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Phycoremediation of Cooum wastewater as nutrient source for microalgal biomass production

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Abstract

Cooum River around Chennai city is polluted atrociously with sewage and requires remedy without ado. Although sewage treatment plant is operational but not adequate for the huge amount of sewage produced and high operational cost. Therefore, we have focused bioremediation of Cooum wastewater (Cww) using microalgae interim get algal biomass with low operational cost. For this, four indigenous microalgae were isolated from various polluted site and identified (two *Oscillatoria* sp, *Chlorella* sp and *Synecocystis* sp.) and used. The Cww was collected, separated the solids and analyzed nitrogen, phosphate and potassium at 27.5, 2.7, 68 mg/L, respectively. The solid free Cww was prepared different concentrations (0%, 1%, 5%, 10%, 15%) in distilled water (v/v) and inoculated microalgae and incubated under light. The biomass was quantified after 15 days and noticed differences in biomass and lipid production. The maximum of 3.1 g/L biomass and 16% lipid production was observed in *Oscillatoria* sp., at 15% Cww, and minimum of 1 g/L biomass and 8% and 5% lipid in *Chlorella* and *Synecocystis* sp., respectively. This result indicates that the Cww alone can support the microalgal growth at considerable amount, thus convalescing commercial production of microalgae using Cww, eventually get clean River and algal biomass.

Keywords: Cooum wastewater; Microalgae; Nutrients; Phycoremediation; Pollution; Sewage

1. Introduction

Water is the most vital resource for all life, therefore, maintaining the water to a high quality is crucial. However, fresh water has become a scarce commodity due to over exploitation and pollution [1]. Untreated sewage and industrial effluents are let off into the nearest water bodies and produce bad smell, infested with water weeds and breeding place of mosquitoes and disease producing pathogens [2].

River Cooum plays an important role in the cleanliness of Chennai City. It starts near Sattarai village in Tiruvallur Taluk and flows through 65 kms, before it joins the sea. It enters the Chennai city near Aminjikarai, runs for about 18 Km through the city and empties into Bay of Bengal [3]. Urban growth on either side of the River, without adequate sanitary sewers has brought about an adverse impact on the River ecosystem. Tuba Raqshan [4] reported that about 1,500 million liters per day (MLD) of sewage was discharged in different zones of Cooum River, which is still conservative, the generation may be much higher. This heavy load of sewage prevents the River from self purification, however around 500 MLD of the sewage is treated by the sewage treatment plants (STPs), nevertheless what happens to the rest of the sewage? Besides, Cww samples revealed almost zero dissolved oxygen and substantial presence of faecal coliform bacteria, nutrients (nitrogen, phosphorous and potassium) and heavy metals and stated Cww is 80 percent more polluted than treated sewer [5, 6]. Polluted water bodies constitute a serious health hazard and lead to bad living conditions for human, animals and plants hence it is desirable to clean up the Rivers by any means right away.

Generally, domestic sewage and industrial effluent is treated by activated sludge process having aerators and allied equipments, which utilize high capital equipment and running cost. It is also desirable to convert the waste into wealth and produce value added product thereby increasing the significance of waste. Developing microalgal based wastewater treatment (phycoremediation) system being considered as economically cheaper, simple, environment friendly and microalgal value added products [7, 8]. However with this added advantage it is not popular and very few algal treatment systems have been implemented in our country so far, due to lack of research. Although some studies used Cww for the algal biomass production as well as nutrients removals [9-13], nevertheless, no research explained the optimum concentration of the Cww for the microalgal growth rather just used the concentrated Cww and ended up less biomass. In this study we isolated different microalgae from various sites including Cww and checked for its growth feasibility on Cww at different dilution and its biomass and lipid production. This study result would be exploited in field scale, eventually clean the Cooum River with algal by product.

2. Materials and Methods

2.1. Collection of Cww and characterization

Two liters of Cww was collected using clean plastic container near Ekkatuthangal, Chennai (Fig. 2) and stored in room temperature for 24 hrs for settlement of suspended solids and then collected one liter of supernatant and stored in 4°C for further use. The chemical parameters of EC, TDS, NH_3, NO_3, PO_4 and K was analysed in supernatant at Mettex lab, Chennai.

