# PROTOTYPE OF AQUATIC ECO-WEB FOR THE BIOMANAGEMENT OF LIQUID EFFLUENTS

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Present paper highlights the role of aquatic eco-system in waste management along with biodiversity conservation. In a study conducted at BRC, authors created a perpetual aquatic eco-web by rearing Tilapias, Carps, and turtles in ferro-cement tanks along with *Spirulina platensis, Oscillatoria princeps, Chlorella vulgaris* and *Lemna gibba*. This aquatic eco-web also exhibited a natural succession of *Spirogyra sp., Azolla filiculoides, Ceratophyllum demersum*, several animalcules, copepods, and insects like dragonfly, butterfly etc. In the study period of six months, this aquatic ecosystem was used for treatment of secondary liquid effluents obtained from a gelatine manufacturing industry using three-tier state-of-the-art biotechnology. The effect of effluents on the stability of the eco-system was monitored periodically. In the process, the effluents exhibited substantial reduction in BOD [from 445 (± 12.45) ppm to 354 (± 12.98) ppm] and COD [from 1832 (± 13.93) ppm to 304 (± 10.30) ppm] values. The acidic pH (6.48 ± 0.138) of the effluents was neutralized (7.3 ± 0.149) after treatment. The organic content of the effluents degraded by inoculated enzymes and microbes were used as nutrients by the floating micro algae and the duckweed. This decreased the TDS content [from 0.272 (± 0.004) % to 0.160 (± 0.008) %]. It was observed that this aquatic ecosystem could effectively treat the obnoxious wastewater without any adverse effect on its biotic components. The treated water can be used for irrigating the neighbouring fields.

**Key words:** aquatic eco-web, effluents, hydrarch succession, *Spirulina*.

Increasing human population needs large number of industries to fulfill their diverse necessities. Then again, industrial activities generate a huge amount of obnoxious waste that has to be released into the environment. Industrial effluents are unbelievably noxious or likely to become so. In addition, their safe disposals pose an inherent problem of creating a financial burden to the industries. In recent past, bio-remedial measures for treatment of wastes are looked upon as more sustainable alternatives in relation to conventional waste treatment technologies. Biological methods such as, constructed wetlands (Babatunde *et al.* 2008), vermiculture treatment (Ghatnekar *et al.* 2009 c), phytoremediation (Meagher 2000) etc. are attracting considerable attention.

Since its inception, Biotechnology Resource Centre (BRC), Mumbai has contributed significantly by developing innovative, bio-safe waste treatment technologies of global importance uniting environment and economy (Ghatnekar and Kavian 1992).

In nature different components of an ecosystem exist inseparably, inter-related and interactive. The present paper describes the role played by different biotic components of an aquatic ecosystem to treat secondary liquid effluents of a gelatine manufacturing industry. The objective of the present study was to monitor the co-existence of these biotic components and their synergistic role in the biomanagement of selected liquid effluents. In addition it focuses perpetual zero discharge

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**Table 1: Chemical and Microbiological analysis of the Liquid effluents before and after treatment.**

| **Parameters** | **Before treatment** | **After treatment*(45 days)** |
|---|---|---|
| pH | 6.48 ± 0.138 | 7.3 ± 0.149 |
| Temperature | Ambient | Ambient |
| Total Solids (TS) % | 0.302 ± 0.010 | 0.428 ± 0.012 |
| Total Dissolved Solids(TDS) % | 0.272 ± 0.004 | 0.160 ± 0.008 |
| Biological oxygen demand | 445 ± 12.45 | 354 ± 12.981 |
| (BOD) - ppm | | |
| Chemical oxygen demand | 1832 ± 13.93 | 304 ± 10.30 |
| (COD) - ppm | | |
| **Microbiological analysis** | | |
| (Total CFU) | 1.992 (± 0.088) × $10^5$ | 2.096 (± 0.034) × $10^7$ |
| *A. flavus* | - | 1.776 (± 0.031) × $10^{1.7}$ |
| *A. niger* | - | 1.878 (±0.033) × $10^{1.85}$ |

* Average of readings recorded from all 5 tanks ± Standard Error.

The secondary liquid effluents were procured from the Effluent treatment plant of I.G.C.L., Vapi, Gujarat. Protease and cellulase enzymes used for treatment of the selected effluents were obtained from Egenix Biotech Pvt. Ltd., Bangalore.

