

# RECLAMATION

*Managing Water in the West*

Technical Memorandum No. MERL-2011-21

## Adhesion Mechanism of Zebra and Quagga Mussels



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**Technical Service Center, Denver, Colorado**  
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**Adhesion Mechanism of Zebra and Quagga  
Mussels**

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## **Introduction:**

Zebra and Quagga mussels are freshwater fouling organisms which began appearing in western US waters in 2007. Multifaceted research is being undertaken by USBR and others to mitigate the realized and potential impacts of these mussels on hydraulic equipment. One aspect of this research involves examining the adhesion characteristics of these organisms to learn how to discourage attachment. This paper is a collective review of literature on the adhesion mechanism of zebra and quagga mussels. Important factors investigated were aspects of the mussel byssus including: morphology, physical structure, mechanical strengths, comparison to marine mussels, biochemical characterization, molecular weights of polyphenolic proteins, extent of crosslinking, and molecular diversity.

## **Byssus Morphology and Physical Structure**

The byssus organ of a mussel is how mussels attach themselves to surfaces. A schematic of the byssus is shown in Figure 1.<sup>1</sup> The root is anchored into muscle tissue in the shell, a stem extends from the root, numerous threads emerge from the stem, and at the distal end of the thread, a disc shaped plaque attaches to the surfaces. It has been suggested that the zebra mussel roots and stems are made of collagen fibers and the threads are made of elastin fibers.<sup>2</sup> The fibers provide tensile strength to the threads and allow the mussel to expand and contract the length of the byssal thread. The plaque appearance is foam-like, with vacuoles (voids) that contain the flocculent material (adhesive material).<sup>1</sup> The average plaque diameter is 200 microns. There may be a correlation between plaque porosity and adhesion strength; marine mussel plaques have greater porosity and greater adhesion strengths to identical substrates than the zebra mussel.<sup>3</sup> Several types of byssi have been observed including: permanent, temporary, and belying. Permanent byssi are normally aligned in straight rows and columns directly beneath the byssus.<sup>4</sup>

Juvenile and adult mussels also form temporary byssi.<sup>4</sup> The biggest difference between temporary and permanent byssi is that temporary byssi are thinner, longer, and attach in a tripod pattern probably for greater stability. Temporary byssi are normally found in juvenile and smaller adult mussels because they tend to move around more and may want to detach from the surface. The temporary byssi are released by secreting an enzyme that disrupts the adhesion mechanism. Currently the enzyme has not been identified, but may potentially be significant in developing materials to resist mussel attachment.

Relocating or swimming juvenile and adult mussels also employ an elongated belying byssus that can be up to 20-30 times the length of the mussel.<sup>2</sup> This belying byssus is initially used to attach the mussel to a surface. The belying byssus has multiple plaques for achieving greater probability to attach to a surface.

Direct observation of thread formation and zebra mussel attachment was conducted in 1990.<sup>4</sup> The mussel foot always was swiped across the substrate surface prior to the byssi attaching to the surface.<sup>4</sup> A liquid thread formed that hardened in contact with water.<sup>4</sup> A few minutes later another thread was formed. It was unclear if the mussel foot secretes an adhesive just prior to the byssi formation, or if the foot was used to clean the surface prior to the byssi formation. The permanent byssi form relatively fast, within a few minutes. Other studies show that the number of byssi formed depends on several environmental factors (water velocity, water temperature, salinity, food availability, etc.), and substrate type.<sup>5,6</sup>

