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Reutilization of Wastewater from Freshwater Ornamental Fish Farm through Chemical and Biological Processes

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Abstract

The possibility of reuse of wastewater from freshwater ornamental fish farm for aquaculture purpose was studied by treating it chemically and biologically. Chemical agents like calcium oxide (quick lime), ferric chloride, ferric aluminum sulphate (ferric alum) and fly ash were used. Highest percentage reduction in ammonia (60.3%), phosphate (59.24%) and chemical oxygen demand (COD) (70.32%) was observed after treatment with quick lime, fly ash and alum respectively. In biological process, micro algae *Chlorella vulgaris* and duck weed *Lemna minor* were used. In micro algal treatment, chemically treated wastewater did not show any significant production of algae despite significant difference ($p<0.05$) among algal growth in different days of algal culture. At the end of culture, highest net photosynthesis and assimilation number obtained from fly ash was 16.36 ± 0.99 and 24.42 g m^{-3} h^{-1} respectively. Level of ammonia and phosphate was drastically reduced from 124.52 to 2.61 μg at-N l^{-1} and 56.52 to 0.168 μg at-P l^{-1} respectively in the wastewater treated with fly ash and micro algal culture. COD level was reduced from 112.80 to 5.1 mg l^{-1} after treating the wastewater with ferric chloride and micro algal culture. No production of *Lemna* was registered from the wastewater treated with lime and ferric chloride. The culture of duck weed drastically reduced the ammonia level from 74.38 to 3.82 μg at-N l^{-1} and 59.66 to 1.84 μg at-N^{-1} in alum and fly ash treated wastewater respectively. No trace of phosphate was recorded in the *Lemna* treated waters. In culture trial of common carp fry, fly ash treated and fly ash cum algal cultured wastewater showed high net growth efficiency. Net growth efficiency varied significantly ($p<0.01$) between chemically treated and biologically treated waters.

Keywords: Wastewater, ornamental fish, aqua farm, *Chlorella vulgaris*, *Lemna minor*

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Introduction

Aquaculture practices are widely carried out in different ways, *viz.*, extensive, semi-intensive and intensive. These practices are undertaken in both inland and coastal environment. The effluents from the aquaculture systems pose a big menace to the aquatic environment. Many farms discharge effluents without any treatment. It results in high biological oxygen demand, eutrophication, turbidity, high level of organic matter and increase in heavy metal load in the environment. Discharge of untreated effluent water brings down the ambient oxygen level to below 3.5 ppm which causes stress to fishes (Boyd & Pillai, 1984). Another major problem is eutrophication *viz.*, repeated bloom of unwanted algae (Welch, 1980) which degrades the water quality and chokes the gills of the fishes.

The problems of inland aquaculture system are entirely different from coastal aquaculture systems. Inland wastewaters can be treated using various chemical and biological processes including filtration. The methods include different kinds of fixed film biological filter (Klemtson & Rogers, 1985) and up-flow plastic bead filters (Losaordo et al., 1994) for the removal of ammonia and other chemical agents like zeolite and lime for removal of sodium, ammonium and potassium ions from the effluent. Unwanted or excessive nutrients can be removed using micro algae like *Chlorella* and *Chlamydomonas* (Tripathi et al., 1991). Other biological methods include use of bio-reefs (Rangaswami, 1993), *Lemna* (Edward, 1996), hyacinth, *Hydrilla* and *Ceratophyllum* (Rajesh et al., 2001), cynobacterium (Dumas et al., 1998) and *Rhodopseudomonas* (Kim et al., 1999), which facilitate the denitrification of wastewater. It is essential to determine suitable methods for wastewater treatment as there is wide variation in the characteristics of effluents generated from aquaculture systems. Ornamental fish farms employ high density culture practices with a stringent water quality control regime. The present study was carried out with the objective of evolving suitable treatment method for wastewater from ornamental fish farms for reuse.

Materials and Methods

Wastewater samples required for the present study were collected from M/s. Redlin fresh water ornamental fish farm, Sawyarpuram, Thoothukudi, Tamil Nadu, India. The collected samples were stored in refrigerated condition and later treated by both chemical and biological methods. In case of chemical treatment, calcium oxide (quick lime), ferric alum, ferric chloride and fly ash were used, whereas in biological treatment a combination of micro-algae *Chlorella vulgaris* and floating duck weed *Lemna minor* was used.

