# THE EFFECT OF SOLID SURFACES UPON BACTERIAL ACTIVITY[1]

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The bacterial population of water samples from the sea, lakes or other sources usually increases during storage. While several different factors may be operative (Prescott and Winslow, 1931), it is noteworthy that the magnitude of the increase is often related to the size of the receptacle in which the water is stored. For example, Whipple (1901) found that the bacterial population of water, which initially contained an average of 77 bacteria per ml., increased to 300 per ml. in a gallon, 900 per ml. in a quart, 7,020 per ml. in a pint and 41,400 per ml. in 2-ounce bottles after 24 hours' incubation under comparable conditions. He attributed this to the greater availability of oxygen in the small receptacles which were not filled to capacity. However, ZoBell and Stadler (1940) have shown that the multiplication and respiration of aerobic bacteria is independent of the oxygen tension within the examined ranges of 0.30 to 36 mgm./liter.

Using oxygen consumption as well as bacterial multiplication in glass-stoppered bottles filled to capacity with sea water as criteria, ZoBell and Anderson (1936) noted that bacteria are generally more active in small than in large receptacles of similar shape. Since the small receptacles present relatively more solid surface per unit volume of stored water than large receptacles, they concluded that solid surfaces are beneficial to bacteria in dilute nutrient solutions. A similar conclusion was reached by Lloyd (1937). The following report is concerned with the ways in which solid or adsorbing surfaces may influence bacterial activity.

## GLASS SURFACES ADSORB NUTRIENTS

Inasmuch as the effect of volume or solid surfaces upon bacterial activity can be demonstrated only in very dilute nutrient solutions and since the effect is more pronounced with colloidal than with dissolved nutrients, ZoBell (1937) suggested that nutrients may be concentrated on solid surfaces. This explanation is supported by more recent observations here and elsewhere. The work of ZoBell and Grant (1943) shows that bacterial activity is directly proportional to the concentration of nutrients when the latter is less than 10 mgm./l. Since sea water ordinarily contains less than 5 mgm./l. of organic matter (Krogh, 1931), only a small part of which is readily attacked by bacteria (Waksman and Carey, 1935a), it follows that any factor which tends to concentrate the organic matter would promote bacterial activity.

Although the small quantity and complexity of the organic content of sea water make it difficult to estimate the amount which is adsorbed by glass or other solid surfaces under different conditions, there are several ways in which

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it can be demonstrated that organic material is adsorbed. The sea water used for this purpose was collected in carboys, passed through a fine sintered-glass filter and stored in a water bath at 0°C. The low temperature inhibits (but does not prevent) bacterial activity while the organic matter is being adsorbed.

The glassware used in the experiments was thoroughly cleaned in hot sulfuric-acid-dichromate-cleaning-solution, treated with trisodium phosphate solution and finally thoroughly rinsed with distilled water. Glass beads and glass wool were heated in a muffle furnace for three hours to free them of organic matter. There is no evidence that the glass carried any nutrients or reactive material. Pyrex glass was used almost exclusively, although no differences were found in the amount of organic matter adsorbed, or in the attachment of bacteria, on Pyrex glass and other kinds of glass.

After standing in sea water at 0°C. for several days, glass slides were removed and stained with methylene blue, gentian violet and carbol fuchsin. Microscopic examination showed the presence of numerous bacteria covering one-fiftieth to one-tenth of the surface. All of the surface was coated with an irregular film of material which stained like complex organic matter. There was little or none of this stained film on slides which were immersed in sea water for only a short period of time. Likewise there was no film on slides immersed in "aged" sea water, the organic content of which had been reduced to less than 0.5 mgm./l.

In other experiments sea water was stored at near 0°C. in 600 ml. bottles partly filled with glass wool, glass beads or with glass tubes to give 2 to 200 cm.$^2$ of glass surface per ml. of solution. After different periods of storage the water was carefully siphoned out of the bottles and 100 ml. of dilute permanganate solution was introduced. While heating the bottles in a boiling water bath, they were manipulated to rinse the interior with the oxidizing agent after which they were cooled and the contents titrated with N/80 thiosulfate. The procedure indicated that from 2 to 27 per cent of the organic content of the sea water had been adsorbed by the glass. The amount adsorbed was roughly proportional to the area of solid surface exposed to the water. Repeating this experiment with dilute aqueous solutions or suspensions of known chemical composition showed that glucose, lactate and glycerol were not adsorbed perceptibly, while lignoprotein, nutrose and an emulsified chitin preparation were adsorbed. Very little peptone was adsorbed.

Using similar methods Stark *et al.* (1938) found that measurable amounts of organic matter accumulate within a few hours on chemically clean glass slides immersed in lake water. They expressed the belief that the accumulation of organic nutrients favors bacterial growth. Corroborative evidence is given by the studies of Heukelekian and Heller (1940) on the relation between food concentration and solid surfaces.

Harvey (1941) noted that small glass tubes immersed in sea water adsorbed enough organic matter to alter the surface tension as shown by capillary attraction. Treating a solution containing 10 mgm./l. of peptone with permanganate before and after being exposed to enough glass wool to give a surface of 50 cm.$^2$/

ml. of solution, he found that from 0 to 7 per cent of the peptone was adsorbed by the glass wool. Although the amount of organic matter adsorbed is barely more than the range of the experimental error, Harvey speculates that there might be enough to give a monomolecular layer of nutrient on the glass which might be replaced as fast as it is utilized by bacteria. He points out that according to Blodgett (1935), layers many molecules thick may build up on solid surfaces. A key to the voluminous literature on the factors influencing the adsorption by solids is given by Adam (1938).

## BIOLOGICAL EVIDENCE OF ADSORPTION

Immediately after its collection and filtration through fine sintered glass filters, sea water was dispensed in 145 ml. glass-stoppered bottles. Part of the bottles were loaded with several pieces of thin-walled glass tubing tightly packed in an upright position, which materially increased the area of solid surface ex-

TABLE 1

*Oxygen consumed by bacteria after different periods of time in sea water stored at near 0°C. and at 22°C. in glass-stoppered bottles, some of which were filled with glass tubes to increase the area of solid surface*

| | GLASS-STOPPERED BOTTLES | | BOTTLES WITH GLASS TUBES | |
|---|---|---|---|---|
| | Ratio of cm.²:ml. | | | |
| | 1.1:1 | | 7.2:1 | |
| | Incubation temperature (°C.) | | | |
| | 0 | 22 | 0 | 22 |
| Oxygen consumed in 5 days (mgm./l.) | 0.02 | 0.37 | 0.01 | 0.46 |
| Oxygen consumed in 10 days (mgm./l.) | 0.12 | 0.78 | 0.20 | 0.91 |
| Oxygen consumed in 20 days (mgm./l.) | 0.26 | 1.02 | 0.38 | 1.29 |

posed to water without appreciably decreasing the volume. The oxygen content of the water in some of the bottles was determined at once by a refined Winkler method which was accurate to ±0.01 mgm./l. Part of the bottles were stored in a water bath at 22°C. and part of them were stored in a water bath at near 0°C. Bottles were removed for analysis after 5, 10 and 20 days' incubation. The protocol of a representative experiment is summarized in table 1.

