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The Efficacy of Polyacrylamide and Related Compounds to Remove Microorganisms and Nutrients from Animal Wastewater

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ABSTRACT

Polyacrylamide (PAM) is an effective flocculent and we hypothesized that it would reduce transport of microorganisms and nutrients in water flowing over and through soil. We measured nutrients, total coliform bacteria, fecal coliform bacteria, fecal streptococci, active and total fungi, and active and total bacteria in water leached through cattle, fish, and swine manure, PAM, PAM + Al(SO$_4$)$_3$, or PAM + CaO mixtures, and 25-cm sand columns. In the laboratory study, PAM, PAM + Al(SO$_4$)$_3$, and PAM + CaO treatments reduced total coliform bacteria, fecal coliform bacteria, and fecal streptococci by 10- to 1000-fold in water leached through sand columns. Polyacrylamide + Al(SO$_4$)$_3$ and PAM + CaO treatments reduced the concentration of NH$_4$, total P, and PO$_4$ by 20 to 60% in leachate when cattle and swine manure were applied. In a field study, after water flowed over all three manure sources and then PAM, PAM + Al(SO$_4$)$_3$, or PAM + CaO in furrows, total coliform bacteria, fecal coliform bacteria, and fecal streptococci were reduced by 10- to 1000-fold in water flowing 1 and 27 m downstream of the treatments compared with the control treatment. When water flowed over cattle manure and then PAM + Al(SO$_4$)$_3$ or PAM + CaO, PO$_4$ concentration was reduced by 50 to 70% and total P concentration was reduced by 0 to 75% in water 27 m downstream of the treatments compared with the PAM alone treatment. Polyacrylamide + Al(SO$_4$)$_3$ and PAM + CaO should be able to reduce the numbers of enteric microorganisms and nutrient concentration in animal wastewater running off animal confinement areas, reducing the amount of these pollutants entering surface and ground waters.

THE number and size of animal production operations in the USA has steadily increased for several decades (USEPA, 1998). Large volumes of animal waste are generated as wastewater from urine and animal washing and as semisolid or solid manure. Pollution of surface flow and ground water from the application of animal waste has been well documented (Mallin et al., 1997; Mawdsley et al., 1995; Khaeel et al., 1980). Liquid-waste discharge onto soil initiates solute and microbe movement into the soil that follows natural ground water drainage patterns and may contaminate adjoining surface water. These same bodies of water are often used for sources of drinking water and/or for recreational activities. Human contact with recreational waters containing intestinal pathogens is an effective method to spread disease. Therefore, it is critical to maintain the quality of our lakes and streams by keeping them free of intestinal pathogens and excess nutrients.

Management practices that are currently used to mitigate the input of pollutants from animal waste to surface and ground water include control of animal numbers (Gary et al., 1985; Jawson et al., 1982), control of animal diet (Diez-Gonzalez et al., 1998), constructed wetlands, and riparian filterstrips (Coyne et al., 1998, 1995; Walker et al., 1990; Young et al., 1980). However, there are several problems with vegetative systems: (i) the establishment of vegetation in wetlands or riparian areas can take from months to years to establish, (ii) vegetative systems are not effective when vegetation is not growing (winter months) and can become nutrient sources rather than nutrient sinks, (iii) riparian filterstrips or constructed wetlands are effective for only small quantities of runoff (relatively infrequent or low-intensity runoff events) since continuous application can quickly overload the system and nullify the ability of the vegetation to take up nutrients, and (iv) vegetative systems cannot be transported to the site of a waste spill or runoff area. Therefore, even when best management practices are used, animal production operations can sometimes contribute large amounts of nutrients and enteric microorganisms to watercourses.

Polyacrylamide has been used in furrow-irrigated agriculture for erosion control and increased infiltration (Lentz et al., 1992; Lentz and Sojka, 1994; Sojka et al., 1998a,b). Lentz et al. (1998) and Lentz and Sojka (1994) reported that PAM treatment reduced sediment loss rate over time with improvement of the runoff water quality parameters ortho-P, total P, nitrate, and biological oxygen demand. Runoff sediment reduction averaged 94% and infiltration increased 15% in a series of studies conducted over 3 yr. Subsequent studies have further documented the capacity of PAM treatment of furrow irrigation water to reduce sediments, nutrients, and pesticides in irrigation water (Agassi et al., 1995; Bahr and Stieber, 1996; Singh et al., 1996; Sojka et al., 1998a,b).

Recently, Sojka and Entry (2000) also documented large reductions in microorganisms in water flowing in 1- to 30-m furrows in irrigated crop land after applying 15 to 30 g of PAM directly to the soil in the first 1.0 m of the furrow. Efficacious treatment for sediment reduction has been possible in most instances with net PAM application rates of only about 1 kg ha^{-1} (1 lb acre^{-1}) applied at a concentration of 10 μg PAM mL^{-1} in the first 1.0 m of the furrow. The objective of this study was to: (i) determine the efficacy of PAM, PAM + Al(SO$_4$)$_3$, and PAM + CaO to remove coliform bacteria, active bacteria and fungi, and nutrients from three types of animal wastewater by sampling leachate draining from sand columns and (ii) determine the efficacy of PAM, PAM + Al(SO$_4$)$_3$, and PAM + CaO to remove coliform bacteria, active bacteria and fungi, and nutrients from three types of animal wastewater in surface runoff in silt loam textured soils.

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Abbreviations: AMD, acryamide monomer; PAM, polyacrylamide.

Table 1. Efficacy of polyacrylamide (PAM), PAM + Al (SO₄)₃, and PAM + CaO to remove total and fecal coliform bacteria, fecal streptococci, active and total bacteria, and active and total fungi through columns filled with clean sand.†

