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## BRIDGES

# EPS (Extracellular Polymeric Substances), silk, and chitin: vitally important exudates in aquatic ecosystems

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**Abstract.** Exudates are ubiquitous in marine and fresh waters. They include Extracellular Polymeric Substances (EPS; including mucus, slimes, and biofilm matrices), silk, and chitin. EPS have many uses for the organisms that produce them—attachment, locomotion, feeding, and protection. They also act as glues to bind other materials, including the egesta within fecal pellets. Silk, an exudate produced only in fresh waters, is used in tube construction, to make nets used in feeding, and as a means of attachment. Chitin is the basis of the peritrophic matrix that surrounds the gut contents of some invertebrates and may act as a binding surrounding their fecal pellets. EPS are found free in the water column and in and on the substratum, as well as in close contact with the organisms that exude them. Free EPS have an essential role in particle formation and aggregation, processes that have been studied primarily in marine systems. Like EPS, silk is highly adsorptive and is found free within substrata, but rarely in the water column. Understanding the role of exudates requires collaboration between marine and freshwater biologists, physical geographers, and chemists, especially those working on surface processes and colloids. The results of these collaborations should expand our understanding of the many vital roles played by exudates in aquatic ecosystems.

**Key words:** EPS, exopolymers, mucus, slime, silk, chitin, peritrophic matrix, marine and freshwater ecosystems.

Exudates are compounds that pass from cells, whether those cells are unicellular organisms or part of the complex structure of multicellular organisms. In describing exudates, I shall include only those that are organic and shall exclude the mineral deposition that creates shells, skeletons, reefs, tubes, and other coverings. Exudates include a wide range of organic polymers formed from chains of simple molecules. They may be arranged into loose associations or into discrete structures. All aquatic biologists are familiar with mucus and slime (2 terms for Extracellular Polymeric Substances [EPS]), and freshwater biologists are familiar with silk. Peritrophic matrices formed from chitin that protect the gut wall of arthropods are less familiar. These exudates have functions for the organisms that secrete them, and, when free from the organisms, they have significant effects on processes in aquatic environments.

This article describes the many roles of exudates and why it is important that we know more about them. Past research has been focused on organisms,

but a wider view of all organic matter is required to understand the functioning of aquatic systems. This wider view would be enhanced by removal of barriers among the subdisciplines of aquatic science and by forging links with other nonaquatic sciences (e.g., analytical chemistry).

### Production of EPS, Silk, and Chitin Forming a Peritrophic Matrix

The composition of EPS varies with the organisms that produce them, but polysaccharides often dominate. EPS are ubiquitous (Wotton 2005), and a key feature of EPS is their power to bind water after secretion (Sutherland 2001). The result is that a small volume of the primary chemical expands to many times its original volume as water becomes incorporated into the matrix, a process that occurs in seconds. In addition, EPS are lubricants that aid locomotion, provide a protective coating against damage by abrasion or harmful chemicals and microorganisms, and have the capacity to adsorb a wide range of organic compounds that may be useful as food (Wotton 2005). Thus, they are very valuable to the

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organisms that secrete them and are vital in many biological processes.

Silk is a proteinaceous secretion produced by some aquatic insects and by aquatic spiders. Fibroin is the main structural protein of arthropod silk, and sericin binds the fibroin fibers (Jin and Kaplan 2003). Silk produced by spiders and silkworms has been studied most, but the structure of silk secreted by caddisfly and midge larvae is known. Limnephilid caddisfly larvae produce silk in different structural forms depending on spinneret aperture and pressure, but ribbon silk (diameter 1–4  $\mu\text{m}$ ) consists largely of fibroin in 2 filamentous cores with other components acting as a glue (Engster 1976). The formation of silk filaments by midge larvae was studied in detail by Case and Wieslander (1992), but they did not give a biochemical analysis of the components. Silk filaments are very strong, and, mass for mass, aquatic-insect silk is the most adsorptive biological material known (measured using polar pesticides by Brereton et al. 1999).

