Evaluation of bioaugmentation and biostimulation on arsenic remediation in soil through biovolatilization

Peng Chen^{1,2} · Jin Li^{1,3} · Hong-Yan Wang¹ · Rui-Lun Zheng⁴ · Guo-Xin Sun¹

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Abstract Arsenic (As) removal through microbially driven biovolatilization can be explored as a potential method for As bioremediation. However, its effectiveness needs to be improved. Biostimulation with organic matter amendment and bioaugmentation with the inoculation of genetic engineered bacteria could be potential strategies for As removal and site remediation. Here, the experiments were conducted to evaluate the impacts of rice straw and biochar amendment, inoculation of genetic engineered *Pseudomonas putida* KT2440 (GE *P. putida*) with high As volatilization activity, on microbial mediated As volatilization and removal from three different arseniferous soils. In general, the addition of rice straw (5%) significantly enhanced As methylation and volatilization in comparison with corresponding non-amended soils. Biochar amendments and inoculation of the GE *P. putida* increased As methylation and volatilization, respectively, but less than that of rice straw addition. The effectiveness of As volatilizations are quite different in the various paddy soils. The combined amendments of rice straw and GE

P. putida exhibited the highest As removal efficiency (483.2 µg/kg/year) in Dayu soil, with 1.2% volatilization of the total As annually. The highest water-soluble As concentration (0.73 mg/kg) in this soil could be responsible for highest As volatilization besides the rice straw and bacteria in this soil.

Keywords Arsenic · Remediation · Biovolatilization · Biostimulation · Bioaugmentation · Genetic engineered *Pseudomonas putida* KT2440

Introduction

Arsenic is ranked first on the Super Fund List of Hazardous Substances by the US Environmental Protection Agency (<http://www.atsdr.cdc.gov/SPL/index.html>). Its mobility and toxicity vary greatly among different chemical species. Inorganic arsenate (As(V)) and arsenite (As(III)) are highly toxic in the natural environment (Gong et al. 2002; Akter et al. 2005; Wang et al. 2014a). Transformations of As species in soil usually involve oxidation, reduction, and methylation. The intermediate products of biomethylation are pentavalent methylated arsenicals and trivalent methylated arsenicals. The former include monomethylarsonic acid (MMAs(V)), dimethylarsinic acid (DMAs(V)), and trimethylarsine oxide (TMAs(V)O), which have low toxicities with LD₅₀ of 1800, 1200, and 10,000 mg/kg, respectively (Feldmann et al. 2004). These trivalent methylated arsenical intermediates, monomethylarsonous acid (MMAs(III)) and dimethylarsinous acid (DMAs(III)), are highly toxic, but they could be easily oxidized to less toxic MMAs(V) and DMAs(V). So, methylated As were supposed to be harmless to human. Arsenic transformations are commonly driven by microbes in soil (Bentley and Chasteen 2002; Rhine et al. 2005; Liao et al. 2011). Several studies stated that As can

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✉ Guo-Xin Sun
gxsun@rcees.ac.cn

¹ State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, People's Republic of China

² University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

³ College of Resources and Environment, Yunnan Agricultural University, Kunming 650201, People's Republic of China

⁴ Research and Development Center for Grasses and Environment, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, People's Republic of China

be volatilized from soil into the atmosphere by microorganisms such as fungi, yeast, and bacteria (Cullen and Reimer 1989; Thomas and Rhue 1997; Turpeinen and Pansar 2002; Meyer et al. 2007; Zheng et al. 2013; Wang et al. 2014a, b, 2015). This process involves sequential conversion of the inorganic As into arsines, including monomethylarsine ($\text{AsH}_2(\text{CH}_3)$), dimethylarsine ($\text{AsH}(\text{CH}_3)_2$), trimethylarsine ($\text{As}(\text{CH}_3)_3$), and AsH_3 (Bentley and Chasteen 2002; Turpeinen and Pansar 2002; Qin et al. 2006). Microbial biomethylation of arsenic is believed to be an activation mechanism (Challenger 1945; Cullen and Bentley 2005). The final product of the methylation is volatile $\text{As}(\text{CH}_3)_3$, which is non-toxic (LD_{50} 1060 mg/L) (Cullen and Bentley 2005). The values of LD_{50} for dimethylarsine and arsine were 6 and 0.2–1.6 mg/L, respectively (Cullen and Bentley 2005), and $\text{AsH}_2(\text{CH}_3)$ and $\text{AsH}(\text{CH}_3)_2$ were identified to be potent genotoxic (Mestrot et al. 2013). Considering the dilution effect of air and relatively short half-lives of arsines (about 8 h) (Mestrot et al. 2011b), the release of these volatilized As into atmosphere would not significantly increase their level and would not be harmful to organisms.

