Short communication

Effects Of Cadmium On A Marine Cyanobacteria

Abstract

Cyanobacteria, being one of the earliest producers on the planet, have developed unique survival strategies. These uniqueness of cyanobacteria are drawing the attention of scientists to study the properties of this organism for the goal of pollution control. Due to industrialisation and other reasons, water bodies across the globe is facing problems of heavy metal toxicity. As a way to remediate water bodies polluted with heavy metals like Cadmium, use of cyanobacteria could be significant. Cyanobacteria have been shown to remove or absorb 10-60% of the contaminating cadmium in different previous studies. Strain AP25 isolated from islands of the Indian Sundarbans have been used for this study. The photosynthetic pigments of AP 25 have been studied in order to determine whether the uptaken heavy metals alter vital cellular processes in cyanobacteria. Along with the photosynthetic pigments, different physiological parameters of AP25 also showed decline with time even when the Cadmium concentration remained a constant of 1000 µM.

Keywords: Cyanobacteria, photosynthetic pigments, bioremediation, physical and physiological changes.

1. Introduction

Heavy metal pollution of aquatic habitats is one of the prime concerns nowadays since the persisting heavy metal ions affect aquatic life and human life alike. Cadmium is extremely potent as a poison. The World Health Organization (WHO) has set the limit of potable water to be 3 μgL^{-1} and for irrigation water to be 0.05 mgL^{-1} for short-term use and 0.01 mgL^{-1} for long-term use [El-Hameed M.M.A., *et al*.,2021; Bon I.C., *et al*.,2021]. In particular, Cadmium carries huge risks as the contamination sources of this highly potent poison, from varied sources like– industrial, agricultural and even natural. As the utilities of heavy metals are rising in several industrial processes, the issue of the improper handling of toxic byproducts released as wastes from such industries is also gradually increasing. The association of toxicity with such metals is not due to the fact that they are toxic agents but because they are capable of forming complex compounds, mostly with water, that are often toxicogenic in nature. Cadmium is one of the most studied heavy metal pollutants in aquatic habitats due to the relatively good solubility of its salts in water. Its sources include wastes from cadmium-based batteries, runoffs from fields treated with phosphate fertilizers that contain cadmium [Matsunaga T,*et al*.,1999; Bon I.C., *et al*.,2021].

Apart from being a Group 1 carcinogen, Cadmium can cause both kidney and liver dysfunctions. Hence, removal of heavy metal from polluted water is a big challenge. Eventually, scientific inquisitiveness has led to an extensive research in the area of bioremediation.

Bioremediation, or the use of a biological system's natural capability of up taking metal ions to lower the level of harmful heavy metals in polluted waters, has earned a lot of attention in the past years. A cyanobacterium is one of the important aquatic organisms with the potential of bioremediation. It has been one of the best studied microorganism in this regard, as its sequestering capability usually outdoes that of other organisms' capable of bioremediation. However, several of their physiological and metabolic processes suffer adverse effects as result of the toxicity exerted by the heavy metal like Cadmium [Garcia-Pichel, F.,2009; Matsunaga T, *et al*.,1999; El-Hameed M.M.A., *et al*.,2021].

Cyanobacteria are majorly found in freshwater and marine habitats. They constitute a large part of marine planktons globally and are found in great numbers mainly along the coastal areas as benthic vegetation, which lies in a zone bounded by the marks of the high tide and the low tide. Freshwater cyanobacteria exist in an extensive range of trophic conditions and can flourish very well in every zone, starting from the shallowest epilimnion zones to the deepest part of the extremely euphotic parts of hypolimnion. Sundarbans, a mangrove forest in India, is rich in biodiversity. The cyanobacteria used in this study have been isolated from Sundarbans [Pramanik A,*et al*,2011].

