsequent decrease to 13 per cent survival after 4 days.

SUMMARY

Great numbers of bacteria enter the sea by way of surface drainage and sewage outfalls but, as one proceeds outward from land, an extremely rapid decrease in the numbers of bacteria in the water is observed. Numerous explanations for the decrease in numbers have been suggested. Dilution, adsorption, and sedimentation are factors of importance but they do not account fully for the rapid disappearance of bacteria that enter the sea. The significance of biological factors has been emphasized by various investigators who observed that the survival of bacteria is greater in heat sterilized sea water than in untreated sea water. Although it has been postulated that predators, bacteriophages, and heat labile toxic substances of biological origin (antibiotics) contribute to the rapid death of bacteria in natural water, the evidence is primarily indirect and, therefore, not conclusive. Hydrostatic pressure, solar radiation, temperature, salinity, pH, oxidation-reduction potential, and nutrient availability are physicochemical factors known to influence the activities of microorganisms, but their significance in maintaining the bacterial population of the sea at a reduced level remains to be evaluated. Likewise, our present knowledge of the interrelationships of microorganisms in marine environments must be extended before the bactericidal action of sea water can be explained.

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