The cost-effective in situ biovolatilization of As from contaminated soil may be an alternative for bioremediation technologies (Brown et al. 2003; Hartley et al. 2009). The As removal from arseniferous soil via biological volatilization has only been explored preliminarily (Wang et al. 2014a). However, the amounts of volatile As generated by indigenous microorganisms were limited and the rates of As volatilization were relatively low (Wang et al. 2014a). It is imperative to explore ways to accelerate the rate of in situ volatilization of As for remediation of As-contaminated soil. It has been reported that As methylation and volatilization in soil is a strictly biological process and driven by microbial activity (Turpeinen and Pansar 2002; Zheng et al. 2013; Wang et al. 2014a, b). The rate and efficiency of As methylation and volatilization can be enhanced through biostimulation and/or bioaugmentation (Wang et al. 2014a). Biostimulation aims at enhancing microbial activities to remove As through methylation and volatilization driven by indigenous microorganisms in the site (Gao and Burau 1997). Bioaugmentation is to improve As remediation by inoculating microorganisms with high activities of As volatilization (Edvartoro et al. 2004; Wang et al. 2014a).

The amendments of organic matter, such as cellulose, cattle manure, rice straw, urine, dried distiller grain, and clover, have shown the substantial enhancement of As volatilization, respectively (Edvartoro et al. 2004; Mestrot et al. 2009; Mestrot et al. 2011a; Huang et al. 2012a, b; Jia et al. 2013b). It is quite popular to return rice straw into paddy fields in China, and rice straw has been proved to be effective in eliciting As volatilization compared to other organic matters such as cow dung (Mestrot et al. 2011a). The application of biochar into agricultural fields offers a number of benefits such as increasing fertility, improving soil structure, and providing a better habitat for microorganisms (Glaser et al. 2002; Lehmann et al. 2003;

Lehmann and Rondon 2006), and decreased the emission of greenhouse gases (Spokas et al. 2009; Novak et al. 2010). A few studies have dealt with the impact of biochar amendment on As mobility and transfer in the plant-soil system (Hartley et al. 2009; Namgay et al. 2010; Zheng et al. 2012). However, there is no report on the effects of biochar on As volatilization.

Arsenic volatilization and remediation efficiency could be improved via inoculation of bacteria into soil. The remarkable enhancement of As volatilization in contaminated soil slurries was observed by inoculating a new strain (*Cytophagaceae*) with an As methyltransferase gene (*arsM*) (Huang et al. 2016). Liu et al. successfully expressed an *arsM* gene in *Bacillus idriensis* and *Sphingomonas desiccabilis* accelerating As volatilization from arseniferous soil (Liu et al. 2011). However, the capacity of As volatilization by natural bacteria is limited. Inoculation of genetically engineered bacteria might be a good option for As remediation via biovolatilization. The *arsM*, which is responsible for As methylation and volatilization (Qin et al. 2009; Yin et al. 2011; Lomax et al. 2012), has been successfully overexpressed in *Pseudomonas putida* KT2440 through synthetic biological tool. This genetic engineered *P. putida* KT2440 (GE *P. putida*) showed high activity of As volatilization (Chen et al. 2013, 2014). However, no information is available on the As volatilization by these genetically engineered bacteria in different soils.

In the present study, we chose GE *P. putida* as exogenous strain for bioaugmentation and rice straw (RS) or biochar (BC) as the major substrate for biostimulation to enhance the rate of As methylation and volatilization in three paddy soils. The objectives of the experiments were to (i) determine the effects of RS and BC amendment on As volatilization and (ii) evaluate the efficiency of As volatilization through the inoculation of the GE *P. putida*.

Materials and methods

Preparation of materials and paddy soil

Three different paddy soils were collected from the plowing layer (0–20 cm) in Dayu, Jiangxi Province, and Zhuzhou and Qiyang, Hunan Province, in southern China, respectively. All soils were air-dried, passed through a 2-mm sieve, and stored in the dark before use.

