



Phycoremediation of Paper and Pulp Mill Effluent using *Planktochlorella nurekis* and *Chlamydomonas reinhardtii* – A Comparative Study

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Abstract

In the present study, the wastewater collected from a paper and pulp mill industry was treated using two microalgae, *Planktochlorella nurekis* and *Chlamydomonas reinhardtii*. The microalgae was grown in paper and pulp mill effluent (PPME) under natural environmental conditions and harvested on the 12th day. Results of the study showed that both *P.nurekis* and *C. reinhardtii* could reduce nitrate (96 % and 86%), phosphate (100% and 88%), COD (92% and 93%) and other physico-chemical parameters after the experiment. The percentage reduction of heavy metals such as Cr, Co, Ni, Cu, Zn, As, Sr and Cd were 100%, 97%, 77%, 71%, 72%, 98%, 88% and 88% respectively by *P.nurekis*. Similarly the percentage reduction of the foresaid heavy metals were 100%, 46%, 44%, 49%, 68%, 57%, 86% and 86% respectively by *C. reinhardtii*. The lipid content of *P.nurekis* was 24% and 20.5% for *C.reinhardtii* was after the experiment. Comparatively, *P.nurekis* exhibited significantly higher phycoremediation capacity as well as lipid production potential than *C. reinhardtii*. It is evident that both microalgae have the potential for the treatment of paper and pulp mill effluent and both the species can be used as good candidates for lipid production.

Keywords: Phycoremediation, paper and pulp mill effluent, Heavy metals, Lipids, *P.nurekis*, *C. reinhardtii*

1 Introduction

Globally, the paper and pulp mill industry is listed as the sixth largest polluter among the various industries which discharge a huge quantity of liquid, solid and gaseous wastes into the environment [1]. Enormous volume of wastewater (~300 m³) is generated for the production of each metric ton of paper depending on the nature of raw material, end product and the extent of water reuse [2, 3, 4]. These untreated wastewater (effluent) causes significant damage to the receiving water bodies since they have high COD, BOD, chlorinated compounds, suspended solids, fatty acids, tannins, resins, acids, lignin and its derivatives, sulphur compounds etc. [1, 5, 6, 7, 4]. Several treatment processes including removal of suspended solids, colloidal particles, floating matters, colors and toxic compounds by conventional and non-conventional methods like sedimentation, flotation, screening, adsorption, coagulation, oxidation, ozonation, electrolysis, reverse osmosis, ultra-filtration and nano-filtration technologies etc. are practiced [8, 9, 10, 11, 12]. The main disadvantages of these conventional processes are high operational cost and increased sludge production. Hence, phytotechnological approach is a viable option for its remediation. Effective in situ phyto remediation (use of plants) techniques have been applied for reducing the heavy metal load from paper mill effluents [4, 11, 13]. Eventhough it seems to be promising, there are some limitations associated with this technique. Phyto remediation requires long term maintenance and it may be effective only seasonally. Moreover, the remediation efficiency was very low

[14, 15, 16]. As an alternative, phycoremediation employing microalgae for the removal of the nutrients from wastewater is gaining much attention [17, 18-23]. Phycoremediation is used to describe treatment of pollutants in a contaminated area using micro and macroalgae [24-28].

Microalgae can be easily cultured in fresh water, marine water, brackish water or on non-arable land. They do not compete with agriculture for existing resources. Microalgae utilize atmospheric carbon dioxide during their photosynthetic process and also have proven their potential to abate greenhouse gases. They reproduce rapidly, achieving faster growth than any energy crop and can be harvested frequently [29]. Microalgae have many applications in the field of pollution abatement, biofuel production and carbon sequestration [30]. Phycoremediation is an eco-friendly low cost technology which serves as an attractive option for pollution control in the developing countries. The spent biomass after phycoremediation can be used for making high worth products such as biodiesel, biogas and other algal metabolites [31, 21, 32].

Studies have reported the ability of microalgae in the treatment process for the removal of nutrients from varied types of wastewater [34-37]. However, not many studies have been conducted on treatment of PPME using microalgae. The use of pulp and paper mill effluent for the cultivation of microalgae is least explored. The conventional treatment system is widely adopted for the treatment of pulp and paper mill effluent. On this background, an attempt has been performed to check the

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feasibility for cultivation of microalgae such as *P.nurekis* and *C.reinhardtii* in PPME for nutrient removal. Hence, the present study aims to evaluate the potential of *Planktochlorella sp.* and *C. reinhardtii* for the treatment of PPME and subsequent production of biomass and lipid with a bio-energy perspective.

2 Material and methods

2.1 Isolation and culturing of freshwater microalgae

Mixed microalgae were collected from Kuttanad wetland ecosystem, Kerala, India and were then cultured in diluted paper and pulp mill effluent (20 times dilution). After 7 days, the dominant species was isolated and sub-cultured for obtaining pure strain. The isolation and purification was done by repeated streaking on nutrient agar plate and later cultured in Bold and Basal medium (BBM). Composition of the medium was KH_2PO_4 1.75g, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.25g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.75g, NaNO_3 2.5g, K_2HPO_4 0.75g, NaClO_4 0.25g, $\text{Na}_2\text{EDTA} + \text{KOH}$ 1g +0.625g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O} + \text{H}_2\text{SO}_4$ 0.498g +0.1ml, H_3BO_3 0.25g and 1 ml trace metals mixed solution (H_3BO_3 2.860g, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 1.810g, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.222g, NaMOO_4 0.079g, $\text{CO}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ 0.0494g, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.079g). The microalga *Chlamydomonas reinhardtii* was obtained from Chlamydomonas resource centre, University of Minnesota (USA). It was cultured in Tris – Acetate Phosphate (TAP medium). The composition of TAP medium was 40 X TAP 25 ml (NH_4Cl 1.5g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.4 g, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 2 g), Phosphate solution 0.375 ml (K_2HPO_4 28.8 g, KH_2PO_4 14.4 g), Hutner's trace element 1 ml (Na_2EDTA 2H₂O 5 g, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 2.2 g, H_3BO_3 1.14 g, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 0.5 g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 0.16 g, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.16 g, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ 0.11 g), Acetic acid 1 ml, Tris buffer 2.42 g.

The isolated pure culture of microalga was subjected to DNA isolation and PCR amplification for identification using eukaryotic forward primer (ss5-5'GGTTGATCCTGCCAGTAGTCATATGCTTG3') and reverse primer (ss3-5'GATCCTTCCGACGGTTCACCTACGGAAACC3'). The PCR products were sequenced and the obtained partial sequence was matched with previously published sequences in the National Centre for Biotechnology Information (NCBI) database using ADVANCED BLAST (www.ncbi.nlm.nih.gov/BLAST) and the percentage similarity with already identified 18S rRNA gene sequences in the GenBank database were determined. Later the 18S rRNA gene sequence of isolated microalga was submitted in NCBI GenBank for the allocation of accession number.

2.2 Growth of microalgal strains and culture conditions

The isolated microalgal species was identified as *Planktochlorella sp.* based on standard literature. Both *Planktochlorella sp.* and *C. reinhardtii* were cultured in 250 ml flasks separately in B B M and T A P medium respectively. The culture was incubated at $28 \pm 1.0^\circ\text{C}$ and maintained at a light intensity of $25 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ using fluorescent tube lights. Carbon dioxide gas was supplied at 10psi/kg for 5min per day. Aeration was provided for preventing the settling down of the cells at the bottom. Growth rate of *Planktochlorella sp.* and *C. reinhardtii* were measured by using Sedgwick rafter cell counting method [38].

