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Eminently affordable, easy to use. Read the paper below for more important information about *Legionella Pneumophila*.

THE BIOLOGY OF

CIRCULATING COOLING WATERS

I. WHY TEST FOR BACTERIA IN COOLING WATERS?

A. Biological Effects in Cooling Waters

Circulating water is used for the purpose of transporting unwanted heat. As a heat transfer agent, water is the most efficient compound on Earth. There are, however, two prominent undesirable effects associated with the use of water in aerated systems:

- Water promotes corrosion and,
- Biological things grow in contaminated water.

Corrosion effects from non-biological causes in cooling water are dealt with elsewhere.

Biological activity is the subject of this paper. The following pertains to problems caused by biological activity in cooling waters, about how to measure biological problems early, and how best to control them.

Classifying Biological Activity in Cooling Waters

There are many ways of categorizing different life forms. For the purposes of this discussion, two classifications of microorganisms are convenient:

1. Freely circulating. This means rods, spheres etc; organisms suspended in the water that have some mobility, but do not, by their own action, attach to surfaces. These include pathogenic organisms---those that cause human disease.

2. Sessile organisms are those that naturally stick to surfaces, accreting mass and forming biofilms.

Circulating organisms

Most bacteria are in this category. They metabolize and reproduce while freely circulating as suspended particles or on the surface of other suspended particles (dirt). Because cooling waters tend to be low in nutrients, and usually contain inhibitory chemicals, freely circulating bacteria reproduce slowly in well-maintained systems.

Sessile organisms

These include the **slime-forming** bacteria. All forms of microorganisms compete for the nutrients that are available in a particular ecosystem. Freely circulating organisms have some limited mobility, being able to swim around and stumble upon food. Slime forming bacteria are sticky, without mobility. They stick to the surfaces and wait for the circulation to bring the food around. In heavily contaminated systems (machining fluids, e.g.;) the freely circulating organisms have an advantage and tend to dominate. In low nutrient circulating environments (cooling towers) where long times are available, the slime forming bacteria have advantages. Slime bacteria attach to a surface over which the water circulates. Whatever other tiny particles that are suspended in the circulation are carried past these sticky cells and some of this mass gets stuck on the slime. Over time, the stickiness causes more and more slime forming cells to accrete. Eventually, a layer of slime (biofilm) covers the surface. This biofilm acts as an excellent insulator so the water can't access the surface of the heat exchanger. The organic acids that are waste products of bacterial metabolism get between the biofilm and the heat exchanger surface and corrode the surface. This is Microbially Induced Corrosion (MIC). Other circulating solids stick in the slime layer (including freely circulating bacteria) and are consumed. In this way the slime-formers can dominate in low nutrient circulating systems.

By this action, bacteria and nutrient are "filtered" from the circulating waters by the successful slime, resulting in a low population density in circulation even when the system is badly fouled by the presence of biofilm.

Freely circulating bacteria have mobility to move around and stumble upon food. In low-nutrient systems where it is a long way between tidbits of food, freely circulating organisms tend to do poorly. Sessile bacteria stick to surfaces and wait for the circulation to bring the nutrient around.

The pathogen ***Legionella Pneumophila*** can prosper in this environment. A brief discussion of this species in this context will be useful in gaining an understanding of the biodynamics of cooling tower waters. *Legionella* was first identified as the causative agent in a lethal outbreak of bacterial pneumonia at an American Legion convention in Philadelphia in 1976 (hence, the designation "Legionnaire's Disease"). A high percentage of the attendees died from this pneumonia. *Legionella* has since been implicated by The Centers for Disease Control (CDC) in some 150,000 deaths per year in the USA and many more throughout the industrialized world.

Legionella is a gram-negative, aerobic, motile, rod. This means that it will not absorb a gram stain, that it needs oxygen, that it can move about, and that it is shaped like a cigar. This description is included here for the purpose of completeness. Such "unfamiliar" terms will be avoided in this work wherever possible. *Legionella* is ubiquitous (See!) in soil and water. Most healthy adults have antibodies to *Legionella,* which means they have been exposed and their immune systems have succeeded in repelling the assault. The victims of *Legionella* are those who are in some way immuno-compromised (hospital patients, drug users, alcoholics, some of the elderly etc;). Normally functioning immune systems can defend against *Legionella* infection.

Legionella has rather complex nutritional requirements which limit the rate at which it can spread in most environments. It does have the ability to invade cooling water systems for reasons that will become clear.

