Evaluation of Dissolved Chitosan for Suspended Solids Removal

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ABSTRACT

In a preliminary study conducted at The Conservation Fund Freshwater Institute (Shepherdstown, WV, USA), dissolved chitosan was added to a recirculating system to determine if the chitosan would coagulate particulate matter and consequently increase solids removal. The recirculating water became visibly clearer and the culture tank total suspended solids (TSS) concentration dropped from 10.7 to 2.9 mg/L within 2 hours after dosing had been initiated. However, fish showed symptoms of distress and the chitosan treatment was discontinued. In subsequent studies conducted to determine the particle capture mechanism associated with chitosan addition, effluent treated with dissolved chitosan was not returned to the system. The results of two jar test studies indicated that dissolved chitosan did not enhance particle capture by settling or by microscreen filtration when mixed with a fish culture system effluent containing *10 mg/L of TSS. However, these jar tests indicated that an additional 44% of TSS could be removed from the water that had already passed through a microscreen filter if this water was treated by a mixing and settling step, even without addition of dissolved chitosan. Additional studies using small-scale fluidized-sand biofilters indicated that the reduction in TSS observed in our initial experiment was due to TSS capture in the fluidized sand biofilter. TSS concentrations were reduced from 5.1-7.4 mg/L at the biofilter inlet to 1.7-2.2 mg/L at the biofilter outlet. Thus, adding dissolved chitosan to water flowing into a fluidized-sand biofilter turned the biofilter into a novel type of upflow 'sludge blanket clarifier,' which appears to be both non-plugging and relatively simple to operate. In addition, dissolved chitosan did not change nitrification occurring within the fluidized-sand biofilter. Therefore, adding a coagulant (such as dissolved chitosan or a

non-toxic polymer) to the flow entering a fluidized sand biofilter has the potential to create a unit process that reduces TSS while simultaneously treating dissolved wastes.

INTRODUCTION

Organic suspended solids encountered in aquaculture systems will contain phosphorus, can contain undesirable organisms, and may cause gill irritation in salmonids (Noble and Summerfelt 1996). Organic matter can also degrade and release ammonia and create a biochemical oxygen demand. Suspended solids must be removed from recirculating aquaculture systems to improve water quality. In addition, suspended solids must also be removed from their effluents in order to meet state and federal effluent discharge limits. Sedimentation and microscreen filtration are the primary mechanisms used to remove particulate matter from coldwater recirculating systems and their effluents. However, sedimentation and microscreen filtration units typically do not remove particles much smaller than about 75 mm (Timmons et al. 2002), which might not be adequate because particles that can contribute to gill irritation and mortality may be in the 5-10 mm range (Chapman et al. 1987). Other options that can be used to increase the removal of fine particles include foam fractionation (Weeks et al. 1992), ozonation (Summerfelt et al. 1997), and possibly the addition of flocculation aids such as ferric chloride, alum, and/or polymers (Ebeling et al. In Review).

Chitosan is an organic, cationic polymer commonly derived from chitin extracted from the exoskeletons of crustacean for use in a variety of commercial applications. Chitosan has been touted as a non-toxic coagulant that is widely applied in wastewater and agricultural applications and that is also being studied for uses in human medicine (Sandford 1989, Elson 1996). Dissolved chitosan has been used at doses of 0.15-1.0 mg/L as a coagulant or coagulant aid to increase solids removal in various surface water treatment applications (Vaidya and Bulusu 1984, Kawamura 1991) and in wastewater treatment and food processing applications (Bough 1976, Wu et al. 1978). Feeding, injecting, and bathing rainbow trout (*Oncorhynchus mykiss*) in chitosan solutions has been shown to be a non-toxic and effective immunostimulant (Anderson and Siwicki 1994, Siwicki et al. 1994).

Chitosan has also been reported to be non-toxic when ingested by fish (Kono et al. 1987). Acidified chitosan that had been dissolved in malic acid was reported to be non-toxic to fathead minnows (*Pimephales promelas*) in a Technical Data Sheet (Sea Klear Chitosan Toxicity Data 11/8/96) provided by Vanson (Redmond, WA, USA). Based on our literature search, we found no indication that dissolved chitosan would be toxic to fish.

The purpose of this research was to determine if low doses of dissolved chitosan would produce coagulation and flocculation of fine particulate organic matter and thus increase solids removal within recirculating aquaculture systems or from their effluent.

