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Reduction in toxicity of arsenic(III) to *Halobacillus* sp. Y35 by kaolin and their related adsorption studies

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ABSTRACT

The growth of *Halobacillus* sp. Y35 has been investigated in HGM hypersaline medium with different doses of As(III) and kaolin. The metabolic heat flux decreases with the increase in As(III) concentration, indicating that strain Y35 lowers their metabolic activity in order to resist the As(III) toxicity. Carbon dioxide flux, cell growth and protein synthesis rates, and total thermal effect have been, for the first time, successfully employed simultaneously to assess the effect of As(III) on strain Y35 in the absence and presence of kaolin. The relative adsorption capacity and adsorption intensity of kaolin for As(III) are higher with strain Y35 than that without strain Y35, demonstrating that it is possible to reduce the toxicity of As(III) to our environment by both using mineral adsorption and biosorption technology. Our work shows the potential application of kaolin and strain Y35 for the removal of As(III) from contaminated groundwater.

1. Introduction

Environmental concern on arsenic contamination has increased in the past few decades due to the increased awareness of its risk to plants, animals, and human health. Arsenic can enter into soils, aquifer sediments and drinking water through various pathways; for instance, the weathering of arsenopyrite is usually associated with iron sulfide ore-deposits. In natural geological and soil environment, the presence of arsenic is usually low [1]; however, higher concentrations were detected in hypersaline (about 0.2 mM), closed basin soda lakes (0.2–3.9 mM) and their remnant brines [2]. Although arsenic is a well known toxicant to most eukaryotes and prokaryotes, some prokaryotes such as *Alcalilimnicola halodurans*, *Halobacillus* sp., *Bacillus halodurans*, *Arsenite-oxidizing bacterium* and *Bacillus arseniciselenatis* have evolved and survived under arsenic by their unique biochemical or physiological mechanisms [3].

The biosphere is so complex that the research of arsenic transport is mainly divided into two different streams. In physical chemistry, the adsorption and desorption of arsenic on mineral, humic acid and soil are usually investigated by parameters such as pH, specific surface area, particle size, cation exchange capacity, electrokinetic response, and ionic strength [4–6]. In microbiology, the four basic processes of microbial arsenic transformation including methylation, demethylation, oxidation, and reduction are investigated [7]. Our group has previously identified some arsenite-oxidizing and arsenate-reducing bacteria can survive even in groundwater and sediment with high concentrations of arsenic [8]. Both arsenate and arsenite have different adsorptive affinities to various common mineral surfaces, *e.g.*, kaolinite and alumina; which strongly affect their concentration in the aqueous phase and thus, interfering the microbial growth and activity. To our knowledge, little is known about the microbial heat flux in a mixed model of arsenic(III) (As(III)) and mineral. As such, simultaneous study on the biosorption process and microbial metabolism in arsenic-contaminated environment is pivotal; and physiological research methods are required to monitor the changes in the concentration of arsenic in aqueous phase.

In this work, the 16S rRNA gene library was initially used to compare *Halobacillus* sp. Y35 (EF533972) with some other microorganisms by the phylogenetic tree. Then the effect of mixed models

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of As(III) and kaolin on *Halobacillus* sp. growth process was assessed and evaluated by using microcosms. The mixed substrates were homogenized to evenly distribute both the bacterial suspensions and mixed substrates; thereby reducing spatial variability. This allows more effective assessment of the poisonous effect of arsenic on microbial growth [9]. The *Halobacillus* sp. metabolic process was monitored by microcalorimetry. Other chemical and biological indicators including carbon dioxide flux (F_{CO_2}), total thermal effect (Q_{total}), cell growth rate (k_{cell}) and protein synthesis rate ($k_{protein}$) were also simultaneously measured to assess the microbial growth. Arsenic concentrations in aqueous phase were determined by inductively coupled plasma-mass spectrometry (ICP-MS).

In this research, we have successfully developed a method to measure the growth activity of *Halobacillus* sp. Y35 (EF533972) in As(III) and kaolin as well as to quantify the adsorption of As(III) in mixed sorbents of kaolin and *Halobacillus* sp. Our findings can provide important information for evaluating the metabolism and mixed adsorption process of extremophiles in kaolin and transport of arsenic in soil, sediment or soda-lake. This information would be helpful for developing a biosorption and chemosorption method to remove arsenic toxicity from contaminated water and soil in our environment.

2. Materials and methods

2.1. Reagents

Highest purity As_2O_3 was purchased from Beijing Chemical Reagents Company (Beijing, P.R. China) and was used without further purification. The stock solution of As_2O_3 was prepared by adding 6.605 g of As_2O_3 in 100 mL sterilized water followed by titration with 10.0 M sodium hydroxide until all As_2O_3 were completely dissolved. The solution was made up to 1.0 L with sterilized water. The final concentration of the As(III) solution was 66.77 mM. Kaolin was kindly provided by the State Key Laboratory of Geological Processes and Mineral Resources, Chinese University of Geosciences (Wuhan, P.R. China). The kaolin particles consist of 95 wt.% of kaolinite, 5 wt.% of mica and traces of halloysites. The kaolin particle size was smaller than 50 μm. All other reagents were of analytical grade or above.

