

## EFFECTS OF “SHELLBOND” ON WATER QUALITY: I. LABORATORY EXPERIMENTS

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*Abstract:* ShellBond is a patented material derived from high temperature treatment of calcareous materials, including egg shells and oyster shells. The abilities of egg and oyster ShellBond to remediate several common water quality pollutants (turbidity, excess phosphate, and excess ammonium) were investigated in laboratory experiments in order to determine how ShellBond acted in aqueous matrices and what dosing levels gave significant responses. ShellBond reduced turbidity over 24 hr to levels below North Carolina water quality standards from levels well above the 50 NTU standard. Egg ShellBond released significant amounts of reactive phosphate, but oyster ShellBond was effective at reducing phosphate concentrations in 24 hr. Oyster ShellBond had no significant effects on ammonium in distilled water. Oyster ShellBond reduced turbidity, phosphate, and ammonium concentrations in diluted swine lagoon liquid. Oyster ShellBond was also very effective at reducing phytoplankton biomass measured as chlorophyll *a*. These results suggest that ShellBond dosing may have promise for water quality remediation, justifying field scale investigations.

*Key Words:* ShellBond; water quality; turbidity; phosphate; ammonium; phytoplankton.

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### INTRODUCTION

Efforts to manage water quality include evaluation of the uses of various soil and water amendments to react with pollutants either before or after they enter surface waters. For example, gypsum (a hydrated form of calcium sulfate,  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ), produced from mining or as a by-product of industrial processes, has been evaluated as a soil amendment for reducing erosion (as phosphogypsum, Agassi and Ben-Hur 1992) and leaching of nutrients from soils and for control of phosphate solubility in aqueous systems (Anderson et al. 1995; Phillips 1998; Bastin et al. 1999; Varjo et al. 2003). Alum (aluminum sulfate,  $\text{Al}_2(\text{SO}_4)_2$ ) is used to precipitate phosphates in drinking water and sewage treatment processes (Linstedt et al. 1974; Omoike and vanLoon 1999), and has also been evaluated as a soil amendment for phosphorus management (Anderson et al. 1995; Ann et al. 1999). Recently synthetic products such as polyacrylamides have been evaluated for soil erosion and nutrient runoff control (Sepaskhah and Bazrafshan-Jahromi 2006; Sojka et al. 2007; Szogi et al. 2007; McLaughlin 2007).

A novel kind of material, termed ShellBond<sup>®</sup>, has been developed and proposed for environmental pollution mitigation (U.S. Patent #7,309,438; issued Dec. 2007). ShellBond is manufactured by a proprietary process employing heating under carefully controlled conditions and subsequent re-hydration of natural shell or bone materials, including oyster shells, egg shells, and

cattle bones from slaughterhouse operations. Initial evaluations suggested that these novel materials might have useful properties and offer valuable uses for what might otherwise be wastes or low-value materials.

We evaluated the utility of two kinds of ShellBond, derived from oyster shells and chicken egg shells, here termed “Oyster ShellBond” and “Egg ShellBond” in remediating water with elevated levels of turbidity, the nutrients ammonium and phosphate, and phytoplankton biomass at “algae bloom” levels. Suspensions and solutions were prepared in the laboratory using distilled water, amended natural pond water, and diluted liquid from a swine waste lagoon.

### METHODS AND MATERIALS

ShellBond was obtained from the manufacturer and prepared for use by rehydrating the original egg shell- and oyster shell-derived powders with distilled water in a 5:1 powder:water ratio by volume. The rehydration step releases heat, so final ShellBond preparations were allowed to cool before further handling. A stock suspension of each type of ShellBond was prepared using 20 g of dried, cooled ShellBond in 100 ml water at the beginning of each experiment. Working stocks of each ShellBond type were then prepared by diluting the stock suspension (1 ml = 0.2 g ShellBond) to desired final concentrations.

