Toxicity of Cadmium (Cd) on microalgal growth, (IC50 value) and its exertions in biofuel production

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ABSTRACT

This study evaluated the IC50 value of cadmium against four different strains of microalgae. Chlorella sorokiniana was able to tolerate 300 mg/L of cadmium. The lipid productivity increased by 6% at 50 mg/l of Cd (II) stress. The decrease in biomass productivity was recorded with increasing concentration of Cd. The results showed that chlorophyll contents, chlorophyll a (Chl a) and chlorophyll b (Chl b) gradually decreased on increasing the concentration of Cd over 100 mg/l. The FAME composition of C. sorokiniana cultivated under Cd (II) stress and control medium were analyzed to determine the quality of the biodiesel produced. The major fatty acids present in the TAGs of the treated microalga were C10:0, C12:0, and C15:0.

Keywords: Cadmium; Stress; Microalgae; Metabolites; Growth; Biofuel.

1. INTRODUCTION

Cadmium (Cd) is the most widely known toxic and hazardous heavy metal environmental pollutant that leads to appalling illnesses in humans including cancer, kidney dysfunction, liver damage and bone diseases [1; 2; 3; 4; 5; 6]. The major causes pertaining to Cd toxicity due to its uncontrolled discharge in the environment by different industries [7].

Thus, Cd toxicity is one of the major environmental concerns globally that has attracted the attention of various researchers to tackle it by utilizing microbial flora such as fungi, bacteria, lignocellulosic materials and microalgae [8]. However, amongst all of the above microalgae have gained the most popular owing to their greater tolerance to Cd in comparison to other organisms.

Microalgae have evolved different intracellular and extracellular mechanisms to resist heavy metal toxicity and also discriminate between the essential and the non-essential heavy metals. This makes them a suitable practical option to tackle heavy metals in wastewaters [9; 10]. The use of both living and dead microalgae for removal of cadmium has been reported [11; 12; 13]. The efficient uptake of Cd has been reported in Phormidium sp. and Spirulina sp.[14].

Microalgae based biofuels have also been projected as world energy security against diminishing fossil fuels. This is because of their simple cultivation requirements and high lipid concentrations of many microalgal species in comparison to the traditional crops used for biofuel production [15;16; 17]. In-fact some researchers have reported Cd to increase lipid productivity [18; 19] in different microalgae which makes it a multipurpose biological agent to solve the issue of heavy metal pollution as well as the energy crisis. Metal induced strain leads to modifications in the lipid profile of the microalgae viz., composition, chain length, cetane number, viscosity, Nox emissions, etc.[20; 21; 22; 23]. Thus, metal-induced stress can be deliberately introduced in order to modify the fatty acid composition in microalgae and produce biodiesel of desirable quality and properties [24].

Keeping in view the identification of the above-mentioned fact of such microalgal species could open up new avenues towards integrating microalgal bioremediation and biofuel generation that too with enhanced lipid yields. Also, the selected microalgae can be further cultivated directly on cadmium polluted wastewaters which is further going to cut down the cost for cultivation media.

An effective hybrid approach has been presented here for cadmium removal complementing with enhanced lipid productivity in oleaginous microalgae viz.,Chlorella singular is, Chlorella sorokiniana, Chlorella minutissima, and Scenedesmus abundans) for biofuel generation. All the microalgae were first analyzed for their cadmium tolerance capabilities and were simultaneously assessed for the cadmium-induced stressed in lipid productivity. The findings of the study indicate this hybrid method as a cleanup approach pertaining to a sustainable environment.

2. MATERIALS AND METHODS

2.1. Materials. The four microalgal species viz., Chlorella singular Chlorella sorokiniana, Chlorella minutissima and Scenedesmus abundans were available in Uttaranchal University, Dehradun, Uttarakhand, India.

2.2. Microalgae cultivation, Growth, Cd IC50 values determination.

The microalgal species were first cultivated in 500 ml flasks containing Bold’s Basal Medium (BBM) [6] for 7 days at 24°C with cool white fluorescent light. Flasks were shaken manually after...
regular intervals of time. The heavy metal tolerance of each of the four microalgae species was determined against CdCl₂. The stock solution of CdCl₂ (10 mg/ml) was prepared and then diluted according to the requirement. After 96 h of microalgal cultivation growth of four microalgae was measured using a spectrophotometer the O.D was measured at 686 nm. The IC₅₀ value is that Cd concentration that reduces the microalgae cell viability by 50% as compared to microalgae cultivated in BBM. The maximum IC₅₀ of Cd was recorded in Chlorella sorokiniana with an IC₅₀ value of 300 μg/ml. The IC₅₀ value is that Cd concentration which reduces the microalgae cell viability by 50% as compared to microalgae cultivated in BBM after 96 h of cultivation [25; 26; 27]. The IC₅₀ value was calculated using linear interpolation analysis and Microsoft Excel 2010.

