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Pilot and farm-scale microbial and chemical treatment of waste

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Abstract

A range of strategies are needed to ensure flexible and effective treatment of waste from catfish ponds at a range of different types of operations and throughout the year. A diverse range of chemical, microbial and biochemical products may be suited for this purpose, including a number that are commercially available, but their efficacy has not been independently tested. The soil bacteria, *Pseudomonas stutzeri*, was isolated from catfish ponds and tested for its ability to reduce NH_4-N loads in waste water. In laboratory tests at Cantho University promising denitrification activity was obtained but this could not be reliably reproduced in studies at Murdoch University or in tests at CLRRI. Commercially available preparations for waste water treatment were found to be variably effective, but the most promising materials assessed were the biological preparations from plant materials, Yucca extract and crushed *Moringa oleifera* seeds. Ozone treatment of the wastewater appears to have a limited role in oxygenation of the wastewater and reducing NH_4-N. Aquatic plants with or without fish such as tilapia were promising options for improving water quality in settling ponds. Despite the diverse range of approaches examined no single technology was developed to a point where scale-up to field or operational scale was feasible.

Part 1: Isolation of *Pseudomonas stutzeri* in wastewater of catfish fish-ponds in the Mekong Delta and its application for wastewater treatment

This work was published in full by Cao et al. (2009)[1] and a copy is appended (Appendix 1). A brief summary of the findings are outlined below.

The presence of nitrogen in wastewater discharge is undesirable for several reasons; free ammonia is toxic to fish and many other aquatic organisms and the presence of nitrite and nitrate ions in drinking water is a potential health hazard (Obaja et al., 2003). High N (especially ammonia) in fish-pond water may be toxic to fish and it may cause eutrophication in canals, streams, small rivers. Ammonia concentration in wastewater from catfish-ponds varies from 0.6 to12.0 mg/L. When concentration is higher 7 mg/L the health of catfish is impaired and at >10 mg/L, catfish will die. However, a low ammonia level (0.2–2.0 mg/L) is beneficial for the health of catfish (Boyd, 1998). Excessively high ammonia levels threaten the quality of water used for household purposes by many rural families.

Nitrification is the process of converting ammonia to nitrate via nitrite and is mediated by two groups of chemolithoautotrophic bacteria, ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (Park and Noguera, 2007). Denitrification is part of the bioenergetic apparatus of the bacterial cell, where the N oxidation of nitrate and nitrite and the gaseous N oxides, NO and N_2O, serve in lieu of O_2 as terminal acceptors for electron transport phosphorylation. *Pseudomonas stutzeri* was proposed in 1983 as model organism for denitrification research (Lalucat et al., 2006). *P. stutzeri* has a wide environmental distribution but is formed mainly in soil and water and it serves as a model for the study of the biochemistry and genetics of denitrification and natural transformation processes (Rius et al., 2001). Nitrogen removal from wastewater is generally achieved by a combination of nitrification and denitrification processes. *P. stutzeri* has been shown to be involved in nitrification and denitrification processes as well as in the degradation of environmental pollutants. *P. stutzeri* strains have been used successfully to treat wastewater in many developed countries (Su et al., 1997, 2001).

The aim of this study was to explore the potential for reducing soluble N load in fishpond wastewater using naturally occurring denitrifying bacteria, *Pseudomonas stutzeri*. Twenty-seven isolates were selected from in wastewater (liquid/solid) of catfish-ponds located along the Tien river, in the Mekong Delta, Vietnam in SW-LB medium (artificial seawater Luria-Britani medium) supplemented with 10 mM NH_4 and NO_3 and twenty-five isolates were identified as *Pseudomonas stutzeri* based on similarity of PCR-16S rRNA using universal primers and specific primers.

To evaluate ammonia removal from wastewater of fish-ponds, the study examined nitrogen removal ability of *P. stutzeri* isolates in wastewater with high ammonia levels in the laboratory [in 50 mL, 10 L, and 500 L containers]. pH, ammonia, nitrite, nitrate, and the population of denitrifying bacteria were recorded during the experiment and BOD (Biological Oxygen Demand), TSS (Total suspended solids), EC, PO4_, TOC (Total of Organic Carbon), population of Escherichia coli and Salmonella were recorded before and after experiments. All experiments were carried out with a completely randomized design with four replications; 4 % inocula

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[1] Cao Ngoc Diep, Pham My Cam, Nguyen Hoai Vung, To Thi Lai, Nguyen Thi Xuan My (2009). . Isolation of Pseudomonas stutzeri in wastewater of catfish fish-ponds. Bioresource Technology doi:10.1016/j.biortech.2009.02.021

were used in four experiments without aeration.

Four isolates were more effective in lowering soluble N (NH_4, NO_2 and NO_3) levels in fishpond water than control solutions and lowered NH_4-N from 10 mg/L to negligible amounts after four days.

Further experiments are undertaken at Murdoch University to determine the fate of N lost from solution and the relative activity of ammonia oxidation, and nitrite and nitrate reduction by *P. stutzeri* isolates. Results proved inconclusive. At 1580 mg NH_4/L, the strains were ineffective in reducing soluble N. At 10 mg NH_4/L, growth of three isolates was poor and no decrease in NH_4-N was detected.

Using cultures of *P. stutzeri* provided by Cantho University, effects of inoculation of fishpond wastewater were investigated at CLRRI. It was found that control wastewater declined in NH_4-N are quickly as the treatments with *P. stutzeri* inoculation. Consequently, it was decided to cease further work on the use of *P. stutzeri* to decrease NH_4 levels in wastewater.

The time spent on exploring this initially promising research lead with *P. stutzeri* ultimately limited the potential to thoroughly explore other options for microbial, biological or chemical treatment of waste water.

Part 2: Chemical, microbial and biological treatment of wastewater:

A range of commercial products are available claiming anti-microbial activties that decrease bacterial counts and improve water quality in catfish ponds. Some of these are in common use as reported in the survey conducted in Cantho and An Giang provinces (Cao and Bell, 2009). An evaluation of commercial bacterial inoculants was undertaken at CLRRI to determine their efficacy in decreasing bacterial counts and soluble N and P in wastewater. These were compared with chemical treatments such as calcium hypochlorite and extracts of the plants, Yucca and *Moringa oleifera* in three successive experiments.

