BIOFILM AND BIOFOULING MANUAL

BY

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1. INTRODUCTION TO BIOFILM

Many bacteria are **planktonic-** they float around in water. Most microbiological work is done using these suspended cultures on water samples. But most of the bacteria that cause problems are **sessile**- attached to a surface. Once bacteria attach to a surface they change. The most obvious change is that they begin to excrete a slimy material ---- hence the source of the derivation of the word *biofilm.* However, research is showing that biofilm is not merely the provision of the excretion of slimy material but rather they are showing that bacteria which attach to a surface turns on " a whole different set of genes which effectively makes it a **significantly different organism to deal with compared to the planktonic material.**

Bacteria living in a biofilm do a number of things differently from the single planktonic cells of the same type of bacteria eg *Pseudomonas aeruginosa* and these are:

- There is a division of labour in a biofilm where some cells utilize the available nutrients to turn on metabolic pathways. Other cells utilize degradation products (suspended solids, corrosion products, dead bacteria / algal cells) to produce new cells that are dispersed into the biofilm environment.
- In biofilms, bacteria (film forming fungi can also form biofilms) employ cell-cell communication which is now termed " quorum sensing " whereby they sense the level of increased cell population density ---- they release and detect hormone-like molecules that accumulate in the surrounding aquatic environment as the bacterial cell density increases.
- The biofilm having achieved this quorum sensing shows vast differences in heterogeneity from the same bacterial species in different environments.
- The biofilm having achieved this quorum sensing status can begin to excrete toxins and polysaccharides, change the properties of the original bacteria cell, and change the shape of the biofilm.

2. CHARACTERISTICS OF BIOFILMS

We will examine a number of the known and researched characteristics of biofilms.

a) Biofilms consist of :

- water (85% to 95% by weight)
- microbial cells
- extracellular polymeric substances (EPS) such as polysaccharides, proteins and other bipolymers

- suspended solids
- corrosion products
- algal material
- fungi
- protozoa

The biofilms grow in micro-colonies embedded in the EPS structure which are interspersed with less dense regions containing highly permeable water channels. Counting of individual micro-organisms in a biofilm is not practical and in addition a number of species in the growing biofilm are non-culturable.

b) Research has shown that there is no difference in the rate of colonization across different types of supporting material (glass, stainless steel, rubber lining). The actual number of viable cells in the biofilm will differ in terms of absolute number of colonies.

c) Biofilm structure is very dependent upon fluid velocity of the water, nutrient load, temperature, pH, electrostatic potential, biocide concentration and biocide contact time. Change a process parameter and the biofilm structure changes. Biofilms can grow across a vacuum!

d) There are 4 categories by which detachment of biofilm from a surface takes place and these are:-

- erosion --- small particles from the biofilm surface being detached into the bulk fluid
- sloughing --- large pieces of biofilm being detached
- abrasion --- detachment by collision of solids
- grazing --- removal of biofilm due to its consumption by higher organisms such as protozoa.

These 4 different methods of detachment each exert a different response in counting microbiological colonies in bulk water samples and they exert different effects on disinfectant / biocide efficacy.

Detachment of biofilm can occur by increasing the flow rate of water to greater than 3-4 m per second. Fluid shear forces cause erosion whilst high fluid velocities cause abrasion and sloughing.
Detachment of biofilm caused by disinfectants / biocides results in sloughing.

Detachment of biofilm is dominated by the electrostatic interaction in cell–cell attachment. Change in electrostatic potential can change the biofilm structure.

The structure of biofilms is a function of the spatial distribution and homogeneity of the biofilm in a water circuit. Hence, the importance of measuring spatial distribution of biofilm.

e) Structure of biofilms : depends upon flow conditions

--- turbulent flow produces homogeneous and slimy biofilms

--- laminar flow produces a scattered biofilm with significant protuberances.

--- laminar flow biofilms are more easily inactivated than turbulent flow biofilms

- turbulent flow biofilms are more active as seen by the increase in respiratory conditions for the micro-organisms, have less EPS but higher protein content. { Proteins which contain glycine, lysine and histidine react with many disinfectants / biocides like chlorine, bromine, ozone, gluteraldehyde, QAC's, peracetic acid products, hydrogen peroxide---- **no reaction with chlorine dioxide**}.
- effect of disinfectants / biocides is related to the age of the biofilm. Younger biofilms are easier to remove but age is relative for each system as age varies from minutes to days.
- slug dosing of a disinfectant / biocide has been demonstrated to be significantly more superior to continuous low level dosing in the removal / detachment of biofilms. In many cases the level of detachment of biofilm changes by factors of 10 to 100 times for slug dosing compared to continuous dosing.
- The decrease in the susceptibility of biofilms to disinfectants / biocides is now been proven to be influenced by phenotypic characteristics of the adherent cells and biofilm rather than biofilm structure. In other words, the various cells in the biofilm of the same bacterial type (that originally formed the biofilm) undergo physical / chemical changes
- due to the formation of the biofilm thereby they exhibit different properties to their planktonic relatives.