The further study we have chosen the Chlorella sorokiniana to analyse the different parameters of microalgae.

2.3. Estimation of photosynthetic pigments.

For estimation of photosynthetic pigments 5 ml of the microalgal culture was centrifuged at 5500 rpm for 5 min on 10th day. For estimation of pigments 5 ml of the microalgal culture was taken on the 10th day of cultivation and centrifuged at 5000 rpm for 5 min [6]. The obtained biomass was suspended in the 3 ml methanol and allow tostand at 45 °C for 30 min. Centrifuged, recorded the absorbances in supernatant and evaluated the pigments according to Kumar et al., [6], subtracting at 750 from other Absorbencies. Chlorophyll a (Chl a), chlorophyll b (Chl b) and Carotenoids (Car) were determined using the formulas given by Lichtenthaler, [28]

$$\text{Percent inhibition} = \frac{\text{Biomass productivity in control medium} - \text{Biomass productivity in treated medium}}{\text{Biomass productivity in control medium}} \times 100$$

3. RESULTS AND DISCUSSION

3.1. IC₅₀ value and growth of microalgae.

All four microalgae species were found tolerant to CdCl₂ with different IC₅₀ values (50-300 μg/ml) for the same.

![Figure 1. Growth of microalgal four species under Cd (II) Stress (100 mg/l). A4- Chlorella sorokiniana, Chlorella minutissima and SA- Scenedesmus abundans, CM- Chlorella minutissima, A2- Chlorella singularis.](image)

The maximum IC₅₀ value to Cd was recorded in Chlorella sorokiniana with an IC₅₀ value of 300 mg/l. During the cultivation of microalgae, it also displayed the fastest adaption amongst the four microalgae with the shortest lag phase of 03 days amongst the four microalgal species (Fig 1). The growth was also uniform throughout the cultivation duration with a gradual increase in the number of cells.

3.2. Effect on photosynthetic pigments.

A decrease in all the photosynthetic pigments was recorded in C. sorokiniana cultivated in the presence of Cadmium (Cd) as compared to those cultivated in non-Cd containing medium (Table 1). The Chl a content decrease from 2.842 to 0.470 μg/mg while carotenoids concentration increase from 0.453 to 0.128 μg/mg at 50 mg/l of Cd. In past studies, researchers reported that Cd stress damage the biosynthesis of chlorophyll [34; 35; 36].

<table>
<thead>
<tr>
<th>Cd Conc. (μg/ml)</th>
<th>30</th>
<th>100</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl a</td>
<td>2.842</td>
<td>0.470</td>
<td>1.383</td>
</tr>
<tr>
<td>Chl b</td>
<td>2.804</td>
<td>0.599</td>
<td>1.052</td>
</tr>
<tr>
<td>Car</td>
<td>0.453</td>
<td>0.128</td>
<td>0.286</td>
</tr>
</tbody>
</table>

3.3. Effect on biomass productivity and lipid yield.

Biomass productivity was decreased in Cd treated cell (300 mg/l) (112 ± 0.01 g/L/d) on 5th day. 2 fold 2.6 fold decreases in C. sorokiniana biomass reported under Cd (300 mg/l) stress (Table 2). Increase in 6 % of lipid content under Cd treated algal cell at 50 mg/l as compared to the control. The increase in lipid content under metal stress conditions is common in algae cells [37; 38].
Under stress, the condition leads to less growth and algal cell metabolism shifts towards the synthesis of triacylglycerol [39; 40].

Figure 2. FTIR graph of A-Cd (II) treated (100 mg/l) and B-control Chlorella sorokiniana.

Table 2. Biomass productivity and Lipid yield under Cd stress.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Cd concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>Biomass</td>
<td>800 mg/l</td>
<td>626 mg/l</td>
</tr>
<tr>
<td>Lipid yield</td>
<td>26.25%</td>
<td>32.5%</td>
</tr>
</tbody>
</table>

3.4. FTIR analysis.

Normal algal cell has high carbohydrate, protein and photosynthetic pigments as compared to Cd treated cell (Fig 2). High peak was observed in 2938 cm\(^{-1}\) means Cd treated cell increase the lipid. 1067 cm\(^{-1}\) region showed high peak means Cd treated cell there is increase in carbohydrates content also.