Experiment 1:

Wastewater, collected at fish farm nearby CLRRI, was kept in 24 flasks of 500 ml each. Five waste water treatments and a control of this experiment as details given below:

1. Benzalkonium chloride (BKC) (80% 2.8.1.1. BKC): for control of bacteria, and algae used in the 1st treatment at concentration of 0.33ppm for T1 (the recommended dosage 0.8 -1.2 L/ 3,000 m^3 water)

2: Protectol (1,5 – Pentanedial): for control of bacteria at a dosage of 0.5 ppm for T2 (the recommended dosage 1 L/1,500 – 2,000 m^3 water)

3. CAP 2000 (11.2 % sodium thiosulfate, 4.8 % sodium lauryl sulfate, 1.02 % ethylene diamine tetraacetatic acid): for flocculation and sedimentation of suspended solids at a dosage of 2 ppm for T3 (the recommended dosage 2 – 3 L/1,600 m^3 water)

4. Chlorine (calcium hypochlorite) is a disinfectant at dosage of 1/1.000 for T4

5. Moringa oleifera (crushed seeds) water treatment at dosage of 1/1.000 for T5

6. Control (no water treatment) for T6

Experiment was laid out in complete randomized design with four replications. Ammonium, nitrite, nitrate anf phosphate were analysed following the methods of Indophenol blue, Greiss Hosvay, salicylate, and molybden blue; respectively. Water samples were taken after 1, 2, 3, 4 and 5 days for analysis of these mentioned characters. In addition to chemical analysis, microbial counts of different treatment was made at the beginning and end of this experiment.

Results

Ammonium concentrations declined gradually over the 5 day period (Table 1). By contrast, there was a 75 % reduction in ammonium with moringa seed and a 30-40% reduction with CAP, Protectol and chlorine. BKC had no initial effect on ammonium but by 5 days had reduced it to half the level in the control. The final ammonium values on day 5 were lowest in Protectol, moringa and chlorine treatments with concentrations less than 20 % of the control values.

Table 1 Ammonium concentration (mg/L) in waste water under different treatments over times

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5

BKC	2.083	1.681	1.447	1.216	0.879
CAP	1.400	0.985	0.701	0.558	0.718
Protectol	1.110	0.950	0.537	0.373	0.323
Moringa Seed	0.506	0.463	0.350	0.344	0.335
Chlorine	1.274	0.833	0.742	0.435	0.275
Control	2.217	2.026	1.994	1.844	1.653
CV %	6.6	7.5	10.0	13.7	19,2
5% LSD	0.171	0.158	1.175	0.198	0.244

The moringa seed preparation was most effective in decreasing solution nitrate, which was almost undetectable within 1 day of application (Table 2). By contrast other treatments only produced a 30-50 % decrease in nitrate concentrations on day 5 compared to the control. Nitrite concentrations were relatively low throughout even in the control and only moringa treatment decreased the levels at day 5 (Table 3).

Table 2 Nitrate concentration (mg/L) in waste water under different treatments over times

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
BKC	0.129	0.096	0.275	0.297	0.229
CAP	0.101	0.101	0.314	0.408	0.324
Protectol	0.098	0.112	0.273	0.258	0.210
Moringa Seed	0.013	0.0096	0.0023	0.0017	0.0043
Chlorine	0.465	0.482	0.420	0.426	0.355
Control	0.442	0.432	0.427	0.419	0.403
CV %	22.9	17.7	16.7	17.1	17.2
5% LSD	0.087	0.066	0.086	0.094	0.025

Table 3 Nitrite concentration (mg/L) in waste water under different treatments over times

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
BKC	0.055	0.095	0.063	0.042	0.034
CAP	0.055	0.185	0.562	1.100	0.046
Protectol	0.056	0.183	0.524	1.104	0.068
Moringa Seed	0.060	0.064	0.051	0.025	0.013
Chlorine	0.086	0.102	0.083	0.063	0.028
Control	0.095	0.066	0.058	0.051	0.051
CV %	35.8	6.4	23.2	25.2	18.0
5% LSD	0.044	0.013	0.095	0.182	0.022

Moringa seed more than doubled solution P but along with Protectol and BKC were the only products to decrease day 5 levels of P (Table 4). Chlorine markedly reduced initial P concentrations, but final levels exceeded those in the control unlike other treatments.

There was no major effect of the treatments on total bacterial counts after 5 days (Table 5). Total bacterial counts remain 6.5-7.5 x 10^6 after treatment, only 30 % less than the control.

Table 4: Phosphate concentration (mg/L) in waste water under different treatments over times

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
BKC	0.404	0.379	0.408	0.349	0.186
CAP	0.247	0.374	0.472	0.314	0.258
Protectol	0.370	0.373	0.416	0.266	0.127
Moringa Seed	0.865	2.727	1.244	0.398	0.092
Chlorine	0.090	0.051	0.094	0.795	0.647
Control	0.368	0.350	0.352	0.343	0.345
CV %	31.5	10.3	16.0	51.9	26.2
5% LSD	0.224	0.133	0.145	0.388	0.131

Table 5. Effect of wastewater treatments on total bacterial counts after 5 days.

Treatment	Bacteria population (CFU/ml)
Control	9.24×10^6
BKC	7.52×10^6
CAP 2000	7.30×10^6
Protectol	7.12×10^6
Moringa seed	6.52×10^6
Chlorine	7.22×10^6

Moringa seed preparation was the most effective treatment in reducing NH_4-N, NO_3-N (1 day) and P (after 5 days) concentrations in wastewater. Calcium hypochlorite was effective in decreasing NH_4-N but slower to act than moringa and ineffective in decreasing NO_3-N or P. BKC which is commonly used by catfish farmers was not effective Protechol was more effective than BKC and CAP200, but none of these were as consistently effective as crushed moringa seed.

Experiment 2

Treatments

1. Vime-Bitech (*Lactobacillus ssp, Bacillus spp, Saccharomycess cerevisiae*, enzyme protease, enzyme amylase,enzyme cellulose, enzyme lipase, enzyme pectinase): for decomposition, digestion enhancement at a dosage of 1kg/250-500kg feed or 1kg/3000-6000m^3 water.

2. Vime- Subtyl (*Bacillus subtilis)*: for N decomposition at a dosage of 1kg/3000-6000m^3 water or 1kg/400-500kg feed

3. Vime- Yucca (Yucca extract): for reduction of NH_3 in fishpond water at a dosage of 1 L/ 3000-4000 m^3 water

4. Zimovac (*Lactobacillus ssp, Bacillus ssp, Nitrosomonas, Nitrobacter*, lactose buffer): for residue decomposition to control ammonia (NH_3), nitrate (NO_3), and hydrogen sulfide (H_2S) at a dosage of 100 g/ 2000 m^3 water

Filtration materials

5. Charred rice husk (Charhusk) at 850 ^{0}C

6. Clay from acid sulfate soil (2 kg clay mix with 0.5 kg rice straw) heated at 500 ^{0}C (Brick 2)

7. Clay from acid sulfate soil (3 kg clay mix with 0.5 kg rice straw) heated at 500 ^{0}C (Brick 3)

8. integrated T1+T2

9. integrated T1+T3

Results

All products initially increased NH_4-N compared to the control but only significantly in Subtyl, Zimovac and Brick 2 and 3 and char husk (Table 6). Control NH_4-N declined by 30 % over 5 days. Brick + char increased NH_4 after 5 days while Bitech, Subtyl, Zimovac, Yucca 0 and Char decreased NH_4-N several fold.