Initial experiments examined the ability of varying concentrations of ShellBond to reduce turbidity. Reagent quality kaolin, a form of clay mineral, was used to prepare turbid suspensions at concentrations of 50 and/or 250 mg/L in distilled water. One hundred ml volumes of kaolin ( $\text{H}_2\text{Al}_2\text{Si}_2\text{O}_8 \cdot \text{H}_2\text{O}$ , USP grade, Fisher Scientific) suspensions were then decanted into 100 ml graduated cylinders, amended with 0.01 to 1.0 ml volumes of stock ShellBond suspensions, with four replicates per treatment plus unamended controls, shaken thoroughly, and allowed to stand for 24 hr. Turbidity of the suspensions was measured using an HF Scientific DRT-15CE turbidity meter that was calibrated for each use with standard formazin at 0.02 NTU.

A second set of experiments examined the effects of varying concentrations of ShellBond on soluble reactive phosphate (SRP) in distilled water solutions and SRP and ammonium in diluted hog waste lagoon liquid. ShellBond suspensions were prepared as above. Phosphate solutions were prepared in distilled water from reagent grade  $\text{K}_2\text{HPO}_4$ . Hog waste lagoon liquid was obtained from a swine producer in Pender County, North Carolina and diluted 10 $\times$  with distilled water prior to use in experiments. SRP and ammonium assays were performed on samples taken before and after 24 hr incubations conducted as above using the methods of Parsons et al. (1984) and Koroleff (1970), respectively, with standards and reagents prepared from deionized water and reagent grade compounds. Analytical precision of the SRP assay was about  $\pm 0.002$  absorbance units, corresponding to  $\pm 0.088 \mu\text{M}$ . Simultaneous measures of the effects of ShellBond on turbidity, SRP and ammonium were conducted as part of this set of experiments as well.

A final set of experiments examined the effects of ShellBond on phytoplankton biomass in a bloom situation. ShellBond suspensions were prepared as above and added to a mixed phytoplankton culture, originally grown from a local storm water pond, that had been amended with nutrients to stimulate growth. Phytoplankton biomass was measured as chlorophyll *a* in samples taken at the beginning and end of 24-hr incubations according to the fluorometric method of Welschmeyer (1994), in addition to measures of SRP and turbidity as above.

## RESULTS

Amendment of kaolin suspensions with egg and oyster ShellBond showed some potential as an option for lowering turbidity in preliminary experiments. Addition of varying amounts of ShellBond to suspensions of 5 mg  $100 \text{ mL}^{-1}$  and 25 mg  $100 \text{ mL}^{-1}$  of kaolin yielded the lowest turbidity after 24 hr at intermediate concentrations (10–20 mg  $100 \text{ mL}^{-1}$ ) of ShellBond

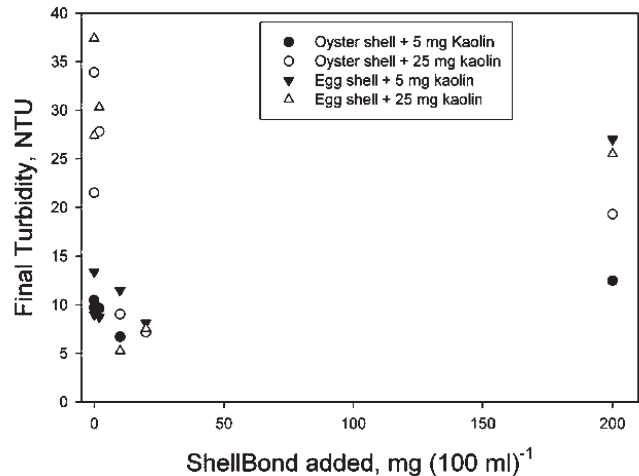


FIG. 1. Effects of added oyster and egg ShellBond (@ 0, 5, 10, 20, and 200 mg  $100 \text{ mL}^{-1}$ ) on turbidity of kaolinite (@ 5 and 25 mg  $100 \text{ mL}^{-1}$ ) suspensions after 24 hr incubation.

(Fig. 1). Higher turbidity in controls (without added ShellBond) indicated that ShellBond had some effect in reducing turbidity, whereas higher turbidities with higher levels of added ShellBond indicated that ShellBond itself contributed to overall turbidity. Comparisons of turbidity at the beginning and end of subsequent incubations with kaolin suspensions demonstrated that ShellBond contributed as much turbidity per added mg of dry material as did kaolin ( $8.41 \pm 1.87 \text{ NTU mg}^{-1}$  ShellBond added vs.  $8.98 \pm 2.46 \text{ NTU mg}^{-1}$  kaolin added). Thus, higher concentrations of ShellBond contributed as much or more turbidity initially as the kaolin, but yielded a correspondingly greater depressing effect on final turbidity values as well (Fig. 2).

