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Aquaculture Solids Management Using A Combination of Sand/Gravel or Unwoven Fabric Bed With *Lolium perenne* Lam as A Plant Biofilter[1]

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Objective This work is an evaluation of the efficiency of a sand-gravel or unwoven fabric bed system and *Lolium perenne* Lam as plant biofilter in the reduction of solids and nutrients removal from aquaculture discharge water. **Methods** The first step consisted of the collection of wastewater in the tank and the distribution at three different hydraulic loading regimes (0.5, 1, 1.5 L/hour) to the different experimental systems. The second step was to evaluate the performance of the different systems. The first system consisted of a bucket filled with a substrate of sand/ gravel (20 cm in depth), on the bottom of which was a 80 mesh/ inch^2 of nylon (S1); the second was similar, but was planted with *Lolium perenne* lam (S2); the third was planted with a grass plate consisting of 7 layers of unwoven fabric planted with *L. perenne* (S3). **Results** The second system showed the best performance in reducing solids as well as in nutrients (TN, TP, and COD) reduction. The removal rates for TS, TN, and TP were negatively correlated with the loading regimes, with 0.5 L/hour being the most efficient and thus taken as the reference. **Conclusions** Solids management using a sand/gravel substrate as bed culture and *Lolium perenne* L. as plant biofilter has proved to be an efficient technique for solids reduction with low operating cost. This grass plays an important role in wastewater eco-treatment by absorbing dissolved pollutants (TAN) as nutrients for its growth.

Key words: *Lolium perenne* lam; Hydraulic loading regimes; Microcosm

INTRODUCTION

Solids and /or dissolved nutrients from intensive aquaculture facilities, if left untreated, have a negative effect on the receiving water bodies. Solids management is becoming an urgent concern in developing countries as well as in industrial ones. Effluents from aquaculture ponds are typically abounding in suspended organic solids, carbon, nitrogen and phosphorus[1]; while the high amount of solids present in waste effluents is potentially one of the most important environmental problems of aquaculture[2]. Suspended matter in aquacultural systems produres negative effects on aquaculture systems and, if released into the environment, is detrimental to aquatic habitats. It has also been demonstrated that other pollutants (nutrients) are associated with elevated total suspended solids (TSS) concentrations[3]. High concentrations of suspended solids should be avoided as they form an additional source of ammonia, which in its unionized form is highly toxic to the fish[4].

Solids removal is accomplished mainly by sedimentation and mechanical filtration. However, these techniques alone are less effective in reducing TN and TP. Improvements in nutrient removal may be achieved by the development of systems that are more actively managed than passive sedimentation ponds[5].

Solids management is often designed to be combined with other unit processes for the control of dissolved nutrients, biochemical oxygen demand (BOD), dissolved gases (O_2 and CO_2), pH, and pathogens[6]. Solids management actually consists of a series of stages and our proposed approach can be considered as one of the unit processes in the efficient removal of solids from aquaculture discharge. We used *L. perenne* due to its tolerance to wide range of environmental conditions and the fact that it is frequently used in China for high-quality grass turf production.

This work is an evaluation of the efficiency of a sand-gravel or unwoven fabric bed system and *Lolium perenne* lam as a plant biofilter in the

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reduction of solids and nutrients removal from aquaculture discharge water.

MATERIAL AND METHODS

The experiment was carried out in the key laboratory (glasshouse) of the Environmental Engineering and Eco-Agriculture Department in Zhejiang University (China) from April to July 2005.

Our design consisted of a system that operated in a down flow mode under constant head pressure. The influent used in the tests was wastewater from a turtle pond while the plant selected was *L. perenne* lam.

The first step consisted of collecting the wastewater into the tank and then distributing it to the different experimental systems for further treatment. The second step was the determination of the performance of the different systems in solids reductions. The loading capacity and the hydraulic retention time were monitored adequately.