2.2. Isolation of Microalgae and purification

Four microalgal water samples were collected in a sterile container from various places (stagnant rain water algae (SRA), Goat shelter algae (GSA) in Pallavaram, Anakapatur River algae (AnA) and Cooum River algae (near Ekkatuthangal), Tamil Nadu. Collected algal samples were inoculated into 500 mL beaker containing 250 ml of Algae Culture Broth (Himedia M342) and incubated for 10 days at 25°C under light (4000 lux) and dark cycle (16 hrs/8hrs). After 10 days, algal growth were observed under light microscope, if its protista algae we used AAS (Antibiotic Antimycotic Solution) for to remove bacterial and fungal contamination according to Aparna et al., [14] if cyanobacteria, used centrifuging and washing for 5 times with sterile media and streaked in Algal culture agar [15]. The purified microalgae were morphologically identified using compound microscope by wet mount method.

2.3. Growth of microalgae in Cww

Ten days old microalgal mats were collected from the Algal culture broth and washed with sterile distilled water (twice) and homogenized with pestle and mortar. Then the small amount (equal to 100 mg dry wt) of algal culture was inoculated into the test tubes contained 10 mL of sterile Cww at different concentration (0%, 1%, 5%, 10% and 15%). The inoculated test tube was incubated at 25°C under light (4000 lux) and dark cycle (16 hrs/8hrs). All the experiment was carried in replication.

2.4. Quantification of microalgae biomass and lipid

After 15 days, microalgal biomass was harvested by vortex and scrapping method and filtered through pre-weighed Whatman No.1 filter paper. The filter paper was washed with deionized water and dried at 60°C for 12 hr then weighed the filter paper and calculated the dry weight. The lipid was extracted from the dried algal biomass (50 mg) using Chlorophorm:methanol (2:1 v/v) extraction method and calculated as per Suresh et al., [16].

3. Results and Discussion

3.1. Isolation and identification of Microalgae

The Fig. 1 shows the microalgal isolates identified from the different samples as two *Oscillatoria* sp., one from SRA and another in CWA sample. However, GSA and ARA samples identified as *Chlorella* sp., and *Synecocystis* sp., respectively. Among four isolates, cyanobacterial genus were dominant than protista algae, interestingly the CWA belongs to cyanobacterial genus of *Oscillatoria.*

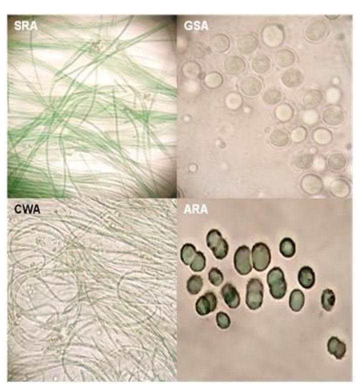

Fig. 1. Microalgal isolates observed and identified under light microscope at 450x magnification. SRA-stagnant rain water algae (*Oscillatoria* sp.); GSA-Goat shed algae (*Chlorella* sp.); CWA-Cooum water algae (*Oscillatoria* sp.); and AnA-Anakaputur River algae (*Synechocystis* sp.).

3.2. Cww characterization

The Fig. 2 shows the collection site and the appearance of the collected Cww. It was black in color and smelled like rotten egg, probably H_2S. It was very difficult to grow algae in this raw Cww due hindrance of light penetration by the suspended solids, therefore removed the solid by natural sedimentation and the supernatant was analyzed for some vital nutrients parameters required for the algal growth (Table 1). The Cww shown very good nutrient source for algal growth as observed 27.5 mg/L available nitrogen, 2.7 mg/L phosphate and 68 mg/L potassium. Moreover it was shown high TDS and EC of 4282 mg/L and 6070 µS/cm, respectively. In 1993, Mary Bai [3] observed that the maximum of 4.3 mg/L nitrate, 1.4 mg/L ammonia and 0.004 mg/L phosphate, which was very much increased at the moment due to urbanization in the respective area. In accordance to our study, Arone Soul Raj et al., [11] observed that the increased nutrient pollution as 20, 12.9, 65 mg/L nitrate, ammonia and potassium, respectively, whereas phosphate level was very low at 0.22 mg/L. In case of EC and TDS was shown at 5300 µS/cm and 3710 mg/L, respectively. In 2017, Aswathy et al., [17] noticed high level of nitrate (160 mg/L) and phosphate (2.5 mg/L) in Cww near Saidapet site, while reduced TDS at 1120 mg/L. This study also declared that the urbanization and the release of untreated sewage to the River, therefore to clean the River by removing its nutrient pollution by using microlage would be very suitable.

