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Bioluminescence testing for rapid detection of microbial contamination in aqueous polymer emulsions

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ABSTRACT

Most aqueous polymer emulsions (APE) used in coatings applications are susceptible to microbial spoilage, which can result in loss of application properties for the end customer. A spoiled batch can potentially cost $50,000 - $250,000; an estimate that does not necessarily include the cost of product replacement, remediation and disposal in addition to labor and other ancillary costs.

Early detection of microbes in APE prior to shipment can reduce this risk. Normally, the typical test method requires 24–72 h to detect most microbial species. Moreover, some species need as many as 7–9 days of incubation for detection, and others might not be detected at all. With these traditional methods, by the time contamination is detected at the manufacturing plant, the material may have already been delivered to a customer. We have validated a reliable and easy-to-use bioluminescence test for microbial detection in most APE. The bioluminescence test is a rapid method for quantification of living cells based on the detection of the molecule adenosine triphosphate or ATP. We found the method to be easy to execute with repeatable results. It also has a good correlation with our standard method and results are obtained in less than an hour. This is the first report on the successful use of ATP detection to assess the microbial quality of APE in the coatings industry. Having a process that facilitates real-time detection, along with triggering immediate action will reduce the risk of delivering contaminated material and reworking costs. This is expected to improve the overall performance of manufacturing plants.

1. Introduction

Biodeterioration (biological deterioration) of aqueous polymer emulsions (APE) can result in malodor, pigmentation, and even loss of application properties. The type of microbe and the rate of spoilage depend on the chemical and environmental state of the material; therefore multiple variables have to be considered when detecting and enumerating microorganisms. Mohan and Srivastava (2010) have recently reviewed polymer biodeterioration. They listed the primary factors contributing to polymer biodegradation susceptibility, including: polymer structure, molecular weight, hydrophobic-hydrophilic characteristics, additive chemistry, synthesis process and environmental conditions. Gu (2003) reported the existence of both aerobic and anaerobic metabolic pathways for polymer biodeterioration and noted that typically, the process begins with exoenzyme-mediated depolymerization, followed by endoenzyme-mediated mineralization and subsequent conversion to biomass and metabolites. APE have the appropriate chemical and physical properties to sustain microbial growth in the form of vegetative cells or biofilms. These properties include a water-based matrix that provides carbon, nitrogen and phosphate for metabolism; in most cases, a pH range of 6–8; and an optimal storage temperature of 25–37 °C. In addition, recent industry trends towards greener chemistries (*e.g.* removal of formaldehyde and lower levels of VOC, residual monomers, and preservative levels) have made this type of product more hospitable to microbes.

In contrast to materials used in food, medical, and personal care industries, architectural and industrial APE are not expected to be

Abbreviations: APE, aqueous polymer emulsions; ATP, adenosine-5′-triphosphate; cATP, cellular ATP; CFU, colony forming units; PCM, plate count method; C_v, coefficient of variation; R^2, coefficient of determination; VOC, volatile organic compounds.

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sterile. However, the introduction of contaminated raw material can severely affect the production or shelf life of customers' end products. Reviewing the biodegradability of polymer emulsions, Gillatt (1990) listed the primary signs and symptoms of biodeterioration: viscosity changes, pH changes, color changes, odor development, visible growth and exoenzyme production. Individually and collectively, these changes result in substantial economic loss. In 2010 the global market for APE was estimated to be approximately 11 million MT, valued at $25 billion (U.S.) (Freedonia, 2010). These statistics mean that every 1% of product rendered unusable due to biodeterioration represents a $250 million (U.S.) loss.

Furthermore, the global demand is forecasted to rise 5.2% per year to 51.6 million MT in 2017, valued at $186 billion (Freedonia, 2013) Depending on batch size and polymer chemistry, an individual spoiled batch of APE can cost $50,000 to $250,000 (U.S.) to handle and replace (unpublished data). This estimate does not include the cost of remediation, disposal, labor, or other ancillary costs at the manufacturing site. Additionally, delivery of compromised or spoiled material directly impacts customers' operations, driving higher costs for supplier and customer. Therefore, early and reliable detection of microbial contamination prior to product delivery is valuable to our business by reducing the cost of handling spoiled or returned product. Customer loyalty is maintained and promoted by providing a reliable, high quality product delivered on time to meet demand.

As recommended by ASTM International, our current method for enumerating microbes in APE relies on the use of microbiological media (ASTM D5588, 2008; D5465, 1993; and D2574, 2006). A known volume of sample is spread on semi-solid nutrient medium that supports the growth of most microorganisms. This medium is then incubated at a specific temperature for 7 days. The appearance of colony forming units (CFU) is monitored and counted. A CFU is the visible microbial growth on a solid surface presumably originated from the reproduction of one cell. This plate count method (PCM) requires delays of 2–7 days between inoculation and final data availability.