As might be expected, bacteria multiplied more rapidly and consumed more oxygen in the water incubated at the higher temperature. At both temperatures more oxygen was consumed in the water which was exposed to the largest solid surface. Previous work has shown that the amount of oxygen consumed is directly proportional to the amount of organic matter oxidized. It requires approximately 1 mgm. of oxygen to oxidize 1 mgm. of organic matter found in sea water. For each milligram of organic carbon which is oxidized to carbon dioxide, 0.45 to 0.65 mgm. of organic carbon is converted into bacterial protoplasm (Waksman and Carey, 1935b, ZoBell and Grant, 1943).

The plate count of the water stored at 0°C. increased progressively throughout the experiment. The plate count of the water stored at 22°C. reached a maximum of several million bacteria per ml. in 5 days and then dropped off sharply after 10 days. Although more oxygen was consumed at 22°C. in the bottles which presented the larger surface area from the 5th to the 20th day, the plate count of this water was smaller than in the bottles having no glass tubes to increase the solid surface. This apparent parodox is explained by the abundance of periphytic or sessile bacteria found attached to the glass. From the direct microscopic examination of the water itself and glass slides immersed in the water, it was calculated that there were more bacteria tenaciously attached to the walls of small glass bottles than the number found in the water.

In earlier experiments ZoBell and Anderson (1936) found 31,000 bacteria per ml. of water and an equivalent of 84,000 bacteria per ml. attached to the glass. Sometimes ten times as many bacteria are found attached to the glass as in the

TABLE 2

*Oxygen consumed by bacteria in 20 days at 22°C. in sea water, which had been stored in 145 ml. glass-stoppered bottles for 5 days at 0°C.*

Part of the water was left in the original bottles in which it was stored and part was transferred to clean bottles and the emptied "original" bottles filled with aged sea water. Aged sea water was incubated in clean bottles as a control.

| | INITIAL DISSOLVED OXYGEN CONTENT | DISSOLVED OXYGEN AFTER 20 DAYS | OXYGEN CONSUMED IN 20 DAYS |
|---|---|---|---|
| | *mgm./l.* | *mgm./l.* | *mgm./l.* |
| Original water in original bottles..... | 7.83 | 6.93 | 0.90 |
| Original water in clean bottles..... | 7.86 | 7.18 | 0.68 |
| Aged water in original bottles..... | 7.62 | 7.23 | 0.39 |
| Aged water in clean bottles..... | 7.61 | 7.47 | 0.14 |

water. The ratio of the number of bacteria occurring in the water and on solid surfaces seemed to be influenced by the concentration and kind of organic matter present, the proximity of the solid surface to the water mass, the time and temperature of incubation and the kinds of bacteria present.

The water in some of the bottles was carefully siphoned into sterile chemically clean bottles after 5 days storage at 0°C. The original bottles were drained free of sea water and refilled with aged sea water which contained less than 0.2 mgm./l. of bacteriologically oxidizable organic matter. The decanted water with which the bottles were filled originally and the aged sea water in the original bottles was then incubated at 22°C. for 20 days. Oxygen determinations revealed that detectable quantities of oxidizable organic matter had been adsorbed by the glass of the original bottles as shown by the data in table 2. The data are the average of quadruplicates.

It will be noted that whereas the original water, which was incubated in the bottles (original) in which it had been stored for 5 days at 0°C., consumed an average of 0.90 mgm./l. of oxygen in 20 days at 22°C., water siphoned from the original bottles into chemically clean bottles consumed only 0.68 mgm./l. of

oxygen. The difference is believed to represent the amount of organic matter adsorbed by the glass walls of the original bottle. This interpretation of results is substantiated by the fact that aged sea water consumed 0.25 mgm./l. more oxygen when incubted in the "original" bottles than when incubated in chemically clean bottles.

Figure 1 shows the number of bacteria found by plate counts in sea water stored in glass-stoppered bottles at 12°C. and the number of attached bacteria. The number of attached bacteria per ml. of water was estimated by counting microscopically those attached per unit area of glass slides submerged in the water and multiplying by the total area of glass exposed to the water. After ten days' incubation the water from part of the bottles was siphoned into "new"

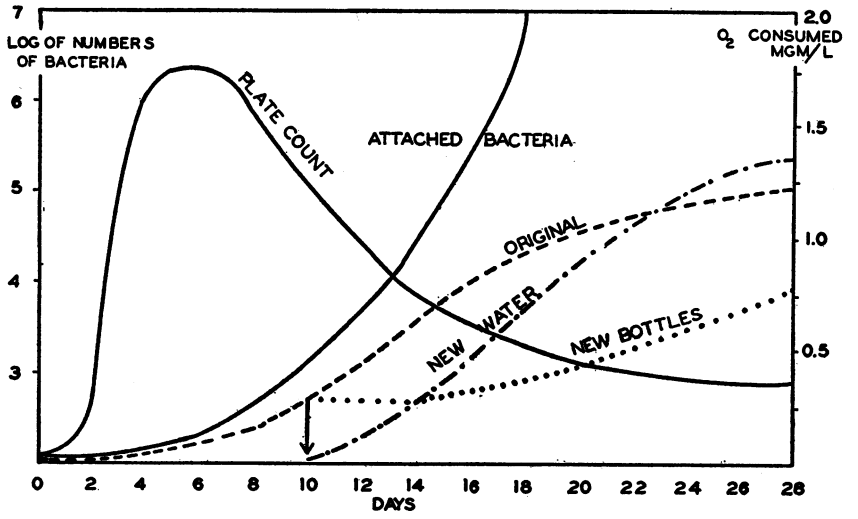


FIG. 1. Number of bacteria found in sea water as indicated by plate counts and the equivalent number per ml. attached to glass surfaces after different periods of incubation at 12°C. (solid lines). The oxygen uptake in the water is also shown (original). After 10 days part of the water was transferred to chemically clean (new) bottles and the emptied bottles were refilled with recently collected (new) water.

chemically clean bottles and the emptied bottles were refilled with "new" recently collected sea water. Determining the oxygen content of water after different periods of incubation showed that little more oxygen was consumed in the decanted or original water in "new" bottles, presumably because a large proportion of its bacterial and organic content had been adsorbed by the original bottle in which it was stored. That both organic matter and bacteria were adsorbed is indicated by the fact that the "new" water in the original bottles consumed more oxygen and consumed it more rapidly than the original water in the original bottles.