Table 6: Ammonium concentration (mg/L) in waste water under different treatments over times

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
Vime-Bitech	0.572	0.038	0.076	0.039	0.052
Vime-Subtyl	0.640	0.032	0.067	0.023	0.056
Zimovac	0.715	0.045	0.089	0.050	0.073
Vime-Yucca 1	0.557	0.512	0.473	0.353	0.360
Vime-Yucca 0	0.443	0.427	0.285	0.090	0.060
Char-Husk	0.387	0.128	0.125	0.051	0.056
Brick 2	0.599	0.236	0.188	0.264	0.247
Brick 3	0.652	0.522	0.349	0.292	0.233
Brick 2 + Char-Husk	0.717	0.387	0.398	0.289	0.304

Brick 3 + Char-Husk	0.553	0.390	0.563	0.217	0.281
Control	0.346	0.140	0.130	0.150	0.202
CV %	27.3	139.0	57.7	46.7	24.1
5% LSD	0.261	0.615	0.245	0.131	0.072

All treatments except Yucca increased NO_3-N and NO_2-N over time and relative to the control (Tables 7, 8).

Table 7: Nitrate concentration (mg/L) in waste water under different treatments over times

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
Vime-Bitech	0.229	0.220	0.273	0.349	0.746
Vime-Subtyl	0.256	0.252	0.411	1.031	1.114
Zimovac	0.152	0.189	0.385	0.960	1.042
Vime-Yucca 1	0.311	0.184	0.234	0.144	0.138
Vime-Yucca 0	0.102	0.048	0.083	0.021	0.003
Charhusk	0.767	0.181	0.316	0.639	0.985
Brick 2	0.676	0.264	0.354	0.623	1.004
Brick 3	0.709	0.216	0.333	0.022	0.976
Brick 2 + Char husk	0.738	0.225	0.322	0.647	0.955
Brick 3 + Char-Husk	0.698	0.374	0.377	0.631	0.961
Control	0.155	0.030	0.107	0.082	0.266
CV %	9.1	29.9	19.9	10.3	8.6
5% LSD	0.068	0.101	0.098	0.092	0.109

Table 8: Nitrite concentration (mg/L) in waste water under different treatments over times

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
Vime-Bitech	0.071	0.281	0.384	0.295	0.714
Vime-Subtyl	0.075	0.386	0.470	0.063	0.102
Zimovac	0.071	0.387	0.397	0.074	0.266
Vime-Yucca 1	0.034	0.080	0.031	0.118	0.026
Vime-Yucca 0	0.016	0.063	0.009	0.068	0.034
Charhusk	0.057	0.180	0.031	0.106	0.182
Brick 2	0.049	0.176	0.063	0.139	0.195
Brick 3	0.045	0.214	0.059	0.110	0.203
Brick 2 + Char husk	0.041	0.191	0.039	0.118	0.175
Brick 3 + Char husk	0.053	0.245	0.071	0.116	0.165
Control	0.069	0.075	0.026	0.071	0.037
CV %	12.0	11.4	34.7	24.6	37.0

5% LSD	0.011	0.04	0.083	0.049	0.120

Phosphorus in solution dropped by about 90% in the control wastewater (Table 9). Most treatments experienced a similar drop in P to the control. Brick as a filtration materials, except when mixed with charhusk, strongly decreased P in solution. Bitech also strongly decreased solution P.

Table 9: Phosphate concentration (mg/L) in waste water under different treatments over times.

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
Vime-Bitech	0.106	0.076	0.040	0.035	0.037
Vime-Subtyl	0.134	0.095	0.060	0.056	0.147
Zimovac	0.134	0.092	0.092	0.077	0.223
Vime-Yucca 1	1.272	0.665	0.762	0.651	0.686
Vime-Yucca 0	0.327	0.171	0.151	0.148	0.212
Charhusk	4.867	2.003	1.767	0.571	0.321
Brick 2	0.081	0.052	0.076	0.076	0.094
Brick 3	0.051	0.038	0.019	0.021	0.057
Brick 2 + Charhusk	3.134	1.727	1.045	0.514	0.275
Brick 3 + Charhusk	4.248	1.384	0.630	0.492	0.251
Control	3.193	2.127	2.012	0.507	0.226
CV %	37.2	47.8	64.1	19.8	49.9
5% LSD	1.012	0.622	0.661	0.096	0.195

There was no effect of Subtyl on total bacterial counts, but all other treatments decreased levels by 30 % (Table 10). However, after treatment levels remained 7-8 x 10^6 CFU/mL.

Table 10: Effect of treatments on total bacterial counts on day 1, 2 and 3.

Treatment	Bacteria population (CFU/ml)		
	Day1	day 2	day 3
Control	$10.24x10^6$	$10.24x10^6$	$10.24x10^6$
Charhusk	$7.43x10^6$	$7.56x10^6$	$7.64x10^6$
Brick 2	$9.46x10^6$	$9.20x10^6$	$9.14x10^6$
Brick 3	$7.70x10^6$	$7.73x10^6$	$7.78x10^6$

Brick 2 + Charhusk	$7.22x10^6$	$7.12x10^6$	$7.43x10^6$
Brick 3 + Charhusk	$6.62x10^6$	$6.50x10^6$	$6.75x10^6$
mean	$8.45x10^6$	$8.43x10^6$	$8.49x10^6$
SD	$1.11x10^6$	$1.07x10^6$	$0.99x10^6$

In conclusion, the filtration of wastewater by brick was ineffective except for lowering P. Only Yucca at the lower rate decreased both NH_4-N and NO_3-N, but it had no effect on solution P. Total bacterial counts were not effectively decreased by any of the treatments by filtration.