2.3 Collection, characteristics and experimental setup

Paper and pulp mill effluent (PPME) was collected from Hindustan Newsprint Ltd. factory in Kottayam district, Kerala, India in 20L plastic containers and stored at 4°C . The collected effluent was filtered separately and preliminary examination to

determine the optimum concentration of wastewater was carried out (70% wastewater: 30% water). Initially, the microalgae were subjected to the growth phase and in the second stage, their ability to substantially reduce nitrate, phosphate, COD and heavy metals was conducted. The experiment was scaled up in 20L glass containers (2 nos.) with actual working volume of 10L. Concentrated uniform suspension (10ml) of *Planktochlorella sp.* and *C. reinhardtii* was separately added to the concerned glass containers. Similarly, equal volume of wastewater was taken in another set of glass tanks without microalgae maintained as control. The glass containers were exposed to natural sunlight and mixing was performed manually to avoid settling of cells. The study was conducted for 12 days in three experimental sets of reactors. Aliquots were withdrawn from the three glass containers for the analysis of physico-chemical parameters before the inoculation of microalgae and subsequently at periodic intervals of 2 days throughout the experimental period. The supernatant obtained after centrifugation at 5000 rpm for 10 min was used for the analysis of pH and COD as per the standard methods of APHA (1998). Analysis of nitrate, phosphate and sulphate were done by ion chromatography (DIONEX ICS-1100). Heavy metal analysis was done by ICP-MS (Thermo iCAPRQ). The nutrient removal efficiency of both microalgae was calculated according to following equation.

$$\text{Removal efficiency (\%)} = \frac{(P_0 - P_t)}{P_0} \times 100$$

where P_0 was the initial concentration of wastewater and P_t was the concentration of wastewater after phycoremediation process.

2.4 Microalgal Cell Counting and Biomass estimation (Dry biomass)

Cell count method was employed for the determination of microalgal cell growth in the culture reactors maintained under the experimental conditions [38]. 1ml of culture medium was taken regularly at an interval of two days and the cells were enumerated with the help of a Sedgwick rafter cell. Measurements were done in triplicate and the mean values were represented in the results. Gravimetric method was employed for the quantitative estimation of biomass [38]. 2ml of culture medium was taken at an interval of two days and filtered through a pre-weighed Whatman No:1 filter paper. It was then oven-dried at 60°C for 1 hour and reweighed. The difference between each observation was calculated as per the following formula;

$$\text{Biomass (Dry weight in g)} = W_2 - W_1$$

where, W_2 is weight of dried filter paper after filtration and W_1 is weight of filter paper.

2.5 Lipid extraction

Cells were harvested by centrifugation at 4500 rpm for 5 min and washed once with distilled water and oven dried. An aliquot (20g) of the biomass was mixed with 100ml of double distilled water and the cells were disrupted using a sonicator (CPX130) at a resonance of 20 kHz for 5 min. This sonicated mixture was blended with 2:1 methanol chloroform solution as per the modified Bligh and Dryer method [39]. The mixture was transformed into a separating funnel and shaken for 5 min. The lipid fraction was then separated from the funnel and evaporated using solvent in the rotary evaporator. Finally the weight of the crude lipid obtained from each sample was

measured using an electronic weighing balance (Shimadzu, Japan).

2.6 Statistical analysis

Analysis of variance (ANOVA) was performed to find out any significant difference in water quality parameters among the culture reactors of *Planktochlorella* sp. and *C. reinhardtii* with the control reactor. The mean and standard deviation within samples were calculated for all cases. The statistical analysis was done by using SPSS 21 version.

3 Results and discussion

3.1 Molecular identification of microalga

The results of BLAST on the NCBI revealed that isolated microalga exhibited 99% similarity with *Planktochlorella nurekis*. The 18S rRNA gene sequence was submitted to GenBank and designated as *Planktochlorella nurekis* CS18 with GenBank accession number: MG811583. Phylogenetic tree of microalga *P. nurekis* shown in figure 1.

3.2 Growth and biomass of the microalgae

Initially, the collected effluent was dark brown in colour but changed to lighter shade after 12 days of microalgal treatment. The results showed considerable increase in both cell count and biomass of microalgal cells after the experiment. The initial cell density of both species was 550 cells/ml. After 12 days, *P. nurekis* showed higher cell density of 4320 cells/ml and *C. reinhardtii* had 3650 cells/ml (Figure.2). At the early stage of the experiment, the cell biomass of *P. nurekis* was 0.042g/L and it was 0.037g/L for *C. reinhardtii* (Figure.3). *P. nurekis* and *C. reinhardtii* showed highest biomass on the 10th day of the study period and after which the biomass of both the species got reduced (Figure.3). The initial biomass increase was proportional to the availability of all the required nutrients upto the 10th day. As the cells grew, the nutrients were taken up

and thus began to decrease in the medium. This insufficient supply of nutrients caused the decline of biomass and cell count after the 10th day. Microalgae growth was directly affected by the availability of light, nutrients, temperature, the initial inoculation density and the growth environment [40].

3.3 Physico-chemical properties of paper and pulp mill effluent

The PPME had dark brown coloration, which might be due to the presence of chlorinated organic compounds produced from lignin degradation during conventional bleaching, wood cooking and alkali extraction of the pulp [41]. The dissolved chemicals especially mercaptans and hydrogen sulphide used during the manufacture of paper resulted in the characteristic pungent odour [42]. The physico-chemical parameters such as pH, nitrate, phosphate, COD, sulphate and heavy metals (Cr, Co, Ni, Cu, Zn, As, Sr and Cd) contents were assessed. The paper and pulp mill effluent was slightly acidic in nature with pH 6.02 which changed to alkaline with a pH of 8.3 after 12 days of treatment with *P. nurekis* and *C. reinhardtii* (Table 1). The results showed that the pH of the water plays an important role especially with respect to metabolism, survival and microalgal growth. The pH of paper mill effluent changed from neutral to alkaline after 12 days of growth of *P. nurekis* and *C. reinhardtii*. The pH between 8.2 and 8.7 was favourable for the growth of the microalgae compared to the neutral and acidic redox-conditions. In the present study, *P. nurekis* showed higher biomass in pH 8.3 (Figure.4) and *C. reinhardtii* showed higher biomass in pH 7.9 (Figure.5). An increase of pH by 35.79% in industrial effluent treated with *Chlorella vulgaris* was reported by Dominic *et al.* [43]. Vijayakumar *et al.* [44] also reported pH increase (alkaline) in the dye effluent treated with *Oscillatoria* sp. Studies of Wurts *et al.* [45] showed the increase of carbonate and bicarbonates in water due to the growth of algal species.