The experiment has been repeated several times with similar results. However, in some lots of water the beneficial effect of solid surface upon bacterial activity is barely perceptible, which may be attributable to the quality and

quantity of organic matter, the kinds of bacteria present, the surface tension of the water, the nature of the electrolytes and other factors which are being investigated. The explanation of the negative or indifferent results like those obtained by Castell and McDermott (1942), for example, require further experimental work.

The amount of adsorbable organic matter present in natural waters might be influenced by the bacterial population of the water as well as by the quality of the organic matter itself. Part of the organic matter is actually in the form of attached bacterial cells. During the early stages of storage amorphous organic matter appears to predominate on submerged surfaces. After prolonged storage, glass surfaces are usually completely covered with attached bacteria which obliterate any amorphous organic matter which might remain. The work of ZoBell and Grant (1943) indicates that, after prolonged incubation, 60 to 70 per cent of the oxidizable organic carbon in dilute solutions is oxidized to carbon dioxide, and 30 to 40 per cent is converted into bacterial protoplasm.

Our work (ZoBell, *et al.*, 1943) on bacteria which oxidize petroleum hydrocarbons has supplied the most outstanding examples of the beneficial effect of adsorbing surfaces. Due to the low solubility and relative immiscibility in water, hydrocarbons are attacked very slowly by bacteria in aqueous media. If, however, the hydrocarbons are made available on the extensive surfaces of adsorbents such as sand, shredded asbestos or infusorial earth, the rate of bacterial utilization is accelerated two- to ten-fold. Söhngen (1913) noted that ignited soil, silica or iron oxide promoted the bacterial oxidation of petroleum. He concluded that the growth of the bacteria was a function of the solid surface. Similarly Greig-Smith (1914) found that kieselguhr accelerated the utilization of paraffin hydrocarbons by bacteria.

## SOME BACTERIA ARE SESSILE

Many species of bacteria grow tenaciously attached to solid surfaces. This is the basis of the buried slide technique of Cholodny (1930) for the direct microscopic study of soil bacteria. It is also the basis of the submerged slide technique of Henrici (1933) for an ecologic survey of water bacteria. The work of ZoBell and Allen (1935), Henrici (1936), Hotchkiss and Waksman (1936), Smith and ZoBell (1937), and Waksman and Vartiovaara (1938) indicates that many water bacteria grow attached to solid surfaces. Kusnetzowa (1937) believes that all water bacteria are capable of attaching themselves to glass. Henrici and Johnson (1935) have described several species of periphytic bacteria which grow only attached to a firm substrate.

Exclusive of exotic species from the terrestrial environment along the littoral zone, most of the bacteria found in the sea appear to be associated with solid surfaces. The microscopic examination of suspended particles reveals the presence of numerous bacteria, whereas very few bacteria are found unattached in sea water. This is in agreement with the observation of Lloyd (1930) that marine bacteria are not planktonic but are attached to solid particles. According to Waksman *et al.* (1933) the bacteria of the sea are usually found attached

to larger plankton organisms, existing "only to a very limited extent in the free water of the sea."

The most obvious reason bacteria are found associated with plankton organisms is because the tissue and organic metabolic products of the latter provide a ready source of bacterial food and energy. Also plankton organisms are often coated with a slimy exudate to which bacteria adhere. However, many bacteria do not depend for attachment upon the adhesive properties of solid surfaces, and they are not entirely organotropic because they attach to chemically clean glass, porcelain, plastic, metal and other surfaces. Bacteria will be found tenaciously attached to glass slides an hour or two after submerging the slides in the sea. Such bacteria seem to be stereotropic or thigmotactic.

Upon isolation from submerged slides some of the bacteria grow only on solid surfaces. We have observed sessile bacteria which form a film on the walls of test tubes or flasks without perceptibly clouding dilute nutrient solutions. The most striking example is a chitinoclastic bacterium (ZoBell and Rittenberg, 1938) which grows on strips of chitin with virtually no bacteria occurring in the liquid medium as indicated by the fact that no bacteria may be found in a 2-mm. loopful of fluid although masses of them can be observed growing on solid surfaces.

*Achromobacter marinoglutinosus*, *Achromobacter membranoformis* and *Flavobacterium amocontactum* are new species of sessile, film-forming or attachment bacteria described by ZoBell and Allen (1935). Dr. Robert S. Breed directs my attention to the fact that since the first two organisms have lophotrichous or polar flagella, according to the revised key they should be designated *Pseudomonas marinoglutinosus* and *Pseudomonas membranoformis*. Among the new species of marine bacteria recently described by ZoBell and Upham (1943) are several sessile forms including *Pseudomonas stereotropis*, *P. sessilis*, *P. periphyta*, *P. coenobios*, *P. membranula*, *Bacillus epiphyticus*, *Micrococcus sedentarius*, *M. sedimenteus*, *Achromobacter stationis*, *A. aquamarinus*, *Bacterium sociovivum* and *B. immotum*.

A total of 96 different cultures of bacteria isolated from marine materials have been used to inoculate sea water in wide mouth bottles enriched with 100 mgm./l. of peptone. Sterile chemically clean glass slides were inserted vertically. After different periods of incubation at 25°C., a slide from each bottle was removed. Without fixation the slides were washed, stained and dried. Large numbers of bacteria were found on slides from 29 of the cultures, virtually no bacteria were found on slides from 47 cultures and a variable number of bacteria were found on slides from 20 cultures. Although the procedure does not differentiate clearly between sessile bacteria and non-sessile ones, it shows differences in the attachment propensities of certain bacterial species which can be duplicated.

No relationship was found between the gram reaction of the pure cultures and their attachment propensities. This is significant because according to Eisenberg (1918), gram-positive bacteria of high lipoid content are adsorbed more readily than gram-negative ones. Rubentschik *et al.* (1936) observed no

sharp dividing line between the adsorptive capacity of gram-negative and gram-positive species, although they did note differences in the sessile habits of various species of bacteria.

