REVIEW PAPER

ATTACHED MICROBIAL GROWTHS—I. ATTACHMENT AND GROWTH

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OCCURRENCE OF MICROBIAL SLIMES

WATER-SOLID interfaces occur universally and are critically important in natural and industrial processes. Microorganisms accumulate at these interfaces, frequently with beneficial effects.

A trickling filter can be described as a fixed bed reactor supplying a large amount of surface area used for the purification of organic waste of domestic or industrial origin. Microorganisms attach to the packing material and form a reactive surface for the adsorption and breakdown of the wastes. The benthos of natural streams carries out an analogous process although this can be a significant drain on the oxygen resources of the stream.

More often, however, microbial attachment is undesirable. Marine fouling is initiated by the development of slimy films on submerged surfaces. The formation of such primary films is believed to be a necessary precursor of heavy fouling (WOOD, 1967) and is, at least in part, the result of microbial activity.

HORBUND and FREIBERGER (1970) present a review of the role of slime films in marine fouling. The slime film which accumulates on non-toxic substrates greatly enhances the desirability of the surface for further attachment. The marine bacterial colony secretes a substance, primarily polysaccharide, which has a slimy consistency and is tacky. The secretion encourages attachment of other organisms and food for other forms. Fouling of heat exchangers and cooling towers by microbial attachment is a continuing problem for industry and, in particular, power generating stations.

Surface attachment is one of the biggest operating problems in laboratory biological reactors and causes difficulties in scale-up. Unless wall growth is accounted for, the laboratory process with high surface-to-volume ratio can give an over-optimistic prediction of necessary residence time and stability of the full-scale process because washout becomes impossible. As HAMER (1972) noted, the walls of a chemostat represent the situation where a surface enables bacteria to grow on a substrate which is otherwise too dilute to support growth. HOWELL *et al.* (1972) developed a simple model which describes scale-up problems and dynamic operating characteristics of biological reactors.

Biological fouling of carbon columns is a constant problem in water and wastewater treatment operations. Attached organisms can increase organic removal rates through the columns for short periods until sloughing begins and effluent quality

deteriorates. Frequently anaerobic conditions due to heavy slime growth occur, resulting in production of sulfide and other undesirable products.

Deterioration of pipeline capacity has been attributed, in many cases to growth of thin attached microbial films. Striking examples of conduits incurring frictional losses of this type are discussed in detail elsewhere (Part II of this review).

Beneficial clinical effects might be observed following oral administration of kaolin, or charcoal that could be attributed to inactivation of adsorbed pathogens. GUNNISON and MARSHALL (1937) suggest however, that adsorption of toxins or enzymes are at least partially responsible.

CHARACTERISTICS OF SLIME

Slime is a generic term used in the context of this review to mean attached microbial growths and associated material. Slime has been defined as the viscous or gelatinous film formed on submerged surfaces, including both living and non-living material (HEUKELEKIAN and CROSBY, 1955). It appears to act as a binder or matrix which holds large colonies of microorganisms together in an aggregate or floc (MAIER, 1967), and is composed primarily of polysaccharides (WILKINSON, 1958).

Slime organisms

Slimes in natural streams are composed of predatory, phototrophic and chemotrophic microorganisms with the initial and primary population consisting of heterotrophs, i.e. completely dependent upon oxidation of organic substrates for energy (SANDERS, 1966).

Samples taken from trickling filters used on wastewater treatment indicate that microbial growth originates in a mixture of slime and zooglyphic bacteria (microorganisms that form gelatinous aggregates). Other microorganisms associated with zooglyphic slime include filamentous bacteria and fungi, protozoa, and algae (MAIER, 1966).

The biological composition of the primary film developed in aerobic and anaerobic locations in sewage treatment plants is similar to films observed in trickling filters. A more diversified fauna is formed in aerobic locations. The bacteria *Beggiatoa*, *Thiotrix* and the zooglyphic bacteria become abundant as the slime develops (HEUKELEKIAN, 1956b).

Chlamydothrix (frequently referred to as "slime bacteria") are sometimes the cause of obstruction in pipes (FROBISHER, 1937). One of the most prominent members of this order, *Sphaerotilus*, is a filamentous eubacteria which grows as a chain of cells surrounded by and enclosed in a delicate, smooth-walled sheath (STANIER *et al.*, 1963). Accumulation of the hydroxides of iron and manganese give the sheaths a yellow or brown color.

