Influence of Interfaces on Microbial Activity

MARK C. M. VAN LOOSDRECHT,† JOHANNES LYKLEMA, WILLEM NORDE, AND ALEXANDER J. B. ZEHNDE

Department of Microbiology and Department of Physical and Colloid Chemistry, Agricultural University, Wageningen, The Netherlands

INTRODUCTION

Bacterial adhesion is important in, e.g., (microbial) ecology as well as biotechnology, biofouling, caries formation, and (aerobic and anaerobic) wastewater treatment. As long ago as 1913, Söhngen (90) reported that the presence of a solid phase can influence a diversity of bacterial processes, such as nitrogen fixation, alcohol oxidation, nitrification, and denitrification. As time went on, more detailed studies on the interaction between bacteria and solid surfaces became available. ZoBell (106) and Heukelekian and Heller (47) showed that bacterial activity increased due to the presence of glass surfaces, in particular when nutrient concentrations were low. Bacterial activity in soils in relation to the presence of clay minerals has been investigated by Stotzky and Rem (91–94). The influence of anion-exchange resins (39–42) or plastics (9, 10, 23, 25, 26, 28) on adhesion and activity of bacteria has also been studied in detail for a variety of systems.

In the laboratory, bacteria are generally cultivated and studied as cell suspensions in liquid media. The natural environment, however, is more complex. Usually a wide variety of surfaces is available for attachment and colonization. For natural environments, it has often been reported that surfaces are the major site of microbial activity. For example, by staining actively respiring bacteria with tetrazolium, Harvey and Young (37) showed that, in a marsh estuary, almost all detectable and respiring bacteria were associated with particles. Glucose mineralization in estuarine waters is also predominantly carried out by adhered bacteria (34). In several ponds and marshes, the contribution of particle-bound bacteria to total heterotrophic activity has been found to be at least four times as high as could be expected from the fraction of attached cells (57). In soils, the degradation of a nonadsorbing compound was found to be essentially carried out by attached organisms only (58).

Adhesion of microbes is often found to be increased during exponential growth, presumably as a result of increased cell wall hydrophobicity during this growth phase (24, 101, 107; T. L. Sie, Ph.D. thesis, University of Hannover, Hannover, Federal Republic of Germany, 1985). This fact makes surfaces a preferred locus for metabolically active bacteria. It has been found that, in a natural population of microorganisms, attached bacteria are often more active than free cells (37, 43, 50, 51, 57, 83).

Although there appears to be a qualitative consensus that surfaces do influence bacterial metabolism, the experimental observations are not always consistent; neither has a general explanation been advanced for this influence (8). The lack of experimental consistency is at least partly due to the great variation in experimental design with respect to the nature of the solid phase, the bacteria, the substrates, sterility, and other experimental conditions. The relevant but disparate literature is summarized in Table 1, wherein it is confirmed that, although generally surfaces do exert an influence, no systematic trends can be observed.

Considerable confusion with regard to the theoretical interpretation has been caused by the fact that most authors did not discriminate between direct and indirect influences of
| Observation | Explanation | Reference(s) |
|-------------|-------------|--------------|
| Increased growth rate | Increased substrate concn at the interface | 47, 106 |
| | More efficient use of proton motive force | 20 |
| | Detoxification of substrate | 19, 73, 91 |
| | Detoxification of inhibitors | 23, 38, 91 |
| | pH buffering by ion exchange | 66 |
| | No explanation | 26, 40, 42, 43, 53, 54, 90 |
| Decreased growth rate | Less cell surface available for substrate uptake | 56, 52 |
| | Higher maintenance coefficient | 56 |
| | Substrate transport limitation | 13 |
| | No explanation | 6, 82 |
| Increased assimilation and decreased respiration rates | No explanation | 10 |
| Decreased assimilation | No explanation | 71, 72 |
| Increased respiration | Change in membrane processes | 71, 72 |
| | pH buffering by ion exchange | 92–94 |
| | No explanation | 5, 78, 89 |
| Increased adhesion of active cells | Increased cell hydrophobicity | 101, 107 |
| | No explanation | 37, 43, 44, 57 |
| Higher activity of attached cells | No explanation | 9, 25, 45, 51, 55, 83, 85 |
| Decreased substrate utilization | Desorption limitation | 17, 22, 32, 65, 93, 95, 96, 104 |
| | Diffusion limitation | 54 |
| | Lower substrate concn | 39 |
| | No explanation | 1, 31 |
| Lower substrate affinity | Diffusion limitation | 10, 40 |
| Change in pH optimum | Proton concn at surface different from the bulk | 39, 42 |
| Difference in fermentation pattern | Surface is electron acceptor | 70 |
| Increase in productivity | Immobilization of biomass | 68, 69, 99 |
| Decreased mortality | Decreased phagocytosis | 36, 48, 103 |
| | Other | 11, 60 |
| No effect | | 31, 71, 75, 76, 87, 100 |

* Given by the authors of reference(s) cited.

surfaces. To the former category belong changes in microbial activities directly resulting from the presence of a surface, e.g., changes in the structure and permeability of the membranes as an immediate consequence of the presence of a nearby surface. These changes may be reversible or irreversible. Indirect influences involve changes in cell activity due to (i) changes in the composition of the medium that, in turn, derive from adsorption or desorption phenomena at the interfaces, (ii) the specific geometry and heterogeneity of the space around an adhered cell, and (iii) the fact that the cells remain in a particular place when they colonize a surface. Examples of indirect influences are changes in substrate availability, pH buffering, water activity, or higher stability of DNA outside the cell and, as a possible consequence, higher rates of DNA transformation (61–63, 80).