2.2. Strains identification and culture medium

Halobacillus sp. Y35 (EF533972) isolated from a hypersaline soil in Yancheng City, Jiangsu Province, P.R. China was provided by the State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University (Wuhan, P.R. China). The nearly full-length 16S rRNA genes were amplified by PCR using universal primers Uni-27F and Uni-1492R [8]. The PCR programme consisted of an initial denaturation for 5 min at 94 °C; 30 cycles of 1 min at 94 °C, 1 min at 50 °C, 1 min at 72 °C; and a final extension step at 72 °C for 5 min. The PCR products were purified by using the UltraPure™ PCR Kit (SBS Genetech, Shanghai, P.R. China). DNA sequencing was performed by the Sunbio Company (Beijing, P.R. China). All the 16S rRNA gene sequences were checked manually and edited for phylogenetic analyses. Sequence alignments were performed using the C_{LUSTAL} W program [10]. B_{LAST} N (for 16S rRNA gene) was used to analyze similarities (www.ncbi.nlm.nih.gov/BLAST). Phylogenetic trees were generated from alignments by the neighbor-joining method and the reliability of inferred trees was tested with bootstrap test using the MEGA3 program (www.megasoftware.net). Some reference sequences from the GenBank were used in generating phylogenetic trees for clarification.

Minimum inhibitory concentrations (MICs) of the arsenic resistant bacteria were determined by inoculating a single colony in triplicate into liquid chemically defined medium (CDM) supplemented with different concentrations of As(III) and shaking at 28 °C for 1 week. The MIC was defined as the lowest As(III) concentration that completely inhibited the growth of *Halobacillus* sp. Y35.

Halobacillus sp. Y35 was cultivated at 28 °C on HGM agar slants and stored at 4 °C. During the microcalorimetric measurements, *Halobacillus* sp. Y35 grew in HGM medium ($g\,L^{-1}$) containing $MgCl_2{\cdot}6H_2O$ (19.5), $MgSO_4{\cdot}7H_2O$ (29.0), $CaCl_2{\cdot}2H_2O$ (1.1), KCl (6.0), NaBr (0.5), $(NH_4)_2SO_4$ (10.0) and NaCl (58.0), and yeast extract (2.0). Tris–HCl buffer (10 mM) was added to maintain the pH at 7.5. In addition, 2% (w/v) agarose was added to HGM solid medium for pure culture in Petri dish. It was then sterilized at high-pressure at 121 °C for 30 min.

2.3. Cultivation experiments

Halobacillus sp. Y35 were cultured in our sterile bioreactor with a working volume of 1.0 L. The growth process of *Halobacillus* sp. Y35 was continually monitored. (This process was similar to the ampoule environment of the microcalorimeter.) The initial pH and cell density of the bioreactor cultures were kept at 7.5 and OD_{700} (absorbance at 700 nm) = 0.2. These experiments were conducted in triplicate. Anaerobic atmosphere was maintained in a glove box by sparging with filter-sterilized nitrogen. No antifoaming agents were used. The various cultivation studies were: (1) t*Halobacillus* sp. Y35 was cultivated in HGM medium without As(III) or kaolin; (2) for study of microbial metabolism and biosorption, strain Y35 was inoculated in HGM medium with five different doses (1.0–5.0 mM) of As(III); and (3) in order to measure the toxicity and absorption kinetics of the mixed models, five different doses (1.0–5.0 $g\,L^{-1}$) of kaolin was added to the HGM medium with or without As(III).

2.4. Microcalorimetric and cell dry weight analyses

Halobacillus sp. Y35 was pre-cultured in HGM medium at 28 °C overnight before microcalorimetric measurements. Then the bacterial suspensions were mixed by inoculating *Halobacillus* sp. Y35 into the same medium to an optical density of 0.2 at 700 nm. A TAM III multi-channel thermal activity microcalorimeter (Thermometric AB, Sweden) was used for metabolic heat flux measurements. In this study, the heat flux measurement was based on the isothermal ampoule method. 4.0 mL-stainless steel ampoules were sterilized to hold the strains. All determinations were performed in triplicate in ampoules containing 3.0 mL bacterial suspensions plus different substrates. In this work, the ampoule containing only 3.0 mL bacterial suspensions was used as the control. The temperatures of the calorimeter system and the isothermal box were maintained at 28 °C. The power–time curves of the growth of isolated strains were continuously recorded by a computer.

The thermokinetic parameters such as Q_{total} and growth rate constant (k) which can reflect the biochemical reactions were determined by analyzing the power–time curves. The growth power–time curves of strains obey the thermokinetic equation at the exponential phase of growth [11,12]:

$$\ln P_t = \ln P_0 + kt \quad (1)$$

where t is the time, P_t is the power output at time t, and P_0 is the power at the beginning of exponential growth phase. Q_{total} was calculated through the integration of each curve at the end of the exponential increase of the signal [13]. At the same time, the effect of mixed medium on microbial metabolic process was determined by a combination of methods. All samples were incubated at 28 °C on a shaker at 200 rpm.

In order to show the results in a more quantitative way, the cell growth rate (k_{cell}) is calculated by the growth rate equation [14]:

$$k_{cell} = \frac{\ln X_t - \ln X_0}{t_t - t_0} \quad (2)$$

where X_t, X_0, are the cell dry weights ($g\,L^{-1}$) at the beginning and end of an incubation interval, and t_t (h) and t_0 (h) are the corresponding times. The inhibitory ratio (I) is defined as [12]:

$$I\ (\%) = \frac{k_0 - k_t}{k_0} \times 100 \quad (3)$$

where k_0 is the growth rate constant of the control and k_t is the growth rate constant for strain Y35 in the presence of an inhibitor (in here, As(III)). In this research, the microbial growth rate constants k and k_{cell} were calculated using the microcalorimetric method [12] and Eq. (2), respectively. k_{cell} was monitored by measuring the cell dry weight (CDW) of 5-day culture. The CDW was determined gravimetrically in quadruplicate after oven-drying at 105 °C. Non-inoculated treatments were used as controls. Each ampoule was filled in a tent with a nitrogen atmosphere and an oxygen content of less than 0.1% (v/v).