The bacterium *Crenothrix* also exudes a tubular sheath. Filaments attach themselves by one end to some solid object. Fission of the uppermost cells takes place in three planes before liberation from the sheaths. The increase in volume at the upper end further complicates the problem in pipelines. *Crenothrix* will form large gelatinous masses that clog pipelines containing water with low dissolved oxygen and high iron concentration.

Another order of bacteria very prominent in slime formation are the stalk-forming *Caulobacteriaceae*. *Gallionella ferruginea* forms stalks encrusted with $\text{Fe}(\text{OH})_3$. Multiplication of the organism by branching of stalks causes complex tangles leading to extensive deposits and incrustation of iron which may eventually occlude the pipe. Iron bacteria thrive in an environment containing Fe^{2+} so the surface of corroding steel is ideal for their growth (PIATEK, 1969). The organisms surround themselves with hydrated oxides that result from the oxidation of Fe^{2+} and these deposits develop into troublesome gelatinous globules which clog pipe passages.

Presence of ammonia in water may give rise to nitrifying bacteria that are also adsorbed in slime (KOOIJMANS, 1966). Nitrification results in high oxygen consumption which lowers oxygen content to a level conducive to growth of iron bacteria.

The biological composition of slimes examined during a survey of slime outbreaks in the United Kingdom is described by CURTIS and CURDS (1971) and compared with biota of other polluted habitats. Slimes were frequently dominated by *S. natans* or zoogloeal bacteria. Interrelationships of the various species were studied using cluster analyses and diversity indices.

Composition of slimes

LAMANNA (1965) describes bacterial slime and capsular material as ergastic or non-living material. It is considered to be an excretion or secretion which has no direct metabolic function. The bulk of the slime material may have different characteristics than the immediately adjacent capsule material as indicated by distinctly different antigenic properties. Composition is reported to be predominantly carbohydrate (dextran, fructosans, lactosans) containing small amounts of nitrogen. The nitrogen is believed to be incorporated in polymers of hyaluronic acids or similar compounds. The carbohydrates are polymerized to produce branched or linear polysaccharides. Polysaccharides from each bacterial species is believed to be unique. For example, the capsular material of *Sphaerotilus natans* has been isolated and purified and analysis has indicated that the major components are fucose, galactose and glucuronic acid in approximately equimolar amounts (GAUDY and WOLFE, 1962).

Cell binding materials may be of low molecular weight and soluble or high molecular weight consisting of a high degree of cross-linkage, i.e. insoluble matrix. The molecular framework may be comprised of polymeric carbohydrates containing anionic and non-ionic functional groups such as $-\text{COOH}$ and $-\text{OH}$ (BUSCH and STUMM, 1968).

WILKINSON (1958), in a comprehensive review of bacterial polysaccharides, states that capsular material is highly hydrated, containing as much as 98 per cent water. Consequently the material is slimy or gelatinous in consistency (STACY and BARKER, 1960) and has approximately the same refractive index as the medium (LAMANNA and MALLETT, 1965). During the growth and autolysis of pneumococcus organisms in a liquid medium, the capsular material is continually going into solution (STACY and BARKER, 1960). KENIS (1968a) and PAVONI (1972) observed a similar phenomenon.

Excellent photomicrographs of attached slime indicate polysaccharide material compacted in areas adjacent to the microorganisms. Cells of this type did not stain heavily suggesting that the dye diffused very slowly through the surrounding material (JONES *et al.*, 1969). This property of slime has important implications in mass transfer through attached biological films.

FACTORS AFFECTING THE GROWTH AND ATTACHMENT
OF SLIME ATTACHMENT

RENN (1956) has stated that "... when water containing low concentrations of organic matter stands undisturbed, the organic matter reorients itself so that energy is lost. This is done by concentration of the matter at the air-water and solid-water interfaces. Work is done through decrease in the interfacial tensions existing at the air and container interfaces." This statement refers to sterile systems but could be applied equally well to biologically active systems. Surface tension depressants or detergents in dilute solutions retard the attachment of films (ZOBELL, 1943). In non-sterile systems, the microorganisms migrate to the interfaces where interfacial trapping and/or preferential growth takes place (ZOBELL, 1936). More bacteria attach during logarithmic growth than during later growth phases (ZOBELL, 1943).

HOOPER and GRZENDA (1957) found that organic matter adsorbed on surfaces regardless of utilization and that slime mass increases the rate of adsorption of organic materials at interfaces.

Bacteria do not attach to glass slides very tenaciously unless left in contact with water for several hours. Then, some are attached so tenaciously that they are not dislodged by washing or staining. This suggests that bacteria grow on, or attach themselves to, solid surfaces rather than merely being passively stuck (ZOBELL, 1943).