In this review, we first give an overview of the theoretical background of bacterium-surface interactions. Thereafter, we critically review the literature on the influence of solid surfaces on microbial activity, thereby discriminating when possible between direct and indirect contributions.

BACTERIAL ADHESION

General Remarks

Microbial colonization of a solid-liquid interface may occur in the following sequence (Fig. 1). First is transport of cells to a surface. Bacteria can reach a surface by three different modes. (i) In diffusive transport, bacteria exhibit a non-negligible Brownian motion (average displacement, 40 μm h⁻¹ [64]) that can be observed under a microscope. This motion accounts for random contacts of small bacteria with interfaces even under quiescent conditions and is responsible for crossing any diffusion layer, across which no convection can take place. Diffusive transport is slow compared with transport by convective flow or transport of motile cells. Under quiescent conditions, sedimentation of bacteria may contribute significantly to bacterial transport and represent the only way bacteria come into contact with a surface. (ii) Convective transport of cells is due to liquid flow and may be several orders of magnitude faster than diffusive transport, but there may exist situations in which the final
part of the route to the surface (passage through the diffusion sublayer) is diffusion controlled. An extensive overview of convective bacterial transport is given by Characklis (16).

(iii) In active movement, once a motile bacterium is in the vicinity of a surface, it may encounter a surface by chance or chemotactically respond to any concentration gradient that may exist in the interfacial region.

The next occurrence is initial adhesion, which is mainly a physicochemical process. It can be reversible or irreversible. The distinction is not sharp, but for practical purposes it can be satisfactorily made by defining reversible adhesion as deposition to a surface in which the bacterium continues to exhibit Brownian motion and can readily be removed from the surface by mild shear or the bacterium's own mobility. Irreversibly adhering bacteria exhibit no Brownian motion and cannot be removed unless by a strong shear force. The process of initial adhesion is discussed in more detail in the next section.

Following initial adhesion is firm attachment. After the bacterium has been deposited on the solid surface, special cell surface structures (e.g., fibrils or polymers) may form strong links between cell and solid surface. Polysaccharides have been shown to be essential for the development of surface films, but not for the initial adhesion of bacteria (2, 29).

Last in the sequence is surface colonization. When firmly attached cells start growing and newly formed cells remain attached to each other, microcolonies or biofilms may develop. In the case of growth of reversibly adhering cells, part of the newly formed cells will be released into the medium (37).

The last two processes are sensitive to the type of organism and are therefore more specific than the first two.

Initial Adhesion

A bacterial suspension may be interpreted as a living colloidal system, and the initial steps of adhesion can, in a first approximation, be described by colloidal chemical theories such as the DLVO theory (102). These theories describe the change in Gibbs energy as a function of the distance between two bodies. If steric effects do not play a role, the total interaction Gibbs energy is obtained from the summation of the Van der Waals and the electrostatic interaction. The Van der Waals interaction is usually attractive. The electrostatic interaction is usually repulsive due to the fact that in nature both bacteria and surfaces are predominantly negatively charged.

Figure 2 shows the electrostatic \( G_{\text{el}} \), Van der Waals \( G_{\text{v}} \), and total interaction Gibbs energy \( G_{\text{tot}} \) as a function of separation \( H \) for a bacterium and a solid surface, at different ionic strengths. At low ionic strength, \( G_{\text{tot}}(H) \) has a positive maximum that constitutes a barrier for adhesion in the primary minimum. The maximum in \( G_{\text{tot}}(H) \) is lower with increasing ionic strength, due to a reduction of the range of repulsion of \( G_{\text{el}} \). At certain intermediate values of the ionic strength, \( G_{\text{tot}}(H) \) is so low that a fraction of the particles may contain sufficient thermal energy to pass the barrier (i.e., slow irreversible adhesion takes place). At even higher ionic strength, (ii) when \( G_{\text{tot}}(H) \) is \( \approx 0 \), all particles can reach the primary minimum.

At longer distances of separation (a few nanometers), another, more shallow minimum in \( G_{\text{tot}}(H) \) exists, the so-called secondary minimum. It is most pronounced at intermediate ionic strengths and is deeper for systems having a larger Van der Waals attraction and for larger particles (102). In this context, microbial cells are considered to be large particles. If the depth of the secondary minimum does not exceed values beyond a few kT per particle \( (1 \text{ kT} = 4 \times 10^{-21} \text{ J} \text{ bacterium}^{-1}) \) \( k \) = Boltzmann constant; \( T \) = temperature in 'K'), the bacteria become reversibly attached. In the case of opposite charges on the interacting particles, \( G_{\text{el}} \), and hence \( G_{\text{tot}} \), is negative at all separations.