Manure	Treatment	Total coliforms	Fecal coliforms	Fecal streptococci	Bacteria		Fungi	
					Active	Total	Active	Total
		bacteria per 100 mL water			µg C per mL water			
Cattle	source material	3.05×10^6a	2.20×10^6a	1.17×10^5a	759a	4727a	317a	283a
	control	2.23×10^5b	1.50×10^5b	4.50×10^4b	88b	697a	0b	0c
	PAM	2.45×10^4c	1.81×10^4c	3.75×10^2d	77b	797b	0b	30b
	PAM + Al (SO₄)₃	1.10×10^4c	2.43×10^3d	7.75×10^2cd	33c	273c	4b	8c
	PAM + CaO	2.75×10^2d	6.72×10^3e	4.75×10^2d	13c	33d	0b	5c
Fish	source material	2.87×10^6a	1.70×10^6a	5.13×10^4a	295a	1995a	0d	0b
	control	1.87×10^5b	1.00×10^5b	2.65×10^5b	97b	532b	1a	1b
	PAM	6.45×10^4c	2.80×10^4c	1.63×10^4b	43c	18d	2a	48a
	PAM + Al (SO₄)₃	1.02×10^4c	9.50×10^3cd	2.50×10^1c	30c	25d	1a	7b
	PAM + CaO	3.25×10^4c	1.43×10^4d	1.80×10^5b	10c	15d	0b	0b
Swine	source material	6.27×10^8a	1.07×10^8a	1.19×10^8a	387a	5850a	43b	43a
	control	2.73×10^7b	2.18×10^7b	1.70×10^6b	330a	5365a	20b	4b
	PAM	2.75×10^6c	1.35×10^6c	9.00×10^5c	195ab	267c	0c	0b
	PAM + Al (SO₄)₃	9.60×10^5cd	8.85×10^5c	1.40×10^5c	20c	117c	10b	1b
	PAM + CaO	3.75×10^4e	3.30×10^5b	1.34×10^5e	13c	190c	0c	0b

† In each column, values followed by the same letter are not significantly different as determined by the least square means test ($P \leq 0.05$), $n = 16$.

MATERIALS AND METHODS

Column Study

Experimental Design

The column study was arranged in a completely random design (Kirk, 1982) consisting of four treatments: (i) PAM, (ii) PAM + Al(SO₄)₃, (iii) PAM + CaO, and (iv) a control having no amendment with three different manure types (cattle, fish, and swine). Forty-eight columns were prepared for the study. There were four replications (columns) with four observations (four samples taken from each leachate sample as sampling error) for each treatment × manure combination (four treatments × three manure types × four replications × four observations per column).

Waste Material

Cattle (*Bos taurus*) and swine (*Sus scrofa*) manure was fine-grained material obtained from animals on local farms given feed rations with dietary supplements. Fish manure was obtained from rainbow trout (*Oncorhynchus mykiss*) raised at the Clear Springs Hatchery, Haggerman, ID, which were fed a blended diet of 40% fish meal, 30% plant protein–carbohydrate, 15% fish oil, 10% nonfish protein, and 5%

binders, vitamins and minerals. Concentrations of total coliform bacteria, fecal coliform bacteria, fecal streptococci, active and total fungi, and active and total bacteria for each manure type are listed as source material in Table 1 for the column study and Table 3 for the surface flow study. Nutrient concentrations in each manure type are listed as source material in Table 2 for the column study and Tables 4, 5, and 6 for the surface flow study.

Column Description

A 2.00-mm wire screen was cut into squares (125 × 125 mm) and secured to the bottom of each 10.0-cm-diameter × 40-cm-long polyvinyl chloride cylinder (Fig. 1). A 12-cm-diameter funnel was placed below each column and the apparatus was secured to a ring stand. Three kilograms of sand was placed in each column (columns were filled to 30 cm) leaving a 10-cm space at the top of each column. Sand in columns was a combination of the following size grains by weight: 0.28% ≥ 3.35 mm, 4.86% ≥ 2.362 mm, 21.64% ≥ 1.379 mm, 11.05% ≥ 1.000 mm, 28.9% ≥ 0.600 mm, 24.26% ≥ 0.246 mm, 6.60% ≥ 0.149 mm, 1.00% ≥ 0.125 mm, and 1.40% > 0.125 mm. Sand in columns was loosely packed and then repeatedly washed with distilled water to flush microorganisms

Table 2. Efficacy of polyacrylamide (PAM), PAM + Al (SO₄)₃, and PAM + CaO to filter nutrients through columns filled with clean sand.†

Manure	Treatment	NO₃	NH₄	Total P	PO₄	K	Ca	Mg
		µg element per mL water						
Cattle	source material	104.83a	490.07a	2630.00a	1596.00a	1630.00a	711.70a	1981.00a
	control	0.07e	1.94c	112.16b	3.52c	22.51c	47.12cd	12.00c
	PAM	0.12e	0.33d	66.86b	1.85d	9.28d	87.89c	2.84d
	PAM + Al (SO₄)₃	0.02e	0.61d	42.47c	1.56d	1.66e	3.66f	3.22d
	PAM + CaO	0.15e	0.44d	8.97d	0.18e	2.73e	2.86f	1.68d
Fish	source material	5.97c	271.50b	1309.60a	1465.30a	86.65b	1428.37b	54.68b
	control	0.04e	0.00e	35.74c	1.29d	1.82e	5.48f	6.89cd
	PAM	0.04e	0.00e	39.68c	1.00d	2.57e	10.49ef	5.54d
	PAM + Al (SO₄)₃	0.03e	0.04e	37.23c	1.70d	3.70e	14.00e	10.37c
	PAM + CaO	0.01e	0.00e	35.38c	1.48d	2.62e	6.70f	4.00d
Swine	source material	10.17b	946.09a	3359.33a	2940.53a	875.06a	69.02c	37.65b
	control	17.94b	0.61d	70.91bc	5.60b	8.19d	126.12c	24.01b
	PAM	5.26c	0.41d	66.53bc	2.04c	2.43e	25.44d	4.99cd
	PAM + Al (SO₄)₃	6.42c	0.20d	44.15c	3.35c	2.82e	42.86cd	11.09c
	PAM + CaO	2.81d	0.08e	48.82c	4.03c	1.97e	19.24e	2.69d

† In each column, in each manure type, values followed by the same letter are not significantly different as determined by the least square means test ($P \leq 0.05$), $n = 16$.