Photosynthetic pigments are complex conjugative structures, primarily composed of isoprene or tetrapyrrole ring backbones, are capable of absorbing lights of different spectra and inducing different pathways. Cyanobacteria have developed such pigments that can control their physiological and metabolic needs. The types of such pigments found in cyanobacteria are mostly the following– chlorophyll, carotenoids (β-carotenes, lutein, fucoxanthin, astaxanthin, etc.), phycobiliproteins (Phycoerythrin, Phycocyanin and Allophycocyanin) and scytonemin [Saini D. K,*et al*. 2018].

Photosynthetic apparatus is majorly altered by Cadmium as it damages both of the photosystems and the light-harvesting complexes. It disrupts the functioning of PS II on its donor and acceptor sides, it interferes with the biosynthesis of chlorophyll and enzymes like RubisCO that are part of the CO_2 fixation pathway, distorts the entire chloroplast structure, etc.

2. Materials

2.1. Strains used in the experiment

AP25 - Cyanobacterial strain AP25 was collected from the biofilms present on the soils of Sagar island of the Indian Sundarbans. Its morphological properties were examined under a light microscope, with *Leptolyngbya boryana* as the reference strain [Pramanik A, *et al*., 2011].

2.2. Culture Conditions

Both AP25 culture was grown in ASN III media, pH 7.2 in 2l Erlenmeyer flasks. It was kept aerobically at room temperature (27 ± 2 ºC) under an alternating 12 h light-12 h darkness condition [Pramanik A, *et al*., 2011].

2.3. Heavy Metal Stock Solution

A 2000 µM Cadmium-contaminated aqueous solution was prepared as stock. The stock was diluted using the growth media to obtain the concentrations required for the experiments.

3. Methods

A large mass of pure AP25 culture was homogenized using a hand-held homogenizer in a sterile condition and 1 ml of it was added to every tube containing a total of 14 ml of ASNIII media and Cadmium of 1000 µM concentration. The tubes were kept aerobically at room temperature (27 ± 2 ºC) under an alternating 12 h light-12 h darkness condition [Pramanik A, *et al*., 2011].

3.1. Measurement of optical density and biomass

The optical density of the supernatant of the culture was measured at 750 nm using a spectrophotometer on Day 0, Day 7, Day 14, Day 21 and Day 29. Clear ASN III media was used as control. The biomass was measured using a digital scale.

3.2. Quantification of total protein

Protein precipitation from the cyanobacterial culture was done using Trichloroacetic acid treatment. For both treated and untreated tubes, supernatants were discarded, retaining only 1 ml media with the culture. They were mixed well and transferred to Eppendorf tubes. 250 µl of hot TCA was added, using concentrations 6% and 24% of it to standardize the concentration that yields best results. The tubes were cooled for 5 minutes and then incubated at 4°C for 10 minutes. The tubes were centrifuged at 14,000 rpm for 5 minutes (at 4°C). After removing the supernatants, 20 µL cold acetone was added to the pellets. They were centrifuged again at 14,000 rpm for 15 minutes (at 4°C). Supernatants were removed and the pellets were dried by keeping the tubes in a heat block for 5 minutes at 55°C. The pellets were dissolved in a 500 µl Tris-HCl buffer. A BSA standard curve was also made and the Folin Lowry method was followed to measure the optical densities at 660 nm.

The procedure was followed for all the time points.

3.3. Quantification of phycobiliprotein

For the quantification of phycobiliprotein, a freeze thaw method was adopted. Treated and untreated cultures from Day 0, Day 7 and Day 14were separately put into tubes and centrifuged at 10,000 rpm for 30 minutes. The supernatant in each tube was discarded and the following were added–Tris-HCl buffer, EDTA and lysozyme. Tubes were kept in alternating conditions of 0°C and room temperature (~35°C) [Schmelling N.M., 2019; Hazra P and Kesh G. S.,2017].