Biofilms do not grow in homogeneous structures. They change their shape, size and other chemical / physical characteristics across any given unit area and across the whole system---- spatial distribution of the biofilm is a major factor in determining the ease of detachment of the biofilm.

f) In potable water distribution systems biofilm formation leads to a deterioration of the microbiological quality of the treated water. Major areas of concern are:-

- Re-growth of coliform s of non-fecal origin
- Multiplication of opportunistic pathogens like *Aeromonas*, *Pseudomonas* and *Legionella* to mention a few examples.
- Increased heterotrophic plate counts
- Color, odor and taste problems
- Microbiologically induced corrosion (MIC)
- Can induce scaling
- Provide protective places for pathogenic bacteria.

Microbial measurement in potable water systems poses special problems mainly related to the low amount of bacteria present, low levels of nutrients in the potable

water and their low activity. Hence, the best suited techniques are those that are very sensitive to these small changes.

We will also report on a number of observations regarding the various research papers that have been written on the impact of disinfectants / antimicrobials / biocides on biofilms.

i) gluteraldehyde has been shown to provide a protective effect on cells against lysis (attack)---- no effect on biofilm at 200 ppm levels
ii) the most widely tested compounds used to control biofilm have been chlorine, hydrogen peroxide, QAC and peracetic acids. <u>These chemicals have been shown to have very poor to no effect on biofilm detachment</u>.
iii) Ozone has been shown to kill cells in the biofilm without any detachment of the biofilm. Re-growth of the micro-organism population 2 to 4 days later is evident with ozone treatment.
iv) Biofilms have been shown to grow across UV lights quite readily.
v) The latest research by G. Gagnon, Dalhousie University in Canada has shown that **chlorine dioxide** and chloramines are very effective in the detachment of biofilms in potable water distribution systems

These characteristics of biofilms are being used to explain " the mechanism of (biofilm) resistance to antimicrobial agents". There is no one mechanism rather researchers believe that there are 3 broad categories:

a) reduction of the antimicrobial concentration in the water surrounding the biofilm
b) failure of the antimicrobial agent to penetrate the biofilm
c) adoption of a resistant physiological (phenotype) by at least a fraction of the cells in the biofilm.

In the first scenario, the antimicrobial agent is depleted to ineffectual levels before it gets to the biofilm. In the second scenario, the antimicrobial agent is delivered to the surface of the biofilm but it does not effectively penetrate the biofilm. In the third scenario, the antimicrobial agent permeates the biofilm but it is unable to kill microorganisms because they exist in a phenotype state that confers reduced susceptibility. { The reduced susceptibility of biofilms has not been attributed to the usual mechanisms of mutation or acquisition of genetic elements that cause specific resistance genes that account for conventional antibiotic resistance. For these mechanisms to explain biofilm resistance, the genetic modification would have to appear in the biofilm but absent in the planktonic state--- this is not happening.

Some research has also shown that the amount of biofilm removed and the reduction in viable cell numbers in the biofilm were not correlated. Some antimicrobial agents cause significant killing but not much removal of biofilm and vice versa. This underscores the fact that biofilm removal and cell killing are distinct processes and both need to be fulfilled to have a successful treatment.

What does all this mean in terms of reality where we need to treat biofilms on a daily basis and explain to the customer what is happening and how we will solve his problems?

1. Take the ice water system in a winery as an example. At "B" Winery a residual of 1 ppm **chlorine dioxide** gives good results but at "V" Winery you only get a good result with 3 ppm residual. The above explains this problem.
2. Research has shown that a slug dose of an antimicrobial will do more damage to the biofilm than a low continuous dose and this is easily explained by the three mechanisms which explain antimicrobial resistance. There is a minimum inhibition concentration (MIC) that any antimicrobial requires before it can inactivate a bacteria cell.
3. From 2, it is obvious that the MIC for the same type of bacteria can differ from site to site. This explains why one begins to get a good result but say after one week the bacteria counts are high again. A slug dose at this point will get on top of the problem.
4. **Chlorine dioxide** is going to be a more effective antimicrobial than most other chemicals----why?------ small molecule; non ionic; a gas; highly soluble in organics; no reaction with polysaccharides; very few chemical reactions; stable in water with a measurable residual.