At short distances of separation, say \( H \leq 1 \text{ nm} \), short-range interactions (for instance, hydrogen bonding, ion pair formation, etc.) are effective, besides steric effects. They determine the strength of adhesion in the primary minimum. The DLVO theory is able to predict whether primary minimum adhesion can occur, but cannot quantify the depth of this minimum. In the presence of extended polymeric molecules on the cell or solid surface or both, steric hindrance (Fig. 3a) or polymer bridging (Fig. 3b) may occur in addition to the interactions described by the DLVO theory.

Generally, initial bacterial adhesion is a reversible process (12, 102) taking place in the secondary minimum (102). This
implies no direct contact between cell and surface. By interference reflection microscopy, a non-zero separation distance between bacteria and a glass surface has been confirmed (27). As expected for secondary minimum adhesion, the distance depended on the concentration and valency of the cations.

Reversible adhesion implies a continuous exchange between free and adhered cells, which makes it difficult to distinguish between the activity of adhered and free cells. Hermansson and Marshall (45) showed experimentally that such an exchange between free and adhered cells did occur. The exchange rate decreased with increasing adhesion strength. The exchange of cells in stirred systems is enhanced due to convective transport of cells (84, 102). In order to prevent exchange between surface and suspended cell populations, or even rule out the presence of suspended cells, bacteria have to be irreversibly attached to the surface (75, 100, 102).

Summarizing, the following statements can be made on the influence of adhesion on bacterial activity measurements. In the case of reversible adhesion, it is difficult to distinguish experimentally between free and adhered cells. Direct influences of surfaces on microbial activity must be expected only for irreversibly adhering cells. For bacteria adsorbed in the secondary minimum, the distance from the surface is so long that the influence of the surface on the local Gibbs energy in the cell surface (and hence on its structure) is negligible.

**CONSEQUENCES OF ACCUMULATION OF CHEMICALS ON SURFACES**

The physical properties of a phase boundary differ markedly from those of the bulk of the adjoining phases. As a result, certain compounds or nutrients may accumulate at interfaces. Although the concentrations of a compound in the bulk and at the interface might be very different, in equilibrium its chemical potential is the same at both locations. A positive influence of surfaces on bacterial activity has often been attributed to the accumulation of nutrients at the surface (9, 28, 47, 55, 106). The increased nutrient and substrate concentration is thought to stimulate bacterial growth.
I growth rate or increase the cell yield. However, the energy available for work from a reaction (called Gibbs energy), in this case, biomass production, is determined (at constant temperature and pressure) by the chemical potentials of the reactants and not by their concentrations. Thus, the simple fact that higher substrate and nutrient concentrations can be found at the solid surface does not explain the often reported positive influence of surfaces on bacterial activities.

For a proper interpretation of possible effects of the presence of solid surfaces on bacterial metabolism, it is necessary also to keep the geometry of the interaction range between cells and surfaces in mind (Fig. 4). Bacteria are 1 to 2 μm in diameter and have cell walls of 20- to 100-nm thickness. Reversibly adhering cells are located at a distance of approximately 5 nm from the surface (102). The thickness of an adsorbed layer of substrates, nutrients, etc., usually does not exceed a few nanometers, which implies that only very small parts of the bacterial surface (<0.1%) is in direct contact with adsorbed materials. It follows that for geometric reasons the influence of any surface is predominantly indirect. Direct influences, if any, are expected only for a small fraction of the bacterial surface. Hence, if direct influences of the presence of a nearby surface do exist at all, they are likely outweighed by indirect ones.

Depending on the conditions, ad- and desorption may influence bacterial activities positively, negatively, or not at all. No effects are expected when substrates do not adsorb to the solid surface. Negative effects may be obtained when the solid particles act as an adsorbent. Addition of such a solid phase will lower the free substrate concentration and as a consequence decrease its bioconversion rate. On the other hand, when most of the substrate is adsorbed to the solid phase and desorption can take place, the adsorbent can act as a source of substrate and so influence the microbial activity positively. In this case, the substance has to diffuse from the surface to the microbial cell. The rate of substrate transport to active cells is determined by the concentration gradient of the substrate, which becomes steeper if a bacterium approaches a substrate-enriched surface. Although high substrate concentrations at the interface may allow adsorbed cells to be more active than free-living cells, it is not the concentration as such which is stimulatory, but the faster mass transfer because of a shorter diffusion distance. Such a mechanism might explain the surface-enhanced utilization of stearic acid as observed by Kefford et al. (55). In case of inhibitor adsorption, the arguments given above may just be reversed.

SURFACE-ASSOCIATED GROWTH

When bacteria are adhered to a surface and start to grow, several patterns may arise, depending on the mode of attachment. (i) Cells are reversibly adhered to the surface and to each other. This will result in an equilibrium distribution between adhered and suspended cells. (ii) Cells are irreversibly bound to the surface (by, e.g., polymers) but not to each other, resulting in the formation of a monolayer of cells on the surface. (iii) Cells are irreversibly attached to the surface and to each other, resulting in biofilm formation.

