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Stabilisation of Wastewater by *Lemna minor*: A Microcosm Study

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Abstract

The present study was undertaken to determine the efficacy of *Lemna minor* (duckweed) in stabilisation of untreated mixed wastewater. It was observed that Lemna can efficiently remove dissolved solids and organic matter in a microcosm operated under sunlight. The removal of BOD_5 and COD was of the order of 50% and 75%, respectively. Microscopic examination of the roots confirmed the role of roots in entrapment of suspended solids and organic matter over the roots. The DO levels in wastewater increased to about three times during the treatment. The biomass, chlorophyll *a* and *b*, and carotenoid content had significant increase, and ratio of chlorophyll *a*/chlorophyll *b* was almost constant. No biochemical or physiological stress of wastewater over *Lemna* could be reported during the study. The study concluded that *Lemna minor* can be used for stabilisation of low strength mixed wastewater, particularly for remote and small communities.

Keywords: *Lemna Minor*; Rhizoremediation; Wastewater Stabilisation; Chlorophyll.

Introduction

Constructed wetlands (CWs) are being used throughout the world for wastewater stabilisation (Gersberg et al., 1986), removal of nutrients (Haritash et al., 2015), suspended solids (Noe et al., 2010; DeBusk et al., 1995), pathogens (Alaerts et al., 1990) and even toxic pollutants (Miranda et al., 2000). Such systems are becoming more popular in developing countries because they require less energy, less technical know-how, and are efficient in removal. The plants growing in wetlands have major functional role in pollutant removal through filtration, absorption, and sedimentation. The treatment takes place in root zone primarily, and is referred to as rhizo-remediation. The plant roots along with sediments act as a matrix for filtration and subsequent decomposition and absorption. The role of sediments/packing matrix and microorganisms can not be overlooked as they play a major role in removal depending on the type of pollutant, its physico-chemical properties, and the conditions prevalent in CWs (Haritash *et al*., 2017). Still, most of the studies designate vegetation to have the major role in treatment. Vegetation in wetlands can broadly be classified as submerged, emergent, and floating depending on the layer of water in which it is present. Submerged and emergent plants have a stable/fixed root system in sediments, whereas the roots of floating macrophytes remain suspended freely in water column. Lab-scale mesocosm studies on floating macrophytes are effective to determine the treatment efficiency of plants exclusively since the packing matrix may be excluded. Most of the studies on floating macrophytes deal with water hyacinth, water pennywort, and duckweed (EPA, 1988; Brix, 1997) etc. Whereas pennywort is confined to North Africa, Europe, and Florida, and water hyacinth is an intensive weed; duckweed is useful because of its wide geographic distribution (Alaerts et al., 1996), high nitrogen and protein content

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(Mbagwu & Adeniji, 1988), and significant treatment efficiency (Tripathi et al., 1991). It is commercially grown in lagoons in south-east Asian regions for the treatment of sewage and industrial wastewater, culturing fish (World Bank, 1992), fodder production (Leng, 1995), and extraction of proteins from it. The present study was undertaken to determine the removal efficiency of *Lemna minor* towards organic matter and dissolved solids in a lab-scale microcosm. *Lemna* removes the organic matter and suspended solids with the help of its roots suspended in water column. Sediments were not included in the microcosms, so as to determine the treatment efficiency of *Lemna* alone. Parallel experiments were undertaken to study the effect / stress of wastewater over physico-biochemical characteristics of vegetation to arrive at the efficacy of *Lemna* towards wastewater stabilisation.

Materials and Methods

The present study was undertaken in microcosm made of plastic tubs. Healthy fronds of Lemna were collected from a pond located in the campus of Delhi Technological University (DTU), Delhi. The pond receives untreated domestic wastewater from residential colonies around it; and a part from the hostels of DTU. The wastewater being used in the study was collected from the inlet of pond and its physicochemical properties were determined immediately after collection using standard methods as prescribed by APHA (1998). The wastewater was classified as low-strength against the value of COD (Almeida, 1999). Two plastic tubs (15 L) were filled with the wastewater to a volume of 10 L, and 200 g (fresh weight) of *Lemna minor* was added to each. One of the tubs was kept in sunlight (average photoperiod of 10 hours), and the other was kept under artificial fluorescent light to study the possibility of treatment under artificial light. Samples were collected after 3 days, and later at regular intervals of 7 days for 21 days to study the degree of treatment with respect to time. The microscopic examination of roots of *Lemna* was also done to confirm their role in filtration and absorption of suspended solids and organic particles from the wastewater.