MATERIALS AND METHODS

Dissolved chitosan stock solution

A 1% chitosan (10,000 mg chitosan/L) stock solution was used in the study. For reasons of material availability, this solution was prepared by one of two methods: (1) 10 g chitosan dissolved in 100 mL of 10% acetic acid and 900 mL distilled water (2) 10 g chitosan dissolved in 10 mL glacial acetic acid and 990 mL distilled water. For the jar tests, further dilutions of the stock solution were prepared to produce uniform 10 mL doses into the 2 L jars. For example, for a 0.1 mg/L final jar concentration of chitosan, the chitosan stock was diluted to produce a 20 mg/L chitosan dosing solution.

Chitosan dosed into a coldwater recirculating system

In a preliminary study conducted at the Conservation Fund Freshwater Institute, dissolved chitosan was added to a recirculating system (Figure 1) to determine if the chitosan would coagulate solids and consequently increase solids removal. The recirculating system (Figure 1) has been described elsewhere (Heinen et al. 1996a). Dissolved chitosan was added to create a concentration of 1 mg/L in the recirculating flow entering the fish culture tanks. The concentration of TSS in the water exiting the culture tank was measured 2 hours after chitosan addition had begun. The experiment was terminated at this point due to chitosan toxicity problems that had become apparent.

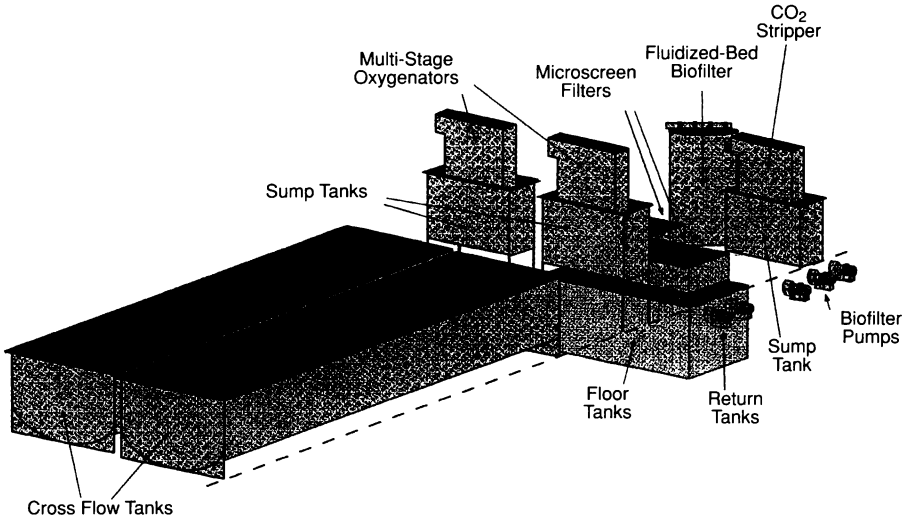

Figure 1: Illustration of the recirculating culture system used in this study (Heinen et al., 1996a)

Jar test studies

The effects of dissolved chitosan on TSS coagulation and flocculation were evaluated using jar test methods. Two series of jar tests were run using water samples that were collected either before or after an 80 mm Hydrotech (Vellinge, Sweden) microscreen filter unit. Both jar tests utilized square cross-sectioned Wagner floc jars (11.5 x 11.5 x 21 cm) with a sampling tap positioned 5 cm from the bottom of the jar. Samples were stirred with a Phipps and Bird six-paddle stirrer (Model 7790-400, Richmond, VA, USA) with a rectangular paddle blade (76 cm x 25 cm).

For the first jar test series, each of the six Wagner floc jars received 2 L of water collected following microscreen filtration. Next, the jars were dosed with the appropriate 10 mL dose to produce 0.025, 0.050, 0.10, 0.20 and 0.40 mg/L chitosan. The jars were then flash mixed at 100 rpm for 1 minute, floc mixed at 30 rpm for 20 minutes and then allowed to settle for 30 minutes. Finally, a 1 L sample was collected through the sampling port from each jar and these samples were analyzed for TSS, color and turbidity using standard methods (APHA 1989). These analyses were also performed on a 1 L unmixed control sample.

The second jar test series examined effluent leaving the fish tanks prior to microscreen filtration. Jars were dosed with 0.0, 0.1 or 0.4 mg/L chitosan. The data from the two replications were averaged. Following

the 1 minute flash mix and 20 minute floc mix, the full 2 L of treated effluent was collected from each jar. The treated effluent was passed through successively smaller nylon net filters and finally through a standard TSS filter paper to capture the remaining solids. Millipore Nylon Net Filters (Bedford, MA, USA) sized 120, 80, 41, 20 and 11 mm and a Gelman Glass Fiber Filter (Pittsburgh, PA, USA) rated nominally at 1 mm were used. The mass of solids on each of the screens was measured using the standard method for TSS analysis (APHA 1989). The screen filters were used to determine if particle size distribution was altered by chitosan addition.

