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Expression of Arsenic Regulatory Protein in *Escherichia coli* for Selective Accumulation of Methylated Arsenic Species

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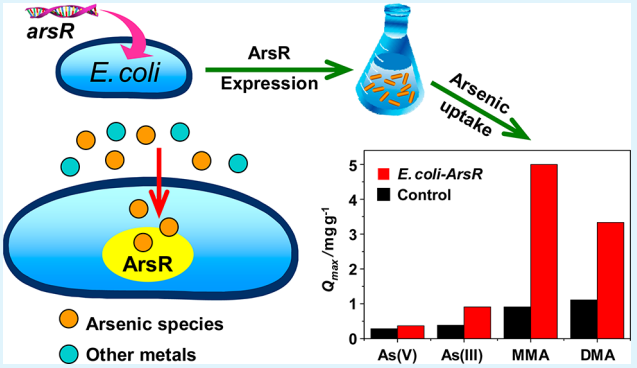
Supporting Information

ABSTRACT: ArsR is a metalloregulatory protein with high selectivity and affinity toward arsenic. We hereby report the expression of ArsR in *Escherichia coli* by cell engineering, which significantly enhances the adsorption/accumulation capacity of methylated arsenic species. The ArsR-expressed *E. coli* cells (denoted as *E. coli-ArsR*) give rise to 5.6-fold and 3.4-fold improvements on the adsorption/accumulation capacity for monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), with respect to native *E. coli* cells. The uptake of MMA and DMA by the *E. coli-ArsR* is a fast process fitting Langmuir adsorption model. It is interesting to note that the accumulation of methylated arsenic is virtually not affected by the presence of competing heavy-metal species, at least 10 times of Cd(II) and Pb(II) are tolerated for the adsorption of 1 mg L^{-1} methylated arsenic. In addition, an ionic strength of up to 2 g L^{-1} Na^+ poses no obvious effect on the sorption of 1 mg L^{-1} MMA and DMA. Furthermore, the accumulation of MMA and DMA is less sensitive to the variation of pH value, with respect to the blank control cells. Consequently, 82.4% of MMA and 96.3% of DMA at a concentration of 50 μg L^{-1} could be readily removed from aqueous medium by 12 g L^{-1} of *E. coli-ArsR*. This illustrates a great potential for the *E. coli-ArsR* for selective remediation of methylated arsenic species in waters, even in the presence of a high concentration of salts.



KEYWORDS: *metalloregulatory protein, ArsR, genetically engineering, methylated arsenic, accumulation, remediation*

INTRODUCTION

Arsenic is a naturally occurring carcinogenic metalloid, and arsenic contamination of drinking water has posed significant health risks to millions of people worldwide.[1] South Asia (e.g., Bangladesh and the Gangetic delta in particular) has been seriously afflicted with natural groundwater arsenic poisoning (>600 000 with diagnosed arsenicosis, >20 million at risk) and the World Health Organisation has labeled it as the greatest environmental calamity in recorded history.[2,3]

It is widely documented that arsenic is found mainly in the inorganic forms (arsenite As(III) and arsenate As(V)) and organic forms (organic monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and others) in environmental waters. Although inorganic arsenic is the predominant existing form of arsenic in natural water, the presence of high concentrations of organic arsenic species has also been reported; 53%−60% of the total dissolved arsenic in river and estuarine waters in southwestern Spain are found to be methyl arsenic compounds.[4] It is also reported that several lakes and estuaries in California were contaminated with methylated arsenic, which accounts for 24% of the total dissolved arsenic.[5] Methylated arsenic species are introduced into the environment mainly due to the industrial and agricultural activities. MMA and DMA are extensively used as weedicides and defoliation agents in cotton fields in the United States and caused problematic contamination in soils and natural waters.[6] In common sense, organic arsenic species are often regarded as less toxic than their inorganic counterparts. However, recent studies have indicated that methylated arsenic is also highly carcinogenic, and some of the methylated arsenic species are even more toxic than inorganic arsenic.[7] In this respect, the effective remediation of organic arsenic is urgently required. However, there are so far only very limited reports on the removal of organic arsenic species, especially for MMA and DMA.[7−10] It is well-known that iron has a very high affinity to arsenic; therefore, iron-based sorbents, which are conventionally used for the uptake of inorganic arsenic, have been applied for the removal of methylated arsenic with a certain extent of success.[7,11−14] TiO_2 has also been employed for the adsorption of methylated arsenic species, followed by their photocatalytic degradation into inorganic arsenic.[8,15,16] Nevertheless, these sorbent materials generally exhibit less affinity toward organic arsenic species, with respect to inorganic arsenic species.[7]

