B−51 CHARACTERIZATION OF A CHEMOLITHOAUTOTROPHIC ARSENITE-OXIDIZING BACTERIUM STRAIN B1 FOR REMOVAL OF ARSENIC FROM GROUNDWATER

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1. INTRODUCTION

Over 137 million people in more than 70 countries are affected by toxicity of naturally occurring arsenic (As) in groundwater[1]. Thus, the drinking water standards for arsenic has been set to be 10 μg/L by WHO since 1993. Therefore, development of cost-effective and environmentally friendly treatment processes for arsenic contaminated groundwater is necessary in the world.

The main arsenic forms in groundwater are arsenate As(V) and arsenite As(III). As(III) is more toxic and less adsorptive than the former one[2]. Adsorption using activated alumina/iron hydroxides (Al/Fe) is the most effective and inexpensive method for arsenic removal. However, this method usually needs pre-oxidation of As(III) to As(V) to enhance the overall arsenic removal. Although chlorine, hydrogen peroxide and ozone are effective oxidants, these chemical oxidants are not always useful in practical applications because of their high cost, inefficient As(III) oxidation and generation of undesirable by-products caused by unspecific reactions [3]. In addition, possible inhibitors for the chemical oxidation of As(III) such as ammonium, iron, phosphorus and humic substances are often found in groundwater.

As illustrated in Figure 1, microbial As(III) oxidation is an attractive alternative because of its specific reaction, low costs and environmentally friendly nature. Arsenite-oxidizing bacteria are classified into heterotrophic and chemolithoautotrophic groups (HAOs and CAOs, respectively). Of the two groups CAOs are more beneficial as remediating agents because their use can reduce remediation costs and the occurrence of secondary contamination associated with the need to add external organic carbon compared with HAOs.

Our previous study was undertaken to isolate CAOs that can oxidize As(III) effectively to As(V) without organic carbon[4]. The objective of this study is, therefore, to investigate characteristics of a facultative CAO, *Hydrogenophaga* sp. strain B1[4]. The organic carbon utilization by stain B1 was studied for its mass cultivation although this facultative CAO oxidizes As(III) without any organic carbon. Arsenite oxidation by strain B1 was also investigated under various arsenite concentrations and pH values.

2. MATERIALS AND METHODS

(1) Media

Bacto™ Trypticase Soy Broth (TSB) and a basal salt medium[4] were used to cultivate strain B1. For growth tests, 20 mM of potassium hydrogen phthalate buffer (pH 5.0), phosphate buffer (pH 6.0), HEPES (pH 7.0-8.0) or CHES (pH 9.0) was added to stabilize the pH value.

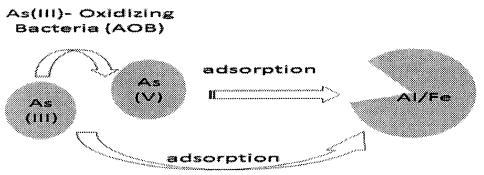

Fig.1. Principles of arsenic removal from groundwater by using As(III) oxidation using AOB and subsequent As(V) adsorption using Al/Fe.

(2) Carbon source utilization tests

Carbon source utilization profiles of strain B1 were determined using Biolog GN2 microplates. Washed cells were suspended in 20 mL 0.85% NaCl solution at OD_{600} of 0.01. Aliquots of 150 μL of the suspensions were transferred to 95 wells on the microplate. After 48-h incubation at 28°C, absorbance at 595 nm (A_{595}) of each well was measured.

(3) Arsenite oxidation tests

Strain B1 was aerobically cultivated in 20 mL of TSB in a 50-mL bottle for 24 hours at 28°C on a rotary shaker at 120 rpm. To examine effects of pH on As(III) oxidation by strain B1, the experiments were done with various pH media, which was prepared as described above. Fresh cells were inoculated into a 300-mL flask containing 100 mL BSM supplemented with 1 mM of As (III) to a final optical density at 600 nm (OD_{600}) of 0.02. To examine effects of the initial As(III) concentration, As(III) was added to BSM at 0.1, 0.5, 1, 5 or 10 mM at pH 6.0 with the bacterial cells inoculated at an OD_{600} of 0.2. The specific As(III) oxidation rate (SAOR) were calculated from the slope of the linearized time course.

