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RESEARCH ARTICLE

Efficient Bioremediation of Aluminium by using Ecofriendly Cyanobacteria from heavy metal contaminated water

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Santhosh Kumar Mehar Chinreddy Subramanyam Reddy Phyto or bioremediation is an effective, economical and ecofriendly technology for metal detoxification from industrial waste water. Elevated aluminium level causes acidifcation of water which may affect the aquatic animal and human health. To dilute the aluminum toxicity levels from industrial waste water phytoremediation is a one of the best approaches. Bioremediation has provided problems solving opportunities in the field of solid waste by detoxifying effluents. Blue green algae is the primatitive life form on the earth, which can be edible and may be used to treat the industrial water containing heavy metal. In the current study the aluminium remediation ability investigated with flamentous Cyanobacteria spirulina and their efficiencies were compared. Results indicated that the spirulina is very efficient to detoxify the aluminium from waste water, significant improvement of photosynthetic pigments and sugars were observed. An integrated eco-friendly approach to the industrial wastewater treatment through live spirulina based phytoremediation is envisaged as part of the environmental management.

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Introduction

The toxicity of heavy metals released into the environment has recently observed a number of studies aiming at the removal of metal ions from aqueous solutions. (Fourest and Volesky, 1997). Environmental pollution becomes an important role of concern for all the nations worldwide. Not only developing countries but the developed nations are also affected and suffering from it. The large number and great structural variety of micro pollutants make it very difficult to assess the adverse effects. Which are not acute but are subtle, chronic effects (Schwarzen, Bachetal, 2006). About 20% of the globally accessible renewable fresh water is used by industry and municipality (Cosgroove et al, 2000). Aluminium is the third most abundant element and the most prevalent metal in

the earth, constituting approximately 8% of total mineral components. Daily consumption of aluminium by human beings through different sources like food, water, occupational exposure to aluminium in industries etc., is about 5-10 mg (AB Edwards RA). An elevated level of aluminium is a potentially toxic and it may lead to mainly three disorders: microcytic anemia, aluminium-induced bone disease, and neurological dysfunction.

Bioremediation has been investigated as an alternative method for treating the metal containing waste water of low concentrations in response to heavy metals micro-organisms have evolved various measures via process such as transport across the cell membrane, biosorption to cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation reduction reaction (Lu and Wilkins, 1995). The use of algae for adsorption of heavy metals has the advantages (Trujillo et al., 1991) of low cost raw materials huge adsorption capacity and no secondary pollution etc.,. Thus, algae is promising for use in treating industrial water containing heavy metals.

The effects of temperature, pH and salinity on the growth and protein content of two locally isolated species of spirulina were studied (Sabbagh, 2006) the effects of Al on Protein and carotenoides content of spirulina.

The present study deals the detoxification of aluminium contaminated water, by using cyano bacteria (Spirulina) and consequences measured. We have successfully removed Al from contaminated water as well as we noticed different growth parameters of spirulina. Al adsorption was significantly increased when the al level was high and also noticed protein, carotinoid contents also measured.

2. Material and Methods

Spirulina was collected from the Bharathidasan University, Tiruchirappalli, Tamilanadu. The algae was first centrifuged and then stored in liquid medium (Zarrouk) details in table 1 for 15 days at 26 ± 2^{0} c under the light generated by a 40 w white fluorescent lamp. The culture flask contained glass beads (2mm, five in each flask) to prevent the cell clumps) in algal growth media, kept in shaker for gentle shaking. Source of heavy metals stock solutions were prepared with AlClO₃ (Al) the concentrations were 0.5 ppm to 8 ppm taken for this study. Fifteen days, cultured 10 mL of spirulina sample was harvested from homogenous algae and supernantatnt was collected by centrifugation (4000g, 15min). The rest sediment containing algal samples were resuspended individually with 10 mL of EDTA (10µM) solution and allowed it for gentle shaking. Samples were once again centrifuged; supernatant was collected for measuring the adsorbed ionic concentration. All three parts i.e. media, EDTA and pellets from low sample were dried, digested with double acid (HNO3:HCLO4 mixture (10:1, v/v) in boiling water bath for 1hour. After cooling the samples, made the volume to 10 mL with distilled water and analyzed for Al level by atomic Absorption Spectrophotometer at 628 nm (validated for analysis of trace metals).

Different concentrations (ppm) of Al levels were subjected to spirulina for 15 days and quantified the Protein (Lowry et al., 1951), Chlorophyll 'a', Chlorophyll 'b', total chlorophylls (Gentyet al., 1989) and Sugars (Dubosis et al., 1956). The Effect of (aluminium) heavy metal on Proteins, Chlorophyll 'a', Chlorophyll 'b', total Chlorophylls and Sugars were assessed. The growth parameters of the stress induced sprirulina data were colleted and statistically analysed with ANOVA as compared to control treatments.