Chitin is a polymer ( $\beta$ -(1,4)-N-acetyl-D-glucosamine,  $\text{C}_8\text{H}_{13}\text{O}_5\text{N}$ ) that forms a significant component of the skeletons of arthropods and is a means of strengthening in many other organisms, including yeasts. In many insects and crustaceans, chitin also is exuded into the gut to form a peritrophic matrix that protects the gut wall from abrasion by the gut contents. Chitin, together with chitin-binding proteins and glycans, is secreted at the junction of the fore-gut and mid-gut, to form a continuous and very thin-walled tube (Hegedus et al. 2009).

### Uses Made of Exudates by Aquatic Organisms

Individual bacteria and algae use EPS to stick themselves to substrata, and the exudates produced by colonies of benthic bacteria and algae often form a biofilm in which individuals live (Lock et al. 1984, Costerton et al. 1994). The matrix of EPS was termed “The House of Biofilm Cells” by Flemming et al. (2007), who stressed that EPS produced by the component organisms includes many compounds in addition to the abundant polysaccharides. These compounds are important in the structure and functioning of the matrix and create a honeycomb structure. Among these compounds is extracellular DNA, previously regarded as the product of lysed cells, and now thought to be an essential exudate that is likely to maintain the structural integrity of the film (Flemming et al. 2007). Biofilms adsorb and retain materials from the water column (Battin et al. 2003), resulting in the acquisition of a store of potential materials for digestion and the retention of exoenzymes secreted to

break those materials into labile components (Flemming et al. 2007, Flemming and Wingender 2010).

Exudates also are used to aid locomotion. Raphid diatoms secrete EPS to move over substrata (Edgar and Pickett-Heaps 1983), and exudation of EPS by some cyanobacteria allows their locomotion through the water column. Flatworms secrete mucus to provide a platform over which they glide using cilia for propulsion (Ruppert and Barnes 1994), a method of locomotion also used by primitive gastropods (Ruppert and Barnes 1994). In more advanced snails, locomotion is by muscular waves on the foot, and EPS ensure good contact with the substratum and a thixotropic surface over which the snail can glide (Ruppert and Barnes 1994). These trails become visible to the naked eye when sediment particles become attached.

Many marine organisms use EPS on tentacles and other projections of the body (termed mucociliary feeding because cilia are also essential) to capture particles, but this feeding method is uncommon in fresh waters (Wotton 1994). Freshwater bivalves use EPS in mucociliary feeding and retain this method from their marine ancestors. Mucus secreted on the gills helps retain particles and acts as a barrier to prevent the ingress of water by osmosis. Mucus on the gills of migratory fishes has a similar function.

Mucus is slippery and makes prey difficult to grasp and, thus, provides defense against predators. Fish mucus also contains antibiotics that prevent microbial attack (Ebran et al. 2000, Subramanian et al. 2008a). However, fish mucus also binds potentially harmful metals, such as Pb (Coello and Khan 1996, Tao et al. 2000a), Cu (Tao et al. 2000b), and Al (Berntssen et al. 1997). The EPS of other organisms fulfill many similar protective functions (Wotton 2005, Ritchie 2006). EPS produced by phytoplankton provide labile substrates for bacteria, which then consume adsorbed viruses that might otherwise attack the algae (Murray 1995), and mycosporine-like amino acids secreted into mucus serve as effective UV light screens (Shick and Dunlap 2002). Other protective functions of EPS are likely to be discovered.

Silk is used to cement the components of cases, tubes, and enclosures constructed by freshwater invertebrates. The best-known case-builders are caddisfly larvae, and the silk used to bind case materials may be flattened to enhance adhesion (Engster 1976). Cases and their components are characteristic of different caddisfly genera (Wiggins and Currie 2008). The silk tubes of chironomid larvae take a variety of forms (Chaloner and Wotton 1996). They may be on the surface of substrata or form effective linings of tubes within the substratum where they

prevent incursion of sediment particles and ensure that the tube is free for irrigation by undulations of the larval body. Tubes adsorb detritus rapidly and become brown with adhesion of mineral fragments and organic matter (including fecal pellets; Wotton and Hirabayashi 1999).