Table 3. Efficacy of polyacrylamide (PAM), PAM + Al (SO₄)₃, and PAM + CaO to remove total coliform bacteria, fecal coliform bacteria, and fecal streptococci from water flowing at 8.6 L min⁻¹ over 8.6 L of dairy manure.†

Manure	Treatment	Distance‡	Total coliforms	Fecal coliforms	Fecal streptococci	Active bacteria	Active fungi
		m	colonies per 100 mL water			µg C mL⁻¹	
Cattle	source material	–	1.87×10^7a	5.00×10^4a	8.23×10^5a	64.63a	268.07a
Cattle	control	inflow	1.74×10^4d	0.00e	37e	5.83c	0.00d
	PAM		1.64×10^4d	0.00e	1.00e	5.41c	0.00d
	PAM + Al (SO₄)₃		1.64×10^4d	0.00e	2.00e	6.82c	0.00d
	PAM + CaO		1.56×10^4d	0.00e	0.00e	6.34c	0.00d
Cattle	control	1.0	2.64×10^5b	3.92×10^3b	9.50×10^3d	10.35b	106.00b
	PAM		1.44×10^4c	6.20×10^3d	3.44×10^2d	5.14c	0.00d
	PAM + Al (SO₄)₃		1.11×10^4c	7.77×10^3d	3.02×10^2d	6.55c	4.93c
	PAM + CaO		1.22×10^4c	3.51×10^3d	8.85×10^2d	5.25c	0.00d
Cattle	control	27.0	2.99×10^5b	9.46×10^3b	2.22×10^4b	9.39b	0.00d
	PAM		1.13×10^4c	2.12×10^2c	1.06×10^3c	5.92c	4.93c
	PAM + Al (SO₄)₃		1.07×10^4c	6.08×10^2c	1.20×10^3c	5.57c	9.87c
	PAM + CaO		8.52×10^4bc	5.67×10^2c	5.59×10^3c	5.25c	0.00d

† In each column, values followed by the same letter are not significantly different as determined by the least square means test ($P \leq 0.05$), $n = 27$.
‡ Distance from treatment. Inflow water was sampled prior to flowing over animal waste.

and nutrients that could be loosely held to sand particles. Columns were allowed to drain for 1 h prior to the start of the experiment. One-centimeter-thick PAM + Al(SO₄)₃ or PAM + CaO mixtures were spread on the surface of each sand column. A solid wet cattle, fish, or swine waste 2.5 cm thick was placed over the control, PAM, PAM + Al(SO₄)₃, or PAM + CaO mixtures. A 6.5-cm head space at the top of each column was left so that distilled water could be poured over the animal waste. A 15.24-cm-diameter funnel was placed under each column and into a 500-mL erlenmeyer flask below (Fig. 1).

Polyacrylamide

The PAM copolymer used was a dry granular material having an approximate molecular weight of 12 to 15 Mg mol⁻¹, with an 18% negative charge density (provided by CYTEC Industries [Wayne, NJ] and marketed under the trade name Superfloc 836A).

Treatments

Polyacrylamide treatments consisted of 5 g of PAM, 10 g PAM + Al(SO₄)₃ [5 g PAM mixed with 5 g Al(SO₄)₃], or 10 g PAM + CaO (5 g PAM mixed with 5 g CaO) spread over the surface of sand surface. Then 100 mL of solid wet animal waste was poured over the various PAM treatments (Fig. 1). The control treatment did not have a PAM mixture placed

on the sand surface. After animal waste was applied over the PAM mixtures, 500 mL distilled water was poured over the top of the animal waste. Water flowed through the animal waste, through the PAM mixtures, and finally through washed sand. Leachate was collected in a 500-mL erlenmeyer flask (Fig. 1). The PAM-only treatment consisted of a layer of 5 g PAM spread over the surface of the sand in the 10-cm diameter (Fig. 1). The PAM + Al(SO₄)₃ treatment consisted of a mixture of 5 g PAM mixed with 5 g Al(SO₄)₃' spread over the surface of the sand, and the PAM + CaO treatment consisted of 5 g PAM mixed with 5 g CaO. After the above described PAM treatments were applied to the surface of the sand in each column, 100 mL of cattle, fish, or swine manure was placed on top of each treatment (Fig. 1.) Five hundred milliliters of distilled water was then applied to each column. Columns were allowed to drain until leachate filled the 500-mL erlenmeyer flask below each column (2–4 h). Survival of total and fecal coliform bacteria, fecal streptococci bacteria, active and total fungi and bacteria, and nutrients contained in leachate from each column were analyzed as described below.

Surface Flow Field Study

Field Study Site

The study was conducted at the USDA Agricultural Research Service's Northwest Irrigation and Soils Research Laboratory in Kimberly, ID. The soil in the test field was Portneuf

Table 4. Efficacy of polyacrylamide (PAM), PAM + Al (SO₄)₃, and PAM + CaO to remove total coliform bacteria, fecal coliform bacteria, and fecal streptococci from water flowing at 8.6 L min⁻¹ over 8.6 L of fish manure.†

Manure	Treatment	Distance‡	Total coliforms	Fecal coliforms	Fecal streptococci	Active bacteria	Active fungi
		m	colonies per 100 mL water			µg C mL⁻¹	
Fish	source material	–	1.87×10^7a	8.00×10^4a	8.23×10^4a	32.00a	0.00c
Fish	control	inflow	1.20×10^4c	8.63×10^3b	6.86×10^2c	11.47d	4.62b
	PAM		6.88×10^3c	1.63×10^3b	1.12×10^2c	10.93d	0.00c
	PAM + Al (SO₄)₃		5.03×10^4c	3.45×10^3b	1.68×10^2c	11.73d	3.08b
	PAM + CaO		2.31×10^4c	1.42×10^3b	2.65×10^2c	11.66d	0.00c
Fish	control	1.0	2.44×10^5b	3.87×10^3b	5.31×10^4a	20.47b	4.63b
	PAM		1.01×10^4c	2.92×10^2c	8.20×10^3ab	14.53c	6.17b
	PAM + Al (SO₄)₃		1.08×10^4c	4.17×10^2c	3.51×10^3b	15.38c	0.00c
	PAM + CaO		8.16×10^3d	1.58×10^2c	3.08×10^3b	16.05c	0.00c
Fish	control	27.0	2.73×10^5b	1.16×10^3b	1.40×10^4a	21.51b	12.34a
	PAM		1.38×10^4c	2.92×10^2c	8.20×10^3ab	14.36c	3.08b
	PAM + Al (SO₄)₃		1.62×10^4c	2.16×10^2c	6.69×10^3b	17.42c	3.08b
	PAM + CaO		1.45×10^4c	2.31×10^2c	6.55×10^3b	16.77c	6.17b

† In each column, values followed by the same letter are not significantly different as determined by the least square means test ($P \leq 0.05$), $n = 27$.
‡ Distance from treatment. Inflow water was sampled prior to flowing over animal waste.