For chemical treatment, commercial grade quick lime (CaO) containing 24.39 g of 100% pure CaO was chosen. It was added at six different concentrations of 100, 200, 400, 600, 700 and 800 mg l^{-1}. Ferric alum and ferric chloride were added individually at concentrations of 50, 100, 200, 400 and 500 mg l^{-1}. Fly ash was added at concentrations of 10, 20, 40, 60, 80 and 100 g l^{-1}. All these chemicals were added individually to 1 l of wastewater and aerated vigorously for one hour. In addition, fly ash treated samples were further thoroughly agitated using a stirrer or shaker since the quantity of fly ash used for effluent treatment was above 10 g l^{-1}. The experiment was conducted in triplicates to avoid sampling error. Levels of inorganic phosphorous, ammonia and COD were determined individually for every trial. Inorganic phosphorous was estimated using the methodology of reduction of phosphomolylodic acid, ammonia (NH_3) using indophenol blue method and chemical oxygen demand (COD) using alkaline permanganate oxidation method (APHA, 1995).

After treating the wastewater with different chemical agents, it was decanted and filtered using membrane filter of 0.45 μ porosity. pH of the treated waters were adjusted before initiating the biological treatment. Wastewater treated with quick lime had pH of above 7.5. It could be achieved with repeated aeration till all calcium hydroxide (CaOH) got converted in to insoluble calcium carbonate ($CaCO_3$). Ferric alum treated water had pH of below 6.5 and hence quick lime was added to raise the pH to 7.5. Water treated with fly ash and ferric chloride did not show any pH variations. The ferric chloride treated water was added with 1 ml of micro nutrients for enhancing micro algal growth.

Chemically treated water samples were again subjected to different biological treatment methods. As a first stage of biological treatment, the raw wastewater and chemically treated wastewaters were aerated well and placed in refrigerator for five days to destroy the existing plankton. After that, 20 ml of micro algae *Chlorella vulgaris* was inoculated and growth of algae was monitored on daily basis for one week. Apart from *Chlorella vulgaris*, duck weed *Lemna minor* was allowed to grow in both raw wastewater and chemically treated waters for a period of seven days. During the experiment, multiplication rate of micro algae and production rate of duck weed was estimated. The prime objective of use of duck weed in the experiment was to compare the growth efficiency of micro and macro vegetation in treated wastewaters. Chlorophyll 'a' and net photosynthesis were determined as per APHA (1995). Assimilation number, which is the ratio of net photosynthesis to Chlorophyll 'a' was worked out as it indicates the physiological status of algal form.

Feed trial experiment was conducted with the objective of determining the suitability of chemically and biologically treated wastewaters for fish culture practices. All the four chemically and biologically treated wastewater samples were taken for this experiment along with freshwater as control. pH was adjusted to 7 - 7.5 using quick lime. The waters were intensively aerated for two days. After aeration, algae settling at the bottom of the containers were removed. Feed trials were conducted in all these six samples in triplicates by introducing 10 common carp frys having average weight of 0.42 ± 0.02 g. The animals were fed *ad libitum* with the standard feed containing 20% protein prepared in the laboratory. This experiment was conducted for 21 days in the laboratory with sufficient water exchange and continuous aeration. During the experiment, the quantity of feed consumed, assimilation and faecal material released were recorded daily. Besides, the growth estimation, dry weight increment, gross growth efficiency (K_1) and net growth efficiency (K_2) were estimated in all growth trials using standard procedures (Santhanam, 1981).

All the data obtained were analyzed statistically using analysis of variance (ANOVA) technique. Student 't' test was used to study the significant effect of various chemical and biological treatments of wastewater and growth performance of species cultured.

Results and Discussion

The physico-chemical characteristics of effluent of fresh water ornamental fish farm are given in Table 1. The effluent contained high organic load and less plant nutrients. The various levels of ammonia removal from the wastewater by different treatments is given in Table 2. The highest level of ammonia (60.3%) was removed by quick lime treatment while it was low (40.26%) in ferric alum treatment. The efficiency of removal of ammonia among different chemical agents was significantly different ($p<0.01$). The lowest level of ammonia (49.435 μg at-N l^{-1}) was registered in quick lime treated water and the highest (74.384 μg at-N l^{-1}) in ferric alum. Earlier studies by Deeba (1998) and Bhaskar (2002) have shown effective removal of ammonia from wastewater using lime. The levels of removal of phosphate in different chemical treatments are given in Table 2. The removal of phosphate was high in fly ash treatment (59.24%) and low in lime treatment (47.68%). Percentage removal of phosphate with different treatments was highly significant ($p<0.01$) except with lime treatment. The lowest and the highest level of phosphate was registered in the treatment of fly ash (23.04 μg at-P l^{-1}) and quick lime (29.57 μg at-P l^{-1}) respectively. In case of phosphate removal, the efficiency of fly ash with quick lime was significantly high ($p<0.002$) followed by alum versus ferric chloride ($p<0.01$) while it was not at significant level between the agents of quick lime and alum.

