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Role of Dominant Green and Red algae of Indian Sundarbans in Nutrient remediation process employing synthetic saline wastewater

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Abstract:

The Sundarbans 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, two abundant Chlorophytes (*Ulva intestinalis* and *Chaetomorpha aerea*) and one abundant Rhodophyte- *Gracialaria* sp. of this region were recorded. 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 both nutrient remediation (8.61 µg/L/d/gB Nitrate-Nitrogen, 0.06 µg/L/d/gB Nitrite-Nitrogen, 5.7 µg/L/d/gB Ammonium Nitrogen and 0.64 µg/L/d/gB Dissolved Inorganic Phosphate). *Gracialaria* sp. showed intermediate nutrient removal capacity (6.9 µg/L/d/gB Nitrate-Nitrogen, 0.048 µg/L/d/gB Nitrite-Nitrogen, 5.01 µg/L/d/gB, Ammonium Nitrogen and 0.55 µg/L/d/gB Dissolved Inorganic Phosphate).*Chaetomorpha aerea* documented nutrient remediation potentials (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)

Introduction:

Waste water cultivation of both micro- and macroalgae is a common practice to remove excess nutrients especially Nitrogen (N) and Phosphorous (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 was also reported by Sauze in southern France (Sauze 1983) In closed mariculture systems, Dissolved Inorganic Nitrogen (DIN) species emanating from fish waste accumulate up to toxic levels. *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 saline waste water. In the current study, three species of green and red algae viz. *Gracilaria* sp., *Ulva intestinalis* and *Chaetomorpha* sp. were collected from Indian Sundarbans 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

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(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:

Collection of Algal strains:

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

Preparation of Synthetic Wastewater:

Synthetic wastewater was prepared using a sea salt formulation of Central Salt and Marine Sciences Institute (CSMCRI) and Calcutta University (Patent no. 12826682.2-1354. 2014). The salt formulation 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

Cultivation of macro algae 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)

Estimation of Nutrient Consumption:

Dissolved Inorganic Nitrogen was measured as the summation of Nitrate-Nitrogen, Nitrite-Nitrogen and Ammonium-Nitrogen along with 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:

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. (0.06g/d/gB). Both *Chaetomorpha aerea* and *Gracilaria* sp. documented maximum biomass after 24 days of cultivation while *Ulva intestinalis* showed maximum biomass yield after 21 days (Fig. 1).

Measurement of nutrient consumption:

Minimum nutrient levels were observed after maximum biomass yield as expected. Subsequently 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) intermediate by *Gracilaria* sp (5.01 µg/L/d/gB) and minimum by *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)

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

Algal Species	Biomass Generated (Fresh Weight)	Number of Days
Enteromorpha intestinalis	583.425	21
Gracilaria sp.	253.75	24
Chaetomorpha aerea	317.38	24

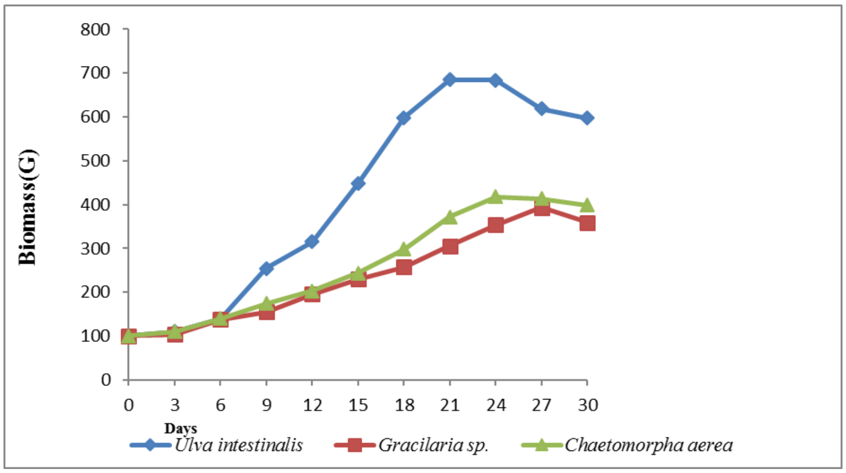

Fig. 1: Figure depicting growth curves of the experimental algae in synthetic saline wastewater

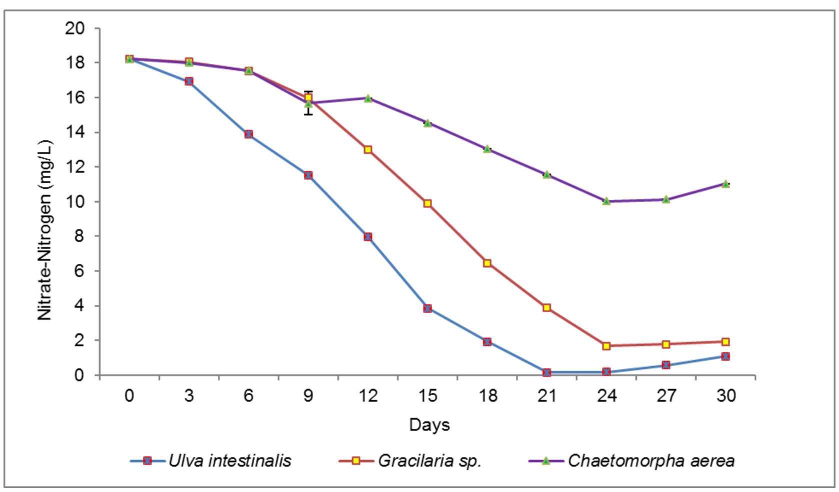

Fig. 2: Figure depicting depletion of Nitrate-Nitrogen by the three macroalgae in synthetic saline wastewater