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As a promising alternative of traditional remediation methods, bioremediation based on the genetically engineered bacteria provides green and effective approaches, and it has been predicted to have an increasing role in the removal of various metals, especially arsenic.[17] Various proteins, e.g., phytochelatins (PC) and arsenic-chelating metallothionein (fMT) found in marine alga,[18,19] have been expressed to improve the accumulation of arsenic by microbes. The expression of phytochelation synthase has been reported to enhance the accumulation capacity of arsenic by increasing the PC production.[20,21] Many microbes evolve resistance mechanisms to heavy metals via the expression of a series of metalloregulatory proteins.[22,23] The regulatory protein ArsR has been utilized to control the expression of proteins responsible for the detoxification of arsenic.[24−26] *Escherichia coli* ArsR could bind specifically toward arsenic and discriminate effectively against phosphate, nitrate, carbonate, sulfate, and cadmium.[27] Because of the high binding selectivity and affinity toward arsenic, ArsR has been used for the design of arsenic cell sensors and the remediation of inorganic arsenic.[27−29] However, ArsR has never been exploited for its potentials in the uptake of organic arsenic species and their removal or remediation.

We hereby report a novel remediation protocol for methylated arsenic species by the expression of arsenic selective metalloregulatory protein ArsR in engineered *E. coli* cells. The accumulation performance for organic arsenic has been thoroughly evaluated by regulating the arsenic uptake rate, accumulation isotherm, the pH dependence uptake, the tolerance of ionic strength, and coexisting heavy-metal species. The engineered *E. coli* cells show significant improvement on the accumulation capacity toward both inorganic and organic arsenic species with high selectivity and affinity. Importantly, the ArsR-expressed *E. coli* cells have the potential to remove organic arsenic species, especially methylated arsenic.

EXPERIMENTAL SECTION

Materials. All chemicals used are at least of analytical reagent grade (G.R.) and obtained from Sinopharm Chemical Reagent Co. (Shanghai, China), unless otherwise specified. 1000 mg L^{-1} of stock solutions of various arsenic species are prepared by dissolving appropriate amounts of sodium arsenite, disodium hydrogen arsenate heptahydrate, MMA and DMA (Wako Pure Chemical Industries, Ltd., Japan) in deionized water. The acidity of the sample solutions is controlled at pH 6 by regulating with 0.1 mol L^{-1} HNO_3 and/or 0.1 mol L^{-1} NaOH. Deionized (DI) water of 18.2 MΩ cm is used throughout.

Strains and Plasmids. In the present study, *E. coli* DH5α was used for plasmids multiplication, and *E. coli* BL21(DE3) was adopted for the expression of metalloregulatory protein, followed by performing uptake of arsenic species of interest. Plasmid pET30a was employed to insert the arsR gene for protein expression.

To construct an expression vector for ArsR, the arsR gene was retrieved from plasmid pMUTin-23 (donated by Dr. Sylvia Daunert, University of Kentucky, Lexington, KY, USA).[30,31] by PCR using the primers arsR for, 5′-GGGAATTCCATATGCTGCTACGGAAATATGAAC-3′, and arsRback, 5′-GCCAAGCTTCATAATAACTCCATGTTC-3′. The amplified fragment was doubly digested with NdeI and Hin dIII and inserted into the corresponding sites in pET30a, creating *pET30a-ArsR*. The map of *pET30a-ArsR* is shown in Figure 1A. The resulting plasmid was transformed into *E. coli* BL21(DE3) for the expression of ArsR and carrying out the ensuing studies.