Table 1. Physico-chemical characteristics of the wastewater of ornamental fish farm

Parameter	Level
Water hardness (mg l^{-1})	46.00
TDS (mg l^{-1})	123.45
Total alkalinity (mg l^{-1})	60.02
pH	8.10
Dissolved Oxygen (mg l^{-1})	9.90
Chemical Oxygen Demand (mg l^{-1})	112.81
Biological Oxygen Demand (mg l^{-1})	70.24
Suspended Solids (mg l^{-1})	51.82
Phosphate (μg-at P l^{-1})	56.52
Ammonia (μg-at N l^{-1})	124.52
Nitrite (μg-at N l^{-1})	2.14
Nitrate (μg-at N l^{-1})	56.52

The removal of COD content from raw effluent was found high (70.32%) in ferric alum treated water and low (52.09%) in ferric chloride treatment (Table 2). The removal of COD was significantly high especially with lime and ferric alum ($p<0.001$) compared to fly ash ($p<0.01$) and ferric chloride ($p<0.05$). The highest and the lowest level of COD was observed in the treatment of ferric chloride (54.05 mg l^{-1}) and ferric alum (33.484 mg l^{-1}) respectively. Significant difference in the removal of COD was observed between fly ash and ferric chloride ($p<0.01$) followed by quick lime and fly ash ($P<0.05$). However, there was no significant difference between alum and quick lime. It became apparent that quick lime (60.3%), fly ash (59.24%) and alum (70.32%) effected highest percent of removal of ammonia, phosphate and COD respectively.

The raw waste water inoculated with micro-algae did not exhibit good development of chlorophyll 'a' during the early phase (Table 3). However, it was good at the end of the culture period. The average chlorophyll content recorded in a week period of culture was 0.661 g m^{-3}. Production of chlorophyll 'a' was not significantly different among the chemically treated waters. However, significant ($p<0.05$) difference in growth of micro algae was observed between days in the treated wastewaters.

At the end of the seventh day, the highest net photosynthesis (16.36±0.099 g m^{-3} h^{-1}) was observed from fly ash treated water, followed by those treated with alum (14.26±0.099 g m^{-3} h^{-1}), ferric chloride (6.02±0.089 g m^{-3} h^{-1}) and lime (5.87± 0.071 g m^{-3} h^{-1}) (Table 4).

The values of assimilation number registered in the different treatments ranged from 11.0 to 24.42. The highest assimilation number was registered from wastewater treated with fly ash (24.42) followed by alum (23.00), lime (11.7) and ferric chloride (11.5). The assimilation number remained above 20 in both alum and fly ash treated wastewater. It indicated the good physiological status of the algae since assimilation number was above 5 (Curl & Small, 1965; Qasim et al., 1969). It could be observed that chemically treated wastewater did not pose any physiological stress to the algae during culture. In the study, wastewaters treated with fly ash and alum highly favoured the process of organic production.

Table 2. Changes in level of ammonia, phosphate and COD in wastewater of ornamental fish farm subjected to various chemical and biological treatments

Parameter	Raw Wastewater	Lime		Alum		Ferric Chloride		Fly ash	
		C	CB	C	CB	C	CB	C	CB
Ammonia (μg at-N l^{-1})	124.52	49.435	13.28	74.384	2.92	69.955	11.21	59.66	2.61
Phosphate (μg at-P l^{-1})	56.52	29.57	0.83	27.47	0.192	29.09	0.718	23.04	0.168
COD (mg l^{-1})	112.81	35.97	6.92	33.484	6.81	54.05	5.1	39.36	6.85

C : chemical treatment; CB : chemical cum biological treatment

Table 3. Production of Chlorophyll 'a' (g m^{-3}) from raw wastewater and chemically treated wastewater of ornamental fish farm