BUT even with these characteristics there is no " standard " level for removal of biofilm.

3. OVERVIEW TO BIOFILM MONITORING

Biofouling is a biofilm problem---- undesired deposition and growth of micro-organisms on surfaces such as heat exchangers, water storage and distribution systems and in medical applications. These biofilms cause significant economic losses. Any strategy which incorporates anti-fouling technologies will be more cost effective if the extent of the biofilm could be monitored on-line in real time without destroying the biomass formation.

Current biofouling monitoring techniques rely on the removal of biomass from the system in the form of coupons that have been exposed to the fluid for a given period of time. These samples are then analyzed which is time consuming and requiring skilled personnel. Furthermore, current biofilm control technologies are based on some of the following :-

- Biofilm is monitored by monitoring the process performance or product quality. The biofilm is detected only after it has already caused economic losses.
- Biofilm monitoring is based on decisions made from the results obtained from bulk water samples. It has been shown above that there is no correlation or relationship between planktonic bacteria and sessile bacteria of the same type.
- Biofilm is usually treated as a " disease" of the plant process water. Thereby, if one kills the organisms in the bulk water one will affect a cure of the disease!

- Disinfectants are used to kill the organisms in the bulk water, however, they will leave dead biomass in the system that accumulates and promotes re-growth of the organisms by using the dead biomass as a nutrient source. (In many instances the real problem is the biomass of the biofilm).
- Some oxidising disinfectants (**like chlorine dioxide**) cleave the bonds between the extracellular polymeric substances (EPS) which are responsible for the attachment of the biomass. This detached biomass needs to be inactivated, by slug dosing, so as to stop the re-growth potential.
- Biofilms are resistant to many disinfectants like chlorine, ozone, peracetic acid because they only cause cell deaths and re-growth of the biofilm is evident. In such instances a " saw tooth curve" of micro-organism levels is evident.
- In most instances the amount of nutrients in a system is not limited. Oxidants like ozone can actually increase the amount of assimilable organic carbon content thereby increasing the biomass quantity.
- From the above we have shown that biofilms are evident some time after formation. Research has shown, as indicated above, that detachment of the biofilm is dependent upon its age, type of disinfectant / biocide used, its concentration and contact time available in the system. The general mode of
- operation is for the significant over use of poorly selected disinfectants / biocides which results in economic, environmental concerns and costs.
- Contemporary biofouling control strategies operate with information from water samples and blindly applying disinfectants / biocides because they kill these organisms in the planktonic state.

Biofouling monitors operate on 4 levels and each type is described below:-

Type 1: Measurement of the kinetics of deposition of material and changes to the physical properties of the deposit . These systems cannot detect the difference between micro-organisms (biotic) and abiotic deposit components like corrosion deposition, suspended solids, scale and non micro-organisms. These units work on a variety of parameters like light scattering; turbidity measurements; electrochemical changes in conductance; redox potential and heat transfer exchange resistance.

Type 2: Systems which can distinguish micro-organisms (biotic) and abiotic deposits in a biofilm. These systems can measure the kinetics of deposition of biofilms and some of these systems can also undertake to measure the spatial distribution of biofilms. These systems can be used to correlate biofilm structure with absorbance for a given set of plant conditions. They can also be used to monitor disinfectant / biocide efficacy by changes in biofilm structure. These systems use infrared sensors, fluorescence or microscopical observations.

Type 3: These systems provide detailed chemical and / or physical composition of the biofilm. These systems use sophisticated spectroscopy and microscopy analysis and currently are only suitable for biofilm research and not for use in industry.

Type 4: These systems can discriminate between living and dead organisms within the biofilm surface. To-date no such equipment exists.

Biofouling monitoring is direct, on-line, in-situ, continuous, non-destructive real time information regarding biofilm in a specific system. Industrial process water or potable water is not a sterile system hence there is a level of biofilm in all systems which is inherently present without causing problems to that system. The difficulty is to determine this " base-line" for each system. Why? Biofouling monitoring is basically a means of monitoring physical / chemical parameter(s) it is not a means of quantifying biofilm function. Currently there is no way of doing this. The reason being that biofilms do not conform to any mathematical model; they vary in thickness, density and physical / chemical composition from point to point in any given biofilm in any given process water system. Biofouling monitoring is a means of measuring and comparing specific parameter(s) in biofilms in a specific process over a period of time. Optimization of the type of disinfectant / biocide to be used, cleaner applications which requires more sophisticated monitoring strategies and different biofouling removal technologies are going to become the state of the art techniques to optimize disinfectant / biocide usage.