Experiment 3

A. Materials

1. Vime Protex (1,5 - Pentanedial (glutaraldehyde) 200g, Alkyldimethylbenzylammonium chloride 152g): a disinfectant for fishponds to control bacteria at a recommended dosage of 1L/2,000 -4,000 m^3 water

2. Vime Yucca (Yucca schidigera extract) used for control of NH_3 in fishpond water at a dosage of 1L/ 3,000 – 4,000m^3 water

*3 Vime Kon (*Potassium monopersulfate 49.400 mg, sodium hexametaphosphate 22.4 mg, malic acid): a disinfectant fishpond at a dosage of 100 g/200-300 m^3 water

4 Calcium hypochlorite ($Ca(OCl)_2$): for oxidation of organic matter at a dosage of 1/10,000

5 Moringa oleifera (crushed seeds): for clearing water by flocculation of suspended solids and a disinfectant at a dosage of 1/1,000

B. Methods

This experiment composed of 6 treatments with 3 replications and followed completely randomized design. Details of treatment was given below:

- NT1: concentration of Vime Protex 0.25ml/500ml wastewater

- NT2: Concentration of Vime Yucca 1ml/500ml wastewater

- NT3: Concentration of Vime Yucca 0.25ml/500ml wastewater

- NT4: Concentration of Vime kon 0.0001ml/500ml wastewater

- NT5: Concentration of Calciumhypoclorite 1/1000 ml wastewater

- NT6: Moringa seed powder of 1/1000 wastewater

Results

Vime Protex strongly increased NH_4-N over the 5 days while Vime Kon, followed by moringa were most effective in decreasing NH_4-N (Table 10). Vime Protex also strongly increased NO_3-N (Table 11). Vime Kon which reduced NH_4-N had the opposite effect on NO_3-N while moringa was effective in decreasing both. Calcium hypochlorite was most effective in reducing NO_3-N concentrations. Nitrite concentrations were all ow except that on Day 1-3, Vime Kon and Vime Protex resulted in above average values (Table 12).

Moringa and Ca hypochlorite were most effective in decreasing solution P after 5 days (Table 13) however, there initial effects were contrasting. Moringa increased P from Day 1-4 while Ca hypochlorite decreased values. Yucca products both increased solution P.

Table 9: Ammonium concentration (mg/L) in waste water under different treatments over times

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
Vime Protex	2.409	3.379	3.403	2.764	3.612
Vime Yucca 1	0.823	0.640	0.629	0.587	0.730
Vime Yucca 2	0.330	0.418	0.359	0.384	0.347
Vime Kon	0.234	0.324	0.396	0.293	0.086
$Ca(OCl)_2$	1.707	1.551	1.679	0.908	0.752
Moringa seed	0.620	0.492	0.433	0.323	0.219
CV %	10.1	6.6	6.1	13.3	8.5
5% LSD	0.187	0.135	0.127	0.212	0.047

Table 11: Nitrate concentration (mg/L) in waste water under different treatments over times

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
Vime Protex	1.830	2.237	4.537	3.458	2.857
Vime Yucca 1	0.220	0.714	0.536	0.251	0.300
Vime Yucca 2	0.147	0.644	0.219	0.097	0.385
Vime Kon	1.508	2.306	2.091	1.935	2.192
$Ca(OCl)_2$	1.653	1.305	1.392	1.162	1.294
Moringa	0.374	0.281	0.255	0.237	0.147
CV %	17.1	11.2	9.0	12.7	18.8
5% LSD	0.298	0.253	0.246	0.274	0.130

Table 12: Nitrite concentration (mg/L) in waste water under different treatments over times

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
Vime Protex	0.113	0.156	0.160	0.069	0.078
Vime Yucca 1	0.060	0.076	0.014	0.029	0.047
Vime Yucca 2	0.032	0.081	0.007	0.013	0.118
Vime Kon	0.596	0.291	0.267	0.009	0.006
$Ca(OCl)_2$	0.095	0.147	0.096	0.072	0.065
Moringa	0.027	0.026	0.022	0.017	0.015
CV %	7.8	69.2	96.3	12.5	13.3
5% LSD	0.022	0.163	0.095	0.008	0.133

Protex and Yucca at 3.5 mg/l were effective in decreasing total bacterial counts while Vime Kon, Ca hypochlorite and moringa were most effective in causing a 1000-fold drop in numbers in wastewater after 5 days (Table 14).

Table 13: Phosphate concentration (mg/L) in waste water under different treatments over times

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
Vime Protex	0.220	0.347	0.225	0.299	0.444
Vime Yucca 1	1.340	1.228	1.163	1.261	1.196
Vime Yucca 2	0.473	0.443	0.446	0.435	0.565
Vime Kon	0.439	0.321	0.317	0.314	0.434
$Ca(OCl)_2$	0.039	0.042	0.054	0.037	0.207
Moringa	0.716	0.616	0.511	0.426	0.274
CV %	16.4	15.1	5.3	18.3	15.3
5% LSD	0.160	0.137	0.044	0.153	0.144

Table 13: Bacteria population in liquid waste of fishpond under different treatments

Treatment	**Bacteria population** (**x10^6CFU/ ml nước ao**)
Control (no chem.)	6.49
Vime Protex 0.5 ppm	6.43
Vime Yucca 3.5 ppm	6.10
Vime Yucca 0.875 ppm	3.40

Vime Kon 3.25 ppm	3.48
$Ca(OCl)_2$ 1000 ppm	3.48
Moringa 1000 ppm	3.18

In conclusion, Moringa seed was promising as a wastewater treatment since is decreased NH_4-N, NO_3-N, P and total bacterial count. All other products were effective against one or more water quality parameters only. Calcium hypochlorite decreased P and total bacterial count but was ineffective in decreasing solution N. On the other hand Vime Kon decreased NH_4-N and total bacterial count but not NO_3-N and P. Indeed this product contains P and hence would be expected to maintain or increase levels.

Combination of biological and chemical measures for treating water from catfish ponds

Vu Ngoc Ut

Cantho University

Researchers at Cantho University examined ozone treatment of wastewater to determine effectiveness in disease control and in the mineralisation of organic compounds in fishponds. Previous research indicates that bubbling ozone through wastewater can be an effective treatment in brackish water shrimp ponds.

The initial experiment involved ± ozone treatment of 500 L tanks with fish stocked in the tanks. Ozone treatment did not raise levels in wastewater above 0.14 mg/L, by contrast with the higher levels of enrichment achieved in brackish water (0.36 mg/L). Effects on water quality were mixed. No evidence of mortality of catfish was observed in the ozone treated water regardless of fish size. Several laboratory-scale experiments carried out to determine the effectiveness of ozone treatment in oxidation of organic matter and disinfection of bacteria in wastewater.

The objective of the study was to combine biological (water hyacinth and fish) and chemical methods (ozone) to reduce nitrate concentration and algae densities in waste water from catfish ponds prior to discharging to environment.