PCR Analysis of the Recombinant Strains. The positive transformants of *E. coli* BL21(DE3) bearing plasmid pET30a-ArsR were selected to propagate in LB medium with 50 mg L^{-1} of kanamycin and their plasmids were extracted by using an AxyPrep

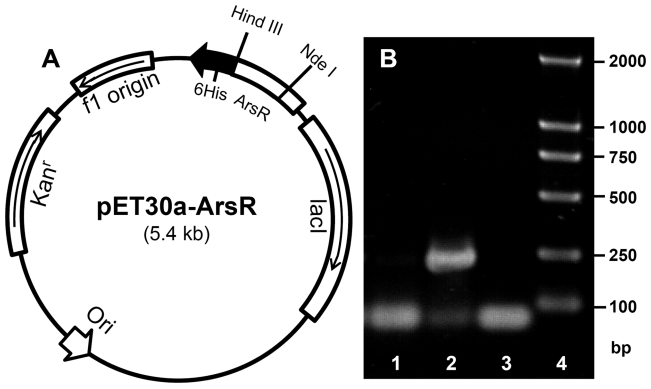

Figure 1. (A) Plasmid map of *pET30a-ArsR*; (B) PCR analysis of the recombinant strains by agarose gel electrophoresis. Lane 1: plasmid pET30a as the template; Lane 2: plasmid pET30a-ArsR as the template; Lane 3: blank as the template; Lane 4: DNA marker DL 2000. Agarose gel: 2%.

plasmid Miniprep Kit (Axygen Biotech., Hangzhou, PRC), according to the manufacturer's instructions for PCR identification. PCR amplifications were performed with arsRfor and arsRback (as described in the previous section) as primers and the plasmid DNA of transformant as the template. The reaction temperatures and times were controlled as in the following: step 1, 94 °C for 3 min; step 2, 94 °C for 30 s, followed by 52.7 °C for 30 s, and then 72 °C for 30 s, and the reactions are repeated for 30 cycles; step 3, 72 °C for 5 min. The PCR fragments were analyzed by 2% agarose gel electrophoresis.

Strain Cultivation and Protein Expression. *E. coli* BL21(DE3)-bearing plasmid *pET30a-ArsR* was precultured aerobically for overnight at 37 °C with shaking at 200 rpm. It was then used to inoculate 100 mL of LB medium and 50 mg L^{-1} of kanamycin. The *E. coli* BL21(DE3) cells bearing plasmid *pET30a-ArsR* were grown until the optical density or absorbance of the cells at a wavelength of 600 nm (OD_{600}) reached 0.6. Afterward, 1 mM of isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce the protein expression. The cells were allowed to grow for a further period of 12 h at 26 °C and then harvested by centrifugation at 5000 rpm for 5 min. The *E. coli* cells expressed with ArsR were stored at 4 °C for further experiments.

The cell pellet was resuspended in 5 mL of lysis buffer (50 mM $NaH_2PO_4{\cdot}2H_2O$, 300 mM NaCl, 2 mM 2-mercaptoethanol (BME), pH 8.0). After being disintegrated by sonication at 150 W for 4 s (with 10 s intervals), the mixture is centrifuged at 12 000 rpm for 30 min. The following experiments were conducted to identify whether the regulatory protein has been expressed as desired:

(1) The protein left in the supernatant was purified and concentrated by adsorbing onto a Ni-NTA-Sefinose Column (Bio Basic, Inc., Canada) followed by stripping with a small amount of eluting buffer (50 mM $NaH_2PO_4{\cdot}2H_2O$, 300 mM NaCl, 250 mM imidazole, pH 8.0);
(2) The cell extract supernatant and the purified/concentrated protein were thereafter analyzed by 15% SDS-PAGE with Coomassie Blue R-250 staining.

The details for SDS-PAGE electrophoresis are shown in the following sections.