The development of micro-colonies on glass slides indicates that some of the sessile bacteria multiply while attached to solid surfaces. In very dilute nutrient solutions more bacteria will often be found growing on the walls of the culture receptacle and on immersed glass slides than in the solution (ZoBell and Anderson, 1936).

## HOW BACTERIA ATTACH

Sessile bacteria do not seem to be covered with a mucilaginous substance which causes them to stick to solid surfaces with which they come into contact. This point has been investigated by immersing glass slides in suspensions of bacteria and removing them after different periods of time for microscopic examination. Very few bacteria attach to slides firmly enough to resist being washed off with running water unless the slides have been left in contact with the bacteria in dilute nutrient solutions for at least a few hours. Likewise only a small proportion of the bacteria transferred to glass slides and air dried, as in the preparation of a smear for staining, adhere to the glass without heat or chemical fixation. However, when the glass slides are left in dilute nutrient solutions of bacteria for several hours, many bacteria adhere so tenaciously that they are dislodged neither by washing with water nor by staining procedures.

The foregoing observations suggest that bacteria grow on, or attach themselves to, solid surfaces rather than merely being passively stuck. Credibility is lent to this conclusion by the fact that regardless of the density of the bacterial population, more bacteria attach to glass slides during the early logarithmic phase of growth than during later growth phases.

Henrici and Johnson (1935) have described a group of bacteria "which secrete stalks by which they are attached to a firm substrate." Their search of the older bacteriological literature has shown that similar types have been observed before. We have found bacteria growing on slides submerged in the sea and in Great Salt Lake (Smith and ZoBell, 1937) which have a definite holdfast, some of which could be described as having "stalks."

As pointed out by Henrici and Johnson (1935), it is often difficult to distinguish bacteria having large polar or adherent lophotrichous flagella from stalked bacteria on slides which, after several days submergence, are covered with various kinds of bacteria besides other sessile organisms and a heterogeneous assortment of particulate and amorphous organic matter. Also in some cases we have not been certain that some of the definitely stalked microorganisms appearing on submerged slides were bacteria although, except for the stalk, they had the morphological appearance of bacteria. However, in spite of the difficulties, careful microscopic study of submerged slides has convinced us that some water bacteria are attached to solid surfaces by means of a filament or stalk which is definitely longer and narrower than the bacterial cell itself. In fact, some stalked bacteria have been observed which appeared to be dividing.

On only three slides out of several hundred examined have we observed bac-

teria with stalks which were sufficiently rigid to hold the bacteria away from the slide. When examined as wet mounts the bacteria appeared to be a few microns away from the slide. It was necessary to focus up and down in order to follow the stalk from the holdfast to the bacterial cell.

Several cultures of sessile bacteria have been isolated from submerged slides. After a few hours submergence in the sea the glass slides were washed with sterile water, transferred aseptically to sterile petri dishes and covered with nutrient agar (ZoBell, 1941). When grown in sea water enriched with 5 mgm./l. each of glucose and peptone, several of these cultures contained bacteria which attached to glass slides immersed in the medium. Six of them had structures which appeared to be stalks, but with all six cultures the bacterial cell, the stalk and the holdfast rested directly upon the glass slide. Upon cultivation on solid media they lose their stalks, although this property is regained after a few passages through sea water containing less than 10 mgm./l. of organic matter.

Only a small percentage of the cells of the six "stalked" cultures found attached to glass slides exhibited stalks. This may be attributed to faulty technique in staining stalks; it may suggest that the stalks develop after the cells have attached, or it is possible that all the cells do not have stalks. Further studies will be necessary to clarify this point.

Although some bacteria are attached by stalks, this is not the commonest attachment structure. Most sessile bacteria are found with the bacterial cells in intimate contact with the solid surface. It is believed that after coming into contact with a solid surface, physiologically active sessile bacteria secrete a cementing substance. When the bacteria are removed mechanically from glass slides to which they have attached themselves, a faintly staining film having the shape and arrangement pattern of the attached cells frequently remains on the slides.

Some bacteria appear to secrete a faintly staining material on the slide which is two or three times the diameter of the cells themselves. Examining slides after different periods of submergence in bacterial cultures reveals that the size of this film increases with age. It is not uncommon to see micro-colonies on "islands" of the film. The smaller islands conform more or less to the shape of the cells but larger islands of the film are quite irregular in outline.

The film appears to be a part of or product of the bacterial cell. The fact that the islands are most pronounced in dilute nutrient solutions makes it seem probable that the film does not consist of organic matter adsorbed from the water. However, it is still indeterminate whether the bacteria are responsible for the presence of the film or if the film of adsorbed organic matter precedes the bacteria thereby providing for their attachment. Microscopic particles of detritus including particles of carbon and indigotin, with which the slides have been treated, stick to the film thereby establishing that the film has adhesive properties. This mucilaginous slime produced by sessile bacteria is believed to be instrumental in the fouling of submerged surfaces (ZoBell and Allen, 1935, ZoBell, 1939). According to Sanborn (1932), the formation of slimy or viscous growths is common in cultures from marine fish.

An effort has been made to characterize the holdfasts of sessile bacteria by

treating slides coated with attached bacteria with different solvents. Dilute HCl or $H_2SO_4$ has little or no effect on the holdfasts, which indicates that the cementing or adhesive material is not calcareous. (Many sessile organisms attach by means of calcareous cements which are dissolved by dilute acids.) Dilute caustic, ammoniacal or bicarbonate solutions were without apparent effect. The holdfast is not destroyed by 95 per cent alcohol. Xylol, chloroform, ether and carbon disulfide seemed to dislodge some of the attached bacteria but not enough to warrant the conclusion that the cement is a lipin. Eisenberg (1918) believed that a high lipin content favored the adsorption of bacteria.

None of the common staining solutions dislodged the attached bacteria. When dessicated, many of the attached bacteria disappear from slides. Recently it has been found that a bacterial chitinase extract dislodges many attached bacteria which suggests that the holdfast is chitinous, but the extract also contains proteolytic and possibly other exoenzymes which themselves might have digested the cementing material.

Dilute solutions of detergents or wetting agents such as sodium taurocholate, sodium ricinoleate, sodium lauryl sulfate (Dreft) or "aerosol" remove part of the attached bacteria, the cells of some cultures being affected more than those of others. The presence of detergents or surface tension depressants in dilute nutrient solutions retards the attachment of bacteria to solid surfaces.

