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Michaelis–Menten kinetics for the simultaneous phytoremediation of Cr(VI) and phenol by determining the chlorophyll content using water hyacinth

Ankur Gupta*, Chandrajit Balomajumder

Department of Chemical Engineering, Indian Institute of Technology Roorkee, India, Tel. +91-8057410290; emails: guptaankur599@gmail.com (A. Gupta), chandfch@iitr.ernet.in (C. Balomajumder)

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ABSTRACT

In this study, the aquatic macrophyte water hyacinth was grown in single and binary solution of Cr(VI) and phenol in an artificial photosynthesis chamber. The growth of the plant was determined by analyzing the chlorophyll content of the water hyacinth leaves using UV spectrophotometer. The chlorophyll content of leaves and root of the water hyacinth is found to be reduced due to the uptake of Cr(VI) and phenol. The specific growth rate of plant was calculated at various concentrations of Cr(VI) and phenol, respectively. A change in the surface morphology of the water hyacinth plant leaves has been observed after the uptake of toxic pollutant Cr(VI) and phenol. Various kinetic models such as Monod, Haldane and sum of kinetic model were applied to the experimental data for the estimation of kinetic model parameters.

Keywords: Chlorophyll; Monod; Phytoremediation; Water hyacinth

1. Introduction

Various technologies are used for the removal of heavy metals and organic compounds such as electrolysis [1], reverse osmosis [2], ion exchange [3], adsorption [4,5], and oxidation-reduction [6] but all these methods are associated with secondary waste generation. The disposal of secondary waste to a suitable place is a major challenge for the industries. Phytoremediation is an eco-friendly, cost-effective method for the uptake of pollutant at the waste effluent disposal site using aquatic macrophytes, thus overcoming generation of large amount of secondary waste [7]. Heavy metals and organic compounds such as Cr(VI) and phenol are discharged simultaneously from process industries such as tanneries, electroplating, steel, paper, textile, paints etc. [8]. According to the guidelines of the World Health Organization (WHO), the maximum permissible limit of discharge of Cr(VI) is 0.05 mg/L [9]. The guidelines set by the US Environmental Protection Agency for the discharge of phenol by process industries is 0.005 mg/L [10]. Several species of aquatic macrophytes have been used for the removal of heavy metals such as water hyacinth (*Eichhornia crassipes*), duckweeds (*Lemna sp.*), water lettuce (*Pistia sp.*), water starwort (*Callitrichecophocarpa Sendtn*), aquatic weed (*Salvinia natans*), and cordgrass (*Spartina argentinensis*) [11–14]. Water hyacinth is an aquatic macrophyte having rapid growth rate resulting in almost double the size within 2 weeks. It can grow in polluted wastewater at a temperature range from 10°C to 40°C [15]. Hairy roots of water hyacinth provide large surface area for uptake of pollutants [16]. Water hyacinth can grow under high heavy metals concentration, at varying nutrient conditions and pH 6–8 [17]. In the process of phytoremediation, plants are utilized to treat the toxic pollutant in aqueous wastewater stream. Several kinetic models are reported in literature to study inhibitory effects of toxic metals and organic pollutants on the growth of aquatic macrophytes. The objectives of the present study are: (a) to study the growth of water hyacinth plant in artificial photosynthesis chamber in the presence of Cr(VI) and phenol for 24 h light and 24 h dark period; (b) to observe the changes in surface morphology of water hyacinth plant leaves due to uptake of Cr(VI) and phenol; (c) to study the effect of initial concentrations of Cr(VI) and phenol on specific growth rate of water hyacinth plant;

* Corresponding author.

and (d) to study the growth kinetics of water hyacinth plant in the presence of Cr(VI) and phenol using Monod, Haldane, and sum of kinetic model.