Arsenic Accumulation and Removal by the ArsR Expressed *E. coli* Cells. *E. coli* BL21(DE3)-bearing plasmid *pET30a-ArsR* were harvested and resuspended in a LB medium with 50 mg L^{-1} of kanamycin controlled at 30 °C. For the ensuing arsenic accumulation studies, a concentration of ArsR expressed *E. coli* cells of 60 mg L^{-1} (calculated by dry cell weight) is used, and the experiments were conducted as described in the following. Portions of 100 μL of cell suspension (~6 mg) were added into a series of 2-mL centrifuge tubes. The cells were separated from the mixture by centrifuging at 4000 rpm for 5 min, followed by carefully removal of the supernatant. Then, 1000 μL of sample solutions containing appropriate amount of arsenic

species were added into the centrifuge tubes for arsenic adsorption by agitating at 30 °C for 1 h. *E. coli* cells harboring *pET30a* without ArsR expression were used as a blank control. The amount of arsenic removed by *E. coli* cells was calculated as $(C_0 - C)v/m$, where C_0 and C represent the arsenic concentration of the supernatant before and after cell accumulation and adsorption, respectively; v represents the volume of arsenic solution; and m represents the dry weight of the *E. coli* cells used.

An atomic absorption spectrophotometry (ETAAS) system (Model SP-3802AAPC, Shanghai Spectrum Instruments, PRC) was used for the determination of arsenic with an arsenic hollow cathode lamp (193.7 nm, Research Institute of China Electronics Technology Group Corporation) being used as a light source operated at 6.0 mA with a 0.7 nm slit width. Pyrolytically coated graphite tubes with L′vov platforms were used with the temperature program for the detection of arsenic as following: drying at 120 °C (10 s/10 s ramp/holding time), pyrolysis at 700 °C (10 s/15 s), atomization at 2500 °C (0 s/3 s), and cleaning at 2600 °C (0 s/3 s). Other heavy metals such as Pb, Cd, Cr, and Cu were also determined using the Model SP-3802AAPC ETAAS system with hollow cathode lamps (Research Institute of China Electronics Technology Group Corporation) with the operating parameters as detailed in Table S1 in the Supporting Information.

RESULTS AND DISCUSSION

Verification of the Recombinant Strains by PCR Amplification. As can be seen in Figure 1B, the PCR products of *pET30a-ArsR* isolated from the positive clone shows a clear band at ∼250 bp, which corresponds to the inserted 210-bp arsR gene segment of *B. subtilis*. This clearly demonstrated the successful insert of the arsR segment into the recombinant *E. coli* harboring *pET30a-ArsR*. In addition, the gene sequencing was also conducted to further confirm the constructed *pET30a-ArsR*.

Expression of ArsR and Protein Analysis. *E. coli* cells harboring plasmid *pET30a-ArsR* and *pET30a* were growing to exponential phase and then induced to express proteins by adding IPTG as the inducer. The cells were further disintegrated by sonication for intracellular protein analysis, using SDS-PAGE electrophoresis. It is seen from Figure 2A (Lane 1, as indicated with an arrow) that, in comparison with the blank control, e.g., *E. coli* cells harboring *pET30a* without ArsR expression, *E. coli* harboring *pET30a-ArsR* (hereafter referenced as *E. coli-ArsR*) exhibits an extra protein band with a molecular weight of 10.3 Da.

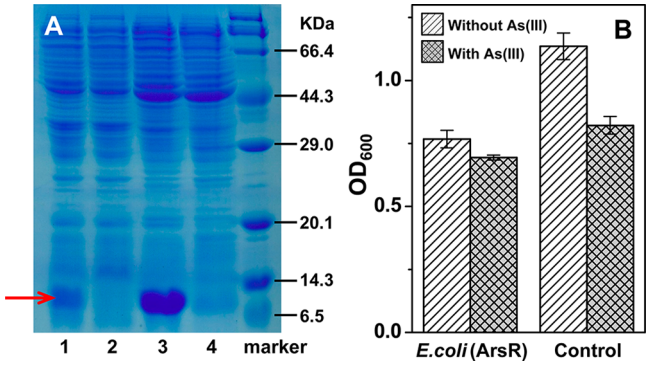

Figure 2. (A) Protein analysis by SDS-PAGE electrophoresis. Lane 1: cell lysate of *E. coli* (*pET30a-ArsR*) with ArsR expression; Lane 2: cell lysate of blank control cell; Lane 3: roughly purified cell lysate of *E. coli* (*pET30a-ArsR*) by a Ni-NTA column; Lane 4: roughly purified cell lysate of blank control cell by a Ni-NTA column. (B) The enhanced resistance of *E. coli* against arsenic after ArsR expression.