When submerged in the sea the number of bacteria found on glass slides increases exponentially with time until the bacteria became too numerous to count or are obscured by other organisms and detritus which attach simultaneously. This suggests that the bacteria are multiplying on the glass or that the attachment of bacteria promotes the attachment of additional bacteria. Probably the adhesive films formed by the first sessile bacteria help other bacteria which come into contact with the surface to gain lodgement long enough to produce their own holdfasts. If only the cumulation of adsorbed nutrients was responsible, the increase in the number of bacteria on submerged slides would probably be arithmetic rather than exponential.

## EFFECT OF ORGANIC NUTRIENTS

Unquestionably organic nutrients on the solid surfaces promote the multiplication of bacteria but there is evidence that adsorbed nutrients are not directly responsible for the attachment of bacteria. Slides have been left in sterile sea water until they have been covered with adsorbed organic matter after which they were submerged in the sea. Significantly more bacteria did not attach to these slides than to chemically clean ones.

However, the organic content of the water has a pronounced effect on the relationship of microorganisms to solid surfaces. Increasing the solid surface area ordinarily does not alter the rate of bacterial multiplication or metabolism in solutions containing more than 5 to 10 mgm./l. of organic nutrients. The beneficial effect of solid surface becomes more pronounced as the concentration of organic nutrients decreases. Heukelekian and Heller (1940) observed that *Escherichia coli* failed to grow when the concentration of organic nutrients was

less than 0.5 to 2.5 mgm./l. except in the proximity of solid surfaces. When the concentration exceeded 25 mgm./l., the activity of *E. coli* was not influenced perceptibly by solid surfaces.

The kind of organic matter is also important. Increasing the ratio of solid surface to volume does not influence the rate of bacterial activity in dilute solutions (1.0 to 10 mgm./l.) of glucose, glycerol or lactate. Many bacteria are found attached to glass slides in such solutions, but in such solutions the bacteria do not seem to depend upon solid surfaces because proportionately more occur suspended in the solution than anchored. Conversely in solutions enriched with 5 mgm./l. of sodium caseinate, lignoprotein or an emulsified chitin preparation inoculated with a few bacteria per ml., a beneficial effect of increased solid surface can be demonstrated. This suggests that there is a relationship between solubility, dispersion or molecule size of the organic nutrient and the surface phenomenon. However, many other factors may be involved and further studies must precede generalizations.

The beneficial effect of solid surface upon the rate of bacterial activity in sea water is much more pronounced with some lots of sea water than with others. Since the inorganic content, gas tension and the pH of sea water have been nearly constant, the differences are attributed to differences in the organic content of the water. A heterogenous assortment of organic matter occurs in sea water but most of it probably consists of large molecules of colloidal dimensions which are fairly refractory to bacteria decomposition. Differences may be expected in the relative quantities of different kinds of organic matter adsorbed on glass, and especially differences in the orientation of molecules. Their availability might be influenced by the angle of adsorption as well as by the polarity, both of which might influence the vulnerability of hydroxy, carboxy, amino or other groups. According to Adam (1938) most organic molecules form zero angles with glass but certain large molecules are "adsorbed on to glass with the long chains outwards." For example, the contact angle of paraffin wax molecules is 105°, the largest found with solids and water.

It is generally believed that the large molecules or particulate materials are hydrolyzed by exoenzymes before the organic matter can be ingested and assimilated by bacteria. In a dilute solution of such organic hydrolytes, containing only a relatively few bacteria, the bacteria would have to be in close contact with the hydrolyte in order for the exoenzymes to be most effective. Otherwise the bacterial exoenzymes would be dissipated throughout the solution. It is conceivable that the hydrolyzates themselves might have a tendency to diffuse away from the bacterial cell responsible for their formation; so, in dilute nutrient solutions of large molecules of organic matter, the bacteria may have difficulty in digesting and absorbing or ingesting enough nutrient to provide for their organic requirements.

However, if the bacterial cell is on a solid surface juxtaposed with nutrient hydrolytes, the solid surface may retard the diffusion of the exoenzymes and the hydrolyzates away from the cell (see figure 2). Besides the physical attraction which might be exerted by the solid surface, an anchored bacterium would not

be influenced by the bombardment of molecules (with the resultant Brownian movement and diffusion) which would tend to separate the bacterium from its exoenzymes and hydrolyzates. Furthermore, the interstices at the tangent of the bacterial cell and solid surface might serve as concentration foci for exoenzymes and hydrolyzates. As other bacteria attach or develop from cell division, more interstitial or capillary spaces would be formed which would tend to retard further the diffusion of materials away from the cells and to favor the absorption or ingestion of soluble nutrients. Also solid surfaces might

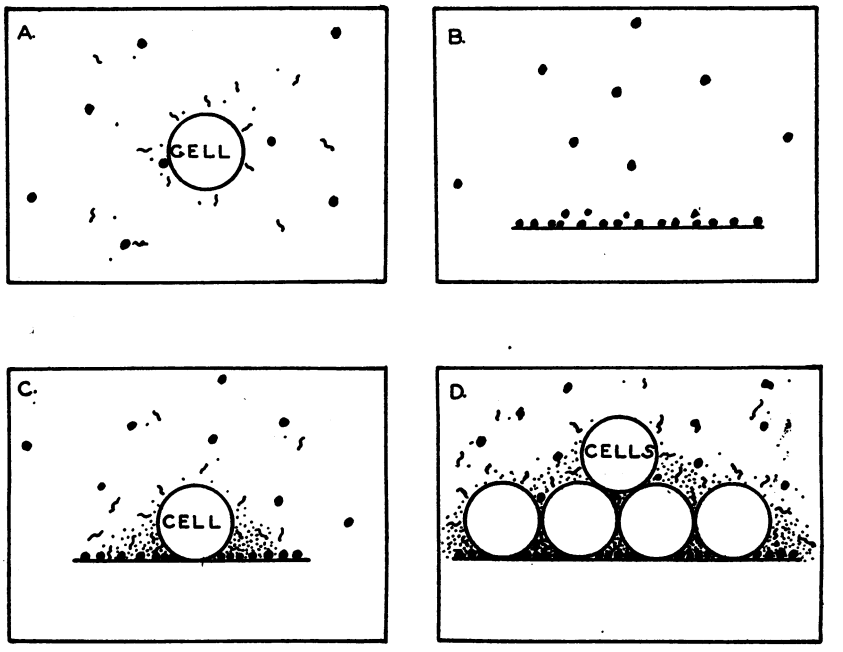


FIG. 2. A, A free-floating bacterial cell surrounded by a few suspended particles of food (dark circles) which must be hydrolyzed by the exoenzyme (helicoidal lines) before the resulting hydrolyzates (dots) can be ingested and assimilated. B, Particles of food concentrated in a monomolecular layer on a solid surface. C, Food particles are more available to the cell on the solid surface where the interstices at the tangent of the bacterial cell and the solid surface retard the diffusion of exoenzymes and hydrolyzates away from the cell. D, Multiple cells form additional interstitial spaces.

facilitate the orientation of bacterial exoenzymes in the most advantageous position (Danielli, 1937). According to Adam (1938), it has been found by Willstätter that adsorption of a soluble enzyme on a neutral inactive support will often increase its stability and activity.