2. Materials and method

Water hyacinth plant used for the remediation of Cr(VI) and phenol were collected from a stagnant area of the river Solani at Roorkee, India. All the plants used for the experiments were 4–5 weeks old and having approximately same biomass. All the plants were washed several times, dried, and weighed. The Hoagland nutrient solution (3% by volume) was used for the growth of water hyacinth plant [18] in the photosynthesis chamber under controlled conditions (30°C ± 1°C, 45 μmol m^{-2} s^{-1} photon flux intensity, 60% relative humidity). Water hyacinth plants were grown in 2 L pots containing 1 L Hoagland nutrient solution and kept in photosynthesis chamber. Single and binary synthetic solution of Cr(VI) and phenol were prepared by adding pre-calculated amounts of Cr(VI) and phenol in Hoagland solution [19]. The plants were grown in a photosynthesis chamber with a photoperiod of 24 h of light and 24 h of dark period. A fixed volume (1 L) of solution inside the pot was maintained by adding the solution of corresponding concentration in the required volumes.

The growth of water hyacinth plant was calculated by measuring the chlorophyll content in the leaves. The water hyacinth was exposed to the various concentrations of Cr(VI) and phenol both in single and binary solution ranging from lowest to highest.

2.1. Determination of chlorophyll

The chlorophyll content of the water hyacinth plant before and after uptake of Cr(VI) and phenol was calculated after 24 h of experiment. The leaves of water hyacinth were cut into small pieces and then precisely weighed. 0.5 g fresh water hyacinth leaves were taken in 25 mL volumetric flasks and filled with 80% acetone. Separate flasks were used for the various concentrations of Cr(VI) and phenol. After preparing this solution, all the experimental proceedings were done in dark. After shaking the flasks for 24 h in incubator shaker, the absorbance of solution was measured in a cuvette with an optical path of 10 mm against 80% acetone as a blank in UV spectrophotometer at 645 and 663 nm. The amount of chlorophyll in plant leaves was calculated by [20]:

$$C_a = \frac{(12.3D_{663} - 0.86D_{645}) \times V}{d \times 1000 \times \mathrm{W}} \quad (1)$$

where C_a is the concentration of chlorophyll *a* (mg g^{-1} FW); *D* is the optical density (OD) at the specific wave length; *V* is the final volume (mL); *W* is the fresh weight of leaf material (g); and *d* is the length of light path (cm).

For measuring the chlorophyll content in the roots of the water hyacinth plant, same procedure was adopted.

2.2. Kinetic models used for the estimation of specific growth rate

The study of kinetics of growth of the plant in the presence of toxic metal and organic pollutant is quiet important. The specific growth rate (h^{-1}) for first-order kinetics and exponential growth phase of water hyacinth plant was calculated by measuring the chlorophyll contents using spectrophotometric method.

The water hyacinth plant was grown at various toxic concentrations of Cr(VI) and phenol both in single and binary solution, and the specific growth rate of the plant is calculated using Monod kinetic model. The specific growth rate (h^{-1}) of the water hyacinth plant was calculated by measuring the chlorophyll content of the plant at various concentrations of Cr(VI) and phenol for both single and binary solution as per the following equation [21–25]:

$$\mu = \frac{In(\mathrm{OD}_2 / \mathrm{OD}_1)}{(t_2 - t_1)} \quad (2)$$

where OD_2 is the OD at time t_2; OD_1 is the OD at time t_1; t_2 is the exponential phase time; and t_1 is the lag phase time.

2.2.1. Monod kinetic model

If Cr(VI) and phenol are non-inhibitory compounds, then Monod non-inhibitory kinetic model is used for the estimation of model parameters μ_m and K_s [26]. The linearized form of the Monod kinetic model is given below, which is also known as line weaver Burk plot:

$$\frac{1}{\mu} = \frac{1}{\mu_m} + \frac{K_S}{\mu_m}\frac{1}{S} \quad (3)$$

When $S >> K_S$, K_S is the half velocity constant, or saturation constant, or Monod constant (g/L).

