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Protocol

DBLSOP06

SLIME FORMING BACTERIA

SLYM-BART™

THEORETICAL

Slime-Forming Bacteria (SLYM) is the name given to bacteria that are able to produce copious amounts of slime without necessarily having to accumulate any iron. These slime-like growths are therefore often not dominated by the yellows, reds and browns commonly seen where IRB are present. Some of the IRB also produce slime but it is sometimes denser and has more texture due to the accumulation of various forms of insoluble iron. SLYM bacteria can also function under different reduction-oxidation (redox) conditions but generally produce the thickest slime formations under aerobic (oxidative) conditions. These can develop in the SLYM-BART™ as slime rings growing around the floating ball in the BART tester. Slime growth can also be seen as a cloudy (fluffy or tight plate-like structures) or as gel-like growths (thick goo) which may be localized or occur generally through the body of water medium. Very commonly the gel-like slime growths form from the bottom up in the test vials. One common check for these types of growth is to tilt the BART tester gently and see that the cloud- or gel- like growths retain their structure and tilt with the tester. Almost all of the slime forming bacteria can produce copious amounts of slime that can contribute to plugging, loss in efficiency of heat exchangers, clouding, taste, and odor problems. This is one of the most sensitive BART™ testers. A positive usually involves a cloudy reaction in the inner test vial often with thick gel-like rings around the ball. A negative test remains clear.

A vast majority of bacteria can produce slime-like growths. The slime is actually formed by a variety of extracellular polymers that are long thread like molecules. These extracellular polymeric substances (EPS) literally coat the cells into a common slime-mass within which large volumes of water become clustered and bound. Often 95 to 99%

of the volume of slime is actually water. Some bacteria produce an EPS that remains tightly bound to the individual cell. These are called capsules. Other bacteria generate such a copious amount of EPS that it envelops whole masses of cells within a common slime.

The role of the slime appears to be protective. If environmental conditions are harsh (e.g., due to shortage of nutrients), the slime layers tend to get thicker. Not only does the slime act as a protectant to the resident bacteria but it also acts as a bio-sponge by accumulating many chemicals that could form either a nutrient base, or be toxic to the cells. EPS may be produced by enzymatic activity (e.g., dextran sucrase or levan sucrase) on carbohydrates. In addition, EPS may be synthesized within the bacterial cells and released to form an enveloping slime.

Location of Slime Forming Bacteria

Slime forming bacteria tend to be aerobic and form slimes at redox fronts. In the BART™ tester, this front may form around the ball causing a slime ring, or deeper down in the liquid medium column to form an observable growth. This growth may be plate-like and appear to float at a specific depth, cloud-like with indefinite edges, form as basal dense slimes in the conical base of the test vial, or be gel-like and maintain its shape even when the vial is tilted. Since slime tends to be formed by bacteria under stress, it is common for the slimes to form after there has been an initial growth that may take the form of a localized or general cloudiness.

Many slime bacteria can produce various pigments that will color the slime. Such growths are usually white, grey, yellow or beige in color. These often darken over time particularly in the presence of daylight. Distinctive colored slimes include red (commonly associated with *Serratia marcescens*) and violet (associated with either *Chromobacterium* or *Janthinobacterium* species). Blackening may also occur particularly after growth. This may be a result of the production of either iron sulfides or carbonates which is commonly associated with the presence of mixed cultures including enteric bacteria in the SLYM-BART.

SLYM-BART™ can be used as a simple presence/absence (P/A) test capable of indicating to some extent the population size and the types of SLYM organisms present in the water sample. Different microorganisms utilize various sites along the redox gradient under a ball to grow and regular careful observations are needed to catch the start of growth so that the time lag can be determined. Slime forming bacteria can cause very serious engineering problems since the slime formation can compromise the engineered specifications into many systems. Primarily, the effects of the slime growths are to reduce hydraulic or thermal conductivity and reduce water quality (generally, the first symptom is increased turbidity followed by taste, odor or color problems. As the slimes slough into the water later during the infestation, it can be expected to see sudden rises in the total organic carbon, increases in aggressivity and reductions in water quality.

Reaction Patterns for the SLYM-BART™ tester

DS -Dense Slime (Gel-Like)
SR -Slime Ring around the Ball
CP -Cloudy Plates layering
CL -Cloudy Growth
BL -Blackened Liquid
TH -Thread-Like Strands
PB -Pale Blue Glow in U.V. Light
GY -Greenish-Yellow Glow in U.V.

Of the above reactions, it is the CL (cloudy) reaction that is by far the most common. Occasionally the CL will be preceded by a CP which will be transient (lasting commonly less than 24 hours). Descriptions of the various reactions are given below:

DS – Dense Slime
This reaction may not be obvious and require the observer to gently rotate the BART™ test at which time slimy deposits swirl up. These deposits may swirl in the form of a twisting slime when the tube is gently rotated. This swirl can reach 40 mm up into the liquid column, or it may rise up as globular gel-like masses that settle fairly quickly. Once the swirl has settled down, the liquid may become clear again. In the latter case, care should be taken to confirm that the artifact is biological (ill-defined edge, mucoid, globular) rather than chemical (defined edge, crystalline, often white or translucent). Generally, these dense slime growths are beige, white or yellowish-orange in color.

CP – Cloudy Plates Layering
When there are populations of aerobic bacteria, the initial growth may be at the REDOX front that commonly forms above the yellowish-brown diffusion front. This growth usually takes the form of lateral or "puffy" clouding which is most commonly grey in color. Often the lateral clouds may be disc-like in shape (plates) and relatively thin (1 to 2 mm). It should be noted that if the observer tips the BART™ slightly, the clouds or plates often move to maintain position within the tube. The edges of the plates are distinct while the edges of the “puffy” forms of layering are indistinct. These formations are most commonly observed 15 to 30 mm beneath the fill line. Cloudy formations will tend to extend to cause an overall cloudiness of the liquid medium (CL). These plates sometimes appear to divide (multiple plating) before coalescing into a cloudy liquid medium.