The *pET30a* is a fusion protein-expression plasmid, resulting in the foreign protein fused to a 6His tag for the purpose of facilitating protein purification. Thus, the recombinant regulatory protein ArsR expressed by *E. coli-ArsR* has a 6His tag and could be selectively purified by the Ni-NTA affinity column. In order to further demonstrate the correct expression of the recombinant ArsR protein, cell lysate of *E. coli-ArsR* was roughly purified by flowing through the Ni-NTA column and concentrated by elution with a small amout of imidazole buffer, followed by performing electrophoresis, as illustrated in Figure 2A (Lane 3). The heavy blue band at 10 KDa clearly indicated the preconcentration of the ArsR as expected, confirming the correct expression of the recombinant ArsR protein.

Enhancement of Arsenic Resistance by ArsR Expression. *E. coli-ArsR* cells were first allowed to grow in a LB medium with 50 mg L^{-1} of kanamycin until OD_{600} reaches 0.5. Afterward, appropriate amount of IPTG was added and the cells were divided into two equal portions. One portion of the cells was cultured in the medium with 200 mg L^{-1} of arsenite, while the other portion was used to grown in the absence of arsenic. In the meantime, a control blank was conducted for each case by following the same procedure. After each category of the cells was grown for 4 h, the OD_{600} values were compared and evaluated. As illustrated in Figure 2B, the OD_{600} values of the *E. coli-ArsR* cells is lower than that of the control blank cells, because of the growth inhibition caused by the expression of ArsR. A decrease of the cell density of ∼30% was also observed for the control blank attributed to the toxic effect of As(III) toward the *E. coli* cells without ArsR expression. It is obvious that the *E. coli-ArsR* was less sensitive to arsenic exposure, corresponding to a ca. 10% decrease of the cell density. This indicates that the expression of ArsR clearly enhanced the resistance toward arsenic species.

Bioaccumulation of Methylated Arsenic by the ArsR-Expressed *E. coli* Cells. *Bioaccumulation Isotherms and Kinetics.* The experiments for recording the isotherms of MMA and DMA adsorption/accumulation were carried out at pH 6 under batch conditions by shaking a mixture of 6 g L^{-1} cells and 10−160 mg L^{-1} arsenic species (30 °C, 200 rpm). Figures 3A and 3B illustrate the adsorption/accumulation isotherms of MMA and DMA by both *E. coli-ArsR* and the native *E. coli* cells, showing significant differences of the sorption capacity for either arsenic species after the expression of ArsR. The time course experiments were carried out for the evaluation of the kinetics for MMA and DMA adsorption/accumulation by *E. coli-ArsR* cells. The results illustrate that the uptake of both organic arsenic species are rapid processes (Figure 3C). The uptake equilibrium was approached within ca. 60 and 30 min for MMA and DMA, respectively. A relatively faster accumulation of DMA, with respect to MMA, might be due to the fact that DMA is easier to penetrate through the liposome structures in the cell membrane, because of its increased organic nature that originated from the substitution of a second methyl group.

In all cases, Langmuir adsorption/accumulation behavior is followed well, as shown in Figure 3D. For the adsorbed arsenic concentration at equilibrium (Q_e, expressed in terms of mg As/g sorbent) and the corresponding arsenic concentration in the aqueous medium (C_e), C_e/Q_e, as a function of C_e, results in a linear plot where the reciprocal of the slope results in the maximum binding capacity, while Q_{max} and the slope/intercept ratio gives rise to the sorption binding constant.

