The Use of Petrifilms to Quantify Aerobic Bacteria in Irrigation Water

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A protocol was developed using Petrifilms™ to quantify the population densities of aerobic bacteria in irrigation water as an onsite monitoring technique. To validate this method, quantification of colony-forming units (CFU/mL) of aerobic bacteria in recirculated irrigation water was compared between Petrifilm and standard culture substrate, with 18 subsamples of 300 mL and 9 log dilutions. A Petrifilm is a dehydrated medium containing guar, xanthan gums, nutrients, and triphenyl tetrazolium chloride on a card with a plastic cover. The comparison method used the APHA Standard Methods (#9215C.6c) for Heterotrophic Plate Count, using Spread Plate Method with R2A substrate. The substrate (Petrifilm or R2A), day (3 or 7), and their interaction significantly affected bacteria CFU/mL. Estimated CFU/mL (×10⁶) using the Petrifilms increased from 50.1 on measurement day 3 to 83.1 at day 7, whereas use of the R2A substrate resulted in higher values of 65.9 at day 3 to 146.2 at day 7 (standard error = 4.0). Comparison of the estimated CFU/mL from Petrifilm to R2A required a conversion factor of 130%, 172%, or 292% when comparing counts from day 3 for both substrates, day 7 for both substrates, or day 7 R2A/day 3 Petrifilm, respectively. The results validate use of Petrifilm as a substrate for quantifying bacterial density in irrigation water, although a calibration curve is needed to interpret the results.
Materials and Methods

Samples were collected from a subirrigation recovery tank in a large-scale commercial greenhouse in Apopka, FL. On 4 Nov. 2011 a total of 18 irrigation samples were randomly collected at 15-min intervals. Samples were collected over a 6-h monitoring period from one subirrigation return tank, during multiple flooding events. Water quality measurements [pH, electrical conductivity, dissolved oxygen, and temperature (°C)] were stable during collection (data not shown). Samples (300 mL) were collected into sterile 500 mL Whirl-pak bags® (Nasco, Ocala, FL) and maintained in temperature-controlled vessels at 22 to 25 °C, similar to the initial sample temperature. Samples were immediately transferred to the laboratory at UF and placed into culture within 6-h of collection.

The Reasoner’s 2A medium (R2A) (Remel Labs, Lenexa, KS) was prepared by dissolving 15.2 g of the dehydrated mix into 1 L of purified boiled water and adjusted to pH 7.2 at 25 °C, autoclaved and cooled to 45 to 50 °C in a water bath prior to pouring into sterile 50-mm-diameter petri dishes (Fisherbrand #09-720-500 (Table 1). Petrifilm (Table 1) preparation followed manufacturer’s instructions (3M, Saint Paul, MN.). Total aerobic bacteria for nursery water samples were quantified using 990.12, Petrifilm Aerobic Count Plate method (AOAC, 1999) or R2A as described by method 988.18, Aerobic Plate Count Method (AOAC, 1999). The sample was distributed over the substrate using the Spread Plate Method 9215 C (APPHA, 1995). Samples were mixed with a vortex shaker and serial diluted from 10⁰ to 10⁻⁸, with sterile saline/peptone diluent, and two replicates per dilution were used. Cultures were transferred using aseptic procedures (APPHA, 1995).

Plates were incubated in darkness ranging from 25 to 27 °C in a growth chamber at 70% RH. Colonies were counted on both media at day 3 and 7, to satisfy the recommended incubation period for Petrifilm and R2A agar, respectively. The paired samples of Petrifilm were compared using ANOVA in PROC GLM using log-transformed data, with mean comparisons using Tukey’s HSD, and linear regression with PROC REG in SAS.

Results and Discussion

There were significant effects of substrate type, (P < 0.001), day (P < 0.001), and their interaction (P < 0.01) on bacteria CFU/mL (with least-square means shown in Table 2). Bacteria CFU/mL increased over time. The R2A substrate demonstrated overall higher bacterial colony counts than Petrifilm. A comparison based on standard recommendations for incubation periods (3 d for Petrifilm, and 7 d for R2A (AOAC, 1999) showed that averaged counts on Petrifilm were lower for most sample times (Table 2). For all methods and measurements, the bacterial CFU/mL greatly exceeded the recommended threshold of 10,000 CFU/mL (Rogers et al., 2003). Both methods indicated a high risk of biofilm formation.

Conversion between bacteria CFU/mL estimates from the two substrates would depend on the measurement day, based on the significant interaction between substrate and measurement day. To convert bacteria CFU/mL from Petrifilm on a given number of days of incubation to the bacteria CFU/mL estimated with R2A, the Petrifilm bacteria count CFU/mL (× 10⁸) could be multiplied by 65.9/50.1 = 1.3 for day 3, or 146.2/83.1 = 1.79 on day 7 using least-square means in Table 2. Based on standard recommendations for incubation periods [3 d for Petrifilm, and 7 d for R2A (AOAC, 1999)], the conversion factor between R2A and Petrifilm would be 146.2/50.1 = 2.92.

| Table 2. ANOVA effects. Effect of substrate (Petrifilm or R2A) and measurement day (3 or 7) on bacteria CFU/mL. Substrate, day, and their interaction were significant at P < 0.001. Letters show Tukey’s HSD comparison at the P = 0.05 level for the substrate × day least-square means. Data were log-transformed for ANOVA analysis, and back-transformed for presentation in this table. |
|-----------------|--------------|--------------|-----------------|
|                  | Day 3 (CFU/mL × 10⁸) | Day 7 (CFU/mL × 10⁸) | Least square mean |
|                  |               |               |                  |
| R2A              | 65.9 B        | 146.2 D       | 98.1             |
| Petrifilm        | 50.1 A        | 83.1 C        | 64.5             |
| Least square mean| 57.4          | 110.2         |                  |
| Std. error       | 2.9           | 2.8           |                  |

Conclusion

This study confirmed that Petrifilm can be used to quantify aerobic bacteria colonies in irrigation water to estimate its microbiological quality. However, given the effect of substrate and measurement day on bacteria CFU/mL, a comparison of counts from Petrifilm to recommended guidelines on R2A required calibration. Based on manufacturer’s suggested incubation time, the counts on Petrifilm after 3 d can be used to estimate the counts on R2A after 7 d. Further validation with a known bacterium or multiple irrigation sources would be helpful to evaluate whether differences in counts between the substrates is consistent for all microorganisms of concern. Differences in CFU/mL between substrates may have resulted from different growth response of aerobic bacteria species to the nutrient formulations and physical environment. One limitation of the Petrifilm is its lack of selectivity on specific plant pathogens. This technique as a non-specific measurement of aerobic bacteria is best suited for quantifying microbial load, estimating a potential for biofilm formation and relative efficacy of water treatment systems along points in an irrigation line, rather than to identify presence or absence of a specific plant pathogen.
Literature Cited


