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ORIGINAL ARTICLE

Chromium biosorption onto a locally isolated Cr (VI) tolerant *Gliocladium viride* ZIC_{2063} and phytotoxicity studies

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Abstract This report is based on the investigations of the effect of initial concentration of Cr (VI) ions, biosorbent dosage and effect of static and agitated conditions on the biosorption of Cr (VI) in aqueous solution using *Gliocladium viride* ZIC_{2063} biomass in a batch biosorption process.The percentage removal of Cr (VI) ions from solutions was maximum at 200 mg/L initial Cr (VI) concentration after 35 min. The removal of Cr (VI) was 100% at biosorbent dose of 3.0 g, but uptake capacity "q" (mg/g) of Cr (VI) ions decreased with further increase in biosorbent dosage. The efficiency of biosorbent increases under agitated conditions. The Langmuir and Freundlich adsorption isotherms were used in equilibrium modeling. The Langmuir isotherm provided the best correlation for Cr (VI) onto the *Gliocladium viride* ZIC_{2063}. Phytotoxicity assays were carried out with treated and untreated wastewater against *Pisum sativum* to provide a preliminary assessment of treated effluent suitability for land application. Results suggested that Cr (VI) toxicity against *Pisum sativum* reduced to 75% after effluent treatment with *Gliocladium viride* ZIC_{2063}

Keywords Biosorption · *Gliocladium viride* ZIC_{2063} · Cr (VI) · Kinetic · Isotherm

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Introduction

The discharge of heavy metals from metal processing industries is known to have adverse effects on the environment. Conventional treatment technologies are not economical and generate huge quantities of toxic chemical sludge. Biosorption of heavy metals is an innovative and alternative technology for removal of these pollutants from aqueous solutions. Batch systems have been mainly used to evaluate biosorption efficiency, optimum experimental conditions, biosorption rate and possibility of biomass regeneration (Vijayaraghavan and Yun 2008). The biosorption of heavy metals is affected by cell surface properties of fungi and physicochemical properties of adsorption medium (Donmez and Aksu 2002). It has been reported that agitation, pH, temperature, initial metal concentration and biosorbent concentrations have an important effect on the efficiency of the biosorption process (Benefield et al. 1982). The increasing biosorption efficiency with increasing total surface area of the biosorbent has also been reported in the literature (Weber 1972). Batch experimental data modeling has an important role in technology transfer from laboratory-to-industrial scale. Appropriate models can help the understanding of process mechanisms, analyze experimental data, accurately predict operational efficiency and optimize processes (Volesky and Holan 1995). The disposal of untreated and treated wastewater on land may have a direct impact on

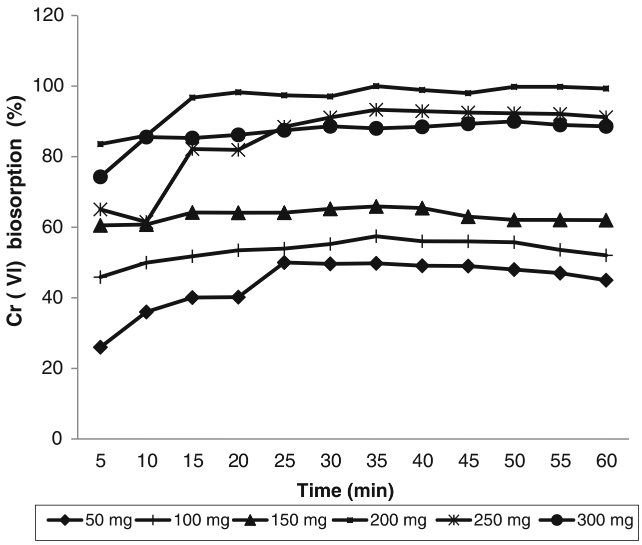

Fig. 1 Biosorption kinetics of Cr (VI) ions by *Gliocladium viride* ZIC_{2063} at various initial Cr (VI) concentrations (mg/L), biosorption conditions: temperature 30°C, pH 3.0, biosorbent 2.0 g (wet weight), agitation 122 rpm, volume of Cr (VI) solution 50 mL in a 25-mL conical flask

soil fertility and agricultural productivity. The presence of heavy metals in the wastewater restricts plants growth and hence its use for agricultural purposes. The phytotoxicity bioassay represents a valuable and necessary tool to provide a preliminary assessment of the treatment process and its suitability for land application (Fuentes et al. 2004; Deepa et al. 2006). To evaluate the phytotoxic nature of treated and untreated wastewater on vegetation, phytotoxicity bioassays were conducted on *Pisum sativum*. This paper aims to quantify the Cr (VI) biosorption ability of *Gliocladium viride* ZIC_{2063} at the best operating conditions such as initial concentration of Cr (VI) ions, biosorbent dosage and effect of static and agitated condition in a batch system. Langmuir and Freundlich adsorption isotherms were applied to analyze adsorption data.