Analyses of the effects of ShellBond on phosphate solubility revealed immediately that egg ShellBond and oyster ShellBond differed significantly. Incubations of

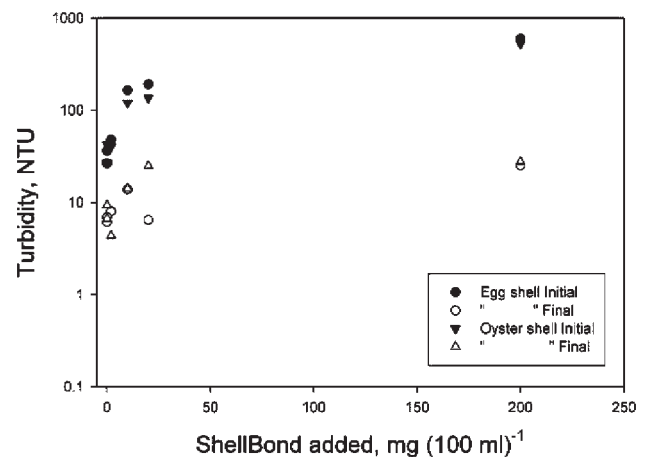


FIG. 2. Effects of added oyster and egg ShellBond (@ 0, 5, 10, 20, and 200 mg  $100 \text{ mL}^{-1}$ ) on turbidity of kaolinite (@ 5 mg  $100 \text{ mL}^{-1}$ ) suspensions at start and end of 24 hr incubation.

Table 1. Response of orthophosphate concentrations to ShellBond amendments (all at 200 mg L<sup>-1</sup>, incubated 24 hr at room temperature (20°C).

Amendment	Initial $\mu\text{M}$ [PO <sub>4</sub> ]	Final $\mu\text{M}$ [PO <sub>4</sub> ]
None (control)	0.0	0.0
	0.088	0.0
	0.0	0.0
	0.0	0.0
	6.78	6.64
K <sub>2</sub> HPO <sub>4</sub>	6.82	6.47
	8.62	8.58
	8.80	8.84
	15.4	53.9
Egg ShellBond	16.4	60.3
	8.84	4.27
Oyster ShellBond	9.33	3.62
	1.28	0.0
Oyster ShellBond (filtered)	3.34	0.0
	13.3	7.44
Oyster ShellBond + K <sub>2</sub> HPO <sub>4</sub>	13.6	7.44

each type of ShellBond (@200 mg L<sup>-1</sup>) in 100 ml distilled water revealed that phosphate concentrations in the egg ShellBond treatment rose substantially over a 24 hr period, from approximately 16  $\mu\text{M}$  to over 50  $\mu\text{M}$  (Table 1). Phosphate concentrations in a suspension of oyster ShellBond declined from approximately 9  $\mu\text{M}$  to approximately 4  $\mu\text{M}$  (Table 1). These findings indicated that egg ShellBond contained a significant amount of soluble phosphate, calculated to be approximately 6 mg PO<sub>4</sub>-P g<sup>-1</sup>. Consequently, egg ShellBond was not used in subsequent experiments. Oyster ShellBond released a lesser amount of soluble phosphate (equivalent to 0.14 mg g<sup>-1</sup>), and appeared to remove some of the initially solubilized phosphate over 24 hr by adsorption

and settling (Table 1). When particulate ShellBond remaining in suspension was removed by filtration prior to final phosphate analyses, much lower levels of soluble phosphate were detected, indicating that particulate ShellBond reacted in the phosphate assay, either through solubilization of particulate ShellBond in the phosphate assay itself (which is conducted at low pH) or through reaction of phosphate adsorbed to particulate ShellBond in the assay. ShellBond amendment was somewhat effective in removing additional soluble phosphate (added as K<sub>2</sub>HPO<sub>4</sub>) from solution, but a significant amount of reactive phosphate remained in solution/suspension without additional filtration. Thus, ShellBond materials have quite variable effects on reactive phosphate concentrations.