ZOBELL (1936, 1937, 1943) suggested that the point of attachment between a bacterial cell and the solid surface may serve as a concentration point for nutrients and also exo-enzymes that promote the assimilation of the nutrient material. Zobell hypothesized that bacterial attachment occurs in two steps: (1) cells adsorb to the surface in the physical sense; (2) attachment then begins with the aid of capsular material or by other methods. JONES' micrographs (1969) lend support to these suppositions. The micrographs show that the slime does not consist of layer upon layer of organisms but instead is comprised of microorganisms imbedded in a gelatinous matrix of polysaccharide and water, the volume of microbial cells representing only a small portion of the total slime volume.

MEADOWS (1971) indicates that mobile Gram-negative bacteria readily attach to and detach from glass slides, remaining attached anywhere from a few seconds to many hours. The cells attach at one end or along their length. Those attaching at one end appear to do so by their polar flagella. Bacteria killed with u.v. radiation attach as readily as living bacteria, but considerably fewer attach when killed by heat or formalin. From this, Meadows concludes that attachment depends on the integrity of the cell wall. He also suggests that under natural conditions, bacteria killed by most forms of physical and chemical stress in the environment are unlikely to attach. Consequently, solid surfaces in natural waters are likely to be initially colonized by viable cells.

Large molecules or particulate materials must be hydrolyzed by exo-enzymes before the organic matter can be ingested and assimilated. In dilute solution, containing few bacteria, bacteria have to be in close contact with hydrolyte for exo-enzymes to be most effective or the enzymes are dissipated in solution. It is conceivable that hydrolyzates might diffuse away from cells responsible for their formation. However, if the cell is on a solid surface juxtaposed with nutrient hydrolytes, the solid surface may retard diffusion of exo-enzymes and hydrolyzates away from cells. Besides the physical attraction which might be exerted by the solid surface, an anchored cell would not be

influenced by the bombardment of molecules which would tend to separate the cell from its exo-enzymes and hydrolyzates. Interstices between cell and solid surfaces might also serve as concentration foci for nutrient molecules (ZOBELL, 1943).

Solid surfaces might also facilitate optimum orientation position of bacterial exo-enzymes (DANIELLI, 1937).

Attachment kinetics. KORNEGAY and ANDREWS (1968) reported growth of biological films in an annular reactor occurring in three distinct phases. The first phase was logarithmic and lasted until the "active" film thickness was reached. At the "active" thickness, substrate utilization reached a steady state value and was not affected by additional film accumulation. During the second phase, film growth was essentially linear and film continued to accumulate until the plateau film thickness was reached. This was the beginning of the third phase in which film accumulation was equal to zero and the newly formed cells were washed from the reactor. It should be noted that HELMSTETTER and CUMMINGS (1963) used the attachment characteristics of cells to advantage in producing synchronized bacterial cultures. When the growth medium flowed past the surface, the only cells appearing in the medium were the new daughter cells which were not involved in the attachment.

SANDERS (1967) describes a model for growth and development of attached stream bacteria. Growth rate of slime bacteria becomes limited when the attaching surface becomes completely covered with one layer of cells. At this point the increase in mass of organisms shifts from logarithmic to linear with time. A second growth limiting factor occurs when the thickness of slime mass exceeds the limiting thickness for diffusion of oxygen. From this point on, oxygen consumption rate is constant. The maximum rate of nutrient removal presumably occurred when the slime thickness equalled the limiting thickness for oxygen diffusion. Subsequent experimental data by the author indicates that the hypothesis is not tenable (SANDERS, 1971).

KALIUZHNY (1962) describes a theory for adsorption of yeast cells on cellulose fibers based on the biophysical state of the cells which could be extended to slime formation. The role of aqueous membranes in yeast cells and the state of the water in the membrane region are emphasized. The effect of depressing and dehydrating substances such as Ca, Mg, and Fe and ethyl alcohol on the aqueous membrane and ionic sphere are also implicated. Water binding is also shown to be related to polysaccharide content of the cells. Yeast flocculation is accompanied by a decrease in free water and increase in bound water. The same process occurs in yeast sorption by cellulose fibers. An analogous process would be the attachment of microorganism to an inert surface with the aid of microbially produced polymer molecules.

Initial microbial attachment to *submerged* surfaces is probably in the form of monolayers. Attachment to *non-submerged* surfaces appears to be different. Thick layers of cells tend to build up at the air-liquid interface and, in effect, the layer of microorganisms so formed constitutes a microbial film reactor (HAMER, 1972).