2.5. Growth activity analyses

The F_{CO_2} was monitored by a Pasco PS-2110 CO_2 gas sensor (Roseville, CA, USA). By selecting between two ranges, 1.0 and 10 $g\,L^{-1}$, the sensor was calibrated before use. The signals of F_{CO_2} was captured and processed by Xplorer GLX Interface PS-2002, serial cables, a power supply, and DataStudio software (version 1.9.8). The physicochemical properties of microbial growth in the mixed substrates were monitored on-line by this sensor. In order to investigate the growth activity of *Halobacillus* sp. Y35 in the mixed models, some quantitative methods were used to measure microbial physiological parameters in logarithmic phase. For instance, the respiration activity of strain Y35 was measured by monitoring the F_{CO_2} and the CO_2 flux rate (k_{CO_2}) ($mg\,h^{-1}$) which is defined as [15]:

$$k_{CO_2} = \frac{V(\ln D_t - \ln D_0)}{t_t - t_0} \quad (4)$$

where D_t, D_0, are the F_{CO_2} ($mg\,L^{-1}$) at time t_t (h) and t_0 (h), respectively and V (L) is the total volume of the mixed solution.

In addition, the protein concentrations of the supernatant were measured by the Bradford method [16], using bovine serum albumin as the standard; and the amount of total protein could then be calculated. The protein synthesis rate ($k_{protein}$) (% h^{-1}) is determined as [17]:

$$k_{protein}\ (\%\,h^{-1}) = \frac{(S_t - S_0)}{S_t(t_t - t_0)} \times 100 \quad (5)$$

where S_0 and S_t are the microbial protein concentrations ($mg\,L^{-1}$) at the beginning and end of an incubation interval, respectively, and $(t_t - t_0)$ is the time elapsed (h) between the two protein samples. The maximal protein synthesis rate (k_{max}) was determined from the maximum $k_{protein}$ value during the whole incubation period.

2.6. Adsorption of arsenic(III) on kaolin with strain Y35

The adsorption of As(III) was investigated during the *Halobacillus* sp. Y35 growth in the mixed medium. After the 5-day culture period, 20.0 mL bacterial suspension was collected and decanted into 40 mL polycarbonate centrifugation tubes at room temperature. These cell suspensions were centrifuged at 10,000 rpm and 10 min, and the supernatant containing As(III) was stored at 4 °C. The arsenic contents in supernatant fluids were determined by a Hewlett Packard HP 4500 Plus Quadrupole ICP-MS system (Waldbronn, Germany). Arsenic content in the supernatant was quantified using a standard calibration curve of As(III). Working As(III) standard solutions within the range 0.0–5.0 mM were prepared daily by diluting 66.77 mM As(III) stock solution with appropriate volumes of 0.25 M HCl. The detection limits of ICP-MS for arsenic are 100–200 ppt. In addition, non-inoculated treatments were used as controls to indicate the adsorption of As(III) on kaolin. The percentage of As(III) adsorption (% As(III) adsorbed) on kaolin with or without *Halobacillus* sp. Y35 was calculated according to the following equation [4]:

$$\text{As(III) adsorbed}\ (\%) = \frac{([\text{As(III)}]_0 - \text{As}(T)_{eqm})}{[\text{As(III)}]_0} \times 100 \quad (6)$$

where $[As(III)]_0$ is the initial As(III) concentration added and $[As(T)_{eqm}]$ is the equilibrium supernatant As(III) concentration after interacting with kaolin in the absence or presence of *Halobacillus* sp. Y35.

Moreover, adsorption isotherm is commonly employed to assess the adsorption characteristics of a solute on an adsorbent. An adsorption isotherm is presented as the amount of solute adsorbed per unit weight of adsorbent as a function of the equilibrium concentration in the bulk solution at a constant temperature. The Freundlich adsorption isotherm is commonly used and expressed as [18]:

$$\log q = \log K_f + \frac{1}{n} \log C_e \quad (7)$$

where C_e is the equilibrium concentration of As(III) in the supernatant (mM), q is the amount of As(III) adsorbed at equilibrium on kaolin (mM/g). K_f and $1/n$ are constants that characterize the adsorption capacity and the adsorption intensity of kaolin for As(III) [19]. As such, the adsorption isotherm of As(III) on kaolin with or without *Halobacillus* sp. Y35 was determined using Eq. (7).

3. Results and discussion

3.1. Identification of strain Halobacillus sp. Y35

After the growth on high salt HGM solid medium, the cell of strain Y35 was identified as gram-positive, forming 1–2 mm round and convex colonies, slightly glossy, yellow to cream and as facultative anaerobic microorganism. The optimal growth of anaerobic ally is at 30 ± 2 °C and pH 7.5 in HGM medium. Strain Y35 was resistant to arsenic with MICs = 5.0 mM As(III). In addition, strain Y35 is able to grow at high salt concentrations: growth was observed in HGM medium containing sodium ion >58 mg mL^{-1} and chloride ion >84 mg mL^{-1}.