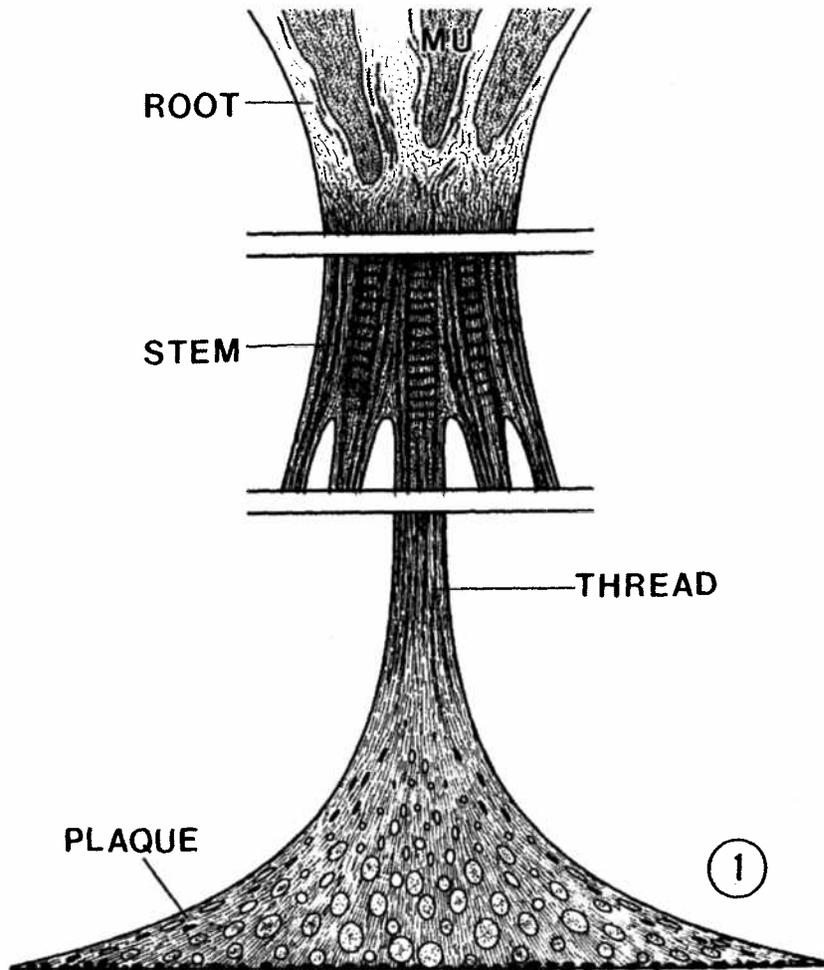


Figure 1. Schematic of the byssus components.<sup>1</sup>

## Mechanics of Adhesion

Adhesion between two materials is achieved through a combination of adsorption, mechanical interlocking, and molecular diffusion across an interface. It is vital to have intimate contact between the two materials. For byssal adhesion, microtopography, viscosity of adhesive, and wetting tendency are important.<sup>5</sup> The byssal adhesion of the zebra mussel is not well understood, but the most probable mechanism is mechanical interlocking. Mechanical interlocking is achieved when the mussel adhesive is secreted into the microscopic pores and crevices of surfaces. The adhesive is also known to have chemical functionality to provide hydrogen bonding, ionic bonding, and covalent chelating bonding with metals and will be further discussed in the Biochemical characterization section. It is known that the adhesive is about 10 nanometers thick between the bottom of the plaque and the substrate.<sup>7</sup> This is the reason that there is limited bulk materials properties data available for the adhesive. The mechanical properties of the adhesive can only be measured by nano-indentation techniques, of which there was no literature found at this time.

## Mechanical Properties of Byssi Threads, Adhesion Strengths, and Comparison to Marine Mussels

The mechanical properties of the zebra mussel byssi were compared to other marine mussels.<sup>1</sup> Zebra and quagga mussels have the shortest and thinnest byssal threads, but they have the highest mechanical properties of all the marine mussels. The tensile strength of zebra mussel and marine mussel threads were found to be 48 MPa, and 13-26 MPa respectively.<sup>8</sup> The tensile modulus (stiffness) for zebra mussels was 137 MPa, other marine mussels had byssi tensile modulus ranging from 35-79 MPa.<sup>8</sup> Zebra mussel threads also had the greatest elongation and extent of recovery. This was a surprising find since it is believed that the marine mussels in greater tidal zone and wave action environments should have had higher strengths.<sup>8</sup> It is believed that the fibers in the threads of marine mussels resemble collagen, whereas the zebra and quagga mussels fibers are elastin.<sup>1</sup> The elastin fiber provides greater elongation without breaking compared to collagen fibers, which are more rigid. The architecture of the zebra mussel byssal thread is also different than marine mussels in that they have lateral filaments connecting between fibers, providing reinforcement to the fibers running parallel with the fiber bundles.<sup>1</sup>