Sweep floc removal of TSS within pilot-scale fluidized-sand biofilters

Three pilot-scale biofilters were used in this study. Each column was 16.2 cm in diameter and 2.5 m tall. Immediately before each trial began, 9 L of actively nitrifying sand was taken from the main system biofilter and was transferred into each of the test columns. After being filled with sand, the pilot-scale biofilters were fluidized and allowed to stabilize for 60 hours prior to dosing. Each of the columns received tank effluent after it had passed through the microscreen filter. Dosing began at 9:00 a.m. and continued for 48 hours. Cole-Parmer (Chicago, IL, USA) peristaltic pumps were used to supply the pilot-scale biofilters with water from the recirculating system. Chitosan doses of between 0.44 and 0.55 mg/L were applied. Columns dosed solely with acetic acid had concentrations between 0.44-0.45 μL acetic acid per liter effluent, which is a concentration equivalent to the acetic acid concentrations in the columns dosed with dissolved chitosan solution. The fluidized bed heights were measured at time 0, 2, 4, 6, 24, 26, 28, and 30 hours. Other biofilter influent water conditions were as follows: average flow = 6.9 L/min, temperature = 15.1, pH = 7.6, alkalinity = 240 mg/L.

Water quality parameters were monitored to determine effects of chitosan dosing on biofilter performance. Equipment used included a YSI Model 58 dissolved oxygen meter (Yellow Springs, CO, USA) and Fisher Scientific Accumet pH meter 915 (Pittsburgh, PA, USA). A DR/2000 spectrophotometer utilizing the Nessler method and Diazotization method were used to test total ammonia nitrogen and nitrite nitrogen, respectively, using methods developed by Hach Company (Loveland, CO, USA). Sampling was conducted at t = 0, 2, 4, 6, 24, 28, 30, and 48 hours. Samples for TSS were collected at t = 0, 1, 3, 6, 24, and 30 hours.

RESULTS AND DISCUSSION

Chitosan dosed into a coldwater recirculating system

In the preliminary study, where dissolved chitosan was added to a recirculating system (Figure 1), the recirculating water had become visibly clearer within 2 hrs of initiation of chitosan addition, and the culture tank TSS levels had dropped from 10.7 to 2.9 mg/L. However, fish began to show symptoms of distress after 2 hrs of exposure to chitosan, so the treatment was discontinued. Mortality of 4.6% was observed over the next 24 hours. Nitrification was not affected by the short-term dose of dissolved chitosan. The toxicity of dissolved chitosan to rainbow trout was a surprise based on the extensive literature review that had been conducted. Following this incident, detailed toxicity trials and histological examinations on rainbow trout indicated that dissolved chitosan concentrations as low as 0.019-0.038 mg/L caused lifting of lamellar epithelium, hypertrophy, and hyperplasia of lamellar epithelial cells while concentrations of 0.075 mg/L caused mortality after 24 hours (Bullock et al. 2000).

The preliminary study did indicate that dissolved chitosan improved TSS removal from the recirculating flow. However, additional tests were required to determine exactly how chitosan improved particle capture. Did dissolved chitosan coagulate particles and increase the rate that they settle or are they removed by microscreen filtration? Or, did chitosan cause particles to stick to the biosolids found in the recirculating system's fluidized-sand biofilter? In either case, the application of dissolved chitosan had now become of interest only from an effluent treatment stand-point. Therefore, in our subsequent studies, we applied dissolved chitosan to water that had been removed from the recirculating system to avoid further exposing fish to chitosan.

Jar test studies

Jar test results are shown in Table 1. A one-way analysis of variance was performed on the data. The TSS, color, or turbidity measurements were not found to be significantly different among levels of chitosan addition. We thought that chitosan may have inhibited particle settling by attaching to the particles and making them nearly neutrally buoyant. Our hypothesis was based on a report by Vaidya and Bulusu (1984) that

dissolved chitosan added to turbid water created a "floc [that] was light and settled slowly."

