## Nutrient Reduction and Productivity Studies in Low Strength Freshwater Aquaculture Wastewater Using Lemna Gibba (Duckweed) System

### M.Karthik1 and R. Nagendran2

1 Scientist, National Environmental Engineering Research Institute, Nagpur --440020

2 Professor, Centre for Environmental Studies, Anna University, Chennai-60025

#### Abstract

The use of macrophytes plants for treatment of wastewater for nutrient removable is addressed. A study was conducted to ascertain the nutrient reduction potential in freshwater aquaculture using Lemna gibba (Duckweed). Experiments were conducted with plastic trays of dimensions 34 x 27 x 6 cm. Wastewater from freshwater aquaculture was collected and experimented under different dilutions. Nutrient removal and productivity of the Lemna gibba system were studied for a period of ten days, the experiments were conducted over a period of 90 days. The nutrient removal patterns were obtained for ammonia-nitrogen, nitrite, nitrate and phosphate. Reductions in nutrient in the Lemna gibba system are higher in raw wastewater compared to dilutions. Ammonia nitrogen had maximum removal efficiency followed by nitrate, nitrite and phosphate. Biomass productivity was also higher in the raw wastewater series than with dilutions. The studies indicated the survival of Lemna gibba in low nutrient loading conditions and also as a potential biomass resource.

Key Words: Duckweed; Lemna gibba; Freshwater aquaculture; Nutrient reduction; ammonia-N, nitrate, nitrite, phosphate; Productivity.

#### Introduction

The demand for food due to increase in population created a spontaneous growth of freshwater and seawater aqua farms. The effluents from aqua farms are rich source of organic materials and nutrients, and their release can upset the dynamic balance of the aquatic ecosystem.

The agencies concerned with water quality control and abatement of pollution of natural waters view aquaculture as a potential source of water pollution (Harris 1981, Alabaster 1982). Among other constituents, aquaculture wastewaters are characteristics in having considerable amount of nutrients such as nitrogen, phosphorous and other compounds. A number of studies have been conducted on the treatment of aquaculture effluents, and a comprehensive review on the topics has been made by Boyd 1990. The role of nutrients and other organic matter on eutrophication is also well established.

The role of aquatic plants in scavenging inorganic and some organic compounds from wastewater has been well documented (Reddy et al. 1987). Effluents thus renovated by the plants are stripped of their pollutants and when released into waterways cause less environmental damage. The principle floating aquatic plants used in aquatic treatment systems are duckweed, water hyacinth and pennywort. Duckweed units clean water so rapidly and effectively that they are now seriously being considered for use as "final polish" in sewage treatment.

The selection of duckweed for wastewater treatment is often the areas, where water is premium and where the infrastructure for sophisticated high technology wastewater treatment systems is not well established or they are not economically viable (Wood *et al.* 1989). Therefore, a real need exist for simple and low cost systems capable of producing desirable results with considerable removal of pollutants. From this viewpoint, technologies such as those employing aquatic plants appear to be viable alternatives in developing countries like India.

#### Materials and Methods

The wastewater from a fresh water fish-rearing pong a. Hydrobiological Research Centre, Chennai was collected at the end of the harvesting period. Mixing was done to ensure the composite nature of the effluent. In the laboratory, the sample was filtered across a blotting cloth having a mesh size of 50 u and stored at  $4^{\circ}$  c. the wastewater samples were analyzed following Standard methods 1995. The general characteristics of the wastewater are given in Table 1.

The experimental setup used for the study is illustrated in Fig. 1. A series of 4 trays consisting of Lemna system and one as control with different ratios of raw wastewater to tap water was used in the study. Series I contains undiluted wastewater, series II contains 75% raw wastewater and 25% tap water, series III contained 50% raw wastewater and 50% tap water and the series IV contained 25% raw wastewater and 75% tap water the experiments were carried out using plastic trays of 34 x 27 x 6 cm with a volume of 5.5 litres. The trays were filled with wastewater up to a height of 5.5 cm (5.05 litres). A free board of 0.5 cm was provided to prevent the washing of duckweed. A temporary shed covered the whole setup.

Plants were acclimatized for 2 weeks before the experiments were conducted. About 15 gm (wet) healthy specimens were selected and transferred to the experimental trays. Each set of experiments was spread over 10 days , the whole experimentation was carried for about 3 months. The biomass productivity was estimated as detailed by Oron *et al.* 1986.

#### Results and Discussion

The wastewater collected from Hydrobiological Research Station, Chennai, from fresh waster rearing pond has high concentration or organics and inorganics as evident from Table-1. The effluent possesses similar characteristics as that of low strength municipal sewage, except for chlorides. Though nutrients are well within the prescribed limit of the statutory boards for disposal, the studies were conducted under low concentration of nutrients to understand their use in tertiary treatment of nutrient removal from wastewater where there is wide fluctuations in the nutrient loading.