If reversibly adhered cells divide, newly formed cells may initially remain attached. However, the adhering cells remain in equilibrium with the suspended cells. As detachment can be a relatively slow process (102), small microcolonies may develop. Such a microcolony formation has actually been observed by Caldwell and co-workers (13, 14) for the growth of *Pseudomonas fluorescens* on glass surfaces.

In continuous culture, growth kinetics on a surface may deviate from that in the bulk phase. Since attached cells are only removed by detachment, surface growth is essentially uncoupled from the dilution rate. Surface growth in fermentors results in an apparent increased productivity (DX) in the fermentor itself, especially at high dilution rates. This is due to the fact that beyond the maximum dilution rate (maximum growth rate of a bacterium) attached microbes still remain in the fermentor (4, 59, 82, 99) (Fig. 5) and detached cells are continuously released from the surface into the suspension. Large amounts of bacteria on surfaces may act as a kind of cell buffer, compensating for losses in biomass as a result of changes in dilution rate (54).

MICROBIAL ACTIVITY AT INTERFACES

General Remarks

During the 1984 Dahlem Workshop on Microbial Adhesion and Aggregation, the discussion group on activity on sur-
FIG. 5. Effect of attached cells on biomass concentration and productivity in a continuous culture as a function of the dilution rate. The curves are obtained by incorporating Monod kinetics in a steady-state mass balance for a continuous culture with attached and suspended cells. This balance yields: \( D \cdot X = \mu (X + k) \) for the biomass concentration \( (X) \) and \( D(S_0 - S) = (\mu (X + k)) / Y \) for the substrate concentration \( (S) \), where \( \mu \) is the specific growth rate (hours\(^{-1}\)), \( Y \) is the growth yield (grams per mole), \( S_0 \) is the incoming substrate concentration (moles per liter), \( D \) is the dilution rate (hours\(^{-1}\)), and \( k \) is a constant determined by the thickness and density of the biofilm and the surface/volume ratio in the culture vessel. Values used: \( Y = 0.5 \) g mol\(^{-1} \); \( \mu_{\text{max}} = 0.8 \) h\(^{-1} \); \( S_0 = 1.0 \) g liter\(^{-1} \); \( k = 0 \) (no attached growth), 0.05 (wall growth, 500 cm\(^2\) liter\(^{-1} \), 10\(^{-4} \) g of biomass cm\(^{-2} \), and 1.0 (growth on suspended particles, 1 m\(^2\) liter\(^{-1} \)). Adapted from Topiwala and Hamer (99) with permission from John Wiley & Sons, Inc.

FIG. 6. Increase in surface population density of a *Pseudomonas* sp. grown on glass slides in a carbon-limited chemostat \( (D = 0.06 \) h\(^{-1} \). Symbols: \( \square \), 50 nM; \( \blacksquare \), 500 nM; \( \bigcirc \), 50 \( \mu \)M; \( \bullet \), 5 nM glycerol; \( \cdots \cdots \cdots \) growth of suspended population on the basis of the dilution rate. After Ellwood et al. (20).

surfaces concluded: "Attachment to a surface can undoubtedly affect the activity of microorganisms, although sometimes in ways that are not readily predictable on our current knowledge" (8). This statement still holds, especially in the context of possible direct effects of surfaces on bacterial physiology. Many experiments have been reported in which a direct influence of adhesion on microbial activity was suggested, but in only one report has an explanation been proposed (20). In the following sections, experimental evidence for influences of solid surfaces on microbes are critically evaluated in the light of the remarks made above. This part of the review is limited to experiments performed under reasonably well-defined conditions and (in this section) to substrates that do not have a strong interaction with the solid surface. Due to the great diversity in experimental setup, it is necessary to discuss each experiment individually.

**Growth Rate**

Ellwood et al. (20) observed an increase of surface population density on glass slides in a continuous culture of a *Pseudomonas* sp. with a dilution rate \( (D) \) of 0.06 h\(^{-1} \). The data of this experiment indicated that, at least initially, the population increase of the adhered cells was greater than the growth of suspended cells. By supposing that only a small part of the surface-associated "growth" was due to newly adhering cells from the suspension, the authors concluded that the faster increase of the surface population was, for a major part, the result of real growth. To explain this conclusion, the authors suggested that, from cells growing in suspension, a small fraction of the protons at the outer side of the membrane leak into the medium. The presence of a solid surface will hinder diffusion of protons away from the bacterium, and as a consequence (i) increase the efficiency of the membrane processes and (ii) create a more energized bacterial membrane, especially in the vicinity of the solid surface where the diffusional transport of protons will be most reduced. It is, however, readily verified that any effect of retardation of proton diffusion on bacterial growth is negligible. First, the production of 1 g of cells costs approximately 0.1 mol of ATP. If this amount of ATP is generated totally by the proton motive force, 0.3 to 0.6 mol of hydrogen ions have to be circulated over the cell membrane. A loss of only 1% of the hydrogen ions will result in a substantial pH shift in the medium (several millimoles of hydrogen ions would be added), which could easily be detected but has never been reported. Second, loss of protons by microorganisms to the medium, for the sake of electroneutrality, has to be compensated for by other cations, thus keeping the electrostatic potential across the membrane unaffected. Third, the creation of a more energized very small part of the cell membrane due to the presence of a surface is not likely, because protons diffuse very easily through the periplasmatic space. Last, microbial cytoplasmatic membranes from adhering cells are too far away from the area where electrostatic influences of the surface may be of any significance (Fig. 2 and 4). In fact, within a distance of 25 nm the electrostatic Gibbs energy \( (G_{el}) \), originating from the surface, diminishes by a factor of about 10\(^3\). From the above we have to conclude that the theory of Ellwood et al. (20) is not suitable to explain possible significant changes in bacterial activity upon adhesion.

