

# MICROBIOLOGY OF LOTIC AGGREGATES AND BIOFILMS

ERIC A. STRAUSS  
Upper Midwest Environmental  
Sciences Center  
La Crosse, Wisconsin

## INTRODUCTION

The microbial community is a vital component of lotic (flowing water) ecosystems and is important for nutrient cycling, organic matter decomposition, downstream transport of materials, and transfer of energy to higher trophic levels. Some of this microbial community is transported downstream as unattached cells; however, much of it is associated with suspended organic particles (i.e., aggregates) or attached to available substrate in biofilms. Research on microbial activity within organic aggregates has mostly been limited to marine and lake systems. As a result, more is known about the microbial communities and microbial processes in marine and lake aggregates compared with aggregates in lotic ecosystems. Conversely, biofilms have been studied in great detail in many systems, including lotic ecosystems. Many excellent and detailed reviews are available on the topics of aggregates and biofilms (1–11). Included here is a general overview of the microbiology and ecology of these topics and how they are relevant to lotic ecosystems.

## LOTIC AGGREGATES

### Origins and Terminology

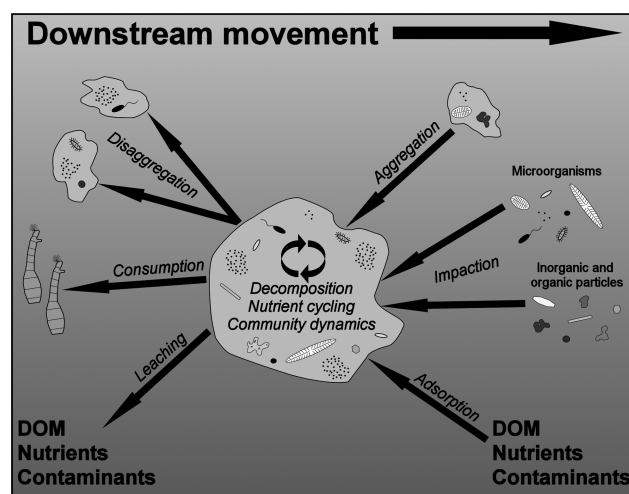
Microorganisms readily colonize substrates in lotic ecosystems to form diverse and active communities. Suspended organic matter is often rich in nutrients and easily metabolized carbon, providing an especially good substrate for microbial colonization and growth and for the subsequent formation of lotic aggregates. Lotic aggregates can be defined as the interactive association between a microbial community and suspended organic matter in a flowing water system. The organic matter that is a central component of lotic aggregates may have a variety of origins including allochthonous and autochthonous particles and byproducts of animal and microbial activity. Allochthonous organic matter (e.g., wood, leaves, and terrestrial debris) readily enters lotic systems through various pathways. Larger particles tend to quickly settle onto the channel bed or get trapped in channel structures, whereas the smallest particles often become entrained in the flowing water. Over time, physical and biological processes fragment the large particles into smaller detrital particles, which can then become resuspended into the water column. Organic matter particles of autochthonous origin (e.g., biofilm slough and aquatic vegetation) also may become fragmented and entrained in the current

in the same manner as those particles of allochthonous origin. Organic byproducts of animal activity such as fecal pellets and discarded invertebrate exoskeletons are common in highly productive systems and can be nutrient-rich substrates for microbial colonization (12). Aggregates also may form in conjunction with dissolved organic matter (13) or colloidal organic polymers that are small (1.5 nm to 0.45  $\mu\text{m}$ ), high-molecular-weight byproducts of organic matter decomposition that remain suspended in the water (14).

Aggregates have the potential to enlarge as they travel downstream by combining with other aggregates (aggregation) and to gather additional materials primarily through impaction (Fig. 1) (15). However, the physical forces exerted by flowing and turbulent water usually fragment larger aggregates (disaggregation), maintaining the aggregates at a smaller size compared with those found in nonflowing systems (16). Although relatively small, the size of lotic aggregates are still highly variable and can be further classified into microaggregates (<5 to 500  $\mu\text{m}$ ) and macroaggregates (>500  $\mu\text{m}$ ) (7). Larger aggregates are also sometimes called “river snow” in keeping with terminology used to describe similar aggregates present in marine (marine snow) and lake (lake snow) ecosystems (17,18). In the field of stream ecology, lotic aggregates may also be called seston, which can be defined as particles entrained in flowing water. A list of common terms and abbreviations used to describe suspended matter in lotic systems is provided in Table 1.