2.2.2. Haldane kinetic model

If the Cr(VI) and phenol are inhibitory compounds for a single substrate solution, then the Haldane inhibitory kinetic model is applied to the experimental data for the estimation of Haldane inhibitory constant K_i. The parameters obtained from the Monod kinetic model are used to calculate the Haldane kinetic model parameter K_i (Eq. (4)) by minimizing the ARE (average relative error) (Eq. (5)) between experimental and calculated values of specific growth rate (h^{-1}) using Solver function in MS Excel 2010 [27]:

$$\mu = \frac{\mu_{\max} S_L}{K_S + S_L + \frac{S_L^2}{K_i}} \quad (4)$$

$$\mathrm{ARE}(\%) = \sqrt{\sum_{i=1}^{N}\left(\frac{q_{e,i}^{\exp} - q_{e,i}^{\mathrm{cal}}}{q_{e,i}^{\exp}}\right)^2} \times \frac{100}{N} \quad (5)$$

2.2.3. Sum kinetic model

For the binary solution of Cr(VI) and phenol, the interaction parameters were determined according to the sum kinetic model for binary inhibitory substrates [28,29].

$$\mu = \frac{\mu_{max,1} S_{1L}}{K_{S,1} + S_{1L} + I_{2,1} S_{2L} + \frac{S_{1L}^2}{K_{1i}}} + \frac{\mu_{max,2} S_{2L}}{K_{S,2} + S_{2L} + I_{1,2} S_{1L} + \frac{S_{2L}^2}{K_{2i}}} \quad (6)$$

3. Results and discussion

3.1. Fe SEM of leaves of water hyacinth before and after uptake of Cr(VI) and phenol

The Field Emission Scanning Electron Microscope (Fe SEM) analysis of water hyacinth leaves was carried out before and after uptake of Cr(VI) and phenol in binary solution. The images of Fe SEM of fresh water hyacinth plant, after uptake of Cr(VI) and after uptake of phenol, are shown in Figs. 1(a)–(c), respectively. A change in the surface morphology of water hyacinth plant leaf before and after uptake of Cr(VI) and phenol was seen. The surface of the leaf became non-homogeneous and irregular after the uptake of Cr(VI) and phenol in contrast to the fresh homogenous leaves [13]. The possible reason could be the accumulation of Cr(VI) and phenol in the plant.

3.2. Growth curve of water hyacinth plant

The growth of the water hyacinth plant was observed by analyzing the chlorophyll content in the leaves of the plant by the method given in section 2.1. The water hyacinth plant was grown in the binary solution of toxic concentration of 5 mg/L of Cr(VI) and phenol. The chlorophyll content of the plant was determined at an equal interval of time of 3 h. The growth curve of water hyacinth plant is given in Fig. 2. It was observed that initially as the plant adopts the environment, the chlorophyll content of the plant is less, which increases exponentially after 15 h of operation up to 42 h as the plant grows using Cr(VI) as nutrient and phenol as

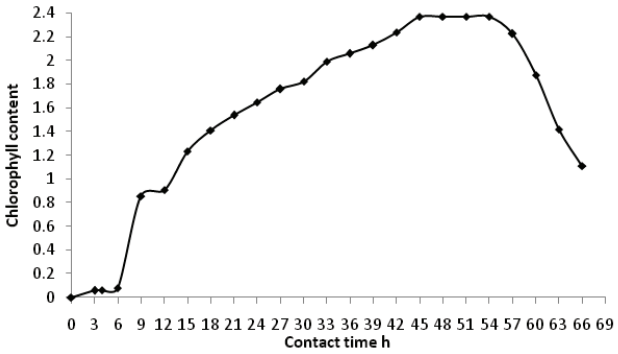

Fig. 2. Growth curve of water hyacinth.