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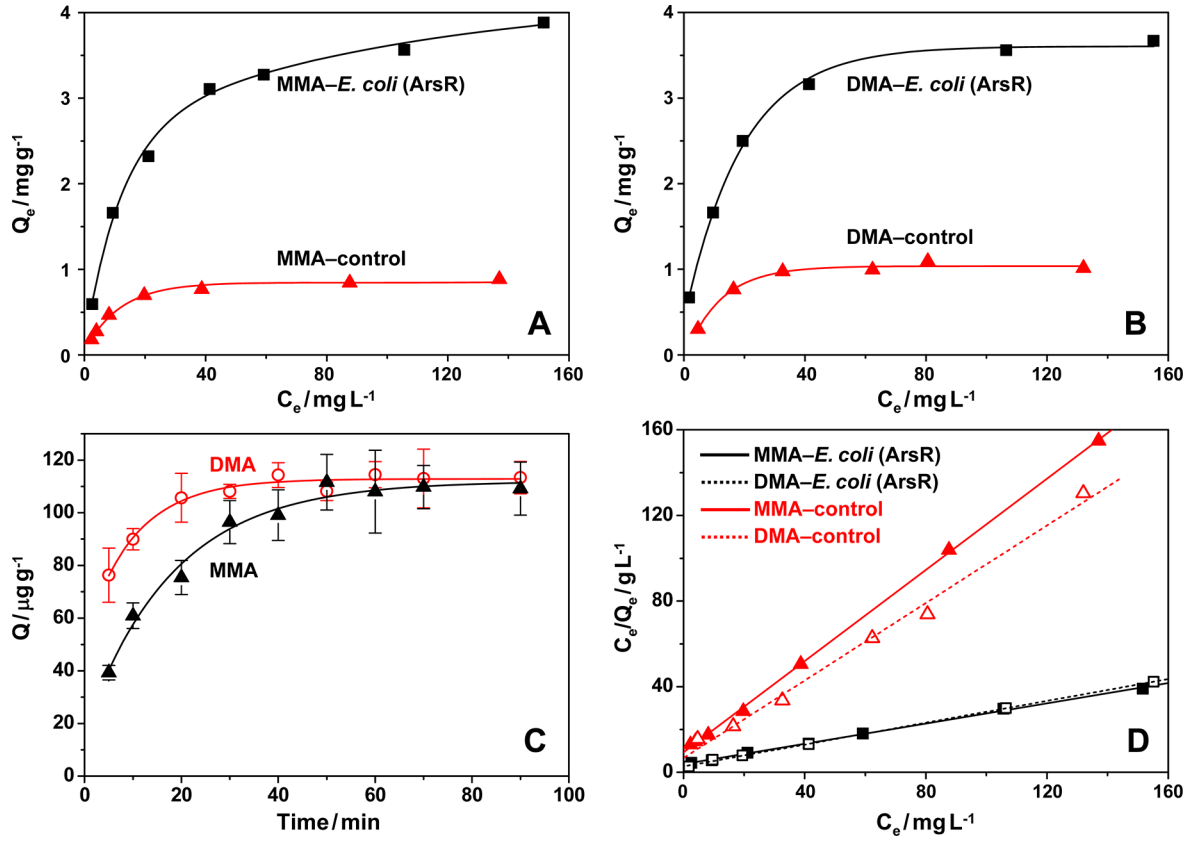

Figure 3. Adsorption/accumulation isotherms for (A) MMA and (B) DMA by *E. coli-ArsR* and the blank control cells. (C) Accumulation kinetics of MMA and DMA (1 mg L^{-1}) by 6 g L^{-1} *E. coli-ArsR* (30 °C, at pH 6). (D) Langmuir isotherm linear fits at 30 °C. (Conditions: 10−160 mg L^{-1} MMA/DMA, pH 6, 6 g L^{-1} *E. coli-ArsR*, incubation time = 1 h.)

The maximum binding capacity of the four arsenic species by the control blank of *E. coli* (without ArsR expression) are derived in the order of As(V) < As(III) < MMA < DMA (see Figure 4). A higher capacity of As(III), in comparison to As(V), may originate from its higher affinity toward the thiol groups of intracellular enzyme and the reductive substances. On the other hand, the methyl substitution results in an increase of the binding capacity for organic arsenic species, with respect to their inorganic counterparts. This observation is opposite to that reported in a recent study, where the methyl replacement leads to a decrease of the binding capacity of arsenic species by iron oxide minerals.[7] The decrease on DMA binding capacity was attributed to the occupation of active binding sites by the two additional methyl groups and the spatial effect. However, in the present case, the binding mechanism may be different. The substitution of methyl group could enhance the hydrophobic/lipophilic interactions between the arsenic species and the organic components inside the cell or on the cell surface, such as proteins, carbohydrates, and liposome.[32,33]

