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Microcalorimetric study the toxic effect of hexavalent chromium on microbial activity of Wuhan brown sandy soil: An in vitro approach ☆

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Received 28 February 2006; received in revised form 2 February 2007; accepted 7 February 2007

Abstract

A multi-channel thermal activity monitor was applied to study soil microbial activity in Wuhan brown sandy soil in the presence of different concentrations of hexavalent chromium ($K_2Cr_2O_7$). In order to stimulate the soil microbial activity, 5.0 mg of glucose and 5.0 mg of ammonium sulfate were added to a 1.20-g soil sample under a controlled humidity of 35%. The results show that the poisonous species of $K_2Cr_2O_7$ at an half inhibitory concentration (IC_{50}) value of 4.27 $\mu g\,mL^{-1}$ against soil microbe, and an increase of the amount of hexavalent chromium is associated to a decrease in the microbial activity of the soil, probably due to an increase in the toxicity of hexavalent chromium, affecting strongly the life in this soil microbial environment. Our work also suggests that microcalorimetry is a fast, simple and more sensitive method that can be easily performed to study the toxicity of different species of heavy metals on microorganism compared to other biological methods.

Keywords: Microcalorimetry; Soil microbial activity; Hexavalent chromium

1. Introduction

Soil can be considered as a multicomponent, forming an open biochemical system where several physical, chemical and biological reactions may occur. In this system, both matter and energy can also be exchanged with the surroundings, which are composed of a conglomerate of solids, liquids and gases (Sposito, 1989; Barros et al., 1997). Soil microorganisms play an essential role in the environment due to their role in cycling nutrients and in the decomposition of organic material (Wardle and Ghani, 1995). Nutrient cycling occurs as a consequence of microbial activity and is especially important in the ecosystems where the input of nutrients is low (Degens et al., 2000). Addition of inorganic and organic matter promotes changes in chemical and physical properties of the soil and the biodiversity of the soil microbial community can be influenced by the added chemicals. Soil microorganisms and their controlled processes are essential for the long-term sustainability of ecological and agricultural systems (Aikio et al., 2000). However, many studies of ecological effects on microbes in the soil are short-term. On the other hand, microorganisms also play an important role in degrading many agrochemicals and accumulating heavy metals. This action in the soil can promote a decrease in the toxicity of many inorganic and organic compounds and influence the health of the soil.

☆This work described in this paper was supported in part by grants from the Sino–Italian Governmental International Science and Technology Cooperation Project (BI-CN/06-07/04 and No. Annex 2-3/2006), National Natural Science Foundation of China (No. 40673065 and No. 40425001), the Specialized Research Fund for the Doctoral Program of Higher Education (20060491508), the Hubei Key International Cooperation Project (2006CA007), the Key Project of Chinese Ministry of Education (107077).

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doi:10.1016/j.ecoenv.2007.02.005

Chromium pollution in some places of Wuhan city has been a serious problem due to the natural geological reasons together with the industrial pollution. Hexavalent chromium is directly correlated to carcinogenicity in human and to acute toxicity of aquatic organisms, while its reduced form, Cr^{3+}, is an essential element for animals (Cary, 1982; Coleman, 1988; Arillo and Melodia, 1991; Richard and Bourg, 1991; Kim et al., 2002). Cr(VI) exists in soils and natural waters predominantly as a soluble anion that may be formed via oxidation of soluble and insoluble forms of less-toxic Cr(III). Chromate is 100 times more mobile and more toxic than Cr^{3+} (Richard and Bourg, 1991). In the body, Cr(V), one of the reduction products of Cr(VI), is a known carcinogen and will lodge in any tissue to form cancerous growth. The annual chromate discharge worldwide is 239,000 tons (Cary, 1982) as a result of numerous industrial activities such as the preservation of wood, leather tanning and metal finishing. Cr(VI) emitted from these industries is transported to the environment, becoming a source of toxicity in the ecosystem. Many studies have shown that hexavalent chromium can be reduced to the trivalent form by reduction reaction with organic and inorganic ions (e.g. elemental iron, divalent iron, sodium bisulfite) and humic substances and thereby the toxicity of chromium is reduced due to the decrease in the concentration of the toxic Cr(VI).