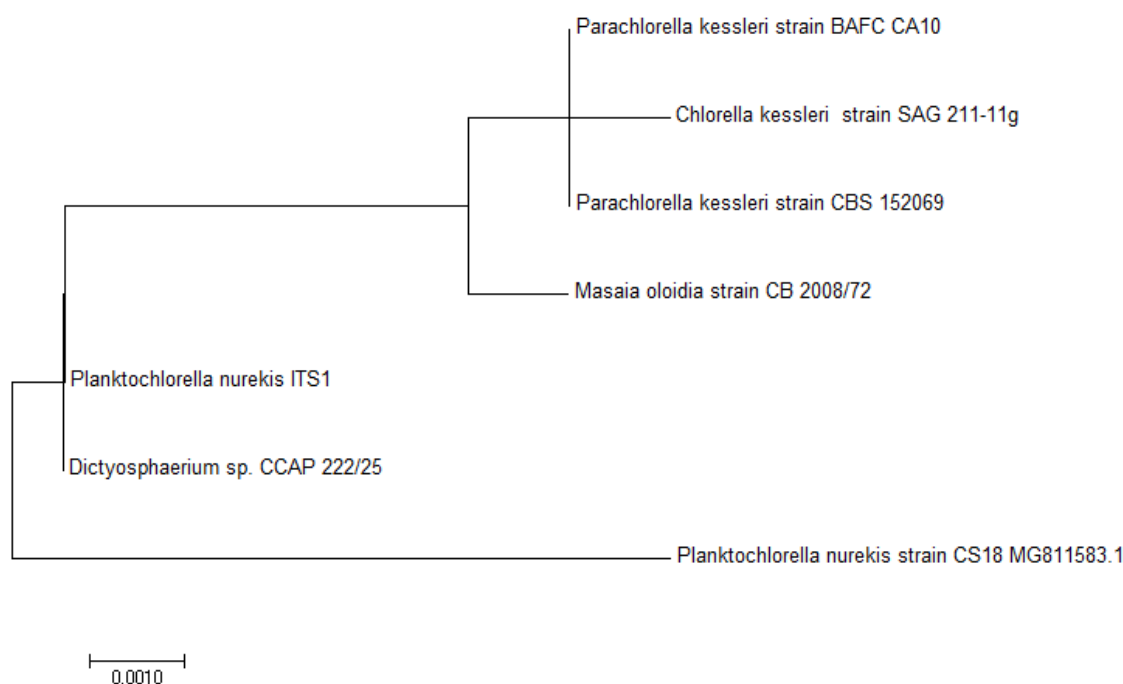
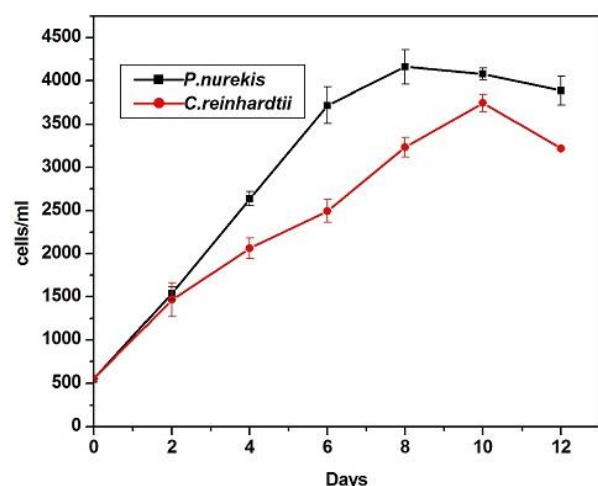
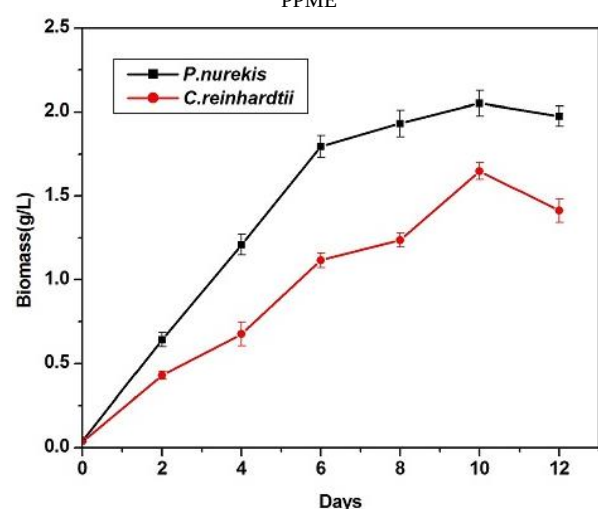
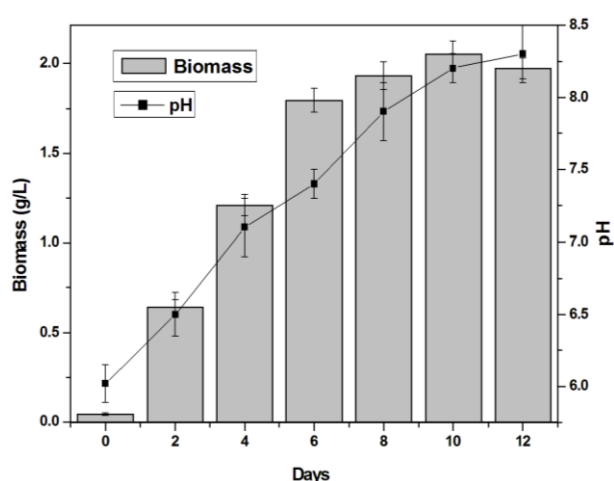
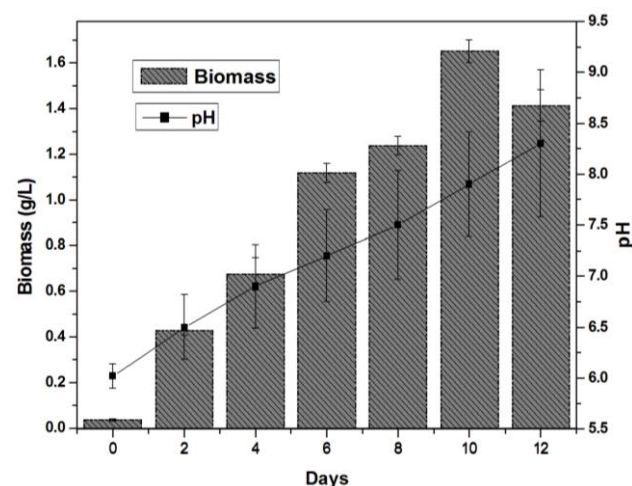


Figure 1: Neighbour-joining phylogenetic tree of *P. nurekis* isolated from the Kuttanad wetland ecosystem

Figure 2: Variation of cell number of *P. nurekis* and *C. reinhardtii* in PPMEFigure 3: Variation of biomass of *P. nurekis* and *C. reinhardtii* in PPMEFigure 4: Variation of pH and biomass of *P. nurekis* cultivated in pulp and paper mill effluent

Total oxidized nitrogen is the sum of the nitrite and nitrate nitrogen. The amount of nitrate was found to be very high in PPME (28mg/L) before experiment. After the experimental period, about 96% of nitrate-N was reduced by *P. nurekis* and 86% was reduced by *C. reinhardtii*. Nitrogen is present in the form of nitrate, nitrite, ammonia and organic nitrogen in the order of decreasing oxidation state in water and wastewater.

Urea and di-ammonium phosphate used in the manufacturing processes were the source of nitrate-nitrogen and phosphate in PPME. Wang *et al.* [46] obtained 62.5% removal of nitrate-N by *Chlorella* sp. grown in effluent from aeration tank of municipal wastewater treatment plant. Sivasubramanian *et al.* [47] reported that 90% of nitrate was removed from soft drink industrial effluents treated with microalgae in outdoor cultivation.