UNO and TANAKA (1970) studied adhesion to glass of particulate pharmaceuticals and small synthetic particles from aqueous suspension at the air-liquid-solid interface. Observations indicate that such particles adhere to glass walls of vessels as thick layers above the surface of the suspending liquid. Particle trapping is the essential first step and the proposed mechanism is one of spreading wetting with surface tension of the liquid film providing the force for entrapment at the liquid-air-particle interface. The number of trapped particles is proportional to the bulk concentration of particles and

particle size. The second step is particle adhesion which is proportional to bulk particle concentration, particle size, wetting interval and frequency, temperature and flow rate of the aqueous film over the glass surface.

Role of biopolymers. ZOBELL (1943) suggested that after coming into contact with a solid surface, physiologically active attached bacteria secrete a cementing substance. The mucilaginous film has high adsorptive properties and is believed to be instrumental in the fouling of submerged surfaces (ZOBELL, 1943). However, it is possible that the mucilaginous substance might arrive at the inert surface first and enhance the bacterial attachment.

PAVONI *et al.* (1972) investigated exocellular polymer production in mixed cultures and indicated a direct correlation between polymer production and biological flocculation. Flocculation probably results when the polymers bond electrostatically or physically and bridge the cells of the dispersion into a settleable matrix.

FRIEDMAN *et al.* (1968a, b, c, 1969) discuss the exocellular polymer material of *Zoogloea* species and its relationship to floc formation. The polymer is primarily polysaccharide in nature and has the ability to concentrate metallic ions and nutrients. The authors suggest that the polymer matrix can serve a nutritional function for the cell by concentration of nutrients from solution. This could explain how slime organisms grow in extremely dilute nutritional aquatic habitats.

CORPE (1970) isolated a bacterium identified as *Pseudomonas atlantica* from slides submerged in seawater. The bacterium produced a viscous polymer believed to be involved in the attachment of the bacterium to solid surfaces. An acid polysaccharide containing glucose, galactose, mannose, galacturonic acid, pyruvic acid and other unidentified constituents, was isolated from peptone-glucose seawater broth.

Effect of inorganic salts. Adsorption by polysaccharide material is not necessarily the only mechanism by which cells attach to solid surfaces. NORDIN (1967) showed that interfacial phenomena are directly involved in adhesion of *Chlorella*, a unicellular alga, to glass surfaces in simple ionic solutions. The strain *Chlorella* was rather unique in that it produced no extracellular slime layer. The principal mechanism governing adhesion appeared to be electrostatic interaction between electrical double layers and various specific surface interactions resulting from surface heterogeneity and ion adsorption. Under most conditions, the algal cells and glass surfaces have negative zeta potentials and adhesion does not occur. When FeCl_3 is added, the zeta potentials change and adhesion occurs. DUGAN (1972) also notes the influence of ionic strength of the liquid on adsorption and flocculation of biologically active cells.

Effect of nutrient on attachment and growth

In general, the amount of slime formed varies directly with concentration of the nutrient material (REID, 1960; HEUKELEKIAN, 1956). Abundant slimes which readily slough are characteristic of all growth in liquids high in oxidizable material (HEUKELEKIAN, 1956), while dense, readily adhering slimes are most characteristic of low concentration nutrient solutions. Nevertheless, KORNEGAY (1968) observed no appreciable change in chemical composition of slime film (carbon, hydrogen, oxygen and nitrogen) with changes in substrate or dissolved oxygen concentration. Increasing solid surface area ordinarily does not alter the rate of bacterial multiplication or metabolism in solutions containing more than $5\text{--}10\text{ mg l}^{-1}$ organic nutrients (ZOBELL, 1943) because most of the uptake is then from organisms suspended in the fluid.

Apparently, the nature or availability of oxidizable material is of as great significance as total concentration. HEUKELEKIAN (1955) noted increased slime growth in sewage when glucose and/or peptone were added. Biochemical Oxygen Demand (BOD) studies have indicated that overall uptake of glucose by bacteria as being the highest among simple compounds (GULEVICH *et al.*, 1967). Glucose and other sugars provide a readily available energy source and easily convertible carbon skeletons which provide the "building blocks" for polysaccharide synthesis.

In the initial stages of attachment, the effect of solid surfaces upon bacterial activity is more pronounced with colloidal rather than dissolved substrate where proportionately more bacteria occur suspended in solution (ZOBELL, 1943).