The tank used was a plastic container (500 L) with a pipe that ends on a spray tap connected to a timer for monitoring wastewater release (Fig. 1). Three settings of the timer were applied in order to monitor the wastewater released in each bucket. The timer was set at 1/60, 1/30, and 1/20 min on/off and each opening released 0.5 L, corresponding to the three different hydraulic loading regimes, 0.5, 1, and 1.5 L per hour.

Three laboratory-scale microcosm systems were used in the tests. The first system consisted of a bucket filled with a substrate of sand/ gravel (20 cm in depth), on the bottom of which was a 80 mesh/ inch^2 of nylon. The second system was similar but was planted with *L. perenne*. The third system was planted grass plate consisting of 7 layers of unwoven fabric planted with *L. perenne* (Fig. 2).

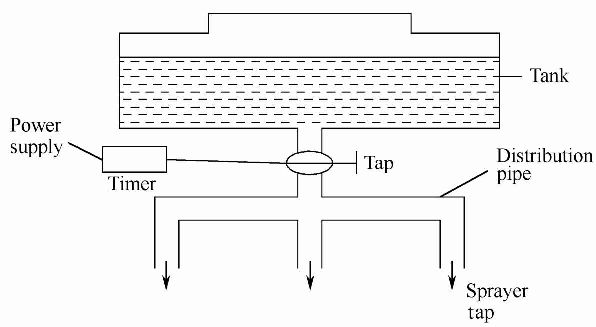


FIG. 1. Design of wastewater collection and distribution.

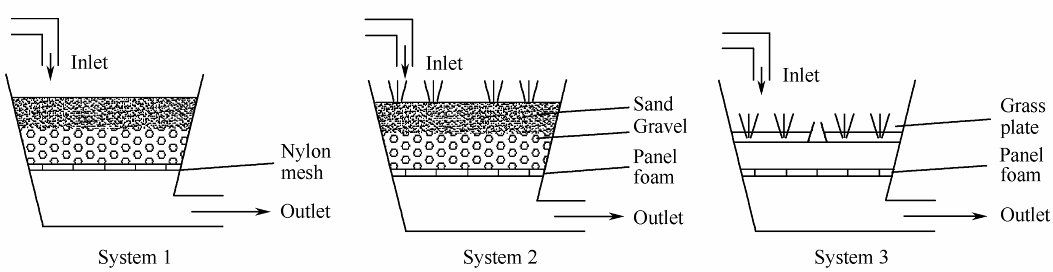


FIG. 2. The three different systems for solids reduction.

The sand and gravel were put in successive layers in order to promote the uniform distribution of the flow. The bottom of each bucket was provided with a hollowed foam panel to allow the outflow join the outlet pipe. The influent wastewater was from the turtle aquaculture pond. Three replicates were done for each experimental system and the duration of the trial was 21 days for each loading capacity. A two-way ANOVA analysis was performed to assess the solids and nutrients removal efficiencies.

The samples of the influent and effluent wastewater were analyzed every three days for TN (total nitrogen), TP (total phosphorus) and TAN (total ammonia nitrogen). Total solids (TS), volatile solids (VS), and suspended solids (SS) of the inflow and outflow were also determined after every two days. TS was determined by gravimetric methods, TVS and TSS were measured using standard methods[7]. TN was determined by alkaline potassium persulfate digestion-UV spectrophotometric method, TAN was measured using Nessler's reagent colorimetric method and TP was determined using ammonium molybdate spectrophotometric method. COD was analyzed using the dichromate method.

RESULTS

The three systems were identified as S1, the bucket without plant, S2, the bucket with sand/gravel as substrate with plants, and S3, the grass plate system with unwoven fabric. Wastewater from the tank was released at three different regimes to the S1, S2, and S3 systems. The three different load regimes were identified as C1 (0.5 L/hour), C2 (1 L/hour) and C3 (1.5 L/hour).

The removal rate was determined by subtracting the influent concentration from the effluent concentration, divided by the influent concentration and multiplied by 100 to express it as a percentage for TS, TN, TP, and TAN.