Black fly larvae (Diptera:Simuliidae) use silk in 2 ways by secreting both filaments and pads (Barr 1984). Filaments are used for return to the substratum if larvae are swept into the drift or as life-lines up which larvae climb if they become dislodged (Wotton 1986). Life-lines must be exceptionally strong because they are only 3  $\mu\text{m}$  in diameter (Wotton 1986). Pads are different in that they show no filaments or other defined structures (Barr 1984). Pads are placed on the substratum and are used for anchorage by feeding larvae and for looping locomotion. Another use of silk occurs in black fly pharate pupae (functionally, larvae), which secrete a meshwork of silk strands attached to the substratum to form the cocoon in which pupation occurs.

Silk is used by some caddisfly larvae to construct a net to capture suspended material from flowing water (Brown et al. 2004). Nets vary in mesh size among taxa, and larvae graze over the net surface. A similar tactic is used by some chironomid larvae (*Rheotanytarsus* spp.), which build tubes of silk with strengthening ridges extended into anterior horns (Kullberg 1988). Silk strands are strung between the horns to form a sticky trap for particles. These particles are ingested with the silk before new strands are secreted.

## The Wider Role of Exudates in Aquatic Ecosystems

### *Fecal pellets*

EPS bind the fecal pellets of many vertebrates and invertebrates. These EPS are secreted by animals, ingested with other food, produced by ingested microorganisms, or produced by microorganisms resident within the gut. The pellets allow waste materials to be carried away from the animal that produces them, and they provide an important mechanism for transfer of material from the water column to the substratum. The significance of EPS-bound pellets for this process in streams was highlighted by the work of Ladle and colleagues (Ladle and Griffiths 1980, Ladle et al. 1987). If animals are found in very high population densities and have a low assimilation efficiency, then it follows that fecal pellets will be abundant over and within the substratum. Freshwater biologists have tended to study feeding and not egestion by animals, so this process is often missed. Nevertheless, sediments may consist largely of pellets from the water column and

pellets produced by bioturbators within the sediments, biodeposition that is much more familiar to marine biologists (Grabowski et al. 2011).

Malmqvist et al. (2001) showed that the production of feces is a very significant process in large, unregulated rivers in boreal regions where huge numbers of benthic suspension feeders are found. Fecal pellets are produced in rapids, sink through the water column in less turbulent sections, and most are deposited in lowland reaches and estuaries (Malmqvist et al. 2001). However, tributaries of these rivers do not export many pellets to the main river (Malmqvist and Wotton 2002), thus reducing an expected flow of organic matter (Wipfli et al. 2007).

The fecal pellets of many planktonic crustaceans, especially copepods, are wrapped in peritrophic matrix after egestion. The membrane encases the pellets, which are densely packed and sink rapidly through the water column. In marine systems, evidence exists that the peritrophic matrix breaks or is broken by animals, and the contents then disperse and sink more slowly, releasing potential sources of nutrients within the photic zone (Poulsen and Kjørboe 2006). Breakdown of the peritrophic matrix after colonization by bacteria also occurs in lakes, and it is likely that copepod pellets reach the substratum only in lakes <70 m deep (Ferrante and Parker 1977). The feces of benthic crustaceans also may be wrapped in peritrophic matrix. Observations on *Gammarus* pellets show that this wrapping breaks apart soon after egestion, whereas the fecal material remains bound tightly with EPS (Joyce et al. 2007). The pellets, like those of black fly larvae, remain intact for >30 d (Joyce et al. 2007). In insects with rapid gut throughput time, contents pass too rapidly to allow formation of a peritrophic matrix around the feces, and broken sections of peritrophic matrix then become incorporated within fecal pellets.

### *Sloughed biofilm, bindings, and mucus trails*

Biofilms are 3-dimensional (Costerton et al. 1994) and pieces can be broken off and transported by water movements. Sloughed biofilm is a component of the particulate matter in water, and it maintains the characteristics of EPS, its principal component. Measuring techniques often focus on particle size or gross chemical composition because these characteristics are convenient for processing samples in bulk. However, the origin of particles suspended in water is considered rarely, and the contribution made by sloughed biofilm, microaggregates, and larger flocs to the total particle load is not known.

