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Removal of Cr(VI) using a cyanobacterial consortium and assessment of biofuel production

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A B S T R A C T

The use of cyanobacteria for the removal of heavy metals from wastewater is gaining interest due to its lower cost of operation and being environmentally benign. As chromium (Cr(VI)) is potentially toxic and carcinogenic for humans, its removal from water and wastewater is obligatory in order to avoid water pollution. In the present study, the capacity of a living cyanobacterial consortium consisting of *Limnococcus limneticus* and *Leptolyngbya subtilis*, collected from East Kolkata Wetland, a wetland of international importance, for removal of Cr(VI) is investigated at different operating conditions. Input variables such as initial concentration of Cr(VI), pH and inoculum size are varied using one factor at a time (OFAT) analysis in the range of 5–30 mg/L, 7–11 and 2–10%, respectively. An optimum removal of 50% is achieved after 12 days of inoculation with initial concentration of 15 mg/L Cr(VI) at pH 9 and with inoculum size 10%. Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS) studies have ascertained the uptake of Cr(VI) by the living consortium. Fourier Transform Infrared Spectroscopy (FTIR) study has revealed that the amine, phosphate and carbonyl groups are involved for binding vis-à-vis biosorption of Cr(VI). The increase in inoculum size improves the percentage removal of Cr(VI). To assess the possibility of biofuel production, the cells are harvested for their dry biomass and lipid content. An increase in both dry biomass and lipid content is observed when living consortium is grown in Cr(VI) contaminated simulated wastewater instead of BG-11 medium. A regression model is developed to predict the interactive effect of four input variables namely initial concentration of Cr(VI), initial solution pH, inoculum size and time with three output variables namely dry biomass, lipid content and percentage removal of Cr(VI). Finally, Response Surface Methodology (RSM) is employed to optimize the process conditions for removal of Cr(VI). The optimum condition obtained from RSM study is initial Cr(VI) concentration:10 mg/L, pH: 9, inoculum size: 4%, time: 9 days and the predicted percentage removal (51%) matches quite well with experimental one (52.7%).

1. Introduction

Heavy metal pollution has become one of the most serious environmental problems of this decade mainly because of the non-biodegradable and recalcitrant nature of the metals. The release of heavy metals into aquatic environments has become a matter of concern over the last few decades as it causes a serious threat to the aquatic biota and the eventual transfer to humans (Dutta et al., 2016a, b). These heavy metals remain in the environment changing from one chemical state to another and ultimately can be accumulated in the food chain (Mane et al., 2011). Although a trace amount of these heavy metals is essential for cell growth, at higher concentrations they are detrimental and can cause serious health hazards including even death. Chromium (Cr), commonly found in wastewater as an effluent from tannery, textile, paint, metal

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cleaning, plating, electroplating and mining industries, is listed among five most toxic heavy metals (Han et al., 2008). Though it exists in several oxidation states due to its electronic structure, Cr(III) and Cr(VI) are the most common chromium compounds (Gupta and Rastogi, 2009). Furthermore, the latter is several times more toxic than the former because of carcinogenic, mutagenic and teratogenic effects (Qu et al., 2014). According to World Health Organization (WHO), the permissible limit for Cr(VI) and total chromium (including Cr(III) and Cr(VI)) in drinking water are 0.05 and 2 mg/L, respectively (Gupta and Rastogi, 2009). It is therefore, mandatory to remove chromium from wastewater before it is released to the environment.

In this regard, several conventional techniques such as chemical precipitation, membrane separation, ion exchange, and solvent extraction are employed for the treatment of chromium laden wastewater (Mukherjee et al., 2013). However, these techniques have the limitations of secondary pollutant generation, high chemical and/or energy requirements and high cost. Furthermore, they are unable to meet the statutory limit where the metal concentration is less than 100 mg/L (Anjana et al., 2007). Biological removal of Cr(VI) using bacteria, yeast and fungi (García-García et al., 2009; Cárdenas-González and Acosta-Rodríguez, 2011; Chatterjee et al., 2011; Qu et al., 2014) is ubiquitous. However, such processes have the problems of solid sludge disposal, susceptibility of contamination, requirement of additional nutrient, etc. As such there is a growing need to find a more sustainable process capable of removal of Cr(VI) in cost-effective and environment friendly manner.