Toxic chemicals have caused great attention due to their effects on the environment and threats to human health. However, agriculture, industry and many other fields need chemicals in various production processes. A practical resolution to these conflicting interests requires accurate toxicological information. The acute toxicity test is very important, because an acute toxicity study can establish relationship between the dose of toxicant and its effect on the tested organism. The toxicity of substances can be expressed as LC_{50}, IC_{50}, or EC_{50} values. Accurate measurement of effects of potential toxic materials depends on reproducibility of acute toxicity tests. Using growth metabolism of microbe as the environmental risk assessment process is attracting more interest.

Bioenergetic investigations should be the most important in ecotoxicology for assessment of harmful properties of substances. These are closely related to the applicability of calorimetry in biology and environmental science because there is scarcely another more valid method to analyze metabolic activities than calorimetry. In a living system, all the metabolic processes occurring within the cells produce heat, thus, metabolic processes of living cells can be studied by monitoring the heat change with a sufficiently sensitive microcalorimeter. The microcalorimetry can directly determine the biological activity of a living system and provide a continuous measurement of heat production, thereby giving much both qualitative and quantitative information (Li et al., 2000; Prado and Airoldi, 2001; Critter et al., 2002; Liu et al., 2003; Critter et al., 2004; Yao et al., 2005). In recent years, the application of calorimetry in biochemistry, biophysics and environmental science has drawn increasing attention. It allows the study of biology at the molecular and cellular levels with the power–time curves generating a lot of kinetic information. By analysis of the power–time curves, kinetic parameters, such as rate constant for bacterial growth, peak power for microbial activity can be evaluated. We have been studying microbial metabolism and the effect of toxic agents on microbes in our laboratory, so as to understand the influence of toxic agents on the environment and human health.

In the present study, a multi-channel thermal activity monitor, which is a kind of heat conduction microcalorimeter, has been applied to investigate the inhibitory effects of different concentrations of hexavalent chromium on microbial activity in a Wuhan brown sandy soil. Our objective is to evaluate the multi-channel thermal activity monitor as an instrument for determining toxicity. Our proposed microcalorimetric method is a fast, simple and more sensitive technique to investigate the toxicity of various species of heavy metals on microorganism; as a result, the understanding on the acute toxicity of heavy metal to the soil microbes can be easily acquired.

2. Materials and Methods

2.1. Reagents

All chemicals such as glucose, ammonium sulfate, potassium chloride, $K_2Cr_2O_7$ were analytical grade. They were supplied by the Shanghai Yuelong Chemical Factory (Shanghai, PR China). $K_2Cr_2O_7$ was used as a toxicant by exposing it to the test soil organism after which microbial activity was measured as described underneath. All the glassware, as well as the polyethylene and polypropylene laboratory ware, were soaked in 10% HNO_3 (v/v) for at least 48 h before use. Deionized water was used throughout the study.

2.2. Soil sample and its physicochemical properties

A Wuhan brown sandy soil from the campus of the China University of Geosciences (30.58°N and 114.28°E) obtained from 5 to 15 cm depth was used. The soil was air dried for 10 days and homogenized by sieving to less than 2 mm, to eliminate roots and large particles. The soil was stored in polyethylene bags at 4 °C and was used for all assays.

Soil organic matter was determined by placing the dry soil (10.00 g) in a muffle furnace (600 °C) and then monitoring the decrease in mass for 24 h (Barros et al., 1999; Prado and Airoldi, 2001). Under these conditions organic matter is combusted, leaving only the inorganic component of the soil. Carbon, nitrogen, hydrogen and sulfur content in the soil were determined with a Leeman Labs Model CE-440 Elemental Analyzer (Kreuztal, Germany). The pH was measured with a Leici PHS-25C pH meter (Shanghai, PR China) in a suspension of 2.0 g of soil sample with 5.0 mL of 1.0 M calcium chloride (i.e. a 1:2.5 soil: solution ratio) (Barros et al., 1999; Prado and Airoldi, 2001; Critter et al., 2002). The analyses were conducted in triplicate.