Increasing solid surface area does not influence the rate of bacterial activity in dilute solutions ($1-10 \text{ mg l}^{-1}$) of glucose, glycerol and lactate. Conversely in solutions enriched with 5 mg l^{-1} sodium caseinate, lignoprotein or an emulsified chitin preparation, a beneficial effect can be demonstrated (ZOBELL, 1943). Bacteria resuspended in dilute solutions of the proteins salmine or albumin attach in fewer numbers than those resuspended in buffer. Dilute protein solutions of casein or gelatin promote greater attachment of bacteria as compared to the buffer (MEADOWS, 1971). ATKINSON and SWILLEY (1967) indicated that the presence of inorganic colloidal material inhibits nutrient uptake by microbial slimes. This suggests a relationship between solubility, dispersion or molecular size of the organic nutrient and surface phenomenon (ZOBELL, 1943). Such a relationship could be invaluable in understanding processes of bacterial attachment.

The growth medium strongly affects production of polysaccharide by bacteria which in turn affects adsorption properties of cells. In suspended microbial cultures, when the level of nitrogen (ammonium salts) is gradually lowered until it becomes growth limiting, the amount of polysaccharide formed per cell increases to a maximum level (WILKINSON, 1958). Similar results were obtained with phosphorous and sulfur in growth-limiting concentrations. CHARACKLIS (1970a) found that the dry weight of *attached* microbial slime also increases with increasing carbon to nitrogen ratio. KENIS (1972) has found that "all" waters tested produced drag-reducing polymers when fortified with glucose and sucrose indicating omnipresence of polymer-forming bacteria.

The nature of the food (physical and chemical properties), as well as its metabolic role, influences attachment and growth of attached microbial films. However, a distinction should be made regarding the influence on growth as opposed to attachment.

Effect of solid surface

Surface roughness is not a significant factor in the total amount of slime formed (HEUKELEKIAN, 1956a; ZVYAGINTSEV, 1959). Surface properties, however, are important in the primary film forming period (HEUKELEKIAN, 1956). Coating the surface with silicones delays initial buildup, but does not reduce the total number of attached organisms (LARSEN, 1964). Surface tension depressants or detergents in dilute nutrient solutions retard the attachment of the film (ZOBELL, 1943). The degree to which glass has been cleaned of organic material has a substantial effect on adsorption capacity of the glass (ZVYAGINTSEV, 1959). These observations again indicate the possibility that organic polymers adsorbed to inert surfaces enhance microbial attachment.

Organic nutrients on solid surfaces promote the multiplication of bacteria but there is evidence that adsorbed low molecular weight nutrients are not directly responsible for the attachment of bacteria. Slides coated by organic matter from sterile seawater show no significant difference in bacterial numbers (ZOBELL, 1943). CORPE (1970), however, isolated an acid mucopolysaccharide from a marine bacterium growing on a surface submerged in seawater. Air-dried smears of the high molecular weight polymer on glass slides accelerated attachment of microorganisms and other debris.

Effect of bacterial cell concentration and species

There is no apparent dependence of cell adsorption on the systematic position of the organism, its Gram reaction, morphology or motility (ZVYAGINTSEV, 1959; LARSEN, 1964; ZOBELL, 1943). Some species of bacteria, especially in concentrated cell suspensions, form continuous monolayers in 10–30 min and if the bacteria are not separated into single cells, adsorption occurs in several layers.

Heterogeneous cultures have shown a marked superiority over pure cultures for slime growth especially when higher life forms are present (REID, 1960). Bacteria, however, are the most important group of organisms. It has been observed in trickling filters that free and colonial Gram negative rods are dominant as zoogeal masses. *Sphaerotilus*, *Beggiatoa* and other unidentified filamentous organisms are also present (TOMLINSON, 1966).

CHARACKLIS (1970b), working with organisms from the species *Bacillus* and *Bacteroides*, observed more slime growth with the latter organism in identical semi-continuous reactors.

In summary, there are certain species of organisms which are more likely to attach and reproduce on surfaces. The degree and rate of attachment however, is also likely to be dependent on the specific environment the species encounters.

Effect of velocity and shear forces. Higher velocities retard primary film formation but once established, faster flows result in greater growth (HEUKELEKIAN, 1956a). Experiments by the MINISTRY OF TECHNOLOGY (1965) indicate maximum slime development occurred between 0.5–0.9 ft s⁻¹ with higher velocities promoting scour and lower velocities unable to produce an adequate supply of nutrients. SANDERS (1966) and CHARACKLIS (1967) grew slimes within the velocity range 0.1–1.0 ft s⁻¹ and obtained maximum growth at the upper limit.