The 16S rRNA gene DNA of strain Y35 was sequenced to determine its phylogenetic position as depicted in Fig. 1. The almost complete 16S rRNA sequence of strain Y35 with 1440 nucleotides was compared with the sequence of 18 alkaliphilic *Bacillus* and 11 neutrophilic species which belong to several groups of genus Bacillus (Fig. 1A). The 16S rRNA sequence of *Halobacillus* sp. Y35 shows 93% similarity to the 16S rRNA sequence of *B. halodurans* C-125 (accession number NC002570) according to the BLAST method (www.ncbi.nlm.nih.gov/BLAST). It has been reported that *B. halodurans* C-125 can grow well at pH 7.0–10.5 even when the sodium ion concentration is high (1–2%) [20,21]. The nucleotide sequence of strain Y35 is not shown here because these different DNA fragments were too dispersed and complex to build any new gene function. Our results suggest some possible functional relationships between the strain Y35 and other microbial species (Fig. 1). The phylogenetic modeling indicates that the relationship between strain Y35, Arr and geobacillus strain is close but has some distance with Aox species (Fig. 1B). This infers that cell metabolism and function are particularly important and closely interrelate with

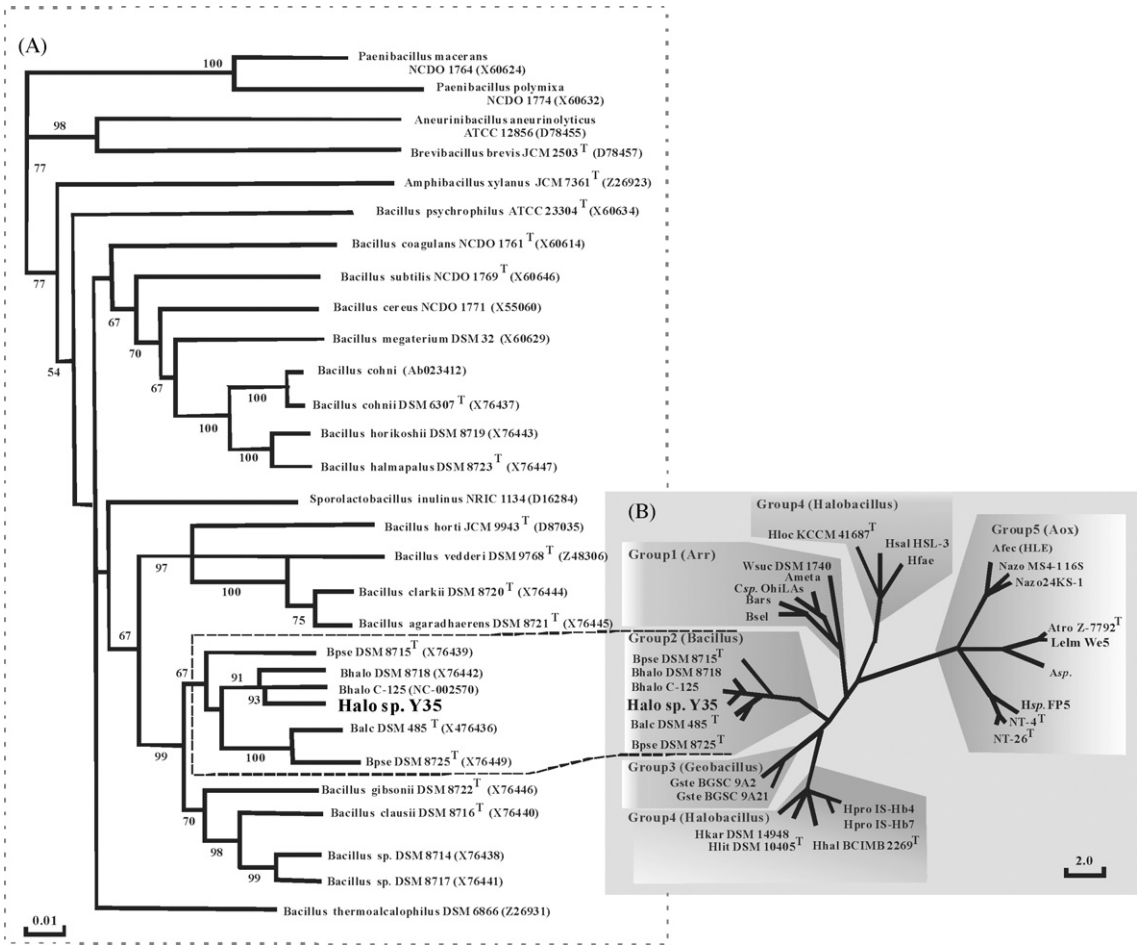

Fig. 1. Phylogenetic tree derived from 16S rRNA sequence data of strain Y35 (EF533972). (A) 16S rRNA gene sequences of strain Y35 is compared with the sequence of 18 alkaliphilic *Bacillus* and 11 neutrophilic species belonging to several groups of genus *Bacillus*. Numbers are bootstrap values greater than 500. Bar 0.01 K_{nuc} unit. (B) 16S rRNA gene sequences of strain Y35 is compared with some other microbial species. The abbreviations for the microorganisms are as follows (GenBank accession numbers of the nucleotide are in parentheses): Wsuc DSM 1740, *Wolinella succinogenes* DSM 1740 (NC005090); Ameta, *Alkaliphilus metalliredigenes* (AY137848); C*sp*.Ohil As, *Clostridium* sp. OhiLAs (DQ250645); Bars, *Bacillus arseniciselenatis* (Arr) (AY660885); Bsel, *Bacillus selenitireducens* (Arr) (AY283639); Bpse DSM8715^T, *Bacillus pseudofirmus* DSM8715^T (X76439); Bhalo DSM8718, *Bacillus halodurans* DSM8718 (X76442); Bhalo C-125, *Bacillus halodurans* C-125 (NC_002570); Balc DSM485^T, *Bacillus alcalophilus* DSM485^T (X76436); Bpse DSM8725^T, *Bacillus pseudofirmus* DSM8725^T (X76449); Gste BGSC 9A2, *Geobacillus stearothermophilus* strain BGSC 9A2 (AY608927); Gste BGSC 9A21, *Geobacillus stearothermophilus* strain BGSC 9A21 (AY297092); Hkar DSM 14948, *Halobacillus karajiensis* DSM 14948 (AJ486874); Hlit DSM 10405^T, *Halobacillus litoralis* sp. DSM 10405^T (X94558); Hal BCIMB 2269^T, *Halobacillus halophilus* NCIMB 2269^T (X62174); Hpro IS-Hb4, *Halobacillus profundi* IS-Hb4 (AB189298); Hpro IS-Hb7, *Halobacillus profundi* IS-Hb7 (AB195680); Hloc KCCM 41687^T, *Halobacillus locisalis* KCCM 41687^T (AY190534); Hsal HSL-3, *Halobacillus salinus strain* HSL-3 (AF500003); Hfae, *Halobacillus faecis rrs* (AB243865); Afec (HLE), arsenite-oxidizing bacterium *Alcaligenes fecalis* (HLE) (AY027506); Nazo MS4-116S, *Natronobacillus azotifigens* strain MS4-1 16S (EU143685); Nazo24KS-1, *Natronobacillus azotifigens* strain 24KS-1 (EU143681); Atro Z-7792^T, *Amphibacillus tropicus* Z-7792^T (AF418602); Lelm We5, *Lake Elmenteita isolate* We5 (X92140); Asp., arsenite-oxidizing bacterium *Agrobacterium* sp. (AF316615) ; Hsp.FP5, *Halobacillus* sp. FP5 (AM295006); NT-4^T, arsenite-oxidizing bacterium NT-4^T (AY027503); NT-26^T, arsenite-oxidising bacterium NT-26^T (AF159423). The tree was constructed by the MEGA3 program (http://www.megasoftware.net). Bar = 2.0 K_{nuc} unit.

Arr and geobacillus [7] have pointed out that these microorganisms can play an important role in the ecology of arsenic transport and cycle. Therefore, our 16S rRNA sequences indicates that strain Y35 can possibly survive in high arsenic content and alkaline condition (pH > 7.5) and seems capable of protecting its cells in toxic environment via mechanisms such as cell wall property [22], Na^+:H^+ antiporter system [23] and cell membrane transport [22].

3.2. Cell activity of strain Y35 in mixed models

The microbial metabolic heat flux, k_{CO_2} and k_{max} (Figs. 2–4) data suggest some decreasing trends of cell activity with the increase in As(III) concentration. The main reason behind this phenomenon is that cells have to reduce normal metabolic activity and to enhance cellular detoxification ability [24,25]. During this process, the cells may have to pump some arsenic to the extracellular environment and/or some biochemical reactions such as methylation, demethylation, oxidation and reduction may have taken place within the cells such that the toxicity of arsenic can be much reduced [7]. In other words, special detoxification function will replace some conventional physiological function so as to resist poisoning. It can be said that these cell physiological properties are very helpful to reflect the substrate toxicology in living experiment [12].

It is also worth to discuss further the effect of different doses of kaolin on the microbial growth activity with the increase in As(III) concentration as illustrated in Figs. 2–4. Although the bacillus species-kaolin interaction has been studied by methods such as electron microscopy and adsorption equilibrium [26,27], our work is the first to investigate the microbial growth process with kaolin in extreme environments using a combination of and continuous methods. Our microcalorimetric results show that the changes in cell heat flow rate are very small (80–100 $\mu W\,mL^{-1}$) when strain Y35 is cultivated in HGM medium with five different concentrations of kaolin in the absence of As(III) (Control curves in Fig. 2). At the same time, k_{CO_2} and k_{max} are 400–570 $mg\,h^{-1}$ (Fig. 3) and 0.12–0.18% h^{-1} (Fig. 4), respectively. These small changes of cell metabolism in the presence of kaolin are consistent with Stotzky's earlier work. Stotzky (1986 and 1989) claimed that DNA can adsorb