4. BIOFILM CONTROL STRATEGIES

We review the factors that will be needed to take cognizance of in the selection of the right disinfectant / biocide and the most cost effective slug dose timing regime. The applied dosing of the appropriate disinfectant / biocide in a biofilm control strategy will need to satisfy the following conditions:-

- Low redox potential
- No hydrolysis or dissociation in water
- Few chemical reactions particularly with polysaccharides, proteins, enzymes and biopolymers.
- High solubility and stability in hydrocarbons
- Identification of biofilm formation, above the level of the baseline biofilm (which is not problematic) so that no time is wasted in remedial action.
- Changes in process conditions alter the rate of colonization and biofilm characteristics. Biofouling monitoring needs to be sensitive to these changes.
- Each system will have different biofilm characteristics even if the same bacteria type is the responsible organism eg slimes formers, SRB's etc. Dosing patterns will vary.
- Detachment of biomass, in most cases, is important without causing process or product contamination. Only killing of cells promotes re-growth. { Soak and disinfect process off-line will achieve these results provided the disinfectant can remove biofilm}.
- Slug dosing in terms of concentration and time between intervals will vary from system to system. The only method of effectively monitoring the cost

effectiveness of this treatment is by using a biofouling monitor which can monitor disinfectant / biocide efficacy.

- Biofilms contain areas of highly permeable water channels. Disinfectants / biocides efficacy requires a diffusion time for the product through these channels. Over a period of time more biofilm is removed and the disinfectant / biocide slug dosing pattern will be reduced.

Biofouling control is a sophisticated science with no standard method to treat similar systems. There is a need for product optimization used in conjunction with a biofouling monitor prior to attaining the desired results but this process will be far more cost effective then blindly adding a disinfectant / biocide in the hope of controlling biofilms. A number of techniques will be needed to be used to achieve the most cost effective treatment program. We outline herein BTC PRODUCTS' program which we believe can become the benchmark in biofouling control strategy for a number of different industrial applications.

5. BIOFOULING CONTROL STRATEGY

The major aspect to our technology package:

- SNiPER® CHLORINE DIOXIDE TECHNOLOGY

We compliment the products used in proper cleaning with a significant **chlorine dioxide** formula with the capability of optimization of the chlorine dioxide efficacy on bacteria and the biofilm. We have developed methods for deployment of chlorine dioxide through a management system that ensures its success in the Food & Beverage Industry, Environment Restoration, Medical Industry, Public Buildings and Industrial water treatment or Municipal Wastewater applications.

1. CHLORINE DIOXIDE TECHNOLOGY

Our Chlorine Dioxide technology is based on a proprietary formula of an RTU that will remain stable and retain its efficacy for an extended period of time, above any other CLO2 product on the market.

A) **RTU** CHLORINE DIOXIDE SOLUTIONS

GER'S PRODUCTS has a chlorine dioxide solution which has 24 months stability. We call this product **R**eady **T**o **U**se (**RTU**) Chlorine Dioxide solution to differentiate it from the so-called stabilized chlorine dioxide products which need to be mixed on site prior to use.

SNiPER® **RTU** Chlorine Dioxide product gives the industry easy access to the powerful chemistry of chlorine dioxide because all that is now required is the necessary deployment in the proper applicator system. The complexity of the dosing system is determined by the customer's needs.

2. GER PRODUCTS BIOFOULING CONTROL STRATEGY

The focus of our biofouling control strategy will be centered on the known presence of microbial contamination and/or the presence of biofilm. SNiPER® releases chlorine dioxide through a mechanical action at the point of contact with a rapid lethal effect of the organisms.

As is evident from all the research on biofilms there is no " standard" method of removal and killing of biofilm. Each system is to be evaluated individually and in terms of the customer's requirements taking into account:

Process performance
Product integrity
Regulatory issues: environmental / discharge regulations, FDA / EPA & EU approvals etc may be required depending on application and claims made.
Cost effectiveness
Microbiological efficacy

To achieve these requirements we will make use of the arsenal at our disposal:

Non-oxidising biocides in combination with chlorine dioxide (an oxidising biocide) will provide maximum insurance against organisms showing resistance to any biocide. This is equally important in cooling towers as well as in the cleaning of poultry houses particularly in the latter case against the spread of the quick mutating avian flu virus which is wreaking havoc in the poultry industry in Asia.

To conclude we wish to reiterate that biofilm control strategies will need to have multiple levels of attack not blindly taking surface water samples and adding a biocide at a rate that the customer deems affordable. To be successful in the application of SNiPER® technology, the representative will need to show the customer their capability in evaluating the problem, recommend the process / application and how to reach the solution.

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