2.3. Determination of biological properties

The number of living microorganisms was estimated by a spread plate method of viable count. A series of 10-fold dilutions for the soil sample was used after a 10.0-g dry soil was suspended in 90 mL sterile water and stirring the contents for 30 min. Aliquots of 1.0 mL of the suspension were taken and added to 9.0 mL sterile water. The sample was further diluted five times with the sterile water. Finally, 0.10 mL serial aliquots of the

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diluted sample suspension were spread over the surface of an agar plate with Martin's medium for fungi, beef extract peptone medium for bacteria and Gause's No. 1 synthetic medium for actinomycetes, respectively. Each sample was plated in triplicates and the plates incubated at 28 °C until the colonies appeared. The colony forming units (CFU) on each plate were counted. The number of bacteria, fungi, varying from 10 to 100 for fungi and 30 to 300 for prokaryotes, and actinomycetes were calculated.

2.4. Calorimeter

The TAM III multi-channel thermal activity monitor (Thermometric, Järfälla, Sweden) is a new model that was primarily designed for assessing properties of technical products, such as toxicity and stability/compatibility of explosives and pharmaceutical compounds. In this instrument each channel is a twin calorimeter where the two units are positioned with one on the top and the other at the bottom. A 4.0 mL glass or stainless steel vessel can be inserted into the top calorimetric unit. The multi-channel twin calorimeters are all inserted into a precise liquid thermostat (water or oil, 15–150 °C, short-term variation $<50\,\mu K$, drift during 24 h within $\pm 100\,\mu K$). The two calorimetric units are separated by a small 'primary' heat sink that is in thermal contact with a surrounding steel tube. The liquid thermostat will thus serve as the main heat sink for all calorimeters. The thermal power detection limit for the twin calorimeters is reported by the manufacture to be 0.1 μW. A regulated change of the thermostat temperature also allows the instrument to be used in slow temperature-scanning experiments. One of the TAM III multi-channel calorimetric units is used as a reference. All vessels are simultaneously introduced into the sample chamber.

In this experiment, the TAM III multi-channel calorimeter was used to measure the output of heat as an indicator of metabolic growth of soil microorganism under various concentrations of hexavalent chromium. The microcalorimetric thermostat was set at 28 °C. The voltage signal was recorded by a computer whereas other performance settings were as specified by the manufacture.

2.5. Microcalorimetric measurement

In order to select the optimal dose of glucose and ammonium sulfate for the growth of soil microbes, 0.60 mL of a solution of 0.0, 1.5, 3.0, 5.0, 6.0 mg glucose, respectively and ammonium sulfate (5.0 mg) was added into 1.20 g of soil. Then the microcalorimetric determinations were taken and done in triplicates.

The thermal effect was obtained using 4.0 mL stainless steel ampoules with Teflon sealing disks to avoid evaporation of the sample solution. All determinations of the thermal effect were performed in ampoules containing 1.20 g of soil and 0.60 mL of a solution made of 5.0 mg glucose together with 5.0 mg ammonium sulfate in the presence of different concentrations of hexavalent chromium. Glucose and ammonium sulfate provided as nutrients, i.e. C and N to stimulate soil microbial activity. Under these conditions, the applied moisture was maintained at 35% to maximize microbial activity (Prado and Airoldi, 2001). All samples were done in triplicate.

3. Results and discussion

3.1. Properties of Wuhan brown sandy soil

The microbial activity in a chosen system can be affected by the soil properties. Thus, the inherent physical and chemical properties such as pH, organic matter content and elemental composition are important features to be considered. The soil used was constituted of $4.35 \pm 0.12\%$ organic matter, $3.97 \pm 0.21\%$ carbon; $0.79 \pm 0.06\%$ nitrogen and $3.56 \pm 0.21\%$ hydrogen. It had a pH of 6.74 ± 0.08 and its microbial population was very sensitive to the addition of nutrients. In general, metabolism is directly promoted by addition of the desired nutrients to the ecosystem. This stimulating source is normally composed of a mixture of glucose, ammonium sulfate and water in variable ratios. The effect of different amounts of glucose and ammonium sulfate (5.00 mg) on soil microbial activity in Wuhan brown sandy soil at 25 °C is displayed in Table 1. Standard errors are commonly employed to assess the accuracy and reproducibility of a proposed method. As such, the standard deviations of the data in Tables 1 and 2 were also determined and presented (Miller and Miller, 2005). The determination of the growth rate constants (k) and other calorimetric data are discussed in the following sections. It was found that 5.0 mg glucose and 5.0 mg ammonium sulfate provided the best growth of soil microbes with the highest growth rate constant. As such, 1.20 g of soil and 0.60 mL of a solution of 5.0 mg glucose together with 5.0 mg ammonium sulfate was chosen to study the soil microbial activity.