Figure 5: Variation of pH and biomass of *C. reinhardtii* cultivated in pulp and paper mill effluent

Phosphorous is present almost solely as phosphates in natural water and wastewater. At the initial stage of the experiment, the phosphate content in PPME was 12mg/L. By the end of the experiment, complete removal (100%) of phosphate from PPME was achieved by *P. nurekis* and 88% reduction was obtained with *C. reinhardtii* (Table 1). Microalgae was able to assimilate phosphorus in excess, which was stored in the cells in the form of polyphosphate granules, potassium and magnesium were co-transported along with phosphate [48]. Mirquez *et al.* [21] reported 70 to 83% and 100% reduction of both phosphate and nitrate from municipal wastewater by mixed microalgae and bacterial culture. Similarly, the complete removal (100%) of phosphate from PPME was observed after 12 days of incubation with *P. nurekis* whereas, *C. reinhardtii* showed 88% removal of phosphate. The optimal inorganic N/P ratio for algal growth was suggested to be in the range of 6.8-10 and it was found that the N/P ratio was much more than the optimal ratio, indicating nitrogen richness. Nevertheless, despite of richness of N/P ratio in the effluent, microalgal growth was found significant till 10 days of cultivation after which the growth decline. Previous studies have reported the decrease of phosphate level in the wastewater due to the growth of algal species [49, 50].

After the treatment process, both *P. nurekis* and *C. reinhardtii* could reduce the sulphate in the PPME from 230mg/l to 46mg/L and 92mg/L respectively (Table 1). 80% sulphate was removed from PPME using *P. nurekis* and *C. reinhardtii* showed 60% removal. High amount of sulphate were present in paper and pulp mill effluent due to sulphate kraft process used in the formation of wood pulp and use of sodium sulphate in the bleaching process [42]. These results are in agreement with the studies of Azarpira *et al.* [51] where more than 90% removal of sulphate in municipal wastewater using blue green algae was observed. Similarly, Ahmad *et al.* [52] also demonstrated significant reduction of SO_4^{2-} using *Chlorella* and mixed algal culture. In the present study, the initial COD of PPME was very high value with 9200 mgO_2/l . After 12 days of treatment, significant reduction of COD was

noted for both microalgae (Table 1). *P.nurekis* could remove 93% COD and *C. reinhardtii* could remove 92% COD from PPME. The reduction in COD is caused by the rapid biodegradation and bioconversion of organic matter due to the growth of microalgae [53]. A total of 98% reduction of COD from sewage water was reported by Ahmad *et al.* [52] in a comparative study of removal of organic and inorganic matter from sewage water using different species of algae like *Spirogyra*, *Chlorella* etc. Elumalai *et al.* [54] also observed substantial decrease in COD in textile wastewater after treating with *Chlorella* and *Scenedesmus* sp. revealed that algal consortium was more efficient. Microalgae released oxygen to the surrounding water during photosynthesis, which is used for the oxidation of organic matter simultaneously reducing the demand of oxygen in the growing medium [55]. Compared to other studies, the present study proved that the proposed microalgae have the potential to remediate the effluent from pulp and paper mill industry (Table 2). The statistical analysis results of COD showed high significant variation ($p < 0.01$). While parameters like nitrate, phosphate and sulphate had shown significance at 0.05 level ($p < 0.05$). The Bonferroni post hoc test showed that the significant variation in nitrate, phosphate, sulphate and COD among *P.nurekis* and control samples at 0.05 level of significance ($p < 0.05$).

3.4 Removal of heavy metals from PPME by *P.nurekis* and *C. reinhardtii*

Green microalgal cells cultured in wastewater with high heavy metals are known to accumulate higher concentrations of metal [56]. In the present study, heavy metals like Cr, Co, Ni, Cu, Zn, As, Sr and Cd were analysed by ICP-MS. The initial concentration of Cr in the PPME was 0.129 ppb. After 12th day of the experiment, both *P. nurekis* and *C. reinhardtii* (Figure.6) showed complete removal of Cr. The amount of Co in PPME was 0.208 ppb. Nearly 97% Co was removed by *P. nurekis* whereas *C. reinhardtii* could remove about 46%. *Chlorella* could efficiently reduce 76%-96% of Cd and 78%-94% of Ni from the medium within 7-28 days when cultured under laboratory condition [57]. The initial value of Ni in PPME was 0.95ppb and was reduced to 0.22 ppb and 0.52 ppb by *P. nurekis* and *C. reinhardtii*, respectively. The initial concentration of Cu was 14.6ppb. Both *P.nurekis* and *C. reinhardtii* showed comparatively less reduction of Cu i.e. 71% and 49%, respectively. About 72% of Zn was removed by *P.nurekis* and *C. reinhardtii* could remove 68%. *Scenedesmus bijuga* and *Oscillatoria quadripunctulata* showed heavy metal removal capacity with 37-50% for Cu, 20-33% for Co, 35-100% for Pb and 32-100% for Zn from the sewage and petrochemical industry effluent [58].

Maximum reduction of As concentration in PPME was done by *P.nurekis* (98%) while it was 57% for *C. reinhardtii*. The initial Sr concentration was 390.2 ppb and *P.nurekis* (Figure. 6) showed 88% reduction of Sr content in PPME while it was 86% reduction by *C. reinhardtii* (Figure.6). After 12 days of the experiment, Cd concentration was reduced from 1.05ppb to 0.13ppb by *P.nurekis* and to 0.12ppb by *C. reinhardtii*. The removal efficiency of the heavy metal from wastewater by microalgae depended on their large surface area and high binding affinity [59]. It was noted that the reactor of *P.nurekis* performed higher ability to consume heavy metals from PPME. Wang *et al.* [60] revealed that heavy metals like Al, Ca, Fe, Mg and Mn could be removed efficiently by *Chlorella* sp. from municipal wastewater. Saunders *et al.* [61] observed that phycoremediation potential of three species of microalgae cultivated in wastewater polluted with heavy metals from coal-fired power plant. All species accumulated high concentrations of heavy metals. Microalgae are very responsive to heavy metal

toxicity and also could eliminate metals through the process of adsorption and absorption. The percentage reduction of Cr, Co, Ni, Cu, Zn, As, Sr and Cd were 100%, 97%, 77%, 71%, 72%, 98%, 88% and 88% respectively by the *P.nurekis* (Figure. 6). Similarly, the percentage reduction of the foresaid heavy metals were 100%, 46%, 44%, 49%, 68%, 57%, 86% and 86% respectively by the *C. reinhardtii* (Figure. 6). No variation was observed in the control reactors.

The metal sorption capacity depended on the type of biosorbent, the availability of concentration of heavy metals [57]. Heavy metal concentration was lower in pulp and paper mill effluent. Heavy metal with lower concentration could easily enter the cells of microalgae through micronutrient transporters and get attached to peptides or proteins and finally moved to specific cellular compartments for detoxification [62]. In this study, it was noted that *P.nurekis* and *C. reinhardtii* was more resistant to the toxicity of concentration of heavy metals from PPME. Both microalgal species exhibited reasonably high potential for phycoremediation for the afore mentioned heavy metals from PPME. This may be due to the lower concentration of heavy metals from PPME. The statistical analysis results showed high significant variation ($p < 0.01$).