The observed increase in growth rate of adhered cells is more likely due to the fact that the cell concentration on a surface in the culture vessel does not depend directly on the dilution rate; i.e., part of the suspended cells leaves the reactor, whereas all cells associated with the surface remain in the reactor. Before equilibrium is reached (in the dis-
cussed case during the first 20 h (Fig. 6), this uncoupling from the dilution rate is presumably responsible for the observations made by Ellwood et al. (20).

Keen and Prosser (53) reported a 25% increased growth rate and a broader pH range for growth of Nitrobacter cells attached to glass. They reasoned that their observation is not due to an accumulation of nutrients or an altered pH at the surface but more likely the result of an extracellular slime layer formed by attached cells. This layer helps to create a microenvironment low in nitrite concentration, which is the substrate of nitrobacters but is toxic at higher concentrations. The interpretation of the surface effect given by these authors is therefore typical for an indirect effect.

**Growth Yield**

Fletcher and co-workers (9, 10, 25, 26, 28) have extensively studied the influence of adhesion on bacterial activity. The results of these studies did not indicate a consistent trend, and the outcome of the experiments depended strongly on environmental conditions and substratum properties. Only in one experiment did Fletcher (26) report a strong positive influence of adhesion on bacterial metabolism. In this experiment, glucose assimilation and respiration by adhering cells of a *P. fluorescens* isolate exceeded that of free-living cells by a factor of 2 to 5 or even more. Analysis of the published data demonstrates that at the end of the experiment all added glucose had been consumed in the case of the free cells. In the case of attached cells, the corresponding calculation was not possible since the total number of attached cells per flask was not reported. However, by making a mass balance, it appeared that much less attached cells were incubated per flask than free cells. The smaller "activity" of the free bacteria could therefore very well be the consequence of substrate limitation (fewer molecules of glucose per bacterial cell). Hence, the observation is not necessarily attributable to a surface effect.

Classical examples indicating a positive influence of solid surfaces on bacterial activity are the experiments described by Heukelekian and Heller (47) and ZoBell (106). These authors stated that solid surfaces stimulate growth, especially at low nutrient concentrations (<10 mg liter⁻¹). As indicated before, this cannot be explained by the adsorption and concentration of nutrients onto the surface, except for macromolecules which may be retained at the surface.

Heukelekian and Heller (47) measured growth of *Escherichia coli* in a glucose-peptone medium with and without glass beads. In the presence of glass surfaces, especially at low nutrient concentrations, a much higher cell yield was obtained (Fig. 7). The cell yield is even greater than indicated in Fig. 7 since adhered cells were not accounted for in the measurement of the total viable cell count. The experiment of Heukelekian and Heller is quoted but has never been confirmed in the literature. Therefore, we repeated this experiment as precisely as possible from the original description. All glassware was cleaned in chronic sulfuric acid and afterwards rinsed several times with distilled water. The results thus obtained are included in Fig. 7 and are averages of three independent measurements. Lower cell yields were found in the presence of glass beads, comparable to the results reported by Jannasch and Pritschard (50). In our experiments, adhered cells were also not accounted for, which explains the lower apparent cell yield in the presence of glass beads.

In contrast to Heukelekian and Heller (47), we never find higher cell counts in suspension in the presence of glass beads, and we do not have a definitive explanation for this difference. Since the cleaning procedure of the glass beads has not been reported by Heukelekian and Heller, the higher cell yield might perhaps be attributed to a contamination of the glass surface with organic carbon compounds. As a matter of fact, without taking specific precautions, it is extremely difficult to prevent such a contamination. The presence of a small fraction of organic material on the glass beads could explain why at low substrate concentrations (between 12.5 and 0.5 mg liter⁻¹) no change in total cell yield was observed. Another remarkable point in the results of Heukelekian and Heller is the peculiar dependence of cell yield on the added amount of substrate. If the substrate concentration is lowered by a factor of 10 (from 100 to 10 mg liter⁻¹), the cell yield decreases by a factor of 100. Finally, it is striking that most of our cell counts are higher by 1 to 2 orders of magnitude. This could be the result of various factors such as the use of different *E. coli* strains (the original strain is not available any more) or different qualities of peptone or glucose. In any case, our results, which are clearly at variance with those of Heukelekian and Heller, do not support the idea of the existence of any surface effect at low substrate concentrations.

**Substrate Conversion**

Addition of an ion-exchange resin has been shown to induce several changes in bacterial activity (39-42, 70). These changes are as follows.