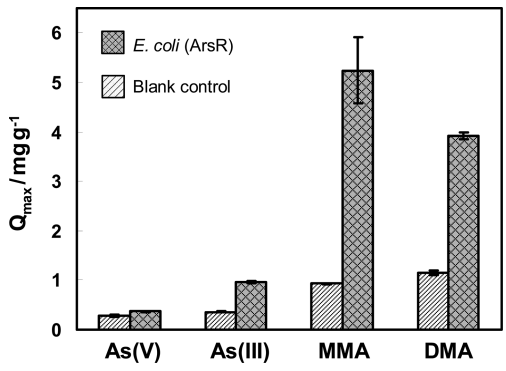

Figure 4. Comparison of the adsorption/accumulation capacity of the arsenic species with sorption by *E. coli-ArsR* and the blank control cells.

With the expression of ArsR, the binding capacity of the arsenic species As(V), As(III), MMA, and DMA, at equilibrium by the engineered *E. coli-ArsR* cells were derived to be 0.37 ± 0.02, 0.95 ± 0.02, 5.24 ± 0.66, and 3.92 ± 0.06 mg g^{-1}, respectively, corresponding to 1.3-, 2.6-, 5.6-, and 3.4-fold improvement, respectively (see Figure 4). It is known that ArsR is specific to the binding of arsenite by forming a pyramidal structure through the three cysteine residues, together with As(III) on the apex.[24,34] Considering that, during the process of adsorption/accumulation, arsenate might be reduced to arsenite by the arsenate reductase inside the *E. coli* cell, it is thus reasonable that the binding capacity for As(V) was also slightly increased after ArsR expression. On the other hand, although MMA and DMA are both pentavalent, the increase of their binding capacity was more pronounced than that for As(V). In previous studies, there was clear evidence showing that MMA(V) and DMA(V) could be reduced to MMA(III) and DMA(III) by arsenate reductase in *E. coli* cells.[35] The trivalent MMA and DMA exhibit higher affinity toward thiol-containing proteins than As(III).[32] This explains the fact that a more pronounced improvement on the accumulation capacity of the methylated arsenic species exists after ArsR expression.

pH-Dependent Accumulation of Arsenic Species. Obvious variations for the adsorption/accumulation of the four arsenic

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species of interest by both *E. coli-ArsR* and the blank control cells were observed with the change of pH values, as illustrated in Figure S1 in the Supporting Information. When considering the control blank, e.g., *E. coli* cells without ArsR expression, virtually no adsorption/accumulation of the arsenic species was observed at pH <2, except for DMA, for which favorable adsorption was encountered within a wide range of pH (2−11). This opposite behavior of DMA might be related to its hydrophobicity, which contributes to the high binding capacity. Meanwhile, it is obvious that a basic medium (pH >7) seems to have a more obvious effect on the uptake of organic arsenic than their inorganic counterparts. A highest adsorption/accumulation capacity was achieved for both MMA and DMA at a pH close to their pK_a values (MMA: pK_{a1} = 4.19, pK_{a2} = 8.77; DMA: pK_a = 6.14).[7] At these pHs, both the methylated arsenic species and *E. coli* cells are negatively charged; thus, electrostatic interaction should not be responsible for arsenic uptake. The accumulation of anionic arsenic species might possibly be the result of active intake by the metabolism process and passive adsorption via hydrophobic interaction and the substitution of the OH− group by the anionic arsenic species.[36] With regard to the ArsR-expressed cells, e.g., *E. coli-ArsR*, roughly similar trends were observed for the pH-dependent adsorption/accumulation of the four arsenic species as those observed for the blank control cells, whereas, in most cases, the variations of adsorption/accumulation of the four arsenic species are not as sensitive as those observed using blank control cells as a sorption medium. In this respect, the expressed ArsR moiety behaves somewhat similar to having a "buffering effect" to the adsorption/accumulation of arsenic species. The less-sensitive binding of arsenic to *E. coli-ArsR* with pH variation in a certain range is important for practical uses. On the other hand, however, the adsorption/accumulation capacities for all the arsenic species were significantly improved, as described in the previous section.