Of note, data from the zero chitosan jar tests (i.e., at the 0.0 mg/L chitosan dose in Table 1) indicated that an additional 44% of the TSS could be removed from the filtered water discharged from the microscreen filter if this water was then treated by a mixing and settling step - even without chitosan addition. While microscreen filtration is important to quickly remove cBOD and ammonia contained in the solids, this research indicates greater particle capture could be achieved by installing a mixing and settling step after the microscreen filter.

After the first jar test studies, we thought that the chitosan and mixing steps might be creating a larger floc that was not settling. To verify our hypothesis, in a second jar test study the full 2 L of water was removed from the jars after the 20 minute flocculation-mixing step was completed. This water was then passed through successively smaller filter screens. The water sample was passed through one screen at a time, starting with the largest, and then through screens with progressively smaller openings. The focus of this series of tests was to determine if chitosan addition changed the particle removal across the different sized screens. If chitosan addition increased the particle removal across the screens with the largest openings, then chitosan addition could be used to enhance solids removal efficiency using microscreen filtration.

The results from passing the flocculated water samples through progressively smaller screen openings indicated that the screen with the largest openings (i.e., 120 mm) captured nearly 80% of TSS in the flocculated water sample (Figure 2). TSS capture did not differ significantly among levels of chitosan addition, i.e., 0.00, 0.10, and 0.40 mg/L of chitosan dose (Figure 2). Therefore, there was no indication that chitosan addition produced a larger floc, which would improve particulate capture across a microscreen filter. Interestingly, these results also suggest that pre-treating water before it enters a microscreen filter with a 20 minute flocculation step could increase the TSS capture efficiency across a 120 μm sieve panel to approximately 80%. In contrast, without a 20 minute flocculation pretreatment step, the microscreen filters that contained 80 μm sieve panels only removed 50-60% of the TSS loading within the recirculating system (Heinen et al. 1996b).

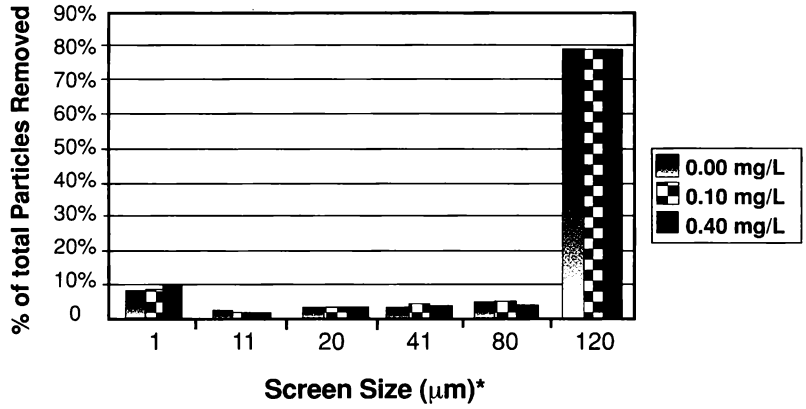

Figure 2: Percentage of total particles removed by each screen (by mass) at the three dose levels of chitosan applied to fish tank effluent.

Sweep floc removal of TSS within pilot-scale fluidized-sand biofilters

After concluding the jar test studies, the use of dissolved chitosan would probably not have been deemed viable for commercial scale aquaculture. However, we observed a large increase in water clarity and reduced TSS concentration following only 2 hours of chitosan addition to the recirculating system. After ruling out the possibility that chitosan increased TSS removal across the drum filter, it was determined that TSS capture within the fluidized-sand biofilter was the most likely explanation of the solids removal that occurred in the preliminary study.

Recirculating system water was pumped through three replicated pilot-scale fluidized-sand biofilter columns to determine if either dissolved chitosan or acetic acid (at a concentration equivalent that in the dissolved chitosan dose) increased TSS capture across the biofilter columns, changed the bed expansion and growth within biofilter columns, or inhibited nitrification activity.

While all columns removed TSS (Tables 2 and 3), addition of dissolved chitosan caused the fluidized-sand biofilter to remove 2-3 times more TSS than the columns dosed with the acetic acid and the columns that had no acetic acid or chitosan addition. The columns dosed with 0.44-0.55 mg/L of dissolved chitosan produced effluent TSS concentrations that were 1.7-2.2 mg/L (Table 2), which indicates the presence of an effective TSS capture mechanism within the expanded bed. In addition, the dissolved chitosan doses applied did not negatively affect the total ammonia nitrogen (TAN), nitrite nitrogen, dissolved oxygen, or pH of the water discharged from the biofilter columns (Tables 4-7).