The best removal rates were observed at C1 in all the systems for TS, TN, TAN, and TP (Table 1). Therefore, more attention was paid to this hydraulic loading regime (C1= 0.5 L/hour) to be applied for further investigations. The system S2 performed well in terms of solids reduction. The efficiency of the systems in nutrients removal varied with the systems applied and the nutrient considered.

TABLE 1

TS, TN, and TP Removal Rates (%) for the Three Systems at Three Different Loading Capacities

Parameters		Loading Regimes→Systems		
		C1	C2	C3
TS	S1	51.55 (1.59)^{c}	33.4 (1.11)^{c}	18.33 (0.51)^{c}
	S2	63.52 (1.60)^{a}	44.4 (1.45)^{a}	32.50 (0.70)^{a}
	S3	56.80 (0.92)^{b}	40.33 (1.25)^{b}	26.50 (0.70)^{b}
TN	S1	43.53 (0.83)^{c}	33.52 (1.66)^{c}	23.73 (0.97)^{c}
	S2	72.52 (1.66)^{a}	51.66 (1.55)^{a}	37.50 (1.35)^{a}
	S3	71.34 (1.15)^{b}	48.63 (1.65)^{b}	33.77 (0.92)^{b}
TP	S1	55.47 (1.65)^{b}	43.4 (1.31)^{b}	40.43 (1.72)^{b}
	S2	80.71 (1.85)^{a}	66.26 (2.10)^{a}	58.5 (0.91)^{a}
	S3	46.6 (1.55)^{c}	38.46 (1.65)^{c}	31.63 (1.65)^{c}
TAN	S1	11.45 (0.69)^{c}	10.53 (0.83)^{c}	10.4 (0.72)^{c}
	S2	84.67 (0.75)^{a}	78.43 (1.26)^{a}	72.69 (1.76)^{a}
	S3	81.83 (0.80)^{b}	76.53 (1.20)^{b}	70.91 (2.89)^{b}

Note. The values in parentheses represent the standard deviations of the means. ^{a,b,c}: significantly different at *P*=1% (Tukey multiple range test).

The removal rates for TS, TN, and TP were negatively correlated with the loading regimes, although the correlation of TAN removal and the loading regime was less pronounced (Fig. 3).

For C1 loading regime, S2 showed the best efficiency in solids reduction for TS, TVS, and TSS (Table 2).

TABLE 2

Solids Reduction in the Three Different Systems at C1

Systems	TS (mg/L)	VS (mg/L)	SS (mg/L)
Influent	865 (10)	415 (5.03)	122.6 (4.16)
Effluent			
S1	420 (5)	397 (4.35)	43.6 (1.34)
S2	317.3 (12.5)	256.3 (18)	24 (3)
S3	372.3 (17.5)	353.6 (5.13)	33.7 (2.31)

Note. The values in parentheses represent the standard deviation of the means.

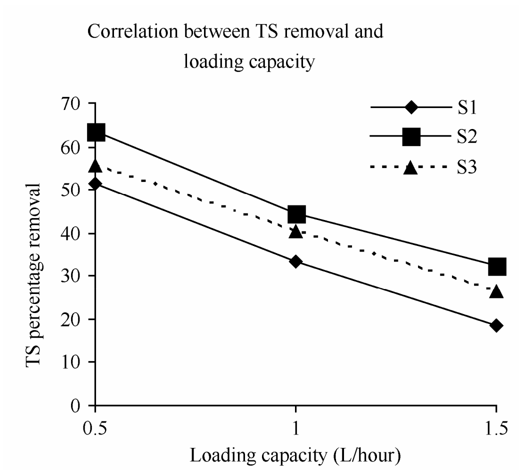
There was a general alleviation of all the nutrients considered (TN, TP, TAN, and COD) for all of the systems, but the reduction was more pronounced for S2 and S3 than for S1 (Table 3).