Table 1. Effluent characteristics from freshwater aquaculture pond

Sr. No.	Parameter	Concentration* (mg/L)
1	pH	8.5
2	Dissolved Oxygen	4.0
3	Conductivity (u s/cm)	1200
4	BOD <sub>5</sub>	53
5	COD	164
6	Chlorides	460
7	Total dissolved solids	650
8	Total suspended solids	280
9	Total solids	930
10	Ammonia	2.53
11	Nitrate	4.00
12	Nitrite	0.35
13	Phosphate	0.87

All values are expressed in mg/L, except pH and conductivity

<sup>\*</sup>All values represent average of 3 sets of readings

The presence of inorganic nitrogen and phosphorous at low concentrations of 0.3 mg/L and 0.01 mg/L respectively is bound to cause eutrophication (Sawyer 1947). The presence of nutrients in the form of ammonia. nitrite and nitrate, signifies the addition of nutrient to the waster during the fish culture. The pH of the wastewater was alkaline range (8.5). The reason being that considerable amounts of nutrients are added that increase the algal growth and the rate of photosynthesis in the aquaculture ponds and increased photosynthetic rate is known to shift the pH of water towards alkaline range (Wood et al. 1989, Picot et al. 1991).

# Nutrient Removal Studies in Lemna gibba System

#### Ammonia Removal

Time trace data of the ammonia as presented in (Fig. 2) suggest that there is a reduction in the concentration of ammonia in all the series. The overall removal efficiency of ammonia at the end of  $10^{th}$  day were 48.2%, 52.1%, 46.1% and 26.6% in the series I, II, III and IV (Fig. 3). Contrary to the expectation series II had greater overall removal efficiency compared with series I, III and IV. Maximum reduction was observed at the end of third day in all the series and the rate of reduction decreased in the subsequent 7th and  $10^{th}$  days. The reduction observed in the control could be attributed to the presence of nitrifiers present in the wastewater. The results are quiet comparable to those available in the literature. Dahab and Lee 1993, Reed *et al.* 1995 and Sarkar 1997 have reported that duckweed remove 25% to 33.9% of ammonia-nitrogen from wastewater. In the present system higher overall removal efficiency was achieved.

The removal pattern and efficiency appear to be dictated by natural chemical processes involving the interaction between ammonia, oxygen and nitrification bacteria. It is likely that removal of ammonia in series IV is more due to its convesion in to nitrate than its Utilization by Lemna gibba. The result is concluded on the basis of less productivity compared to the series I, II, III.

#### Nitrite Removal

Time trace data for nitrite removal is shown in Fig. 2. the nitrite concentration in the raw wastewater was quiet low in all the series. The removal efficiency was highest in series I (42.9%, 36.0%, 22.2%, and 16.7%) (Fig. 3). It was observed that rate of reduction for nitrite was maximum at the end of 7<sup>th</sup> day. There was not significant reduction after the 7<sup>th</sup> day in series II, III and IV. Nitrite being a unstable species between ammonia and nitrate, it becomes difficult to establish accurate relationship between the formation and removal of nitrite in the present system. The role of microorganisms in the reduction of nitrite could not be ruled out, and the absence of studies of this parameter makes it quiet complicated for any conclusion.

#### Nitrate Removal

Time trace data of nitrate is shown in Fig. 2. the overall removal efficiency obtained were 30.7%, 32.4%, 36.2%, and 55% in the series I, II, III and IV respectively (Fig.3). The increasing trend shown in the series is contrary to the expectation of obtaining low efficiency. The productivity was low in the series IV and increased removal efficiency of nitrate could mean that; due to low concentrations of ammonia in wastewater, the preference of nitrate to ammonia by the system. Such observations have also been reported by Landolt 1996. Martin et al. 1978 and Whitehead 1987 reported that nitrate removal efficiency to be higher at lower concentrations. The nitrate removal efficiency was much lower than obtained for ammonia. This could be attributed to the continuous conversion of nitrate by interaction microorganisms with the plant. The present values for removal efficiency are comparatively higher than those reported for Lemna major used in the treatment of eutrophicated ponds by Sarkar 1997. It could signify that Lemna gibba is better adapted to nitrate removal than Lemna major.

#### Phosphate Removal

Time trace data for phosphate reduction in the Lemna gibba system is given in Fig.2. the removal efficiency at the end of  $10^{th}$  day for all the series I , II , III , and IV were 29.9%, 21.9%, 14%, and 18.8% respectively (Fig.3). unlike the species of nitrogen, the efficiency in phosphate removal was much less. The rate of reduction for the 3 periods 0-3, 0-7 and 7-10 day in all the series was insignificant. It was observed that reduction was proceeding in the control system; which could signify that precipitation of phosphate to the bottom productivity was found to be lower with respect to series III.

Rejmankova, 1982 reported that reduction of removal efficiency of phosphate by duckweeds, when concentration of phosphate goes below 4 mg/L. Very low concentrations of phosphate in the wastewater under study could be a reason for low removal efficiency. Boniardi *et al.* 1994 have reported very high removal efficiencies ranging from 43 to 75% for phosphate from wastewaters. However, the initial concentration maintained by then was over 9 folds fore than the initial concentration in the wastewater used presently.