Materials and methods

Propagation of biomass

Gliocladium viride ZIC_{2063} was cultivated at 28°C in potato dextrose liquid medium containing, per 500 mL: potatoes 300 g and dextrose 1.5 g in a 1-L fermenter (Bioengineering, Switzerland). Seven-day-old slant culture was used as inoculum. Mycelium was harvested in the stationary growth phase by filtration through a filter paper and washed three times with deionized water.

Batch studies on biosorption kinetics of chromium (VI)

A volume of 100 mL of metal ion solution $Cr_4\ (SO_4)_5(OH)\ _2$ with varying initial metal ion concentrations (50–300 mg/L) was placed in 250-mL conical flasks. Different amounts of biomass 1.0–5.0 g (wet weight) with particle size of 100 µm were then added to the solution to obtain a suspension. The suspensions were adjusted to pH 3.5. A series of such conical flasks was then shaken in an orbital shaker at a temperature of 30°C. The effect of static and agitated

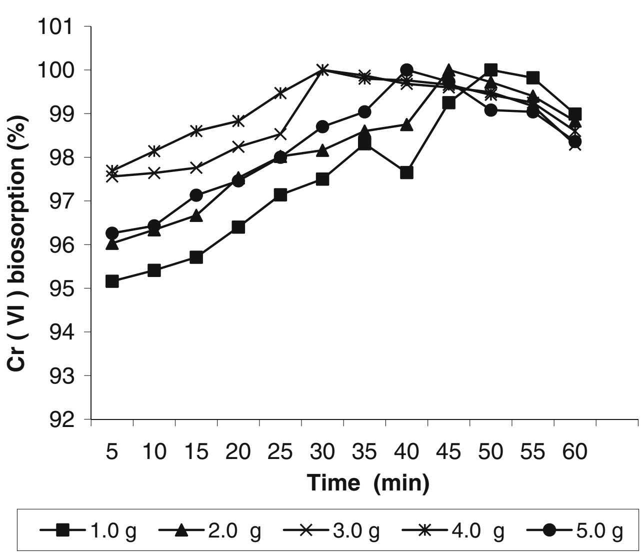

Fig. 2 Biosorption kinetics of Cr (VI) ions by *Gliocladium viride* ZIC_{2063} with various biosorbent dosages, biosorption conditions: Cr (VI) initial conc. 200 mg/L, temperature 30°C, pH 3.5, agitation 122 rpm ,volume of Cr (VI) solution 50 mL in a 250-mL conical flask

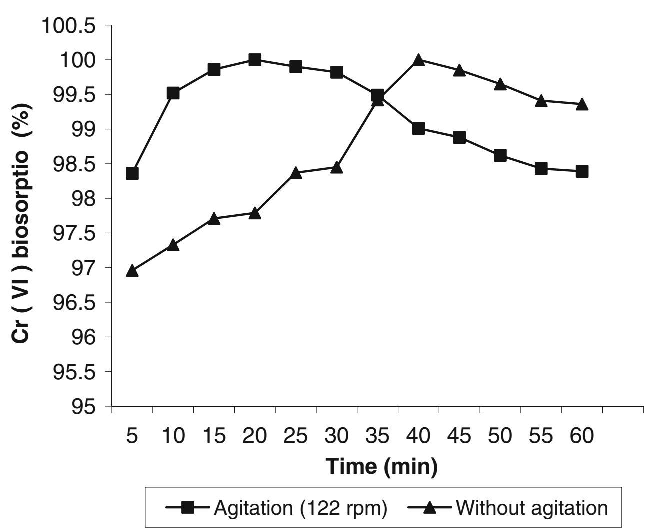

Fig. 3 Biosorption kinetics of Cr (VI) by *Gliocladium viride* ZIC_{2063} under static and agitated conditions, biosorption conditions: Cr (VI) initial conc. 200 mg/L, temperature 30°C , pH 3.5, biosorbent 3.0 g (wet weight), volume of Cr (VI) solution 50 mL in a 25-mL conical flask

conditions (122 rpm) on Cr (VI) ion removal was also assessed. All experiments were run in triplicate. The samples were withdrawn every 5 min during the first 1 h biosorption. The suspension was filtered using Whatman filter paper and then centrifuged at 2,800 *g* for 5 min. The supernatant was analyzed for Cr (VI) ions concentration using Polarized Zeeman Atomic Absorption Spectrophotometer Z-5000 (Perkin Elmer Analyst 300 Hitachi, Japan).

