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Role of Dominant Green and Red algae of Indian Sunderbans in Nutrient remediation of synthetic saline wastewater

Sarban Sengupta and *Ruma Pal

Phycology laboratory, Department of Botany, University of Calcutta; 35, Ballygunge Circular Road, Kolkata-700019, West Bengal, India. ***Corresponding author:**rpalcu@rediffmail.com

Abstract:

The Sunderbans is the single largest block of mangrove habitat in the world with a wide diversity of algal forms and high rate of Eutrophication. In our regular survey of this region, two abundant Chlorophytes, *Ulva intestinalis* , *Chaetomorpha aerea* and one Rhodophyte, *Gracialaria* sp. were recorded in blooming conditions in different areas. These three algal genera were collected and cultivated in synthetic wastewater to investigate nutrient remediation potentials. *Ulva intestinalis* was found to be the most potential genus for nutrient remediation process, removing 8.61 µg/L/d/gBiomass Nitrate-Nitrogen, 0.06 µg/L/d/gBiomass Nitrite-Nitrogen, 5.7 µg/L/d/gBiomass Ammonium Nitrogen and 0.64 µg/L/d/gBiomass Dissolved Inorganic Phosphate along with maximum biomass growth. On the other hand, *Chaetomorpha aerea* documented minimum nutrient remediation potentials among the three cultivated genera (3.42 µg/L/d/gB Nitrate-Nitrogen, 0.047 Nitrite-Nitrogen, 4.97 µg/L/d/gB Ammonium Nitrogen and 0.53 µg/L/d/gB Dissolved Inorganic Phosphate) with medium growth rate .Overall the *Gracialaria* sp. showed intermediate nutrient removal capacity with minimum biomass yield in experimental synthetic waste water.

Introduction:

Waste water cultivation of both micro and macroalgae is a common practice to remove excess nutrients especially N and P and other pollutants. Green algae especially *Chlorella* sp. is used extensively for wastewater remediation (Wang *et al*. 2009).The use of macroalgae for wastewater treatment is also well recognized in parts of Europe (Schramm 1991). Ryther *et al*. (1972, 1979) reported the use of seaweeds *Gracilaria tikhaviae* and *Agardhia subulata* for the treatment of secondary municipal effluents in the USA. Apart from this, macroalgae are also utilized as nutrient traps in Japan (Hirata and Xu 1990) and Israel (Cohen and Neori 1991). Cultivation of *Ulva intestinalis* and *U. lactuca* in sewage enriched brackish water were also reported by Sauze in southern France (Sauze, 1983).Generally in closed mariculture systems, Dissolved Inorganic Nitrogen (DIN) species emanating from fish waste accumulate up to toxic levels and *Ulva lactuca* has successfully been utilized as an efficient biofilter for the removal of nitrogenous species especially ammonia (Cohen and Neori 1991). Buschman et al. (2005) reported efficient capturing of nutrients by *Gracilaria chilensis* from salmon tanks in Chile. The algae cultivated in the salmon tanks also yielded high quality agar.

In the present investigation the experimental media was formulated with known salt concentration with nitrogen and phosphorus enrichment, comparable to waste water and named as synthetic waste water. In our regular survey of Indian Sunderbans, three species of green and red algae viz. *Gracilaria* sp., *Ulva intestinalis* and *Chaetomorpha* aerea were collected from Indian Sunderbans and grown in synthetic wastewater to study their nutrient remediation potentials together with useful biomass production. Variation in salinity and nutrient concentrations induces variation in algal growth (Gorain *et al*. 2013). Hence, a constant salinity of 8ppt was employed and initial nutrient concentrations were kept the same for all the three algae.

Materials and Methods:

a. Collection of Algal strains:

All the three species of macroalgae, (recorded as abundant species) were collected from the brackish water region of Indian Sunderbans, located between 21° 31´N and 22° 53´N, and between 88° 37´ and 89° 09´E in the South 24 Pargana district,India. Samples were collected in transparent plastic bags and sealed.

In the laboratory, they were washed with water to rid of epiphytes and grown in liquid media in uni-algal conditions. Samples were identified according to proper monographs (Prescott, 1962; Krisnamurthy, 2000, Algaebase).

a. **Preparation of Synthetic Wastewater:** Synthetic wastewater was prepared using natural sea salt formulation of Central Salt and Marine Chemicals Research Institute (CSMCRI) and Calcutta University for better algal growth (Patent no. 12826682.2-1354. 2014). The salt mixture was dissolved in tap water to achieve a salinity of 8ppt. Sodium Nitrate and Ammonium Chloride were added to the media to achieve a total Dissolved Inorganic Nitrogen (DIN) concentration of 30.4 mg/L with 18.23 mg/L of Nitrate-Nitrogen and 12.05 mg/L of Ammonium-Nitrogen. Nitrite-Nitrogen was found to be a minor constituent (0.125mg/L)of the DIN species.

