

On the following pages you will find our PolySoft test results for *Determining Resistance of Synthetic Polymetric Materials to Fungi* test carried out by AMS Laboratories Pty Ltd.

The study/test was undertaken to determine the resistance of PolySoft Polyolefin plastomer granule to the growth of fungicide.

The results show the PolySoft test specimen demonstrated resistance to fungi by displaying zero growth after 28 days of incubation which is a fantastic result. PolySoft does not contain suitable nutritive medium to support the growth of fungi and is therefore judged as fungistatic (having an inhibiting effect on the growth of fungi).

Certificate of Analysis
Dated: 03/01/2012

CLIENT: POLY SOFT PTY LTD.
PO Box 4215,
East Gosford NSW, 2250

ATTN: Graham Brown
graham@polysoft.com.au

SAMPLE DESCRIPTION:
Polyolefin Plastomer granules(green sample)

OUR REF: 1116297

ORDER NO: -

DATE RECEIVED: 16/11/11

DATE COMMENCED: 02/12/11

EXAMINATION: *Determining Resistance of Synthetic Polymeric Materials to Fungi*
ASTM G21-09

Test specimens were contaminated with a mixture of fungal spore suspension in the presence of incomplete nutritive medium. If the test specimens contain no nutritive component, the fungi will not proliferate and deterioration of the material will not occur.

METHODS:

1. Test organisms were grown and sporulated for 14 days; each of the fungal spore suspensions were made comprising approx. 2×10^5 - 1×10^6 CFU/mL. Inoculum concentration was confirmed by assay.
2. 50x50mm portions of the sample were cut to prepare the specimens.
3. Approximately 3-6mm thick plates of Nutrient-Salt-Agar were prepared.
4. Specimens were mounted on the surface of the agar facing upwards.
5. Aliquot of 1.0mL of composite spore suspension were spread evenly onto the surface of the agar and test specimen so that the entire surface is moistened with spore suspension. (Figure 1)
6. Positive control was made by inoculating 0.1mL of composite spore suspension onto 3 pieces of sterile 25 x 25mm filter paper to check the viability of the control. (Figure 1A, 2A.)
7. Both, test specimen plates and positive control plates were incubated in an upright position in a 28-30°C incubator. Positive control plates were incubated for 7 days and test plates were incubated for 28 days.
8. The test samples were regularly monitored for the fungal growth up to 28 days.
9. Obvious fungal growth was observed on the control plates after 7th day of incubation.
10. Fungal growth was assessed in accordance with Table 1. (Taken from G21-09 criteria)

CONDITIONS:

Test organisms:	1. <i>Aspergillus brasiliensis</i>	ATCC 9642
	2. <i>Aureobasidium pullulans</i>	ATCC 15233
	3. <i>Chaetomium globosum</i>	ATCC 6205
	4. <i>Trichoderma virens</i>	ATCC 9645
	5. <i>Penicillium funiculosum</i>	ATCC 11793

Inoculum Level: Approx. 2×10^5 - 1×10^6 Colony Forming Units (CFU)/mL

Incubation period: Maximum 28 days (Terminate at the appearance of growth)

Incubation Temperature: 28 - 30°C

OUR REF: 1116297 cont'd

RESULTS:

Table 1: Assessment of fungal growth on the control and test sample

REPLICATE	Observed growth on specimens (Rating)	
	Control <i>(25 x 25 mm sterile filter paper)</i>	Sample <i>(50 x 50 mm test specimen)</i>
Replicate 1	Heavy growth (60% to complete coverage) – rating 4	No apparent growth– rating 0
Replicate 2	Heavy growth (60% to complete coverage) – rating 4	No apparent growth– rating 0
Replicate 3	Heavy growth (60% to complete coverage) – rating 4	No apparent growth– rating 0
Observation of fungal growth on Nutrient-Salt Agar	Scattered growths of sporulated fungal colonies were observed on the surrounding medium of three replicate plates (test plates and control plates).	