Application of biosorption isotherms

Langmuir and Freundlich adsorption isotherms were applied to the data to analyze its applicability for wastewater treatment bioreactors. The chromium biosorption coefficient (q) used for the construction of the sorption isotherms was calculated from the initial concentration (C_i) and the final or equilibrium concentration (C_f) in every flask, as follows:

$$q = \frac{V(C_i - C_f)}{M} \quad (1)$$

where V is the volume of the chromium solution in the flask and M is the dry mass of biosorbent (Cossich et al., 2002). The Langmuir sorption model was chosen for the estimation of maximum metal biosorption by the biosorbent. The Langmuir isotherm was expressed as:

$$q = \frac{q_{\max} b C_f}{(1 + bC_f)} \quad (2)$$

where b is a constant related to adsorption/desorption energy, and q_{max} is the maximum biosorption upon complete saturation of the surface (Cossich et al. 2002). The linearized equation for the Langmuir's isotherm is

$$1/q = 1/q_{\max} + 1/b*1/C_f \quad (3)$$

The Langmuir's constant b was calculated from the initial slope of the linear plot of $1/q$ versus $1/C_f$ (Mala et al. 2006) using SPSS v.12.0 (SPSS, Chicago, USA).

The Freundlich model was represented by the equation:

$$q = kC_f^{(1/n)} \quad (4)$$

where k and n are constants (Cossich et al. 2002). The plot of log C_f versus log q was employed to generate the intercept value of K and the slope of n (Arica and Bayramoglu 2005).

Results and discussion

Effect of initial Cr (VI) ions concentrations

The effect of initial Cr (VI) ions concentrations on biosorption kinetics was investigated in the range of 50–300 mg/L (Fig. 1). Cr (VI) removal rates of 49.78, 57.46, 65.91, 100, 93.3, and 88.03% were obtained for 50, 100, 150, 200 and 300 mg/L, respectively. The *Gliocladium viride* ZIC_{2063} was also found to remove 178.87 mg of Cr (VI) per gram of adsorbent from an aqueous solution (200 mg/L).

The Cr (VI) adsorption capacity increased with time and then leveled off toward the equilibrium adsorption capacity. At all the concentrations, the biosorption equilibrium time was obtained after 35 min. The maximum value of biosorption coefficient (3,450 mg/g) was calculated at 200 mg/L Cr (VI) ions concentration. The metal uptake is greater at high metal ions concentration due to a concentration gradient between binding sites and metal ions (Vijayaraghavan and Yun 2008). Fungi can tolerate high metal ions concentration and its biosorption capacity increases with increasing concentration (Lee et al. 2000; Gupta et al. 2001; Bennet et al. 2002).

Effect of biosorbent doses

The percentage removal of Cr (VI) ions was studied by varying the adsorbent dose (1.0–5.0 g wet weight) at initial Cr (VI) ions concentration of 200 mg/L. A gradual increase in

Table 1 Comparison of Langmuir and Freundlich isotherms constants along with correlation co-efficient (r) at different initial concentrations of Cr (VI) ions

Initial Cr (VI) ions concentration (mg/L)	Langmuir isotherm constants q_{max}	b	Correlation coefficient (r)	Freundlich isotherm constants k	n	Correlation coefficient (r)
50	623	−0.063	0.985	4.554	−1.256	0.997
100	1,436.5	−0.284	0.991	4.536	−0.846	0.998
150	2,454	−0.014	0.908	4.445	−0.614	0.914
200	3,450	−0.088	0.949	4.375	−0.467	0.974
250	3,272.25	−0.024	0.904	5.468	−0.941	1.0
300	3,502	−0.055	0.996	6.197	−1.203	1.0

Table 2 Comparison of Langmuir and Freundlich isotherms constants along with correlation co-efficient (*r*) at different biosorbent dose (g)