(1) There is a decreased rate of substrate oxidation (39). Hattori and Hattori (39) attribute it to a reduction of the surface area of the adhered cell that is exposed to the liquid and thus to substrate and oxygen. In addition, they mention that the removal of some essential cofactors by the resin could also be responsible for the observed inhibition. Since all experiments have been performed with a high amount of ion-exchange resin (1 g m⁻³), the diffusion of substrate and oxygen to the cells might in fact become rate limiting. In our classification, this is an illustration of an indirect effect.
(ii) An upward shift in the pH optimum due to addition of anionic resin and a downward shift due to cationic resin (39) occur. The shift in pH optimum due to the presence of an anion-exchange resin, was explained by Hattori and Hattori (39) in the following way. The slight negative surface charge resulting from the bound anions on the anion-exchange resin attracts protons, and as a consequence adsorbed cells are surrounded by a higher concentration of hydrogen ions as compared with the bulk liquid. According to these authors, the adsorbed cell is therefore exposed to a pH that is markedly different from its suspended counterpart. However, as already discussed above, it is very questionable that protons accumulated on the surface will have any significant effects on cell activity (Fig. 4). It is more likely that the pH of the medium itself is changed upon addition of the resin due to exchange of chloride ions with hydroxyl or phosphate ions.

(iii) There is a shift from more reduced to more oxidized end products of glucose fermentation by E. coli. According to Morisaki, this result is due to the fact that the resin acts as an electron acceptor (70). However, it is more likely that the selective binding of certain anionic fermentation products is the reason for the observed shift in dissolved chemicals in the medium. In conclusion, in these examples no compelling evidence for a direct effect of the surface on bacterial metabolism has been offered.

An increased respiration rate and decreased glucose consumption rate by resting cells of E. coli in the presence of a C_{12} or C_{13} alkane-water interface have been reported by Morisaki (72). With C_{6-11} and C_{14-18} alkanes, respiration and glucose consumption did not markedly differ from the control, to which no alkanes were added. Similar effects with the same organism and experimental setup were observed upon addition of solids. The presence of styrene-diphenylbenzene copolymer, polytetrafluoroethylene, and pyrophylite (consisting of silicic acid and alumina) particles in the culture gave a higher respiration/uptake ratio as compared with the submerged cultures without the solids. No variations in this ratio or in the absolute rates was measured with nine other solids, namely, anion-exchange resin, cation-exchange resin, polyacrylate, kaolinite, montmorillonite, alumophane, silicic acid, Celite, and alumina (71). It is difficult to give an explanation for these seemingly inconsistent results. With both the solids and alkanes, only a very small fraction of bacteria was associated with the interfaces, so that the consequences of direct influences of the phase boundary, if any, are outweighed by indirect effects. By calculating from Morisaki’s data (71, 72) the ratio between glucose resired and glucose assimilated, it is found that in the control experiments the assimilated glucose fraction is unusually high, about 89% in the experiments with the solids and about 84% when alkanes were used as the second phase. This fraction decreases to 60% with dodecanes and to 69% with styrene-diphenylbenzene copolymer. In a well-growing culture of E. coli, only about 50% of the glucose is assimilated (33). Since all experiments were done with resting cells, in a medium containing glucose only, this high amount of substrate assimilation is very unexpected.

Humphrey and Marshall (49) showed that a surfactant-like impurity in dialysis membranes had a similar effect on bacterial activity as reported by Morisaki (71, 72). Hence, there might be some kind of effect due to the presence of a surfactant rather than an interface on the bacteria. Thus, with respect to the distinction between direct and indirect influences by the presence of a third phase, these results are also inconclusive.

### Table 2. Indirect effects of the presence of a solid phase on microbial activity

| Effect                                | Reference(s) |
|---------------------------------------|--------------|
| pH buffering                          | 66, 67, 92-94|
| Protection against                    |              |
| Desiccation                           | 11, 64       |
| Viruses                               | 50, 86       |
| Chlorination                          | 36, 45, 103  |
| Radiation                             | 7, 77        |
| Productivity increase at high dilution rates | 4, 14, 59, 68, 69, 99 |
| Increased DNA transformation          | 62           |

### Substrate Affinity

A decrease in substrate affinity (or increase in the half-saturation constant, $K_s$) for adhered cells has been reported regularly (10, 13, 40). According to Bright and Fletcher (10) there are two possible explanations: (i) the difference is due to changes in the environment of the cell (i.e., mass transport limitation), or (ii) the higher $K_s$ values for surface-associated cells is a reflection of a real difference in assimilation behavior. The former is an indirect effect in our classification, whereas the latter would correspond to a direct influence. In general, the first alternative seems to be the most realistic since the change in $K_s$ is independent of the type of attachment surface (10). The determination of the "apparent" $K_s$ values is, in fact, used to determine mass transport limitations to adhered cells or in biofilms (18, 79). Caldwell and Lawrence (13) demonstrated the occurrence of a glucose diffusion-limited growth of adhered cells, even at glucose concentrations of 0.1 g l^{-1}. The decrease in growth at high surface population density as observed by Ellwood et al. (20) (Fig. 6) might also be caused by substrate diffusion limitation. Thus, substrate affinity changes might all be attributed to indirect effects.