Methodology

Experiment was designed with 3 treatments in which waste water after being treated with ozone was exposed to water hyacinth and tilapia for further treatment. The hypothesis was ozone will transform nitrogen from NH_3/NH_4^+ into NO_2^- and NO_3^-, and NO_3^- will be absorbed by water hyacinth or algae. In the tilapia treatment, algae will be taken up by the fish. Water after being treated will have low concentration of nitrate and densities of algae that is considered clean water before coming to the water ways.

Three treatments with 3 replicates each included:

- Control: no water hyacinth and tilapia
- Tilapia
- Water hyacinth and tilapia

Water from catfish ponds was treated in a 4000 L tank with ozone for 48 h. Ozone was injected into the tank with an ozone generator of 8 g/h through a venturi pump for full dispersion. Ozone concentration was measured every 6 hours together with some water parameters including temperature, pH, TSS, DO, COD, TAN, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$, PO_4^{3-} and total bacteria. Bacteria were checked before and after treating ozone.

After 48 h, ozone-treated water was pumped to different treatments in 500 L tanks. The water settled for 2 days and water hyacinth and tilapia were placed in tanks according to treatments. In the tilapia treatment, 5 fish with a mean weight of 50 g were distributed in each tank. Water hyacinth covered only 50 % of the water surface of the tanks. Fish were not fed during the experiment duration.

Sampling was implemented every 5 days. Water was sampled for determination of TSS, COD, DO, TAN, N-NO_2^-, N-NO_3^-, PO_4^{3-} and algae density. Temperature and pH were measured daily. The experiment was terminated after 20 days.

Results

1. *Water parameters in the ozone treatment unit*

Ozone concentration increased from 0.08 mg/L after 6 hours to 0.15 mg/L after 48 hours of injection (Table 1). Temperature slightly increased after 6 hours and was stable until the end. Similarly, pH also increased after 6 hours and did not change afterwards. Dissolved oxygen concentration increased significantly after 6 hours of treatment (from 3.6 mg/L to 7.68 mg/L) and became stable until 36 hours and increased again at the end. TSS was highly variable but tended to decrease toward the end of treatment period.

Concentrations of TAN decreased with time together with increased nitrate concentration (8.3 times compared to the initial level). Phosphorus concentration also increased with time (Table 2).

Table 1: Ozone dissolution and fluctuation of water parameters during duration of ozone treatment in 4 m^3 tank.

Time (hour)	Ozone concentration (mg/L)	Temperature (^oC)	pH	DO (mg/L)	TSS (mg/L)
0	0.00	27.5	6.4	3.60	74.0
6	0.08	28.1	7.4	7.68	94.0
12	0.10	28.3	6.9	8.24	72.0
24	0.12	28.6	7.4	8.08	78.0
30	0.12	28.8	7.1	8.40	48.0
36	0.12	28.8	7.2	8.40	60.0
48	0.15	28.8	7.2	9.28	50.0

1. *Bacteria counts*

Before and after treating with ozone, samples of water were isolated for total bacteria counts. The samples were incubated for 24 hours and the bacteria were counted. Number of bacteria was substantially reduced. A total of 99 % of bacteria were killed after 48 hours of ozone treatment.

2. *Fluctuation of water parameters after transferring to biological treatment*

Temperature varied depending on the weather but was not significantly different among treatments (Table 3).

Table 2: Fluctuation of nitrogen and phosphorus parameters during ozone treatment

Time (hours)	COD (mg/L)	TAN (mg/L)	NO_2^- (mg/L)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)
0	9.20	4.19	0.19	1.09	1.92
6	10.8	4.65	0.21	2.02	1.95
12	11.2	4.48	0.16	3.61	1.96
24	12.4	3.87	0.42	3.81	2.13
30	10.8	3.91	0.06	8.23	2.11
36	17.2	3.01	0.27	8.50	2.11
48	12.4	1.13	0.80	9.08	2.01

Table 3 : Temperature in different treatments

Time (days)	**Control**	**Tilapia**	**Water hyacinth + tiplapia**
0	28.9±0.00	28.9±0.00	28.9±0.00
5	33.3±0.34	33.6±0.42	31.7±0.45
10	26.4±0.07	26.4±0.42	26.4±0.45
15	29.3±0.07	29.4±0.03	28.9±0.03
20	27.6±0.13	27.6±0.12	27.4±0.14

pH in the control and tilapia treatments increased with time, whereas pH in tilapia + water hyacinth decreased (Table 4). In tilapia + water hyacinth treatment, algae did not grow well as light was limited due to shading by water hyacinth and nutrient limitation. Better algae growth in the control and fish tanks resulted in increased pH.

In the control, TSS decreased obviously after 5 days and slightly increased again after that. TSS concentration in the control ranged from 21.4-55.5 mg/l. Variation of this factor in the tilapia treatment was relatively high and tended to increase from 34.7 to 107.8 mg/l. Treatment of fish + water hyacinth had less fluctuation in TSS and varied only from 46.7-76.3 mg/l (Figure 1). There was no significant difference between treatments in TSS concentrations. Increased algae densities in the control and tilapia treatments may result in high TSS. In addition, the activities of tilapia in tanks may have deterred sedimentation causing increased TSS. Decrease of densities of algae in tilapia + water hyacinth toward the end of the experiment may have contributed to lower TSS during these periods in this treatment.

Table 4: pH fluctuation in different treatments

Time (days)	**Control**	**Tilapia**	**Water hyacinth + tilapia**
0	7.20±0.00	7.20±0.00	7.20±0.00
5	9.63±0.35	8.33±0.55	7.20±0.26
10	8.83±0.25	8.33±0.55	6.63±0.26
15	9.93±0.25	9.70±0.75	6.77±0.06
20	9.30±0.30	9.73±0.35	6.23±0.06

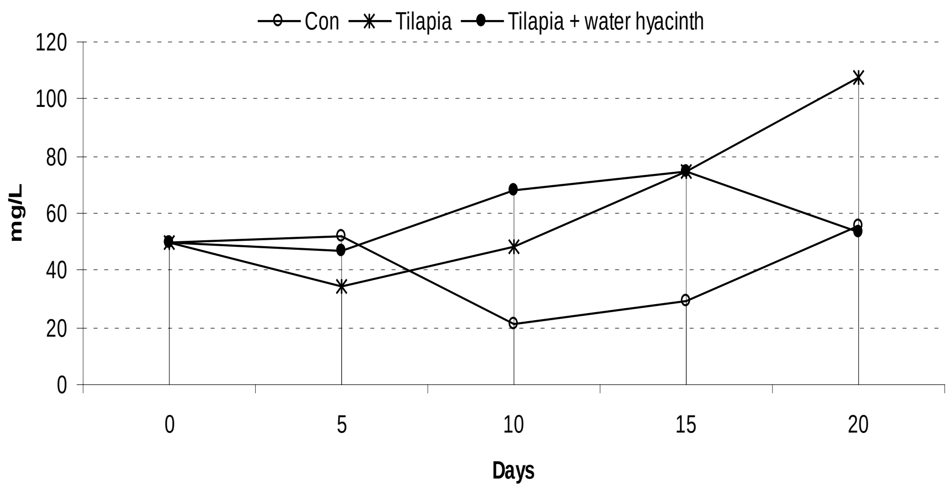

Figure 1: Fluctuation of TSS in different treatments during 20 days of experiment