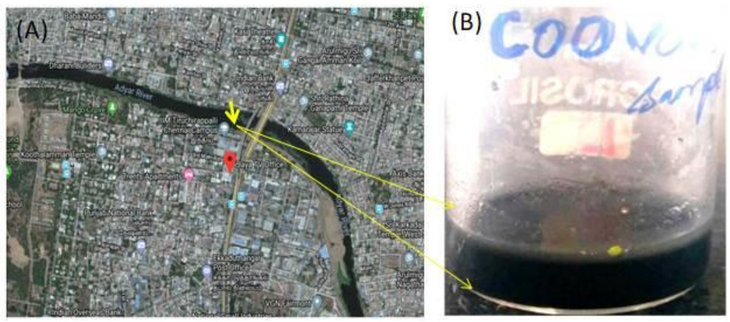

Fig. 2. The Google map showing the collection site of Cooum wastewater (A) near Ekkatuthangal, Chennai and the sample of Cooum wastewater (B).

Table 1. Analysis of different chemical parameters of Cooum wastewater collected from Ekkatuthangal, Chennai.

Parameter	Average ± SD
EC (µS/cm)	6070 ± 2072
TDS (mg/L)	4282 ± 1495
NH_3 (mg/L)	11.5 ± 1.5
NO_3 (mg/L)	16 ± 3.8
PO_4 (mg/L)	2.7 ± 0.3
K (mg/L)	68 ± 31

3.3. Microalgal growth in Cww

Fig. 3 shows the growth of microalgae in Cww at different concentration in distilled water and it revealed that the *Oscillatoria* sp., from SRA and CWA sample shown copious growth at 5 to 15% Cww in compare with other two microalgae of *Chlorella* sp., and *Synecocystis* sp. Among the *Oscillatoria* sp., CWA shown the more growth than the SRA. Fig. 4 illustrates the agar plate assay of microlagal isolate of CWA and clearly suggested that the 10% Cww promoted the algal growth, whereas 15% displayed diminutive growth, however unlike result was observed in broth assay (Fig. 3) where 15% was shown copious growth than the 10%. In plate assay, 15% Cww was shown more bacterial growth which perhaps restrained the algal growth. Tripathy and Sumathi, [9, 10] observed that the protista algae of *Chlorella* sp., (1.34g/L) and *Scenedesmus sp.,* has shown considerable growth and removal of nutrients from Cww, whereas Dhamotharan et al., [13] found that the prokaryotic algae of cyanobacteria was efficient in growth as well as nutrient removal in Cww. Arone Soul Raj et al., [11] found that the *Botryococcus braunii* has reached OD_{680} value at maximum of 1.4 in undiluted Cww. Kumar et al., [12] stated that the marine *Chlorella* sp., has grown considerable amount (chlorophyll$_a$ reached from 4 to 23 µg/L) in undiluted Cww by utilizing its nutrients. Previous studies shown algae could be grown using Cww as cheap source of nutrients medium eventually remove the pollutents, however, no studies have attempted to find the optimum dilution and dry weight biomass for the Cww in certain area. Therefore, it was not feasible to apply phycoremediation of Cww at available data at large scale.

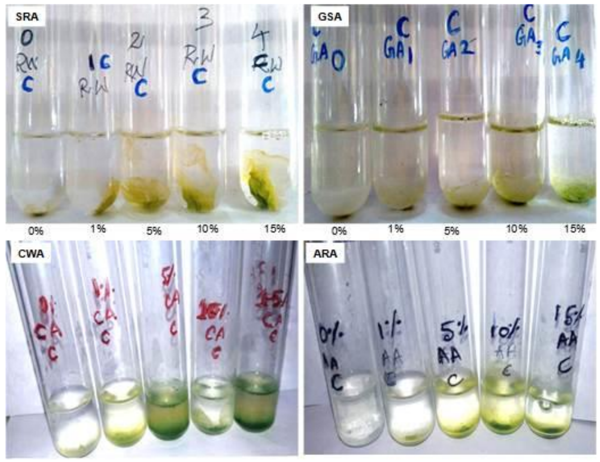

Fig. 3. Growth of microalgal isolates in Cooum wastewater at different concentration for 15 days under fluorescent lamp. SRA-stagnant rain water algae (*Oscillatoria* sp.); GSA-Goat shed algae (*Chlorella* sp.); CWA-Cooum water algae (*Oscillatoria* sp.); and AnA-Anakaputur algae (*Synechocystis* sp.)