RS was air-dried and ground with a mortar to powder. BC was obtained from dried and milled rice straw by pyrolysis at 500 °C in the absence of oxygen in Muffle furnace (Vulcan3-1750, Neytech, USA) for 4 h (Fellet et al. 2011).

The GE *P. putida* KT2440 was cultured in a 500-mL flask containing 200 mL LB medium (5 L) at 30 °C on the shaker (150 rpm) for 2 days with optical density at 600 nm (OD_{600}) at about 0.8 and harvested by centrifugation ($8000\times g$ for

10 min). The pellets from 5 L culture medium were resuspended with 600 mL sterile deionized water.

Experiments of As volatilization

The apparatus used for trapping volatile As in the laboratory consisted of a ground Erlenmeyer flask (250 mL) attached to a pressure pump and a trapping tube through platinum-cured silicone tubing (Mestrot et al. 2009; Huang et al. 2012a, b). A total of 150 g of air-dried soil were placed into each flask, with the amendment of 5% RS or 5% BC (w/w) in combination with or without the inoculation of 15 mL resuspended bacterial solution and homogenized by hand. The number of inoculated bacteria was about 6.7×10^8 cell/g soil by calculation based on the estimate (10^9 cells/mL for $OD_{600} = 1.0$) (Crawford et al. 2012). The non-amended soil was used as a control. The flasks inoculated with 15 mL resuspended bacterial solution were supplemented with 135 mL deionized water per flask, and others with the addition of 150 mL deionized water (about 2–3 cm layer of standing water). There were triplicate samples for each treatment. All treatments for each soil were as follows:

1. Soil (150 mL deionized water),
2. Soil + BC (applied 5% biochar and 150 mL deionized water),
3. Soil + RS (applied 5% rice straw and 150 mL deionized water),
4. Soil + GE *P. putida* (inoculated 15 mL of GE *P. putida* solution and 135 mL deionized water),
5. Soil + BC + GE *P. putida*, and
6. Soil + RS + GE *P. putida*.

The flasks were air-tight with ground glass stoppers equipped with air inlet and outlet tubes. Polytetrafluoroethylene filters (0.22 μ m, Millipore, USA) were placed on outlets and inlets to minimize possible interference from dust in air. The inlet tube was attached to an air pump (Bldxbly, 3-W power). The trapping tube containing 2 g of silica gel beads was wrapped in aluminum foil to prevent illumination by ambient light and connected to the outlet tube. The silica gel beads were preimpregnated with $AgNO_3$ as described by Mestrot et al. (2009) with a little modification. Briefly, silica gel (1.0–1.5 mm) was kept in 5% HNO_3 overnight, washed with ultrapure water (Millipore Milli-Q), impregnated with 6% $AgNO_3$ solution (w/v) overnight, and then oven-dried at 70 °C to constant weight.

The filtered air was circulated through the flask at a flow rate of 3 L/min at intervals (8 h) for 30 min each time. The flasks were weighed every day; deionized water was added if necessary to maintain the water content. All apparatus were arranged in a completely randomized design in the laboratory at room temperatures (25 °C). This experiment lasted for 3 months and the trapping tubes were replaced monthly with new ones to avoid the inactivation of $AgNO_3$ by reducing gas generated in flask or sunlight. At the end of this experiment, soils were

sampled from each flask for extraction of water-soluble As, which is the most active, mobile, and available fractions.

Sample preparation and chemical analysis

Volatile arsenic

The silica gel in each trapping tube was taken out, homogenized, and divided into two parts. One part was put into 50-mL polyethylene tubes with 2 mL concentrated nitric acid, and kept overnight, and then all samples were digested in a microwave (MARS, Matthew Inc., USA) (Sun et al. 2010). The digested samples were diluted to 50 mL with ultrapure water. The supernatant were filtered by a 0.45- μ m filter (Millipore) and stored at 4 °C until analysis. For quality control, three blanks were prepared at the same time. The concentrations of total As were determined by inductively coupled plasma mass spectrometry (ICP-MS; 7500, Agilent Technologies).