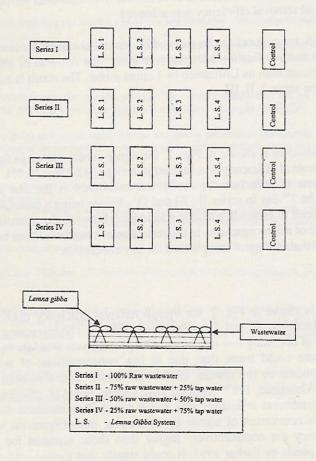


Figure 1. Experimental setup

## **Biomass Productivity**

Data in the production of *Lemna gibba* in the experimental treatment system are presented in Fig. 4. A significant growth was observed in all the series. Maximum growth rate was achieved at the end of the 7<sup>th</sup> day in series I and II, whereas the series III and IV showed maximum growth at the end of 3<sup>rd</sup> day with decreasing trend (Fig. 5). It could be concluded that lower levels of nutrients present in the series III and IV might have hampered growth rate. Symptoms of nutrient deficiency from all the *Lemna gibba* system were observed after the 7<sup>th</sup> day. Loss of pigment, chlorophyll and discoloration were severe after the 10<sup>th</sup> day. The die off's were observed quiet early in series III and IV (after 7 days) than series I and II. Much study is needed to ascertain the growth rate after 7 days. *Lemna gibba* in the system doubles after 3 days in series I, whereas kin series II, III. and IV the doubling is observed in between 7 and 10 days. Duckweeds are known to double their fronds every 4 days (Sherwood *et al.* 1995). It is evident from Fig. 2 to 5 that maximum growth rate as well as biomass yield was obtained in series I, where undiluted wastewater was used.

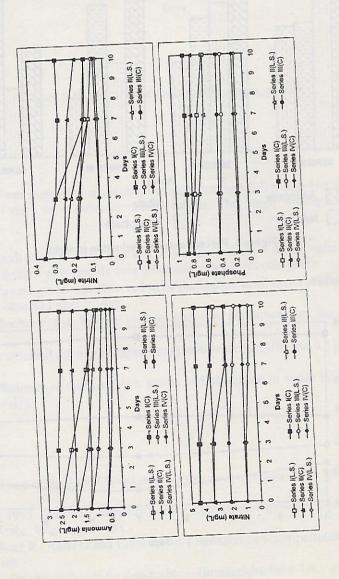


Figure 2. Time trace data for ammonia, nitrite, nitrate and phosphate

### Scale Up Studies for Field Application

Proportionate scale up studies of the tray system indicate that  $18.36 \text{ m}^2$  surface area is required to treat  $1 \text{ m}^3$  of the wastewater, with effective depth of 5.5 cm. Field studies indicated a effective depth of 6.0 cm to replicate the results obtained from the tray system. Maximum absorption by the plant is carried in the root zone by *Lemna gibba* (duckweeds), with an average root length of 9 mm.

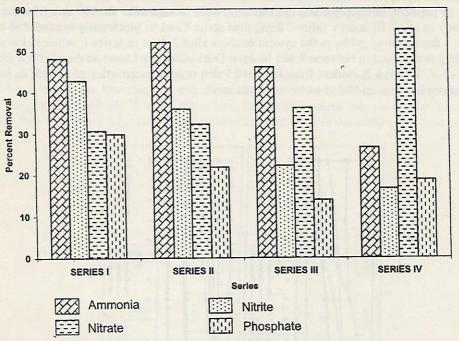


Figure 3. Percent nutrient reduction in series

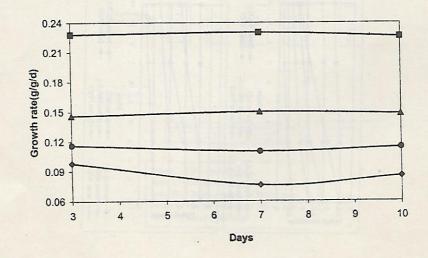


Figure 4. Biomass production of *Lemna gibba* system, wetgram

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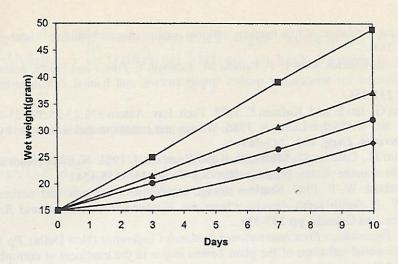


Figure 5. Productivity of Lemna gibba system, g/g/day

#### Conclusion

Studies on the removal of nutrients from aquaculture wastewaters using *Lemna gibba* system was carried out in trays for a period of 90 days in 4 different concentrations. The major findings of the study have been summarized below

- The trend of overall removal efficiency was ammonia > nitrate > nitrate > phosphate in all the series. It suggests that preference of ammonia over other nutrients by > Lemna gibba
- Growth rate of *Lemna gibba* was observed to be the highest in all series during 3 to 7 days. Decrease in growth rate was observed after 7<sup>th</sup> day
- Production of biomass and highest growth rate was maximum in the series I containing raw wastewater
- The growth rate obtained during the experiments was between 0.076 and 0.230 g/g/day
- The removal efficiency of nutrients, viz. nitrate and phosphate in series IV was quiet high (55% and 18.8%, respectively).

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