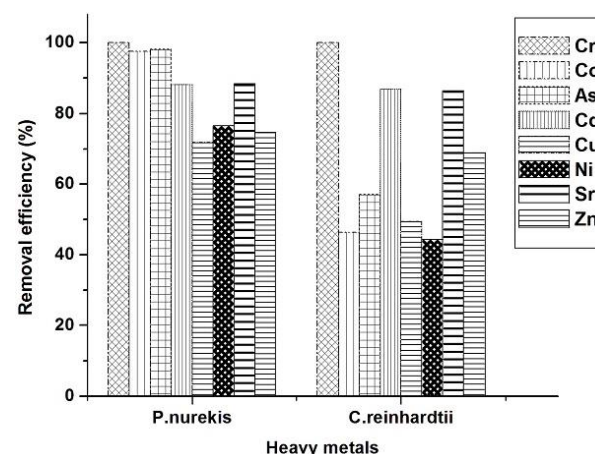


Figure 6: Heavy metals (ppb) removal of *P.nurekis* and *C. reinhardtii* in PPME before and after treatment

3.5 Lipid content – A bioenergy perspective

Lipids are the substances that are insoluble in water, related biosynthetically or functionally to fatty acids and their derivatives. The biodiesel production from the lipid content of microalgae is a promising technology [72] and is considered as carbon neutral [30]. Lipid content obtained from *P.nurekis* was 24% and that from *C. reinhardtii* was 20.5% lipid (Table 3). Comparatively, *P.nurekis* showed higher yield of lipid than *C. reinhardtii* (Figure.7). Malla *et al.* [73] reported 20.69% and 28.32% total lipid content when *C.minutissima* was grown on IARI and CETP wastewater. Dried biomass is typically a prerequisite for biodiesel production from microalgae as moisture interferes with the base homogenous catalyst used in the transesterification reaction [74]. In order to prevent the denaturation of the intracellular lipid, a low temperature (typically less than 100°C) is used to dry the biomass [75]. In the study, the algal biomass was subjected to oven dry at 80°C until the water content got evaporated and the lipid was weighed. Hempel *et al.* [76] reported that *Chlorella* sp.589 achieved 30.2% lipid, *Chlorella* sp.800 achieved 24.4% lipid and *Chlorella saccharophila* 477 achieved 27.6% lipid. A comparative analysis of the lipid content obtained from the present study to the other reported studies is given in Table 3.

Table 1: Physico-chemical characters of PPME during the study period

Parameters (unit)	Initial values	Experiment reactor	Period of experiment (days)					
			2 nd day	4 th day	6 th day	8 th day	10 th day	12 th day
pH	6.02	<i>P.nurekis</i>	6.5 ± 0.15	7.1± 0.2	7.4 ± 0.1	7.9± 0.20	8.2 ± 0.1	8.3± 0.2
		<i>C. reinhardtii</i>	6.5± 0.32	6.9± 0.41	7.2± 0.45	7.5±0.53	7.9± 0.51	8.3± 0.72
		Control	6.2±0.1	6.4±0.2	6.7±0.1	6.8±0.3	6.9±0.2	6.8±0.3
Nitrate(ppm)	28	<i>P.nurekis</i>	18.4 ± 0.23	12± 0.54	9.6 ± 0.23	4.8 ± 0.45	1.1 ± 0.52	0.98 ± 0.95
		<i>C. reinhardtii</i>	19.8± 0.15	15± 0.23	10± 0.32	7±0.36	5± 0.02	3.2± 0.02
		Control	21.4±0.73	20.7±0.8	20.0±0.62	19.8±0.53	19.2±0.71	18.8±0.42
Phosphate(pp m)	12	<i>P.nurekis</i>	6.0± 0.15	4.1± 0.20	2 ± 0.25	1.0 ± 0.13	0.4 ± 0.01	0.0
		<i>C. reinhardtii</i>	6.4± 1.57	5.2±1.24	4.1± 1.36	3.7± 1.24	2.9± 1.12	1± 0.90
		Control	7.7±0.12	7.4±0.13	6.9±0.39	6.5±0.43	6.1±0.53	6.0±0.91
Sulphate(ppm)	230	<i>P.nurekis</i>	190±1.52	153 ± 1.39	118±1.23	88 ± 1.51	63 ±1.64	46 ±1.12
		<i>C. reinhardtii</i>	202± 1.36	184± 1.52	175± 1.45	152± 1.67	120± 1.62	92± 1.23
		Control	224±1.5	216±1.6	210±2.1	205±2.2	198±0.92	195±0.96
COD (mgO ₂ /L)	9200	<i>P.nurekis</i>	8125 ± 7.63	6200 ± 5.24	4350 ± 4.23	1125 ± 6.84	804± 2.46	800± 2.38
		<i>C. reinhardtii</i>	9045± 6.36	8630± 5.12	6240± 5.73	4320± 2.63	2100± 3.64	900± 1.22
		Control	11155±3.1	11100±3.5	11000±3.4	10990±3.1	10980±2.1	10985±2.2

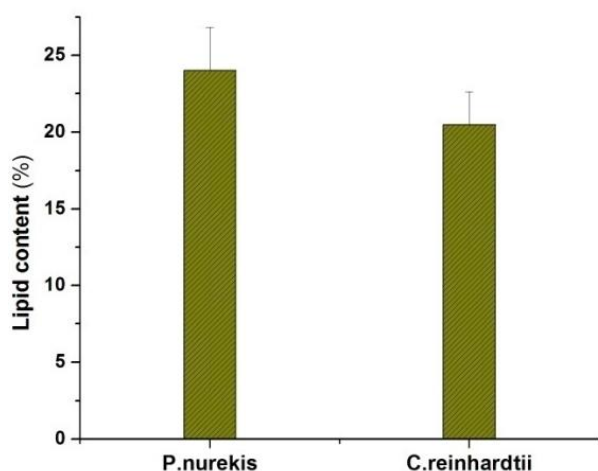
Table 2: Wastewater removal efficiency of different treatment methods used in pulp and paper mill industries

Methods	Technology/ organism used	Days	EC (%)	TDS (%)	Nitrate (%)	Phosphate (%)	Sulphate (%)	COD (%)	Reference
Anaerobic process	Anaerobic reactor	7	nr	nr	nr	nr	nr	88	[63]
Aerobic process	Membrane bioreactor	nda	nr	nr	nr	nr	nr	80	[64]
Hybrid system	USAB electrochemical	25	nr	nr	nr	nr	nr	80	[65]
Wet air oxidation	Heterogeneous catalyst	2 hr	nr	nr	nr	nr	nr	83	[66]
Phytoremediation	<i>Lemna minor</i>	28	nr	50.5	nr	nr	nr	40.5	[13]
Phytoremediation	<i>Trapa natans</i> L.	60	nr	81.8	93.8	90.4	nr	82	[4]
Phytoremediation	<i>Vallisneria spiralis</i>	45	57.1	57.5	nr	nr	nr	74.66	[67]
Phytoremediation	<i>Eichhornia crassipes</i>	45	65	78	nr	nr	nr	60	[68]
Phytoremediation	<i>Eichhornia crassipes</i>	20	90.06	91.94	91.36	91.68	49.89	92.96	[42]
Phytoremediation	<i>Pleurotus</i> spp.	7	nr	nr	nr	nr	nr	57.2	[69]
Phycoremediation	<i>Chlamydomonas reinhardtii</i>	15	nr	nr	93.18	90.71	nr	nr	[70]
Phycoremediation	Mixed culture of <i>Scenedesmus</i> sp.	28	nr	nr	65	71.2	nr	75	[71]
Phycoremediation	<i>Planktochlorella nurekis</i>	12	90.1	87.8	95.6	100	80	92.8	Present study
Phycoremediation	<i>Chlamydomonas reinhardtii</i>	12	91.7	47.69	85.6	87.5	60	91.9	Present study