3. RESULTS AND DISCUSSION

(1) Carbon source utilization by strain B1

Table 1 shows the carbon source utilization profile of strain B1. Strain B1 can well utilize some of phosphate compounds (d,l-α- glucosephosphate, α-d-glycerol phosphate) and sugar alcohols (xylitol, 2-aminoethanol, 2,3-butaediol), etc. as carbon sources. Those carbon sources can be used for mass cultivation of strain B1.

(2) Effects of intial As(III) concentration on As(III) oxidation by strain B1

Strain B1 took 2, 4, 4, 24 and 48 hours to completedly oxidized 0.1, 0.5, 1, 5 and 10 mM of the initial As(III) concentration, respectively, as shown in Figure 2.

As seen in Figure 3, SAOR increased as the initial As(III) concentration increased from 0.1 to 10 mM, and reached the maximum value of 2.8 micromol As(III)/mg-cell/h at 10 mM of the initial As(III) concentration.

Table 1. Carbon source utilization of strain B1 detected as absorbance of Biolog GN2 microplat after 48 hour incubation

monosaccarides		**carboxylic acids**		**amino acids**		**surfactants**	
N-Acetyl-DGalactosamine	-	Pyruvic Acid Methyl Ester	+	Glucuronamide	-	Tween 40	-
N-Acetyl-DGlucosamine	-	Succinic Acid Mono-Methyl-Ester	-	L-Alaninamide	-	Tween 80	-
L-Arabinose	+	Acetic Acid	+	D-Alanine	-		
D-Fructose	-	Cis-Aconitic Acid	-	L-Alanine	-	**sugar alcohols**	
L-Fucose	-	Citric Acid	-	L-Alanyl-glycine	-	Adonitol	-
D-Galactose	-	Formic Acid	-	L-Asparagine	-	D-Arabitol	-
D-Mannose	-	D-Galactonic Acid Lactone	-	L-Aspartic Acid	-	i-Erythritol	-
β-Methyl-D-Glucoside	-	D-Galacturonic Acid	-	L-Glutamic Acid	+++	m-Inositol	+
D-Psicose	-	D-Gluconic Acid	+	Glycyl-Laspartic Acid	-	D-Mannitol	-
L-Rhamnose	-	D-Glucosaminic Acid	-	Glycyl-L-Glutamic Acid	-	D-Sorbitol	-
α-D-Glucose	-	D-Glucuronic Acid	-	L-Histidine	-	Xylitol	+++
disaccarides		α-Hydroxybutyric Acid	+++	Hydroxy-LProline	-	2-Aminoethanol	+++
D-Cellobiose	+++	β-Hydroxybutyric Acid	+++	L-Leucine	-	2,3-Butanediol	+++
Gentiobiose	-	γ-Hydroxybutyric Acid	+++	L-Ornithine	-	Glycerol	+
α-D-Lactose	-	p-Hydroxy Phenylacetic Acid	+	L-Phenylalanine	-		
Lactulose	-	Itaconic Acid	-	L-Proline	-	**nucleotides**	
Maltose	-	α-Keto Butyric Acid	+++	L-Pyroglutamic Acid	-	Inosine	-
D-Melibiose	-	α-Keto Glutaric Acid	-	D-Serine	-	Uridine	+++
Sucrose	-	α-Keto Valeric Acid	-	L-Serine	++	Thymidine	-
D-Trehalose	-	D,L-Lactic Acid	+	L-Threonine	+		
trisaccarides		Malonic Acid	-	D,L-Carnitine	+	**amines**	
Turanose	-	Propionic Acid	+++	γ-Amino Butyric Acid	-	Phenyethylamine	-
polysaccarides		Quinic Acid	+++	Urocanic Acid	+	Putrescine	+
D-Raffinose	-	D-Saccharic Acid				**phosphate compounds**	
α-Cyclodextrin	-	Sebacic Acid	-			D,L-α-Glycerol Phosphate	-
Dextrin	++	Succinic Acid	-			α-D-Glucose-1-Phosphate	+++
Glycogen	+	Bromosuccinic Acid	+			D-Glucose-6-Phosphate	+++
		Succinamic Acid	-				