Table 5. Efficacy of polyacrylamide (PAM), PAM + Al (SO₄)₃, and PAM + CaO to remove total coliform bacteria, fecal coliform bacteria, and fecal streptococci from water flowing at 8.6 L min⁻¹ over 8.6 L of hog manure.†

Manure	Treatment	Distance‡	Total coliforms	Fecal coliforms	Fecal streptococci	Active bacteria	Active fungi
		m		— colonies per 100 mL water —		— µg C mL⁻¹ —	
Swine	source material	–	3.80×10^6a	4.53×10^4a	1.10×10^5a	48.86a	28.72c
Swine	control	inflow	9.80×10^5b	6.90×10^3ab	1.80×10^3c	12.64c	0.00f
	PAM		7.35×10^5b	1.82×10^3b	6.03×10^3c	13.16c	9.34e
	PAM + Al (SO₄)₃		1.04×10^6ab	8.12×10^3b	2.00×10^3c	13.99c	0.00f
	PAM + CaO		5.16×10^5b	2.00×10^4a	4.15×10^3c	11.69c	0.00f
Swine	control	1.0	1.84×10^6a	1.36×10^4a	1.24×10^4b	21.30b	18.68d
	PAM		7.85×10^5b	1.06×10^5b	4.90×10^3c	18.57b	266.19a
	PAM + Al (SO₄)₃		1.55×10^5b	3.34×10^3b	2.75×10^3c	12.63c	121.42b
	PAM + CaO		1.28×10^5b	7.02×10^2c	2.17×10^3c	12.72c	32.69c
Swine	control	27.0	9.90×10^5a	4.24×10^4a	1.73×10^4b	26.98b	3.11e
	PAM		2.95×10^5b	3.71×10^3b	5.96×10^3c	13.98c	90.41b
	PAM + Al (SO₄)₃		4.07×10^5ab	2.06×10^3b	1.03×10^3c	13.91c	60.43c
	PAM + CaO		4.90×10^5b	1.41×10^3b	1.01×10^3c	13.28c	23.35d

† In each column, values followed by the same letter are not significantly different as determined by the least square means test ($P \le 0.05$), $n = 27$.
‡ Distance from treatment. Inflow waste was sampled prior to flowing over animal waste.

silt loam (coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcid), with 10 to 21% clay and 60 to 75% silt, and organic matter of approximately 13 g kg⁻¹. Saturated paste extract electrical conductivity (EC) of this soil ranges from 0.7 to 1.3 dS m⁻¹, with exchangeable sodium percentage (ESP) of 1.4 to 1.7, pH of 7.6 to 8.0, and a CaCO₃ equivalent of 2 to 8%. Slope on this site was approximately 1.5%.

Experimental Design

The surface flow study was also arranged in a completely random design consisting of four remediation treatments: (i) PAM, (ii) PAM + Al(SO₄)₃, (iii) PAM + CaO, and (iv) control (no treatment). Each remediation treatment had three different manure types superimposed (cattle, fish, and swine) corresponding to three different dates (Kirk, 1982). Cattle manure was used as a waste source on 24 Aug. 1998, fish manure was used as a waste source on 8 Sept. 1998, and swine manure was used a waste source on 16 Sept. 1998. Surface flow was sampled at three distances along the furrows (at the inflow point and at 1 and 27 m down slope of the above treatments; Fig. 2) and at three times during flow (0.5, 3.5, and 6.5 h). We analyzed three subsamples for each microbial and nutrient parameter (stated below) from each sample to account for sampling error (Kirk, 1982). We took 324 samples for the study (four treatments × three manure types × three sampling points along each furrow × three sampling times during each

water application × three samples from each water collection as sampling error).

Treatment Application

Furrows were prepared with weighted 75° shaping tools. Furrows were 0.1 m wide × 0.1 m deep × 30 m long. We placed 9.5 L of solid wet cattle, fish, or swine waste 0.3 m from the water inflow point in a 1.0-m-long area (Fig. 2). Immediately downstream PAM or PAM mixtures were placed in a 1.0-m long-area (Fig. 2). Water flowed 0.3 m from the inflow point and then flowed over a 0.1-m-wide × 0.1-m-deep × 1.0-m-long area containing 9.5 L of solid wet cattle, fish, or swine manure and then over a 0.1-m-wide × 0.1-m-deep × 1.0-m-long area containing the PAM, PAM + Al(SO₄)₃, and PAM + CaO mixtures or no chemical (control) treatment (Fig. 1). Application of PAM, PAM + Al(SO₄)₃, and PAM + CaO involved the spread of granular PAM on the surface of the soil. Application amounts were 35 g PAM, a mixture of 35 g PAM + 350 g Al(SO₄)₃, and a mixture of 35 g PAM + 350 g CaO. Irrigation water came from the Snake River via Twin Falls Canal Company, through a storage pond to 10-cm-wide × 10-cm-deep × 40-m-long furrows at a rate of 7.6 L min⁻¹ in spigoted plastic pipe. Experience from other experiments has shown that water flowing over these patches of PAM granules results in PAM concentrations of 5 to 15 g m⁻³ during the first 20 to 40 min during the flow of water,

Table 6. Efficacy of polyacrylamide (PAM), PAM + Al (SO₄)₃, and PAM + CaO to filter nutrients in cattle wastewater flowing at 8.6 L min⁻¹ over 8.6 L of manure.†

Manure	Treatment	Distance‡	PO₄	Total P	NO₃	NH₄	K	Ca	Mg
		m			— µg element per mL water —				
	source material	inflow	1596.00a	2630.00a	104.83a	490.07a	1630.00a	711.70a	1981.00a
Cattle	control		0.025c	1.72e	0.23c	0.08d	2.11c	20.87b	4.42b
	PAM		0.025c	2.37e	0.38c	0.09d	2.12c	20.27b	4.52b
	PAM + Al (SO₄)₃		0.022c	1.88e	0.32c	0.10d	2.12c	20.24b	4.59b
	PAM + CaO		0.022c	1.79e	0.25c	0.04d	2.04c	20.63b	4.44b
	control	1.0	0.50b	16.01b	0.44c	0.34b	5.66c	15.78b	5.66b
	PAM		0.46b	14.90b	0.47c	0.52b	6.67c	15.96b	6.67b
	PAM + Al (SO₄)₃		0.07d	3.58d	0.34c	0.92b	5.87c	20.75b	3.93b
	PAM + CaO		0.60b	18.39b	0.32c	0.37b	8.21b	15.65b	8.21b
	control	27.0	0.21c	10.49c	0.93b	0.07d	4.06c	16.21b	3.77b
	PAM		0.27c	11.07c	1.28b	0.20c	3.78c	16.92b	5.08b
	PAM + Al (SO₄)₃		0.07d	5.31d	0.72b	0.14c	3.82c	16.14b	3.70b
	PAM + CaO		0.11d	8.71cd	0.89b	0.15c	4.66c	16.35b	3.74b

† In each column, values followed by the same letter are not significantly different as determined by the least square means test ($P \le 0.05$), $n = 27$.
‡ Distance from treatment.

declining to much lower concentrations after 6 h (Sojka, unpublished data, 1999).