Nr – not reported, nda- no data available

Table 3: Comparison of Lipid produced with previous studies

Sl. No.	Species	Lipid Content (%)	References
1.	<i>Chlorella vulgaris</i>	14- 50	[77]
2.	<i>Chlorella sp.</i>	13.6	[46]
3.	<i>C.vulgaris</i>	14-22	[78]
4.	<i>C. pyrenoidosa</i>	2.0	[79]
5.	<i>C. sorokiniana</i>	19.0–22.0	[80]
6.	<i>C. fusca</i>	9.68	[81]
7.	<i>N. vigensis</i>	19.29	[82]
8.	<i>Ankistrodesmus sp.</i>	30.0	[83]
9.	<i>Scenedesmus sp.</i>	31.0	[84]
10.	<i>Chlamydomonas reinhardtii</i>	25.25	[85]
11.	<i>C. saccharophila</i>	18.10	[86]
12.	<i>Scenedesmus obliquus</i>	12-14	[87]
13.	<i>P.nurekis</i>	22.0	Present study
14.	<i>C. reinhardtii</i>	20.0	Present study

Figure 7: Lipid content of *P.nurekis* and *C. reinhardtii* after experiment

4 Conclusions

Paper and pulp mill effluent contains large amount of organic and inorganic nutrients. The study indicated that the treatment of PPME by microalgae is very efficient. Based on the results of the physico-chemical analysis, *P.nurekis* could significantly reduce nitrate, phosphate, sulphate, COD etc. from PPME. The percentage reduction of Cr, Co, Ni, Cu, Zn, As, Sr and Cd were 100%, 97%, 77%, 71%, 74%, 98%, 88% and 88% respectively by the *P.nurekis*. Similarly the percentage reduction of the foresaid heavy metals were 100%, 46%, 44%, 49%, 68%, 57%, 86% and 86% respectively by the *C. reinhardtii*. At the end of the experiment, 24% of lipid was obtained from *P.nurekis* and 20.5% lipid was obtained from *C. reinhardtii*. Comparatively, *P.nurekis* showed higher yield of lipid than *C. reinhardtii*. It could be concluded that the indigenous microalgae, *P.nurekis* and *C. reinhardtii* have immense phycoremediation capacity for the treatment of paper and pulp mill effluent. Comparatively *P.nurekis* exhibited significant phycoremediation capacity as well as lipid production potential than *C. reinhardtii*. The subsequent biomass and lipid can be used for the production of valuable products such as biodiesel. Considering the short period of time and low external inputs, phycoremediation is a viable option for the treatment of paper and pulp mill effluent.

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Ethical issue

The authors are aware of and comply with the best practices in publication ethics specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. The authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

Competing interests

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution for data collection, data analyses, and manuscript writing

References

- [1] Ali M, Sreekrishnan TR. Aquatic toxicity from pulp and paper mill effluents: A review. *Adv. Environ. Res.* 2001; 5:175-196.
- [2] Thompson G, Swain J, Kay M, Forster CF. The treatment of pulp and paper mill effluent: a review. *Bioresour Technol* 2001; 77: 275-286.
- [3] Garg A, Narayana VVVS S, Chaudhary, P, Chand S. Treatment of pulp and paper mill effluent *J Sci Industr Res.* 2004; 63: 667-671.
- [4] Kumar V, Chopra AK. Reduction of pollution load of paper mill effluent by phytoremediation technique using water caltrop (*Trapa natans*L.). *Cog Environ Sci.* 2016; 2: 1-12.
- [5] Bishnoi NR, Khumukcham RK, Kumar R. Biodegradation of pulp and paper mill effluent using anaerobic followed by aerobic digestion. *J Environ Bio* 2006; 27(2): 405-408.
- [6] Sureshvarr K, Bharathiraja B, Jayakumar M, Jayamuthunagai J, Balaji L. Removal of azo dye compounds from paper industries wastes using phytoremediation methodology. *Int J Chem Sci.* 2010. 8 (1): 687-700.
- [7] Razali MAA, Ahmad Z, Ariffin A. Treatment of Pulp and Paper Mill Wastewater with Various Molecular Weight of PolyDADMAC Induced Flocculation with Polyacrylamide in the Hybrid System. *Adv in Chem Eng and Sci.* 2012; 2: 490-503.
- [8] Azimvand J, Mirshokraie SA. Assessment of physico-chemical characteristics and treatment method of Paper Industry Effluents : a review. *Intl Res J Appl Basic Sci* 2016; 10 (1): 32-43.

