A Look at Biofilms in the Brewery

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Brewers know that beerstone can wreak havoc on beer flavor. Many brewers do not realize, however, that microorganisms can still be present even when the equipment appears to be spotless. The term used to describe this condition is biofilm.

What is a biofilm? How does one know if a biofilm exists? If a biofilm does exist, how does one rid the brewery of it? This article focuses on the mechanisms of attachment and survival that organisms use to create and survive in a biofilm. Cleaning and sanitizing chemicals and methods for the prevention and removal of biofilms are also discussed.

A biofilm is a group of organisms that have attached to a surface and entombed themselves in an impenetrable polysaccharide (sugar) coating. Although microbiologists have known about biofilms for decades, it has only been since the advent of scanning electron microscopy (SEM) that biofilms have been researched in detail.

A scientific approach to microbial methodology is identification and analysis of the single cell- from mixed culture to pure culture- in a free living, free floating state. This bacterium whose physical and chemical activities are believed to be independent of neighboring cells is referred to as “planktonic”.

The planktonic cell, however, has the ability and propensity to physically attach itself to any surface, from irregular, soft body tissue to hard, smooth surfaces (including stainless steel). It takes about one minute for complete attachment of a planktonic cell and 1 to 2 days for an array on non-genetic, structural and biochemical changes in progeny cells. The attached microorganism is referred to as “sessile”. Depending on conditions, equilibrium of a biofilm is reached in several days to several weeks.

Most microbial testing can easily quantify numbers of organisms floating in liquids. Once organisms attach to a surface and a biofilm is established, on the other hand, only a scanning electron microscope can truly detect its presence. The reason biofilms can only be seen in this way is because the attachment process occurs on a molecular level. To understand biofilms, it is important to know the process organisms use to attach to surfaces.

There are three stages organisms go through to form a biofilm. The three-step process for the formation of a biofilm is:

1. Adsorption
2. Adhesion
3. Adherence

1. Adsorption

Adsorption should not be confused with absorption. In absorption, a substance is taken up into or throughout a system. Adsorption is defined as the process in which molecules are taken up on the surface of a solid by chemical or physical action. Some common concepts of attraction used in physical chemistry to be aware of here are:

Ionic bonds: The loss or gain of electrons when unions of opposite charges take place.

Covalent bonds: Occurs when two atoms share external electrons.

Metal bonds: These electrons move and act as conductors

Van der Waals forces: Atoms or neutral atoms are united when close to each other. (This bond is weak and fragile).

Hydrogen bonds: The hydrogen atom also creates a fragile union between close molecules.[1]

In their planktonic form, bacteria move around and bump into surfaces, substances and other organisms. Bacteria carry electrical charges and their surface proteins have a specific polar location. This enables bacteria to selectively choose or move away from surfaces and substances. The equation that is used to summarize this ionic activity in the adsorption phase is VT=VA+VR. VT is potential energy, VA the force of attraction, and VR is repulsion. The adsorption phase is reversible. By simply changing the pH or viscosity of the medium, the behavior of organisms can be radically changed.[2]

2. Adhesion

The second reversible stage of the evolution of a biofilm is adhesion. In this stage, the organisms recognize elements that promote adherence. The bacteria join up with a host called a cellular receptor using the laws of Michaelis. The resulting reaction is written as:

R meets B = R <-> Reversible B, Adhesion

R signifies the receptor and B, the bacteria. During the exchange, a weak “RB” complex is formed. Irreversible adherence is achieved only if bacteria have or are capable of producing anchoring appendages. The process for irreversible adherence is described as:

[1] Jean F. Brisou, BIOFILMS: Methods for Enzymatic Release of Microorganisms 1995 CRC Press, Inc., Boca Raton, FL, page 17,19
[2] “Why Bacteria Survive Your Sanitizers”, FOOD QUALITY, (March/April), 2000, pages 56-68

Lectins are an essential part of the adhesion process. The term lectin is derived from latin lego, legere which means, “recognize, choose, sort”. Lectins are glycoproteins that typically have two binding sites. Bacteria with pili or fimbriae contain lectins that are able to differentiate substances such as polysaccharides, single sugars, and other carbohydrates, etc., thereby facilitating adhesion. Lectins are divided into two groups, structural and soluble or viscous.[3]

Fimbriae are extremely thin filaments that surround certain microbes (like E. coli, for example). Hundreds of fimbriae can be counted on a single E. coli cell. The cell walls of organisms provide their protection and are made-up of polysaccharides. Polysaccharides also cover fimbriae. Fimbriae tend to be lipophilic (fat loving). Enzymes and proteins also accumulate during the adhesion step of biofilm formation. These enzymes and proteins react with the polysaccharides to form complex biopolymers. The less stable biopolymers are sloughed off in ambient temperatures while the more stable ones remain intact. The resulting biofilm grows from the inside out rather than planktonic organisms attaching and promoting the growth from the outside.

The biofilm grows exponentially at the beginning of the formation and grows the fastest when many mutually beneficial organisms are present. Growth continues until the thickness of the biofilm impedes the flow of nutrients to the organisms or the force of water passing over the biofilm causes the upper layer to be sloughed off as quickly as it is produced below. As the biofilm organisms are shed into the surrounding environment, the bacterial counts taken are simply an indication of severity of the biofilm itself.

Only about 5-25 percent of the total volume of a typical biofilm is made up of organisms. The remaining majority of volume is over 95 percent water. The dry portion is made up of acidic exopolysaccharides excreted by the organisms. Lipopolysaccharides, which are fatty carbohydrates exist near the organisms. These lipopolysaccharides are more hydrophobic than exopolysaccharides. The exopolysaccharide/water mixture forms a gelatinous substance when sufficient calcium ions replace acidic protons of the polymers.