3.2. Growth power–time curves

The metabolism of microorganisms in the Wuhan brown sandy soil in the absence and presence of distinct amounts of hexavalent chromium is shown in a series of curves (Fig. 1). As illustrated, the power–time curves are recorded for different amounts of hexavalent chromium, in which a great variability of the activity can be visualized. The

Table 1
Effect of different amounts of glucose in 0.6 mL solution containing 5.0 mg ammonium sulfate on soil microbial activity in Wuhan brown sandy soil at 25 °C

Quantity of glucose (mg)	k (min^{-1})	Q_{total} (J)	t_{max} (min)	P_{max} (μW)	Correlation coefficient
0.0	0	–	–	–	–
1.5	0.01820 ± 0.0023	0.28 ± 0.01	199.3 ± 2.19	19.43 ± 0.12	0.9987
3.0	0.02660 ± 0.0031	0.51 ± 0.02	214.5 ± 3.20	32.19 ± 0.22	0.9999
5.0	0.03070 ± 0.0009	0.72 ± 0.02	255.7 ± 1.27	43.45 ± 2.86	0.9996
6.0	0.02392 ± 0.0012	0.93 ± 0.03	543.4 ± 3.36	54.00 ± 1.31	0.9992

Note: k, the growth rate constant; Q_{total}, the total thermal effect for the microbial metabolism generated by the microbial population in the whole process; t_{max}, the time of the maximum peak-heat output power; P_{max}, the maximum peak-heat output power.

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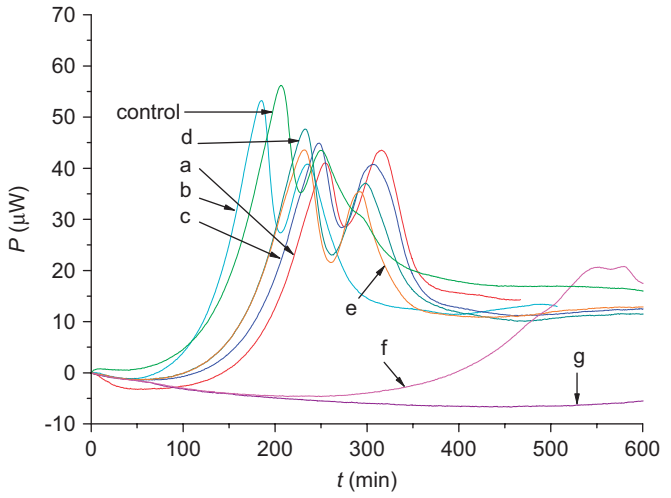

Fig. 1. The power–time curves of soil microbial activity at a moisture content of 35% in 1.20 mg of soil. The soil samples were incubated with 0.60 mL of solution containing 5.0 mg glucose together with 5.0 mg ammonium sulfate and different concentrations of Cr(VI). All samples were incubated at 25 °C. The concentrations of Cr(VI) were (a) 0.2; (b) 0.4; (c) 0.8; (d) 1.6; (e) 2.4; (f) 5.0; (g) 6.0 μg $K_2Cr_2O_7$ per mL. The control treatment did not have any Cr(VI) added.