Adhesion strengths of zebra mussels and marine mussels have been measured on numerous substrates. However, there is no data correlating the number of byssi, which influence the actual adhesion strength of mussels. Therefore the data is usually represented as relative adhesion strength. Zebra mussels relative adhesion strength was the weakest of all the marine mussels.<sup>1</sup> The strongest relative adhesion strengths was the *Mytilus californianus* at 60 N/ animal on smooth steel.<sup>1</sup> The blue mussel *Mytilus edulis* was 10-36 N/ animal.<sup>1</sup> The zebra mussel adhesion strength was 1.6 N/ animal on smooth steel.<sup>1</sup> The explanation given was that the zebra mussel plaque was not as porous as the *Mytilus* species. This makes sense because

greater porosity allows greater mechanical interlocking. The duration or number of byssi was not mentioned in this reference.

## Biochemical Characterization

All fouling mussels have a common amino acid protein called 3,4-dihydroxy phenylalanine (DOPA). It appears that DOPA is a versatile but key component in the adhesive proteins of mussels. DOPA can also be formed from a precursor protein called tyrosine 4-hydroxyphenylalanine in the presence of catechol oxidase.<sup>9</sup> Oxidation caused by catechol oxidase can cause DOPA to further oxidize to a quinone structure.<sup>9</sup> The DOPA quinone is known to cause the byssal tanning (provides the brown color of thread). DOPA can also chelate (bonding with central atom) with metal ions, typically calcium, iron, and aluminum are the most common.<sup>9</sup> The chelated DOPA acts as a crosslinking agent which is reversible.<sup>9</sup> Figure 2 shows the chemical structure of DOPA, tyrosine, DOPA quinone, chelated DOPA, and crosslinking DOPA.

The exact chemical composition of the adhesive material is unknown for zebra or quagga mussels at this time. The most studied mussel is the blue mussel *Mytilus edulis*, in which 6 different polyphenolic proteins have been identified.<sup>9</sup> Each polyphenolic protein is composed of different amino acids with varying molecular weights and DOPA content. Currently, only 3 polyphenolic proteins have been identified in the zebra mussel and only 2 in the quagga mussel. It makes the most sense to discuss the proteins found in the blue mussel at this time to get a more detailed understanding of chemical and physical properties of the most studied mussel prior to describing the zebra or quagga mussel proteins currently discovered.