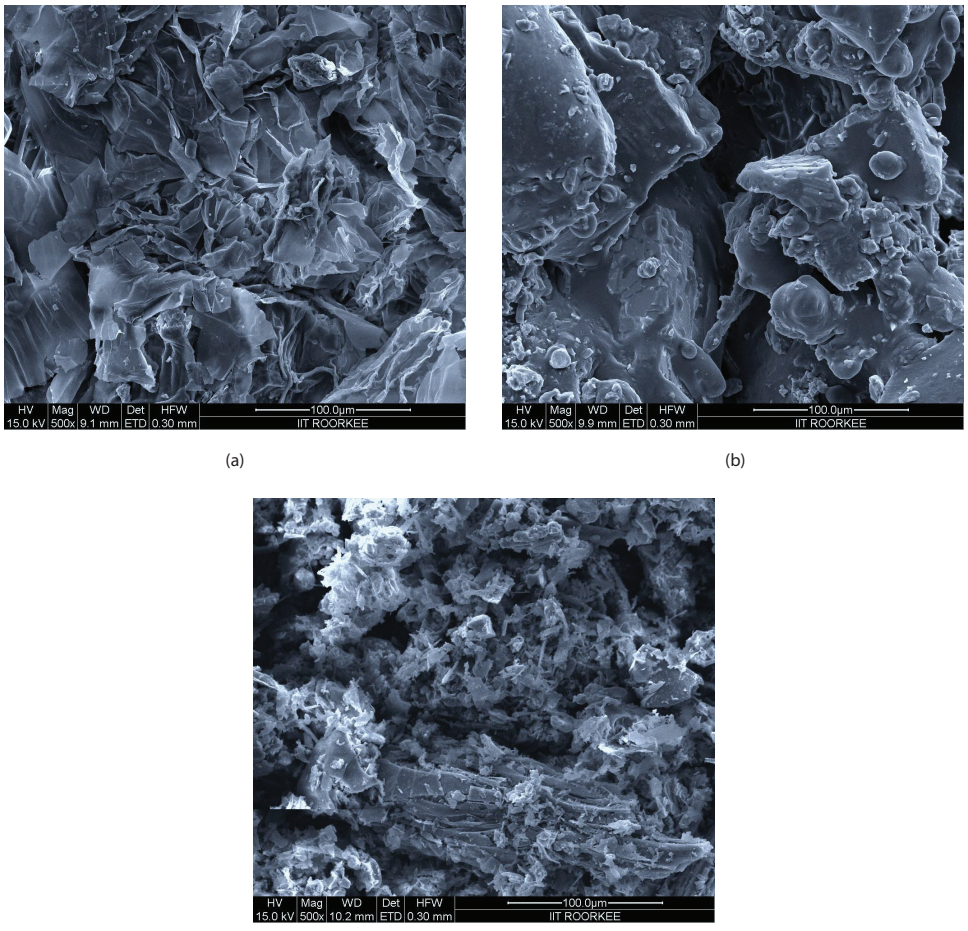

Fig. 1. Fe SEM image of leaf of water hyacinth: (a) before uptake (b) after uptake of Cr(VI), and (c) after uptake of phenol.

carbon source [6,7,13,15]. After 42 h of operation, the chlorophyll contents of the plant become constant up to 54 h and then start to decrease thereafter. The possible reason behind this fact may be due to the decrease in the nutrient or ageing of the plant.