On the substratum, EPS from algae, bacteria, and other microorganisms are the main binding agents of

stabilized sediments (Droppo et al. 2007, Lundkvist et al. 2007, Hubas et al. 2010). This binding ability results from the sticky quality of EPS aided by other biogeochemical processes, such as divalent cation binding (Decho 2000, 2010, Bhaskar and Bhosle 2005, de Brouwer et al. 2005). The strength of binding is likely to be greater in marine and estuarine environments, where cations are found in much higher concentrations than in fresh waters. However, Gerbersdorf et al. (2009) found high levels of EPS in river sediments. A comparison of the stability of river sediments with those in estuarine and marine intertidal zones, where conditions are good for algal and microbial growth and for chemical binding of fine sediments (Grabowski et al. 2011), would be interesting. Silk from large populations of aquatic insects binds mineral particles in streams (Johnson et al. 2009) and, thereby, increases the stability of coarse-grained sediments at normal current velocities.

Silk pads produced by black fly larvae remain on the substratum for up to 3 wk (Kiel 1997), but signs of breakdown begin in days. Nevertheless, population densities are  $>10^5$  larvae/m<sup>2</sup> at some locations (Wotton 1987), so silk must be abundant on/in the substratum. Colonization of silk by microorganisms, aided by the powerful adsorptive capacity of the material, is likely to occur rapidly and could result in significant trapping and processing of nutrients in and around dense larval aggregations.

Mucus on the substratum is highly adsorptive (Brereton et al. 1999) and is colonized by microorganisms, so trails and other attached exudates are good feeding grounds for animals. For example, marine snails graze over their mucus trails after these become colonized with their food and fertilized by nutrients from excreta (Wotton 2005), a behavior known as gardening. Snails can achieve high population densities in fresh waters (Hall et al. 2006), so large amounts of snail mucus on the substratum may result in gardening in these habitats. Caddisfly larvae garden using silk (Ings et al. 2010), so this behavior is not just a feature of the exudation of EPS.

#### *Free EPS and flocculation*

EPS are found free in the water column, some in colloidal form, and thus, are part of the diverse array of colloids found in all natural water bodies. Animals capture and digest EPS and their adsorbed coatings (Decho and Moriarty 1990, Couch et al. 1996, Hoskins et al. 2003), and harpacticoid copepods, at least, assimilate a high proportion of the microbial EPS that they ingest (Decho and Moriarty 1990). Free exudates also form particles by spontaneous assembly of

colloids (Chin et al. 1998). This process is likely to occur initially by coagulation (binding by charge neutralization) followed by flocculation (cementing of components by adhesive bridges of EPS). In lakes and rivers, the assembly of colloids may be governed by mechanisms that differ from those in marine systems because most fresh waters do not contain high concentrations of the divalent cations that facilitate aggregate formation via coagulation. Nevertheless, Weilenmann et al. (1989) demonstrated that coagulation can be a significant process in lakes. They stressed that divalent cations were the main agents promoting coagulation but natural organic matter (humic compounds from a variety of sources) retarded this process. Fresh waters with low cation concentrations, such as those where underlying rock strata are igneous in origin, are likely to have low coagulation rates, which may contribute to their low productivity because microbial breakdown of organic substrates in the small numbers of aggregates will be limited. Flocculation and permanence of aggregates in fresh waters depend on free EPS, just as in the sea.

Kerner et al. (2003) generated large quantities of microaggregates experimentally. Up to 25% of dissolved organic matter (DOM) in sterile river water condensed to form these particles. Addition of phytoplankton exudates increased microaggregate production but made a negligible contribution to particle mass, so the exudates appear to have been acting as triggers for the abiotic aggregation of other matter (Kerner et al. 2003). The system of classification of microaggregates developed by Buffle et al. (1998) stresses the involvement of many compounds in the process of aggregate formation.

Coalescence of microaggregates over time may occur to produce transparent exopolymer particles (TEP). The literature on TEP in surface marine waters and their role in forming marine snow is extensive (reviewed by Passow 2002), but much less has been published on lake and river snow. Grossart and Simon (1993) concluded that lake snow resulted largely from wind-induced turbulence, but the concentration of cations, particulate organic matter, and TEP also are important factors (Grossart et al. 1998). In lakes and oceans, aggregates are important means for transfer of organic matter from surface waters to the substratum, and this mechanism is increasingly recognized as important in rivers (Droppo et al. 1997, Droppo 2001, Bungartz et al. 2006, Wotton 2007). One could argue that the movement of flocs and fecal pellets is predominantly vertical and secondarily horizontal in lakes and the sea, and predominantly horizontal and secondarily vertical in streams and rivers.