### Microbial Community

Bacteria and other microorganisms secrete a viscous, slimy mixture of exopolymers (i.e., chains of polysaccharides) called glycocalyx. In general, the properties of exopolymers can contribute many beneficial functions to organisms



**Figure 1.** Conceptual diagram illustrating processes affecting lotic aggregate size and composition. DOM = dissolved organic matter.

**Table 1. Common Terms, Abbreviations (in parentheses), and Sizes Used to Classify Suspended Matter in Flowing Water Systems**

Term	Size
Dissolved organic matter (DOM)	<0.45 $\mu\text{m}$
Particulate organic matter (POM)	>0.45 $\mu\text{m}$
Course particulate organic matter (CPOM)	>1 mm
Fine particulate organic matter (FPOM)	0.45 $\mu\text{m}$ to 1 mm
Aggregate	>0.45 $\mu\text{m}$
Macroaggregate	>500 $\mu\text{m}$
Microaggregate	<500 $\mu\text{m}$
Colloid	1.5 nm to 0.45 $\mu\text{m}$
Floc	>0.45 $\mu\text{m}$
Seston	>0.45 $\mu\text{m}$

The size ranges listed here are generally accepted definitions, but they can vary somewhat depending on application.

including assisting in locomotion and adhesion to surfaces, and protection from abrasion, dehydration, and nutrient loss. More specifically, in lotic aggregates, the exopolymers bind the contents of aggregates together and assist in the capture of additional nutrients, organic matter, and other microorganisms. Much of the exopolymers associated with lotic aggregates are likely produced by microorganisms within the aggregate, but they also may accumulate via impaction with suspended exopolymers.

Microbial communities thriving within the fragile matrix of exopolymers and particles consist of a diverse heterogeneous assemblage of bacteria, algae, fungi, protozoa, and sometimes zooplankton. Compared with marine and lake ecosystems, relatively few studies have examined the microbial community structure of aggregates in lotic systems. However, many studies on marine aggregates have demonstrated that the concentration of algae, zooplankton, protozoa, and bacteria within aggregates is much greater, often 2–3 orders of magnitude, than in the surrounding water (7). Larger aggregates often support larger microbial communities, but smaller particles may be relatively more densely populated per unit surface area (19). Detailed microscopic analyses have shown that bacteria are not uniformly distributed within or on the surface of aggregates but form microcolonies (7). These microbial communities have temporally dynamic compositions that change quickly in response to conditions both inside and outside of the aggregate and can also exhibit seasonal patterns (17,20).

Inorganic (nitrogen, phosphorus, and silica) and organic (carbohydrates, amino acids, and dissolved organic carbon) nutrients are often 1–2 orders of magnitude more enriched in aggregates compared with surrounding water (7). In addition, many heavy metal and organic contaminants also readily adsorb to the aggregate matrix. As a result of these enriched conditions, downstream movement of aggregates is an important mechanism of transport for nutrients, organic materials, and contaminants. Moreover, these nutrient-rich substrates result in high growth efficiencies of heterotrophic bacteria associated with the lotic aggregates, which suggests that these aggregates are hot spots for microbial processes and the transfer of organic matter into bacterial biomass (20). Enzymes

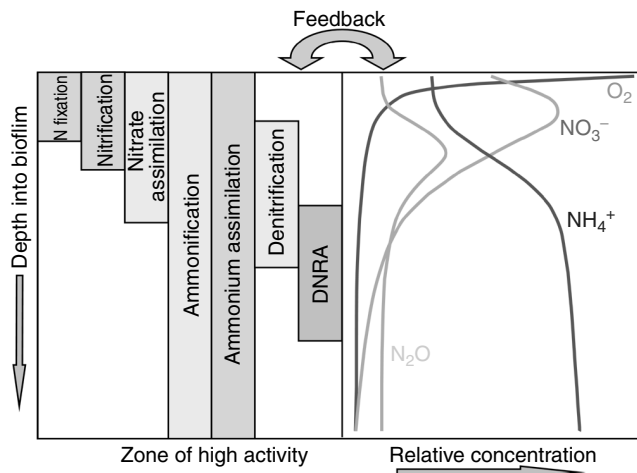
produced by aggregate-bound bacteria solubilize organic matter 1–2 orders of magnitude faster than the bacterial cells can assimilate the dissolved matter (21). This decomposition results in aggregate mass loss and leaching of dissolved organic matter back into the flowing water. Finally, consumption of lotic aggregates by many species of detritus- and filter-feeding organisms is a vital link of energy transfer between microbial and higher trophic levels (Fig. 1).

