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ANALYSIS OF ANTIMICROBIAL ACTIVITY OF SEMCO AVRON 46™ COATING DESIGNED FOR USE IN HVAC SYSTEMS

ANTIMICROBIAL COATING
AVRON 46

CREATED FOR FLÄKTGROUP SEMCO BY THE DEPARTMENT OF BIOLOGY ENVIRONMENTAL RESEARCH CENTER AT GEORGIA STATE UNIVERSITY

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Analysis of Antimicrobial Activity of SEMCO AVRON46 Coating Designed for Use in HVAC Systems

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Executive Summary

A proprietary antimicrobial coating developed by SEMCO Inc. and marketed under the trade name AVRON46™ was challenged with common bacterial culture Pseudomonas aeruginosa to determine its antimicrobial effectiveness. Testing was also completed to assess the impact of water immersion, thought to present the most adverse environment impacting the effective life of the product during normal operation.

The results of the testing suggest that the AVRON46™ coating offers an effective antimicrobial coating for ductwork and other related products. The antimicrobial effectiveness was found to be in excess of 95% for the organism investigated. This level of effectiveness was not impacted by simulated life cycle testing which involved 13 days of immersion in water.

Background:

The increased awareness and concerns regarding microbial (mold and bacteria) infestation of air conditioning systems, particularly air distribution systems, has highlighted the need for anti-microbial coatings that could be applied to the interior of HVAC ductwork. SEMCO Inc. has developed an effective anti-microbial coating that is applied onto the ductwork, after fabrication, which utilizes an active ingredient registered by the EPA for such use.

Georgia State University has performed a series of tests designed to assess the anti-microbial effectiveness of this coating using a common, relatively resistant form of the bacterial culture *Pseudomonas aeruginosa.*

The first series of tests were designed to assess the anti-microbial effectiveness of the SEMCO coating. The second series of tests were designed to evaluate the antimicrobial activity of the coating after repeated water washing to simulate the effectiveness of the product over time. A summary of these test procedures and final results are provided below:

Test 1:

Objective: To document the antimicrobial activity and effectiveness of a proprietary surface coating applied to base materials used by SEMCO to fabricate its spiral ductwork, duct fittings, panels, acoustical enclosures and other products.

Experimental Approach: Cell suspension of *P. aeruginosa* GSU-3 was appropriately diluted in phosphate buffered saline with 0.05% Tween-80 (PBST) to achieve approximately 10^3 and 10^4 cells/ml, and 1.0 ml of this suspension was combined with 20 to 25 ml of PBST. The suspension was filtered through a 0.22 μm filter. Membranes containing cells were placed on the surface of metal coupons and were held for 4 h. These membranes then were transferred individually to a cellulose pad saturated with 1.8

ml growth medium in a Petri dish. Developing colonies from cells on the membranes were enumerated over a 24-hour incubation period at 35 °C.

Both metal with the antimicrobial coating and the same base metal without the antimicrobial coating were analyzed. The samples were examined microscopically following various culture periods to determine the number of live organisms remaining.

Procedure:

Bacterial Inoculum Preparations & Harvests

1. Culture *P. aeruginosa* GSU-3 on TSB at 35^0C for 18-19 hours
2. Harvest in Phosphate buffered saline with Tween-80 (PBST)
3. Adjust cell density and obtain optical density reading using a spectrophotometer. Approximate spectrophotometer readings for ~$2x10^8$ cfu/mL bacterial cultures are 0.10
4. Dilute this suspension to 10^3 and 10^4 cfu/mL by transferring 0.1 mL suspension to tube A with 10 mL PBST, then transferring 0.1 mL suspension from tube A to tube B with 20 mL PBST (10^4 cfu/mL). Remove 1.0 mL from tube B to tube C with 9.0 mL PBST to obtain a cell density at 10^3 cfu/mL.

Filter bacteria onto membrane

1. Sterile 0.22 μm filters were placed in the filter holders.
   a) 20 to 25 ml of sterile PBST was added to each unit.
   b) 1.0 ml of cell suspension was added to the buffer with the aid of a 1000 μl automatic pipette.
   c) Turn on the vacuum and the sample was filtered

Exposure and determination of recovery

1. Using sterile forceps, the membrane filters were transferred to the surface of metal coupons and held for 4 hours. 0.5 mL PBST was added to each filter to help attachment to metal surface.

2. Controls

Control A – recovery of inoculum: One membrane filter with bacteria was transferred to a 60 mm Petri dish with cellulose pad containing 1.8 ml of TSB.