3.3. Effect of initial concentrations onto the specific growth rate of water hyacinth

Graphs are plotted between specific growth rate (h^{-1}) vs. initial concentration of Cr(VI) solution (mg/L), specific growth rate vs. initial concentration of phenol, and specific growth rate vs. binary solution of Cr(VI) and phenol as shown in Figs. 3(a)–(c), respectively. From figures, it is observed that the specific growth rate (h^{-1}) decreases with the increase in initials concentrations for both Cr(VI) and phenol. This shows that both Cr(VI) and phenol are the inhibitory type of substrates. The concentration of both Cr(VI) and phenol was varied from 5 to 50 mg/L. The specific growth rate of water hyacinth plant decreases very rapidly after 20 mg/L of Cr(VI) concentration, and no growth of water hyacinth was observed at 25 mg/L of Cr(VI). In case of phenol, the specific growth rate decreases very slowly up to 20 mg/L of phenol. After 20 mg/L of phenol, the growth rate decreases rapidly, and at 40 mg/L phenol concentration, the plant growth inhibits. For binary solution of Cr(VI) and phenol, the specific growth rate obtained at all concentrations was more than that of single component solution of Cr(VI) and single component solution of phenol. It could be due to the fact that phenol is used as energy source by the plant, which overcomes the toxic effect of Cr(VI). Therefore, it is concluded that phenol is less toxic than Cr(VI).

3.4. Explanation of the results of kinetic model

3.4.1. Monod kinetic model

Values of μ_m and K_s can be evaluated from $1/\mu$ vs. $1/S$ plot (where $S = S_0$ at the start of exponential growth). This plot gives a straight line with the intercept $1/\mu_m$ and slope K_s/μ_m. The line weaver plot for the uptake of Cr(VI) and phenol is shown in Figs. 4(a) and (b), respectively. The parameters of Monod kinetic model obtained are shown in Table 1. The value of specific growth rate as given in Table 1 was found to be more for phenol in comparison with Cr(VI), which shows that Cr(VI) is more toxic than phenol. The value of Monod constant K_S is more for Cr(VI) than phenol, which depicts that Cr(VI) more inhibits the growth of water hyacinth plant. Therefore, both Cr(VI) and phenol were found to be inhibitory type of substrate for the growth of water hyacinth. As the specific growth rate of plant was more in the presence of phenol than Cr(VI), therefore, it can be concluded that phenol can be used as carbon or energy source by the plant.

3.4.2. Haldane kinetic model

The curve of specific growth (h^{-1}) rate calculated according to Haldane kinetic model vs. initial concentration for Cr(VI) and phenol is given in Figs. 5(a) and (b), respectively. The specific growth rate (h^{-1}) for both Cr(VI) and phenol