Biosorbent dose (g)	Langmuir isotherm constants		Correlation coefficient (*r*)	Freundlich isotherm constants		Correlation coefficient (*r*)
	q_{max}	b		k	n	
1.0	7,822.5	−0.007	0.998	3.972	−0.338	1.0
2.0	4,222.25	−0.003	0.989	3.801	−0.234	0.997
3.0	3,057.83	−0.00	0.981	3.592	−0.087	0.999
4.0	2,261.12	−0.001	0.915	3.611	−0.103	0.999
5.0	1,784.5	−0.002	0.979	3.685	−0.157	0.999

Cr (VI) removal was observed with increase in biosorbent dose from 1.0 to 3.0 g biosorbent. Maximum Cr (VI) removal rate (91.73%) was obtained with 3.0 g biosorbent dosage and afterward no significant change was recorded (Fig. 2).The equilibrium time was found to be 35 min. Several researchers have reported that increasing biosorbent dosage increases the metal removal efficiency due to easy access to adsorption sites (Ahalya et al. 2005; Arica and Bayramoglu 2005). The sorption capacity was steady above 3.0 g *Gliocladium viride* ZIC_{2063} dosage due to the screening effect. At high sorbent dosage, interference started between available active sites and metal ions resulting in low metal ion uptake (Hammaini et al. 2006; Tangaromsuk et al. 2002). Biosorption coefficient values for different adsorbent dosages (1.0–5.0 g wet weight) were also evaluated (data not shown).

Effect static and agitated conditions

Agitation (150 rpm) of Cr (VI) solution gave a high removal rate (93.14 within 20 min, while the reaction mixture under static conditions resulted in lower adsorption of Cr (VI) ions (Fig. 3). Agitation of the reaction mixture is essential to produce effective results of biosorption (Ahalya et al. 2005; Lua et al. 2007). This might be due to clump formation of biosorbent under static conditions that reduced the surface area for binding. At equilibrium, biosorption coefficients for agitated mixture were larger (3,104.66 mg/g) than static ones (2,579.66 mg/g). It was also interesting to observe that biosorption efficiency of *Gliocladium viride* ZIC_{2063} under static conditions was larger than with agitation after 35 min contact period.

Application of biosorption isotherms

The equilibrium data for Cr (VI) adsorption by *Gliocladium viride* ZIC_{2063} was applied to Langmuir and Freundlich isotherms at various Cr (lV) concentrations, biosorbent dosages and agitation and static conditions. These empirical models are simple mathematical relationships and describe the experimental behavior of different operating conditions (Esposito et al. 2002). Biosorption was evaluated for different initial Cr (VI) ions concentrations (50–300 mg/L). The Langmuir isotherm was best fitted with linear trend. The data of Table 1 show the value of q_{max} and b with correlation coefficients (*r*) which were in the range of 0.904 to 0.996. The Freundlich isotherm showed a non-linear pattern with k equal to 5.468 and n equal to −0.941 above 250 mg/L. The correlation coefficient (*r*) value was nearly 1.0. Lower values of constant 'b' indicated the high affinity of the biosorption of Cr (VI) with *Gliocladium viride* ZIC_{2063}. For different biosorbent dosages (wet weight), the Langmuir model performed better than the Freundlich isotherm. The Langmuir model described the surface phenomenon of binding sites and Cr (VI) metal ions concentration in biosorption medium. The Freundlich isotherm showed that the metal binding energy to any adsorption site was independent of its availability.

Biosorbent dosages (1.0–5.0 g) showed a linear trend for the Langmuir adsorption isotherm with q_{max} ranging from 1,784 to 7,822.5 mg/g and 'b' equals to −0.007 (Table 2). Non-linearity of the Freundlich isotherm showed that it is worst fitted to the data. Langmuir and Freundlich isotherm constants were also compared for

Table 3 Comparison of Langmuir and Freundlich isotherms constants along with correlation co-efficient (*r*) with agitation and without agitation

Parameter of biosorption media	Langmuir isotherm constants		Correlation coefficient (*r*)	Freundlich isotherm constants		Correlation coefficient (*r*)
	q_{max}	b		k	n	
Agitation (122 rpm)	3,104.66	−0.000	0.982	3.573	−0.072	0.941
Without agitation	2,579.66	−0.006	0.991	3.926	−0.311	1.0

Table 4 Comparative analysis of the Cr(VI) biosorption by different biosorbents reported in literature