Greater growth of slime is observed at higher velocities past the film surface (CHARACKLIS, 1967; HEUKELEKIAN, 1956a; SANDERS, 1966). This effect has been attributed to the better transport of food molecules from the bulk fluid to the surface of the bacterial cells (HEUKELEKIAN, 1956a; HARTMANN, 1967).

Kornegay found that the "plateau" film thickness decreased with increasing shear force at the walls. "Active" film thickness, however, was independent of shear force (70 μ m) with glucose concentration at 215 mg l⁻¹. The films were much more uniform at high mixing speeds.

Slime films can withstand shear forces exceeding 10–15 dyn cm⁻² (CHARACKLIS, 1967; ZVYAGINTSEV, 1959). Slimes grown at higher velocities adhered to the surface more firmly (CHARACKLIS, 1967).

It should be noted that species distribution of organisms in the film varies with the level of turbulence (CHARACKLIS, 1971).

Effect of temperature. The published temperature effects regarding slime attachment

and growth are confusing at best. The quantity of microbial slime formed varies inversely with temperature or occurs predominantly at the optimum growth temperature. Several optimum temperatures for slime growth have been reported:

REID (1960)	27°C,
GREEN (1965)	20–30°C,
SANDERS (1966)	20–40°C.

The quantity of film in a trickling filter decreases with increasing temperature (HEUKELEKIAN, 1945).

FARRELL (1967) points out that synthesis of polysaccharides is often greater at sub-optimum temperatures than that for growth of the microorganism. *E. coli* at 15–20°C produces 25 times the amount of polysaccharide produced when it is grown at 37°C, its optimum growth temperature. WILKINSON (1958) reports similar results with *E. coli* and other members of the *Enterobacteriaceae*. Polymer production by *K. aerogenes*, on the other hand, was relatively unaffected by temperature. It is conceivable that polysaccharide synthesis could account for the observed optimum temperatures for slime growth being somewhat lower than optimum temperatures for suspended bacterial growth. However, FRIEDMAN *et al.* (1968b) indicates that growth temperature is critical for synthesis of the zoogeal matrix and KENIS (1972) indicates that maximum drag-reduction for several bacteria occurs at the optimum growth temperature.

GREEN *et al.* (1965) report the percentage of dry matter in slime to range between 3.5 and 6.5 per cent between temperatures of 5–30°C. Higher temperatures yielded slimes with higher dry weight.

Effect of pH. Slime growth is maximum when pH is maintained near neutrality. REID (1960) suggested pH 7.2 as an optimum. Interestingly, maximum polysaccharide production by a bacterial culture occurs at neutral pH (WILKINSON, 1958). Maximum polysaccharide production by *D. pneumoniae* takes place at pH 6.5 with good production occurring from pH 5 to pH 8.

Effect of dissolved oxygen. KORNEGAY (1968) found the “active” film thickness to be independent of dissolved oxygen (DO) concentration. DO had no effect on substrate removal rate at concentrations from 2.5 to 15 mg l⁻¹ for glucose concentration of 215 mg l⁻¹. SANDERS *et al.* (1971) measured oxygen profiles in slimes and found that oxygen existed below the “active” layer of slime indicating that DO was not the rate-limiting factor. BUNGAY *et al.* (1969) and WHALEN *et al.* (1969) report similar results and apply traditional diffusion equations to steady state DO profiles and transient response data to estimate diffusivity of oxygen and oxygen uptake rates in microbial slimes. Slimes up to 200 μ m thick were always aerobic in 500 mg l⁻¹ nutrient broth containing 7–8 mg l⁻¹ DO (SANDERS, 1971).

A possible explanation for these observations is that substrate diffusion is limiting the removal rate but further research in this area is necessary to explain the observed results.

RATE CONSIDERATIONS FOR NUTRIENT UPTAKE

Fundamental approaches to nutrient uptake have led to several models for fixed biological film reactors and have concentrated on mass transfer reaction kinetics and the relative importance of the two processes.

ATKINSON *et al.* (1963) mathematically analyzed the removal of a soluble organic compound flowing over a flat plate under laminar and turbulent conditions with the biochemical reaction occurring throughout the liquid phase, i.e. the liquid flows through a spongy or fibril mass. The development assumed a slow first order, irreversible reaction which limits the uptake rate of soluble substrate.

SWILLEY (1963, 1964) studied the film flow reactor (flat plate) for different controlling mechanisms and the following generalized reaction:

The reaction scheme may be written $A + B \rightarrow C$ where

A $\equiv$ incoming organic substrate,
 B $\equiv$ Exogenous electron acceptor,
 C $\equiv$ desired product.