3. Results and Discussions

3.1 Assessment of Aluminium detoxification from heavy metal Aluminium water

High concenterations (8ppm) of aluminium cause acidification of water which would affect the aquatic and human health. Present study revealed that the spirulina significantly absorbed the Al in higher concentrations in supernatant as compared to EDTA and pellets (fig.4). The finding high amount of aluminium in the supernatant indicates the absorption of the heavy metals by the spirulina. The absorption was proportionally increased with Aluminium concentrations i.e., when the aluminium concentration increased the absorption level aslo increased.

The growth parameters such as chlorophyll 'a', chlorophyll 'b' and total chlorophylls content showed its healthy growth with various concentrations of aluminium stress. The present work elucidated, spirulina has a great potential to absorb the Al heavy metal at various concentration 0.5 ppm, 1ppm, 2ppm, 4ppm, 6ppm and 8ppm. The algae Spirulina rapidly adsorbed appreciable amount of the heavy metal Al was detected the supernatant indicates absorbption capacity and the healthy growth conditions indicates its stress toleranceat at elevated Al concentrations. Thus these results clearly indicate, the spirulina is a potent eco friendly tool for the remedial for Al.

AAS Methods	Concentrations of Aluminium							
	0.5 ppm	1 ppm	2 ppm	4 ppm	6 ppm	8 ppm		
Supernatant	$\begin{array}{c} 0.77 & \pm \\ 0.05 & \end{array}$	$\begin{array}{cc} 1.01 & \pm \\ 0.05 \end{array}$	$\begin{array}{c} 1.12 \pm \\ 0.03 \end{array}$	1.29 ± 0.05	1.39 ± 0.07	1.64 ± 0.03		
EDTA	$\begin{array}{c} 0.36 \\ 0.04 \end{array} \hspace{0.1in} \pm \hspace{0.1in}$	$\begin{array}{cc} 0.46 & \pm \\ 0.06 \end{array}$	$\begin{array}{cc} 0.46 & \pm \\ 0.01 & \end{array}$	$\begin{array}{ccc} 0.53 & \pm \\ 0.03 & \end{array}$	$\begin{array}{ccc} 0.58 & \pm \\ 0.06 & \end{array}$	$\begin{array}{cc} 0.79 & \pm \\ 0.06 \end{array}$		
Pellets	0.19 ± 0.08	0.39 ± 0.09	$\begin{array}{cc} 0.42 & \pm \\ 0.05 & \end{array}$	$\begin{array}{ccc} 0.45 & \pm \\ 0.04 & \end{array}$	$\begin{array}{cc} 0.45 & \pm \\ 0.06 & \end{array}$	$\begin{array}{ccc} 0.59 & \pm \\ 0.05 & \end{array}$		

Table-4: Estimation of remaining Aluminium in the media and Spirulina in different concentrations (mg l⁻¹)

Fig.-4: Estimation of remaining Aluminum in the media and Spirulina in different concentrations of Aluminium



3.2. Estimation of the Protein content in Spirulina

Protein content was significantly decreased when the algae exposed to aluminum stress. When the Al concentration increased simultaneously the content of Protein was decreased. The data was collected and analysed however the values followed the concentration dependent course. Table-1 reveled that Aluminium (AlClO₃) treatment had negative effect on the Protein content.

Table-1: Estimation of the Protein content in Spirulina in different concentrations of Aluminium(mg ml⁻¹)

Parameter	Control	Concentrations of Aluminium							
		0.5 ppm	1 ppm	2 ppm	4 ppm	6 ppm	8 ppm		
Protein Content	419.09 ± 4.07	411.13 ± 1.29	401.58 ± 3.45	371.23 ± 0.52	366.01 ± 1.23	352.14 ± 18.45	301.44 ± 2.19		

During the control conditions the Algae contains highest Protein content (419.09 \pm 4.07). Where as, algae in Al stress, it is negatively affected on the protein content i.e., the content of Protein is decreased. At the 8 ppm concentration of Aluminium exerted the lowest Protein content (301.44 \pm 2.19) in the algal cells.

Al at 8 ppm concentration more negatively affected on the protein content than, the other treatments (i.e. 0.5, 1, 2, 4 and 6 ppm) and broadly the effect was concentration dependent (Table-1). These results also clearly depicted in figure-1.