After 48 of ozone injection, DO concentration was very high (9.28 mg/l). DO concentrations increased in the control and tilapia treatments, however, in the tilapia + water hyacinth treatment, DO was decreasing. Final DO concentrations in the control, tilapia and tilapia + water hyacinth were 8.32-11.9 mg/l, 7.30-13.3 mg/l and 3.33-9.28 mg/l, respectively (Figure 2). DO concentrations depend mainly on algae densities in the tanks. Algae densities were lowest in tilapia + water hyacinth treatments (see more details in section of algae growth) due to limitation of light. In other treatments, algae were growing better resulting in higher DO concentrations.

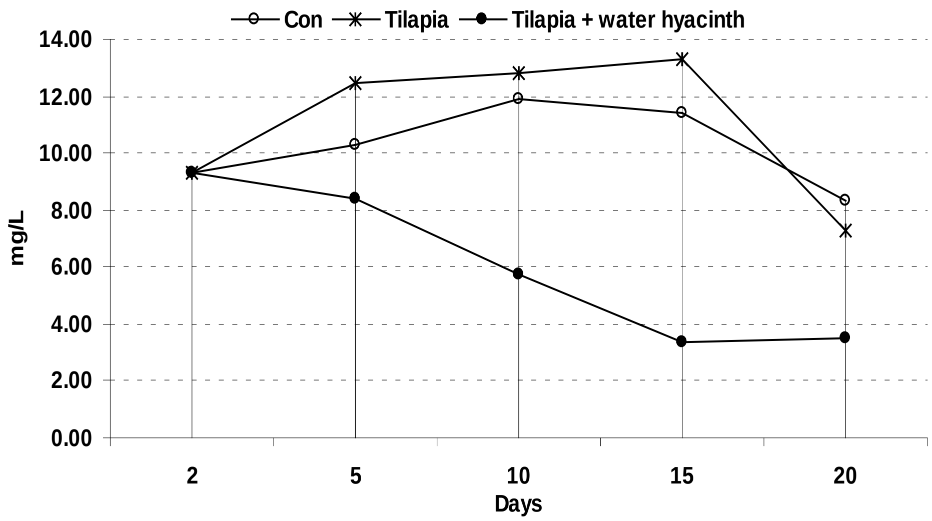

Figure 2: Fluctuation of DO in different treatments

COD concentrations tended to increase during the culture period and maintained at high levels in tilapia (9.2-25.5 mg/l) and tilapia + water hyacinth (9.2-23.9 mg/l) treatments. In the control treatment, COD was lower (9.2-16.4 mg/l) (Figure 3). Similar to other experiments, COD always increased after ozone injection, even in brackish water. This may be due to increased organic matter contents in tilapia and tilapia + water hyacinth caused by the activities of fish as well as their secretions. Although, increased COD concentrations was recorded, they were still in acceptable ranges (less than 30 mg/l).

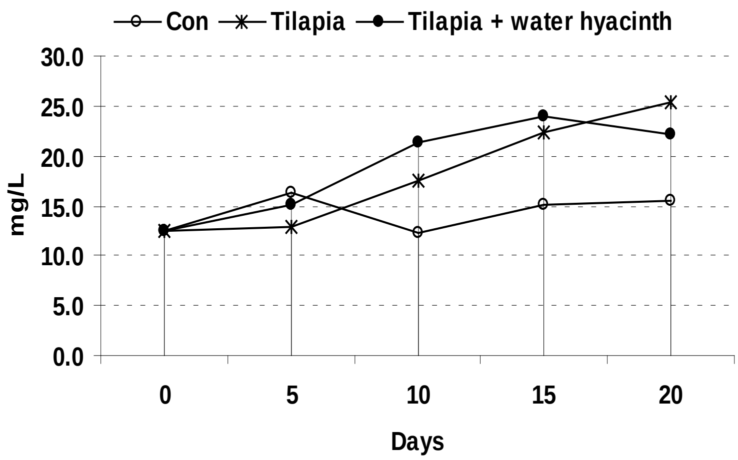

Figure 3: Fluctuation of COD in different treatments

The concentrations of TAN decreased significantly in all 3 treatments. The results indicated that TAN concentration in the catfish waste water decreased even in the control (Figure 4).

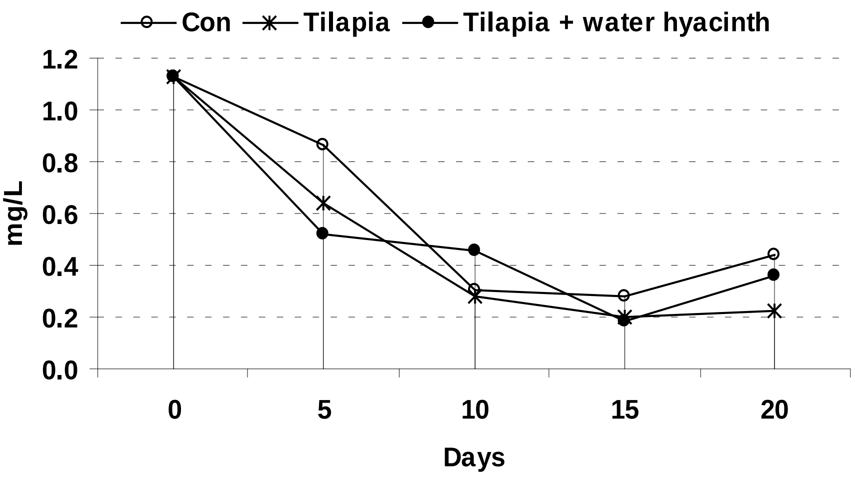

Figure 4: Fluctuation of TAN in different treatments

In all 3 treatments, TAN concentrations were absorbed directly by plants and subsequently decreased dramatically. High algae densities in the control and tilapia treatments reduced the amount of TAN (under form of NH_4^+), whereas in tilapia + water hyacinth treatments, water hyacinth played a important role in reducing TAN. There was no significant difference in TAN concentrations between treatments but within treatment, significant difference was found between sampling periods.