TABLE 3

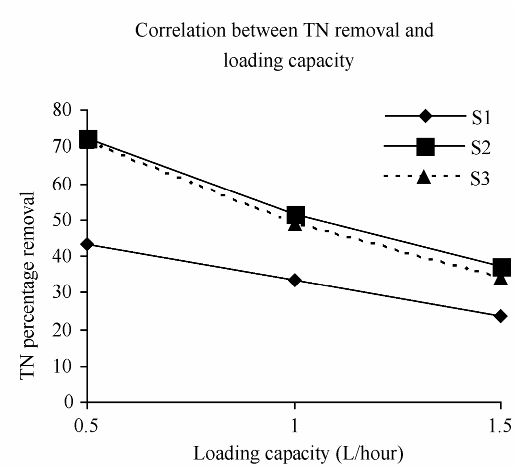
Nutrients Fluctuations in the Three Different Systems at C1

Systems	TN (mg/L)	TP (mg/L)	TAN (mg/L)	COD (mg/L)
Influent	17.1 (1.3)	4.7 (0.5)	7.1 (0.21)	196 (9.6)
Effluent				
S1	9.6 (0.8)	2.1 (0.09)	6.3 (0.3)	118 (7.6)
S2	4.7 (0.5)	0.9 (0.1)	1.1 (0.4)	71.3 (5.8)
S3	4.9 (0.7)	2.5 (0.09)	1.3 (0.3)	74.7 (6.3)

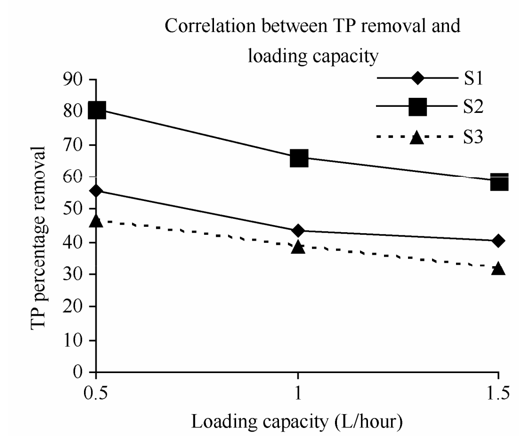
Note. The values in parentheses represent the standard deviation of the means.



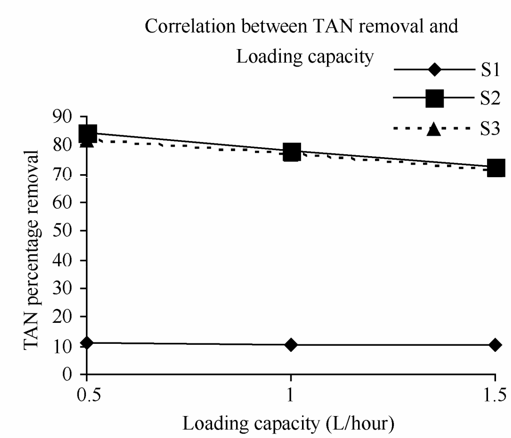
TS: S1: y=-16.61x+67.64; R^2=0.997
S2: y=-15.51x+77.82; R^2=0.982
S3: y=-14.65x+70.17; R^2=0.999



TN: S1: y=-9.9x+53.39; R^2=1
S2: y=-18.78x+88.81; R^2=0.985
S3: y=-17.51x+88.91; R^2=0.987



TS: S1: y=-7.52x+61.47; R^2=0.891
S2: y=-11.1x+90.68; R^2=0.970
S3: y=-7.48x+53.86; R^2=0.999



TS: S1: y=-0.5x+11.77; R^2=0.845
S2: y=-5.99x+90.56; R^2=0.999
S3: y=-5.31x+86.93; R^2=0.999

FIG. 3. Correlation between TS, TN, TP, and TAN and loading regimes.