Parameter	Before jar test	Following jar test at each dose of dissolved chitosan (mg/L) 0.000	0.025	0.050	0.10	0.20	0.40
TSS (mg/L)	9.8±0.7	5.5±0.2	5.4±0.3	5.4±0.3	6.1±0.3	6.1±0.2	6.3±0.3
True color (Pt-Co)	18±1	17±1	17±1	17±1	17±1	16±1	16±1
Turbidity (NTU)	3.3±0.3	2.6±0.1	2.5±0.1	2.4±0.1	2.6±0.1	2.6±0.1	2.6±0.1

Table 1: TSS, color, and turbidity levels (Mean ± SE) of water samples taken from the recirculating system (after the microscreen filter) both before and after the samples had been jar tested at each dissolved chitosan dose.

		Effuent of fluidized-sand biofilter		
	Influent	No dose	Acetic acid only	Dissolved chitosan
Trial 1	7.4 ± 0.3	5.0 ± 0.4	not tested	2.2 ± 0.2
Trial 2	5.1 ± 0.2	4.0 ± 0.1	4.1 ± 0.2	2.2 ± 0.3
Trial 3	5.5 ± 0.2	3.2 ± 0.2	3.2 ± 0.2	1.7 ± 0.2

Table 2: Mean (± SE) fluidized-sand biofilter influent and effluent TSS concentrations (mg/L) measured from 1 to 48 hours after the initiation of chitosan or acetic acid dosing.

	No dose	Acetic acid only	Dissolved chitosan
Trial 1	33 ± 7	not tested	70 ± 7
Trial 2	20 ± 4	22 ± 3	62 ± 5
Trial 3	44 ± 3	44 ± 3	72 ± 5

Table 3: Mean TSS capture efficiency (% ± SE) across the fluidized-sand biofilter columns measured from 1 to 48 hours after the initiation of chitosan or acetic acid dosing.

		Effuent of fluidized-sand biofilter		
	Influent	No dose	Acetic acid only	Dissolved chitosan
Trial 1	10.6 ± 0.06	7.0 ± 0.08	not tested	6.9 ± 0.08
Trial 2	10.6 ± 0.04	7.7 ± 0.04	7.6 ± 0.05	7.6 ± 0.05
Trial 3	10.3 ± 0.06	7.2 ± 0.09	6.9 ± 0.05	6.8 ± 0.04

Table 4: Mean (% ± SE) dissolved oxygen across the fluidized-sand biofilter columns measured from 1 to 48 hours after the initiation of chitosan or acetic acid dosing.

		Effluent of fluidized-sand biofilter		
	Influent	*No dose*	*Acetic acid only*	*Dissolved chitosan*
Trial 1	7.4 ± 0.3	5.0 ± 0.4	not tested	2.2 ± 0.2
Trial 2	5.1 ± 0.2	4.0 ± 0.1	4.1 ± 0.2	2.2 ± 0.3
Trial 3	5.5 ± 0.2	3.2 ± 0.2	3.2 ± 0.2	1.7 ± 0.2

Table 5: Mean (% ± SE) fluidized-sand biofilter influent and effluent pH measured from 1 to 48 hours after the initiation of chitosan or acetic acid dosing.

		Effluent of fluidized-sand biofilter		
	Influent	*No dose*	*Acetic acid only*	*Dissolved chitosan*
Trial 1	0.42 ± 0.01	0.04 ± 0.00	not tested	0.04 ± 0.01
Trial 2	0.36 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
Trial 3	0.39 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01

Table 6: Mean (% ± SE) fluidized-sand biofilter influent and effluent TAN measured from 1 to 48 hours after the initiation of chitosan or acetic acid dosing.

		Effluent of fluidized-sand biofilter		
	Influent	*No dose*	*Acetic acid only*	*Dissolved chitosan*
Trial 1	0.020 ± 0.000	0.005 ± 0.000	not tested	0.003 ± 0.000
Trial 2	0.021 ± 0.001	0.006 ± 0.000	0.005 ± 0.000	0.003 ± 0.000
Trial 3	0.027 ± 0.001	0.007 ± 0.001	0.005 ± 0.000	0.003 ± 0.000

Table 7: Mean (% ± SE) fluidized-sand biofilter influent and effluent nitrate concentrations (mg/L) measured from 1 to 48 hours after the initiation of chitosan or acetic acid dosing.