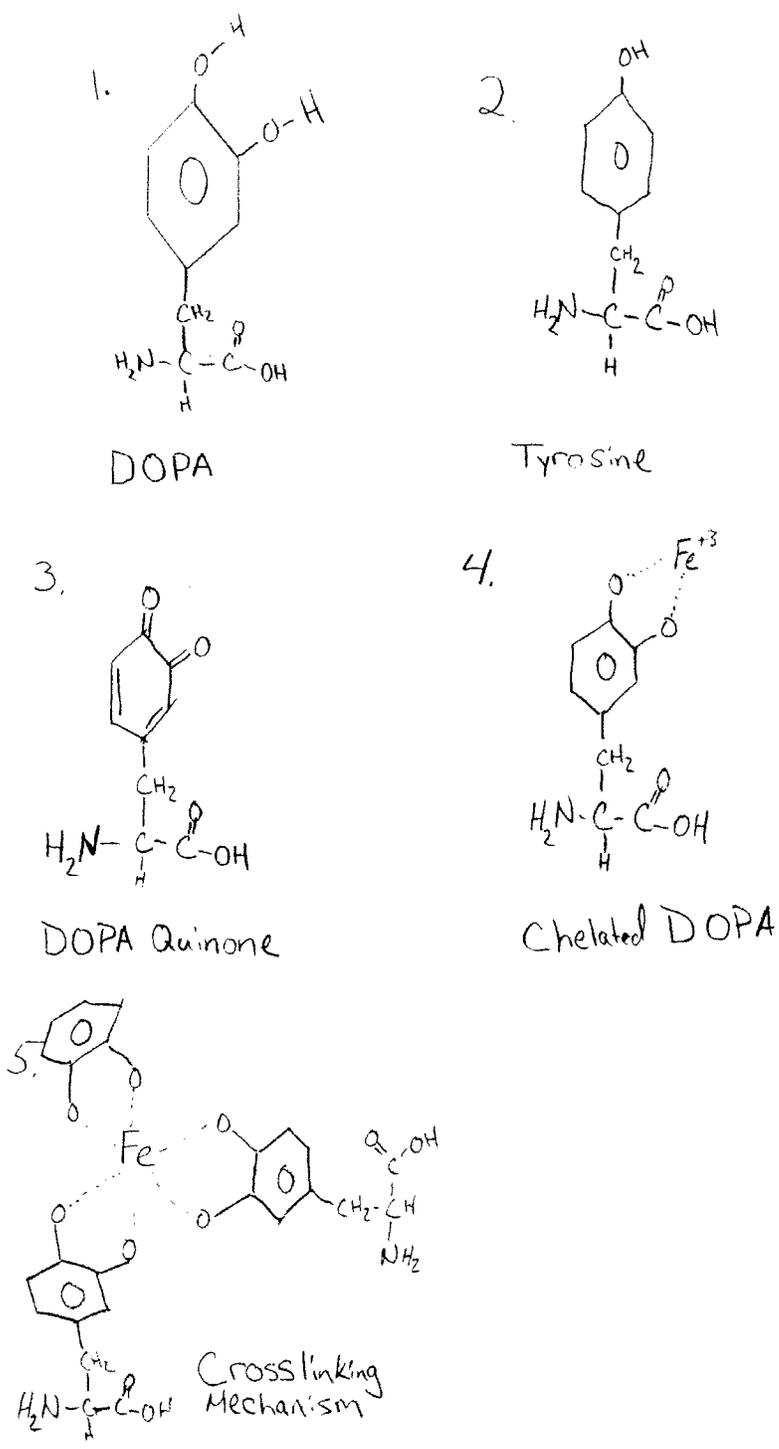


Figure 2 1. DOPA chemical structure, 2. Tyrosine chemical structure, 3. DOPA quinone chemical structure, 4. Chelated DOPA with metal ions, 5. Crosslinking mechanism tris-DOPA- $Fe^{+3}$  complex.

## Blue Mussel *Mytilus Edulis*

The byssal thread polyphenolic protein, labeled Mefp-1, which stands for *Mytilus edulis* foot protein 1, was first characterized in 1981. This protein is primarily found in the byssal thread; however, the plaque also contains approximately 5% of this protein.<sup>9</sup> The Mefp-1 is a large protein containing 897 amino acids with a molecular mass of 115 kDa.<sup>9</sup> Of the 897 amino acids, approximately 60-70% are hydroxylated amino acid, with 10-15% being DOPA.<sup>9</sup> It appears the main function of this protein is for the cuticle (sheath) around the thread and plaque of the byssi.<sup>10</sup> The protein is highly crosslinked with  $\text{Fe}^{+3}$  and  $\text{Ca}^{+2}$  and provides a protective layer around the threads.<sup>11</sup>