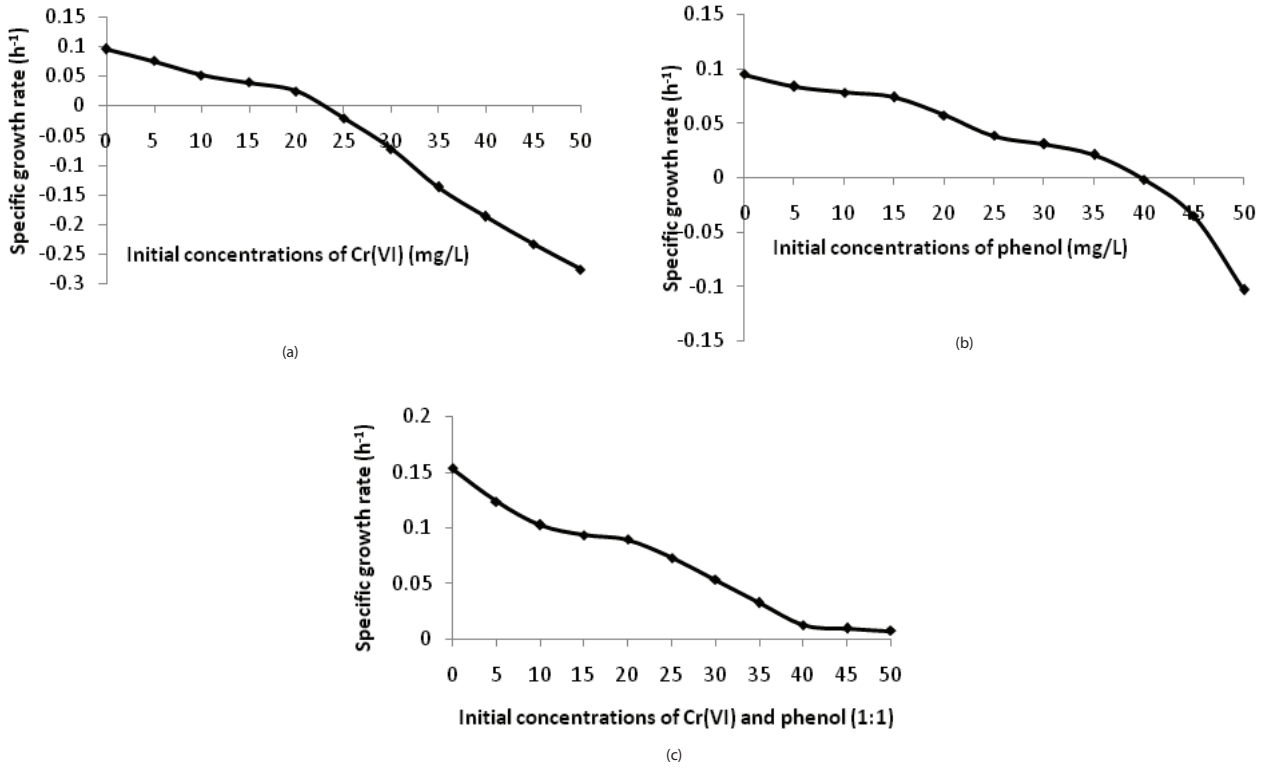

Fig. 3. Effect of initial concentrations of: (a) single component solution of Cr(VI), (b) single component solution of phenol, and (c) binary solution of Cr(VI) and phenol onto the specific growth rate of plant.

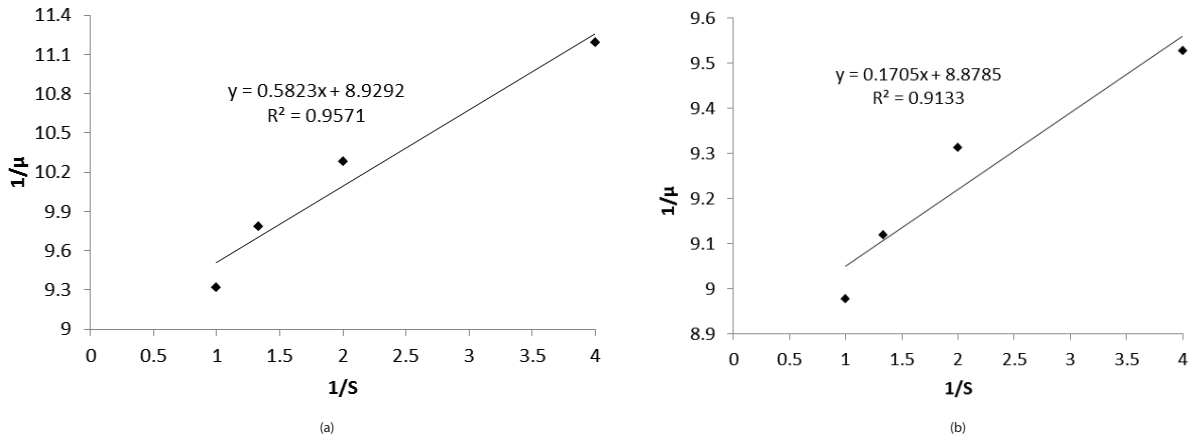

Fig. 4. Line weaver Burk plot: (a) Cr(VI) and (b) phenol.