To study the biochemical stress of wastewater over *Lemna*, dilutions of the order of 20%, 40%, 60%, and 80% alongwith a control and raw wastewater (100%) were prepared in separate plastic pots of 2.0 litres capacity. One litre of respective dilution was transferred to each pot alongwith 0.5 g of *Lemna*. The physico-biochemical parameters like root length (cm), fresh weight (g), Chlorophyll 'a' (mg / ml), chlorophyll 'b' (mg / ml), and carotenoids (mg / ml) were determined in the beginning and after 10 days to estimate the stress of wastewater over *Lemna*, if any. Determination of chlorophyll was done as described by Arnon (1949) using the following equations

Chlorophyll a (mg / ml) $=12.7(A_{663}) - 2.69(A_{645})$

Chlorophyll b (mg / ml) $= 22.9 (A_{645}) - 4.68 (A_{663})$

Carotenoids (mg / ml) $= [1000A_{470} - 3.27(\text{chl a}) - 104(\text{chl b})] / 227$

Results and Discussion

Based on the results obtained in present study, duckweed was found to have the capacity for wastewater stabilisation and removal of organic matter in the presence of sunlight. The experiment under artificial light failed since duckweed could not survive under the artificial light. During the treatment, the pH shifted from near neutral (7.2) to slightly alkaline (8.1) confirming to addition / production of basic species during stabilisation (Table 1).

Table 1: Characteristics of wastewater during its stabilisation by *Lemna minor*

Day	pH	EC (mS/cm)	TDS (mg/l)	Cl^- (mg/l)	DO (mg/l)	BOD (mg/l)	COD (mg/l)
0	7.2	6.82	1280	278	1.8	23	200
3	8.2	7.15	800	234	2.5	20	180
7	8.1	7.74	800	179	4.8	18	70
14	8.1	6.51	760	154	5.0	15	50
21	8.1	6.35	700	140	6.0	12	50

High concentration of organic matter may also contribute to reducing / slightly acidic conditions as a consequence of reduced dissolved oxygen (DO) levels. Degradation / mineralization of organic carbon results in production of CO_2, a part of which dissolves in water in near neutral / slightly acidic conditions resulting in formation of bicarbonate and subsequently carbonate, both of which add basicity to water. The degradation of organic matter in a duckweed based system may also result in production of such species having basic nature. The degradation / removal of organic matter results in improved level of DO with time as is observed in the present study. The DO concentration increased almost three times from 1.8 mg / l to 6.0 mg / l during the treatment. BOD_5 and COD were significantly reduced with a removal efficiency of 50% and 75%, respectively, in the treatment period of three weeks (Table 1). COD removal of the similar order (73-84%) has been observed in lab scale experiments treating municipal wastewater in Turkey (Ozengin & Elmaci, 2007) and Netherlands (Korner et. al, 1998). The low BOD and COD removal efficiency of duckweed may be attributed to its growth over water surface, which inhibits diffusion of atmospheric oxygen in water, and comparatively reduced photosynthetic activity in water column because of reduced sunlight penetration, resulting in low DO levels underneath the duckweed layer (Brix & Schierup, 1989). However, transport of oxygen from leaves to roots, lead to formation of an aerobic zone in rhizosphere (Alaerts et al., 1996). The major pathway for degradation of organic matter was addition of DO to wastewater through the roots of duckweed, and action of microbes associated with roots, over the organic matter. The mineralised matter may be easily absorbed by duckweed / associated microbes for growth and metabolism. Apart from it, the dissolved nutrients (nitrate and phosphate), and micronutrients (Na, K, Fe etc.) are also absorbed from wastewater. Microscopic examination of roots of *Lemna* revealed the association / entrapment of suspended solids and organic particles on sheath of extra cellular enzymes particularly over root-tip (Figure 1).

