Microbial Monitoring in Metalworking Fluids
(Water-based coolant in industrial baths and cooling circuits)
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Introduction
Water-based cooling lubricant emulsions, lacquer baths and cooling circuits can be heavily contaminated by microorganisms leading to massive loss in product quality. A new quantitative ATP-metry (adenosine Triphosphate) test was used in industrial metalworking fluids, baths and cooling circuits to quantify microbial proliferation and improve the efficiency of any chemical treatment. Biomass proliferation of the cooling installation of a metal rolling process in a steel plant was controlled using this new method. Excessive microbial growth causes seven Principal types of problems: Deterioration of working or process fluids; generation of odors, fouling of lines, valves and filters; acceleration of corrosion; poor quality and production performance environmental deterioration including immediate workplace and other areas such as possible contributions to skin and/or respiratory irritation.

Quench-Gone Organic™ kits (Aqua-tools, France) are a dedicated tool to identify in 5 minutes, the best treatment strategy to be implemented on a metalworking fluid installation. As the microbial proliferation zones are identified precisely, efficiency of treatments can be measured immediately after their implementation. Moreover, the kit can be used in an effort to reduce environmental burden by biocides and reduce investment and operation costs.

Presentation of the technology
Active biomass quantification was performed using a second generation of ATP-assay - Quench-Gone Organic (QGO M™) kit measurements (developed by LuminUltra™ and commercialized in Europe & MEA by Aqua-tools)

ATP molecule can be found in all living organisms and is indicative of their metabolic activity and/or the viability of the biomass present in environmental samples. ATP is an energy transporter inside living cells and in implicated in all biological functions, such as nutrition or reproduction.

- Intracellular ATP (cATP) = living organisms

Quench-Gone Organic M™ (QGO M™) kits are designed for low-solids water applications and use filtration to separate biomass from the sample. Filtration makes it possible to concentrate the active biomass and to separate the extracellular ATP in the sample coming from dead bacteria and 90 to 95% of the inhibitors present in the sample. UltraLyse™ makes it possible to lyse the bacteria retained on the filter and subsequently extract the intracellular ATP (cATP™). This parameter is an indicator of total microbiological load in the water. UltraLyse™ has been optimized to maximize ATP extraction and has been shown to extract more than 3 times the ATP recovered by other extraction agents.

QGO M™ kits measure only active biomass in all the types of water using a single analysis. The results from a QGO M™ measurement can be converted into Microbial Equivalents including bacteria and fungi (i.e. the total number of microorganisms per mL of sample).

The chemical reaction used in the method is the same as in nature, when Fireflies produce light. The principle of the measurement relies on the count of photons produced by the action of an enzyme, luciferase, emitting photons in the process of hydrolysis of an ATP molecule: this phenomenon is called bioluminescence. The intensity of emitted light is measured with a luminometer:

$$\text{ATP} + \text{luciferine} + \text{O}_2 \xrightarrow{\text{Mg}} \text{AMP} + \text{PPi} + \text{oxylyciérine} + \text{Light}$$

Luciferase

The sample volume for the assay is 5 ml.

The quantity of produced light is directly proportional to active biomass: in fact, the principle interest of this second generation of ATP kits is the possibility to convert results expressed in RLU (Relative Light Unit) in pg ATP/ml or Equivalent microbials/ml. There is a scientific consensus that 1pg of ATP corresponds to 1,000 microorganisms. A calibrated added volume of ATP (Ultracheck™), stable over time, gives the possibility to compare results in ATP rather than in RLU.

QGO M™ is optimized to detect microbial equivalents ranging between 500 and 67,000,000 per ml (0.5 to 6.7x10^4 pg ATP/mL). QGO M™ is designed for water-based solutions containing organic contaminants, such as organic particles or petrochemicals or water with high oil content.
Threshold for good metalworking fluids by quality, preventive and corrective actions, based on Intracellular ATP can be established for water installations (Table 1).