Pure cultures of *Aspergillus flavus* and *A. niger* were obtained from the culture bank of BRC. Thereafter, the effluent resistant isolates of these species *viz., A. flavus* (BRC-27) and *A. niger* (BRC-28) were cultured in flasks and were maintained at 30 ºC and 90 rpm in the rotary shakers (Orbitek). Later these isolates were multiplied in semi-automated fermentors (BRC- Bio-Boom). Axenic cultures of *Spirulina platensis*, *Oscillatoria princeps*, and *Chlorella vulgaris* were obtained from the culture bank of BRC. Thereafter the effluent resistant isolates of these species *viz., S. platensis* (BRC-45), *O. princeps* (BRC-46), and *C. vulgaris* (BRC-47) were cultured and acclimatized in rectangular plastic tubs (0.45 × 0.32 × 0.12 m); (Plate 4). *Lemna gibba* were collected from stagnant water points of the adjacent nullah and later reared at BRC.

The experimental tanks were filled with 250L nullah water to which the effluents at 50% concentration (250L) were introduced. Selected enzymes (protease and cellulase) and microbial cultures were inoculated in it. After three days, effluent acclimatized strains of selected algae were introduced and soon after their stabilization (Plate 2), *L. gibba* were also introduced into the tanks. Later, fish *viz*., Mozambiq Tilapias, Indian Major Carps and turtles were also introduced in the tanks.

The tanks were aerated (45 minutes, 3 to 4 times daily) using an air compressor (2H.P. Fauzi). The tank water was maintained at constant level by adding the nullah water to check evaporation losses.

The experiment was conducted for the

**Table 2.** Growth rate of algae, and *L. gibba* in terms of Optical Density of tank water and fresh weights of the harvested biomass.

| Number of days | Optical Density* of tank water (at 560 nm) | % I | Fresh weight of algal biomass * (g $L^{-1}$) | Fresh weight of *L. gibba** (g $L^{-1}$) |
|---|---|---|---|---|
| 0 | 0.542 ± 0.01 | - | - | - |
| 15 | 0.690 ± 0.008 | 27.3 | 10.44 ± 0.31 | 20.54 ± 0.7 |
| 30 | 0.817 ± 0.005 | 50.7 | 11.32 ± 0.41 | 21.71 ± 1.79 |
| 45 | 0.878 ± 0.006 | 61.9 | 11.48 ± 0.46 | 24.92 ± 0.9 |

* Average of readings recorded from all 5 tanks ± Standard Error % I percentage increase in absorbance in relation to zero day reading.

period of six months at natural light and temperature regime. During the experimental period, the physico-chemical characteristics of the selected effluents were monitored before treatment and after a period of 45 days (Table 1). Thereafter, 75% of the treated water (375 L) was replaced with fresh load of effluents at same concentration.

The effect of effluents on the biotic components of the ecosystem was closely observed in terms of growth of inoculated algal population, *L. gibba* and fish besides, other aquatic flora *viz*., *Spirogyra sp., Azolla filiculoides, Ceratophyllum demersum* and *Hydrilla verticillata*, that were introduced while addition of nullah water into the tank.

The growth rate of selected algal species in the experimental tanks was monitored in terms of Optical Density (O.D.) of the cultures recorded at an interval of 15 days for period of 45 days using 215 D visible Spectrophotometer (Chemito) at 560 nm (Table 2). The algal biomass was harvested using a stratified sieve of different mesh size. The harvested matter was washed thoroughly with clean water and its fresh weight was recorded (Table 2). The microbial growth in the treated effluents was analyzed in terms of Colony Forming Unit (CFU) using standard dilution plate technique (Aneja 2003).

Data collected were analyzed using the statistical package described in Schaum's Outline Series (Stansfield 1969) and a software package available online at http://-www.graphpad.com/quickcalcs/ttest2.cfm.

## RESULTS AND DISCUSSION

The physico-chemical analysis of selected liquid effluents before treatment (Table 1) revealed their acidic nature (pH, 6.48 ± 0.138). They exhibited very high BOD [445 (± 12.45) ppm] and COD [1832 (± 13.93) ppm] values. Total suspended solid (TSS) (0.302 ± 0.010%) and Total dissolved solid (TDS) (0.272 ± 0.004%) constituted the major organic and inorganic content of the effluents. Ghatnekar *et al.* (2009 b) reported that the gelatine industry wastes are rich in nitrogen and other minerals like calcium, phosphorus and silica.