DISCUSSION

Statistical analysis showed that S2 (grass planted sand/gravel system) presented the highest efficiency in percentage removal for TS, TN, and TP. This system was followed by S3 (grass plate on unwoven fabric) and lastly S1 (sand/gravel system). The three systems showed different capabilities in TS, TVS, and TSS reduction. The undissolved solids produce new pollutants consisting of nitrogen (N) and phosphorus (P) as major components[8]. Therefore, the removal of TS and TSS from the system corresponded with the reduction of TN and TP concentrations. Cripps *et al.*[6] reported that about 7%-32% of the TN and 30%-84% of the TP are in the particulate fraction while the remainder is transported out of the farm in the dissolved fraction. The decrease in TN, TP, and TAN concentrations also reflects the possibility that most pollutants (or nutrients) are absorbed by the grass for growth and metabolic needs during the experimental period. However, Rababah *et al.*[9] pointed out that the removal of most of phosphorus in the system is due to surface sorption.

TAN removal rate is mainly related to the planted systems (S2 and S3). The reduction of TAN may result from the plant assimilation rather than from the systems themselves. *L. perenne* plays an important role in wastewater eco-treatment by absorbing dissolved pollutants as nutrients for growth. However, Yang *et al.*[10] suggested that the main removal mechanism for TAN is nitrification, which can be enhanced by the root zone effect in the vegetated gravel-bed wetland systems, while NO^{3-}-N is removed mainly by denitrification and plant uptake in vegetated systems. Thus, an obvious reduction in TN was observed.

In addition, *L. perenne* can be utilized as a culture media for the removal of undissolved solids from wastewater by trapping back some solids. When the grass is cultured on the system, the dense grass roots system acts as a filter, allowing water to pass through while some undissolved solids agglomerate around the root. The root network increases with plant age and the performance of the system is enhanced by the density of the root system. Since *L. perenne* is a perennial plant it can continuously grows as long as climatic conditions are favorable. Adler *et al.*[11] reported that a biweekly harvest by cutting grass (*Phalaris arundinacea* L., *Agrostis alba* L. and *Poa trivialis* L.) to a uniform 7.6 cm height not only promotes root expansion but also increases nutrient removal; thus we recommend a periodical grass clipping for the same purpose.

The root zone effect (RZE) in vegetated gravel-bed wetland systems not only helps to transfer more oxygen down into the stratum media, but also allows more aerobic microbes to grow in the porous gravel media near the root zone[10]. The decrease in COD concentration is a result of the removal of a great fraction of organic compounds from the system. Since little dissolved COD was expected to be removed by physical mechanisms, the increased removal of dissolved COD within the system cells was likely due to better microbial treatment that occurred as the water percolated down through the sand and gravel layers of the vertical flow wetland[12].

The efficiency of solids adsorption and binding to the surface particles depends on some characteristics of the medium[13]; therefore after some time, a possible clogging may be expected. However, the accumulated large particle size solids consist of protein-rich organic matter, which, when broken down, releases large amount of ammonia[14], that is taken up by *L. perenne*. Although the system is efficient in terms of TAN removal and solids reduction, it needs to be refreshed regularly with a new set of plant filter bed. The relative replacement period is determined both by the total solids load of wastewater and the plant age.

The removal rate efficiency was negatively influenced by the increasing hydraulic loading regime. We observed a decrease in solids reduction with gradual loading regime increase and the nutrients removal following the same trend as that of the solids. In fact, the loading regime determines the up and down flow speed through the different established systems. In addition, increasing the wastewater loading regime decreased the hydraulic retention time of the different systems. This hydraulic loading strategy is recommended to alternate flooding and drying intervals to enhance plant growth and sludge stabilization by air- and photo-oxidation[12].

CONCLUSIONS

This system is an appropriate technology for wastewater treatment in rural areas and small communities. However, the system requires the plant filter bed to be renewed regularly. Solids management using a sand/gravel substrate as bed culture and *Lolium perenne* L. as plant biofilter has proved to be an efficient technique with low operating cost. The solids removal (TS, TSS, and TVS) as well as the reduction of TP and TN in wastewater eco-treatment are enhanced by *L. Perenne*, which absorb dissolved pollutants as nutrients for its growth. The effectiveness of this technique is negatively correlated with the increasing hydraulic loading regime from the inlet.

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