power–time curves show that the shapes of the metabolic thermogenesis curves changed little when the low concentration of hexavalent chromium was in the soil suspension. But when higher than 5.0 μg mL^{-1} hexavalent chromium were added, the shapes changed obviously. The microbial activity curves have two typical peaks. The microorganism's metabolism is so complicated that different peaks may arise from the growth of different microbial communities, or same microorganisms may utilize different carbon sources, for example, they can use glucose first and follow with galactose. Another possible interpretation could be related to soil microorganism adapting to aerobic metabolism for the first peak, and the second peak for soil anaerobes adapting to anaerobic conditions. The presence or absence of oxygen can be very important for the growth of bacteria influencing microbial metabolism. The volume of the measuring cell in the ampoule is 4.0 mL, leaving only 2.0 mL air after adding the soil sample. At the beginning of the experiment, there is a little oxygen available for soil microbe, which initially favors aerobes. When the oxygen is consumed, anaerobes are favored. This may explain why there are two typical peaks. The set of distinguishable profiles obtained from the power–time curves with distinct amounts of hexavalent chromium added to the soil show the variation in activity, as changing the amount of hexavalent chromium applied to the microorganisms. Table 2 displays the effect of hexavalent chromium on the numbers of living microorganisms consisting of bacteria, actinomycetes and fungi in the sandy soil samples. The higher the concentration of the hexavalent chromium added to the soil samples, the smaller the number of the microorganisms was found. This indicates that hexavalent chromium can suppress the growth of these microorganisms in the soil.

3.3. Growth rate constant of soil microbial, generation time, inhibition ratio and half inhibitory concentration

The power–time activity curves of soil microbes show that microbial cell growth is exponential in the log phase of growth. If the cell number is n_0 at time 0, and n_t at time t,

$$n_t = n_0 \exp(kt), \quad (1)$$

where k is the growth rate constant (Wang et al., 2003). If the power output of each cell is w, then

$$n_t w = n_0 w \exp(kt). \quad (2)$$

If the heat output power is P_0 at time 0, and P_t at time t, then

$$P_0 = n_0 w \quad \text{and} \quad P_t = n_t w \quad \text{giving}$$
$$P_t = P_0 \exp(kt) \quad \text{or} \quad \ln P_t = \ln P_0 + kt. \quad (3)$$

The growth power–time curves of the log phase correspond to Eq. (3). So, using the data $\ln P_t$ and t taken from the curves to fit a linear equation, the thermokinetic equation for the soil microbial activity at different concentrations of hexavalent chromium at 25 °C can be obtained and the correlation coefficients are displayed in Table 2. All the correlation coefficients are larger than 0.9950, indicating a good reproducibility and correlation. The growth rate constant (k) and the generation time (t_G), which equals $(\ln 2)/k$, were also obtained. The corresponding k, t_G and correlation coefficient r are shown in Table 2. The values in Table 2 show the generation time (t_G) decrease with the increasing concentration of hexavalent chromium. This means that the generation time becomes longer with the increasing concentration of hexavalent chromium due to the increasing toxicity of this compound.

Inhibitory ratio is commonly defined as concentrations of hexavalent chromium that will inhibit soil microbial activity. The inhibitory ratio I is obtained by

$$I = [(k_0 - k_C)/k_0] \times 100\%, \quad (4)$$

where k_0 is the rate constant of the control, and k_C is the rate constant for soil microbial activity inhibited by an inhibitor whose concentration is C. When the inhibitory ratio I is 50%, the corresponding concentration of inhibitor is called the half inhibitory concentration (IC_{50}). According to the k–C equations, the value of IC_{50} can be obtained using Eq. (4), the IC_{50} was calculated to be 4.27 μg $K_2Cr_2O_7$ per mL in this instance. The changing regulation of the values of I is similar to the generation time and the growth rate constant.

3.4. Relationship among the growth rate constant k, the number of microorganism and different concentrations of hexavalent chromium

Table 2 clearly depicts that the growth rate constant (k) and the number of microorganisms, especially the number

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Table 2
Results of the effects of the concentration of hexavalent chromium on microbial activity in Wuhan brown sandy soil