-: $A_{595} < 0.01$, +: $0.01 \leq A_{595} < 0.05$, ++: $0.05 \leq A_{595} \leq 0.1$, +++: $0.1 \leq A_{595}$

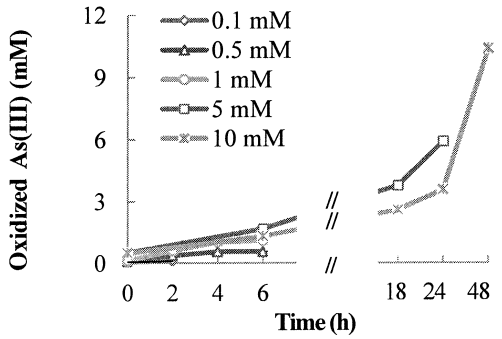

Fig. 2. The As(III) oxidation in the whole cell assay of strain B1 under various initial As(III) concentrations. Error bars indicate the standard deviation obtained from three independent experiments.

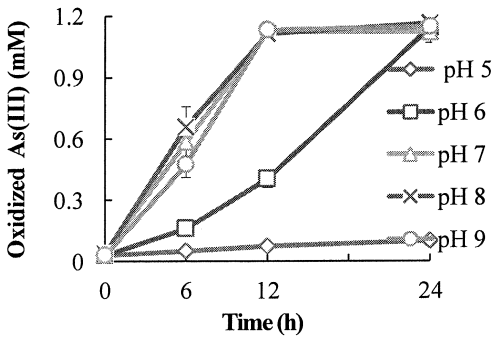

Fig. 4. The As(III) oxidation in the growing cell assay of strain B1 under various pH conditions. Error bars indicate the standard deviation obtained from three independent experiments.

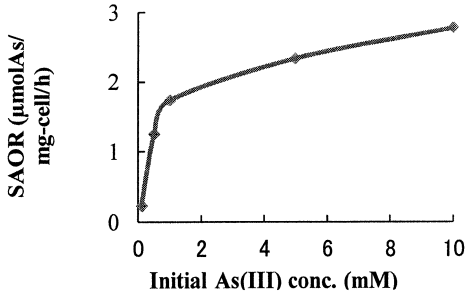

Fig. 3. Effects of initial As(III) concentrations on the specific As(III) oxidation rate (μmol As(III)/mg-cell/h) in the whole cell assay of strain B1. Error bars indicate the standard deviation obtained from three independent experiments.

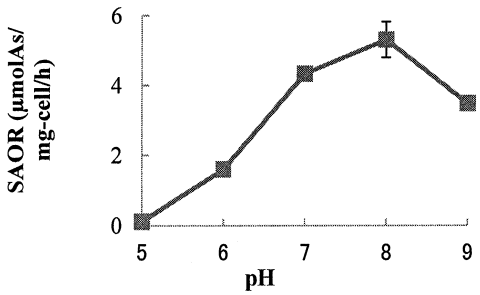

Fig. 5. Effects of pH on the specific As(III) oxidation rate in the growing cell assay of strain B1. Error bars indicate the standard deviation obtained from three independent experiments.

(3) Effects of pH on As(III) oxidation by strain B1

Figure 4 shows that 1 mM of As(III) could be completely oxidized to As(V) by strain B1 at pH 6.0 – 9.0. At pH 5.0, significant As(III) oxidation was not observed. As seen in Figure 5, the highest SAOR was 5.3 μmol As(III)/mg-cell/h at pH 8.0.

4. CONCLUSION

Some of phosphate compounds or sugar alcohols can be utilized by strain B1 as carbon sources. Those carbon sources can be used for mass cultivation of strain B1 although strain B1 does not need any organic carbon in As(III) oxidation. Strain B1 was effective for oxidation 0.1-10 mM As(III) under the autotrophic conditions. Strain B1 is applicable to treat groundwater in neutral and slightly alkaline conditions.

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