The ability of oyster ShellBond to reduce phosphate concentration in a more complex medium, swine waste lagoon liquid (diluted for these experiments 1:10 with distilled water), was examined initially in a simple experiment using one concentration of ShellBond. Results indicated that the combination of hog waste and ShellBond initially yielded a higher concentration of soluble phosphate than in hog waste alone, but that after 24 hr there was a clear decline in phosphate concentrations, particularly when suspensions were filtered prior to phosphate analyses (Table 2). The amount of phosphate released by ShellBond itself initially was approximately 15  $\mu\text{M}$  higher than found in hog waste alone, and about twice as much as was apparently released in distilled water alone (Table 1), suggesting that hog waste dissolved some reactive phosphate from ShellBond initially, but that added ShellBond reduced reactive phosphate over 24 hours, particularly after filtration removed remaining particulates. However, reactive phosphate concentrations in all treatments with hog waste and/or ShellBond remained

Table 2. Effects of oyster ShellBond (all at 200 mg L<sup>-1</sup>) on phosphate concentration in hog waste lagoon liquid (10:1 dilution), incubated 24 hr at room temperature Results of 1-way ANOVA compare initial and final values; "ns" = no significant difference @ p=0.05.

Amendment	Initial $\mu\text{M}$ [PO <sub>4</sub> ]	Final $\mu\text{M}$ [PO <sub>4</sub> ]	ANOVA
None (control)	0.0	0.0	ns
	0.0	0.0	
	0.0	0.0	
K <sub>2</sub> HPO <sub>4</sub>	8.58	8.49	ns
	8.76	8.54	
	8.71	8.58	
	55.1	63.7	
Hog waste	54.8	63.8	F=17.25, p=0.01, df=1,4
	46.6	63.7	
	69.5	53.1	
Oyster ShellBond + Hog waste	64.6	54.6	F=53.5, p=0.0019, df=1,4
	64.8	54.5	
	32.8	27.5	
	52.8	33.3	
Oyster ShellBond + Hog waste (filtered)	55.6	50.9	ns