b. **Cultivation of macroalgae in synthetic wastewater and determination of growth:**
In each experimental set, 100 gms of biomass was taken in 30 liters of experimental medium to start open tank the cultivation process. The tanks were kept in open sunlight with regular aeration. Fresh Weight of the algal biomass was measured at regular intervals of three days on a digital weighing machine after thorough but gentle soaking on blotting paper. The results were carefully recorded in terms of per gram per day biomass production (g/d/gB)

c. **Estimation of Nutrient Consumption:**
Dissolved Inorganic Nitrogen was measured as the summation of Nitrate Nitrogen, Nitrite Nitrogen and Ammonium Nitrogen along with total Dissolved Inorganic Phosphate (DIP). The individual parameters were determined according to standard protocols (APHA 2000). These parameters were estimated every three days in order to determine their nutrient consumption.

Results:

a. **Growth and Biomass yield of the algae in synthetic wastewater:** Among the three algal species, *Ulva intestinalis* documented the highest biomass yield (0.28g/d/gB) followed by *Chaetomorpha aerea* (0.13g/d/gB) and *Gracilaria* sp. (0.12g/d/gB). Both *Chaetomorpha aerea* and *Gracilaria* sp had maximum biomass after 24 days of cultivation while *Ulva intestinalis* showed maximum biomass yield after 21 days (Table -1;Fig. 1).

b. **Measurement of nutrient consumption:** Minimum nutrient levels were observed after maximum biomass yield as expected. Subsequently after growth phase nutrient leaching from degraded biomass was observed. Maximum Nitrate-Nitrogen remediation potential was observed for *Ulva intestinalis* (8.61µg/L/d/gB) followed by *Gracilaria* sp. (6.9 µg/L/d/gB) and *Chaetomorpha aerea* (3.42 µg/L/d/gB).The green alga *Ulva intestinalis* also removed maximum Nitrite-Nitrogen from the experimental media (0.06mg/L/d).*Gracilaria* sp. (0.048 mg/L/d) and *Chaetomorpha aerea* (0.047 µg/L/d/gB) showed almost similar rates of removal. Maximum Ammonium-Nitrogen remediation potential was also observed for *Ulva intestinalis* (5.7 mg/L/d/gB) followed by *Gracilaria* sp (5.01 µg/L/d/gB) and *Chaetomorpha aerea* (4.97 µg/L/d/gB). Maximum removal of phosphate was again showed by *Ulva* (0.64 µg/L/d/gB) followed by *Gracilaria* sp. (0.55 µg/L/d/gB) and *Chaetomorpha aerea* (0.53 mg/L/d/gB) (Fig. 2-5 and tables 1 and 2).

Algal Species	Biomass Generated (Fresh Weight, G)	Number of Days
Enteromorpha intestinalis	584.45	21
Gracilaria sp.	253.75	24
Chaetomorpha aerea	317.38	24

Table 1. Table depicting the biomass yield of the three algae in synthetic saline watewater

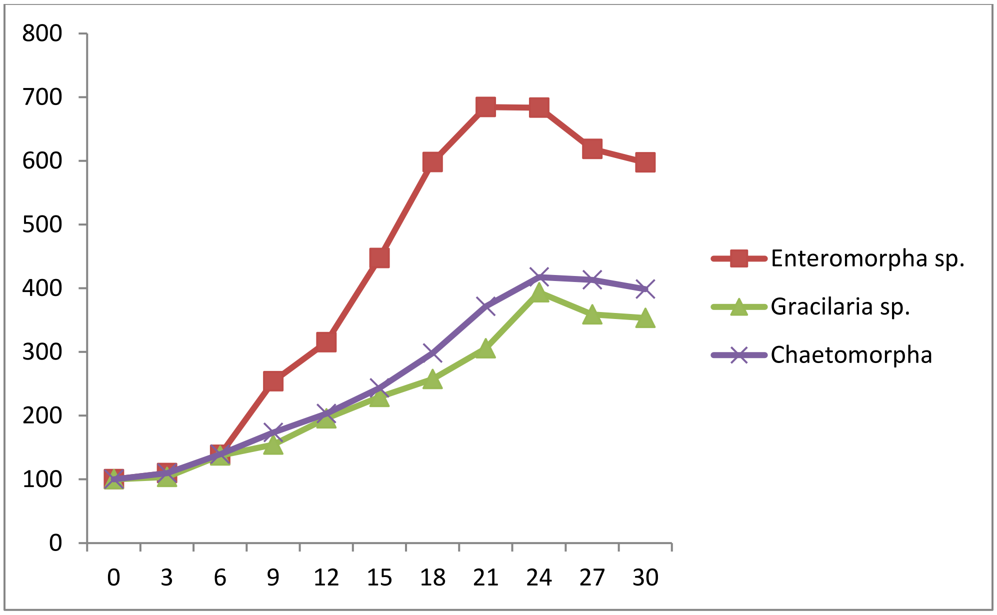

Fig. 1: Figure depicting growth curves of the experimental algae in synthetic saline wastewater