Fig.-1: Effect of Aluminium on the Proteincontent inSpirulina

3.2 Estimation of the Sugars content in Spirulina

Sugars contenet between control and treatments with Aluminium has statistically significant. However the values followed the concentration dependent course. Table-2 reveled that Aluminium (AlClO₃) treatment had negative effect on the Sugars content. However, the concentration of Al increased simultaneously the content of Sugars is decreased.

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Table 2. Estimation	of the Sugar	contont in Chim	ling in different	concentrations of	(1)
Table-2: Estimation	i of the Sugars	content in Spiri	нипа на опнегени	сопсеннганонs ог	A0000000099
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Parameter	Control	Concentrations of Aluminium						
		0.5 ppm	1 ppm	2 ppm	4 ppm	6 ppm	8 ppm	
Sugars Content	$\begin{array}{c} 241.57 \ \pm \\ 1.03 \end{array}$	237.09 ± 3.59	239.22 ± 1.66	232.27 ± 2.05	226.10 ± 3.05	221.17 ± 2.05	216.47 ± 2.75	

During the control conditions in the Algae contains highest Sugars content (241.57 \pm 1.03). When, Algae treated with Al, it is negatively affected on the Sugars content i.e., the content of Sugars is decreased. At the 8 ppm concentration of Aluminium exerted the lowest Sugars content (216.47 \pm 2.75) in the algal cells.

Al at 8 ppm concentration more negatively affected on the Sugars content than, the other treatments (i.e. 0.5, 1, 2, 4 and 6 ppm) and broadly the effect was concentration dependent (Table-1). These results also clearly depicted in figure-2.



Fig.-2: Effect of Aluminium on the Sugars content inSpirulina

3.3. Estimation of the Chlorophyll content in Spirulina

Content of Chlorophyll 'a', Chlorophyll 'b' and Total chlorophylls between control and treatments with Aluminium has statistically significant. However the values followed the concentration dependent course. From table-3 it is obvious that Aluminium (AlClO₃) treatment had negative effect on the Chlorophyll 'a', Chlorophyll 'b' and Total chlorophylls content. It is clear that, if the concentration of Al increased then, simultaneously the content of Chlorophyll 'a', Chlorophyll 'b' and Total chlorophylls were decreased.

Table-3: Estimation of the Chlorophyll content in Spirulina in different concentrations of Aluminium(µg ml⁻)

Do no m o fons	Control	Concentrations of Aluminium						
rarameters		0.5 ppm	1 ppm	2 ppm	4 ppm	6 ppm	8 ppm	
Chlorophyll 'a'	31.07 ± 0.10	$\begin{array}{rrr} 30.34 & \pm \\ 0.17 & \end{array}$	28.85 ± 0.01	26.23 ± 1.43	25.27 ± 1.45	23.28 ± 2.00	16.62 ± 4.10	
Chlorophyll 'b'	55.87 ± 0.18	55.06 ± 0.21	52.27 ± 0.05	$\begin{array}{rrr} 45.38 & \pm \\ 0.49 & \end{array}$	$\begin{array}{rrr} 43.28 & \pm \\ 0.52 & \end{array}$	$\begin{array}{rrr} 47.01 \pm \\ 0.95 \end{array}$	38.79 ± 1.28	
Total Chlorophylls	86.94 ± 0.27	85.40 ± 0.29	81.12 ± 0.06	71.61 ± 1.09	68.55 ± 1.02	70.29 ± 1.05	55.41 ± 2.82	

In the control conditions Algae contains highest content of Chlorophyll 'a' (31.07 ± 0.10) , Chlorophyll 'b' (55.87 ± 0.18) and Total chlorophylls (86.94 ± 0.27) . When, Algae treated with Al, it is negatively affected on the Sugars content i.e. the content of Sugars is decreased. At 8 ppm concentration of Aluminium exerted the lowest Chlorophyll 'a' (16.62 ± 4.10) , Chlorophyll 'b' (38.79 ± 1.28) and total chlorophylls content (55.41 ± 2.82) in the algal cells.

Al at 8 ppm concentration more negatively affected on the Chlorophyll 'a', Chlorophyll 'b' and Total chlorophylls content than, the other treatments (i.e. 0.5, 1, 2 and 4 ppm) and broadly the effect was concentration dependent (Table-3). But Chlorophyll 'b' and total Chlorophylls (47.01 ± 0.95 and 70.29 ± 1.05) are slightly increased at the 6 ppm concentration. These results are clearly presented in figure-3.



Fig.-3: Effect of Aluminium on the Chlorophyll content inSpirulina

Conclusion: In this study we had successfully removed aluminium by using economical, ecofriendly cyano bacteria, spirulina. Further we can extend this technique by using spirulina to eliminate the toxic heavy other metals like uranium, chromium etc.

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