From their studies on the relation of solid surfaces to bacterial activity, Heukelekian and Heller (1940) conclude that "Surfaces enable bacteria to develop in substrates otherwise too dilute for growth. Development takes place either as a bacterial slime or colonial growth attached to the surfaces. Once a biologically active slime is established on surfaces, the rate of biological reaction is greatly accelerated."

## KINDS OF SOLID SURFACES

In order to appraise the effect of surface upon bacterial activity the solids used must be chemically inert. Glass has proved best for this purpose because, besides being virtually insoluble, it is available in a great variety of forms and shapes, and transparent glass slides lend themselves readily to the direct microscopic studies of attached materials. However, glass is by no means the only substance which can be used to demonstrate the attachment of bacteria and the growth-promoting properties of solid surfaces.

When submerged in the sea, bacteria attach to celluloid, cellophane, vinylite, lucite, bakelite and other plastics both transparent and opaque. There is a marked difference in the rate of attachment of bacteria to, the accumulation of organic matter on, and the growth-promoting properties of different kinds of plastics. Details can be given only after war-time restrictions are lifted because these studies may have direct bearing upon the "fouling" of ships' bottoms and other submerged surfaces (ZoBell, 1939). There is evidence that some plastics are attacked biochemically by bacteria.

Bacteria attach less readily to solid surfaces which are rendered hydrophobic by the application of greases, waxes and certain kinds of lacquers than to hydrophilic surfaces of low surface tension which are more wettable. Likewise positively charged surfaces are more conducive to the attachment of bacteria than negatively charged ones. Neither color, plane or angle of immersion nor the degree of roughness or smoothness of a surface seems to influence the attachment of bacteria. For unexplained reasons bacteria generally attach more readily near the edge of glass slides than in the center regardless of the position in which the slides are immersed. Bacteria attach to gold and silver surfaces but clean silver, copper, aluminum and iron surfaces appear to exert an adverse oligodynamic effect.

Porcelain surfaces also promote bacterial activity in dilute nutrient solutions, unglazed porcelain particularly. Ignited sand, asbestos fibres, emery grit and kieselguhr are likewise beneficial. It has been demonstrated that these substances adsorb organic matter from sea water, and proportionately many more bacteria are associated with the solid particles than are found in similar volumes of water. The bacteria are usually so tenaciously attached to the solid particles that oxygen consumption or similar tests must be used to demonstrate their presence and activity. Indifferent results have been obtained with bentonite, talc, kaolin and various samples of marine clays although they adsorb organic matter. This may be because particles smaller than bacteria adsorbed on bacteria may be inimical as suggested by the work of Peele (1936).

Conn and Conn (1940) found that bentonite, kaolinite, beidelite and illite stimulated bacterial activity, which they attributed to increased surface and other factors. Bigger and Nelson (1941) found that ignited asbestos, barium sulfide, barium hyposulfite, barium sulfite, tricalcium phosphate, ferric phosphate, ferrous silicate, kaolin, kieselguhr, magnesium silicate, manganese dioxide, permutit, silica, silver sand, soil, unglazed porcelain, talc and zirconium silicate

make it possible for coliform bacteria to grow in very dilute nutrient solutions (less than 0.4 mgm./l. of organic nutrient). Although some of the substances used by Bigger and Nelson may be active in other ways, they attribute the beneficial effect to the concentration of nutrients by the substances.

Studies with sand, kieselguhr and similar substances reveal that another factor is involved, namely, the distribution of the solid surface in the solution. For example, a layer of sand or kieselguhr 2 to 3 cm. thick in the bottom of a bottle accelerates bacterial activity little more than does a layer only 2 to 3 mm. thick, although the thicker layer presents ten times as much surface area, presumably because little more of the solution is in intimate contact with the sand in one case than in the other. In order to be most effective the solid surface must be distributed throughout the solution. This has been demonstrated by using different shapes of glass.

Enough 2 mm. glass beads were placed on the bottom of 145 ml. glass-stoppered bottles to provide the same surface area as provided by a dozen pieces of 6 mm. glass tubes standing upright in similar bottles. In the latter arrangement none of the water was more than 3 mm. from a solid surface whereas in bottles partly filled with glass beads much of the water was considerably more than 3 mm. from a solid surface. Bacteria consumed oxygen more rapidly in the bottles with the glass tubes than in those having a similar area of glass beads. In fact, the glass tubes stimulated bacterial activity more than a 2- to 3-cm. layer of silica sand in the bottom of the bottles. Although the latter presented much more solid surface than the glass tubes, it was not in close proximity to the water.

Glass wool has been used to provide large areas of solid surface throughout the bottles of water, but it has not proved to be entirely satisfactory for experimental purposes because it is difficult to wash free of impurities, and it is virtually impossible to free the glass wool of adherent gas bubbles.

According to Prescott and Winslow (1931), Kohn conducted experiments which suggested that the multiplication of bacteria during storage in glass receptacles is attributable partly to the solution of certain constituents from glass which favor bacterial life. While this may be true of certain waters, there is no evidence that the waters which we have examined dissolve any growth-promoting substance. The beneficial effects of surface observed with Pyrex glass have been comparable to those obtained with various kinds of soft glass. When stored at 0°C. in contact with large surface areas of different kinds of glass including glass wool and glass beads, the water is rendered less growth-promoting rather than more so which, as discussed above, is due to the adsorption of organic nutrients. That nothing toxic is dissolved from the glass is shown by the fact that such water regains its growth-promoting properties upon the addition of a fraction of a mgm./l. of glucose. It is not a reflection upon the work of Kohn to suppose that the growth-promoting constituents dissolved from glass were organic compounds because, as emphasized by Adam (1938), it is very difficult to prepare chemically clean glass surfaces. Soft glass is more difficult to clean than harder glasses, and significantly, Kohn noted the greatest

bacterial increases in bottles made of softer glasses. As noted by Esty and Cathcart (1921) unbuffered media have a tendency to become more acid during heat sterilization in Pyrex glass and more alkaline in soft glass tubes, but in all of our observations on the effect of glass surfaces we have used well buffered media.