Because microbes have diverse nutritional and environmental requirements for optimal growth, laboratory growth conditions can also be limiting. This is especially true in the case of wild species that have adapted to a unique environment – for example: a bulk tank filled with an APE. This scenario often results in delayed detection of contaminated material due to slower microbial growth rates or false negatives, as it is in the case of viable species that cannot be cultivated under lab conditions. Retaining inventory until test results are available also has adverse economic consequences, including the capital expense of inventory and risk of post-production contamination. Consequently, there is considerable value in being able to reduce the delay between test initiation and completion from days to minutes.

Adenosine-5′-triphosphate (ATP) bioluminescence, among other rapid testing techniques, has become increasingly popular in manufacturing facilities for food, beverages and pharmaceuticals due to the relative simplicity with which it can be integrated into daily operations (Graham, 2014). ATP is a multifunctional nucleotide used to transport chemical energy within cells for metabolism. One molecule of ATP contains an adenine ring, a ribose sugar and three phosphate groups. All healthy, living cells contain ATP. Crombrugge and Waes (1991) estimated that one bacterial cell has ~1 fg of ATP. Miller and Galston (1989) estimated that fungal cells can have up to 100 fg of ATP. Therefore, detection and quantification of ATP gives a direct correlation of the number of living microbial cells in any given material.

ATP measurement using the luminescence from the reaction of the enzyme-substrate pair – luciferin and luciferase – was first described by Strehler and McElroy (1957). During the reaction, ATP is dephosphorylated to adenosine monophosphate (AMP) and pyrophosphate. This reaction releases light. The reaction is shown in the following equation:

$$\mathrm{ATP} + \mathrm{O_2} + \text{luciferin} \xrightarrow{\mathrm{Mg{+}{+}\ \&\ luciferase}} \mathrm{AMP} + (\mathrm{PO_4})_2 + \text{oxyluciferin} + \text{light} \quad (1)$$

Holm-Hansen and Booth (1966) adapted the method for use in determining ATP biomass in seawater samples, and additional test method refinements have followed (Hamilton and Holm-Hansen, 1967; Stanley and Williams, 1969; Karl, 1993; Pivovarenko et al., 2006). However, none were free from a variety of interferences that have historically limited the utility of ATP testing as a microbial contamination condition monitoring tool. Previously, Passman et al. (2009) have described the unique properties of the new method that eliminates ATP test method interferences. The protocol was subsequently adopted as standard test methods to detect microbial contamination in water-miscible metalworking fluids (ASTM E2694, 2011) and fuels (ASTM D7687, 2011). The current investigation validates the use of this protocol for the detection of microbial contamination in APE. This is the first report that evaluates the detection of microbial ATP in coatings and establishes ATP-based microbial quality criteria for APE in manufacturing.

2. Materials and methods

2.1. ATP quantification

Samples were prepared for ATP quantification by using the Quench-Gone Organic Modified (QGOM-XLPD™) test kit from LuminUltra Technologies Ltd (Fredericton, New Brunswick, Canada) following the manufacturer's guidelines. Briefly, an aqueous polymer sample was diluted 1:10 in a proprietary dilution buffer and pressure filtered through a 0.7 μm nominal pore size, glass fiber, in-line filter, to trap microbial cells. The filter was then washed with 5.0 mL of a proprietary rinsing agent and air dried. Next, 1.0 mL of a proprietary lysing reagent was used to extract ATP into a 15 mm × 150 mm reaction tube. The 1.0 mL cellular ATP (cATP) concentrate was then diluted by adding 9.0 mL of a proprietary buffer reagent.

To measure bioluminescence, 0.1 mL of diluted cATP extract was transferred to a 12 mm × 50 mm cuvette to which 0.1 mL of Luminase™ XL, stabilized luciferin-luciferase reagent, had been added. The cuvette was then placed into a Lumitester C-110 luminometer (Kikkoman Biochemifa Company; Chiba, Japan) and bioluminescence was observed as relative light units (RLU). Using a Microsoft Office Excel™ worksheet template, the instrument-specific RLU results were converted to pg ATP mL^{-1} by using the following equation: $(RLU_{sample} \div RLU_{standard}) * (10{,}000\ pg\ ATP \div mL_{sample}) = pg\ ATP\ mL^{-1}$, where the $RLU_{standard}$ is the RLU generated from reacting 0.1 mL of 1000 pg ATP mL^{-1} reference solution with 0.1 mL Luminase ™ XL, and 10,000 pg ATP is derived from the 1 to 10 reference solution dilution factor (0.1 mL x 1000 pg ATP mL^{-1} = 10,000 pg ATP).

2.2. Microbial growth recovery

For quantification of culturable microbes we followed a variation of the plate count method (PCM) in ASTM D5588 (2008) by making serial dilutions of the sample in Dilu-Lok™ Butterfield's phosphate buffer (Hardy Diagnostics, CA, USA) and spreading 0.1 mL of sample on trypticase soy agar plates (TSA; Becton, Dickinson and Company, NJ, USA) in duplicate. The plates were