Effect of *Anthrobacter creatinolyticus* on phytoremediation of arsenic contaminated water by *Echinodorus cordifolius*

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Abstract

Arsenic (As) contamination in the environment is a worldwide problem. Many technologies such as oxidation, membrane technology, adsorption, electrokinetics, ion exchange have been used to remove As from contaminated water. However, these technologies are required high costs and skillful labor for control and maintenance. Phytoremediation, using plant to remove pollution, is a cost effective and eco-friendly technology. Nevertheless, this technology still brings about limitations such as high phytotoxicity of As. Application of microorganisms to enhance heavy metal removal by plant might be an effective technology. Some plant species such as *Echinodorus cordifolius*, *Cyparus alternifolium*, *Acrostichum aureum*, *Coolocasia esculenta*, and *Echinodorus cordifolius* were screened for As removal efficiency. From our screening plants revealed that *E. cordifolius* was the highest efficiency species for As removal. Combination between *E. cordifolius* and microorganisms for As removal was studied. *Bacillus subtilis* and *Anthrobacter creatinolyticus* (isolated from arsenic contaminated soil from Nakhon Si Thammarat province) were used to compare As removal efficiency. The result showed that at 1st and 2nd cycle of experiment, As removal was no significantly different between non-inoculated *E. cordifolius* and inoculated *E. cordifolius*. After that at 3rd and 4th cycle of experiments, *E. cordifolius* inoculated with *A. creatinolyticus* had the highest As removal efficiency compared to other conditions. This might due to *A. creatinolyticus* tolerated to As concentration greater than *B. subtilis*. In addition, *A. creatinolyticus* also produced ammonium for enhancing plant growth. Therefore, *E. cordifolius* inoculated with *A. creatinolyticus* was suitable to apply in As contaminated water.

Keyword: Phytoremediation, Arsenic, *Echinodorus cordifolius*, *Anthrobacter creatinolyticus*

I. INTRODUCTION

Arsenic (As) is a toxic metalloid which can pollute water, soil, crops. The presence of As in environments is a worldwide problem. Over 70 countries have problem with As contaminated ground water of an estimated 150 million people. In estimated, 110 million of this people live in south and south-east Asia. In Bangladesh, higher than 100 million people drink water up to 1 mg/L of As levels that is higher than 100 times the World Health Organization drinking water guideline value (WHO, 2011). As could be found in environment as the result of the dissolution of minerals from volcanic, sedimentary rocks, manufacture of lasers, semiconductors, industry waste, agricultural activities, pharmaceutical products, and human activities (Khang *et al*., 2012). It is rarely found as its native form because of its bonding affinity with other elements (Islam *et al*., 2015). In the environment, As exists in many oxidation states – arsenate (As (V)), arsenite (As (III)), arsenic (As (0)), and methyl group of arsenic. Arsenate (As (V)) is the thermodynamically stable state of arsenic in toxic waters, while arsenite (As (III)) predominates in reduced redox conditions (Pell *et al*., 2013). As is highly toxic to plants and microorganism (Alvarado *et al*., 2008), especially it can affect human health such as skin damage, increase lung, bladder, and kidney cancers, nerve damage in humans (Molin *et al*., 2015). According to World Health Organization (WHO, 2011) As standard in drinking water has 0.01 mg/L. The industrial effluent standard of As in Thailand is not more than 0.25mg/L (PCD).

There are many techniques used for As remediation such as oxidation, membrane technology, adsorption, electrokinetics, ion exchange, adsorption, and coagulation flocculation(Roy *et al*., 2015). These techniques can effect to levels of arsenic contaminants and high removal efficiency (Roy *et al*., 2015). However, these techniques have several drawbacks, principally their technological complexity, high cost, maintenance, and the need skill for technicians (Rahman *et al*., 2014).

Phytoremediation, a plant-based green technology, can be considered as a simple, effective, and eco-friendly technology for As from water (Rahman *et al*., 2011). Using some submerged aquatic saprophyte and floating plants in the process of phytoremediation is more commonly known as phytofiltration (Rahman *et al*., 2011). Moreover, the application of microorganisms to enhance As removal by plant might be an effective technology.