## DISCUSSION

Conn and Conn (1940) speculate that besides concentrating nutrients, solid surfaces may adsorb toxic products and increase the availability of oxygen. These two factors may be operative under certain conditions but it is doubtful if either factor helps to account for the beneficial effect of solid surfaces in extremely dilute solutions.

For many years more or less inert solids such as chalk, glass beads, glass wool, sand, kaolin, clinkers, sponge, animal tissue, charcoal, iron nails, cellulose, etc., have been employed in liquid media to promote the growth of bacteria (see literature cited by Breden and Buswell, 1933). Some of the substances probably provide a favorable oxygen tension and others may serve as a support for bacteria. Breden and Buswell (1933) point out that in continuous fermentation processes (vinegar, sewage beds, etc.), inert materials support organisms, "preventing their loss with removal of spent liquor and making possible the heavy inoculation of fresh substrates." They found that shredded asbestos improved methane fermentation. Numerous methane-producing organisms grew on the asbestos, small pieces of which could be used to inoculate successfully new batches of soluble substances. Butterfield (1937) attributed the beneficial effect of activated sludge to the adsorption of dissolved and colloidal materials.

Peele (1936) investigated several factors, including electric charges and base-exchange complex, which influence the adsorption of bacteria by soils. He found that the addition of soil to bacterial suspensions sometimes retarded the evolution of carbon dioxide, which he attributed to the adsorption of bacteria by soils. Similar results were obtained by Chudiakow (1926). Clay particles considerably smaller than bacteria predominated in the soils used by Peele and Chudiakow, so it is conceivable that such particles surrounding the bacteria retarded the assimilation of nutrients or other normal bacterial activities. McCalla's (1940) observation on the large number of active adsorption sites on a bacterial cell, which may have an affinity for minute positively-charged particles, supports such a concept. According to Dianowa and Woroschilowa (cited by Peele, 1936), "the decrease in biochemical activity when a portion of soil was shaken with a culture of bacteria was much greater when the soil was made up of very fine particles, such as silt and clay, than when the soil contained a larger proportion of sand." Also the adsorption of bacteria by soil probably caused them to settle to the bottom of the medium where they would be less active than when distributed throughout the medium. Peele used *Azotobacter chroococcum* as the test organism.

On the other hand Söhngen (1913) records that the activities of hydrocarbon-oxidizing bacteria are accelerated by adsorption by soil. According to Rubent-

schik *et al.* (1936) the activity of some bacteria (nitrifiers) is retarded while that of others (sulfate reducers) is increased by adsorption by lake mud.

In their studies on the adsorption of bacteria by inert reagents, Gunnison and Marshall (1937) found no evidence that the beneficial clinical effects which sometimes follow the oral administration of kaolin, charcoal, Fuller's earth, etc., could be attributed to the inactivation of adsorbed pathogens. They believe that the adsorption of toxins or enzymes is more likely to account for the reported clinical improvements than the removal of bacteria by adsorption. In their experiments, like those of Peele (1936) and Chudiakow (1926) with soils, the particles used were mostly smaller than bacteria and the concentration of nutrients was high. In our experiments a beneficial effect of solid surface has been observed only in very dilute nutrient solutions and on solid surfaces which are large in comparison to the size of bacteria.

Further studies on the influence of particle size, food concentration and composition, the behavior of different kinds of bacteria, electric charge, surface tension, electrolytes, pH and other factors which influence the relation of bacteria to solid surfaces are indicated.

## SUMMARY

Minute but demonstrable quantities of organic nutrients are adsorbed from sea water by glass.

In dilute nutrient solutions such as sea water the organic matter concentrated by adsorption on solid surfaces enhances bacterial activity.

Many of the bacteria found in sea water are sessile, growing exclusively or preferentially attached to a solid surface.

Most sessile bacteria appear to grow on solid surfaces by exuding a mucilaginous holdfast. A few have stalks. The exudate may aid in concentrating nutrients in the vicinity of the attached bacteria.

The beneficial effect of solid surfaces is usually evident only in very dilute nutrient solutions (less than 10 mgm./l). The beneficial effect is more pronounced with colloidal or poorly soluble substances than with those which are very soluble.

It is believed that besides concentrating nutrients by adsorption and providing a resting place for sessile bacteria, solid surfaces retard the diffusion of exoenzymes and hydrolyzates away from the cell thereby promoting the assimilation of nutrients which must be hydrolyzed extracellularly prior to ingestion.

Glass, plastics, porcelain, sand, kieselguhr and other inert particulate materials are surface active. Particles larger than bacteria are more beneficial than smaller particles. Particles smaller than bacteria may be harmful.

In order to be most effective the solid surface must be distributed throughout the dilute nutrient solution.

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## REFERENCES

ADAM, N. K. 1938 The Physics and Chemistry of Surfaces. Clarendon Press, Oxford, 402 pp.

BIGGER, J. W., AND NELSON, J. H. 1941 The growth of coliform bacilli in distilled water. J. Path. Bact., **53**: 189–206.

BLODGETT, K. B. 1935 Films built by depositing successive monomolecular layers on a solid surface. J. Am. Chem. Soc., **57**: 1007–1022.

BREDEN, C. R., AND BUSWELL, A. M. 1933 . The use of shredded asbestos in methane fermentations. J. Bact., **26**: 379–383.

BUTTERFIELD, C. T. 1937 The oxidation of sewage by bacteria in pure culture. J. Bact., **34**: 207–219.

CASTELL, C. H., AND McDERMOTT, L. A. 1942 Multiplication of bacteria in water and its significance in food spoilage. Food Research, **7**: 244–253.

CHOLODNY, N. 1930 Über eine neue Methode zur Untersuchung der Bodenflora. Arch. Mikrobiol., **1**: 620–652.

CHUDIAKOW, N. N. 1926 Über die Adsorption der Bakterien durch den Boden und den Einfluss derselbe auf die mikrobiologischen Bodenprocessen. Zentr. Bakt. Parasitenk., II, **68**: 345–358.

CONN, H. J., AND CONN, JEAN E. 1940 The stimulating effect of colloids upon the growth of certain bacteria. J. Bact., **39**: 99.