Studies on freshwater flocs have been confined largely to investigations of their importance in the water industry because the activated-sludge method of sewage treatment depends on flocculation. When organically enriched water is circulated by paddles or aeration in large tanks, bacterial floc forms in massive quantities. When circulation ceases, flocs are removed, usually by settling, leaving water of much better quality. Research into activated sludge flocs has extended to characterizing component EPS and their adsorptive properties (e.g., Urbain et al. 1993, Li and Yang 2007, Subramanian et al. 2008b, Yang and Li 2009). The other major methods of sewage treatment are by trickling filters (dependent on EPS in biofilms) and by passing effluent into wetlands (where EPS help in adsorption of organic matter to sediments and in providing conditions that release plant nutrients).

EPS are useful as a means of removing organic matter that results in potential eutrophication, and the adsorptive capacity of EPS ensures trapping of conservative pollutants like pesticides and heavy metals (Bremer and Loutit 1986, Brereton et al. 1999, Bhaskar and Bhosle 2006). Coagulation, flocculation, and the use of biofilms also feature in making water safe to drink (Wotton 2002).

#### **Making Progress in Studying the Importance of Exudates**

EPS, silk, and peritrophic matrices of chitin are vitally important, and they should be better studied. Such research will require integration of oceanography, coastal marine biology, estuarine biology, and freshwater biology and links to hydrogeology, hydrology, and geomorphology. Research collaborations are being built, but education of the next generation of aquatic scientists continues to be centered on courses defined by the subdisciplines within aquatic science. For example, freshwater biology usually is taught separately from marine biology, in part, because the flora and fauna are different and our thinking has been dominated by organisms, not processes.

Some areas of aquatic science, including the importance of bubbles and gas–water interfaces (Wotton 1996, Wotton and Preston 2005) seem to have been neglected by all subdisciplines. We know that wind-induced turbulence is important in aggregation processes (Grossart and Simon 1993), but wind also promotes the formation of bubbles, which hugely increase the area of highly active gas–water interface, albeit for short time intervals. Bubbles implode rapidly, but they promote aggregation of compounds over their surface and this aggregation is maintained by flocculation (Wotton 2005). Aggregates formed by

bubbling air through sea water allow a rapid increase in microbial respiration rates (Kepkay and Johnson 1989) because microorganisms become bound with materials they can break down. If this process is important in the surface waters of oceans and lakes, it also must be very significant in fast-flowing rivers where many aggregates (previously regarded just as particles) in transport and ingested by suspension feeders may have this origin (Wotton 1996). Suspension feeders then release large numbers of fecal pellets bound, in part, by the same exudates that bind flocs.

It would be interesting to follow the fate of labile, semilabile, or recalcitrant organic molecules in a variety of water bodies, as has been discussed for microbial organic matter in oceans (Jiao et al. 2010). Jiao et al. (2010) stress that a significant fraction of recalcitrant organic C in the oceans comes from bacterial lysis or exudation, and the microbiota of the deep ocean appear to be specially adapted to breaking down molecules in this fraction. Do parallels exist in fresh waters, or does very little recalcitrant organic matter of microbial origin exist in lakes, streams, and rivers? Do microorganisms in fresh waters receiving high humic inputs have a similar high exoenzyme activity to those in the deep ocean?

Perhaps the most important link to be forged is that between aquatic biologists and chemists, including those working on colloid and surface chemistry. In the last 20 y, analytical chemistry techniques have been refined and a better understanding has developed regarding which organic compounds to study. Of these, the components of flocs, aggregates, biofilms, and the binding of sediments are among the most important. Use of in-situ hybridization and lectin-binding analysis (FISH-LBA) has advanced our capability to study aggregates (Bockelmann et al. 2002), and confocal laser scanning microscopy now enables visualization of the distribution of different EPS within aggregates (Neu 2000). Many other analytical approaches are being used by chemists, molecular biologists, and mathematicians to deepen our understanding of many processes in organisms. A similarly multifaceted approach would help us understand the importance of exudates in the functioning of aquatic systems.

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