Microbial Sample Collection

Three separate water samples were collected from the surface to a 3.0-cm depth at the inflow point, 1 m and 27 m below the PAM, PAM + $Al(SO_4)_3$, and PAM + CaO treatments. Samples were collected 0.5, 3.5, and 6.5 h after water flow was initiated. Samples were analyzed for total coliform bacteria, fecal coliform bacteria, fecal streptococci, active and total fungi and bacteria, NO_3, NH_4, PO_4, total P, K, Ca, and Mg. Water was collected and stored in air-tight and water-tight 125-mL glass bottles and prepared for coliform testing within 2 h of collection (Greenberg et al., 1992). Water samples tested for active bacteria and fungi were stored at 4°C and analyzed within 24 h of collection (West et al., 1986) to minimize the effects of storage on microbial activity. Subsamples tested for nutrients were stored at 4°C prior to testing.

Coliform Procedures

Total coliform, fecal coliform, and fecal streptococcal bacteria were analyzed using the membrane filter technique (Greenberg et al., 1992). Preliminary water samples from test runs taken 1 to 3 d prior to each test were analyzed to determine each dilution before bacteria were counted. One gram of water was diluted in a series of 2 to 5. One hundred milliliters of final dilution of each sample was vacuum-filtered through a sterile 0.45-μm filter and placed on Em endo medium to determine total coliform bacteria, FC medium to determine fecal coliform bacteria, and KF streptococcus medium to determine fecal streptococci. Total coliform bacteria and fecal streptococci were incubated at 39.5 ± 0.02°C. Fecal coliform bacteria were incubated at 44.5 ± 0.02°C for 24 h.

Microbial Biomass Measurements

Active and total bacteria and fungi in leachate and surface flow were determined for each treatment using methods described by Ingham and Klein (1984). Active fungi were estimated by taking a 1.0-mL water sample diluted in 9 mL of a phosphate buffer (pH 6.0) and shaken at approximately 120 rpm for 5 min. A 1-mL aliquot was removed and stained with 1 mL of a 20 μg mL⁻¹ fluorescein diacetate (FDA) solution in a 0.2 M phosphate buffer (pH 7.0) for 3 min. One milliliter of 1.5% agar in a 0.1 M phosphate buffer (pH 9.5) was added to the FDA suspension. The sample was mixed and an aliquot placed on a microscope slide containing a cavity of known volume (Ingham and Klein, 1984). Immediately after preparation, slides were examined for FDA-stained hyphal length by
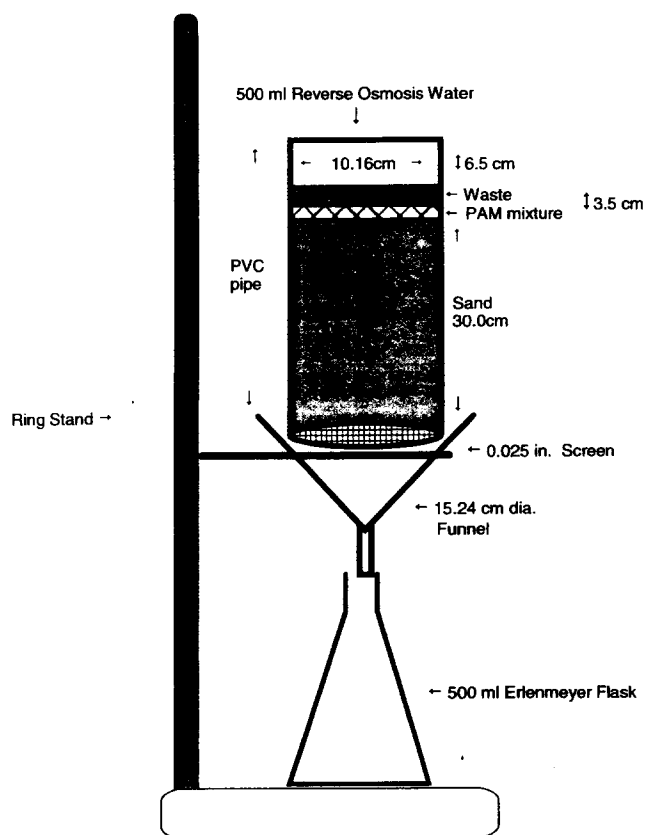

Fig. 1. Diagram of column apparatus showing distilled water poured over animal waste that overlies the polyacrylamide treatment draining through washed sand into an erlenmeyer flask.

epifluorescent microscopy. Total fungal biomass was estimated by measuring the length and diameter of hyphae in 3 to 60 fields with phase-contrast microscopy. Three slides were evaluated from each sample and 10 fields per slide were evaluated with phase contrast microscopy for total hyphal length. Three transects were evaluated for FDA-stained (active) hyphal length at 160× total magnification.

Iodonitrotetrazolium (INT) stain was used for counting active bacteria (Stamatiadis et al., 1990). A 1-mL sample of initial soil suspension was diluted to a final dilution in a mixture of 0.2 mg soil and 4 mL buffer. The suspension was incubated with 4 mL of filtered INT buffer for 60 min in the dark at 20°C. Total bacteria per milliliter of water were estimated from the mean number of bacteria (fluorescent and nonfluorescent
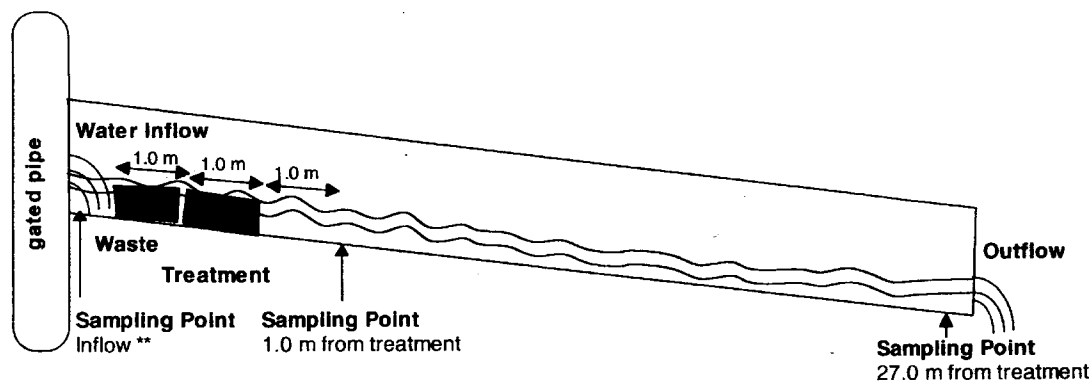