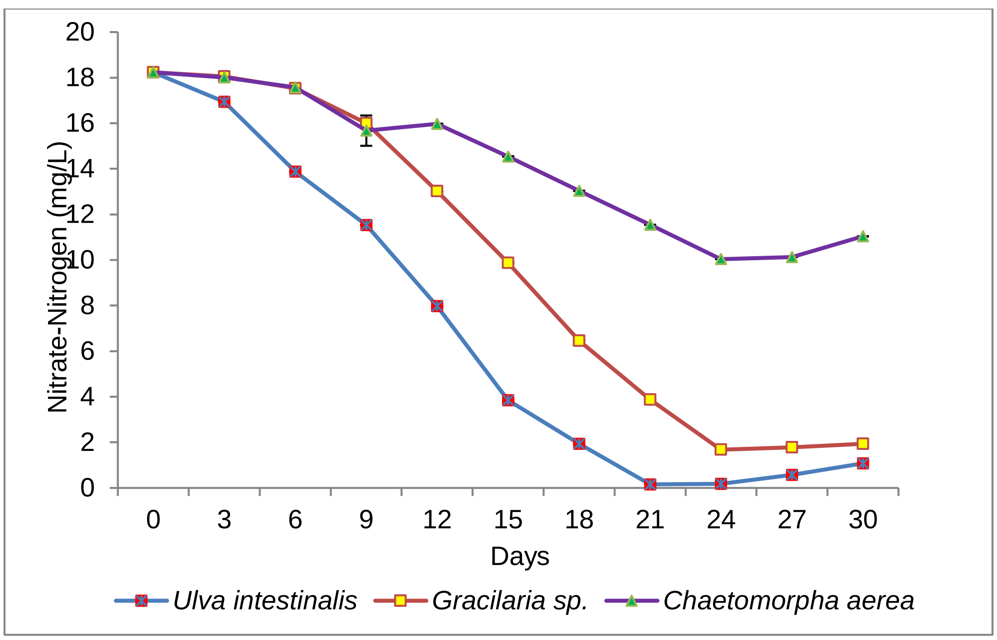

Fig. 2: Figure depicting depletion of Nitrate-Nitrogen by the three macroalgae in synthetic saline wastewater

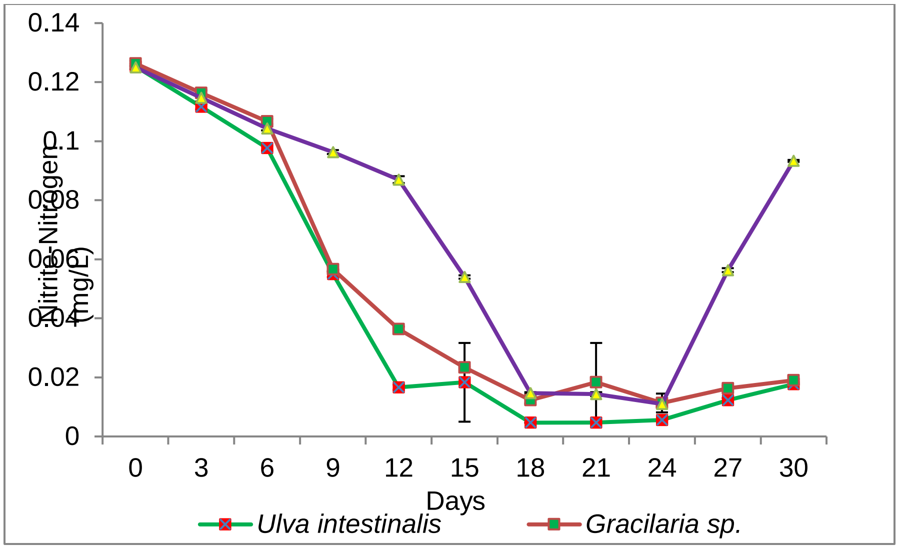

Fig. 3: Figure depicting depletion of Nitrite-Nitrogen by the algae in synthetic saline wastewater