The byssal plaque polyphenolic protein, labeled Mefp-2 is found exclusively in the plaque matrix, consisting of 25-40% of the total plaque protein.<sup>9</sup> Mefp-2 has a molecular weight of 47 kDa with only 2-3% DOPA.<sup>9</sup> The Mefp-2 is primarily the solid foam structure of the plaque that provides cohesiveness to the matrix and is lightly crosslinked by DOPA.<sup>9,10</sup>

The Mefp-3 is a protein found in the plaque interface; it's the smallest byssal protein identified with a molecular weight of 5-7 kDa.<sup>9</sup> Mefp-3 does not have repeat units and contains 20-25% DOPA.<sup>9</sup> It is believed that this protein is a primer like function for adhering to substrates.<sup>9,10</sup>

The Mefp-4 is another protein found in the plaque with a molecular weight of 79 kDa and contains 4% DOPA.<sup>9</sup> They believe the main function is a coupling agent in the collagen fibers in the thread/plaque junction.<sup>9,10</sup>

The Mefp-5 is also found in the plaque; it is a small protein with a molecular weight of 9.5 kDa. It contains 27% DOPA and has the presence of phosphoserine.<sup>9</sup> "Phosphoserine is known to occur in acidic mineral-binding motifs of proteins that bind to calcareous materials."<sup>9</sup> It is believed that this protein plays a significant role in adhesion.<sup>9,10</sup> The chemical structure of phosphoserine is shown in Figure 3.

The Mefp-6 was also found in the plaque; it is a small protein with a molecular weight of 11.5 kDa. It contains a large amount of tyrosine and a small amount of DOPA.<sup>9</sup> The authors believe this protein may provide a link between the plaque structure and the DOPA rich proteins.<sup>9</sup>

It appears that Mefp-3 and Mefp-5 contain the highest concentration of DOPA and are the main proteins involved in the adhesion mechanism. These are also the smallest proteins with very low molecular weights and probably have the lowest viscosity to optimize wetting of surfaces. Mefp-5 also contains a unique amino acid, phosphoserine which binds to calcareous materials.

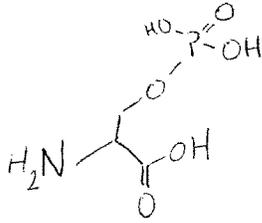


Figure 3. Chemical structure of phosphoserine

## Zebra Mussel *Dreissena polymorpha*

The proteins in the byssi of the zebra mussel have not been completely identified. Currently there are only 2 proteins characterized and 1 protein identified, but not characterized. The zebra mussel byssi also contain significant levels of carbohydrates.<sup>12</sup> The carbohydrate most prevalent is galactosamine; the significance of this carbohydrate is unknown at this time.<sup>12</sup> This is not observed in marine mussels. The most common amino acid is asparagines, with much higher levels than marine mussels.<sup>12</sup> The glycine levels were lower in zebra mussels than marine mussels. Also the zebra mussels lack hydroxyproline which indicates there are no collagen fibers in the threads.<sup>12</sup>

Byssal thread polyphenolic protein Dpfp-1 has a molecular weight of 76 kDa with DOPA concentration of 6.6%.<sup>10</sup> Tyrosine was found to be at a concentration of 8.5%, which can be oxidized to DOPA in the presence of catechol oxidase. It appears the main function of this protein is for the cuticle (sheath) around the thread and plaque of the byssi.<sup>10,13</sup> The protein is highly crosslinked with  $Fe^{+3}$  and  $Ca^{+2}$  and provides a protective layer around the threads.<sup>11</sup> Further details on the actual amino acid concentration and sequencing can be found in references 10, 12, and 13.

The byssal plaque protein Dpfp-2 has a molecular weight of 26 kDa with DOPA concentration of 7%.<sup>13</sup> Tyrosine was found to be at a concentration of 13%, which can be oxidized to DOPA. The Dpfp-2 is primarily the solid foam structure of the plaque that provides cohesiveness to the matrix and is lightly crosslinked by DOPA.