On the basis of activity measurements on attached and free-living *Vibrio* sp., Jeffrey and Paul (52) suggested that not only the apparent substrate affinity but also the maximum substrate conversion rate of attached cells are different from suspended cells. According to these authors, the presence of a solid surface hinders substrate uptake for approximately 20% of the cell surface and hence the maximum conversion rate is reduced, just as proposed by Hattori and Hattori (39); 20% is probably a much too high estimate (Fig. 4).

### Miscellaneous Effects

By modifying the physicochemical environment of the microbes, or the interaction between a microbe and its surroundings, surfaces may have various indirect influences on bacterial activities (Table 2). Among the many examples, the effects of clay minerals have been extensively studied, particularly by Stotzky (91). From some 100 samples of clay minerals and various particles which possess some of the characteristics of clays, only montmorillonite virtually stimulated the respiration of bacteria, basically by maintaining the pH of the environment at a level suitable for growth. The stimulating effect of montmorillonite was confirmed with more than 20 bacterial species differing in morphology, motility, Gram reaction, stage of growth, etc. (91-94). The maintenance of a favorable pH was found to depend on the
initial pH of the system, the relative basicity of the cations on the clay, and the buffer capacity of the clay particles. The positive effects of zeolite (10 g liter⁻¹) on the efficiency of the aerobic wastewater treatment, in particular, nitrification (89), might also be the result of a more optimal pH. However, the data presented by Sims and Little (89) do not allow us to draw clear-cut conclusions. Many other explanations are also possible, e.g., increase of the retention time in the treatment plant of ammonium or nitrifiers adsorbed to zeolite.

Survival of bacteria in soils has been found to be related to the presence of, especially, montmorillonite-like clay minerals (64). Particles of these clays can form a coating on the bacterial cell surface and protect the cell against protozoa grazing (48) or virus attack (50). The action of viruses can be neutralized in two ways: at low ionic strength, bacteria are protected against viruses by a clay envelope; at high ionic strength, however, the viruses themselves adhere strongly to soil or sediment particles (36, 86).

The clay envelope can also protect bacteria from excessive desiccation. Bushby and Marshall (11) found that the resistance of fast-growing rhizobia to dryness was improved by the presence of montmorillonite. Based on an examination of water adsorption isotherms on fast-growing rhizobia, these authors suggested that the sensitivity of these bacteria to desiccation in the absence of montmorillonite is related to their relatively high state of internal hydration at low vapor pressures. Since at low vapor pressure substrate availability is, in general, limited, under such conditions enzyme activities should be reduced to assure the survival of a microorganism. The high state of internal hydration does, however, prevent the reduction of enzymatic activity. As montmorillonite has a higher affinity for water than the microbial cell, the presence of a clay envelope may protect a bacterium by reducing its internal hydration status just to a level at which most enzyme activities cease but protein denaturation does not yet occur.

Absence of Effects

Many experiments on the relation between activity of bacteria and solid surfaces were performed with clay minerals as the solid phase. Filip and Hattori (23) and Stotzky (91) showed that there was no evidence of any direct influence of the mineral surface since addition of clays to a bacterial suspension promoted growth irrespective of whether the clay was applied directly to the solution or separated from it by a dialysis bag.

Several publications exist which show no (in)direct effects of addition of a solid surface to a bacterial culture (Table 1). For instance, Gordon et al. (31) used microcalorimetric and respirometric techniques to detect changes in activity upon adhesion of Vibrio alginolyticus to hydroxyapatite. These authors clearly showed that bacterial activity (i.e., heat and CO₂ production) on glucose or glutamate was not affected by the presence of particles, regardless of whether the bacteria, the organic nutrient, or both were associated with the surface. Also, with Saccharomyces cerevisiae (ethanol production from glucose) or Arthrobacter simplex (prednisolone production from cortisol), no significant difference in the specific activity of irreversibly attached cells could be measured (75, 87, 100).

Conclusion

The conclusion of this section is that, in the majority of the cases studied, the influence of a solid on microbiological activity is an indirect feature, because the solid influences the medium rather than the bacterium. Even in some experiments that have been quoted as proof of direct alterations in the bacteria, closer inspection revealed that indirect influences would probably prevail.

BIOCONVERSION OF ADSORBED MOLECULES

A summary of the literature related to the bioconversion of adsorbed substrates is given in Table 3. Below, some aspects are discussed in more detail. A distinction is made between small molecules and macromolecules (biopolymers) because these different molecules are not only taken up by cells in different ways but, in general, also behave differently on surfaces.

Small Molecules

Introduction of an adsorbing solid phase into a liquid medium may decrease the substrate concentration in solution. The result may be a reduced substrate utilization rate. Examples are the reduction of ammonium oxidation in the presence of different clay minerals (30) and the reduction of succinate assimilation in the presence of an anionic resin (39). Ogram et al. (81) showed that, in a soil slurry with 2,4-dichlorophenoxyacetic acid, only substrate in solution is
degraded by the attached and suspended bacteria. Thus, 2,4-dichlorophenoxyacetic acid must first desorb before degradation can take place.

