Application Note
F13005

GreenLight® System for Determination of Microbial Load

Assessment of Bacterial Load in Spray-Dried Cereal Products

Introduction:

Spray drying is commonly used in industry for the production of heat sensitive products, with the goal of producing high quality powders with precise specifications and possibly coatings.

As a critical point in the process, the dryer requires continuous inspection and strict adherence to CIP (clean-in-place) protocols. Typically, the product or the dryer is tested for bacterial contamination on a routine timeframe in order to trigger CIP activities. One limitation of the bacterial testing is the time taken to yield results using the reference methods or other incubation and plating technologies.

In a comparative study on a soy protein product for the food industry, the MOCON® GreenLight system was used to help reduce time-to-result, improve sample preparation time and decrease process variability in the dryer samples.

The GreenLight system uses a unique oxygen sensor to determine microbial load in a food sample. Oxygen is depleted as aerobic microbes grow and respire. GreenLight records fluorescence from the oxygen sensor in its unique APCheck™ vial. No serial dilutions or plate counts are required.

Assay Setup and Protocols:

The cereal product was semi-soluble in dilution. A method was sought to release bacteria into the diluent for both plating and for GreenLight while avoiding interference from non-soluble particles. The chosen method was to use a bench-top centrifuge.

THE SCOPE OF THE TRIAL WAS TO SHOW CORRELATION TO TRADITIONAL METHODS AND TO DETERMINE IF THERE WAS A DIFFERENCE BETWEEN PRE AND POST CENTRIFUGE RESULTS. THE TRIAL USED SAMPLES IN DUPLICATE OR TRIPLICATE WITH DIFFERENT BACTERIAL LOADS.

The plate count method used for comparison was 3M® Petrifilm™.

Initial Sample Preparation:

• WEIGH OUT A DESIRED AMOUNT OF SAMPLE TO MAKE A 1:10 DILUTION. ADD CORRESPONDING AMOUNT OF BUFFERED PEPTONE WATER IN A STERILE CONTAINER, AND SHAKE UNTIL A HOMOGENOUS MIXTURE IS ACHIEVED.

• PIPETTE SAMPLE PREPARATION INTO A CENTRIFUGE TUBE AND RUN AT LOW SPEED (3300 RPM) AND SHORT TIME PERIOD (2 MINUTES). TIME AND SPEED MAY VARY DEPENDING ON CENTRIFUGE.

• ALIQUOT OFF SUPERNATANT INTO AN APCHECK™ VIAL FOR GREENLIGHT TESTING AND A DILUTION BLANK FOR CONCURRENT PLATING.

• RUN BOTH TESTS AND COMPILE THE RESULTS WHEN PLATE INCUBATION PERIOD ENDS AND PLATES ARE READ.

Results and Discussion:

Sample ID	Petrifilm result before centrifuge log(CFU/g)	Petrifilm result after centrifuge log(CFU/g)	Time to Result on GreenLight 930 (Hours:Min)
6-1	5.398	5.447	4:00
6-2	5.431	5.431	3:54
6-3	5.398	5.176	4:19
5-1	4.820	4.771	6:19
5-2	4.799	4.875	6:13
3-1	2.799	2.833	10:08
3-2	3.799	3.820	9:31
3-3	2.756	2.623	10:02

Table 1: Three sets of samples were used with estimated counts of 10^3, 10^5, and 10^6 CFU/g. (Sample ID 3-x, 5-x and 6-x) Three replicates were tested except for the 5-x series.

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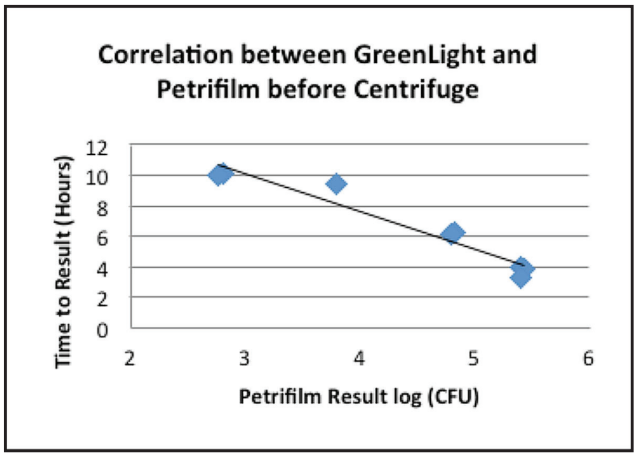