## BIOFILMS

### Biofilm Structure

Microbial biofilms are the thin slime layers that are observed on surfaces and substrates (e.g., rocks, sediment, vegetation, leaves, wood) in aquatic systems. Biofilms (sometimes called microbial mats) are similar to aggregates and possess many of the same general structural and functional attributes. For example, biofilms (like aggregates) contain microbial communities that are vital to lotic ecosystem function and are responsible for many key production, decomposition, and nutrient cycling processes (22). Highly developed biofilms are generally a few millimeters thick and can develop on virtually any available surfaces in natural lotic systems, provided the system has sufficient nutrients. Biofilm development is especially rapid on surfaces that contain or leach nutrients (e.g., particulate organic matter, POM) (2). Available surfaces are still colonized in nutrient-poor or oligotrophic systems, but biofilms are generally less developed. Under oligotrophic conditions, biofilm bacteria are often able to obtain limited nutrients by concentrating organics on the exopolymer matrix, using waste products of nearby microorganisms, and by pooling their biochemical resources (i.e., enzymes) to break down organic particles (11).

Historically, biofilms were thought to be a relatively homogeneous distribution of bacteria in an exopolymer matrix and nutrient and material delivery throughout the biofilm would be dominated by diffusion (2). However, recent advances in the analytical tools used to study biofilms have allowed scientists to more accurately characterize the structure or architecture of biofilms. For example, the application of confocal scanning laser microscopy has allowed nondestructive inspection of intact biofilm samples. This technique has revealed the physical structure of established biofilms to consist of a diverse microbial assemblage arranged in microcolonies and interspersed within a heterogeneous exopolymer matrix containing protrusions and a network of pores and channels (2,4). Microsensors, another relatively recent research tool, have been used to examine chemical properties in spatially explicit areas within biofilms and have found distinct differences in dissolved oxygen concentrations between the pores and channels in the matrix and the matrix itself. These differences suggest that such structures may facilitate water movement and nutrient delivery within the biofilm. Microsensors have also been used to quantify vertical chemical gradients in biofilms that have important implications



**Figure 2.** General patterns of nitrogen cycling (left) and relative concentrations of nitrogen constituents (right) in a lotic biofilm [redrawn and modified from Paerl and Pinckney (3)]. Feedback between the chemical and biological components facilitates the formation and equilibrium of the gradient (i.e., the chemical environment within the biofilm affects microbial processes and vice versa). DNRA = dissimilatory nitrate reduction to ammonium.

for microbial community structure and biogeochemical processing (Fig. 2). Chemical gradients will be discussed further in the next section.

The physical stress caused by the turbulent flow of water in lotic systems can have significant effects on biofilm structure and organization. Many studies have observed that biofilms subjected to fast flowing water tend to be thinner, smoother, denser, more stable, and have lower surface sinuosity than biofilms in slower or nonflowing water (23,24). Cells occupying the surface of lotic biofilms have been observed to be arranged in formations that can withstand the higher shear stress; examples of these formations include hexagon-shaped structures and ripples positioned parallel to flow direction (24). Freely oscillating streamer-like structures are also sometimes observed adhered to the surface of lotic biofilms (8). Streamers are predominately single species colonies that elongate over time in the absence of disturbance.

### Microbial Processes

The rates and types of microbial processes at work in any particular biofilm are largely regulated by the physical and chemical characteristics of the lotic environment. Several types of algae and cyanobacteria flourish in biofilms if sufficient nutrients are available and the overlying water is shallow and clear enough to allow adequate light penetration to the substrate. Oxygen ( $O_2$ ) generated by these primary producers can reach very high levels and is a key factor supporting aerobic biofilm processes. In fact, under high-light conditions, the surficial area of well-lit biofilms can produce  $O_2$  concentrations 2–3 times greater than atmospheric concentrations (25). Much, if not all, of the oxygen is respired as it is transported and diffused deeper into the biofilm creating a vertical gradient

of oxygen concentrations throughout the biofilm (Fig. 2). Chemical gradients are common in biofilms and control which microbial processes are able to occur at certain depths. The opposite is also true; microbial processes control the availability of certain ions. The feedback relationship between these two factors quickly forms a relatively stable gradient equilibrium that is particularly important for the transformations and cycling of certain nutrients, primarily nitrogen, carbon, and sulfur.