Because the biofilm is hydrophobic, it resists being washed away by water. This means that even a near boiling water rinse will not completely rid a surface of a biofilm. The uppermost layer of the biofilm sloughs-off, but the biolfim itself will still remain intact. A common characteristic of biofilm bacteria is that a direct physical contact, such as brushing or scrubbing, is necessary to penetrate and remove the microcolony from its attached surface.

3. Adherence

Over a period of time, (ranging from hours to days) the internal biofilm structure becomes highly defined with one or more microcolonies (pure and mixed culture) and formation of fluid channels also remove microbial waste products from the inside. Whereas planktonic bacteria appear to function as independent cells, the biofilm takes on an appearance of a complex but primitive tissue or organ. It has been observed that transport of nutrients and dissolved oxygen through the fluid channels is not by diffusion but convective flow, further indicating a coordinated and cooperative action amongst the biofilm cell population for a stable coexistence. Each microcolony within the biofilm structure establishes its own metabolic activity according to

[3] Dana Johnson, “Removing Beerstone”, Modern Brewery Age, March, 1998, page 37.

environmental conditions; for example, both aerobic an anerobic species have been isolated from the same structure.

Biofilm bacteria are not trapped within this relatively impermeable exopolymer outer covering. As a function of time, population densities, or environmental conditions, viable cells are shed from the structure in an apparent, regulated fashion where released cells can attach to nearby or distant surfaces and establish additional biofilm structures. If the discharged bacterium ends up in a fluid nutrient medium, the phenotype may revert back to the planktonic state. Except for random mutations, there are no genetic changes from planktonic to sessile (biofilm) to planktonic, etc. However, it is important to note that biofilm bacteria, much like their planktonic counterparts, do engage in an exchange of genetic material (i.e. conjugation) so that daughter cells from this gene transfer will express permanent phenotypic changes.

An organic molecule from biofilm cultures has been isolated in the laboratory and when added to a suspension of planktonic bacteria causes a derepression of the bacterial genome. This molecule alone, without cell attachment to any surface, promotes changes in the expression of 40 genes for exopolymer production, reduction in cell size, cell wall chemistry, and reduction in metabolic activity. All of these factors, separately or as a group, pose biofilm problems in brewing operations such as:

- Difficulty to remove from attached surfaces without direct physical contact, such as hand scrubbing;
- Difficulty to isolate individual bacteria by many established laboratory techniques;
- Increased impermeability to surfactants and other cleaners;
- Increased resistance to sanitizer and disinfectants.

Biofilm Removal

Where possible, some form of physical contact such as hand scrubbing is recommended on contact surfaces and in areas where biofilm development is most likely to occur. But, care must be given to these surfaces in order to prevent scratches and pits within which microcolonies can be established that further avoid the scrub brush or green pad.

For clean-in-place (CIP) applications, the use of strong oxidizers has been shown to be effective to remove biofilms. Phosphoric/nitric acid blends with a compatible surfactant tend to work well on helping remove beerstone from aging, serving and bright beer tanks. Oxygenated noncaustic cleaners now have proven track record in the craft brewing industry, as do chlorinated caustic solutions. Peracetic Acid (PAA) and chlorine dioxide sanitizers have also shown effectiveness against biofilms.

Prevention

A key in keeping biofilms from forming is to properly clean and then sanitize equipment after each use, especially surfaces that come in contact with product over long periods of time (i.e. conditioning tanks, kegs, serving tanks, tap lines, etc.) During cleaning, closely monitor the pH and concentration of the solutions closely. The acid solution must be below pH 2 and caustic solutions must be maintained above pH 12 to hydrolyze the polysaccharide coating that protects the biofilm. Here again, however, care needs to be taken to ensure that pitting of the stainless steel does not occur.

Sanitizing

Research has shown that regulated post-rinse sanitizer concentrations will have very, very limited efficacy on biofilm bacteria. What BIRKO suggests is that a higher concentration be applied to these areas (such as 1,000 ppm quat for floors and drains) but don't rinse with potable water. Instead, rinse with the sanitizer at the prescribed concentration to displace the more concentrated chemical at the appropriate post-rinse level.

Testing

Testing for biofilms requires very costly, very cumbersome equipment so that information to date has been tabulated by environmental simulations in the research laboratory. However, enough data and evidence on these microforms have been accumulated over the last 70 years to indicate, circumstantially, that brewing operations are highly susceptible to biofilm contamination.

A visual inspection of surfaces that we use to judge the cleanliness of a surface is to watch the sheeting action of rinse water. Rinse water will continuously wet a clean surface, while residual soil (and potential biofilm area) will appear as a dry island on the surface. Caution is called for here, however. Caustic solutions tend to attach to soil whether or not the soil is removed, given the sheeting appearance of a clean surface.[5]

Conclusion:

Beer is a wonderful beverage. The hops, pH, alcoholic content, and carbonation make an inhospitable environment for most organisms. At the present time, there are no known pathogens that can survive in beer. Still, beer can develop off flavors and shelf life problems if equipment is not kept in pristine condition. If a beer is having bacteriological problems, it may very well be the result of a biofilm contamination. Feel free to contact us if you would like more information.

[5] Fred Holzhauer, Dana Johnson, "Noncaustic Cleaning in the Brewery", The New Brewer, March/April, 1996, page 78

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