3.5. FAME composition.

The feasibility of microalgal biomass cultivated for detoxification of Cd and its effect on biodiesel production was evaluated by analyzing the total lipid profile and fatty acid profile and compared to BBM (Fig. 3A and 3B). Microalgal lipids can be majorly categorized into structural/polar and storage/non-polar lipids [41]. The FAME composition of C. sorokiniana cultivated in Cd stress cell and BBM medium was analyzed to determine the quality of the biodiesel produced. The major fatty acids present in the TAGs of the treated microalga were C10:0, C12:0, and C15:0 (Fig. 3B, Table 3 and 4). SFA (%) composition was good in both the biodiesel obtained from Cd treated and control microalgal biomass (table 5). PUFA were not reported in Cd treated biomass biodiesel. The value of cetane number was good in both the diesel. High cetane number is good for complete combustion and smooth functioning of engine.

Table 3. GC-MS profile of biodiesel of microalgae cultivated in control medium.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Area %</th>
<th>RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonanoic acid, methylester</td>
<td>C9</td>
<td>0.19</td>
</tr>
<tr>
<td>Decanoic acid, methylester</td>
<td>C10</td>
<td>0.56</td>
</tr>
</tbody>
</table>
Table 4. GC-MS profile of biodiesel of microalgae cultivated under Cd stress.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Area %</th>
<th>RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dodecanoic acid, methylester</td>
<td>C10</td>
<td>2.65</td>
</tr>
<tr>
<td>Dodecanoic acid, methylester</td>
<td>C12</td>
<td>12.65</td>
</tr>
<tr>
<td>Heptadecanoic acid, methylester</td>
<td>C17</td>
<td>0.77</td>
</tr>
<tr>
<td>Pentadecanoic acid, methylester</td>
<td>C15</td>
<td>7.38</td>
</tr>
<tr>
<td>11-Octadecanoic acid, methylester</td>
<td>C18:1</td>
<td>1.33</td>
</tr>
<tr>
<td>Eicosanoic acid, methylester</td>
<td>C20</td>
<td>0.25</td>
</tr>
<tr>
<td>Docosanoic acid, methylester</td>
<td>C22</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Table 5. Biodiesel properties of Cd treated and control algal biomass.

<table>
<thead>
<tr>
<th>Biodiesel Properties</th>
<th>Cd Treated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA: Saturated Fatty Acid (%)</td>
<td>21.810</td>
<td>21.620</td>
</tr>
<tr>
<td>MUFA: Mono Unsaturated Fatty Acid (%)</td>
<td>1.330</td>
<td>0.000</td>
</tr>
<tr>
<td>PUFA: Poly Unsaturated Fatty Acid (%)</td>
<td>0.000</td>
<td>0.970</td>
</tr>
<tr>
<td>DU: Degree of Unsaturation</td>
<td>1.330</td>
<td>1.940</td>
</tr>
<tr>
<td>SV: Saponification Value (mg/g)</td>
<td>59.180</td>
<td>47.870</td>
</tr>
<tr>
<td>IV: Iodine Value</td>
<td>1.196</td>
<td>1.757</td>
</tr>
<tr>
<td>CN: Cetane number</td>
<td>138.258</td>
<td>159.921</td>
</tr>
<tr>
<td>LCSF: Long Chain Saturated Factor</td>
<td>0.250</td>
<td>6.760</td>
</tr>
<tr>
<td>CP: Cloud Point (°C)</td>
<td>-4.992</td>
<td>-4.992</td>
</tr>
<tr>
<td>PP: Pour Point (°C)</td>
<td>-12.240</td>
<td>-12.240</td>
</tr>
</tbody>
</table>

4. CONCLUSIONS

The IC50 values obtained was 300mg/l maximum for C. sorokiniana. An increase in lipid content by 6% was recorded in algal cells cultivated at 50mg/l concentration of Cd. Further increase in concentration decreased the lipid content. A decrease in photosynthetic pigments over 100 mg/l concentration of Cd was noted. The findings of this study will help develop protocols to remediate Cd from contaminated water and the biomass can be used in biodiesel production.

5. REFERENCES

13. Pereira, S.; Micheletti, E.; Zillel, A.; Santos, A.; Moradas, F.P.; Tamagnini P.; De Philippis, R. Using extracellular polymeric substances (EPS)-producing cyanobacteria for the bioremediation of heavy metals: do cations compete for the EPS.
6. ACKNOWLEDGEMENTS

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