The nitrogen cycle is a complex group of microbial processes that transform, oxidize, or reduce nitrogen ions among the various oxidation states. These processes occur under different environmental conditions, all of which can be located in distinct locations along the chemical gradients found in biofilms (Fig. 2). Nitrogen fixation, which is the conversion of atmospheric dinitrogen gas ( $N_2$ ) to biologically available ammonium ( $NH_4^+$ ), is predominately done by cyanobacteria; thus, it occurs in the upper, high-light areas of the biofilm. Nitrification, which is the oxidation of  $NH_4^+$  to nitrate ( $NO_3^-$ ), also occurs in the upper areas because it is an aerobic process. Bacteria that have the enzymes to reduce  $NO_3^-$  for assimilation likely occur throughout the biofilm profile, but they can only use  $NO_3^-$  where it is present. Organic matter is ubiquitous throughout the biofilm; therefore, ammonification (the release of  $NH_4^+$  from organic matter) and the resulting  $NH_4^+$  are also widespread. Ammonium assimilation into microbial biomass is not oxygen-dependent and can take place wherever  $NH_4^+$  is present. Denitrification, which is the anaerobic reduction of  $NO_3^-$  to nitrite ( $NO_2^-$ ), nitrous oxide ( $N_2O$ ), and  $N_2$ , occurs deeper in the biofilm where oxygen is low and  $NO_3^-$  is high. Dissimilatory  $NO_3^-$  reduction to  $NH_4^+$  is strictly an anaerobic process that occurs in the lowest areas of the biofilm where  $NO_3^-$  is still available.

It is generally accepted that bacterial activity and production of biofilm biomass is resource limited in lotic systems. Thus, nutrient availability and the quantity and quality of the dissolved organic carbon in the water have strong positive effects on bacterial activity in the biofilm (26,27). Total bacterial activity also often correlates well with primary production in biofilms because of the influx of oxygen and high-quality organic carbon provided by algae (27,28). Finally, vertical hydrodynamics of stream channels (i.e., upwelling and downwelling) can be an important means of nutrient and dissolved organic matter (DOM) delivery through the biofilm, which can subsequently increase microbial activity (29).

Under certain chemical or physical conditions, specialized biofilms that are dominated by unique groups of microorganisms may develop. For example, orange-colored iron-oxidizing biofilms are prevalent in areas where groundwater with high concentrations of reduced iron ( $Fe^{2+}$ ) is discharged into oxygenated waters (30). Another example is the colorful biofilms of thermophilic bacteria and cyanobacteria that are commonly associated with water flowing from hot springs (Fig. 3). The colors of these biofilms depend primarily on the chlorophyll content in the cells, but also on the ratio of chlorophyll to carotenoids (yellow to red pigments) (31).



**Figure 3.** Orange-colored biofilm associated with hot spring outflow draining into the Firehole River, Yellowstone National Park, Wyoming. The bright orange color is because of pigments in thermophilic cyanobacteria and bacteria in the biofilm.