Assuming an excess of component B, the rate of reaction is equal to $K C_A$ where C_A is the concentration of component A, and K is the reaction rate coefficient or possibly a mass transfer coefficient. In homogeneous systems, organic substrate flows through the microbial population reacting with microorganisms at all points in the liquid phase. In the heterogeneous case, liquid flows over the biological film and reaction occurs only at the surface of the biomass. Limiting cases of reaction and diffusion controlled models were formulated for the heterogeneous case. Results for glucose indicated that diffusion of substrate in the liquid phase was the limiting mechanism in removal of organic substrates.

Experimental work by ATKINSON and SWILLEY (1967) involved a study of the effect of non-biological suspended material on the removal of a soluble substrate (glucose) in the film flow model. Test carried out adding FeCl_3 to the feed indicated a decreased efficiency in glucose removal. Another investigation (ATKINSON *et al.*, 1967) dealt with response of a film flow reactor to extended periods of biological growth without any smoothing of the film. Results indicated that increased removal rates were due to increased surface area of the microbial mass. In fact, observed increases could be due to existence of micro-turbulence caused by surface irregularities (see below). The authors also concluded that mathematical models derived on a heterogeneous basis are applicable and that liquid phase diffusion has considerable influence on glucose uptake rates by the film. Similar experimental work by MAIER (1967) agrees with conclusions of ATKINSON and SWILLEY (1967). It should be noted that Swilley's work utilized NO_3 as an electron acceptor to eliminate possible rate limitations due to oxygen diffusion.

From additional experimentation, Maier concluded that there was no effect on removal of glucose with an increase in microbial film thickness greater than $480 \mu\text{m}$. Consequently, the model assumes only a surface reaction and no diffusion or reaction in the slime. The assumption obviously biases the results and therefore obscures the conclusions to a degree.

All of the experimental models for mass transfer are dependent on the flow regime. For laminar flow, mass transfer from the liquid phase to the slime is by molecular diffusion. In turbulent flow, eddy currents control mass transfer. BUSCH (1968)

presents a model that can be used to represent eddy mixing in the liquid film. Comparisons of data calculated from the model and experimental data for an inclined plate indicate that liquid film flow is not laminar at the slime-liquid interface. Oxygen microprobe measurements also suggest that micro-turbulent flow conditions exist in the region near the slime-liquid interface (SANDERS *et al.*, 1971). Similar turbulent fluctuations below the slime indicate that there may be some active dispersion as well as diffusion in the top layers of the slime.

LEE (1968) investigated the rate of oxygen utilization by a slime film growing on the inside wall of a glass tube. Because a thin slime layer was maintained (maximum thickness was $97\text{ }\mu\text{m}$ by indirect measurement), the film was assumed to have negligible thickness, i.e. removal of DO was due to a surface reaction. Note that Kornegay found removal of DO occurring through $70\text{ }\mu\text{m}$ of bacterial slime. Surface reaction and liquid phase diffusion were considered in the theoretical development. Using untreated sewage as substrate, Lee concluded that the reaction kinetics between DO and the thin slime are zero order. The mathematical model for the general case of cylindrical tube reactor system with a catalytically active wall was applicable to a thin microbiological slime system. However, Lee recommends that the constitutive equations be written for the slime layer and that, coupled with the fluid equations, it would be possible to consider thicker slimes which are still growing and to account for anaerobic layers.

GULEVICH (1967, 1968) attempted to determine the role of diffusion in nutrient uptake by microbial films attached to a rotating disk, the simplest surface from the standpoint of mass transfer, having been used frequently in studies of heterogeneous chemical kinetics. Results indicate that the uptake rate of glucose is diffusion-limited in the laminar flow regions. The theoretical development considered the slime as a surface with negligible thickness but because no observations of slime mass or thickness were presented the results are difficult to interpret. The rotating disk method can also indicate reaction order and reaction rate constant if experimental measurements are precise.

CHARACKLIS (1967) considered the constitutive equations for the slime layer and solved the time dependent equation for diffusion and reaction in the slime including a boundary condition for mass transfer at the slime-liquid interface. The model would be useful if it were combined with a fluid model of the liquid boundary layer.

KORNEGAY and ANDREWS (1967) used a completely-mixed continuous flow annular reactor to investigate the kinetics of a fixed bed reactor by observing removal of substrate (glucose) and growth of microorganisms which were maintained on the interior wall of the reactor. A mass balance on the liquid phase related conversion of glucose to production of microorganisms. Experimental results indicate that an increase in reaction rate did not occur for microbial film thickness greater than $70\text{ }\mu\text{m}$.