3.4. Quantification of chlorophyll

Chlorophyll was quantified using methanolic extraction on Day 21 and Day 29. From cultures of treated and untreated tubes supernatants were discarded and in each tube only 2 ml media were retained. The cultures were mixed well and added to 1 ml in Eppendorf tubes (4 in total; 2 for untreated culture and 2 for treated culture). The samples were centrifuged at 14,000 rpm for 5 minutes (at 4°C). 900 µl of the supernatant was discarded from each tube, 900 µl of 100% methanol was added and mixed well by vortexing. The tubes were incubated in the dark at 4°C for 30 minutes, after which they were centrifuged at 14,000 rpm for 5 minutes (at 4°C). The optical densities were measured at 665 nm by making the volume to 2 ml for both the treated and untreated cultures. 90% methanol was used as control [Schmelling N.M., 2019; Hazra P and Kesh G. S.,2017].

The chlorophyll content (in µg/ml) is estimated using the equation–

Chl [µg/ml] = OD_{665nm} x 13.9 [µg/ml] x dilution factor of culture [Schmelling N.M., 2016]

4. Results and Discussion

4.1. Optical density and biomass

The following data were obtained by measuring the optical density of the supernatant of the cultures and the biomasses of the cultures for the treated media.

Day	$O.D._{750}$	Biomass(in g)
0	0.051	1.909
7	0.013	0.734
14	0.003	0.500
21	0.000	0.220
29	0.002	0.074

Table 1. Optical Density (O.D.) of the supernatant of cadmium treated sample (AP25) and biomass at different time intervals.

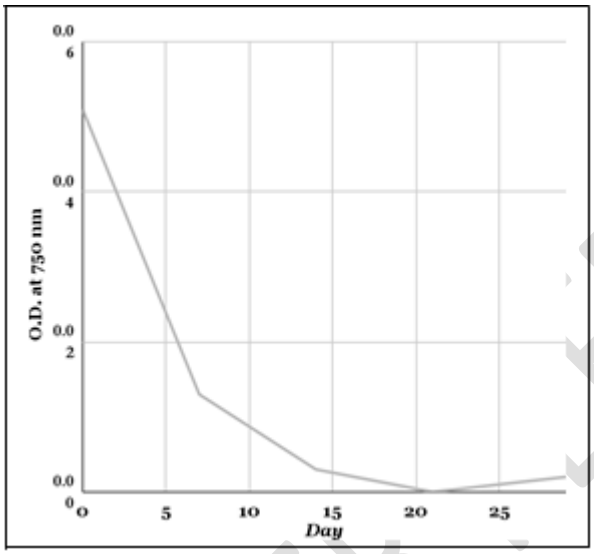

Figure 1. Optical density at 750nm for Day 0, Day 7, Day 14, Day 21 and Day 29

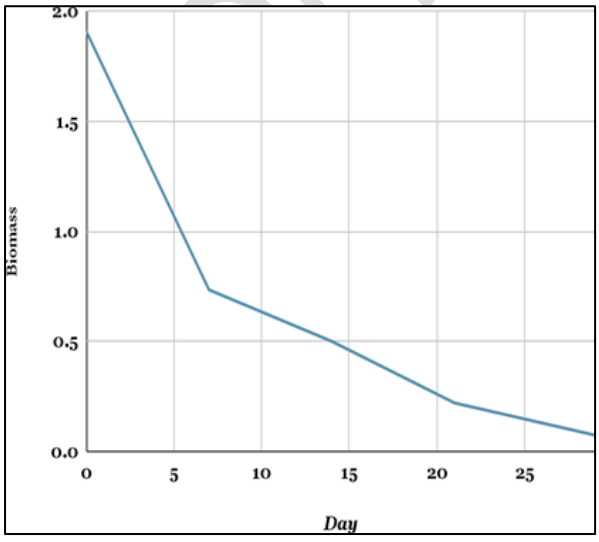

Figure 2. Biomass (in g) for Day 0, Day 7, Day 14, Day 21 and Day 29

The optical density of the supernatant showed a decrease in value, until Day 29, where the optical density increased slightly. The cyanobacteria cultures showed a definite decline in the biomass.