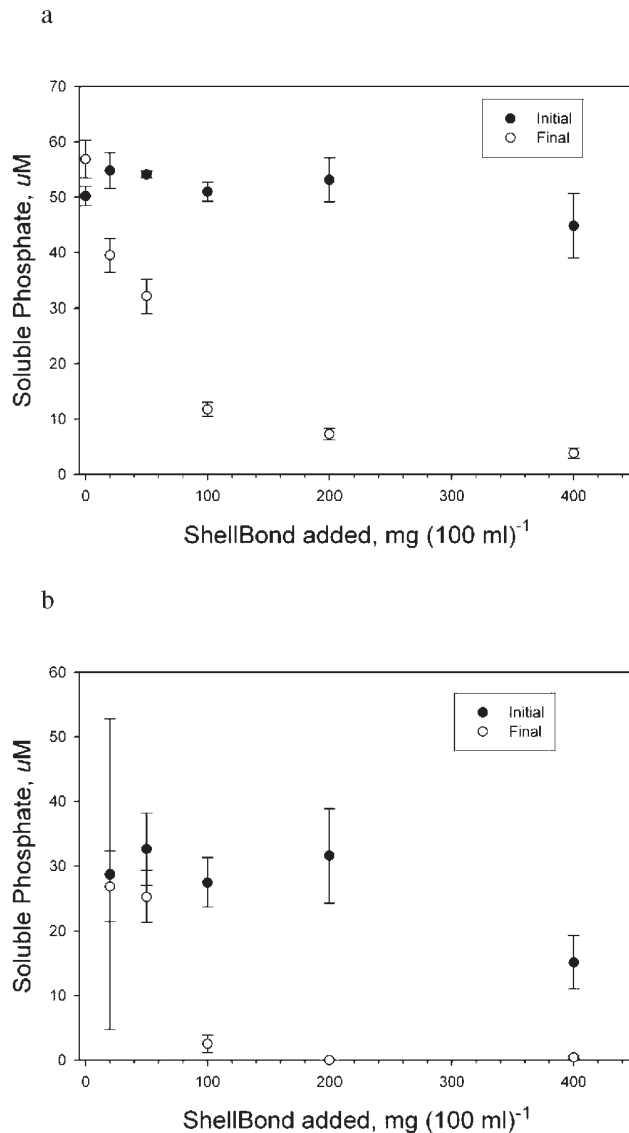


FIG. 3a. Effects of added oyster ShellBond on reactive phosphate concentrations in diluted (10:1) swine waste at start and end of 24 hr incubation. All initial-final differences were significant at  $P \leq 0.00$  using one-way ANOVA; note that control (no added ShellBond) yielded an increase in [phosphate].

FIG. 3b. Effects of added oyster ShellBond on soluble reactive phosphate concentrations (after removal of particulates by filtration) in diluted (10:1) swine waste at start and end of 24 hr incubation. Initial-final differences at ShellBond concentrations  $\geq 100$  mg L<sup>-1</sup> were significant at  $P \leq 0.005$  by 1-way ANOVA.

elevated at this initial concentration of added ShellBond.

The next experiment demonstrated that higher concentrations of added oyster ShellBond reduced phosphate concentrations in hog waste lagoon liquid more effectively (Fig. 3a), and that the effects were greater when remaining particulates were removed by filtration prior to phosphate analyses (Fig. 3b). Thus, ShellBond amendment is capable of substantial effects

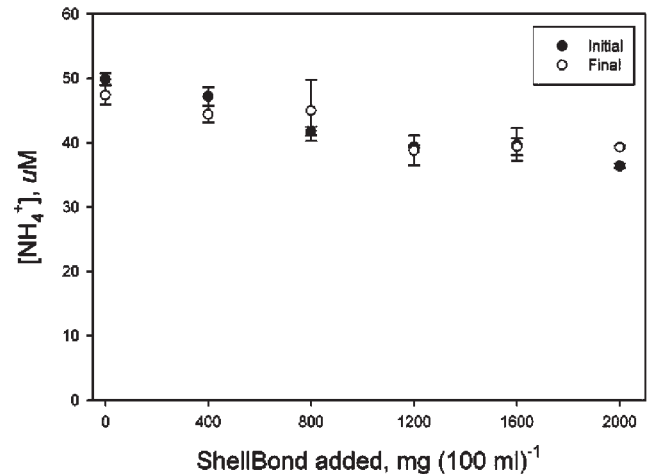


FIG. 4. Effects of added oyster ShellBond on ammonium concentration in diluted (10:1) swine waste at start and end of 24 hr incubation. Differences between initial and final values were significant ( $P < 0.05$ ) only at 2000 mg L<sup>-1</sup> ShellBond addition, but both initial and final ammonium concentrations declined significantly ( $P < 0.0001$ ,  $df = 1, 16$ ,  $F = 118.3$  and 24.8, respectively) with increases in added ShellBond.

on reactive phosphate, but only at doses that previously demonstrated higher residual turbidity.

Amendment of diluted hog waste with ShellBond yielded an immediately noticeable odor of ammonia. When varying concentrations of oyster ShellBond were added to diluted hog waste, there was an immediate significant decline in ammonium concentrations corresponding to dosage of ShellBond, but no significant change over 24 hours (Fig. 4). This result suggests that the basic character of ShellBond shifts pH upward and rapidly releases ammonia, but that other effects of ShellBond on diluted swine waste had little other effect on ammonium concentrations.

An experiment evaluating the ability of varying concentrations of ShellBond and of gypsum (@200 mg L<sup>-1</sup>) to reduce turbidity, phosphate and ammonium concentrations in diluted swine waste showed effects on all three parameters by ShellBond (Table 3). As before, ShellBond initially contributed substantially to initial turbidity levels, but after 24 hours turbidity had declined to levels well below North Carolina standards (50 NTU in fresh water); gypsum treatment was not quite as effective, but also yielded declines in turbidity. As before, ShellBond appeared to contribute additively to the initial values of reactive phosphate, but was very effective at reducing reactive phosphate concentrations over 24 hr, and much more so than gypsum treatment at comparable concentrations. Addition of ShellBond reduced ammonium concentrations immediately compared to controls, but may have had a slight additive effect after 24 hr at higher concentrations as well, whereas gypsum had essentially no effect on ammonium concentrations.

