

Title: Biological Validation of a Chemical Effluent Decontamination System

Supplemental Materials

Supplemental Methods and Results

Evaluation of non-pesticide labeled sodium hypochlorite in the chemical EDS.

Biological validation of the chemical EDS was conducted using a concentrated stock of sodium hypochlorite (12.5%, Buckman's Inc.). The chemical EDS treatment tanks were filled with potable water and sodium hypochlorite (stock, 12.5%) was added following the chemical EDS operating instructions to achieve the free chlorine concentration based on conductivity. For this biovalidation run, the free chlorine concentration was not measured in the laboratory. Subsequent biovalidation runs were conducted adding potable water and 12.5% sodium hypochlorite based on volume-concentration calculations to achieve a free chlorine concentration of 8000 ppm. Laboratory prepared spore packets were suspended in the treatment tank at the top, middle, and bottom. Following the 2 hour contact time, the spore packets were removed and used to inoculate broth as described in the Materials and Methods. Test results are presented in Table S1. The data presented as the presence of bacterial growth following culture in broth. No growth (-) indicates the inactivation of the spores. Growth (+) indicates a failure to inactivate spores. Inconsistent inactivation was observed with this product by this method.

Evaluation of pesticide product labeled sodium hypochlorite in the laboratory.

EPA pesticide product labeled sodium hypochlorite solutions were purchased from two sources (Brenntag and Univar USA, Inc.). These products were diluted in the laboratory and free chlorine concentrations determined using the Hanna Free Chlorine meter as described in the Materials and Methods. Laboratory-prepared spore packets were suspended for a 2 hour contact time and subsequently evaluated for inactivation of spores as described in the Materials and Methods. Clorox[®] germicidal bleach was included as a positive control. Test results are presented in Table S2. Culture growth was detected in all samples using the EPA pesticide product labeled sodium hypochlorite under the conditions tested. The only product that provided complete inactivation of 6 logs of spores was Clorox[®] germicidal bleach at free chlorine concentrations ≥ 5400 ppm. The Brenntag product did not inactivate spores at free chlorine concentrations of ≤ 9000 ppm and a 2 hour contact time.

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Supplemental Tables

Table S1. Testing Results using Sodium Hypochlorite (12.5%, Non-Pesticide Labeled) in the Chemical EDS.

Source	PPM	Culture Growth		
		Top	Middle	Bottom
Buckman's Inc.	ND	-	+	+
	8000	-	-	-
	8000	-	-	+
	8000	-	-	-

Table S2. Testing Results using Pesticide Labeled Bleach Products in the Laboratory.

Product (Source)	PPM	pH	Culture Growth
Sodium hypochlorite, 12.5% (Brenntag)	3000	11.7	+
	6000	12.07	+
	9000	12.1	+
LiquiChlor, 12.5% solution (Univar USA)	5800	ND	+
Clorox® Germicidal Bleach (Clorox)	5400	10.72	-
	6600	10.92	-
	7400	11.1	-

ND, not determined