Control B – recovery of inoculum after 4 h: One membrane filter with bacteria was transferred to a 100 mm Petri dish and held for 4 h, then it was transferred to a 60 mm Petri dish with cellulose pad containing 1.8 ml of TSB.

Control C – coated agents released from metal surface: One membrane previously soaked with PBST was placed on a metal coupon for 4 h, another membrane filter

with bacteria was transferred to a 100 mm Petri dish and held for 4 h. Both membranes were transferred to a 60 mm Petri dish with cellulose pad containing 1.8 ml of TSB, with the membrane containing bacteria on the top.

3. At the end of 4-h exposure, the membrane filters were transferred to Petri dishes containing sterile pads which had previously been saturated with 1.8 ml of TSB.

4. Dishes were then incubated at 35 °C.

5. Dishes were examined over a 48-hour period to ascertain cell recovery and the bacterial colonies on the sample membranes were analyzed microscopically, counted and the number recorded.

Results:

1. Sample description

Sample Number	Control	#46494
	No coating	SEMCO AVRON46™ antimicrobial coating
Surface pH		6
Thickness (mil)		0.5
Coating material		proprietary antimicrobial coating
Base material	uncoated metal	

2. Recovery of organism

Inoculum Check

Control	CFUs in 0.1ml of 10^x dilution				Inoculum /membrane
	x = 0		x = -1		
CFUs	**288**	**239**	**38**	**35**	**3.7E+03**

Recovered CFUs after 4 h exposure

Sample #	Control	#46494	antimicrobial effectiveness
Recovered CFUs	TNTC	105	> 97%
	TNTC	301	> 92%
Average	TNTC	203	>95%

Note 1: - Control: Uncoated metal only

Note 2: - TNTC reflects too numerous to count

Summary

Repeated tests comparing the SEMCO AVRON46™ antimicrobial coating with the control confirmed the antimicrobial effectiveness averaging in excess of approximately 95%.

Test 2:

Objective: To compare antimicrobial activity of the SEMCO AVRON46™ antimicrobial coating against *Pseudomonas* (GSU3) after repeated submersion in water to evaluate the anticipated effective life of the product when applied to duct systems.

Experimental Approach: Cell suspension of *P. aeruginosa* GSU-3 was appropriately diluted in phosphate buffered saline with 0.05% Tween-80 (PBST) to achieve approximately 10^3 cells/ml, and 1.0 ml of this suspension was combined with 20 to 25 ml of phosphate buffer. The suspension was filtered through a 0.22 μm filter. Filter membranes containing cells were placed on the surface of metal coupons and were held for 4 h. These membranes were transferred individually to a cellulose pad saturated with 2.0 ml growth medium (TSB) in a Petri dish. Developing colonies from cells on the membranes were enumerated over a 48-hour incubation period at 35 °C.

Procedure:

Test A.

Coupon preparation --- (six day exposure)

2 coupons were immersed in sterile distilled water overnight and air-dried.

1. 2 coupons that had been used to challenge GSU 3 on 3/4/04 were immersed in sterile distilled water 6 days and air-dried.

2. 2 coupons were not immersed in water.

3. Exposed to $5.0x10^3$ cells/coupon

Bacterial Inoculum Preparations & Harvests

1. Culture *P. aeruginosa* GSU-3 on TSB at 35^0C for 18-19 hours
   (in this test on 4/5/04, GSU-3 grown on TSA plate for 3 days)

2. Harvest in Phosphate buffered saline (PBS)

3. Adjust cell density and obtain optical density reading using a spectrophotometer. Approximate spectrophotometer readings for ~$2x10^8$ cfu/mL bacterial cultures are 0.10

4. Dilute this suspension to $5x10^3$ cfu/mL by transferring 0.1 mL suspension to tube A with 10 mL PBS, then transferring 0.1 mL suspension from tube A to tube B with 10 mL PBST ($2x10^4$ cfu/mL). Remove 2.0 mL from tube B to tube C with 6.0 mL PBS to obtain a cell density at $5x10^3$ cfu/mL.

Filter bacteria onto membrane

1. Sterile 0.22 μm filters were placed in the filter holders.

   a) 20 to 25 ml of sterile PBST was added to each unit.
   b) 1.0 ml of cell suspension was added to the buffer with the aid of a 1000 μl automatic pipette.
   c) Turn on the vaccum and filter the sample

Exposure and determination of recovery

1. Using sterile forceps, the membrane filters were transferred to the surface of antimicrobial coated metal coupons and held for 4 hours. Approximately 0.1 mL of sterile water was added to each filter to help attachment to metal surface.