Table 2: Guide to rating of fungal growth on specimen

Observed growth on specimens <i>(sporulating or non sporulating , or both)</i>	Rating
None (no apparent growth under the microscope)	0
Traces of growth – less than 10%	1
Light growth (10-30%)	2
Medium growth (30-60%)	3
Heavy growth (60% to complete coverage)	4

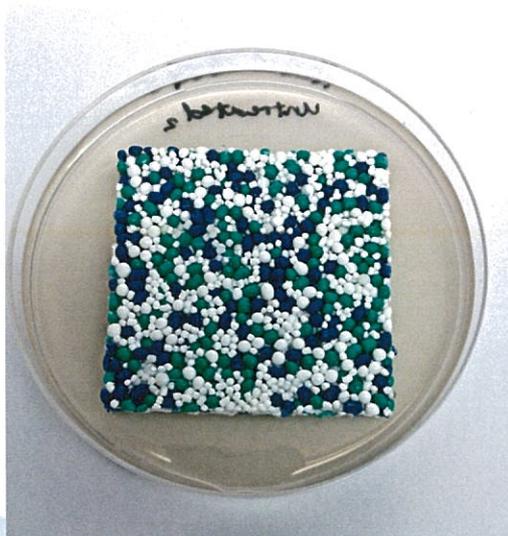
OUR REF: 1116297 cont'd

Table 3: Challenge inoculum spore counts

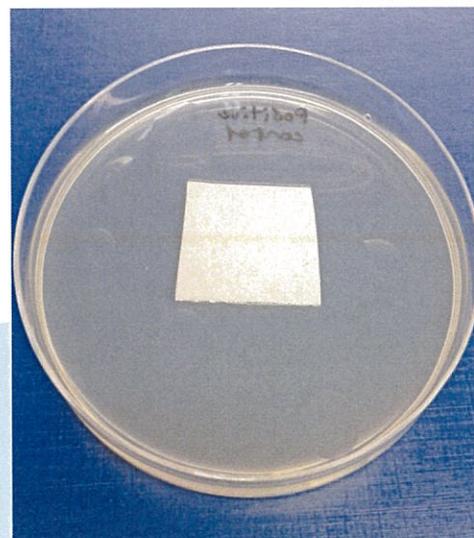
Challenge organisms	Inoculum count (CFU/mL)	volume of inoculum used
<i>Aspergillus brasiliensis</i>	3.5×10^5	3mL
<i>Aureobasidium pullulans</i>	3.9×10^5	3mL
<i>Chaetomium globosum</i>	5.1×10^5	3mL
<i>Trichoderma virens</i>	2.4×10^5	3mL
<i>Penicillium funiculosum</i>	5.0×10^5	3mL
Composite spore count	2.5×10^5	

Figure 1: Positive control and Test specimen inoculated with composite spore suspension.

Sample A



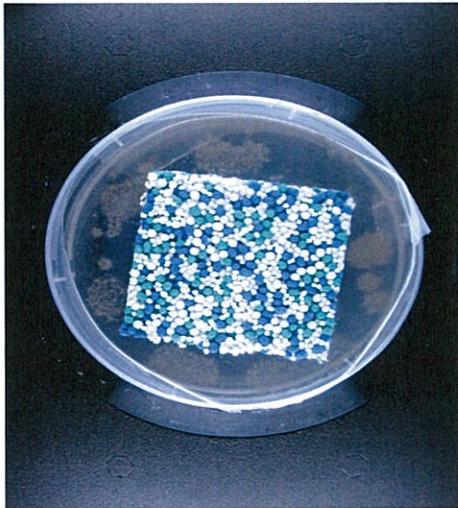
Positive control



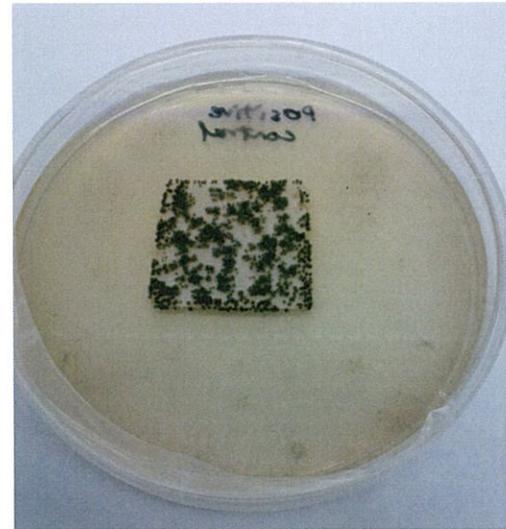
OUR REF: 1110294 cont'd

Figure 2: Positive control and test specimen inoculated with composite spore suspension after 28 days of incubation.

Sample A



Positive control



COMMENTS:

The study was undertaken to determine the resistance of Polyolefin plastomer granules to fungal attack. The test specimen demonstrated resistance to fungi by showing zero apparent growth after 28 days of incubation upon inoculation with approx. 2.5×10^5 CFU/mL of composite fungal spore suspension. The specimen does not contain suitable nutritive medium to support the growth of fungi and is therefore judged as fungistatic.

Signed:



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Sterile Products Testing Manager