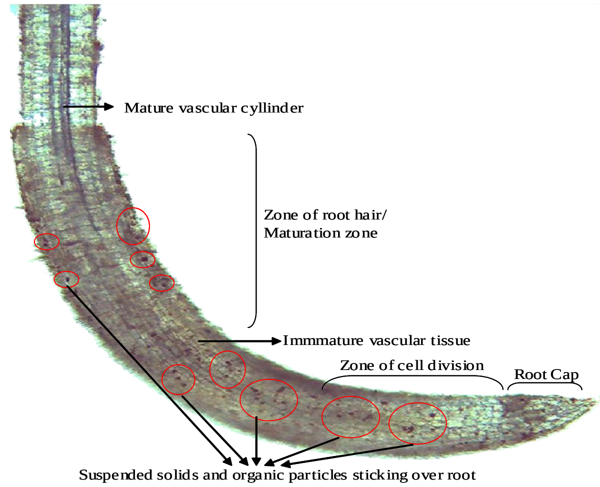

Fig. 1: Root tip of *Lemna* with a sheath of extracellular enzymes and organic particles sticking over it (Resolution: 10*40; Motic, China)

Change in redox state and dynamic chemical equilibrium, too, results in natural precipitation of dissolved impurities. As a result, constantly decreasing total dissolved solids (TDS) and electrical conductivity (EC) values were observed confirming to removal of other dissolved species as well.

The experiments on determination of stress revealed that duckweed underwent no biochemical stress during wastewater treatment. Since wastewater is rich in nutrients (CNP), the plant had easy availability of nutrients and improved growth and metabolism. Significant increase in biomass (fresh weight) of duckweed is an indicator of assimilation of extraneous carbon, water, and other nutrients from wastewater. Although accumulation of biomass was observed, decrease in root length was observed for all the dilutions with no regular trend (Table 2).

Table 2: Biochemical characteristics of *Lemna minor* at different dilutions of wastewater

Dilution of waste water (%)	Root length (cm)		Fresh weight (g)		Chlorophyll 'a' (μg/ml)		Chlorophyll 'b' (μg/ml)		Carotenoid (μg/ml)	
	Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	*Initial*	*Final*	*Initial*	*Final*
Control	2.9	1.6	0.5	1.02	9.87	5.16	5.59	3.15	0.074	0.035
20	2.9	2.0	0.5	1.34	9.87	6.31	5.59	4.60	0.074	0.048
40	2.9	1.3	0.5	1.19	9.87	5.82	5.59	4.26	0.074	0.048
60	2.9	2.0	0.5	1.54	9.87	6.94	5.59	6.03	0.074	0.066
80	2.9	1.5	0.5	1.03	9.87	13.60	5.59	8.48	0.074	0.069
100	2.9	1.2	0.5	0.73	9.87	18.87	5.59	14.78	0.074	0.078

It may be ascribed to presence of some other pollutant (heavy metals) in the collected wastewater, or the degeneration of soft outer tissue of roots due to physical stress during transfer of duckweeds to experimental tubs. Significant improvement in chlorophyll 'a' and chlorophyll 'b' concentration over the control was an indicator of improved plant health, which may be ascribed to higher nutrient availability with increasing fraction of wastewater. Dilution of raw wastewater resulted in comparatively lower levels of chlorophyll and carotenoids. The Chl'a' / Chl'b' ratio revealed no stress over duckweed. Decreasing trend of Chl'a' / Chl'b' ratio may be an indicator of stress (Wolf, 1956). Since the ratio was almost constant, and total chlorophyll had increased in all the dilutions, no stress over duckweed may be reported. Similar increasing trend for carotenoid confirmed the observation that duckweed was not exposed to any stress during the treatment of domestic wastewater.

Conclusion

The study concluded that *Lemna minor* can be an effective option for the stabilization of untreated low-strength wastewater. It could efficiently remove the organic load in terms of BOD_5 and COD in the presence of sunlight to a level of 50% and 75%, respectively, in a period of three weeks. The retention period, however, may be reduced by increasing the pond area. The roots of *Lemna* were found to have major role in removal of suspended and organic particles from wastewater. The use of raw wastewater (without dilution) is recommended since it favors easy availability of nutrients, and improved growth of plants. The duckweed-based stabilization ponds may be used for remote small rural communities, and the stabilized wastewater can be utilized for aquaculture, horticulture etc.

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