2. Control - Aluminum foil coupons without any coating were used

3. At the end of 4-h exposure, the membrane filters were transferred to Petri dishes containing sterile pads which had previously been saturated with 2.0 ml of TSB.

4. The inoculum suspension was verified by spread plate count (TSA)

5. Dishes were then incubated at 35 °C.

6. Dishes were examined over a 48-hour period to ascertain cell recovery.

   a) Dishes with no cell recovery (no growth) were recorded as (0)
   b) Dishes with discrete colonies numbering < 150 per membrane were counted and the number recorded.
   c) Dishes with discrete colonies numbering > 150 per membrane were counted or estimated and the number recorded.
   d) Dishes with confluent growth were recorded as (Too Numerous To Count, TNTC)

Results

1. Sample description

Sample Number	Control	#46494-M-1	#46494-M-2	#46494-M-3
	No coating	Antimicrobial Binder System		
Surface pH		6		
Thickness (mil)		3	3	3
Coating material		Modified	Modified	Modified
Immersed in water	No	6 days	1 day	No
Base material	uncoated metal			

2. Recovery of organism

Inoculum Check

Control	CFUs in 0.1ml of 10^x dilution				Inoculum /membrane
	x = 0		x = -1		
Filter	245	219	31	22	2.65E+03
Agar plate	378	365	47	38	4.25E+03

Recovered CFUs after 4 h exposure

Sample #	- Control	#46494-M-1	#46494-M-2	#46494-M-3
Water rinse?	NO	6 days	1 day	No
Recovered CFUs	TNTC	138	129	93
	TNTC	174	254	307
Average	TNTC	156	192	200

Note 1: - Control: uncoated metal only

Note 2: TNTC - Too Numerous To Count

Test B.

<u>Coupon preparation</u> --- (seven additional days of exposure)

1. The 2 coupons that had been used to challenge GSU 3 on 4/5 and 4/7/04 (test A) were once again used as positive control.

2. 2 coupons that have been used to challenge GSU 3 on 3/4/04, and then been immersed in sterile distilled water for 3 days and air-dried (4/2/04 – 4/5/04), and again been used to challenge GSU 3 on 4/5 and 4/6, respectively, were immersed in sterile distilled water for 7 days (4/13-4/20) and air-dried. A total immersion of 13 days was used to challenge these samples.

3. 2 non-coated metal coupons were used as control.

4. All other procedural steps were followed as previously listed for Test B

Results

1. Sample description

Sample Number	Control	#46494-M-1*	#46494-M-3**
	No coating	Antimicrobial Binder System	
Surface pH		6	
Thickness (mil)		3	3
Coating material		Modified	Modified
Immersed in water	No	13 days	No
Base material	uncoated metal		

* These coupons have been used to challenge GSU3 on 3/4/04, and then been immersed in sterile distill water for 3 days and air-dried (4/2/04 – 4/5/04), and again been used to challenge GSU 3 on 4/5 and 4/6, respectively, Prior to this 7 day test.

**These coupons have been used to challenge GSU3 on 4/5 and 4/6/04, respectively.

2. Recovery of organism

Inoculum Check

Control	CFUs in 0.1ml of 10^x dilution				Inoculum /membrane
	x = 0		x = -1		
Filter	TNTC	TNTC	195	179	1.87E+04
Agar plate	TNTC	TNTC	219	174	1.97E+04

Recovered CFUs after 4 h exposure

Sample #	- Control	#46494-M-1	#46494-M-3
Water rinse?	NO	7 days	No
Recovered CFUs	TNTC	127	312
	TNTC	208	295
Average	TNTC	168	304

Note 1: Control: uncoated metal only

Note 2: - TNTC - Too Numerous To Count

Summary

Coating #46494 made with the SEMCO AVRON46™ antimicrobial coating showed equivalent inhibitory activity against *P. aeruginosa* after being immersed in water for 1 day, 3 days, 6 days and 13 days. No reduction in the antimicrobial effectiveness was observed over the 13 days of immersion in water. This extreme life cycle test (the coating when deposited on the internal surface of a duct would seldom be immersed in liquid water) in addition to the data published by the manufacturer of the active ingredient would suggest that the active life of this product should be extended.

According to SEMCO, as applied the product comprises approximately an 80,000 ppm concentration of the active ingredient within the AVRON46™ antimicrobial coating. EPA testing has confirmed that a concentration of approximately 1 ppm is effective against most common fungi. Based on the EPA environmental half-life data SEMCO has projected a conservative worse case effective life of more than 6 years and a likely effective life of as much as 20 years.