## BIBLIOGRAPHY

- Johnson, B.D., Kranck, K., and Muschenheim, D.K. (1990). Physico-chemical factors in particle aggregation. In: *The Biology of Particles in Aquatic Systems*. R.S. Wotton (Ed.). CRC Press, Boca Raton, FL, pp. 57–81.
- Costerton, J.W. et al. (1995). Microbial biofilms. *Ann. Rev. Microbiol.* **49**: 711–745.
- Paerl, H.W. and Pinckney, J.L. (1996). A mini-review of microbial consortia: their roles in aquatic production and biogeochemical cycling. *Microbial Ecol.* **31**: 225–247.
- Davey, M.E. and O'Toole, G.A. (2000). Microbial biofilms: From ecology to molecular genetics. *Microbiol. Molecular Biol. Rev.* **64**: 847–867.
- Kjørboe, T. (2001). Formation and fate of marine snow: Small-scale processes with large-scale implications. *Scientia Marina* **65**: 57–71.
- Paerl, H.W. and Kuparinen, J. (2002). Microbial aggregates and consortia. In: *Encyclopedia of Environmental Microbiology*. G. Bitton (Ed.). John Wiley & Sons, New York, pp. 160–181.
- Simon, M., Grossart, H.-P., Schweitzer, B., and Ploug, H. (2002). Microbial ecology of organic aggregates in aquatic ecosystems. *Aquat. Microbial Ecol.* **28**: 175–211.
- Stoodley, P., Sauer, K., Davies, D.G., and Costerton, J.W. (2002). Biofilms as complex differentiated communities. *Ann. Rev. Microbiol.* **56**: 187–209.
- Turley, C.M. (2002). The importance of 'marine snow'. *Microbiol. Today* **29**: 177–179.
- Zimmermann-Timm, H. (2002). Characteristics, dynamics and importance of aggregates in rivers—an invited review. *Int. Rev. Hydrobiol.* **87**: 197–240.
- Prakash, B., Veeragowda, B.M., and Krishnappa, G. (2003). Biofilms: A survival strategy of bacteria. *Curr. Sci.* **85**: 1299–1307.
- Wotton, R.S. and Malmqvist, B. (2001). Feces in aquatic ecosystems. *BioScience* **51**: 537–544.
- Kerner, M. et al. (2003). Self-organization of dissolved organic matter to micelle-like microparticles in river water. *Nature* **422**: 150–154.
- Thurman, E.M. (1985). *Organic Geochemistry of Natural Waters*. Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands, p. 497.
- Wotton, R.S. (2004). The ubiquity and many roles of exopolymers (EPS) in aquatic systems. *Scientia Marina* **68**: 13–21.
- Wotton, R.S. (1996). Colloids, bubbles, and aggregates—a perspective on their role in suspension feeding. *J. N. Amer. Benthol. Soc.* **15**: 127–135.
- Böckelmann, U., Manz, W., Neu, T.R., and Szewzyk, U. (2000). Characterization of the microbial community of lotic organic aggregates ('river snow') in the Elbe River of Germany by cultivation and molecular methods. *FEMS Microbiol. Ecol.* **33**: 157–170.
- Neu, T.R. (2000). *In situ* cell and glycoconjugate distribution in river snow studied by confocal laser scanning microscopy. *Aquat. Microbial Ecol.* **21**: 85–95.
- Berger, B., Hoch, B., Kavka, G., and Herndl, G.J. (1996). Bacterial colonization of suspended solids in the River Danube. *Aquat. Microbial Ecol.* **10**: 37–44.
- Grossart, H.P. and Ploug, H. (2000). Bacterial production and growth efficiencies: Direct measurements on riverine aggregates. *Limnol. Oceanogr.* **45**: 436–445.
- Smith, D.C., Simon, M., Alldredge, A.L., and Azam, F. (1992). Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature* **359**: 139–142.
- Battin, T.J., Kaplan, L.A., Newbold, J.D., and Hansen, C.M. (2003). Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature* **426**: 439–442.
- Liu, Y. and Tay, J.-H. (2001). Metabolic response of biofilm to shear stress in fixed-film culture. *J. Appl. Microbiol.* **90**: 337–342.
- Battin, T.J. et al. (2003). Effects of current velocity on the nascent architecture of stream microbial biofilms. *Appl. Environ. Microbiol.* **69**: 5443–5452.
- Fenchel, T., King, G.M., and Blackburn, T.H. (1998). *Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling*. Academic Press, San Diego, CA, p. 307.

26. Tank, J.L. and Webster, J.R. (1998). Interaction of substrate and nutrient availability on wood biofilm processes in streams. *Ecology* **79**: 2168–2179.
27. Fischer, H. (2003). The role of biofilms in the uptake and transformation of dissolved organic matter. In: *Aquatic Ecosystems: Interactivity of Dissolved Organic Matter*. S.E.G. Findlay and R.L. Sinsabaugh (Eds.). Academic Press, San Diego, CA, pp. 285–313.
28. Espeland, E.M., Francoeur, S.N., and Wetzel, R.G. (2001). Influence of algal photosynthesis on biofilm bacterial production and associated glucosidase and xylosidase activities. *Microbial Ecol.* **42**: 524–530.
29. Battin, T.J. (2000). Hydrodynamics is a major determinant of streambed biofilm activity: From the sediment to the reach scale. *Limnol. Oceanog.* **45**: 1308–1319.
30. Emerson, D. and Revsbech, N.P. (1994). Investigation of an iron-oxidizing microbial mat community located near Aarhus, Denmark: Field studies. *Appl. Environ. Microbiol.* **60**: 4022–4031.
31. Brock, T.D. (1994). *Life at High Temperatures*. Yellowstone Association for Natural Science, Yellowstone National Park, Wyoming, p. 31.