DANIELLI, J. F. 1937 The relations between surface pH, ion concentrations and interfacial tension. Proc. Roy. Soc. (London), B 122: 155–174.

EISENBERG, P. 1918 Ueber spezifische Adsorption von Bakterien. Zentr. Bakt. Parasitenk., II, **81**: 72–104.

ESTY, J. R., AND CATHCART, P. H. 1921 The change in the hydrogen-ion concentration in various mediums during heating in soft and pyrex glass tubes. J. Infectious Diseases, **29**: 29–39.

GREIG-SMITH, R. 1914 The destruction of paraffin by *Bacillus prodigiosus* and soil organisms. Proc. Linnean Soc. N. S. Wales, **39**: 538–541.

GUNNISON, J. B., AND MARSHALL, M. S. 1937 Adsorption of bacteria by inert particulate reagents. J. Bact., **33**: 401–410.

HARVEY, H. W. 1941 On changes taking place in sea water during storage. J. Marine Biol. Assoc. United Kingdom, **25**: 225–233.

HENRICI, A. T. 1933 Studies of freshwater bacteria. I. A direct microscopic technique. J. Bact., **25**: 277–286.

HENRICI, A. T., AND JOHNSON, DELIA E. 1935 Studies of freshwater bacteria. II. Stalked bacteria, a new order of Schizomycetes. J. Bact., **30**: 61–93.

HENRICI, A. T. 1936 Studies of freshwater bacteria. III. Quantitative aspects of the direct microscopic method. J. Bact., **32**: 265–280.

HOTCHKISS, MARGARET, AND WAKSMAN, S. A. 1936 Correlative studies of microscopic and plate methods for evaluating the bacterial population of the sea. J. Bact., **32**: 423–432.

HEUKELEKIAN, H., AND HELLER, A. 1940 Relation between food concentration and surface for bacterial growth. J. Bact., **40**: 547–558.

KROGH, A. 1931 Dissolved substances as food of aquatic organisms. Rapports et Proces-Verbaux des Reunions, **75**: 7–36.

KUSNETZOWA, Z. I. 1937 Methode der Ausscheidung der Plankton- und Periphytonbakterien und ihre Anwendung zum Studium der Dynamik der bakteriologischen Prozesse im Wasserbecken. Arbeiten der Limnologischen Stat. Zu Kossino, **21**: 89–103 (Russian with German summary).

LLOYD, BLODWEN 1930 Bacteria of the Clyde Sea area: A quantitative investigation. J. Marine Biol. Assoc. United Kingdom, **16**: 879–907.

LLOYD, BLODWEN 1937 Bacteria in stored water. J. Roy. Tech. Coll. (Glasgow), **4**: 173–177.

MCCALLA, T. M. 1940 Cation adsorption by bacteria. J. Bact., **40**: 23–32.

PEELE, T. C. 1936 Adsorption of bacteria by soils. Cornell Univ., Agr. Expt. Sta., Memoir **197**: 1–18.

PRESCOTT, S. C., AND WINSLOW, C. E. A. 1931 Elements of Water Bacteriology. John Wiley & Sons, New York, 219 pp.

ROLL, H. 1939 Zur Terminologie des Periphytons. Arch. Hydrobiol., **35**: 59–69.

RUBENTSCHIK, L., ROISIN, M. B., AND BIELJANSKY, F. M. 1936 Adsorption of bacteria in salt lakes. J. Bact., **32**: 11–31.

SANBORN, J. R. 1932 Marine bacteria commonly found on fresh fish. J. Bact., **23**: 349–351.

SMITH, W. W., AND ZOBELL, C. E. 1937 Direct microscopic evidence of an autochthonous bacterial flora in Great Salt Lake. Ecology, **18**: 453–458.

SÖHNGEN, N. L. 1913 Einfluss von Kolloiden auf mikrobiologische Prozesse. Zentr. Bakt. Parasitenk., II, **38**: 621–647.

STARK, W. H., STADLER, J., AND MCCOY, E. 1938 Some factors affecting the bacterial population of freshwater lakes. J. Bact., **36**: 653–654.

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

WAKSMAN, S. A., AND CAREY, C. L. 1935b Decomposition of organic matter in sea water by bacteria. II. Influence of addition of organic substances upon bacterial activity. J. Bact., **29**: 545–561.

WAKSMAN, S. A., REUSZER, H. W., CAREY, C. L., HOTCHKISS, M., AND RENN, C. E. 1933 Studies on the biology and chemistry of the Gulf of Maine. III. Bacteriological investigations of the sea water and marine bottoms. Biol. Bull., **64**: 183–205.

WAKSMAN, S. A., AND VARTIOVAARA, U. 1938 The adsorption of bacteria by marine bottom. Biol. Bull., **74**: 56–63.

WHIPPLE, G. C. 1901 Changes that take place in the bacterial contents of waters during transportation. Tech. Quart., **14**: 21–29.

ZOBELL, C. E. 1936 Periphytic habits of some marine bacteria. Proc. Soc. Exptl. Biol. Med., **35**: 270–273.

ZOBELL, C. E. 1937 The influence of solid surface upon the physiological activities of bacteria in sea water. J. Bact., **33**: 86.

ZOBELL, C. E. 1939 Primary film formation by bacteria and fouling. Collecting Net, **14**: 105–106.

ZOBELL, C. E. 1939 The role of bacteria in the fouling of submerged surfaces. Biol. Bull., **77**: 302.

ZOBELL, C. E. 1941 Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. J. Marine Research, **4**: 42–75.

ZOBELL, C. E., AND ALLEN, ESTHER C. 1935 The significance of marine bacteria in the fouling of submerged surfaces. J. Bact., **29**: 239–251.

ZOBELL, C. E., AND ANDERSON, D. Q. 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 RITTENBERG, S. C. 1938 The occurrence and characteristics of chitinoclastic bacteria in the sea. J. Bact., **35**: 275–287.

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

ZOBELL, C. E., AND GRANT, C. W. 1943 Bacterial utilization of low concentrations of organic matter. J. Bact., **45**: 555–564.

ZOBELL, C. E., GRANT, C. W., AND HAAS, H. F. 1943 Marine microorganisms which oxidize petroleum hydrocarbons. Bull. Am. Assoc. Petroleum Geol., In press.

ZOBELL, C. E., AND UPHAM, H. C. 1943 A list of marine bacteria including descriptions of sixty new species. Bull. Scripps Inst. Oceanogr., tech. ser., **5**: 239–345.