

The Bucket Company
Microbiological Analysis Report

Project No: **12453**
Date Received: 8/3/2017
Date of Analysis: 9/20/2017

Subject: Bacteria Resistance Test in Hydrophobic or Polymeric Surfaces

Protocol

JIS Z 2801:2010, Japanese Industrial Standard Test for Antimicrobial Activity and Efficacy in Antimicrobial Products, was followed. Specifics of the test method applied to this project are described below.

Overview of Test Protocol

This test method is designed to evaluate (quantitatively) the antimicrobial effectiveness of agent(s) incorporated or bound into or onto mainly flat (two dimensional) hydrophobic or polymeric surfaces.

Specifics of the Test

Sample Identification

Lab ID	Sample ID
12453-1	2%
12453-2	4%
12453-3	6%

Test Organism

Escherichia coli ATCC# 8739

Test Protocol

Submitted samples were inoculated with 0.4 ml of a 0.2% nutrient broth seeded with a standardized culture of the test organism in triplicate. The inoculated samples were covered with an inert film and incubated at $36 \pm 2^\circ\text{C}$ in a humidity chamber for 24 hours. Surviving microorganisms were recovered via elution of the broth inoculum from the test sample into neutralizing broth. Microbial counts of the samples were determined and the percent reduction of microorganisms (treated versus untreated samples at timepoint) was calculated.

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Results

Tables 1 and 2 show the microbial resistance test results.

Conclusions

The Bucket Company sample 2% showed antimicrobial activity against *Escherichia coli* after 24 hours incubation in the JIS Z 2801 test method with 99.95% reduction and an antimicrobial activity value of 3.33. Sample 4% showed 99.97% reduction and an antimicrobial activity value of 3.59. Sample 6% showed 99.99% reduction and an antimicrobial activity value of 4.24. Percent reduction and antimicrobial activity were calculated against the control at the 24 hour timepoint.

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 Project No: **12453**

Test Method: JIS Z 2801:2010

Table 1. Recovered Organisms from controls at Time = 0 hours

Lab Identification	<i>Escherichia coli</i> ATCC# 8739 cells/cm ²			
	Replicate 1	Replicate 2	Replicate 3	Average
Control	1.54 x 10 ⁴	1.64 x 10 ⁴	1.56 x 10 ⁴	1.58 x 10 ⁴

Table 2. Recovered Organisms from controls and samples at Time = 24 hours

Lab Identification	<i>Escherichia coli</i> ATCC# 8739 cells/cm ²				% Reduction	Antimicrobial Activity
	Replicate 1	Replicate 2	Replicate 3	Average		
Control	1.29 x 10 ⁶	7.00 x 10 ⁵	4.93 x 10 ⁵	8.25 x 10 ⁵	n/a	n/a
12453-1	1.06 x 10 ²	20.9	15.9	47.7	99.99%	4.24
12453-2	93.8	5	1.05 x 10 ³	3.83 x 10 ²	99.95%	3.33
12453-3	2.89 x 10 ²	1.25 x 10 ²	2.16 x 10 ²	2.10 x 10 ²	99.97%	3.59