- [9] Ashrafi O, Yerushalmi L, Haghighat F. Wastewater treatment in the pulp and paper industry: A review of treatment process and the associated greenhouse gas emission. *J of Env Manag.* 2015; 158: 146-157.
- [10] Saravanan V, Sreekrishnan TR. Bio-physico-chemical treatment for removal of colour from pulp and paper mill effluents. *J Scient. And Industr. Resear.* 2005; 64:61-64.
- [11] Kumar BA, Jothiramalingam S, Thiagarajan SK, Hidayathullakhan T., Nalini R. Phytoremediation of heavy metals from paper mill effluent soil using *Croton sparsiflorus*. *Inte Lett of Chem Phy and Astro.* 2014; 36: 1-9.
- [12] Hossain K, Ismail N. Bioremediation and Detoxification of Pulp and Paper Mill Effluent: A Review. *Res J Environ Toxicol.* 2015; 9 (3): 113-134.
- [13] Ali Z, Siddiqui A. Analysis of Paper Mill Effluent (Wastewater) and its Phytoremediation of Free Floating Macrophytes (Lemna minor). *Res J Chem Environ Sci.* 2016; 4: 08-12.
- [14] Doran PM. Application of Plant Tissue Cultures in Phytoremediation Research: Incentives and Limitations. *Biotechnol Bioeng.* 2009;103: 60–76.
- [15] Oh K, Cao T, Li T, Cheng H. Study on Application of Phytoremediation Technology in Management and Remediation of Contaminated Soils. *J of Cle Ener Techno.* 2014; 2: 216-220.
- [16] Pradhan S, Al-Ghamdi SG, Mackey HR. Greywater treatment by ornamental plants and media for an integrated green wall system. *Inter.Biodet. & Biodeg.* 2019; 145: 1-9.
- [17] Rasoul-Amini S, Montazeri-Najafabady N, Shaker S, Safari A, Kazemi A, Mousavi P, Mobasher MA, Ghasemi Y. Removal of nitrogen and phosphorus from wastewater using microalgae free cells in bath culture system. *Biocat and Agri Biotech.* 2014; 3: 126–13.
- [18] Ajayan KV, Selvaraju M, Unnikannan P, Sruthi P. Phycoremediation of Tannery Wastewater Using Microalgae *Scenedesmus* Species *Int J Phytoremediation.* 2015;17(10):907-16.
- [19] Whitton R, Mevel AL, Pidou, M, Ometto, F, Villa R, Jefferson B. Influence of microalgal N and P composition on wastewater nutrient remediation *Water Research.* 2016; 91: 371-378.
- [20] Balaji S, Kalaivani T, Sushma B, Pillai CV, Shalini M, Rajasekaran C. Characterization of sorption sites and differential stress response of microalgae isolates against tannery effluents from ranipet industrial area-An application towards phycoremediation. *Int J Phytoremediation.* 2016; 18(8):747-53.
- [21] Mirquez LD, Filipa L, Behnam T, Dominique P, Nitrogen and phosphate removal from wastewater with a mixed microalgae and bacteria culture. *Biotech Reports.* 2016; 11: 18 – 26.
- [22] Junping LV, Feng J, Liu Q, Xie S. Microalgal Cultivation in Secondary Effluent:Recent Developments and Future Work. *Int J Mol Sci.* 2017; 79: 1-18.
- [23] Ajayan KV, Harilal CC, Selvaraju M. Phycoremediation resultant lipid production and antioxidant changes in green microalgae *Chlorella* Sp. *Int J Phytoremediation.* 2018; 20(11):1144-1151.
- [24] Pathak VV, Singh DP, Kothari R Chopra AK. Phycoremediation of textile wastewater by unicellular microalga *Chlorella pyrenoidosa*. *Cell Mol Biol.* 2014; 60(5): 35-40.
- [25] Amit, Ghosh UK. An approach for phycoremediation of different wastewaters and biodiesel production using microalgae. *Environ Sci Pollut Res.* 2018.
- [26] Bansal A, Shinde O, Sarkar S. Industrial Wastewater Treatment Using Phycoremediation Technologies and Co-Production of Value-Added Products. *J Bioremediat Biodegrad.* 2018; 9:1.
- [27] Kumar PK, Krishna SV, Verma K, Pooja K, Bhagawan D, Himabindu V. Phycoremediation of sewage wastewater and industrial flue gases for biomass generation from microalgae. *Sou. Afri. J. of Chem. Eng.* 2018; 25 : 133-146.
- [28] Sunday ER, Uyi OJ, Caleb OO. Phycoremediation: An Eco-Solution to Environmental Protection and Sustainable Remediation. *Journal of Chemical, Environmental and Biological Engineering;* 2(1): 5-10.
- [29] Chisti Y. Biodiesel from microalgae beats bioethanol. *Trends Biotechnol.* 2008; 26: 126-131.
- [30] Thomas DM, Mechery J, Paulose SV. Carbon dioxide capture strategies from flue gas using microalgae: a review. *Environ Sci Pollut Res.* 2016; 23: 16926–16940.
- [31] Ji M, Abou-Shanab RAI, Kim S, Salama E, Lee S, Kabrad AN, Lee Y, Hong S, Jeona B. Cultivation of microalgae species in tertiary municipal wastewater supplemented with CO2 for nutrient removal and biomass production. *Ecol Eng.* 2013; 58: 142 –148.
- [32] Chandra R, Amit, Ghosh UK. Effects of various abiotic factors on biomass growth and lipid yield of *Chlorella minutissima* for sustainable biodiesel production. *Environ Sci Pollut Res.* 2018; 1-14.
- [33] Apandi N, Mohamed RMSR., Al-Gheethi A, Latiffi A, Arifin SNH, Gani P. Phycoremediation of Heavy Metals in Wet Market Wastewater. : *Ear. and Environ.Sci.* 2017;140: 1-6.
- [34] Gani P, Sunar NM, Matias-Peralta H, Latiff AAA, Parjo UK., Razak ARA. Phycoremediation of Wastewaters and Potential Hydrocarbon from Microalgae: A Review *Adv. in Env. Bio.* 2015; 9:20, 1-8.
- [35] Segura MB, Rodríguez LH, Ospina DP, Bolaños AV, Pérez K. Using *Scenedesmus* sp. for the Phycoremediation of Tannery Wastewater. *Tecciencia.* 2016; 69-75.
- [36] Singh AK, Sharma N, Farooqi H, Abidin MZ, Mock T Kumar S. Phycoremediation of municipal wastewater by microalgae to produce biofuel, *Inter J of Phyto.* 2017; 19(9): 805-812.
- [37] Nayak JK and Ghosh UK. Post treatment of microalgae treated pharmaceutical wastewater in photosynthetic microbial fuel cell (PMFC) and biodiesel production. *Bioma and Bioene.* 2019; 131: 1-10.
- [38] Bellinger EG, Sigeo DC. *Freshwater Algae Identification and Use as Bioindicator* John Wiley and Sons London: 2010; 2-40.
- [39] Bligh EG, Dyer WJ. ARapid Method for Total Lipid Extraction and Purification. *Cana J Biochem Physio.* 1959; 110-115.
- [40] Ding J, Zhao F, Cao Y, Xing L, Liu W, Mei S, Li S. Cultivation of microalgae in dairy farm wastewater without sterilization. *Int J Phytoremediation.* 17 (1-6) : 222-7.
- [41] Ali Z, Rahman M. Physico-chemical characteristics of pulp and paper mill effluent. *Res. and Environ. Life Sci.* 2008;1:59 –60.
- [42] Mishra, S., Mohanty, M., Pradhan, C., Patra , H.K., Das, R., Sahoo, S. Physico-chemical assessment of paper mill effluent and its heavy metal remediation using aquatic macrophytes—a case study at JK Paper mill, Rayagada, India. *Environ.Monit. Assess.* 2013; 185: 4347–4359.
- [43] Dominic VJ, Murali M, Nisha MC. Phycoremediation Efficiency Of three micro algae *Chlorella vulgaris*, *Synechocystis salina* and *Gloeocapsa gelatinosa*. *Acade Rev.* 2009;138-146.
- [44] Vijayakumar S, Thajuddin N, Manoharan C. Role of cyanobacteria in the treatment of dye industry effluent. *Pollu Res.* 2005; 24 (1): 69 -74.
- [45] Wurts WA, Durborow RM. 1992. Interactions of pH, Carbon Dioxide, Alkalinity and Hardness in Fish Ponds. *SRAC Publication No.* 464.
- [46] Wang LM, Min L., Yecong C, Paul C, Yifeng. Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. *Appl Biochem Biotechnol.* 2010; 162 (4): 1174-86.
- [47] Sivasubramanian V, Subramanian VV, Muthukumaran M. Phycoremediation of effluent from a soft drink manufacturing industry with a special emphasis on nutrient removal—a laboratory study. *J Algal Biomass Utiln.* 2012; 3(3) :21–29.
- [48] Bitton G (1990) *Wastewater Microbiology.* 3rd edn Wiley-Liss, New York.
- [49] Znad H, D Al Ketife AM, Judd S, AlMomani F, Vuthaluru HB. Bioremediation and nutrient removal from wastewater by *Chlorella vulgaris*. *Ecolog Eng.* 2018; 110: 1-7.
- [50] Linares LCF, Barajas CG, Páramo ED. Assessment of *Chlorella vulgaris* and indigenous microalgae biomass with treated wastewater as growth culture medium. *Bioresour Techno.* 2017; 244: 400-406.
- [51] Azarpira H, Behdarvand P, Dhumal K, Pondhe G. Comparative studies on phycoremediation of sewage water by using blue green algae. *Inter J Bio* 2014; 58-64.
- [52] Ahmad F, Khan Khan, AU Yasar, A. Comparative phycoremediation of sewage water by various species of algae. *Pro Paki Aca of Sci.* 2013; 50: 131-139.
- [53] Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM.. Microalgae and wastewater treatment. *Saudi J Bio Sci.* 2012;19: 257-275.

