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slowly added, producing a clear solution. After sitting at 50° C. for 24 hours, the solution was analyzed to determine the presence of a peracid.

To determine the presence of a peracid, a kinetic iodometric titration similar to the method disclosed in Sully and Williams ("The Analysis of Per-Acids and Hydrogen Peroxide," *The Analyst*, 87:1037, p. 653 (August 1962)) was used. This method has demonstrated a lower detection limit of about 0.3 ppm for POAA. Given the molecular weight ratio of POAA to the perspective percarboxylic acid of PC-48, the detection limit was estimated to be about 1.4 ppm ( $3.93 \times 10^{-6}$  M). No peracid formation was observed. This is equivalent to a percarboxylic acid formation constant (K<sub>eq</sub>) less than 0.002, suggesting substantially no peracid was formed.

Alternatively, formation of the peracid was determined using <sup>13</sup>C NMR (D<sub>2</sub>O). Using this technique, no carbonyl resonance signal from the peracid was observed.

Other α-sulfonated fatty acid sources such as Alpha-Step PC-48 and Alpha-Step BSS-45 were also reacted with H<sub>2</sub>O<sub>2</sub> in a similar manner, and in both cases, no corresponding peracids were detected.

Non-α-sulfonated fatty acids were also tested to determine the likelihood of peracid formation. For the sulfonated oleic acid discussed above, the measured formation constant was 1.42. The sulfonated undecenoic acid was collected as a stable solid powder, so the formation constant was not measured. Although the formation constant of the peracid of sulfonated oleic acid is significantly lower than that of the most common commercialized peracid, peroxyacetic acid (K<sub>eq</sub>=2.70), it is still high enough to make practical yields.

Overall, without wishing to be bound by any particular theory, it is thought that the α-sulfo group prohibits the oxidation and/or perhydrolysis of the carboxylic acid group by H<sub>2</sub>O<sub>2</sub> to the corresponding peracid. This may in part be due to its strong electron withdrawing effects.

### Example 9

#### Clean in Place Sanitizing Compositions

A study was run to determine the efficacy of compositions of the present invention as sanitizers used in a clean in place cleaning method. A composition including about 5.85 wt % of the sulfonated peroleic acid product, and about 11.6% hydrogen peroxide, about 1 wt % of a chelating agent, about 12.75 wt % of H<sub>2</sub>SO<sub>4</sub>, about 13.6 wt % NAS-FAL (sodium octane sulfonate), and about 1.5 wt % of SXS (commercially available from the Stepan Company) was prepared. Synthetic hard water was used to dilute the test composition to the desired peracid concentration. The peracid was tested at concentrations of 1000 ppm, 750 ppm and 500 ppm. The pH of the use solutions were as follows:

Concentration of Peracid in Use Solution	pH
500 ppm peracid	1.65
750 ppm	1.46
1000 ppm	1.38

The use solutions were tested against *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442. The organic soil used was 5% Fetal Bovine Serum. The exposure time of the test was 5 minutes at a temperature of 20±1° C. A neutralizer screen was also performed as part of the testing to verify that the neutralizer adequately neutralized the product and was not detrimental to the tested organ-

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isms. The plates were incubated at 35° C. for 48 hours with the test systems prior to exposure to the peracids. The results are shown in the table below.

TABLE 16

Test Substance	# Negative Tubes/# Carriers Tested	
Staphylococcus aureus ATCC 6538		
1000 ppm Peracid Composition	60/60	
Pseudomonas aeruginosa ATCC 15442		
1000 ppm Peracid Composition	60/60	
Test Controls		
Control	Test System	Results
Negative Carrier		1 negative of 1 tested
Positive Carrier	Staphylococcus aureus ATCC 6538	1 positive of 1 tested
Positive Carrier	Pseudomonas aeruginosa ATCC 15442	1 positive of 1 tested
Organic Soil		1 negative of 1 tested
Neutralization (1000 ppm)	Staphylococcus aureus ATCC 6538	6 positive of 6 tested
Neutralization (1000 ppm)	Pseudomonas aeruginosa ATCC 15442	6 positive of 6 tested
Culture Enumeration	Staphylococcus aureus ATCC 6538	9.0 × 10 <sup>8</sup> CFU/mL
Culture Enumeration	Pseudomonas aeruginosa ATCC 15442	1.0 × 10 <sup>9</sup> CFU/mL
Carrier Enumeration	Staphylococcus aureus ATCC 6538	1.0 × 10 <sup>6</sup> CFU/mL
Carrier Enumeration	Pseudomonas aeruginosa ATCC 15442	1.0 × 10 <sup>7</sup> CFU/Carrier
Carrier Enumeration	Pseudomonas aeruginosa ATCC 15442	2.3 × 10 <sup>6</sup> CFU/mL
Carrier Enumeration	Pseudomonas aeruginosa ATCC 15442	2.3 × 10 <sup>7</sup> CFU/Carrier

Test Substance	# Negative Tubes/# Carriers Tested	
Staphylococcus aureus ATCC 6538		
500 ppm Peracid Composition	59/60	
750 ppm Peracid Composition	60/60	
Pseudomonas aeruginosa ATCC 15442		
500 ppm Peracid Composition	58/60	
750 ppm Peracid Composition	60/60	
Test Controls		
Control	Test System	Results
Negative Carrier		1 negative of 1 tested
Positive Carrier	Staphylococcus aureus ATCC 6538	1 positive of 1 tested
Positive Carrier	Pseudomonas aeruginosa ATCC 15442	1 positive of 1 tested
Organic Soil		1 negative of 1 tested
Neutralization	Staphylococcus aureus ATCC 6538	3 positive of 3 tested
Neutralization	Pseudomonas aeruginosa ATCC 15442	3 positive of 3 tested
Culture Enumeration	Staphylococcus aureus ATCC 6538	1.0 × 10 <sup>9</sup> CFU/mL
Culture Enumeration	Pseudomonas aeruginosa ATCC 15442	1.0 × 10 <sup>9</sup> CFU/mL
Carrier Enumeration	Staphylococcus aureus ATCC 6538	7.2 × 10 <sup>5</sup> CFU/mL
Carrier Enumeration	Staphylococcus aureus ATCC 6538	7.2 × 10 <sup>6</sup> CFU/Carrier
Carrier Enumeration	Pseudomonas aeruginosa ATCC 15442	2.0 × 10 <sup>6</sup> CFU/mL
Carrier Enumeration	Pseudomonas aeruginosa ATCC 15442	2.0 × 10 <sup>7</sup> CFU/Carrier

As can be seen from these results, the use solutions tested were effective disinfectants against both *Staphylococcus aureus*, and *Pseudomonas aeruginosa* at the concentrations tested.

Another study was run to determine the sanitizing efficacy of the test solution against *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 11229 after a 30 second exposure time. For this experiment the solutions were diluted