A mathematical model was developed which describes nutrient uptake by a saturation function which incorporates the effects of diffusion in the slime and growth rates.

The development yields a continuous function which describes the rate of substrate removal from zero order at high concentrations to first order at low concentrations.

Results indicate that substrate removal and organism growth increased with substrate concentration but approached a maximum. The authors also conclude that the biochemical reaction is not so rapid that it may be considered instantaneous and that it is more than a simple surface reaction. However, the liquid film and biological film do offer diffusional resistance.

In summarizing the kinetic studies, it is important to consider the main assumptions that have been employed in the models. If a surface reaction is assumed (i.e. the film has no thickness) any mass transfer limitations that are observed must occur in the liquid film. There are several reasons for questioning the validity of these results:

1. Kornegay and Sanders "active" film thicknesses up to 70 μm indicated that mass transfer limitations could occur in the microbial film.
2. MATSON *et al.* (1972) and others have calculated that even under the worst conditions, limitations in the liquid film around biological floc accounts for only a small fraction of the resistance to mass transfer.

An experimental system which allows for measurement of mass transfer resistance in the liquid and microbial film must be adopted to clarify concepts regarding the kinetics of substrate uptake by attached microbial growths.

CONCLUSION

Attached bacterial growths are ubiquitous in aquatic systems and frequently are utilized to our advantage as follows:

1. Trickling filters for wastewater treatment.
2. Essential portion of the assimilative capacity of natural bodies of water.
3. As research tools in the study of biological oxidation processes.

In other cases, slimes are nothing more than a nuisance. Examples are:

1. Cause of marine fouling of submerged surfaces.
2. Cause of increased frictional resistance in water-carrying pipelines.
3. Fouling of heat-exchange surfaces and adsorption surfaces.
4. Contribute to scale-up problems in the design of biological reactors.

This review was an attempt to point out the important role that slimes play in aquatic systems, both natural and man-made, and to indicate inconsistencies and contradictions in our knowledge concerning them. Further research is necessary to determine better control methods for nuisance growths and to determine techniques for utilizing attached growths to the fullest advantage.

Recommendations

Research is necessary to develop a predictive model for microbial attachment on wetted surfaces that relates such deposition to chemical water quality parameters. For example, there is an apparent relationship between biodegradation rate, solubility, molecular size and surface activity of the organic substrate and its effect on microbial attachment. The influence of the ionic strength on electrostatic interactions between the cell and the inert surface emphasizes the role of the inorganic constituents. Insufficient amounts of inorganic nutrients, such as phosphorous and nitrogen stimulate the production of microbial polysaccharides which influence attachment.

Initial deposition of organisms is related to the characteristics of the attachment surface and the shear force at the surface. It would be useful for control purposes to understand the mechanism of attachment for different materials and shear force. The following formulations might be useful in elucidating mechanism (61):

1. Nucleation site controlled attachment applies to the initial stages of attachment on a fresh surface and frequently exhibits a lag or induction period. Promotion may be by physical adherence to small pits which provide shelter from fluid shear forces and anchors for microorganisms. Alternatively, there may be preferential areas for chemical adsorption due to local material composition or surface configuration. Once attachment occurs over a significant area, organisms attach to other organisms and this mechanism is no longer valid. It is proposed that the length of the induction period is inversely proportional to the number of nucleation sites available.
2. Diffusion controlled attachment could become limiting for low velocities or low microbial mobility where attachment is rapid. A general equation form is

$$\text{Attachment rate} = K (C_b - C_s)$$

where C_b , C_s cell concentrations in the bulk and at the surface respectively.

$K \equiv$ mass transfer coefficient.

Attachment by this mechanism increases with velocity (K is a function of the Reynolds and Schmidt number). However, above a certain velocity, it only serves to transport fouling material to the surface where another mechanism may determine attachment rate.

In considering initial attachment, due regard must be given to the role of biopolymers, which is obscured by the contradictory results that have been reported.

After initial attachment, the organisms adhere to each other in the film and reproduce within the film. The role of shear forces in determining the thickness of the slime is important from two standpoints:

1. Physical removal of newly formed layers to keep the slime thickness constant.
2. Transport of substrate, nutrients and suspended cells to slime film.

Finally, a consistent explanation for the sloughing of bacterial slime from surfaces must be determined that includes the effect of the physico-chemical environment.

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