- [54] Elumalai S, Saravanan GK, Ramganes S, Sakthival R, Prakasam V. Phycoremediation of textile dye industrial effluent from tirupur district, Tamil Nadu, India. *Int J Sci Innova Dis*. 2013; 3: 31-37.
- [55] Acién FG, Gómez-Serrano, Morales-Amaral MM, Fernández Sevilla, JM, Molina Grima E. Wastewater treatment using microalgae: how realistic a contribution might it be to significant urban wastewater treatment?. *Appl Microbiol Biotechnol*. 2016;100 (21): 9013–9022.
- [56] El-Sheekh MM, Farghl MM, Galal HR, Bayoumi HS. Bioremediation of different types of polluted water using microalgae. *Rend Fis Acc Lincei*. 2016; 27: 401–410.
- [57] Rehman A, Shakoori AR. Tolerance uptake of cadmium and nickel by *Chlorella* sp. isolated from tannery effluents. *Pakistan J Zool*. 2004; 36(4): 327-331.
- [58] Ajayan KV, Selvaraju M, Thirugnanamurthy K. Growth and heavy metal accumulation potential of microalgae grown in sewage waste water and petrochemical effluents. *Pak J Biol Sci*. 2011;14(16): 805-811.
- [59] Roy D, Greenlaw PN, Shane BS. Adsorption of heavy metals by green algae and ground rice hulls. *J Environ Sci Health Part A*. 1993; 28: 37–50.
- [60] Wang LM, Min L., Yecong C, Paul C, Yifeng. Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. *Appl Biochem Biotechnol*. 2010; 162 (4): 1174-86.
- [61] Saunders RJ, Paul NA, Hu Y, de Nys R. Sustainable sources of biomass for bioremediation of heavy metals in wastewater derived from coal-fired power generation. *PLoS ONE*. 2012; 7 (5): 1-8.
- [62] Liu, J, Sun Z, Gerken H. Potential Applications of Microalgae in Wastewater Treatments. *Recent Advances in Microalgal Biotechnology OMICS Group eBooks*. Foster City, USA 2016; 9-18.
- [63] Singh, Thakur IS. Colour removal of anaerobically treated pulp and paper mill effluent by microorganisms in two steps bioreactor. *Bioresour Techno*. 2006; 97: 218-223.
- [64] Lerner M, Stahl N, Galil NI. Comparative study of MBR and activated sludge in the treatment of paper mill wastewater. In *Forest industry wastewaters VIII* IWA publishing, London, United Kingdom, 2007; 23-29.
- [65] Buzzini AP, Pires EC. Evaluation of upflow anaerobic sludge blanket reactor with partial recirculation of effluent used to treat wastewaters from pulp and paper plants. *Bioresour Techno*. 2007; 98: 1838-1848.
- [66] Garg A, Narayana VVVS S, Chaudhary, P, Chand S. Treatment of pulp and paper mill effluent. *J Sci Industr Res*. 2004; 63: 667-671.
- [67] Singhal V, Kumar A, Rai JPN. Phytoremediation of pulp and paper mill distillery effluents by channel grass (*Vallisneria spiralis*). *J of Scient And Indstr Reser*. 2003; 62: 319-328.
- [68] Kaushal J, Bhardwaj P. Potential of *Eichhornia crassipes* and *Lemna minor* for degrading Pulp and Paper Mill Effluent using Phytoremediation technique along with enhancers. *Int J of Adv Reser in Sci and Eng*. 2017; 6: 1924-1932.
- [69] Ragunathan R, Swaminathan K. Biological treatment of a pulp and paper industry effluent by *Pleurotus* spp. *Worl J of Micro & Biotech*. 2004; 20: 389–393.
- [70] Ferdowshi Z. 2013. Screening of fresh water microalgae and Swedish pulp and paper mill wastewaters with the focus on high algal biomass production. *Chalmers university of technology Gothenburg, Sweden*.
- [71] Usha MT, Chandra TS, Sarada R, Chauhan VS. 2016. Removal of nutrients and organic pollution load from pulp and paper mill effluent by microalgae in outdoor open pond. *Bioresour Techno*.
- [72] Chisti Y. Biodiesel from microalgae. *Biotechnol. adv*. 2007; 25: 294–306.
- [73] Malla FA, Khan AS, Rashmi, Sharma GK, Gupta N, Abraham G. Phycoremediation Potential of *Chlorella minutissima* on primary and tertiary treated wastewater for nutrient removal and biodiesel production. *Ecolo.Eng*. 2015; 75: 343-349.
- [74] Sukhija PS, Palmquist DL. Rapid method for determination of total fatty acid content and composition of feedstuffs and faeces. *J Agric Food Chem*. 1988; 36: 1202–1206.
- [75] Zhang X, Rong J, Chen H, He C, Wang Q. Current status and outlook in the application of microalgae in biodiesel production and environmental protection. *Front Ener Res*. 2014; 2: 32.
- [76] Hempel N, Petrick I, Behrendt F. Biomass productivity and productivity of fatty acids and amino acids of microalgae strains as key characteristics of suitability for biodiesel production. *J Appl Phycol*. 2012; 24: 1407–1418.
- [77] Gouveia L, Oliveira AC. Microalgae as a raw material for biofuels production. *J Ind Microbiol Biotechnol*. 2009; 36: 269–74.
- [78] Demirbas D. Importance of algae oil as a source of biodiesel. *Energ Convers Manage*. 2010; 52: 163–170.
- [79] Ghasemi Y, Rasoul-Amini S, Naseri AT, Montazeri-Najafabady N, Mobasher MA, Dabbagh F. Microalgae biofuel potentials (Review). *Appl Biochem Microbiol*. 2012; 48: 126.
- [80] Ngoc LDB, Adenan MF, Bato BA, Mansor N, Mahadzir S. Biomass and Lipid Yield of Locally Isolated Microalgae. *Inter J Chem Eng Appl*. 2013; 4 (4): 262-265.
- [81] Aravantinou AF, Theodorakopoulos MA, Manariotis ID. Selection of microalgae for wastewater treatment and potential lipids production. *Bioresour Techno*. 2013; 147: 130–134.
- [82] Sukkrom K, Bunnag B, Pavasant P. Enhancement of Lipid Production from *Ankistrodesmus* sp. *Int J Chem Eng and Appl*. 2015; 6 (2): 111-114.
- [83] Xin L, Hong-ying H, JiaY. Lipid accumulation and nutrient removal properties of a newly isolated freshwater microalga, *Scenedesmus* sp. LX1, growing in secondary effluent. *Nw Biotech*. 2010; 27 (1): 59–63.
- [84] Kong QX, Li L, Martinez B, Chen P, Ruan R. Culture of microalgae *C. reinhardtii* in wastewater for biomass feedstock production. *Appl Biochem Biotechnol*. 2010; 160: 9–18.
- [85] Chinnasamy S, Bhatnagar A, Hunt RW, Das KC. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresour Technol*. 2010; 101: 3097–3105.
- [86] Bruton T, Lyons H, Lera Y, Stanley M, BoRasmussen M. A review of the potential of marine algae as a source of biofuel in Ireland. *Sustainable Energy, Ireland*. 2009; 88.