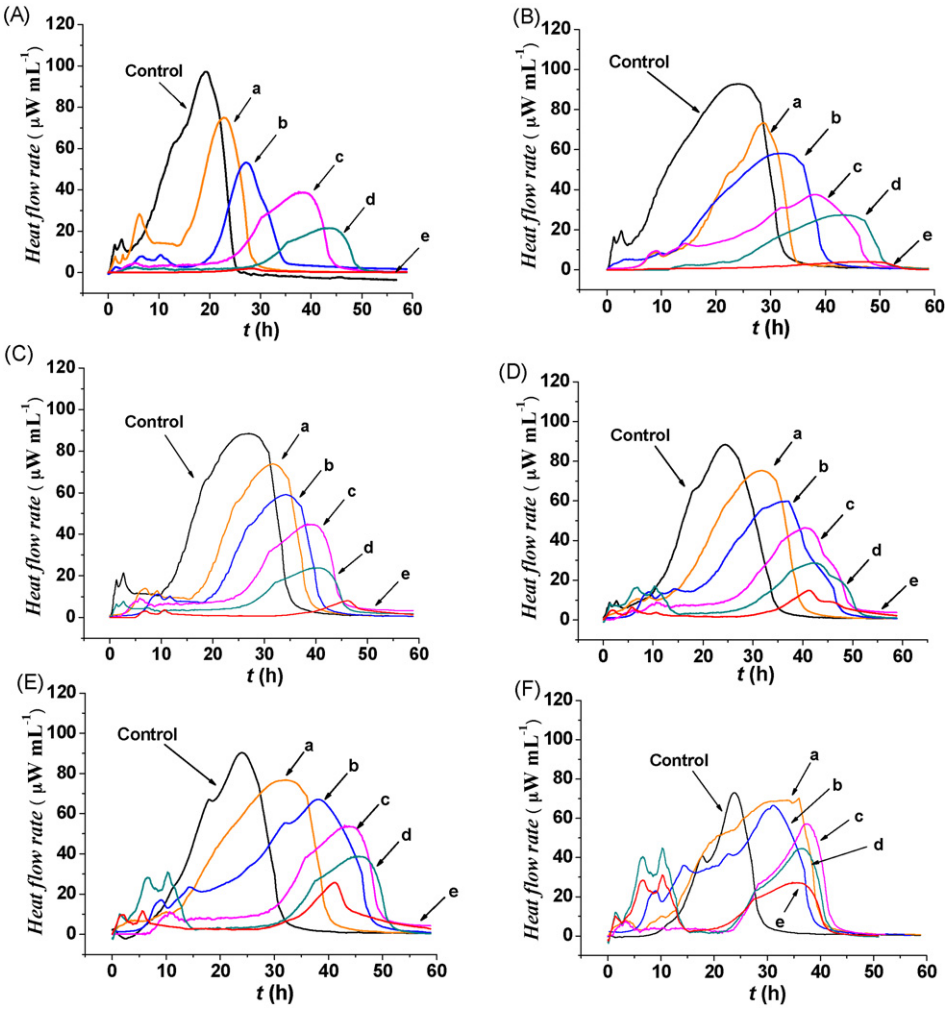

Fig. 2. The power–time curves of strain Y35 at different concentrations of kaolin and As(III). The incremental doses of kaolin are (A) 0.0, (B) 1.0, (C) 2.0, (D) 3.0, (E) 4.0, and (F) 5.0 g L^{-1}. The concentrations of As(III) are (a) 1.0, (b) 2.0, (c) 3.0, (d) 4.0, and (e) 5.0 mM. The ampoule only contained 3.0 mL of bacterial suspensions (initial OD_{700} = 0.2) and different doses of kaolin without any As(III) were used as the controls.

and bind on kaolin, protecting DNA against degradation or contamination by nucleases but not eliminating the ability of bound DNA to transform to cells [28,29]. This implies that kaolin does not affect normal cell physiological function. Cells can maintain their activity in the presence of kaolin [30]. Table 1 displays the microcalorimetric and CDW data of strain Y35 in the absence and presence of As(III) and/or kaolin at various concentrations. The data show that although As(III) can inhibit the cell physiological functions to

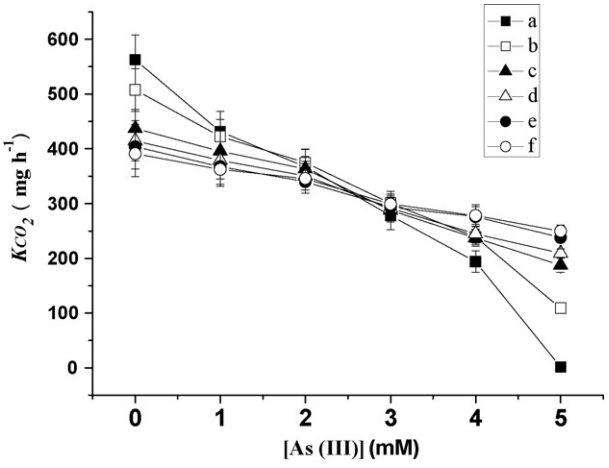

Fig. 3. Cell metabolic activity of strain Y35 in mixed medium. The effect of As(III) concentration on the CO_2 flux rate (k_{CO_2}) at various doses of kaolin: (a) 0.0, (b) 1.0, (c) 2.0, (d) 3.0, (e) 4.0, and (f) 5.0 g L^{-1}.

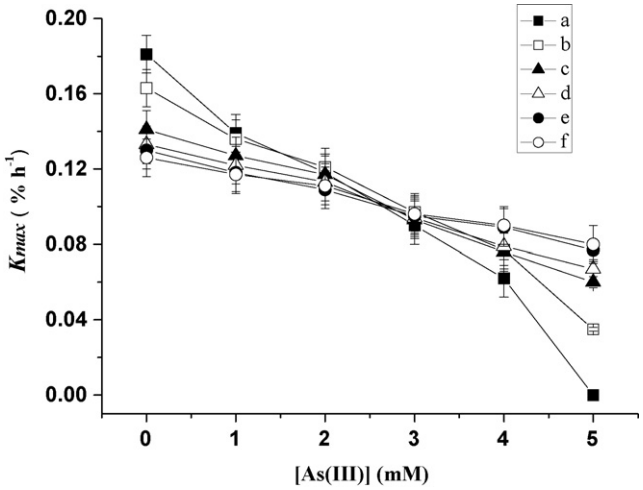

Fig. 4. The effect of As(III) concentration on the maximal protein synthesis rate (k_{max}) at various doses of kaolin: (a) 0.0, (b) 1.0, (c) 2.0, (d) 3.0, (e) 4.0, and (f) 5.0 g L^{-1}.

Table 1
The cell growth parameters of strain Y35 (initial OD_{700} = 0.2) at the log phase growth of the cells at 28 °C.