When tissue sections taken from victims of Legionnaire's disease were examined, the *Legionella* were found to be growing inside macrophages (cells that eat other cells). Not only were the *Legionella* inside the phages, they were inside vesicles (a cell within a cell within a cell). In this manner, *Legionella* was protected from the outside world, and somewhat immune to biocide attack. When circumstances allow the *Legionella* to multiply sufficiently that a high concentration exists inside the host phage, the host phage is lysed (burst open), releasing the *Legionella* into the extracellular environment.

In cooling water systems, this same drama is played out with the slime forming cells that make up the biofilm taking on the role of the phages. *Legionella* "hides" inside vesicles inside the slime forming cells. This makes *Legionella* in cooling waters remarkably resistant to biocides such as Kathon and Chlorine. So, the biofilm is implicated in the production and preservation of *Legionella* in two ways: it nurtures *Legionella* by providing a nutrient rich environment, and it shields *Legionella* from traditional biocides. Without a biofilm, *Legionella* is severely limited in cooling waters; control the biofilm and the *Legionella* will be restricted to negligible levels.

Legionella is spread to humans by aerosol misting. When the biofilm on cooling water heat exchanger surfaces is allowed to prosper, it catches *Legionella* cells, which migrate to the interior of the slime cell and reproduce. When enough reproduction has occurred, a fairly large population of Legionella can be released into circulation where it is misted into the environment for unlucky humans to inhale. This is a particularly insidious occurrence when the intake vents for the building being serviced happen to be in the vicinity of the water surface, and the prevailing air currents push the vapors into the buildings being serviced.

Hospitals are especially vulnerable, in that they have many infectious agents (Chlamydia, Staph, e.g.;) present and a population of humans whose immune systems are weakened. Hospital devices used in inhalation therapy have become contaminated with Legionella, serving as sources of serious outbreaks.

To summarize, slime-forming bacteria represent the main source of problems in cooling towers. They have a tendency to prosper in low nutrient recirculating cooling water systems. Prevent the slime formers and the three prominent biological problems in cooling waters (heat exchanger fouling, MIC, and *Legionella* fears) are controlled. Population levels of *Legionella* sufficient to endanger humans seem to be coincident with successful formations of biofilm comprised of slime forming bacteria. Controlling biofilm formation suppresses the level of *Legionella* to below danger thresholds.

B. Controlling the Biology in Cooling Waters

Controlling the biology in circulating cooling waters is usually done by adding biocides. The popular biocides used are typically broad spectrum, meaning that they can kill a wide range of cell types. From the earlier discussion, it is seen that biofilm formation is the culprit in the problem aspects of cooling waters: Biofilm is the mechanical agent that fouls heat exchange surfaces, making them inefficient; it induces corrosion; and, it can act as host to promote the growth of *Legionella*. In well-managed systems where biofilm formation is preventively controlled, *Legionella* will likewise be unable to grow to high concentrations. When systems are not managed, allowing biofilm to grow to gross proportions, it is likely that a wide spectrum of contaminating organisms will have success.

In addition to expense considerations, care should be taken that the proper dosage of biocides is administered. If too much is added there is danger to humans. If too little is added, cell mutation problems may occur

II. Testing

The best way to make efficient use of biocides is to monitor the systems with biological tests and administer the biocides as dictated by the test results. There are essentially two testing methodologies that may be used: **Growth Methods** and **Biochemical Methods**. Growth methods require placing a bit of the sample onto a nutrient mixture and incubating for 1-3 days. Viable cells from the sample may grow into colonies that are visible and these are counted as colony forming units per milliliter of sample (Cfu/ml). Growth methods provide an estimate of the count of cells that were in the original sample without saying what they were doing there.

Biochemical methods measure the presence of some marker molecule that results from biological activity. For example, the HMB test measures the enzyme Catalase which is produced by aerobic cells when they metabolize. Another biochemical method measures the level of the energy producing molecule ATP. As such, these methods are not counts, but measures of activity. Under certain conditions activity measurements and counts may be correlatable, but, in general, they won't be. Typically, results are available from biochemical methods in just a few minutes.

Because of the availability of immediate results, testing is usually done less frequently when biochemical methods are used. This saves money. Growth methods provide a count in 1-3 days, saying nothing about what the organisms are doing in the system; biochemical methods provide results in minutes, giving repeatable, digital information about how much the organisms are doing, but do not register organisms unless they are metabolically active.