SR - Slime Ring
A slime ring, usually 2 to 5 mm in width forms on the upper side of the ball. The appearance is commonly mucoid and may be a white, beige, yellow, orange or violet color that commonly becomes more intense over time on the upper edge.

CL – Cloudy Growth
Solution is very cloudy and there may sometimes be a poorly defined slime growth around the ball. Sometimes a glowing may be noticed in at least a part of the top 18mm of the liquid medium. This glowing is due to the generation of U.V. fluorescent pigments

by some species of *Pseudomonas*. The common pigments doing this are a pale blue (PB) or a yellowish green (YG) color. Note that this glowing may not be readily observable unless a U.V. light is used. The occurrence of the glowing in a U.V. light means that there is a probability of potentially pathogenic species of *Pseudomonas* and confirmatory testing is recommended.

BL – Blackened Liquid
This is commonly a secondary or tertiary reaction rather than an initial reaction. It is recognized as a clear, often colorless, solution that is surrounded by large blackened zones in the basal cone and up the walls of the test vial. The BL often parallels the BL reaction in the IRB when the two BARTs™ are used together to test the same water sample.

TH – Thread-Like Strands

On some occasions, the slime forms into threads that form web-like patterns in the liquid medium. Sometimes these threads may interconnect from the ball to the floor of the inner test vial.

RPS (Reaction Pattern Signatures) for the SLYM:

DS -CL Dense slime forming bacteria producing copious EPS, facultative anaerobes dominate

SR -CL Aerobic slime forming bacteria (such as M*icrococcus*) dominating with some facultative anaerobes

CP -CL Motile facultatively anaerobic bacteria dominate (e.g., *Proteus*)

CL -SR Mixed bacterial flora including some aerobic slime-formers

CL -BL Slime formers dominated by Pseudomonads and Enteric bacteria

CL -PB *Pseudomonas aeruginosa* dominant member of the bacterial flora

CL -GY *Pseudomonas fluorescens* species group present in the flora

TH -CL Aerobic bacteria dominant which are able to generate slime threads (e.g., *Zoogloea*)

Slime forming bacteria are amongst the fastest growing aggressive consortia (communities) and the medium used in this BART tester is very enriching and causes a wide variety of bacteria to be able to grow rapidly. However, when the bacteria do not grow quickly this indicates a very low population of aggressive bacteria. As a result of this, the time lags of between 3 and 6 days show a rapid decline in populations when compared to the IRB- or SRB-BART™ tests.

The Relationship between Time Lag and the Population For Slime Forming Bacteria

Time lag (days)	12°C	22°C	28°C	36°C
1	1.3M	8.8M	11.8M	16M
2	284T	632T	403T	261T
3	61T	70T	28T	12T
4	13T	11T	3.3T	1.1T
5	3T	2T	613	199
6	586	666	161	53
7	122	228	56	19
8	25	93	24	9

Note: M indicates millions of p.a.c./ml; T indicates thousands while other numbers are direct; see BART QuickPop on the web site for further interpretation.

Risk Potential Assessment – SLYM-BART

The slime forming bacteria are complex consortia involving many bacteria. These consortia inhabit a common "growth" of slime that acts as a communal chamber. Within these slimes, the bacterial cells are commonly dispersed and occupy only a small part of the total volume (<0.1%). Most of the slime is water bound to the organic polymers that bind the slime together. The SLYM-BART reflects the activities of bacteria that are present in the water as a result of the sloughing from the slime. As a result of this, the test may exhibit a complex set of reactions depending upon precisely which bacterial species are present in the water sample. Like the other BART™ tests, the shorter the time lag to the SLYM-BART displaying a reaction then the greater becomes the aggressivity and the more urgent the need to treat. Not all reactions are equally important in determining the aggressivity of the slime forming bacteria (and therefore the need to treat). Below is a list of the reactions described above and their relative importance in relation to the need to treat. Concern can be expressed through the shortness of the time lag (in days) as:

1. Very aggressive (treatment should be started as early as convenient)
2. Aggressive (treatment should be considered in the near future before the condition degenerates further)
3. Moderately aggressive (treatment may not be required but vigilance through ongoing testing should be practiced)
4. Normal Background Levels (routine testing is recommended)

Relationship between the Time Lag to the Reactions in a SLYM-BART™ and the Aggressivity of the Slime Forming Bacteria

Reaction	Aggressivity (time lag range in days)			
	Very High	Significant	Moderate	Background
DS	<1	2	3-7	>7
SR	<1	2-3	4-6	>6
CP	<0.5	1-2	3-6	>6
CL	<1	2	3-6	>6
BL	<1	2-4	5-8	>8
TH	<2	3-4	5	>6
PB	<1	2-4	5-8	>8
GY	<1	2-3	4-8	>8

Some remedial treatments should be considered urgently where the time lag (in days) shows aggressivity to be at the very aggressive or aggressive (1 or 2) levels. Where there has been an RPS (sequence of reactions to form a signature), then the aggressivity should be considered to be equivalent to the most aggressive of the reactions using the above table.

Hygiene Risk Considerations – SLYM-BART

The most significant hygiene risk generated by this test is the BL reaction that indicates that Pseudomonads and enteric bacteria are present. If this reaction occurs within eight days then a fecal coliform test should be performed on that water to determine the hygiene risk directly. Where PB or GY reactions are observed, this should be confirmed using the FLOR-BART™.

August 13, 2006