C, $K_2Cr_2O_7$ ($\mu g\,mL^{-1}$)	0.00	0.20	0.40	0.80	1.60	2.40	5.0	6.0
k (min^{-1})	0.03070 ± 0.0009	0.02967 ± 0.0012	0.02942 ± 0.0008	0.02854 ± 0.0011	0.02516 ± 0.0008	0.02319 ± 0.0016	0.01208 ± 0.0022	0
I (%)	0	3.36 ± 0.26	4.17 ± 0.07	7.04 ± 0.29	18.05 ± 0.13	24.46 ± 0.31	60.65 ± 0.22	100
t_G (min)	22.58 ± 0.12	23.36 ± 0.18	23.56 ± 0.11	24.29 ± 0.12	27.55 ± 0.11	29.89 ± 0.23	57.38 ± 0.37	–
Q_{total} (J)	0.72 ± 0.09	0.17 ± 0.05	0.32 ± 0.03	0.51 ± 0.05	0.52 ± 0.08	0.48 ± 0.06	0.07 ± 0.02	–
P_{max1} (μW)	56.14 ± 2.86	41.02 ± 1.13	53.18 ± 3.91	44.83 ± 2.19	47.61 ± 3.20	43.51 ± 2.75	20.64 ± 2.33	–
t_{max1} (min)	207.2 ± 3.06	255.3 ± 1.22	185.9 ± 2.30	247.2 ± 4.70	233.3 ± 2.14	231.7 ± 1.75	551.4 ± 4.32	–
P_{max2} (μW)	43.45 ± 3.78	43.54 ± 2.56	40.76 ± 2.39	40.68 ± 1.09	36.95 ± 4.11	35.44 ± 2.01	20.76 ± 1.38	–
t_{max2} (min)	250.2 ± 1.92	316.4 ± 3.14	235.8 ± 2.77	305.9 ± 3.03	297.5 ± 2.26	292.2 ± 4.21	577.6 ± 1.72	–
r	0.9966	0.9965	0.9975	0.9981	0.9969	0.9931	0.9985	–
IC_{50} ($\mu g\,mL^{-1}$)				4.27 ± 0.13				
Number of microorganisms ($CFU\,g^{-1}$)								
Bacteria ($\times 10^7$)	3.01 ± 0.38	2.78 ± 0.14	2.43 ± 0.28	2.01 ± 0.08	1.57 ± 0.21	0.82 ± 0.16	0.31 ± 0.09	–
Actinomycetes ($\times 10^6$)	4.67 ± 0.17	3.98 ± 0.55	2.23 ± 0.23	3.37 ± 0.11	1.88 ± 0.09	1.37 ± 0.04	1.69 ± 0.14	–
Fungi ($\times 10^5$)	0.51 ± 0.03	0.87 ± 0.13	0.49 ± 0.10	0.31 ± 0.07	0.41 ± 0.03	0.26 ± 0.02	0.19 ± 0.02	–

Note: "–", no detection; k, the growth rate constant; I, the inhibitory ratio; t_G, the generation time; Q_{total}, the total thermal effect for the microbial metabolism generated by the microbial population in the whole process; P_{max1}, the first peak-heat output power; P_{max2}, the second peak-heat output power; t_{max1}, the time of the first peak-heat output power; t_{max2}, the time of the second peak-heat output power.

change of bacteria, decrease with increasing concentration of hexavalent chromium, further indicating that the soil microbial activity has been inhibited according with increasing concentrations of hexavalent chromium. The power–time curves of the soil microbial activity is a good illustration as shown in Fig. 1. When the $K_2Cr_2O_7$ concentration reached 6.0 $\mu g\,mL^{-1}$, the soil microorganisms failed to grow and the numbers of microorganisms dropped to almost zero. Although the number of microorganism decreased with increasing concentration of hexavalent chromium, it is not a linear relationship as depicted in Fig. 2 (slope a). However, in the concentration range of 0–6.0 $\mu g\,mL^{-1}$, there is a linear negative relationship between k and C as displayed in Fig. 2 (slope b). Slope b in Fig. 2 indicates obvious inhibition of hexavalent chromium on the soil microbial activity and fits the equation: $k = 0.0309 - 0.00364C$, $r = -0.9951$.