Figure 1: The GreenLight time-to-result and plate count results were compared on an x-y plot and a linear regression determined with an R^2 value. A formula ($y = -2.44x + 17.41$, $R^2 = 0.93$) was generated for subsequent use in product testing, where results would be displayed in CFU/g. The pre centrifuge data set shows good correlation to the Petrifilm results. The maximum test time is about 10 hours for a Log (CFU/g) value of 2.756.

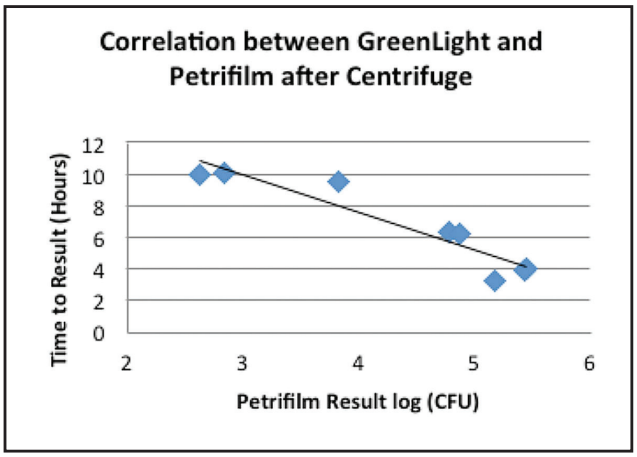

Figure 2: The GreenLight time-to-result and plate count results were compared on an x-y plot and a linear regression determined with an R^2 value. A formula ($y = -2.39x + 17.12$, $R^2 = 0.90$) was generated for subsequent use in product testing, where results would be displayed in CFU/g. The Post centrifuge data set also shows good correlation to the Petrifilm results. The maximum test time is about 10 hours for a Log (CFU/g) value of 2.623. the data sets show similar slopes and intercepts.

Table 2 shows the mean and range (max-min) for each sample set. The bacterial recovery after centrifuge is very good, with minimal increases in variance after the spin step.

Correlation to plate count was generally unchanged after the centrifuge step (more than R^2=0.90).
GreenLight respiration profiles (not shown) were normal showing no effect of semi-soluble particles on the GreenLight oxygen sensor.

Along with excellent correlation, the control of CIP verification testing was improved. For a total of 8 samples tested, the maximum GreenLight time-to-result was 10 hours, versus 48 hours for the reference method. Therefore, for a company with a 3-hour sampling strategy yielding 8 tests per 24-hour period, GreenLight gave results within 4 sampling cycles. The GreenLight method produces shorter test times the higher the bacterial load. Therefore it is possible for out-of- specification results to be achieved well within 8 hours (3 sampling cycles).

Conclusions

The MOCON GreenLight system can enhance high volume spray drying processes by delivering bacterial test results in much shorter times Therefore, CIP activities can be optimized and final product released faster. Additionally the GreenLight process can be used across many product types and points in the production cycle, including coated and uncoated products.

GreenLight can be moved close to the dryer or CIP operation and does not have to be located in a microbiology laboratory. Sample preparation, manual operations, and required media are much reduced in comparison to the plating method which eases the burden of maintaining aseptic sampling.

Sample ID	Petrifilm result before centrifuge (CFU/g)	Petrifilm result after centrifuge (CFU/g)
6-1	5.398	5.447
6-2	5.431	5.431
6-3	5.398	5.176
Mean	5.409	5.351
Range	0.033	0.271
5-1	4.820	4.771
5-2	4.799	4.875
Mean	4.810	4.823
Range	0.021	0.052
3-1	2.799	2.833
3-2	3.799	3.820
3-3	2.756	2.623
Mean	3.118	3.092
Range	1.043	1.197

Table 2: The plating method is compared for pre and post-centrifuging in order to demonstrate potential loss of bacterial load in the diluent

Reference Documents:
FDA Bacteriological Analytical Methods, Chapter 3

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