Fig. 2. Diagram of rill showing irrigation water flowing over manure then a polyacrylamide treatment and collection points at 1 and 27 m from treatments.

bacteria), their average diameter, and length per field. Three slides were evaluated for each sample and 10 fields per slide were evaluated using epifluorescent oil-immersion microscopy to determine numbers and size of fluorescent and total bacteria (Lodge and Ingham, 1991). Bacterial volume was computed from the number of soil bacteria per gram of soil considering that bacterial spheres were 1 μm in diameter (Jenkinson and Ladd, 1981). A carbon to volume conversion factor of 120 μg C mm^{-3} was used for both bacteria and fungi, assuming 1.1 g cm^{-3} wet density, 20% dry matter content, and a 0.41 g g^{-1} carbon content in the bacterium or fungus (Jenkinson and Ladd, 1981).

Soil Chemical Analysis

Nitrate and ammonium were determined using a Lachat autoanalyzer (Lachat Quickchem Systems, Milwaukee, WI) using methods described in Keeney and Nelson (1982). Phosphate and total P were determined using methods described in Olsen and Sommers (1982). Potassium, Ca, and Mg were determined using an atomic absorption spectrometer.

Statistical Analyses

All dependent variables were tested for normal distribution. Number of total and fecal coliform bacteria were transformed using logarithms to achieve normal distributions. Data were then analyzed using general linear models (GLM) procedures for a completely random design with Statistical Analysis Systems (SAS Institute, 1996). For the column study, statistical comparisons were made of total coliform bacteria, fecal coliform bacteria, fecal streptococci, active and total bacteria and fungi, NH_4^+, NO_3^-, total P, PO_4^{-3}, K, Ca, and Mg in water by PAM mixtures × manure type because the interaction was significant in nearly all of the GLM models. In the surface flow study, statistical comparisons were made for total coliform bacteria, fecal coliform bacteria, fecal streptococci, active and total bacteria and fungi, NH_4^+, NO_3^-, total P, PO_4^{-3}, K, Ca, and Mg by PAM mixtures × manure type × distance from inflow because GLM models showed these interactions were significant at $p \leq 0.05$ (Snedecor and Cochran, 1980; Kirk, 1982). In all analyses, residuals were equally distributed with constant variances. Differences reported throughout are significant at $p \leq 0.05$, as determined by the Least Squares Means test. Total coliform, fecal coliform, and fecal streptococci bacteria are reported in untransformed numbers.

RESULTS

Numbers of both total coliform, fecal coliform, and fecal streptococci were usually 10-fold higher in source waste than the control treatments in both the column and surface flow study due to dilution by the addition of deionized or irrigation water (Tables 1, 3, 4, and 5). The concentration of measured nutrients was several orders of magnitude higher in source waste than the control treatments in the column and surface flow study (Tables 2 and 6, 7, and 8, respectively).

Column Study

Columns filled with sand reduced populations of total and fecal coliform bacteria and fecal streptococci in leachate by approximately 10-fold (90% reduction) in all three manure sources compared with the source manure (Table 1). The PAM, PAM + Al(SO$_4$)$_3$, and PAM + CaO treatments reduced populations of total and fecal coliform bacteria and fecal streptococci in leachate from 10- to 1000-fold (90 to 99.9% reduction) in all three manure sources compared with the control treatment and from 100- to 10 000-fold (90 to 99.99% reduction) compared with the source manure. The PAM, PAM + Al(SO$_4$)$_3$, and PAM + CaO treatments reduced the numbers of active and total bacteria and active and total fungi in leachate compared with the control treatment. The PAM + Al(SO$_4$)$_3$ and PAM + CaO treatments reduced the concentration of active bacteria in leachate when cattle and swine manure, but not fish manure, was applied to sand columns.

The PAM, PAM + Al(SO$_4$)$_3$, and PAM + CaO treatments reduced NH_4^+, total P, PO_4^{-3}, K, Ca, and Mg concentrations in leachate when cattle and swine manure, but not fish manure, was applied to sand columns (Table 2). When cattle manure was applied, the PAM + Al(SO$_4$)$_3$ and PAM + CaO treatments reduced PO_4^{-3}, K, Ca, and Mg concentrations in leachate compared with the PAM-only treatment. When swine manure was applied, the PAM + CaO treatment reduced the concentration of NO_3^-, NH_4^+, K, Ca, and Mg in leachate

Table 7. Efficacy of polyacrylamide (PAM), PAM + Al (SO$_4$)$_3$, and PAM + CaO to filter nutrients in fish wastewater flowing at 8.6 L min^{-1} over 8.6 L of manure.†

Manure	Treatment	Distance‡	PO$_4$	Total P	NO$_3$	NH$_4$	K	Ca	Mg
		m				μg element per mL water			
	source material	inflow	1309.60a	1465.30a	5.97a	271.50a	86.63a	1428.37a	54.68a
Fish	control		0.01b	3.50b	0.24e	0.14e	2.32c	28.28c	7.40c
	PAM		0.00b	1.93b	0.25e	0.18e	2.32c	29.16c	7.36c
	PAM + Al (SO$_4$)$_3$		0.10b	2.00b	0.23e	0.19e	2.30c	29.13c	7.34c
	PAM + CaO		0.00b	2.76b	0.23e	0.16e	2.31c	29.30c	7.35c
	control	1.0	0.02b	3.22b	0.36d	0.69b	3.93b	44.57b	10.70b
	PAM		0.00b	3.11b	0.44d	0.65b	3.68b	44.77b	10.61b
	PAM + Al (SO$_4$)$_3$		0.00b	3.00b	0.43d	0.48c	3.74b	43.33b	10.52b
	PAM + CaO		0.03b	3.12b	0.33d	0.52c	3.63b	44.41b	10.44b
	control	27.0	0.03b	3.46b	1.13c	0.30d	4.47b	44.59b	10.91b
	PAM		0.02b	2.80b	1.17c	0.28d	4.00b	45.35b	10.87b
	PAM + Al (SO$_4$)$_3$		0.02b	2.89b	0.87c	0.30d	4.38b	44.64b	10.86b
	PAM + CaO		0.03b	2.89b	0.81c	0.39cd	4.32b	44.96b	10.69b

† In each column, values followed by the same letter are not significantly different as determined by the least square means test ($P \leq 0.05$), $n = 27$.
‡ Distance from treatment.

compared with the PAM-only and PAM + Al(SO$_4$)$_3$ treatments.