A subset of the samples with high As content was analyzed for As speciation using the other parts of the silica gel. The silica gel was eluted with water and extracted in the same microwave digestion system. The extraction program was as mentioned above. Concentrated H_2O_2 (0.2 mL) was added to oxidize As(III) to As(V) due to the close retention times of TMAO and As(III) from the chromatographic column. Volatile arsine (AsH_3), $AsH_2(CH_3)$, $AsH(CH_3)_2$, and $As(CH_3)_3$ were quantitatively converted to monomethylarsonic acid (MMAs(V)), dimethylarsinic acid (DMAs(V)), and trimethylarsine oxide (TMAsO) during the trapping or digesting process (Mestrot et al. 2009). Arsenic species in the samples were quantified by high performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS; 7500, Agilent Technologies) as previously described (Zhu et al. 2008). Indium (In) was used as an internal standard and all samples were randomized prior to analysis. Arsenic (As^{75}) and indium isotopes and selenium isotopes Se^{77} , Se^{78} , and Se^{82} were measured simultaneously to monitor possible polyatomic argon chloride interferences; no polyatomic interference was observed in all samples. A PRP-X100 10- μ m anion exchange column (250 $\times$ 4.1 mm) (Hamilton, China) connected to a precolumn was used. The mobile phase was a mixture of 10 mM diammonium hydrogenphosphate ($(NH_4)_2HPO_4$) and 10 mM ammonium nitrate (NH_4NO_3), adjusted to pH 6.2 using nitric acid. The mobile phase was pumped through the column isocratically at 1 mL/min and the injection volume was 100 μ L. Arsenic species in the samples were identified by comparing their retention time with the standards, and quantified by external calibration curves with peak areas.

Water-soluble arsenic

Soil samples collected from each flask at the end of the trapping of volatile As were dried and accurately weighed into a

polypropylene centrifuge tube (1 g, 50 mL). Thirty milliliters of double-distilled water was added to each tube and shaken for 16 h. The supernatant was obtained by centrifugation (3000 rpm) for 15 min and filtered through a 0.45- μ m filter (Shiowatana et al. 2001).

Soil properties

The contents of C and N in soils were detected on an element analyzer (Vario EL III, Elementar, Germany). Particle size distribution was determined using Mastersizer 3000 laser particle size analyzer. The pH of soils was measured with a digital pH meter in deionized water using a 1:2.5 (w/w) ratio. The pH of biochar and rice straw was determined in a soil/water ratio 1:20 (w/v) following Cheng and Lehmann (2009). The content of organic matter (OM) in soil was measured by the $K_2Cr_2O_7$ oxidation-reduction titration method. Dissolved organic carbon (DOC) was determined by a total organic carbon analyzer (Liquic TOC, Elementar, Germany).

Soils were digested by AIM-500 Block Digester for determination of total As, Cd, Pb, and Zn concentrations. Briefly, soil samples (0.25 g) were weighed into block digestion tubes (100 mL) with 5 mL aqua regia (HCl:HNO₃ 3:1 v/v) and kept overnight, and heated at 120 °C for 12 h. Then, concentrated HClO₄ (2 mL) was injected to each tube, heated at 140 °C for 24 h. The cooled digests were diluted to 50 mL with ultrapure water and filtered. For quality control, three blanks and three samples of SRM for soil (GBW07405) were prepared at the same time. The concentrations of As, Pb, and Zn were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES), and the Cd concentration was analyzed by ICP-MS.

Statistical analysis and quality control

One-way analysis of variance (ANOVA) using Tukey's test was used to identify significant differences ($P < 0.05$), while Pearson correlations were used to check the relationship between different parameters. Statistical analyses were carried out using SPSS version 16.0 software. The recoveries of As and Cd for soil were 104 and 118%, respectively. To assess the column recovery, the recovery of the sum of species compared to the total As was within 90–110%.

Results

Soils and amendments

The three soils varied widely in total As concentrations with 16.4 mg/kg (Zhuzhou), 40.3 mg/kg (Dayu), and 47.8 mg/kg (Qiyang), respectively (Table 1). The corresponding water-soluble As concentrations were 0.30 mg/kg (Zhuzhou), 0.73 mg/kg (Dayu), and 0.37 mg/kg (Qiyang), accounting

for 1.8, 1.8, and 0.8% of total As concentration in these soils. Soil pH varied from 4.8 to 6.2. DOC of the three soils was relatively low (60–100 mg/kg). RS and BC used in this study as exogenous OM showed a low As concentration at 1.3 and 0.8 mg/kg, respectively. The BC has higher pH but much lower DOC levels than these of RS (Table 1).