Biosorbent	Biosorption capacity	Initial Cr (VI) concentration	References
Rhizopus nigricans (polyacrylamid)	21.22 mg/g	100 mg/L	Bai and Abraham 2003
Phanerochaete chrysosporium	15.85±0.95 mg/g	100 ppm	Marandi 2011
Rhizopus arrhizus	8.40 (mg/g)	125 mg/L	Nourbakhsh et al. 1994
Trichoderma viride	4.66 mg/g	175 mg/L	Hala et al. 2009
Gliocladium viride ZIC_{2063}	178.87 mg/g	200 mg/L	Present study

agitated and static conditions (Table 3). The correlation coefficient (*r*) of the Langmuir isotherm obtained from 1/(q) versus 1/C plot was 0.98. The Langmuir isotherm gave the best fit to the biosorption data of the Cr (VI) ion to the *Gliocladium viride* ZIC_{2063}. The Langmuir sorption model was chosen for the estimation of the maximum metal biosorption by the biosorbent. The higher 'b' value of Langmuir model showed a higher affinity of biomass for metal ions (Langmuir 1918). The value of 'b' decreased in the following order for initial Cr (VI) concentrations: 150 mg/L>250 mg/L>300 mg/L>50 mg/L> 200 mg/L>100 mg/L, for biosorbent dosages: 3.0 g> 4.0 g>5.0 g>2.0 g>1.0 g and for agitated conditions: under agitation > under static condition. The value of q_{max} shows the number of active sites on the biomass surface. The qmax value of 3,502 mg/g for *Gliocladium viride* ZIC_{2063} indicated a greater number of active sites for Cr (VI) uptake.

Cr (VI) biosorption is well studied. A comparative analysis of the present study with previous works reported by other researchers by various biosorbents is summarised in Table 4. The uptake values obtained for Cr (VI) biosorption in this study are highly encouraging and were found to be higher than that of many corresponding biosorbents.

Phytotoxicity assay

A germination and growth bioassay was carried out to determine the phytotoxicity of untreated and treated effluent against *Pisum sativum* (Table 5). Untreated effluent was found highly toxic to seed germination. Relative seed germination of *Pisum sativum* was found to be 50% for untreated effluent and 75% for treated effluent. The Germination Index (GI) was 23.0 and 64.5% for untreated and treated effluents, respectively. In the case of the Effluent Tolerance Index (ETI), seeds grown in treated effluent were more tolerant (ETI=0.86) than untreated effluent (ETI=0.46). It was observed that Cr (VI) ions toxicity was less for growth parameters of *Pisum sativum* seedling than seed germination. The root length of *Pisum sativum* seedling was 10.45 mm for untreated effluent and 19.53 mm for treated effluent. Similarly, the shoot lengths for untreated and treated effluents were 3.08 and 6.35 mm, respectively. Total biomass production of *Pisum sativum* seedling was also affected. There was a very strong positive correlation between the control, treated and untreated parameters as shown by the correlation coefficient values of nearly 1.0. The reduction of Cr (VI) ions toxicity was highly significant ($p<0.001$).

Conclusions

Our results indicated that *Gliocladium viride* ZIC_{2063} can be potentially used as an efficient biosorbent because of its rapid kinetics and remarkable biosorption capacity observed during this study. The biosorption performance of *Gliocladium viride* ZIC_{2063} was found to be affected by initial metal concentration and agitation of the process. The phytotoxicity assays of Cr (VI) against *Pisum sativum* verify its hazardous nature and reduction of toxicity in treated effluent.

Table 5 Phytotoxicity bioassay of treated and untreated waste water against *Pisum sativum*

Treatments	No. of seeds germinated	Shoot length (mm)	Root length (mm)	Shoot biomass (mg)	Root biomass (mg)	Total biomass (mg)	ETI	RSG (%)	RRG (%)	GI (%)	*p* value	Correlation coefficient (*r*)
Control	8/8	8.74	22.69	7.09	5.62	12.71	1.00	100	100	100	<0.001	0.923
Untreated effluent	4/8	3.08	10.45	4.34	3.28	7.62	0.46	50	46	23	<0.001	0.986
Treated effluent	6/8	6.35	19.53	6.35	7.72	14.07	0.86	75	86	64.5	<0.001	0.961

Growth conditions: pH 4.0, temperature 30°C, light, 10 days germination, 10 days growth period

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