Surface Flow Study

Concentration of fecal coliform bacteria in inflow water varied and was often above concentrations approved for drinking water standards (USEPA, 1998; Greenberg et al., 1992) (Tables 3, 4, and 5). As water flowed down the furrow (control treatment), total and fecal coliform bacteria and fecal streptococci numbers were reduced by approximately 10-fold (90% reduction) compared with source populations. After water flowed over all three manure sources and then PAM, PAM + Al(SO$_4$)$_3$, or PAM + CaO, total and fecal coliform bacteria and fecal streptococci were reduced by 10- to 1000-fold at 1 and 27 m downstream of the treatments compared with the control treatment. However, there was no difference in the numbers of total and fecal coliform bacteria or fecal streptococci in water at 1 m downstream compared with the organisms in water 27 m downstream of the treatments, indicating that microorganisms were removed from water fairly rapidly.

Concentrations of NH$_4^+$, PO$_4^{-3}$, total P, K, Ca, and Mg in inflow water (prior to flowing over manure) did not vary among treatments (Tables 6, 7, and 8). When surface water flowed over cattle manure and then PAM + Al(SO$_4$)$_3$ or PAM + CaO, concentrations of PO$_4^{-3}$ and total P in water flowing at 1 and 27 m downstream and NH$_4^+$ at 27 m downstream of the treatments were reduced compared with the PAM-only treatment (Table 6). When surface water flowed over fish manure and then PAM, PAM + Al(SO$_4$)$_3$, or PAM + CaO, concentrations of NH$_4^+$, total P, PO$_4^{-3}$, K, Ca, and Mg at 1 and 27 m downstream of the treatments were not reduced compared with the control treatment (Table 7). When surface water flowed over swine manure the PAM + Al(SO$_4$)$_3$ or PAM + CaO, PO$_4^{-3}$, total P, and NH$_4^+$ concentrations in water flowing at 1 and 27 m downstream of the treatments were reduced compared with the PAM-only treatment (Table 8).

DISCUSSION

Contamination of surface and ground water resources by pathogenic bacteria poses a potential health hazard when animal waste is applied to agricultural lands in excess quantities. Animal manure contains many different types of organisms, some that are pathogenic to humans and animals. Large scale deposition of animal manure can result in contamination of surface and ground water and potential transfer of disease-causing enteric bacteria to animals or humans (USEPA, 1998; Mallin et al., 1997; Mawdsley et al., 1995). Despite the ability of soil to filter and kill pathogenic bacteria contained in human and animal waste, land application rates exceeding the soil's capacity to adsorb and process these materials results in contamination of water resources. Laboratory and field studies have shown that enteric microorganisms can be transported long distances through soil (Smith et al., 1985; Chen, 1988; Van Elsas et al., 1991; Huysman and Verstraete, 1993). The explanation that has been given is that preferential flow of water transports bacteria through soil macropores, cracks, and fissures (McMurry et al., 1998; Abu-Ashour et al., 1994).

The water-soluble PAMs developed for use in erosion control are very large anionic molecules that have been shown to be safe for a variety of food, pharmeceutical, and sensitive environmental applications (Barvenik, 1994). They should not be confused with gel-forming cross-linked PAM, or evaluated with other PAM formulations, especially cationic PAMs, which have known safety concerns related to their specific chemistries (Barvenik, 1994). Environmental regulation, safety, and toxicity issues related to PAM use have been extensively reviewed (Seybold, 1994; Barvenik, 1994; Deskin, 1996; Barvenik et al., 1996).

Although the precise mechanism is not fully understood, PAM compounds are used in many industrial processes to accelerate flocculation. Polyacrylamide has been used in irrigated agriculture for erosion control and increased infiltration (Aase et al., 1998; Lentz et al., 1992; Lentz and Sojka, 1994; Sojka et al., 1998a,b).

Table 8. Efficacy of polyacrylamide (PAM), PAM + Al (SO$_4$)$_3$, and PAM + CaO to filter nutrients in hog wastewater flowing at 8.6 L min^{-1} over 8.6 L of manure.†

Manure	Treatment	Distance‡	PO$_4$	Total P	NO$_3$	NH$_4$	K	Ca	Mg
		m	µg element per mL water						
	source material	inflow	6440.53a	3359.33a	10.17a	946.05a	815.06a	60.02a	37.65a
Hog	control		0.00d	0.27b	0.01e	0.14c	3.21b	43.17b	11.28b
	PAM		0.01d	0.58b	0.05e	0.28c	3.20b	42.85b	11.03b
	PAM + Al (SO$_4$)$_3$		0.06d	0.42b	0.03e	0.34c	3.22b	43.16b	11.04b
	PAM + CaO		0.00d	0.24b	0.09e	0.09c	3.20b	42.96b	11.23b
	control	1.0	0.22b	0.00c	0.59bc	0.36c	4.56b	43.50b	11.56b
	PAM		0.18c	0.13c	0.50bc	0.52c	4.26b	44.03b	11.44b
	PAM + Al (SO$_4$)$_3$		0.01d	0.12c	0.25d	1.23b	5.16b	46.71b	11.78b
	PAM + CaO		0.02d	0.00c	0.23d	0.5b	4.32b	43.48b	11.26b
	control	27.0	0.13b	0.00c	0.47bc	0.31c	4.97b	44.55b	11.14b
	PAM		0.08c	0.00c	0.72b	0.30c	4.18b	44.38b	10.77b
	PAM + Al (SO$_4$)$_3$		0.04d	0.00c	0.43c	0.50c	5.20b	45.29b	10.98b
	PAM + CaO		0.06d	0.00c	0.83c	0.53c	5.67b	44.12b	10.57b

† In each column, values followed by the same letter are not significantly different as determined by the least square means test ($P \leq 0.05$), n = 27.
‡ Distance from treatment.