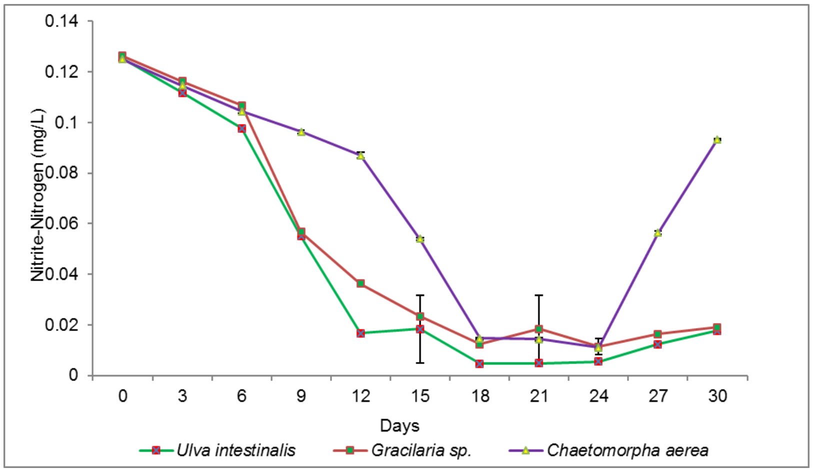

Fig. 3: Figure depicting depletion of Nitrite-Nitrogen by the algae in synthetic saline wastewater

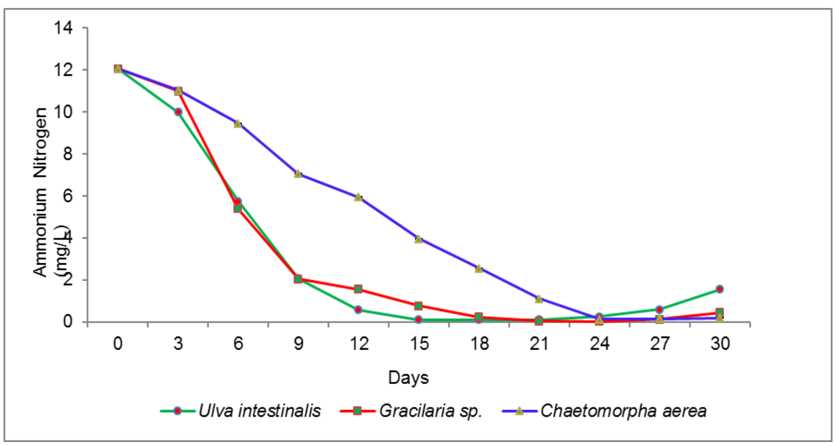

Fig. 4: Graph depicting depletion of Ammonium-Nitrogen by the three algae in synthetic saline wastewater

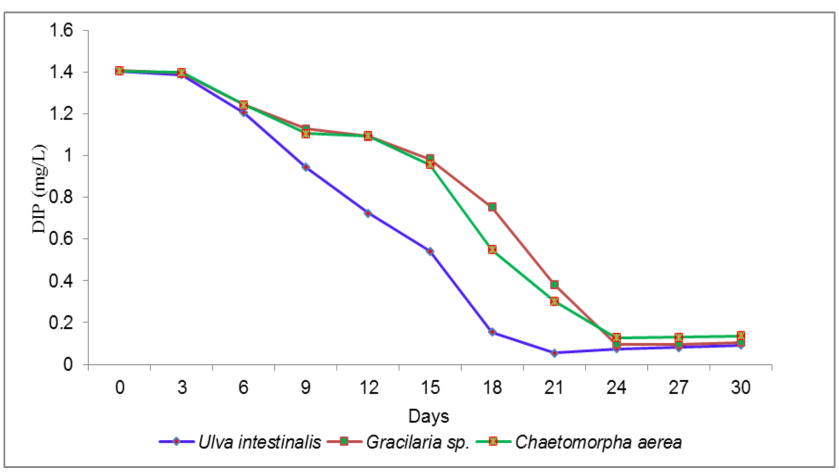

Fig. 5: Graph showing depletion of Dissolved Inorganic Phosphate (DIP) by the algae in synthetic saline wastewater

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)
Ulva 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:

From our results, it became evident *Ulva intestinalis* is the most potential alga for remediation of excessive nutrients from formulated waste water. *Chaetomorpha aerea* documented the least nutrient removal capacity while *Gracilaria* sp. revealed intermediate prospective. 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 detoriation of water quality, degradation of aquatic habitats, displacement of native communities etc (Deegan *et al*. 2012). Eutrophication is a common problem in the Indian Sundarbans and several studies have been carried out (Pal and Chatterjee 1992, Satpati *et al*. 2012). 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).

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