Assessment of As volatilization from As-contaminated soil

We measured As concentration in each trapping tube and deduced the averaged flux during 3-month experiment. The fluxes of As volatilization in different soils are quite different with 0.04 μ g/kg/year in Zhuzhou soil, 0.55 μ g/kg/year in Qiyang soil, and 1.11 μ g/kg/year in Dayu soil (Fig. 1). The addition of RS (5% w/w) significantly enhanced As volatilization from the paddy soils. The flux of As volatilization with rice straw was hundreds of times higher than corresponding non-amended soil (control). The high flux of arsines from paddy soils in the presence of rice straw was observed in Dayu soil (195.9 μ g/kg/year), which was much higher than that in Qiyang soil (66.1 μ g/kg/year) and Zhuzhou soil (30.6 μ g/kg/year). The amendment of biochar (5% w/w) alone also showed some positive effect on As volatilization although not significant (Qiyang).

Genetic engineered *P. putida* KT2440 bearing *arsM* gene exhibited high capacity of As volatilization in previous experiments (Chen et al. 2013, 2014). The inoculation of GE *P. putida* KT2440 in soils improved the As volatilization but not significantly except for Zhuzhou soil. The As flux in soil with inoculation of GE *P. putida* KT2440 was in the range of 0.40–1.64 μ g/kg/year, a bit higher than that of control (0.04–1.11 μ g/kg/year). The combined inoculation of BC or RS with bacteria into the soil was used to investigate As volatilization. In the treatment of BC + GE *P. putida*, only the Dayu soil showed a significant stimulation, up to 39.6 μ g/kg/year, which was much higher than non-amended soil (1.11 μ g/kg/year), BC amendment alone (2.18 μ g/kg/year), and GE *P. putida* amendment alone (1.64 μ g/kg/year) (Fig. 1a). With the application of rice straw and GE *P. putida*, arsine fluxes were also the highest in Dayu soil (483.2 μ g/kg/year), followed by Zhuzhou soil (79.3 μ g/kg/year) and Qiyang soil (29.3 μ g/kg/year). The combination of RS + GE *P. putida* significantly enhanced the As flux in different soils except Qiyang soil, which is lower than RS amendment alone (Fig. 1b).

The application of rice straw (soil + RS and soil + RS + GE *P. putida*) resulted in high rates of As volatilization. These samples were analyzed for As speciation. As(CH₃)₃ was proved to be dominant As species (65.9–84.2%), followed by AsH₃ (14.1–30.7%) (Fig. 2). Tiny amount of AsH(CH₃)₂ (0–1.8%) and AsH₂(CH₃) (0–3.4%) were detected as well. Inoculated GE *P. putida* alone slightly (but not significantly) increased the percentage of methylarsines, and reduced the percentage of inorganic arsine (AsH₃) in total volatilized As,

Table 1 Characteristics of soils and organic amendments

Parameters	Soils			Organic amendments	
	Dayu	Qiyang	Zhuzhou	Biochar	Rice straw
Soil classification	Silt loam	Silt	Silt loam	–	–
Sand (%)	25.4	10.2	33.4	–	–
Silt (%)	70.2	81.4	61.6	–	–
Clay (%)	4.3	8.3	5	–	–
pH	5.86	6.18	4.83	10.3	5.64
OM (%)	1.9	3.2	2.8	22.1	24.4
DOC (mg/kg)	66.2 ± 9.1	94.7 ± 11.2	61.9 ± 4.3	41.8 ± 4.6	2113.5 ± 45.4
Total C (%)	1.0	2.3	1.4	43.6	39.9
Total N (%)	0.1	0.3	0.2	1.7	1.0
As (mg/kg)	40.3 ± 0.4	47.8 ± 3.2	16.4 ± 2.0	0.78 ± 0.09	1.39 ± 0.10
Cd (mg/kg)	0.78 ± 0.02	1.32 ± 0.01	4.06 ± 0.14	0.24 ± 0.01	0.15 ± 0.00
Pb (mg/kg)	52.5 ± 1.5	41.2 ± 0.7	909.1 ± 8.9	8.00 ± 0.05	0.97 ± 0.03
Zn (mg/kg)	196.0 ± 1.7	168.0 ± 4.2	1463.2 ± 51.3	–	–
Water-soluble As (mg/kg)	0.73 ± 0.02	0.37 ± 0.02	0.30 ± 0.04	–	–