Both methodologies are useful. But, none of this matters unless the sample tested can give information that can be used to treat the system in a meaningful way. As the earlier discussion shows, this information is not in the circulating waters. It is on the heat exchanger surfaces. Since quantifying this growth is a difficult if not insoluble problem, the solution is to use a surrogate. The test must produce information after the organisms have grown to detectable levels, but before any damage can be done to the system. The successful test procedure and associated products **(pat. pending 11/99**) are simple and elegant. They are described below. The goal of providing reliable testing information that allows the operator to treat in such a way as to prevent the fouling of the surfaces by biofilm, is achieved by this system.

III. How to Perform the HMB Test

Coupon Test for Slime Formation

This is the most important test for cooling towers. The coupons are installed on one service visit and read on a subsequent service visit. The test is simple: just drop the coupon into one of the test tubes, add a few drops of 50R reagent and 15 minutes later, read the result. After the reading, the coupon is cleaned and reinstalled. This test requires the appropriate coupons and the XMC test kit. See the operating procedure below for interpretations. Products required: HMB device, test kit, coupons.

IV. Interpreting Test Results in Cooling Water Samples

See the procedures for the test being performed.

HMB TEST PROCEDURE---BIOFILM IN COOLING WATERS

Notes: This test uses specially made coupons of a non-metallic material that have been installed in sidestream circulation for an appropriate amount of time. Use test materials from the BioTech 50XMC test kit.

1. Remove the coupon from the rack and without touching the surface of the coupon, place it into one of the predispensed test tubes from the 50XBC test kit. This tube contains a measured amount of diluent and reactants without which test results will not be valid.
2. Add 10 drops of HMB 50R reagent, dispensing it with the tube held at an angle so the reagent runs down the side.

CAUTION: **HMB 50R IS A STRONG OXIDANT. AVOID CONTACT WITH EYES AND SKIN. WEAR PROTECTIVE EYEWEAR AND CLOTHING. IN CASE OF CONTACT WITH EYES, IMMEDIATELY FLUSH WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST FIFTEEN (15) MINUTES. CALL A PHYSICIAN. CONTACT WITH SKIN WILL CAUSE A SLIGHT BURNING AND BLEACHING OF THE SKIN. THIS WILL DISAPPEAR IN A FEW MINUTES.**

3. Without agitating the tube unduly, insert the stopper securely into the test tube.
4. Using the needle apparatus provided in the kit, pierce the stopper for 1-2 seconds so that the head space within the tube is normalized to local conditions.
5. Holding an index finger on the stopper, shake the tube a couple of times to mix the contents. Place the tube in upright position for 15 minutes.

   <u>CAUTION</u>: VERY HEAVILY CONTAMINATED SAMPLES MAY CAUSE PRESSURES TO INCREASE IN THE HEAD SPACE SUFFICIENTLY TO BLOW THE STOPPER OUT OF THE TUBE. KEEP THE TUBE POINTED AWAY FROM THE OPERATOR OR OTHER PERSONNEL. IF THE STOPPER BLOWS, THAT TEST IS TO BE REGARDED AS VERY HEAVILY CONTAMINATED, REQUIRING ACTION.

6. After 15 minutes, repeat the mixing, and tap the tube on a solid surface to assure that no liquid is trapped in the stopper.
7. Turn on the HMB IV, making sure it sets itself to 0.00 BMR within 3-4 seconds.
8. Holding the HMB IV vertically, in the left hand, impale the test tube on the HMB IV needle.
9. The reading will flash once, then stabilize in about one second.
10. Remove the tube from the HMB IV, remove the stopper and discard the tube and its contents.

<u>INTERPRETATIONS OF THE READINGS</u>: This procedure is intended for use on site for the purpose of assessing the need for biocide adjustment/addition. It is expected that service personnel will perform the test on a regularly scheduled service visit. Since the frequency of visits is a variable, <u>relative</u> numbers, not <u>absolute</u> numbers have the most significance. This means that records on each installation should be kept, and when a particular installation's results change, it is a call to action. Nevertheless, some general guidelines are worthwhile:

- Readings less than 0.27BMR have no significance---should always be regarded as null.
- Readings greater than 4.0 BMR are heavily contaminated and normally require action (clearly, this depends on how long the coupon has been in the system, and on other factors).

Read the operator's manual for further discussion.