Nitrite concentrations in the control, tilapia and tilapia + water hyacinth were 0.07-3.36 mg/l, 0.25-4.6 mg/l and 0.02-4.97 mg/l, respectively. The concentration increased during the first 10 days and decreasing substantially towards the end of experiment (Figure 5). Nitrite concentrations in the treatment of water hyacinth + tilapia were lower than those in the control and tilapia treatments. However, they all had the same trend over time. After 15 days, nitrite was reduced significantly by 86, 67 and 98 % in the control, tilapia and tilapia + water hyacinth treatments, respectively.

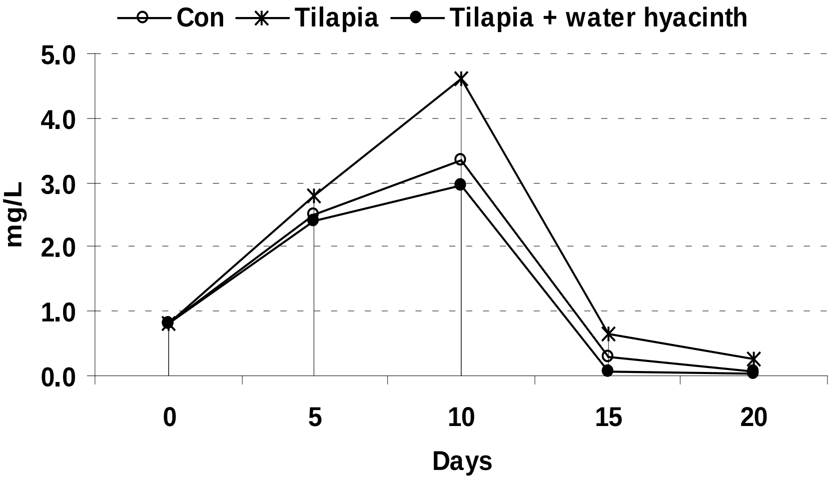

Figure 5: Fluctuation of NO_2^- in different treatments

Nitrate concentrations were decreasing throughout the experiment varying in the ranges of 0.91-10.3 mg/l, 0.16-10.3 mg/l and 0.61-10.3 mg/l for the control, tilapia and tilapia + water hyacinth treatments, respectively (Figure 6). Nitrate concentrations dropped dramatically by day 15 due to strong uptake of algae and water hyacinth. Percentages of nitrate removal by algae and water hyacinth in the control, tilapia and tilapia + water hyacinth treatments were 91 %, 86 % and 97 %.

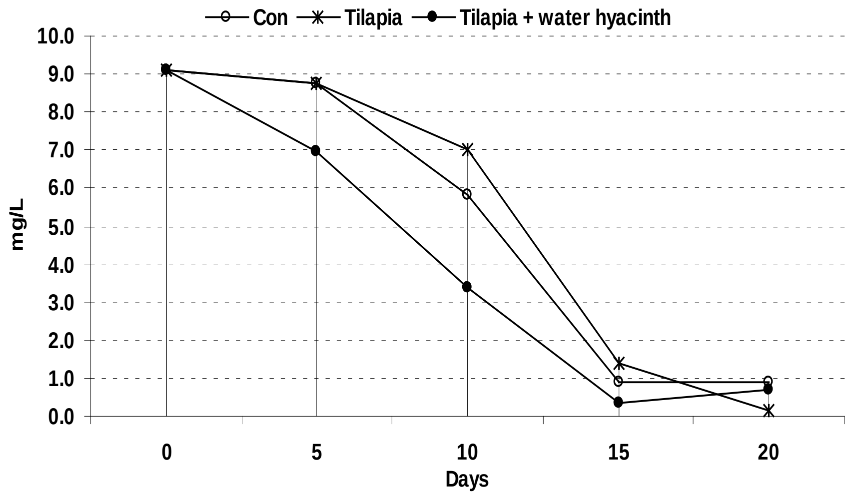

Figure 6: Fluctuation of NO_3^- in different treatments during 20 days of experiment

Soluble phosphorus concentrations in all treatments were also reduced with time, ranging from 0.55-2.05 mg/l, 0.32-2.05 mg/l and 1.06-2.05 mg/l in the control, tilapia, and water hyacinth + tilapia, respectively (Figure 7). Phosphorus absorption of algae had resulted in reduction of phosphate concentrations in the control and tilapia treatments . In water tilapia + hyacinth treatment, as algae did not grow well due to the shading by water hyacinth, the reduction of phosphate was lower in this treatment. In the control and tilapia treatments, there was enough light for algae growth, the amount of phosphate was significantly absorbed from solution.

Green algae were dominantly growing in all treatments, accounting for up to 90 % of the total algae population. Blue-green algae developed in the tanks were mainly filamentous algae. Higher percentage of blue-green algae was recorded in the control rather than in treatments containing fish.

In tilapia treatment, algae density increased up to 8.7 million cells/L (80.9 %) at the end of experiment. Algae density in the control did not increase significantly (only 35.7 %). Lowest density of algae was found in tilapia + water hyacinth treatment. Toward the end of experiment, algae did not grow but decreased in concentrations in this treatment (Figure 8). As discussed earlier, due to shading, algae could not grow well in this treatment. In addition, excretes released from the fish could be a nutrient source for algae growth.