Biosorption is a physiochemical process that occurs in both living and dead systems which allows the binding of contaminants onto its cellular wall structure. The amount of contaminants, that a sorbent can remove via biosorption, is dependent on the composition of the sorbent's cellular surface. The functional groups present on the cell wall are primarily responsible for binding of pollutants vis-à-vis biosorption (Onyancha et al., 2008). Khelaifia et al. (2016) have listed biosorption capacity of Cr(VI) in different raw biosorbent and indicated that date stones has the maximum adsorption capacity of 70 mg/g at pH 2. Cyanobacterium, often referred to as blue-green algae, is believed to have some added advantages over other microorganisms such as fungi, yeast and mosses because of higher mucilage volume with greater binding affinity, larger surface area, and simple nutrient requirements (Anjana et al., 2007). Kumar et al. (2015) has recently reviewed 250 studies on the removal of heavy metals using algal strains out of which almost 200 studies report using non-living algal biomass. It is therefore evident that most researches have been done with non-living biomass. This is because of several reasons namely, requirement of less time for removal, no nutrient requirement and easy maintenance. However, the main disadvantage using dead algal biomass is to acquire them in sufficient amount suitable for removal of pollutants by growing them in large scale a-priori. On the contrary, a living cyanobacterial strain grows in wastewater and concomitantly removes the pollutants (Rawat et al., 2011). Therefore, such a process curtails the number of steps and makes the final remediation easier. Moreover, phycoremediation using living cyanobacteria can play a dual role of treating industrial wastewater and can lead to growth of more biomass (Kushwaha et al., 2014). The enhanced biomass can efficiently be used for biofuel production such as alcohol by fermentation of biomass, producer gas by gasification of biomass, biodiesel from its lipid content (Sharif Hossain and Salleh, 2008; Demirbas, 2011). Application of cyanobacteria for remediation of contaminants and an additional production of large amount of biomass for biofuel therefore, seems a profitable option. Additionally, capability of food production by photosynthesis process and utilization of waste material as nutrient increases the net energy ratio (NER) (Sturm and Lamer, 2011). The extracellular productions of biopolymers by cyanobacterial strains are notable due to their metal binding properties and their capability to provide protection to the cyanobacteria against desiccation and stress (Kiran and Thanasekaran, 2011a). Sharma et al. (2011) have explored the utilization of waste cyanobacterial biomass of *Nostoc linckia* for the biosorption of Cr(VI). The biosorbent is able to remove 80–90% chromium from solutions with initial metal concentration of 10–55 mg/L. Focardi et al. (2012) have isolated and characterized a Cr(VI)-resistant bacterial strain from polluted marine sediments as whole cell and cell free extract to reduce Cr(VI) for bioremediation of chromium-contaminated sites. The cell free extract has reduced Cr(VI) with a maximum of activity at pH 6.5 and at temperature of 28 °C. The reduction of Cr(VI) to Cr(III) has been demonstrated by Ahmad et al. (2013) using *Acinetobacter haemolyticus* in the presence of sugarcane bagasse. The microorganism has reduced Cr(VI) contamination (92–95%) in wastewater systems using agricultural waste as nutrient. Though a number of researches are available on Cr(VI) removal using dead algal biomass (Onyancha et al., 2008; Gupta and Rastogi, 2009), the utilization of a living cyanobacterial biomass for removal of Cr(VI) and assessment of biomass for lipid production is relatively new. This constitutes the motivation of using a cyanobacterium consortium to remove Cr(VI) from wastewater in this study.

In the present study, a living cyanobacterial consortium consisting of *Limnococcus limneticus* and *Leptolyngbya subtilis* has been used to remove Cr(VI) from synthetic wastewater. Initially one factor at a time (OFAT) analysis has been done to find out optimum condition for maximum removal. Three variables namely initial concentration of Cr(VI), initial solution pH and inoculum size have been varied individually during OFAT analysis. Response Surface Methodology has also been used to optimize the removal of Cr(VI). In addition to the three variables as used in OFAT analysis, time has also been considered as input factor during RSM analysis. RSM shows almost similar trends that obtained from OFAT analysis. It is seen that though slightly more removal is achieved at optimum condition, analyzed through OFAT (58%) than through RSM (51%), the time and inoculum requirements are higher in the former case. An empirical model is proposed to see the effect of four variables on three responses namely, removal of Cr(VI), dry biomass and lipid content. Since, cyanobacterial biomass can be used for production of biofuel, the present study leads to the direction of an integrated approach of phycoremediation of Cr(VI) from wastewater and biofuel production from enhanced cyanobacterial biomass, produced during removal of Cr(VI).

2. Materials and methods

2.1. Collection, identification and culture of cyanobacterial strain

In the present study, the cyanobacterial strains were collected from East Kolkata Wetland (EKW) in Kolkata, West Bengal (20°25′ N - 22°35′ N; 88°20′ E – 88°35′ E), a Ramsar site as per 2002 convention (Kundu et al., 2008). The geographical location of EKW ensures a hot and humid climate throughout the year for which it becomes a natural incubator for a diverse group of phytoplanktons (dominated by cyanophyceae) and bacterialphyla (Raychaudhuri et al., 2008). The shallow water-basin allows full vertical circulation of water to the surface where algal blooms occur naturally. After primary treatment, the effluent from several small scale industries, namely tannery, paint and pigment, battery and electroplating containing heavy metals such as lead, chromium and copper are disposed off in a fishpond which constitutes the wet land and naturally cleaned by the rich source of bioremediants (Kundu et al., 2008; Raychaudhuri et al., 2008). Therefore, the