Days	Raw wastewater	Level of Chlorophyll 'a' (g m^{-3}) in different treatments			
		Lime	Alum	Ferric chloride	Fly ash
Initial	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.01
1	0.29 ± 0.02	0.19 ± 0.00	0.21 ± 0.01	0.20 ± 0.02	0.21 ± 0.00
2	0.41 ± 0.01	0.21 ± 0.01	0.29 ± 0.00	0.22 ± 0.01	0.31 ± 0.02
3	0.54 ± 0.02	0.27 ± 0.01	0.39 ± 0.01	0.30 ± 0.02	0.45 ± 0.01
4	0.64 ± 0.01	0.30 ± 0.01	0.45 ± 0.01	0.41 ± 0.01	0.52 ± 0.02
5	0.71 ± 0.00	0.35 ± 0.01	0.52 ± 0.02	0.48 ± 0.02	0.57 ± 0.01
6	0.89 ± 0.00	0.45 ± 0.01	0.59 ± 0.00	0.49 ± 0.03	0.61 ± 0.01
7	0.94 ± 0.00	0.50 ± 0.02	0.62 ± 0.01	0.52 ± 0.00	0.67 ± 0.01

After biological treatment with micro algae, highest and lowest level of ammonia and phosphate was recorded in the treatment of quick lime (13.28 μg at-N l^{-1}; 0.83 μg at-P l^{-1}) and fly ash (2.61 μg at-N l^{-1}; 0.168 μg at-P l^{-1}) respectively (Table 2). The level of COD was high in quick lime (6.92 mg l^{-1}) and low in ferric chloride (5.1 mg l^{-1}). Adoption of algal culture practices in processing of wastewaters is reported (Manimaran et al., 1990; Manimaran & Ramadhas, 2002). The growth of micro algae *C. vulgaris* negligible in lime and ferric chloride treated water. Nevertheless, fly ash and alum treated water supported the micro algal growth. Algal growth facilitated removal of nitrogen and phosphorus from the chemically treated wastewater by absorbing them for their growth. *Chlorella* performed well in removal of phosphate from the wastewater since it requires large quantity of phosphorous for its growth (Chu, 1943).

In the biological treatment of raw and chemically treated wastewaters with duck weed *Lemna minor*, good production rate of *L. minor* was registered. The raw wastewater yielded high production (7133 kg ha^{-1} week $^{-1}$) followed by fly ash (6573 kg ha^{-1} week $^{-1}$) and alum treated water (5256 kg ha^{-1} week $^{-1}$). No growth of weed was observed in lime and ferric chloride treated waters. It has been reported that, in general, the growth of *L. minor* in wastewaters is doubled within four days. Manimaran et al. (1997) reported 380 t ha^{-1} y^{-1} of *L. minor* production from septic tank water. However, in the present study, the production of *L. minor* in freshwater ornamental fish farm was lesser (370.9 t ha^{-1} y^{-1}) than septic tank water. After the *L. minor* culture, the level of ammonia registered in raw wastewater, alum treated and fly ash treated water were 82.62 μg at NH_3-N l^{-1}, 3.82 μg at NH_3-N l^{-1} and 1.84 μg at- NH_3 -N l^{-1} respectively. There were no

Table 4. Levels of net photosynthesis and assimilation number of chemically treated wastewater of ornamental fish farm after biological treatment

Days	Lime	Alum	Ferric chloride	Fly ash
Initial	2.02 ± 0.061 (11.1)	2.02 ± 0.061 (11.0)	2.025 ± 0.061 (11.0)	2.025 ± 0.061 (11.0)
1	2.19 ± 0.025 (11.13)	4.38 ± 0.053 (21.41)	2.23 ± 0.043 (11.15)	4.85 ± 0.065 (22.91)
2	2.35 ± 0.024 (11.21)	6.377 ± 0.061 (21.62)	2.53 ± 0.049 (11.25)	7.12 ± 0.077 (22.98)
3	3.10 ± 0.023 (11.32)	8.67 ± 0.076 (21.84)	3.40 ± 0.055 (11.30)	10.64 ± 0.081 (23.40)
4	3.44 ± 0.041 (11.44)	9.87 ± 0.081 (21.95)	4.67 ± 0.069 (11.38)	12.19 ± 0.089 (23.46)
5	4.119 ± 0.058 (11.54)	11.79 ± 0.089 (22.59)	5.49 ± 0.070 (11.41)	13.63 ± 0.09 (23.92)
6	5.26 ± 0.061 (11.64)	13.74 ± 0.091 (22.95)	5.61 ± 0.085 (11.45)	14.76 ± 0.095 (24.21)
7	5.87 ± 0.071 (11.70)	14.26 ± 0.099 (23.00)	6.02 ± 0.089 (11.50)	16.36 ± 0.099 (24.42)