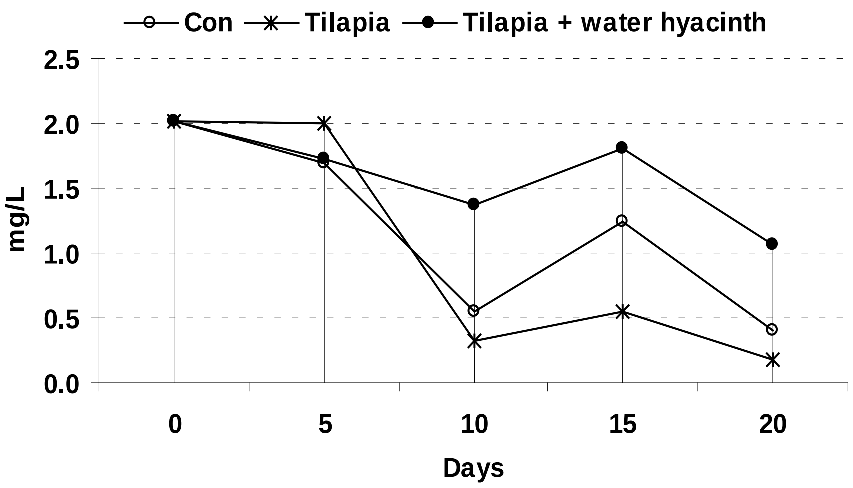

Figure 7: Fluctuation of PO_4^{3-} in different treatments

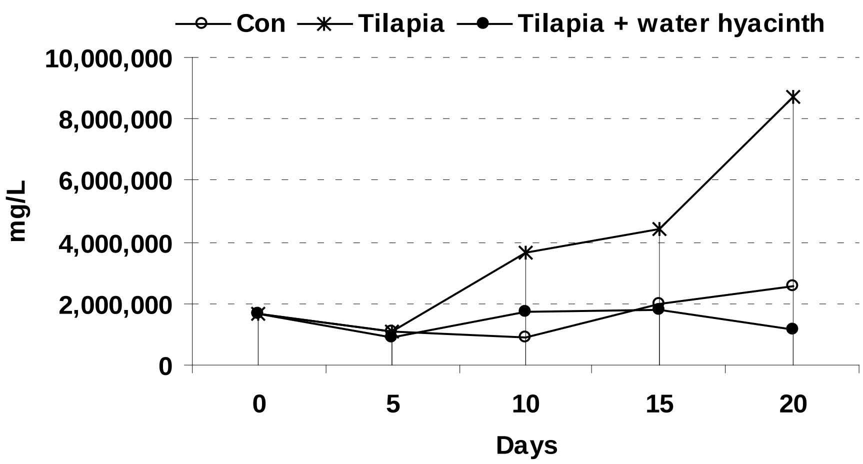

Figure: Algae densities in different treatments during 20 days of experiment

Conclusions

Up to 99 % of bacteria in catfish waste water were killed after treating with ozone for 48 hours in a 4000 m^3 tank. During the ozone treatment, ammonium (TAN) concentrations decreased but nitrate and phosphate increased with time due to the oxidation by ozone. Nitrate and phosphate concentrations were rapidly absorbed by algae and water hyacinth and concentrations decreased in the water. However, algae in the fish treatments were high and not meet the water quality standards. Whereas, in the treatment combining fish and water hyacinth, not only were nitrate and phosphate significantly reduced but algae were not able to develop.

These preliminary findings suggests tilapia + water hyacinth was the best treatment as it reduced nutrient and deterred development of algae. Combination of chemical (ozone) and biological (tilapia + water hyacinth) methods could be applied in treating catfish waste water to reduce nitrogen and phosphorus and algae densities before discharging to the environment. This approach may have application in settling ponds to enhance the treatment of water before discharge or further use.

Use of aquatic plants to improve water quality:

Aquatic plants has long been known on their potentials to reduce water pollution by withdrawing nutrients from water. Boyd (1974) reviewed papers on the potential of aquatic plants, especially water hyacinth, for removal of nutrients from polluted water. According to Brink (1969) the amount of N and P were taken up by aquatic plants to a distance of 8.4 km downstream from the sewage entry. Water hyacinth could potentially remove about 300 g of heavy metals from 1 ha of polluted water per day (Wolverton, 1975). Duckweed was used to remove P in sewage effluent held under static conditions (Sutton and Orne, 1975). In Vietnam, Nguyen & Nguyen (2003) indicated that *Lemma gibba* could be used for domestic sewage treatment and reuse as irrigation water for horticulture. Le (2006) constructed wetland for treatment of waste water from fishpond in the Mekong delta.

Two experiments using 3 aquatic plants (water hyacinth, Enydra fluctuans and *Jussisaea repens*) and two ferns (*Pistia stratiotes* and *Salvinia cucullata)* to improve waste water quality from fishpond to meet standards for discharge were carried out at net house of CLRRI from January to September 2008. Control, having wastewater only, was used as check in both experiments. The first experiment simulated conditions in a settling pond (static system) and the second had a system of continuous flow of waste water.

Experiment 1 (static system):

Experiment was carried out in greenhouse of Soil Science Department of CLRRI during January-August, 2008. Every treatment was kept in one EPS foam box with 60 L wastewater from fishpond. Foam box having dimension of 60x40x60 cm (LxWxH) was lined with nylon sheet to prevent water loss by permeability. There were seven treatments with 3 replications. Treatments composed of 6 aquatic plants (150 g/box) and one control with details given below:

-T1 : water hyacinth;

-T2 : *Enydra fluctuans*;

-T3 : *Jussiaea repens;*

-T4 : Pistia stratiotes;

-T5 : Salvinia cucullata ;

-T6 : aerated by bubling with oxygen ;

-T7 : control (no treatment)

Results of the first experiment indicated that all aquatic plants grew very fast on waste water and reached maximum fresh weight after 30 days but ferns took 45 days in both experiments to reach maximum fresh weight. pH of all treatments varied from 6.0-8.5 and EC of all treatments was significant decreased from 0.250 to around 0.100 mS/cm except in the control. Ammonium, nitrite, nitrate, total N and P in all treatments were reduced to below the limit allowable for discharge to surface water (TCVN 5942-95). COD of all treatments were also decreased but they were still higher than the standard limit. TSS values of treatment with aquatic plants and ferns were decreased after 15 days and then increased again to exceed the initial values due to decomposition of debris of senesced plant parts. However, the control tank had lower TSS than standard limit value.

Experiment 2 (continous flow system)

Number of treatments in the 2nd experiment was almost similar to experiment 1 except treatment 6. In this flow system, everyday wastewater was supplied on top and removed at box-base by tubing. Water flow velocity was control by adjusting valve on every tube so that inflow equal to outflow. Wastewater sample was collected from every box was fixed at 15 L .i.e, total outflow (15 L) was adjusted for 3, 4, 5, 6, 7 and 8 hours interval for the 1st, 2nd, 3rd, 4th, 5th and 6th succeeding days, respectively.

In the second experiment, results were similar to the first experiment in which *Jussisaea repens* seemed to be the best in this experiment. *Salvinia cucullata* was quite effective in reducing TSS but .Water hyacynth was not effective in this system to reduce COD; however, it could absorbed soluble phosphate in wastewater effectively. *Enydra fluctuans* could reduce ammonium only but not effective in Ec and phosphate treatment. Outflow at slower rate proved to be better than fast flow in reducing TSS, COD and nutrients in wastewater.

Thus settling pond in combination with *Jussisaea repens* could effectively treat wastewater from catfish pond; moreover this plant can also be used as vegetable in Vietnamese food. Flow system can be applied effectively for wastewater treatment in settling pond having large area where water in fishpond is frequently added or removed.

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