4.2. Quantification of total protein and phycobiliprotein

Day	TCA%	Sample	O.D.$_{660}$
0	6	Treated1	0.018
		Treated2	0.021
7	6	Treated1	0.005
		Treated2	0.001
		Untreated1	0.005
		Untreated2	0.015
	24	Treated1	0.009
		Treated2	0.018
		Untreated	0.019
		Untreated2	0.001
14	6	Treated1	0.000
		Treated2	0.001
		Untreated1	0.001
		Untreated2	0.001
	24	Treated1	0.000
		Treated2	0.003
		Untreated1	0.001
		Untreated2	0.003

Table 2. Optical density of treated and untreated AP25 at different TCA concentrations and different time intervals.

Both the experiments for total protein estimation and phycobiliprotein quantification were discontinued after Day 14as neither the protein could be well precipitated using the TCA

treatment, nor could the cells be lysed for phycobiliprotein quantification following the standard protocols of freeze thawing.

4.3. Quantification of chlorophyll

The amount of chlorophyll was calculated by the formula using the optical densities obtained at 665 nm –

Day	Sample	$O.D._{665}$	Concentration(μgmL^{-1})
21	Treated	0.294	4.087
	Untreated	0.427	5.935
29	Treated	0.001	0.014
	Untreated	0.505	7.020

Table 3. Optical density of cadmium treated and untreated AP25 at 665nm and their chlorophyll concentrations

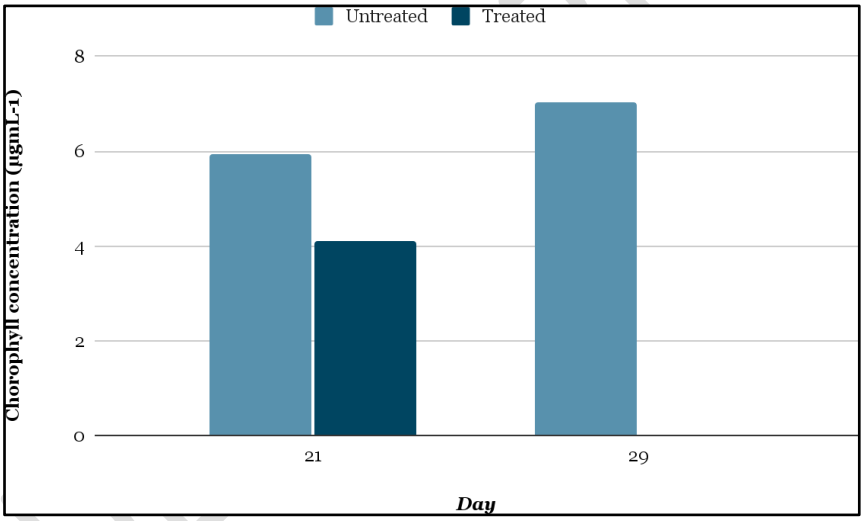

Figure 3. Graphical representation of chlorophyll concentration (μgmL^{-1}) for Day 21 and Day 29

The amount of chlorophyll that could be extracted from the untreated cyanobacteria cultures showed a steady increase from Day 21 to Day 29. The treated culture had lower levels of chlorophyll on Day 21, the concentration being almost zero on Day 29.

5. Conclusion

The decreasing biomass of AP25 culture, with time indicated that an increasing number of cells failed to survive while adapting to the cadmium they kept on taking from the media. The increasing difference in the concentrations of photosynthetic pigments between the treated and the untreated cultures indicated the gradual degradation of the pigments due to the harmful effect of the Cadmium ions on the physiological processes of the cyanobacteria.

Our study result shows harmony with previously reported results. As the strain used in this study was not well studied in many aspects of physiology and biochemistry, the data on the

effects of cadmium on the photosynthetic pigments add a valued contribution. Moreover, the concentration of cadmium used in this study is way high compared to all other previous studies [El-Hameed M.M.A., *et al*., 2021; Matsunaga T, *et al*., 1999; Ahad, R.I.A., *et al*., 2017].

As stated before, the area of study concerning phytoremediation is extremely vast and requires extensive research. Though this study does not include quantification of amount of cadmium removed or absorbed, this study would lead to elaborate research in this field of bioremediation by marine cyanobacteria.

6. References

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