The value was mean ± SE and $n = 3$

which is reasonable due to high capacity of As methylation and volatilization of GE *P. putida* (Chen et al. 2013, 2014). These results confirm the fact that As(CH₃)₃ is the dominant final product of As methylation, as shown in previous studies (Woolson 1977; Mestrot et al. 2009, 2011a). The production of AsH₃ has also been observed (Cheng and Focht 1979; Meyer et al. 2007; Yuan et al. 2010; Mestrot et al. 2011a), and the amendment of rice straw into soils could improve AsH₃ production (Mestrot et al. 2011a). Arsine is highly toxic as a hemolytic toxicant (Pakulska and Czerczak 2006; Gbaruko et al. 2008). Even low concentration (32 mg/m³) exposure may exert a hazardous or lethal effect (Pakulska and Czerczak 2006). Process for in situ removing arsine from gas should be considered with adsorbents such as cuprous oxide (Quinn et al. 2006; Petit et al. 2010).

Effects of amendments on water-soluble As

The water-soluble As in these soils was tested as bioavailable As to investigate its possible relationship with volatile As (Fig. 3). The water-soluble As concentration was 450–4000 µg/kg, and it was obvious that the levels in Dayu (738–3765 µg/kg) and Qiyang soils (494–3942 µg/kg) were much higher than that in Zhuzhou soil (450–1589 µg/kg) (Fig. 3a–c). Obviously, the amendments of RS, BC, and bacterial inoculation significantly enhanced As solubility in all kinds of soils. For each of the three soils, the treatment of RS with strain exhibited the highest water-soluble As, followed by the treatment of RS. These results showed similar trends with As volatilization, which showed highest As volatile flux in the treatment of RS and/or RS + GE *P. putida* (Fig. 1).

In the water-soluble As, inorganic As is the major As species and methylated As were minor (<5%) in the non-amended or BC amended soil. Although the percents of methylated As have increased in other treatments (amended with RS or inoculated GE *P. putida*) in comparison with control, inorganic As was still predominant As species (>50%). The treatment of RS + GE *P. putida* in Qiyang soil is an exception with the percents of methylated As up to 79% (Fig. 3d–f). Most of the organic As are DMAs(V) and tiny amount of MMAs(V) was detected, while TMAO was not detected. With the inoculation of GE *P. putida* into soil, the As concentration and percentage of methylated As in the solution were generally higher than those without inoculation treatments. There was no clear relationship between methylated As in the water solution and the volatile methylarsines, but a positive correlation ($P < 0.01$) was observed between As(III) or inorganic As in the solution and the amount of volatile AsH₃ (Fig. 4). It is known that As(III) methylation and subsequent volatilization is a strictly biological process and driven by microbial activity through successive reduction and methylation (Bentley and Chasteen 2002; Turpeinen and Pantisar 2002; Qin et al. 2006). AsH₃ can be formed under anoxic conditions, but the mechanism is still unknown (Cheng and Focht 1979; Pakulska and Czerczak 2006; Meyer et al. 2007; Yuan et al. 2010).

Discussion

Arsenic methylation and volatilization in situ, which can be enhanced through biostimulation and bioaugmentation, is considered as the perspective approach to soil remediation (Wang

Fig. 1 Flux of volatilized As during the whole period ($n = 3$). Error bars represent the standard error based on three distinct samples. BC 5% of biochar amendment, RS 5% of rice straw amendment. GE *P. putida* *Pseudomonas putida* KT2440 was endowed by the *Rhodopseudomonas palustris arsM* (*RparsM*) gene. The value was mean $\pm$ SE and $n = 3$

et al. 2014a). In Dayu soil, As volatilization was significantly increased, much higher than that in Qiyang and Zhuzhou soils. Our results showed that soil type is a very important factor influencing As methylation and the removal from soil via volatilization (Table 2). Arsenic volatilization flux was highly related to water-soluble As, its species, or clay. Indigenous microorganisms are major contributor for As volatilization. Studies have shown wide difference of As methylation and volatilization in different soils (Mestrot et al. 2009). These are possibly due to different soil properties, the abundance, biodiversity, and activity of microorganisms responsible for As

Fig. 2 Speciation of the volatilized arsenic in three different paddy soils amended with rice straw, non-inoculated or inoculated GE *P. putida*, expressed as the percentage of the sum of all species ($n = 3$)

volatilization in the different paddy soils (Akins and Lewis 1976; Sanford and Klein 1988; Rhine et al. 2005; Huang et al. 2011; Mestrot et al. 2011a; Chen et al. 2014).