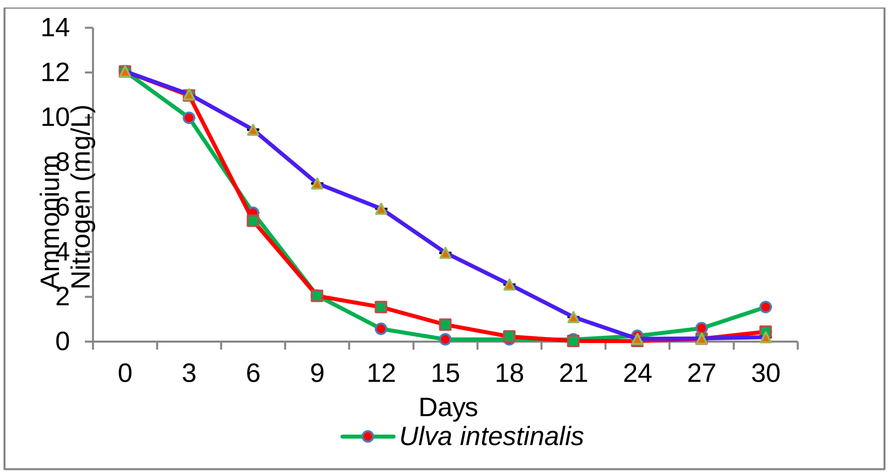

Fig. 4: Graph depicting depletion of Ammonium-Nitrogen by the three algae in synthetic saline wastewater

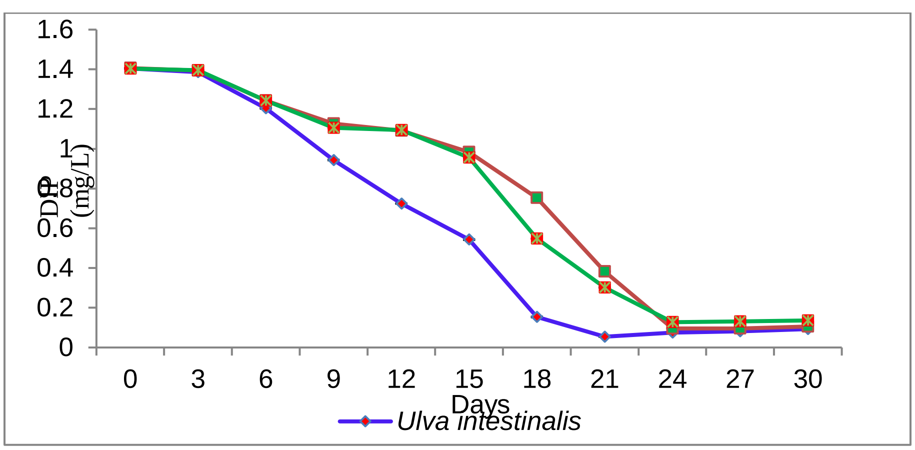

Fig. 5: Graph showing depletion of Dissolved Inorganic Phosphate (DIP) by the algae in synthetic saline waste water.

Table 2: Table depicting the nutrient remediation potentials of the three macroalgae (in µg/L/day/gB)

Algal Species	Nitrate-Nitrogen Remediation Potential (µg/L/d/gB)	Nitrite-Nitrogen Remediation Potential (µg/L/d/gB)	Ammonium Nitrogen Remediation Potential (µg/L/d/gB)	Dissolved Inorganic Phosphorus (DIP) remediation Potential (µg/L/d/gB)
Enteromorpha intestinalis	8.61	0.06	5.7	0.64
Gracilaria sp.	6.9	0.048	5.01	0.55
Chaetomorpha aerea	3.42	0.047	4.97	0.53

Discussion:

Different anthropogenic activities have increased the supply of nutrients several fold especially Nitrogen and Phosphorus into estuarine and marine ecosystems (Smith 2003). This increase in the nutrient content is accompanied by different problems including deterioration of water quality, degradation of aquatic habitats, displacement of native communities etc (Deegan *et al*. 2012). Eutrophication is a common problem in the Indian Sunderbans and several studies have been carried out (Pal and Chatterjee 1992, Manna *et al*. 2010). The macroalgae selected for the experiment have been designated as abundant species of this region (Satpati *et al*. 2012, 2013).The use of marine vegetation as a sink of atmospheric CO_2 is currently a topic of intensive research. Cultivation of macroalgae in saline wastewater serves the dual purpose of remediating the wastewater as well as creating a means of generating usable biomass. The harvestable biomass created in the process has potential use in various fields such as the phycocolloid industry (Kaladaharan *et al*. 2009), fish feed in aquaculture (Khatoon *et al*. 2010) or as a lipid feedstock (Barman *et al*. 2012). Moreover, these algae form the base of the food web and support a wide variety of living organisms in their natural environments. Furthermore, purposeful cultivation of these macroalgae can also serve as a deterrent to the occurrence and spread of Harmful Algal Blooms (Tang and Gobbler 2011, Yang *et al*. 2015). From our results, it became evident that *Ulva intestinalis* is the most potential alga for remediation of excessive nutrients. Therefore, being the most abundant and productive species of this region ,it also takes active part in pollution amelioration process of this brackish water fish producing region of Indian Sunderbans.

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