The result obtained from the analysis of liquid effluents after treatment are presented in Table 1. It clearly indicates the biodegradation potential of selected effluents by the biotic components of aquatic eco-web created in the present study. In the process the effluents exhibited substantial reduction in BOD to 354

PLATE 1. EXPERIMENTAL TANKS AT BRC'S FARM, BADLAPUR, MAHARASHTRA

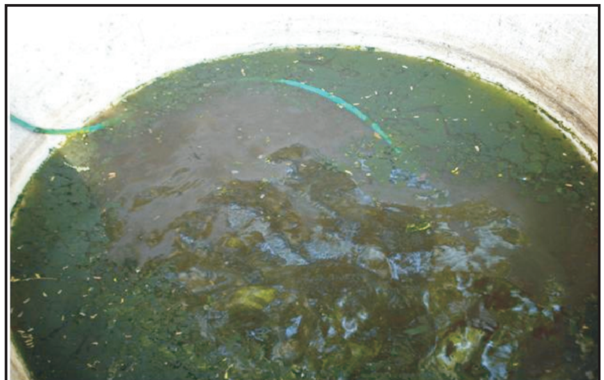

PLATE 2. SATURATION OF ALGAL POPULATION IN EFFLUENTS TREATED WITH SELECTED MICROBES AND ENZYMES.

PLATE 3. CO-EXISTENCE OF ALGAL POPULATION WITH *L. GIBBA*.


PLATE 4: PLASTIC TUBS USED FOR THE ACCLIMATIZATION OF SELECTED ALGAE AND *L. GIBBA* IN LIQUID EFFLUENTS

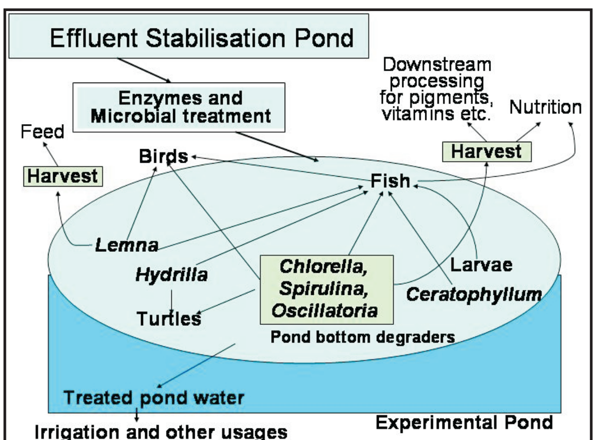


**Figure 1. The prototype model of aquatic eco-web for biomanagement of effluents**

(± 12.98) ppm and COD value to 304 ± (10.30) ppm. The acidic pH of the effluents was also neutralized (7.3 ± 0.149) after the treatment.

The enzymes and microbial cultures degraded the complex organic matter in the proteinaceous waste water into simpler forms. As a result the TDS content in the effluents decreased [from 0.272 (± 0.004)% to 0.160 (± 0.008)%]. The degraded matter was used as nutrients by the micro algae and the duckweed for their growth. The percentage increase in the O.D. (61.9%) and fresh weights of the harvested biomass (Table 2) was indicative of favourable growth of selected algae and *L. gibba* in the treated effluents.

The combination of enzymes, microbes and the aquatic plants thus, formed the principle components of the three-tier, state-of-the-art biotechnology (Ghatnekar and Kavian 1992) used for biotreatment of selected liquid effluents.

It is a known fact that the last two hundred years of industrial revolution has taken a serious toll on the environment mainly due to the tremendous amount of waste generated by the industries. The global concern about environment and resource conservation has led to increasingly stringent waste disposal legislation. The problem of waste management without disturbing nature is becoming increasingly intense and severe for industries in particular and society in general. Industries should consider that they are part of the environment and not stand out against it (Ghatnekar 1999).

In this context BRC, proposes a sustainable and bioethical work-model of waste treatment process. This process involves simulation of an aquatic eco-web for perpetual waste management. The experimental tanks used in the present study served the purpose of aquatic ecosystem.

The entire process of liquid waste treatment in the present study can be viewed as a form of hydrach succession (Ghatnekar 1983). The microbial cultures along with the micro algae symbolized the pioneers of succession which laid favorable conditions for the subsequent natural successors. This aquatic ecosystem exhibited invasion and dominance of even other aquatic flora *viz*., *Spirogyra sp., Azolla filiculoides, Ceratophyllum demersum* and *Hydrilla verticillata* that were introduced while addition of nullah water into the tank. Krivtsov *et al.* (2000) used model simulation to demonstrate the possibility of influencing a species dominant at a later stage of ecological succession by alleviating growth limitation of a different species, dominant at an earlier stage. Such delayed relationships are characteristics of ecological systems.