Samples		Microcalorimetric method				CDW method		
		Q_{total} (J mL^{-1})	k (h^{-1})	I (%)	r^2	k_{cell} (h^{-1})	I (%)	r^2
(A) Y35 + 0.0 g L^{-1} kaolin								
As(III) (mM)	0.0	2.383	0.248	0.00	0.996	0.238	0.00	0.986
	1.0	1.449	0.195	21.13	0.950	0.177	25.64	0.876
	2.0	1.133	0.163	34.02	0.976	0.154	35.42	0.909
	3.0	0.970	0.123	50.42	0.954	0.117	50.73	0.830
	4.0	0.507	0.088	64.36	0.980	0.079	66.72	0.924
	5.0	0.042	0.001	99.60	0.919	0.00	100	0.855
(B) Y35 + 1.0 g L^{-1} kaolin								
As(III) (mM)	0.0	1.988	0.226	8.566	0.980	0.212	11.12	0.924
	1.0	1.612	0.190	23.23	0.984	0.175	26.61	0.939
	2.0	1.395	0.180	27.35	0.994	0.144	39.40	0.978
	3.0	0.999	0.138	44.40	0.996	0.122	48.72	0.986
	4.0	0.541	0.111	55.11	0.990	0.095	59.97	0.963
	5.0	0.158	0.051	79.56	0.998	0.044	81.70	0.994
(C) Y35 + 2.0 g L^{-1} kaolin								
As(III) (mM)	0.0	1.919	0.204	17.54	0.978	0.174	27.15	0.917
	1.0	1.523	0.183	26.14	0.970	0.159	33.24	0.887
	2.0	1.356	0.178	28.20	0.976	0.136	42.76	0.909
	3.0	0.964	0.137	44.81	0.950	0.112	52.96	0.816
	4.0	0.507	0.106	57.33	0.912	0.099	58.46	0.893
	5.0	0.191	0.071	71.39	0.895	0.091	61.98	0.800
(D) Y35 + 3.0 g L^{-1} kaolin								
As(III) (mM)	0.0	1.699	0.191	22.99	0.982	0.168	29.71	0.932
	1.0	1.432	0.180	27.31	0.974	0.148	38.02	0.902
	2.0	1.336	0.170	31.39	0.984	0.134	43.98	0.939
	3.0	0.922	0.130	47.64	0.972	0.122	48.76	0.894
	4.0	0.509	0.110	55.56	0.938	0.101	57.53	0.876
	5.0	0.227	0.080	67.64	0.950	0.100	58.04	0.816
(E) Y35 + 4.0 g L^{-1} kaolin								
As(III) (mM)	0.0	1.677	0.189	23.56	0.966	0.160	32.94	0.873
	1.0	1.432	0.173	30.06	0.944	0.144	39.45	0.896
	2.0	1.331	0.162	34.38	0.972	0.131	44.86	0.894
	3.0	0.917	0.130	47.39	0.948	0.123	48.26	0.809
	4.0	0.546	0.117	52.61	0.938	0.121	49.31	0.876
	5.0	0.256	0.090	63.84	0.956	0.116	51.53	0.837
(F) Y35 + 5.0 g L^{-1} kaolin								
As(III) (mM)	0.0	1.761	0.188	24.20	0.954	0.151	36.84	0.830
	1.0	1.449	0.174	29.66	0.970	0.139	41.59	0.887
	2.0	1.344	0.170	31.15	0.982	0.129	45.99	0.932
	3.0	0.933	0.134	46.06	0.972	0.125	47.71	0.894
	4.0	0.581	0.120	51.35	0.962	0.120	49.77	0.858
	5.0	0.318	0.098	60.32	0.986	0.117	50.99	0.947

a certain extend, the cell heat flow rates can be even higher when higher dosages of kaolin are used. For instance, the Q_{total} and k are 0.318 J mL^{-1} and 0.098 h^{-1} under the highest kaolin concentration (5.0 g L^{-1}) and As(III) dose (5.0 mM) as compared to 0.042 J mL^{-1} and 0.001 h^{-1} at 5.0 mM As(III) without kaolin, respectively. Similarly, the k_{CO_2} and k_{max} data (Figs. 3 and 4) demonstrate that kaolin can help maintain the microbial activity in the presence of As(III). In addition, the highest thermal effect outputs are extended and some of the peaks appear in an earlier stage with increasing concentration of mixed As(III) and kaolin (Fig. 2). Our results prove that kaolin can remove the toxicity of As(III) to microorganisms and maintain its growth in As(III)-contaminated environment.

3.3. Adsorption of arsenic(III) on kaolin with strain Y35

In order to study the earlier thermal output peaks, detoxification role of microorganism, and kaolin and strain Y35 in the mixed medium, the adsorption capacity of kaolin for As(III) was investigated and shown in Fig. 5. Several possible mechanisms for metal biosorption have been scrutinized and systematic studies of the binding of heavy metals to biomass and protein, particularly bacterial and fungal cell, have been reported in the literature [31,32]. The biosorption gradually becomes smaller when the cell metabolic activity has been inhibited by As(III). In this work, the adsorption of mixed adsorbent (*i.e.*, kaolin and strain Y35) was compared and displayed in Fig. 5A (without strain Y35) and Fig. 5B (with strain Y35). The adsorption of kaolin for As(III) significantly increases with increasing kaolin loading. The As(III)-adsorption of kaolin at 5.0 g L^{-1} also increases with the increase in As(III) concentration in the aqueous phase, possibly more kaolin are available to adsorb As(III) under high kaolin loading. In the presence of mixed adsorbent (kaolin and strain Y35), the As(III)-adsorption is remarkably enhanced (Fig. 5B) as compared to sole kaolin under the same As(III) dose (Fig. 5A). These results indicate that kaolin is the main adsorbent in the mixed adsorption and strain Y35 may play certain assisting role in the As(III)-adsorption process. In addition, the toxicity of metal (in here, As(III)) to microorganisms was lessened with the use of kaolin. Actually, some reports claimed that the adsorption capacity of kaolin or clay mineral mainly depends on the surface chemistry structure of the mineral, *i.e.*, crystal edges or broken bonds and is irrelevant to toxicant concentration [5]. As this adsorption process mainly occurs in the early stage of incubation with some thermal outputs, this is why these peaks occur in the early stage as shown in Fig. 2.