Table 1: Recommended thresholds based on QGO M™ kit for metalworking Fluid controls

<table>
<thead>
<tr>
<th>Process</th>
<th>Parameter</th>
<th>Good Control</th>
<th>Preventive Action Required</th>
<th>Corrective Action Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metalworking fluids</td>
<td>cATP (pg/mL)</td>
<td>&lt;1,000</td>
<td>1,000 to 10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>Oily water</td>
<td>cATP (pg/mL)</td>
<td>&lt;100</td>
<td>100 to 1,000</td>
<td>&gt;1,000</td>
</tr>
</tbody>
</table>

Presentation of the study

Industrial lubricants are increasingly providing a rich environment for microbial growth and proliferation. Most of the knowledge on lubricant biodeterioration has been extrapolated from field and laboratory experience with metalworking field. Compositionally more complex than most lubricants, metalworking fluids are either solutions or emulsions of 5% to 10% coolant concentrate to water. Microbes are most prevalent on system surfaces where condensation co-mingles with lubricant to support development. The microbes inhabiting the biofilms that form on these surfaces act like fixed-film biological reactors. Objective of contamination control by QGO M™ is primarily to prevent biodeterioration. Secondary objective is to minimize biomass accumulation. ATP method quantifies viable, cultivable bacteria and viable not cultivable bacteria. This measurement includes microbes that are active in the lubrication system but do not form colonies on culture media and other microbes that might be dormant in the lubricant.

All metalworking-fluid formulations share the common problem of susceptibility to microbial attack. However the challenge for both formulators and metalworking facility operators using water-based fluids, is to minimize the adverse economic impact of uncontrolled microbial contamination in metalworking.

For many years the importance of microorganisms in these films was unappreciated because only a fraction of a film is active biomass. Another aspect of microbial films that has only recently been observed is their ability to protect bacteria from the action of biocides, which is a major problem with most biocide treatment programs.

Biocides that are effective against free-living microbes may never come into contact with microbes living in the film clinging to the system surfaces.

Consequently shortly after a pulse dose, when the biocide concentration falls below toxic levels bacteria from the film rapidly regrow in the fluid. Today, biocide manufacturers and formulators are aggressively seeking methods for making their products more effective against the microbes living within the film layers.

Frequently, organisms that will not grow on nutrient media can be identified under the microscope. Special staining procedures enable the technician to distinguish between live and dead cells.

Other sophisticated as culture plate procedures are commonly used for identifying or confirming the presence of certain organisms.

Each colony ostensibly comes from a single organism. However, aggregates of microbes will form single colonies. Moreover, any given nutrient medium will favor the growth of some microbes and inhibit the growth of others. Under the best conditions in the laboratory, plate counts give only about 10% recovery (define). In the field, recoveries are in the 0.01- to 0.1 percent.

Since microbial populations in a metalworking fluid may increase two to three times during the course of one cycle through the system, this delay in receiving data could have catastrophic consequences.

Results of QGO M™ on metalworking fluids

<table>
<thead>
<tr>
<th>Volume of sample</th>
<th>Relative Luminescence Unit (RLU)</th>
<th>cATP (pg/mL)</th>
<th>Equivalent microorganisms /ml</th>
<th>Target value</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 ml</td>
<td>169518</td>
<td>930.59</td>
<td>930 590</td>
<td>To be treat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;1000 pg cATP /ml</td>
</tr>
<tr>
<td>15 ml</td>
<td>15000</td>
<td>90</td>
<td>90 000</td>
<td>Under control</td>
</tr>
</tbody>
</table>

Conclusion

QGO M™ is a new generation of ATP quantification method that allows any operators to control in 5 minutes microorganisms proliferation in the way to protect against disease. Any strategy of biocide should be validated in order to improve treatment efficiency on viable cultivable or not cultivable microorganisms.

The QGO M kit is a useful tool for optimization of concentration, frequency and injection points for biocides, to identify polluted zones and risk areas and verify discernment of cleaning actions on fixed biofilm.