3.5. Relationship among the total thermal effect in whole process Q_{total}, the number of microorganism and the concentration of hexavalent chromium

From the power–time curves shown in Fig. 1, the total thermal effect of microbial metabolism Q_{total} generated by the microbial population was obtained through integration of each curve from the beginning to the end. Thermal effect values are displayed in Table 2. It was found that the thermal effect decreased with the increase in the concentration of hexavalent chromium. This trend indicated toxic effect on the soil microbial activity due to the addition of hexavalent chromium. However, this trend is not linear since the concentration of 0.2–0.4 μg $K_2Cr_2O_7$ per mL has abnormal phenomena with the total thermal effect of microbial metabolism Q_{total} not following the general trend as shown in Fig. 3. The thermal effect is the sum of catabolic and anaerobic processes that occur during inorganic and organic material degradation and accumulation. It reflects the ability of the community present in soil to facilitate these processes. So, the total thermal effect of microbial metabolism can be one of the indices of soil microbial activity.

3.6. Relationship between the two peak powers (P_{max1}, P_{max2}), or the times in the peak powers (t_{max1}, t_{max2}), and the number of microorganisms or concentration of hexavalent chromium

In order to show the results in a more quantitative fashion, the two peak-heat output powers (P_{max1}, P_{max2}) and the times of the peak of powers (t_{max1}, t_{max2}) were calculated from the power–time curves (Fig. 1). The values are also listed in Table 2 and it is apparent from these values that there is not pronounced correlation between the concentration of hexavalent chromium and the peak-heat output power (P_{max}) or the time of the peak of power (t_{max}) as depicted in Figs. 4 (slopes a and b) and (slopes c and d), respectively. The peak in Fig. 1 reflects the time of soil

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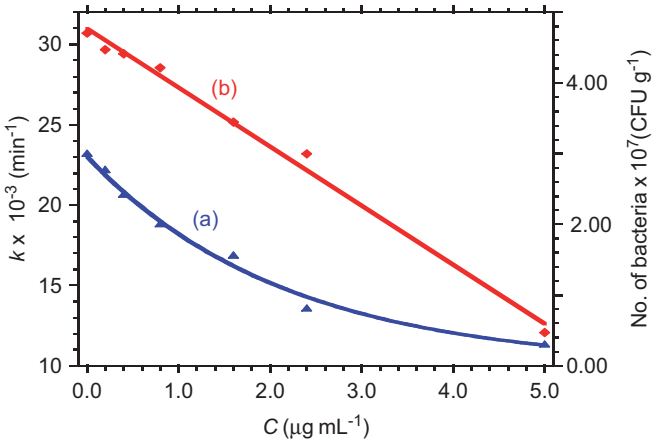

Fig. 2. (a) Plot of the number of bacteria against concentration of $K_2Cr_2O_7$ (C). (b) Relationship between the growth rate constant k and C. The linear relationship between k and C is $k = 0.0309 - 0.00364C$ with $r = -0.9951$. The growth rate constant k decreases with the increase in concentration of $K_2Cr_2O_7$ (C).

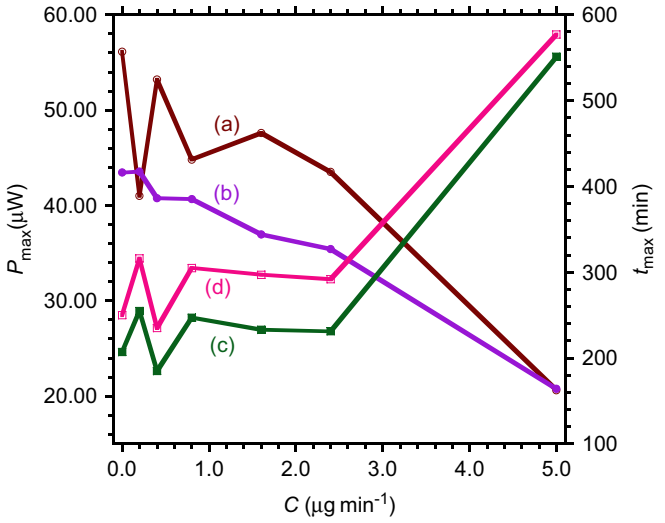

Fig. 4. Relationship between the two peaks (a) $P_{max\,1}$, (b) $P_{max\,2}$, (c) $t_{max\,1}$, (d) $t_{max\,2}$ and C.

