CLEANING LABORATORY EVALUATION SUMMARY

SCL #:	2021
DateRun:	08/31/2021
Experimenters:	Alicia McCarthy, Zoe Lawson, Aditi Patel, Jaimie Thibeault
ClientType:	Cleaner Manufacturer
ProjectNumber:	Project #2
Substrates:	Solution
PartType:	Part
Contaminants:	Bovine Serum Albumin Fraction V (BSA Fraction V)
Cleaning Methods:	Immersion/Soak
Analytical Methods:	Time-tested Colorimetric Assay (e.g. Bradford Protein Assay)
Purpose:	To evaluate the efficacy of Pollet's Bacillus subtilis strain's ability to continuously degrades proteins (Bovine Serum Albumin Fraction V) over a 14-day period utilizing a modified Bradford Protein Assay protocol to characterize microbial activity.

Experimental Procedure:

Testing utilized a modified version of the Bradford Protein Assay (Bradford, 1976). The Bovine Serum Albumin Fraction V (BSA) is a protein that is mimicking dirty conditions. The calibration curve of the BSA solution used 10g/L and the assay used 100g/L of BSA. Both solutions were filtered (0.22µm), vortexed, and stored at +4°C before use.

Calibration Curve of BSA

Serial Dilutions of BSA and water test solutions were prepared for the BSA calibration before measuring the following mixtures at an OD of 595nm. Samples were run against a blank after five minutes.

	Sample solution (µl)	Reagent Blank (µl)
Solution Sample	30	
Demineralized Water		30
Bradford Solution	1500	1500

Assay Solutions and Timeline

The assay inoculum was prepared using a stock solution of peptone media (10g/500ml) and autoclaved (15 minutes at 121°C). A mixture of 125ml peptone media and 250 mg (+/- 10mg) Bacillus subtilis strain was incubated at 37°C for 15h at 150rpm. Bacteria was centrifuged for 10 minutes at 3500 rpm and the pellet was recovered in 10ml of PBS solution. The inoculum after being diluted at 1/100 (in PBS) had an OD (600 nm) around 0.5nm. After preparing the assay inoculum with BSA solution and demineralized water, the optimum OD (600 nm) of the mixture was around 0.9nm

The final concentration of the 50ml test solutions of the assay inoculum and BSA control was 4g/L of BSA. Inoculum control test solution was prepared with inoculum (1ml) and demineralized water (QS 50ml). The solutions were made at room temperature (22.5°C +/- 2.5°C) at 150 rpm at the timing of the assay.

The three solutions were filtered (0.45 µm) to produce 1ml at each time point. The filtrate was used to make the same mixtures mentioned in the table for the calibration curve (30 µL of filtrate, 1,500 µL of Bradford reagent added, mix and run for optical density after five minutes against a blank as described previously) and measured three times. The measurements were taken and averaged for each solution at the following time points (hours) and graphed: 0, 72, 144, 168, 240, 264, 288, 312, and 366.

References:

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1), 248-254.

Results:

Calibration Curve:

BSA Conc. *(g/L)*	**Avg Reading** *(OD) 595nm*
5	1.737
2.5	1.412
1.25	0.972
0.625	0.632
0.31	0.375
0.155	0.210

Pollet Protein Consumption Assay Results:

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Solution	Time *(Hours)*								
	0	*72*	*144*	*168*	*240*	*264*	*288*	*312*	*336*
Inoculum Assay	0.746	0.755	0.063	0.043	0.029	0.024	0.040	0.073	0.028
BSA Control	0.746	0.956	1.145	1.123	1.065	0.948	0.889	0.789	0.720
Inoculum Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Summary:

Substrates:	Solution				
Contaminants:	Bovine Serum Albumin Fraction V (BSA Fraction V)				
Company Name:	**Product Name:**	**Conc.:**	**Efficiency:**	**Effective:**	**Observations:**
Pollet	Bacillus subtilis	250 mg (+/- 10mg) in autoclavedc 125ml peptone media (10g/500ml)	0.00	☑	

Conclusion: The BSA protein drops precipitously in the presence of the Bacillus subtilis.