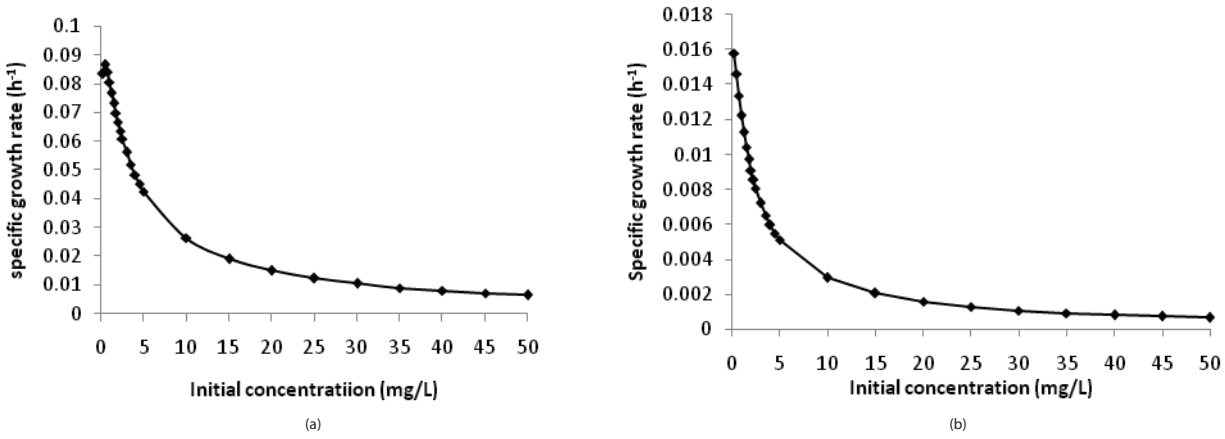

Fig. 5. Haldane kinetic model: (a) Cr(VI) and (b) phenol.

Table 1
Kinetic model parameters for Cr(VI) and phenol

Compound	Kinetic model parameters			
	μ_{max} (h^{-1})	K_s (mg/L)	K_i (mg/L)	I_{12} and I_{21}
Cr(VI)	0.1119212	0.065213	3.08085	1.07×10^9
Phenol	0.11263164	0.0192037	1.815666	1.99×10^8

was decreased with the increase in initial concentration for both Cr(VI) and phenol. The decrease in specific growth rate (h^{-1}) was very less up to the concentration of 5 mg/L, then rapid with increasing Cr(VI) and phenol concentration. The parameters obtained by Haldane kinetic model are given in Table 1. The value of Haldane constant K_i is more for Cr(VI) than phenol, which shows that Cr(VI) is a stronger inhibitor as compared with phenol.

3.4.3. Sum kinetic model

The parameters of single substrate Monod and Haldane kinetic model were used to evaluate the interaction parameters I_{12} (inhibitory effect of Cr(VI)) and I_{21} (inhibitory effect of phenol) for binary solution of Cr(VI) and phenol by using Eq. (6). Interaction parameters $I_{i,j}$ determine the degree to which substrate i affects the uptake of j. Here, the interaction parameter value I_{12} for Cr(VI) (1.07×10^9) was more than that of I_{21} for phenol (1.99×10^8); therefore, Cr(VI) acts as a inhibitory substrate for the growth of plant while phenol helps in the bioaccumulation of Cr(VI) as phenol was used as a carbon source or energy source when the plant was at more stressed condition [4,5,25,30]. The value of kinetic model parameters is given in Table 1.

4. Conclusions

In this study, water hyacinth is proved to be a potential aquatic macrophyte for the simultaneous uptake of Cr(VI) and phenol up to 20 and 40 mg/L, respectively.

The growth of the water hyacinth was more in the presence of phenol because phenol was utilized as carbon source or energy source by the water hyacinth as the specific growth rate obtained at all concentrations was more for phenol as compared with Cr(VI).

The specific growth rate of the plant was decreased continuously with the increase in the concentrations of both Cr(VI) and phenol.

Various kinetics models were applied to the experimental data, which was in good agreement. The value of Monod constant K_s, Haldane constant K_i and interaction parameters obtained from sum kinetic model was more for Cr(VI) than phenol, which indicates that Cr(VI) is more inhibitory type of substrate than phenol for water hyacinth.

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