* Level of net photosynthesis (g m^{-3} h^{-1})
** Assimilation number (in paranthesis)

Table 5. Growth of common carp frys grown in different chemical and chemical cum biological treated wastewater of ornamental fish farm

Parameters	Control	Lime		Alum		Fly ash		Ferric chloride	
		C	CB	C	CB	C	CB	C	CB
Initial average weight (W_1) g	0.42	0.46	0.42	0.40	0.40	0.41	0.41	0.42	0.41
Final average weight (W_2) g	0.52	0.59	0.55	0.54	0.55	0.55	0.56	0.55	0.54
Weighed mean ($W = W_2-W_1/2$) g	0.47	0.53	0.48	0.47	0.48	0.48	0.36	0.48	0.48
Production ($P= W_2-W_1$) g	0.10	0.13	0.13	0.15	0.14	0.14	0.15	0.13	0.13
Food consumption (C) g	1.07	1.30	1.26	1.35	1.35	1.40	1.4	1.32	1.22
Faecal output (F) g	0.12	0.13	0.10	0.17	0.10	0.16	0.14	0.13	0.05
Assimilation (A=C-F)	0.95	1.17	1.16	1.18	1.25	1.24	1.26	1.19	1.17
Metabolism (R=A-P)	0.85	1.04	1.03	1.04	1.09	1.09	1.11	1.06	1.04
Assimilation efficiency (A) %	88.43	89.57	91.64	87.00	91.96	88.24	89.86	89.46	95.33
Gross growth efficiency K_1 (P/C) %	9.56	9.81	10.31	10.31	11.05	10.52	11.07	9.85	10.82
Net growth efficiency K_2 (P/A) %	10.81	10.95	11.25	11.85	12.01	11.92	12.31	11.01	11.34
Relative growth rate day^{-1} (P/W/No. of days)	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01

C: chemical treatment ; CB: chemical cum biological treatment

traces of phosphate in both alum and fly ash treated waters. However, 4.56 µg at-PO_4-P l^{-1} of phosphate was registered in the raw wastewater. Production of duck weed was almost nil in the wastewater treated with other two chemicals.

Growth indicators of common carp fingerlings grown in different chemically treated wastewater and chemical cum biologically treated waters are given in Table 5. In chemically treated water, the percentage of net growth efficiency of fry was high in fly ash (11.92%) followed by alum (11.85%), ferric chloride (11.01%), lime (10.95) and control (10.81%). In micro algae treated water, the net growth efficiency was high in fly ash (12.31%) followed by alum (12.01%), ferric chloride (11.34%), lime (11.25%) and control (10.81%). The gross and net growth efficiency recorded in the four chemically treated and consequent biologically treated wastewaters were higher than the control.

Net growth efficiency varied significantly ($p<0.01$) between chemically treated and biologically treated waters. The highest net growth efficiency was observed in wastewater treated with chemical cum biological methods. The assimilation efficiency was high in biologically treated ferric chloride treated water (95.33%) followed by alum (91.96%), lime (91.64%), fly ash (89.865%) and control (88.439%). In contrary, the assimilation efficiency registered in the chemically treated wastewater was high in lime (89.57%), followed by ferric chloride (89.46%), fly ash (88.24%), control (88.43%) and alum (87.0%).

Common carp frys exhibited high growth rate in fly ash treated water and biologically treated water after fly ash treatment. It could be due to high level of removal of phosphate and ammonia from the wastewater treated. Alum treated water favoured the growth of test animals and ranked next to the fly ash treated water. Uniform growth rate could be registered in the test animals cultured in the lime and ferric chloride cum biologically treated waters. Nevertheless, lowest gross and net growth efficiency was recorded in lime treated water. The higher growth rate observed in common carp fingerlings grown in fly ash and alum treated waters is similar to the findings of Manimaran et al. (1997) in Koi carp fingerlings fed with *L. minor* incorporated feed.

All the four chemical treatments tried in the study, yielded good results in treating the wastewater of the ornamental fish farm. Either one of the methods can be used to treat the wastewater for its reuse. Fly ash encouraged the algal growth during biological treatment. Similarly, one of the biological methods may be adopted for treating the wastewater before releasing it into the adjoining aquatic systems. Biological treatment of wastewater after chemical treatment encourages its further use in aquaculture practices.

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