Gijzen and Veenstra (2000) described duckweed as a key step in waste recycling due to the fact that it forms the central unit of a recycling engine driven by photosynthesis. Therefore the process becomes sustainable, energy and cost efficient, and applicable under a wide variety of rural and urban conditions. Development of *Lemna* layer on the surface discouraged the increase of mosquito larvae. *L. gibba* cover on the surface of water, restricted light and limited oxygen supply to submerged components. However, its occasional harvest and aeration to the tank water eased the development of other species (Plate 3). Frequent aeration also prevented the development of total anaerobic conditions in the deeper parts and caused uniform circulation of dissolved nutrients.

The Carps and Tilapias in the aquatic ecosystem accomplished their role of consumers and controlled extensive growth of plants and larvae. Turtles and pond bottom degraders (Product literature of BRC) controlled the build up of detritus matter at the base of the tank. The fish and turtles thrived harmoniously in a polyculture system. In addition, several tiny animalcules, copepods, insects, dragon fly, moths, etc also formed the conspicuous members of the ecosystem. Various birds such as mynah, wag tail, weaver bird, crane, warbler etc. were periodic visitors of this aquatic ecosystem.

In fine, there was no observable adverse effect on the biotic components of the aquatic ecosystem when the suitably treated effluents were introduced in the system. Moreover, each of the experimental tanks supported an aquatic eco-web that could perpetually treat the waste water (Fig. 1). Then again, it maintained the species diversity and hence ideally formed a prototype model for waste management and biodiversity conservation. The tank water obtained after the treatment was used for irrigating neighbouring fields.

The micro algae, *S. platensis*, *C. vulgaris, and O. princeps* were selected for the above study due to their commercial importance. Nutraceutical properties of *S. platensis* has projected it as a coveted probiotic of 21$^{st}$ Century (Ghatnekar *et al*. 2009 a). Besides, it finds tremendous application in aquaculture and animal husbandary. Sharma *et al.* (2008) suggested the perpetual cultivation of *Spirulina* in aquaculture systems.

The ability of algal species to grow in impure water is being exploited by biotechnologists for dual application *viz.*, to reduce their production cost and for the biomanagement of industrial wastes. Thus, wastes from the industries can be polished along with production of nutritional food supplement by application of suitable technology (Ghatnekar and Kavian 1992).

Ghatnekar *et al.* (2009 d), described the biomanagement of secondary liquid from gelatine manufacturing industry using *S. platensis*, in combination with *A. flavus* and *A. niger,* wherein the liquid effluents were mixed in different concentrations with BRC-Spiro medium (Tamhane and Ghatnekar 2004). Findings from their study suggested that the effluents at 50% concentration mixed with BRC-Spiro medium yielded optimum growth of *S. platensis* and efficient biodegradation of the effluents under study. In the present study, the occurrence of *C. vulgaris, S. platensis* and *O. princeps* was constantly maintained in the tanks by adopting the same protocol.

Earlier, Ghatnekar *et al.* (2009 b) had used 'Three-tier vermi-culture biotechnology' for the treatment of solid effluents obtained from the gelatine manufacturing industry. In their study the effluents were treated with enzyme protease and microorganism *viz., A. flavus, A. niger and Bacillus licheniformis.*

In recent times, the synergistic effect of algae and higher plants in waste recuperation is widely used. Yolanda *et al.* (1998) used *S. platensis* and *Phaseolus* for the biosorption of Antimony and Chromium from natural and industrial waters. Findings in the present paper also highlight this aspect.

Oron *et al*. (1987) reported the COD removal efficiency of 66.5% by *L. gibba* cultured on raw waste water in 20 cm deep ponds. Mandi (1994) observed COD removal efficiency of *L. gibba* to be 72.1% when cultured on 14 cm deep ponds. Korner and Vermaat (1998) conducted live laboratory-scale experiments to assess separately the contributions of duckweed itself, attached and suspended bacteria as well as algae to N- and P-removal in domestic wastewater.

Efforts for mitigating the problems of safe disposal of solid and liquid wastes by the industries are the most needed ones. Different components of an aquatic ecosystem contribute to the environment purification process in their respective niche. The aquatic eco-web used here is a prototype model to serve as a 'significant tool' for waste treatment without compromise in biodiversity. BRC now plans to emulate this model in several states of the country.

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