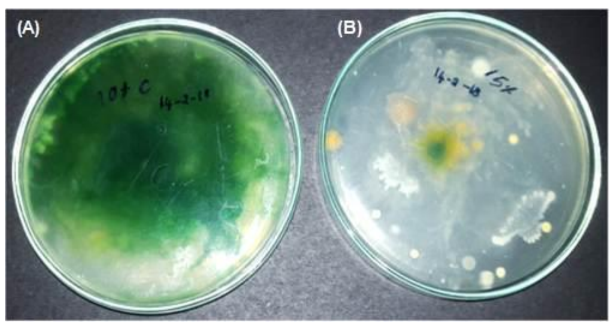

Fig. 4. Growth (10 days) of CWA in agar medium containing different concentration of Cooum wastewater (A; 10%, B; 15%)

In our study, Fig. 5 explained that the microalgal biomass produced in terms of dry weight and clearly suggested that the 10-15% Cww supported the algal growth at considerable amount. The maximum of 3.1 g/L algal biomass was observed in *Oscillatoria* sp. from CWA sample at 15% Cww, followed by *Oscillatoria* sp., from SRA (2.2 g/L), whereas in *Chlorella* (GSA) and *Synecocystis* (ARA) isolates produced about 1 g/L biomass. This study reached high biomass than the any other previous study, because algae used was from the wastewater and optimum dilution of Cww rather than the concentrated Cww. In terms of biomass for value added purpose we analysed the lipid content in the Cww grown algae (Fig. 5). The maximum of 16% lipid (dry weight) content was observed in *Oscillatoria* sp., of CWA, followed by the 10% in *Oscillatoria* sp., from SRA sample. The least amount of 5% lipid was observed in *Synecocystis* sp, while *Chlorella* contained 8% lipid accumulation. Obviously, the results indicated that the Cww supports the algal growth ultimately supports the phycoremdiation of Cww, however the optimum conditions of the wastewater dilution for specific value added products will be studied in future.

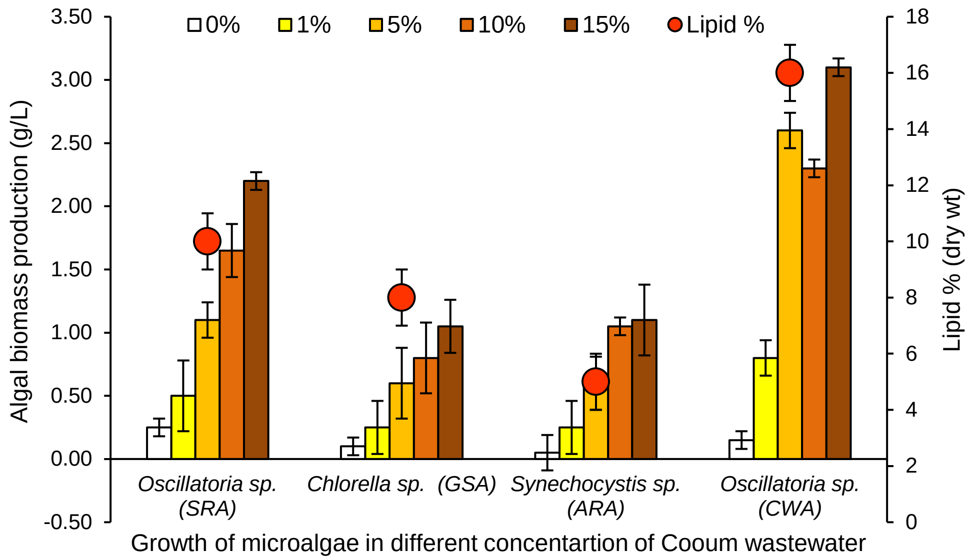

Fig. 5. Biomass and lipid production of microalgal isolates grown in Cooum wastewater at different concentration

4. Conclusion

In this study concluded that the Cww was very much suitable for the growth of microalgae as it contains considerable amount of major nutrients (NPK). Isolated microalgae of cyanobacteria genuses were dominant in samples and produced maximum of 3.1 g/L biomass in 15% Cww by *Oscillatoria* sp., (CWA) with 16% lipid, however the *Chlorella* sp., produced only 1 g/L biomass with 8% lipid. Moreover, each microalga has specific concentration of Cww that found out be more than 15% v/v. Further research is needed for the phycoremediation Cww with more concentrated as nutrients source and utilization of biomass, simultaneously cleans the River at ease, the same could be applied in industrial wastewater.

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