Table 3. Effects of oyster ShellBond and gypsum (mg/100 ml) on turbidity, phosphate, and ammonium concentrations in diluted (1:1) hog waste with 24 hr incubation. "DI" = distilled water controls, "I" = initial, "F" = final.

Amendment	Liquid	Turbidity (NTU)		Phosphate ( $\mu$ M)		Ammonium ( $\mu$ M)	
		I	F	I	F	I	F
Control (0)	Hog Waste	24.8	23.5	60.3	56.3	50.2	52.6
Control (0)	Hog Waste	25.6	23.1	62.2	57.2	51.9	56.7
Control (0)	DI	0.14	0.2	0.04	0.0	0.06	0.09
ShellBond, 20 mg L <sup>-1</sup>	Hog Waste	165	29.7	59.6	49.4	49.6	41.1
ShellBond, 20 mg L <sup>-1</sup>	Hog Waste	170	29.3	62.7	48.6	52.6	38.8
ShellBond, 20 mg L <sup>-1</sup>	DI	58.3	4.7	2.4	0.02	0.0	4.3
ShellBond, 100 mg L <sup>-1</sup>	Hog Waste	440	16.7	60.8	19.4	41.9	38.7
ShellBond, 100 mg L <sup>-1</sup>	Hog Waste	395	6.6	61.8	14.4	42.6	35.9
ShellBond, 100 mg L <sup>-1</sup>	DI	255	15.0	10.2	0.62	7.0	0.64
ShellBond, 200 mg L <sup>-1</sup>	Hog Waste	680	6.76	64.3	5.54	48.6	35.5
ShellBond, 200 mg L <sup>-1</sup>	Hog Waste	700	3.06	62.6	7.26	45.1	36.3
ShellBond, 200 mg L <sup>-1</sup>	DI	463	21.5	16.4	1.23	0.12	1.15
ShellBond, 400 mg L <sup>-1</sup>	Hog Waste	1106	2.87	68.9	5.06	46.2	36.3
ShellBond, 400 mg L <sup>-1</sup>	Hog Waste	1160	1.74	65.0	4.27	43.2	39.1
ShellBond, 400 mg L <sup>-1</sup>	DI	1256	37.6	34.1	1.54	0.67	0.73
ShellBond, 800 mg L <sup>-1</sup>	Hog Waste	1225	2.34	71.7	3.70	43.7	38.5
ShellBond, 800 mg L <sup>-1</sup>	Hog Waste	1123	2.65	75.1	3.30	43.5	38.8
ShellBond, 800 mg L <sup>-1</sup>	DI	1350	24.5	32.8	1.41	0.76	0.55
Gypsum, 200 mg L <sup>-1</sup>	Hog Waste	161	27.3	56.3	54.1	52.8	42.2
Gypsum, 200 mg L <sup>-1</sup>	Hog Waste	175	24.7	58.7	55.2	42.3	43.0
Gypsum, 200 mg L <sup>-1</sup>	Hog Waste	115	24.3	41.8	57.4	41.8	43.4
Gypsum, 200 mg L <sup>-1</sup>	DI	101	8.65	24.3	19.7	0.61	0.33

Amendment with ShellBond of a mixed phytoplankton culture demonstrated effects on turbidity and reactive phosphate levels similar to those observed before (Table 4). The effects of ShellBond amendments on phytoplankton biomass as chlorophyll *a* concentrations were particularly dramatic, however, with >99% reductions in 24 hr (Table 4).

## DISCUSSION

Laboratory results demonstrate significant but not immediate effects on turbidity, in all cases reducing final turbidity levels below the turbidity standard used in North Carolina, 50 NTU for fresh waters (NC DWQ reference). However, kaolin may be atypical of the suspended solids contributing to overall turbidity in field situations, so further testing with more representative but less uniform materials would be necessary, and the initial substantial spike in turbidity caused by

addition of ShellBond itself could be considered problematic. Dosing of ShellBond in these closed systems would not necessarily reflect dosing needed to accomplish comparable results in open systems in which fresh inputs of target materials occur continuously. Finally, the susceptibility of particulates settled by ShellBond treatment to resuspension in turbulent conditions remains to be addressed.

