Riboflavin Testing – A Valuable Evaluation Tool

Dairy FOODS

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Riboflavin Testing is a procedure utilized to evaluate the effectiveness of spray devices to apply cleaning solutions used in CIP (Clean-In-Place) process vessels. This procedure is only a spray device coverage test and should not be confused with being a test to determine the cleanability of a vessel.

3-A Sanitary Standards and Accepted Practices define CIP cleaning as "The removal of soil from product contact surfaces in their process position by cleaning solely by circulating, spraying, or flowing chemical solutions and water rinses onto and over the surfaces to be cleaned." The hygienic process industries commonly utilize spray devices installed in the interior of vessels or ductwork to apply the detergent solutions and water rinses used during CIP to remove process residues and bio-burden, rendering a clean surface for subsequent use. The effectiveness and repeatability of the overall cleaning procedure relies on assuring the cleaning solutions are delivered to surfaces being cleaned with repeatable flow rates and pressures to the spray devices.

The use of riboflavin testing originated about 30 years ago in the pharmaceutical and biotech industries where validation requirements led to its use to assure repeatable cleaning procedures including spray device coverage. Other process sectors have found that riboflavin testing can be useful to assure that vessel sprays are capable of applying cleaning solutions onto and over difficult to clean surfaces. These could include baffles, mixer and agitator blades including underside surfaces, access ports, and certain tank nozzles that have potentially narrow annular spaces due to instrumentation.

It is important to note that any surfaces to be evaluated for spray coverage must be free of fabrication related residues such as oils and adhesives from protective coverings. A simple Water Break Test, performed by applying deionized water from a laboratory squirt bottle, can be used to assure a clean, oil free, condition. If the DI water forms "rivers" or droplets instead of freely spreading over the surface, further cleaning is indicated.

Riboflavin (Vitamin B_2) in dilute solution with water (0.015-0.025% w/w)(weight of riboflavin to weight of water) is fluorescent under UV-A Light in a range of 365nm to 650nm wavelengths. Optimal observation will apply UV-A with intensity at a minimum of 4,000 micro-watts per square centimeter at a distance of 38 centimeters (15 inches). This characteristic allows riboflavin solutions (low cost, easy to prepare, and GRAS affirmed) to be used as indicators for

effectiveness of coverage by spray devices. There are, however, certain aspects of riboflavin solution preparation that must be followed to prevent false positive test results. Riboflavin is commonly sourced in dry powder form. Complete dissolution in water can be accomplished using water heated to at least 70°C (158°F) with light to moderate mixing.

The Riboflavin Test is often accomplished using a clean garden type pump sprayer to apply the riboflavin solution onto all interior surfaces of a vessel. This includes all nozzles and interior features as noted above. Without allowing the riboflavin solution to dry, the vessel is then subjected to one or more fresh water rinses of approximately 30 seconds duration using the CIP System and vessel spray device and allowing for complete drainage to occur between rinses. This is important to prevent atomizing and redepositing riboflavin droplets by ricochet of spray streams. Following the final rinse and drain time, the vessel surfaces are inspected using a portable UV-A light source. Any riboflavin residues are noted by appearance of a yellow-green fluorescence (Refer to Fig. 1 Photo).

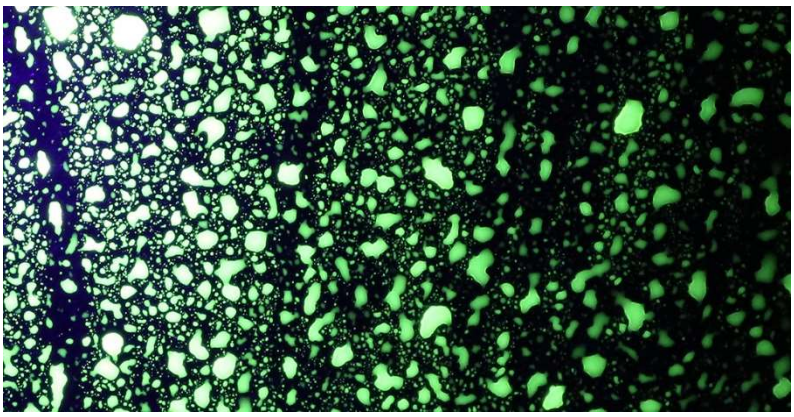

Figure 1. Fluoresence of Riboflavin Droplets on a stainless steel surface. (ESC 2019)

Presence of residual riboflavin droplets are indicative of a potential failure of the spray device to adequately rinse riboflavin from any surface. Care must be exercised to prevent false positive results as a result of opening the vessel access cover(s) or nozzles and allowing riboflavin to flow from the fittings or cover gaskets. Use of the UV-A light can also be helpful to determine if gaskets and seals are not completely closing against mating surfaces. No amount of rinsing can remove residues from cracked gaskets or loose fittings.

Final inspections can include photos of areas with any residual riboflavin as a means to report a failure of spray coverage.

Use of Riboflavin Testing as an evaluation tool for CIP spray coverage should be considered as a cost effective means to assure appropriate spray device coverage function.

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