The byssal adhesive protein Dpfp-3 sequence has not been fully identified, but has a lower molecular weight of 4.5-7 kDa. The exact concentration of DOPA is unknown. However, in characterizing the molecular weight, it was discovered that the molecular weights increased upon aging the mussel byssus, indicating the conversion of tyrosine to DOPA after initial measurements.<sup>10</sup>

## Quagga Mussel *Dreissena Bugensis*

Byssal thread polyphenolic protein Dbfp-1 has a molecular weight of 76 kDa (similar to Dpfp-1), however had a much lower DOPA concentration of 0.6%.<sup>13</sup> However, Dbfp-1 has a much higher concentration of tyrosine compared to Dpfp-1, which is converted to DOPA after oxidation with catechol oxidase.<sup>13,14</sup> Dbfp-1 also has a higher glycine and lysine concentration than Dpfp-1.<sup>13,14</sup> These amino acids form ionic charges to promote ionic interactions and secondary structures such as  $\beta$  sheets and double or triple helix. The secondary structures occur naturally in nature, for instance spider silk or silk worm silk obtain greater strength from the secondary structures opposed to the polymer chemistry. Ionic and hydrogen bonding allow the polymer strands to have greater tensile strength and elongation while having the capability to fully recover. It appears the main function of this protein is for the cuticle (sheath) around the thread and plaque of the byssi.<sup>10,13</sup> The protein is highly crosslinked with  $\text{Fe}^{+3}$  and  $\text{Ca}^{+2}$  and provides a protective layer around the threads.<sup>11</sup> Further details on the actual amino acid concentration and sequencing can be found in references 13 and 14.

The byssal plaque protein Dbfp-2 has a molecular weight of 35 kDa with DOPA concentration of 2%.<sup>13</sup> However, Dbfp-2 has a much higher concentration of tyrosine compared to Dpfp-2, which is converted to DOPA after oxidation with catechol oxidase. The Dbfp-2 is primarily the solid foam structure of the plaque that provides cohesiveness to the matrix and is lightly crosslinked by DOPA.

## Mussel Adhesive Bonding Methods

It is believed that both zebra and quagga mussels have similar high concentration of DOPA for the actual adhesive layer even though it has not been fully identified. We know that DOPA's primary modes of bonding are hydrogen bonding and covalent bonding with metals. Keeping this in mind, one may ask why mussel's adhesive adheres well to epoxy and polyurethane coatings. The answer is hydrogen bonding; there are numerous sites for hydrogen bonding in both epoxy and polyurethane coatings based upon the chemical structure of these coatings. Hydrogen bonding can occur with oxygen, nitrogen, and fluorine. In most coating systems there are free oxygen, nitrogen, or fluorine functional groups at the surface, mussel adhesives can form hydrogen bonding with this functionality. However, some coating chemistries can be designed to have no hydrogen bonding sites available at the surface. These include polyethylene, polypropylene, polybutadiene, and polystyrene. It is believed that the mussel adhesive forms a mechanical bond into the porosity or microcracking of the surface of these polymers.

## Biocide-Free Foul-Release Coating Properties

The theory, chemical, and physical properties required for formulating a biocide free foul release coating system is discussed in this section. The adhesion mechanism at the point of contact between the mussel plaque and the surface of the coating is critical in order for the mussel to firmly secure itself to a surface. The adhesive must be able to have intimate contact with the surface, wetting or spreading across the surface is essential. One physical property that can help prevent the wetting of the mussel adhesive on the surface is low critical surface energy.<sup>15</sup> Low surface energy is a key property in a coating system. Fluorine and silicone based polymers are the lowest surface energy polymers known at this time.