The fluidized-sand biofilter bed exposed to the 0.44-0.55 mg/L of dissolved chitosan feed initially contracted (Figure 3). However, because of the higher TSS capture rate within the chitosan-dosed column, the fluidized bed depth in the chitosan-dosed column grew faster and eventually equaled the depth of the other two treatments at the end of the experimental period (Figure 3). It remains to be investigated what will happen to the solids over a longer dosing period and how those solids will be managed.

The dissolved chitosan appears to have adsorbed to particles in the fluidized-sand biofilter, which created a novel type of upflow 'sludge blanket clarifier' utilizing the biosolids blanket contained in the fluidized bed. With dissolved chitosan creating particle coagulation, the fluidized-

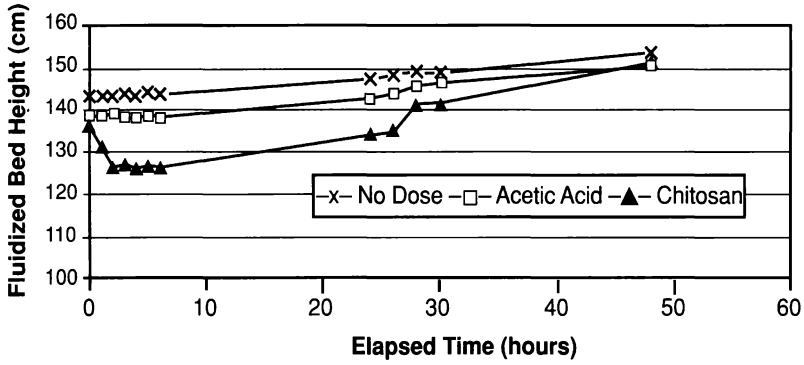

Figure 3: Expanded bed height measured within the pilot-scale fluidized-sand biofilter columns after chitosan or acetic acid dosing had begun, i.e., at time = 0.0 hours.

sand biofilter performed as an upflow 'sludge blanket clarifier' somewhat similar to a solids contact unit that recirculates settled solids, as described by Culp et al. (1978). This preliminary study indicates that fluidized-sand biofilters could be used as a type of upflow 'sludge blanket clarifier' to remove both dissolved and particulate wastes from the effluent of a recirculating aquaculture system. Use of a fluidized-sand biofilter in this application would have advantages because the expanded bed would be non-plugging and the unit would be relatively simple to operate because it would never require backwashing. Biosolids captured in the expanded bed would be simply siphoned off of the top of the bed in a manner similar to that which is used to remove bed growth in commercial fluidized-sand biofilters (Summerfelt et al. 2001).

CONCLUSIONS AND RECOMMENDATIONS

Chitosan was observed to be acutely toxic to rainbow trout at low levels (<1 mg/L). Therefore, dissolved chitosan should not be added to aquaculture systems containing rainbow trout. It is unknown whether dissolved chitosan is as toxic to other aquatic species. Although dissolved chitosan addition was not effective at removing solids when evaluated in jar tests, dissolved chitosan did show promise in an unexpected manner. When dissolved chitosan was added to the water discharged from a recirculating system before passing this flow through a fluidized-sand biofilter, the dissolved chitosan increased the capture of TSS within the expanded bed. Thus, adding dissolved chitosan to water flowing into a fluidized-sand biofilter turned the biofilter into a novel

type of upflow 'sludge blanket clarifier,' which appears to be both non-plugging (because it is a fluidized bed) and relatively simple to operate because it would never require backwashing. In addition, the dissolved chitosan did not affect the nitrification across the fluidized-sand biofilter. Therefore, there is potential for the over-topping effluent from a recirculating system to be treated for both dissolved wastes (e.g., TAN and soluble BOD) and TSS by adding low levels (~0.5 mg/L) of dissolved chitosan to the flow before it passes through a fluidized-sand biofilter.

Additional work is necessary to:

- Determine the effects of dissolved chitosan addition on long-term fluidized-sand biofilter operation, especially to validate the non-effect on nitrifying bacteria and to identify suitable bed management routines.
- Estimate the cost of chitosan addition in a full-scale commercial application.
- Ascertain how much dissolved chitosan passes through a fully developed fluidized-sand biofilter to determine if there would be potential for chitosan toxicity problems encountered after this effluent is discharged.
- Identify other polymers that are non-toxic to fish and humans and would coagulate TSS within fluidized-sand biofilters.
- Test alternate solvents for chitosan such as formic acid or malic acid, because chitosan dissolved with malic acid has been reported to be non-toxic to fathead minnows (Technical Data Sheet Sea Klear Chitosan Toxicity Data, 11/8/96, Vanson, Redmond, WA, USA).

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