Lentz et al. (1998) and Lentz and Sojka (1994) reported that PAM treatment reduced sediment loss rate over time with improvement of the runoff water quality parameters ortho-P, total P, nitrate, and biological oxygen demand. Subsequent studies have further documented the capacity of PAM treatment to reduce sediments, nutrients, and pesticides in irrigation water (Agassi et al., 1995; Singh et al., 1996; Sojka et al., 1998a,b). We hypothesized that PAM flocculates microorganisms attached to soil particles as well as microorganisms suspended in water. The PAM used in this study (Superfloc A836) is an extremely large negatively charged molecule (Lentz and Sojka, 2000; Barvenik, 1994). When PAM is combined with either $Al(SO_4)_3$ or CaO and then added to water, $Al(SO_4)_3$ or CaO should quickly disassociate, freeing Al^{+3} and Ca^{+2} to bind with anionic nutrients such as $H_2PO_4^-$ and NO_3^-. Free Al^{+3} and Ca^{+2} most likely bind with anionic sites on the PAM molecule, forming a bridge with anionic nutrients such as $H_2PO_4^-$ and NO_3^-. The anionic charges on PAM would not only flocculate microorganisms, but also positively charged nutrients in wastewater.

Polyacrylamide degradation in soil is approximately 10% yr^{-1} (Barvenik, 1994). Degradation of the acrayamide monomer (AMD) is fairly rapid (Kay-Shoemake et al., 1998a; Shanker et al., 1990; Lande et al., 1979). Acryamide monomer was completely degraded within 5 d after applying 500 mg PAM kg^{-1} garden soil (Shanker et al., 1990). Lande et al. (1979) applied 25 mg PAM kg^{-1} soil and reported that half life of an AMD in agricultural soils was 18 to 45 h. Enrichment cultures showed that bacteria are capable of using PAM as a sole source of N, but not C (Kay-Shoemake et al., 1998b). The effect of PAM application to water or soils has been shown to both increase and decrease soil microbial biomass (Nadler and Steinberger, 1993; Steinberger et al., 1993; Kay-Shoemake et al., 1998a,b).

Riparian filterstrips are currently one of the few management practices that can mitigate the input of fecal coliform bacteria from animal waste to surface and ground water (Coyne et al., 1995, 1998; Schellinger and Clausen, 1992; Walker et al., 1990; Young et al., 1980). Forest or grass filterstrips can take from months to years to establish. Therefore, the development of new technologies and management strategies to rapidly mitigate the input of nonpoint-source pollutants to surface and ground water is necessary to avoid contamination of water in many circumstances. These strategies may be especially important during filterstrip establishment phases, or when filterstrips alone cannot provide complete protection.

Sojka and Entry (2000) found reductions in active and total bacteria and fungi transferred down irrigation furrows treated with small amounts of PAM for erosion control. This initial finding prompted a closer look at improved strategies for microbial sequestration. Polyacrylamide alone significantly reduced the number of active and total bacteria in waste water. When present, PAM alone also removed active and total fungi. The efficiency of PAM to remove bacteria and fungi from

water in this study is similar to values reported by Sojka and Entry (2000). Polyacrylamide reduced populations of total and fecal coliform bacteria and fecal streptococci in cattle, fish, and swine wastewater leachate and surface runoff by approximately 10-fold (90% reduction) compared with no treatment. The PAM + $Al(SO_4)_3$ and PAM + CaO mixtures reduced populations of total and fecal coliform bacteria and fecal streptococci in cattle, fish, and swine wastewater leachate and surface runoff by approximately 100- to 1000-fold compared with no treatment. Since PAM, PAM + $Al(SO_4)_3$, and PAM + CaO seem to be indiscriminate to the type and species of microorganisms removed from water, it might be expected that these mixtures could effectively remove a large range of pathogenic bacteria and fungi as well as parasitic protozoa including *Cryptosporidum parvum* and *Giardia lamblia* from flowing water. The PAM mixtures should not be expected to sterilize water, but they should be able to substantially reduced the numbers of pathogenic bacteria in wastewater prior to entering public water systems.

Polyacrylamide + $Al(SO_4)_3$ and PAM + CaO compounds also significantly reduced amounts of NH_4^+, PO_4^{-3}, and total P in cattle and swine wastewater leachate and surface runoff. These compounds should be able to reduce these pollutants from wastewater flowing from animal confinement areas. Polyacrylamide + $Al(SO_4)_3$ and PAM + CaO compounds did not remove significant amounts of NH_4, PO_4, and total P from fish wastewater leachate or surface runoff. However, there were substantially lower concentrations of NO_3^-, NH_4^+, PO_4^{-3}, total P, K, Ca, and Mg in fish waste than cattle or swine wastewater. If nutrient concentrations in wastewater are low there is less chance that they will contact binding sites on the PAM molecule or Al^{+3} and Ca^{+2}. Therefore, PAM + $Al(SO_4)_3$ and PAM + CaO may be unable to remove nutrients from wastewater when nutrient concentrations are extremely low.

In general, washed sand should filter bacteria much less efficiently than most soils because water flows more freely through it than soil because there are fewer adsorptive sites and less surface area in sand compared with most soils. One might expect PAM + $Al(SO_4)_3$ and PAM + CaO added to a soil surface to reduce the populations of total and fecal coliform bacteria, fecal streptococci, NH_4^+, PO_4^{-3}, and total P in leachate even more than in a washed sand column. To achieve maximum benefit, we speculate that these compounds need only be spread in a narrow strip around an animal confinement area during periods of risk prior to each rainfall. If the compounds accumulated on the soil surface, they could be removed from the soil surface and composted. Sojka (unpublished data, 1999) has incorporated up to 5600 kg PAM ha^{-1} into soil without noticeable ill effects on soil properties or plant growth.

The potential benefits of PAM + $Al(SO_4)_3$ and PAM + CaO compounds to animal production operations are: (i) they would be inexpensive, (ii) they could be spread on the soil surface immediately, and (iii) they could be used along with other techniques and manage-

ment strategies such as riparian vegetation (Hubbard et al., 1998; Snyder et al., 1998; Jordan et al., 1993; Lowrance et al., 1984) and denitrification walls (Schipper and Vojvodic-Vukovic, 1998; Robertson and Cherry, 1995) to reduce the input of pollutants from animal confinement areas to water resources. Obviously, cost analysis and testing of the use of these two compounds in various animal operations is necessary, but this research demonstrates the potential of PAM + Al(SO₄)₃ and PAM + CaO as a valuable tool to allow animal producers to control pollution from their operations. Animal production operations should not expect the development of PAM + Al(SO₄)₃ and PAM + CaO or any new technology to allow them to suspend best management practices or common sense. Best results of new pollution mitigation technologies will most likely be achieved by combining them with best management practices and sound animal management practices.

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