The fracture mechanics between the mussel plaque and the coating surface is also an important property. There are 3 different types of fracture: tensile, shear, and peel fracture mechanisms. Peeling requires the lowest amount of energy.<sup>15</sup> It has been proven that low elastic modulus of coatings aids in the peel type bond failure between the mussel adhesive and the coating surface.<sup>15</sup> The low elastic modulus allows for flexibility and chain mobility, which does not allow the adhesive to form a strong mechanical bond.<sup>15</sup> Higher elastic modulus allows a more rigid surface so the mussel forms a stronger mechanical bond into the surface roughness of the coating. The lower elastic modulus does have a drawback; the low modulus typically causes the coating to be weak and not very tough or durable.

In recent years, scientists have tried to develop more durable foul release coatings. There are a few commercially available silicone-epoxy hybrid foul-release coatings and fluorinated polyurethane foul-release coatings; however, the initial test results show that these coatings experience fouling greater than the silicone foul release coatings. These durable foul release coatings are easier to clean than traditional epoxy or polyurethane coatings. With advances in polymer synthesis, coatings chemistry, and formulation, it is just a matter of time before a durable foul release coating performs as well as the silicone foul release coating systems.

Current silicone-epoxy foul release coatings that we have tested are believed to slowly leach silicone out of the coating matrix. The leaching results in the surface properties to change and become more epoxy like when immersed in water. MERL is in the process of testing another silicone epoxy, but does not have any results at this time to determine if the silicone will leach out over time.

Current fluorinated polyurethane foul release coatings contain a perfluoro alkyl chain that is surface active. The perfluoro alkyl chain greatly reduces the surface energy. However, the surface has a higher modulus (more rigid) due to the perfluoro alkyl chain compared to the bulk properties of the coating. This may be why the mussels still attached to the surface.

## Applying knowledge of Mussel Adhesion to Coating Development

- All fouling mussels utilize various proteins to create a bond between the plaque and substrate
- While the complete protein structure of the zebra and quagga mussel has yet to be characterized, DOPA is believed to play a significant role.
- The nature of the bond is both chemical and mechanical.
- The chemical bond is comprised of both ionic and covalent bonds, as well as hydrogen bonding. Surface energy controls the thermodynamic driving force for bond creation. Substrates with lower surface energy have a reduced driving force which results in a lower bond strength and interface area.
- The mechanical bond is affected by the surface morphology with rough surfaces providing increased bond strength due to increased surface area.
- The mechanical bond is affected by the substrate's elastic modulus. Peeling fractures require less energy for separation than cleavage fractures and are promoted by low modulus substrates.

It may be possible to use information about mussel adhesion in a manner beneficial to the development of foul-release coatings. The approaches presented here outline several avenues which may lead to the successful development of a durable foul release coating. Formulating a durable foul release coating may be possible if the surface of the coating has properties that contain a low elastic modulus and low surface energy while the bulk properties may be more durable. The surface chemistry should not have any hydrogen bonding sites for the mussel adhesive to hydrogen bond with. At this time, it appears durable foul-release coatings surface properties change over time in immersion service. What initially seems to work changes after a period of time in immersion service. If the surface properties could be maintained then the coating would exhibit better long-term performance.

Mussels rely on their adhesive to wet out the surface and form mechanical bonds to surfaces. If a coating was used that had very low surface texture the mussels may not adhere well. Some such classes of low surface textured coatings are antimicrobial coatings. A few companies which produce these ultra smooth coatings have recently been identified. It is planned to incorporate antimicrobial coatings into the Reclamation's Zebra/Quagga mussel coatings study.

Another approach to developing a coating would be to identify the enzyme that causes the byssal plaque to release. This would take a considerable amount of time to first identify the enzyme, and then to formulate a coating that slowly releases the enzyme to detach the mussels from surfaces. It is unknown if the zebra and quagga mussel enzymes have similar composition and if the compounds would work for both species.

DOPA has been identified as the key ingredient of the mussel adhesive. It may be possible to find a chemical compound that would reduce or neutralize DOPA into a compound that would not be able to form hydrogen bonding to a surface. Again it would take significant time and funding to find a compound capable of reversing the oxidation process that would not react with other chemicals and ions in the water.

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