Egg ShellBond contains a significant amount of phosphorus (~0.6% by weight) that is readily solubilized, so it would be an inappropriate amendment for nutrient reduction purposes, but might have value as a soil amendment in situations when added phosphorus is desirable. Oyster ShellBond was rather effective in reducing high concentrations of reactive phosphate over time, but complete removal of particulate matter to which some reactive phosphate adsorbed would be necessary to reduce reactive phosphate levels low enough to be useful as an effective control on

Table 4. Effects of oyster ShellBond on nutrient-enriched pond water for 24 hr. "I" = initial, "F" = final.

Amendment	Liquid	Turbidity (NTU)		Phosphate ( $\mu$ M)		Chlorophyll <i>a</i> ( $\mu$ g L <sup>-1</sup> )	
		I	F	I	F	I	F
ShellBond, 200 mg L <sup>-1</sup>	Pond	1270	11.4	41.9	11.3	342	1.95
ShellBond, 200 mg L <sup>-1</sup>	Pond	1210	16.9	46.9	11.1	345	1.18
ShellBond, 200 mg L <sup>-1</sup>	Pond	1257	13.4	46.6	10.6	328	1.97
None (Control)	Pond	15.2	13.4	28.2	23.7	374	285

phosphorus loading. Residual reactive phosphate concentrations after ShellBond treatments were on the order of 3–4  $\mu\text{M}$ , or  $\sim 120$ – $150$  micrograms  $\text{P L}^{-1}$ , high enough to support aquatic plant growth to bloom levels in shallow aquatic ecosystems (Correll 1998). The biological availability of settled, particle-bound phosphate in an aquatic ecosystem also remains to be determined, but studies have shown that such phosphate is available to sediment organisms (Estey 2004).

ShellBond's basic chemical properties, likely a result of calcium hydroxide formation during the hydration step in its preparation, help degas ammonia from solutions enriched in it, but the equilibrium reaction (de-protonation of  $\text{NH}_4^+$  ion to  $\text{NH}_3$ ) driving this release does not proceed far enough to drive ammonium levels low enough to manage water quality impairment by ammonia-nitrogen loading. Residual ammonium concentrations in diluted swine waste remained above 30  $\mu\text{M}$ , or 420  $\mu\text{g}$  ammonia-N  $\text{L}^{-1}$ , high enough to support algae blooms (Mallin et al. 1998, 2001). Volatilized ammonia would, of course, represent a potential problem via wet and dry deposition processes.

ShellBond had a particularly dramatic effect on phytoplankton biomass at bloom levels, driving a decline of >99% in chlorophyll *a* levels to well below the standard for defining an algae bloom in North Carolina (40  $\mu\text{g chl } a \text{ L}^{-1}$ , NC DEHNR 1996). This is a limited but very interesting result, as ShellBond appears to function by a combination of coagulation and (perhaps) nutrient sequestration. As a treatment for algae blooms, ShellBond might offer a non-toxic alternative to plant poisons and algicides, most of which are toxic to non-targeted organisms as well, e.g., copper sulfate. Many problematic algae are macroscopic forms, such as filamentous algae, so further testing of ShellBond on these bloom-forming algae would be warranted.

ShellBond amendment demonstrates potential for treatment of water quality impairment by turbidity, nutrient loading, and algae blooms through a combination of chemical reactions and adsorptive and/or coagulant behaviors. It was much more effective than gypsum, another amendment sometimes used in water quality remediation (Anderson et al. 1995; Phillips 1998; Bastin et al. 1999; Varjo et al. 2003). Additional comparisons between ShellBond and better tested amendment materials, such as polyacrylamides (Sepaskhah and Bazrafshan-Jahromi 2006; Sojka et al. 2007; Szogi et al. 2007; McLaughlin 2007) would be useful, particularly in quantifying relative performances in complex mixtures, such as swine waste and algae blooms. Extrapolations from laboratory results to field-scale obviously depend on results of further testing, but the effectiveness of ShellBond in some water quality remediation applications at concentrations well below

one part per thousand suggest the potential of this novel material.

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