On the other hand, in the case of toxic substances, a lowering of the substrate concentration due to adsorption can lead to an increased microbial activity. The degradation of toxic amounts of, e.g., aldehydes (91), benzylamines (96), and phenol (19, 73) has been found to be stimulated upon addition of montmorillonite or activated carbon. Also, the adsorption of specific inhibitors, and, as a consequence, their removal from the solution have been shown to promote bacterial growth (38, 97).

Should only the substrate in solution be converted by bacteria, as found for the system studied by Ogram et al. (81), then the bioconversion of strongly adsorbing (mostly hydrophobic) substrates may become desorption limited. Bioconversion rates will then become dependent on the solid surface area present in a system. Thomas et al. (98) conducted a study of the relationship between the dissolution rates of organic compounds that are sparingly soluble in water and their rates of biodegradation. Bacterial growth caused a reduction in the concentration of naphthalene or 4-chlorobiphenyl in solution. Upon depletion of these compounds in solution, the bacterial growth rate decreased abruptly. Similar results have been found for the bioconversion of hexachlorocyclohexane (H. H. M. Rijnaarts, A. Bachmann, J. C. Jumelet, and A. J. B. Zehnder, Environ. Sci. Technol., in press) and n-alkylamines (105). Although desorption seemed in all of these studies to become rate limiting for biodegradation, the biodegradation rate was still greater than the rate of desorption in sterile systems. Apparently, the actual desorption rates in sterile systems differ from those in nonsterile systems, which may be an indication of a steeper concentration gradient near the surface as a consequence of microbial activity.

In the case of irreversible substrate adsorption, bioconversion is expected to be inhibited. This has actually been reported for aspartate, cysteine (17), diquat (104), and several proteins (91).

Macromolecules (Biopolymers)

Biodegradation of adsorbed macromolecules is complicated by the fact that macromolecules are (i) usually irreversibly adsorbed, (ii) have first to be degraded by exoenzymes to small molecules before they are taken up by the cell, and (iii) often undergo structural changes upon adsorption (especially proteins). The earliest experiments on the biodegradation of macromolecules have been reported by Zobell, who incubated nonsterile seawater in a range of glass bottles with different surface (glass wall)/volume ratios (106). In small bottles with high surface/volume ratios, the greatest oxygen consumption and the most pronounced increase in bacterial counts were measured. Zobell's explanation for these observations was: "It is believed that besides concentrating nutrients by adsorption and providing a resting place for sessile bacteria, the solid surface retards the diffusion of exo-enzymes and hydrolyzates away from the cell, thereby promoting the assimilation of nutrients which must be hydrolyzed extracellularly prior to ingestion" (106). This view is supported by an experiment of Hermansson and Dahlbäck (44), who investigated protein degradation at the air-liquid interface. At a low surface coverage (0.06 mg m⁻²) with proteins, all proteins were converted by cells adhering to the air-liquid interface. At a higher surface coverage (0.15 mg m⁻²), however, a higher amount of CO₂ was released in the bulk solution. The authors' explanation was that more protein is hydrolyzed at the interface than can be utilized by the adhering bacteria. The hydrolyzates left over then diffuse into the bulk liquid, where they are subsequently degraded by suspended bacteria.

Although the presence of surfaces has been shown to enhance the bioconversion of some macromolecules, such as casein, ligninprotein, chitin, and lysozyme (21, 106), in general degradation of polymers is found to be slowed down when a system contains biologically inert surfaces. A decreased degradation was observed for proteins (22, 65, 91) and for DNA (35, 62, 63, 80). This decrease could be the result of various factors such as desorption limitation, structural changes making the polymers less susceptible for enzymes, or adsorption and inactivation of exoenzymes. A possible influence of the conformation of a polymer on its biodegradation rate was shown by Marshman and Marshall (65), who studied bacterial growth on proteins (gelatin, bovine serum albumin, and lysozyme) in the presence of different amounts of clay minerals (montmorillonite and kaolinite). Depending on the protein/clay ratio, growth of bacteria was or was not affected. At a high protein/clay ratio (most of the protein is in solution), no effect on growth was measured; at intermediate protein/clay ratios, the growth rate was reduced but not the final cell yield; and at low protein/clay ratios (all of the protein is adsorbed to the clay), the protein was unavailable for microorganisms.

The conclusion of this review on the biodegradation of adsorbed molecules is that all observed features can be fully understood without assuming any direct influence of the solid phase on bacteria.

CONCLUDING REMARKS

The presence of surfaces may positively or negatively (or not at all) affect microbial substrate utilization rates and growth yields. The results often depend on the nature of the organism, the kind and concentration of substrate, and the nature of the solid surface. In interpreting the effect of surfaces on bioconversion processes, all possible physical and chemical interactions (e.g., diffusion ad- and desorption, ion-exchange reactions, conformation changes, etc.) of a given compound and its possible metabolites with a given surface have to be considered before general conclusions can be drawn.

On the basis of this review, it can be stated that so far there is no conclusive evidence that adhesion directly influences bacterial metabolism, in the sense that the bacteria undergo a structural change due to the adhesion. Observed differences between adhered and free cells could, depending on conditions, all be attributed to an indirect mechanism, i.e., a mechanism by which the surroundings of the cells are modified due to the presence of surfaces but not the cell itself.

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