The Dependence of Arsenic Removal on the Biomass Amount. The concentration of arsenic in drinking water is usually very low, e.g., at the μg L^{-1} level; in this respect, it is important and somewhat meaningful to investigate the removal of various arsenic species at low concentrations. In the present study, the possibility of utilizing *E. coli-ArsR* for methylated arsenic removal was exploited by the adsorption/accumulation of 50 μg L^{-1} of MMA and DMA with different amounts of the ArsR-expressed cells. The sorbent was allowed to adsorb/accumulate arsenic species for 1 h and then the removal efficiency was evaluated. Figure S2 in the Supporting Information shows an obvious improvement in the removal efficiency by increasing the amount of the biomass (*E. coli* cells). It was also noted that the improvement in the removal efficiency of DMA is more obvious than that for MMA. When a small amount of the sorbent, e.g., 0.75 g L^{-1} dry weight of *E. coli*, was used, only 44.4% of MMA and 24.3% of DMA were removed. By increasing the sorbent amount to 12 g L^{-1}, favorable removal efficiencies of 82.4% and 96.3% were achieved for MMA and DMA, respectively. These observations indicated that the ArsR-expressed cells, e.g., *E. coli-ArsR*, exhibit great potentials for the remediation of methylated arsenic in drinking waters.

Binding Selectivity toward Arsenic Species. In order to demonstrate the binding selectivity of the ArsR-expressed *E. coli* toward arsenic species, the binding or adsorption of some commonly emcountered heavy-metal species in drinking water, e.g., Cr(III), Cu(II), Cd(II), and Pb(II), was conducted under the same experimental conditions as those for the adsorption of arsenic species. Figure S3 in the Supporting Information illustrated that the accumulation amount of 10 mg L^{-1} for both inorganic and organic arsenic species were significantly enhanced after the expression of ArsR, and 1.4-, 3.1-, 4.9-, and 2.2-fold improvements were recorded for As(V), As(III), MMA, and DMA, respectively. For the case of Cr(III), Cu(II), Cd(II), and Pb(II), no improvement on their sorption was observed by accumulation with *E. coli-ArsR*. However, in contrast, a slight decline on the accumulation capacity was achieved, with respect to those obtained using the blank control cells. Further studies indicated that the presence of a 10-fold amount of of cadmium and lead poses no influence on the effective removal of both inorganic arsenic and methylated arsenic species with accumulation by the engineered *E. coli-ArsR*. This well-illustrated the binding specificity of ArsR toward arsenic species.

In practice, the removal of toxic metal species from water bodies is carried out in the presence of various coexisting anionic and cationic species. From this point of view, it is important to evaluate the potential interfering effect of ionic strength on the adsorption/accumulation of MMA and DMA by the *E. coli-ArsR*. Thus, the sorption of MMA and DMA was carried out in the presence of sodium chloride and its concentration was gradually increased, and the accumulation capacity was evaluated. As illustrated in Figure S4 in the Supporting Information, the presence of up to 3 g L^{-1} Na^+ poses no obvious effect on the accumulation of DMA. While a slight decrease of ∼10% of the accumulation capacity for MMA was recorded with the increase of Na^+ concentration up to 2 g L^{-1}. These results clearly demonstrated that the ArsR-expressed *E. coli* cells have the capability to remediate methylated arsenic pollution in saline water samples.

CONCLUSIONS

In the present study, arsenic regulatory protein ArsR was expressed in *E. coli* cells for developing a selective medium for the accumulation of methylated arsenic species. With respect to the blank control cells, significant improvements on the accumulation capacity were achieved for both MMA and DMA. On the other hand, virtually no change in the accumulation of other coexisting heavy metal species was observed, which well-confirmed the specificity of ArsR toward arsenic species. In addition, the uptake of MMA and DMA by the ArsR-expressed *E. coli* cells is insensitive to the variation of ionic strength up to 2 g L^{-1} Na^+ and the change of pH value within a wide range of 4−11, facilitating selective sorption of methylated arsenic species from waters, even in the presence of high concentration of salts. The successful removal of MMA and DMA at low level by the ArsR-expressed *E. coli* cells demonstrates a great potential for methylated arsenic remediation.

ASSOCIATED CONTENT

Supporting Information

Figures and tables presenting the effects of experimental variables, comparisons of heavy-metal accumulation by *E. coli-ArsR* and the blank control cells, and the operating conditions for ETAAS determination of some metal species are shown in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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