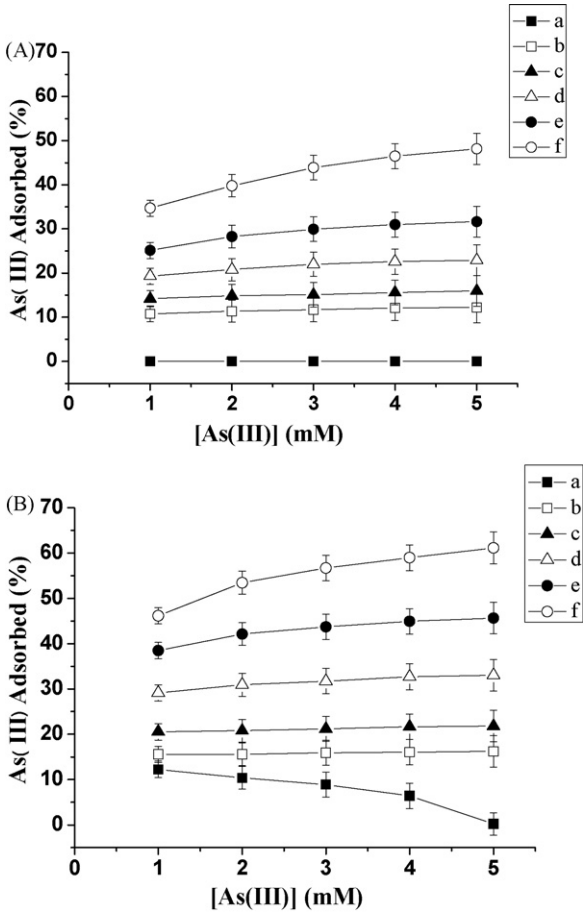

Fig. 5. The effect of As(III) concentration on the As(III) adsorption (A) without and (B) with strain Y35 at various doses of kaolin (a) 0.0, (b) 1.0, (c) 2.0, (d) 3.0, (e) 4.0, and (f) 5.0 g L^{-1}.

Furthermore, the adsorption characteristics of kaolin without and with strain Y35 from the Freundlich isotherm equation are listed in Table 2. Table 2 shows that the adsorption capacity and adsorption intensity for As(III) are indeed higher with the use of both kaolin and strain Y35 than that of sole kaolin. It is obvious that the adsorption capacity of kaolin for As(III) can be enhanced in conjunction with strain Y35, demonstrating their potential use for the removal of As(III) in contaminated groundwater.

Table 2
The Freudnlich equation parameters for As(III) adsorption on mixed adsorbents (a) without and (b) with strain Y35 at 28 °C.

Samples		Freundlich		
		K_f	$1/n$	r^2
(a) Without strain Y35				
Kaolin (g L^{-1})	1.0	2.314 ± 0.079	0.886 ± 0.048	0.840
	2.0	2.022 ± 0.085	0.435 ± 0.044	0.815
	3.0	1.831 ± 0.080	0.309 ± 0.021	0.790
	4.0	1.503 ± 0.068	0.181 ± 0.017	0.877
	5.0	1.348 ± 0.030	0.144 ± 0.036	0.910
(b) With strain Y35				
Kaolin (g L^{-1})	1.0	2.804 ± 0.103	0.985 ± 0.048	0.912
	2.0	2.417 ± 0.092	0.444 ± 0.040	0.901
	3.0	2.205 ± 0.079	0.377 ± 0.031	0.982
	4.0	1.774 ± 0.074	0.242 ± 0.022	0.926
	5.0	1.487 ± 0.075	0.178 ± 0.014	0.854

The standard errors are estimated at $p < 0.05$.

3.4. Comparison of microcalorimetric to cell dry weight methods

The coefficient correlations (r^2) obtained from the microcalorimetric method (>0.895) are higher than from the CDW method (>0.800), inferring that microcalorimetry is a better method (Table 1). At the same time, smaller growth activity has not been detected by the CDW method under the highest As(III) concentration (5.0 mM). For example, the Q_{total} and k obtained from the microcalorimetric method are not 0.0 while the k_{cell} determined by the CDW method is 0.0. In addition, Fig. 3 shows that k_{CO_2} is 1.137 mg h^{-1} whereas the protein synthesis is 0.0 (Fig. 4) at 5.0 mM As(III). These results infer that microcalorimetric and CO_2 sensor methods can provide online, precise and more accurate data [33,34] as compared to the traditional CDW method.

4. Conclusion

In summary, it is observed that *Halobacillus* sp. Y35 can survive in high salt and alkaline conditions via the 16S rRNA sequence and other experimental analyses. This study successfully evaluates the effect of As(III) on *Halobacillus* sp. Y35 cell metabolic activities and heat flux by continually monitoring the microbial cell growth, CO_2 flux and maximal protein synthesis rates. In order to investigate the mixed adsorption of As(III) on *Halobacillus* sp. Y35 and kaolin, the microbial physiological response and biosorption function were determined. Our experimental design and method can reflect the reaction process of *Halobacillus* sp. Y35 cell against As(III) pollution in high-salt medium. This opens new possibilities for the research of mixed adsorption of other toxic metals using combined microbial growth and mixed adsorption processes. Moreover, our work can provide useful information for As(III) control, transport and removal in geochemical and environmental applications by mixed mineral adsorption and biosorption techniques.

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