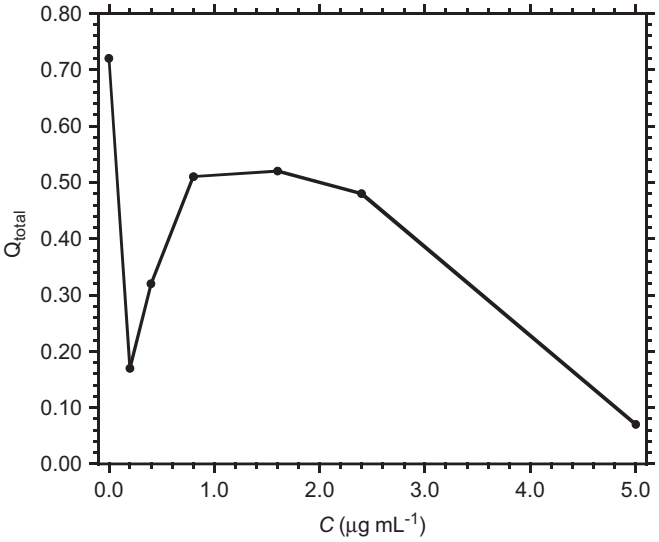

Fig. 3. Relationship between the total heat output in the whole process Q_{total} and C.

microbial activity of different microbial communities and degradation of part of the inorganic and organic material. In general, the two peak-heat output powers decreased with the increase in hexavalent chromium. The times of the peak of powers increased with the increase in hexavalent chromium. This also indicates that the inhibitory effect of Cr(VI) on the growth of microorganisms in the sandy soil decreased with the increase in hexavalent chromium. The mechanisms of action of Cr(VI) originates from its direct action as an oxidizing agent and from the formation of free radicals during its reduction to Cr(III) inside the cell. Therefore, the disappearance of soil microbial activity and other manifestations of toxicity at some concentration of Cr(VI) may be due to the higher rate of its entry and its capacity to cause oxidative damage to cells, as well as to the subsequent toxicity of its reduction product Cr(III). That higher concentrations of Cr(III) required causing toxicity may be due to the low rate of entry of Cr(III) and its ability to cause only part of the damage compared to the damage caused by Cr(VI).

4. Conclusion

Microcalorimetric data show changes in the soil microbial activity in the metabolism processes affected by the different concentrations of hexavalent chromium. All these calorimetric information on the soil can give considerable contributions to important understanding features related to the effect of other toxic compounds on soil. A calorimetric curve of soil microbial activity can be presented with high thermal effect output and a rapidly decrease or with profiles of different minor initial thermal effect production, but with a slow decrease after the peak time. Thus, the area under the curve (in J) obtained by the thermal power effect production in W as a function of time (in s) can show the intensity and the time necessary for the development of activities of a specific soil system. As a consequence, the area under the peak time can be taken as good indicators of microbial activity.

In this research, the microcalorimetric method was successfully used to analyze the inhibitory effect of different concentrations of hexavalent chromium. The thermokinetic parameters obtained from the metabolic power–time curves could act as a quantitative indicator of sensitive the toxic effect of toxicant to soil microbial activity. Our work confirmed the poisonous species of $K_2Cr_2O_7$ at an IC_{50} value of $4.27\,\mu g\,mL^{-1}$ against soil microbe. Our work also provides a new thermochemical method (microcalorimetry) for the investigation of toxicity of different species of heavy metal on microorganism. The

Please cite this article as: Yao, J., et al., Microcalorimetric study the toxic effect of hexavalent chromium on microbial activity of Wuhan brown sandy soil: An in vitro approach, Ecotoxicol. Environm. Safety (2007), doi:10.1016/j.ecoenv.2007.02.005

microcalorimetric detection system is very sensitive with a detection limit of 0.1 μW and baseline stability (over a period of 24 h) is 0.2 μW. Thus the little change in metabolic growth of microbes under different concentrations of different species can be monitored efficiently by changes in thermokinetic parameters, which can capture little differences in toxicity. Compared with other biological methods, microcalorimetry is more sensitive and can be easily performed. It is anticipated that it is a promising technique to be applied in other biological and environmental research fields, such as studying the toxicity of different species of heavy metals.

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