Rice straw addition significantly increased As methylation and volatilization from the investigated paddy soil with a percentage of 0.14–0.49% per year, consistent with previous studies (Mestrot et al. 2011a; Jia et al. 2013a, b) and higher than the results treated with cattle manure, clover, or dried distiller grain (0.07–0.16%) (Mestrot et al. 2011a; Huang et al. 2012a, b). The decomposition of amended RS would introduce large amounts of low molecular weight organic acids into soils, including many functional groups such as $-\text{COOH}$, phenol, catechol, and $-\text{OH}$ (Zheng et al. 2013). These groups lead to the increase of As solubility and bioavailability, which was supported by previous reports (Jia et al. 2013a, b; Wang et al. 2016), though competing with negatively charged As for adsorption sites (Redman et al. 2002; Weng et al. 2009). The increase of bioavailable As might be one possible explanation for stimulation of As volatilization. In addition, RS could serve as an energy source to support the growth of indigenous heterotrophic microbes, including As methylators which facilitate As methylation and volatilization (Mestrot et al. 2009; Solaiman et al. 2009; Thomas et al. 2010; Huang et al. 2012a, b; Jia et al. 2013a, b).

Fig. 3 Arsenic concentration in water solution in paddy soil from **a** Dayu, **b** Qiyang, and **c** Zhuzhou and amended with rice straw (RS) or biochar (BC), inoculated GE *P. putida* ($n = 3$). Arsenic speciation in extractable solution at the end of the incubation experiment, **d** Dayu, **e** Qiyang, and **f** Zhuzhou soils ($n = 3$), expressed as the percentage of the sum of all species. Error bars represent the standard error based on three distinct samples

Biochar addition in paddy soils significantly increased As bioavailability (Fig. 3), which was similar to the significant increase of As concentration in pore water (Zheng et al. 2012, 2015). Biochar addition in soil increased the abundance of Fe-reducing bacteria. The increase of copy numbers in respiratory As reducing (*arrA*) and detoxifying reducing genes (*arsC*) suggested microbial reduction of As(V) adsorbed on Fe minerals to As(III) and increase of As release (Wang et al. 2016). It was acceptable to believe that the inoculation of GE *P. putida* in soil significantly increased the concentration of water-soluble As

(Fig. 3). Because *P. putida* KT2440 has been reported to bear arsenate reductase gene (*arsC*) in its chromosome and rapidly reduce As(V) to As(III) in soil (Chen et al. 2013, 2014).

The GE *P. putida* have exhibited a high capacity for As methylation and volatilization in As-contaminated soil (Chen et al. 2014). When the GE *P. putida* was inoculated alone or combined with BC, the bioaugmentation effect on As volatilization was not significantly improved. The GE *P. putida* is the aerobic bacteria and needs oxygen for growth, but the flooded paddy soils are oxygen limited and BC contains a

Fig. 4 Relationship between the observed volatile fluxes of AsH_3 and the concentrations of **a** As(III) and **b** inorganic As in extractable solution in three different paddy soils amended with rice straw, non-inoculated or inoculated *GE P. putida*, ($n = 3$)

little labile nutrients (Beesley et al. 2011, 2013). Thus, the relatively low nutrients and oxygen limit the bacterial growth and As volatilization. Besides, low As bioavailability in soil constraints the ability of As methylation and volatilization by *GE P. putida*.

Rice possesses unique characteristics in rice root by excreting oxygen and oxidants into the rhizosphere to form an oxidizing microenvironment (Liu et al. 2006; Huang et al. 2012a, b). *P. putida* KT2440 belongs to rhizobacteria that has the capacity to colonize a wide range of plants. *P. putida* KT2440 would be colonized on the rice root or in the rhizospheric soil. The accumulation of inorganic As in rice grain would be reduced because of the decreased level of inorganic As in rhizosphere via As volatilization or methylation of *GE P. putida* KT2440.

In comparison with soil + RS in Qiyang soil, the As flux of soil + RS + *GE P. putida* was decreased (Fig. 1b). It seemed that the presence of *GE P. putida* did not enhance As removal, like other two soils. The Qiyang soil has higher silt and clay content (silt, 81.4%; clay, 8.3%) than those in other soils (Dayu, 70.2 and 4.3%; Zhuzhou, 61.6 and 5.0%, respectively) (Table 1), which means that the Qiyang soil tends to have higher soil compaction and lower gas permeability than other soils (Jenkinson 1981; Groffman and Tiedje 1991). Although much methylated As was exerted in the presence of *GE P. putida* in the treatment of soil + RS + *GE P. putida*, relatively poor soil permeability limited the emission of gaseous As from Qiyang soil (Fig. 1b) and relatively more methylated

Table 2 Correlation analysis of original soil data, water-soluble As in the soil samples after experiments, and As, $\text{As(CH}_3)_3$, and AsH_3 flux in the treatment of soil + RS + *GE P. putida*

	Sand	Silt	Clay	pH	DOC	As	RWSA	RAAs(III)	RAAs(V)	WSA	As flux	$\text{As(CH}_3)_3$ flux	AsH_3 flux
Sand	1												
Silt	-0.992**	1											
Clay	-0.808**	0.725*	1										
pH	-0.888**	0.921**	0.530	1									
DOC	-0.866**	0.823**	0.862**	0.702*	1								
As	-0.875**	0.899**	0.558	0.994**	0.727*	1							
RWSAs ^a	0.023	0.078	-0.488	0.427	-0.249	0.419	1						
RWSAs(III) ^b	0.121	-0.020	-0.562	0.341	-0.318	0.336	0.993**	1					
RWSAs(V) ^c	-0.040	0.142	-0.437	0.480	-0.202	0.469	0.997**	0.981**	1				
WSA ^d	-0.801**	0.850**	0.385	0.983**	0.613	0.976**	0.564	0.484	0.611	1			
As flux	0.275	-0.178	-0.659	0.192	-0.428	0.192	0.958**	0.980**	0.937**	0.341	1		
$\text{As(CH}_3)_3$ flux	0.253	-0.156	-0.643	0.214	-0.410	0.214	0.963**	0.983**	0.943**	0.362	1.000**	1	
AsH_3 flux	0.344	-0.249	-0.706*	0.119	-0.487	0.119	0.938**	0.966**	0.912**	0.271	0.997**	0.995**	1

* $P < 0.05$, ** $P < 0.01$

^a Water-soluble As in original soils

^b Water-soluble As(III) in original soils

^c Water-soluble As(V) in original soils

^d Water-soluble As in soil samples at the end of experiments

As was kept in water (~80%; Fig. 3b) than other soil (25–35%). This is one plausible reason for low As volatilization in Qiyang soil.

Nutrients are enhanced in these soils in the presence of rice straw, which substantially support the bacterial growth, including GE *P. putida* (Mestrot et al. 2009). This may explain why the high efficiency of volatilizing As in the inoculated GE *P. putida* soil amended with RS. The levels of As flux of soil + RS + GE *P. putida* in Dayu soil (483.2 µg/kg/year) were much higher than that in Qiyang soil (29.3 µg/kg/year) and Zhuzhou soil (79.3 µg/kg/year). This may be attributed by the relatively high water-soluble As concentration in soil (Table 1) (Rhine et al. 2005; Mestrot et al. 2011a; Chen et al. 2014).

The highest As flux (483.2 µg/kg/year) was observed in the Dayu soil in the presence of RS and GE *P. putida*, with annual As volatilization of 1.2%, much higher than reported results (Edvantoro et al. 2004; Mestrot et al. 2011a; Huang et al. 2012a, b). These can be developed and applied to manipulate As biovolatilization, both for bioremediation and mitigating the health risks of As accumulated in food crops.

Conclusions

In summary, biostimulation with rice straw amendment and bioaugmentation with GE *P. putida* KT2440 inoculation are potentially feasible strategies for As volatilization and removal in some kinds of agricultural soil. Arsenic emission was significantly enhanced from these paddy soils amended with 5% rice straw or inoculated with the GE *P. putida*. The combination of genetically engineered bacteria with rice straw addition exhibited the highest efficiency of As volatilization (483.2 µg/kg/year) and could be a promising strategy for bioremediation processes of As-contaminated environments.

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