The aim of work is to studies the interaction plant with microorgaism for remove arsenic from water. The aquatic plants such as *Echinodorus cordifolius*, *Cyparus alternifolium*, *Acrostichum aureum*, *Coolocasia esculenta*, and *Echinodorus cordifolius* have been selected for a screening to find plant species with the highest As removal efficiency. *Bacillus subtilis* (Adams, 1973; Giri, et al., 2012) and *Arthrobacter creatinolyticus* (screened from arsenic contaminated soil at Nakhon Si Thammarat province, Thailand) were selected in this work. In addition, the combination of plant and microorganism will be applied to study As removal from wastewater.

II. METHODOLOGY

1. Screening plants for removal arsenic concentration

The aquatic plants species such as *Echinodorus cordifolius, Cyparus alternifolium, Acrostichum aureum,* and *Coolocasia esculenta* were applied to As remediation. They were washed carefully with tab water to remove any contaminated substances from their roots. The similar weights in each species in triplicate are used for screening.

After plants preparation, each plant was placed in small pots with triplicate for each treatment. The arsenic concentration was prepared 2 ppm inside the small pots with water 400 ml. The solutions were sampling at time 1, 3, 5, and 7 days. The solution was analyzed for amount of remaining As by an inductively coupled plasma spectrophotometer (ICP JY2000).

2. Phytoremediation of As solution extracted from As contaminated soil

The research was observed the efficiency of plant *E. cordifolius* inoculated with microorganism *Bacillus subtilis* or *Anthrobacter creatinolyticus* from arsenic solution abtained from arsenic contaminated soil solution. There are many reports revealed that *Bacillus subtilis* could resistant to high concentrations of arsenic (Adams, 1973; Giri *et al.*, 2012). Meanwhile, *Arthrobacter creatinolyticus* was screened from arsenic contaminated soil at Nakhon Si Thammarat province; Thailand will be grown in NB and inoculate into treatment conditions. 100-120g of *E. cordifolius* inoculated with 10% (v/v) *B. subtilis* or *A. creatinolyticus* were grown in small pot (400 mL of arsenic solution) and used as treatment. *E. cordifolius* without inoculation and As contaminated solution extracted from soil were used as control conditions. Initial As concentration was 2 ppm from As contaminated soil solution and the experiment was studied for 4 cycles with 3 days per cycle. All conditions were prepared triplicates for each treatment. As concentrations were through filtered and determined by inductively coupled plasma (ICP JY2000).

3. Removal efficiency

Arsenic removal efficiency (%) for this experiment is defined by the following equation:

$$As\ removal(\%) = \left[\!\left[\frac{C_{control} - C_{treatment}}{C_{control}}\right]\!\right] X\ 100$$

$C_{control}$ and $C_{treatment}$ are the arsenic concentration in the water from control (unplanted) and treatment (planted) small pots, respectively.

4. Analysis and statistic method

Data were analyzed by one way analysis of variance (ANOVA) using Statistical Program for Social Science (SPSS) version at 95% confidence.

III. RESULTS AND DISCUSSION

Screening result revealed that *E. cordifolius* had the highest efficiency species for As removal 98% (TABLE 1). Therefore, *E. cordifolius* was selected to study As removal from water by inoculated with microorganism.

TABLE1. EFFECIENCY OF As REMOVAL BY VARIOUS PLANTS FOR 5 DAYS

Conditions	As removal (%)	System pH
As contaminated wastewater (Control)	13	6.86±0.13
Acrostichum aureum	62	7.03.±0.20
Cyperus alternifolius	18	6.20±0.43
Echinodorus cordifolius	98	6.82±0.07
Colocasia esculenta	66	6.85±0.17

1. Arsenic removal by E. cordifolius inoculated with B. subtilis or A. creatinolyticus

The result showed that phytoremediation of arsenic contaminated soil solution with *E. cordifolius* inoculated with *A. creatinolyticus* had the highest removal efficiency (Fig.1).

In cycle 1^{st} and 2^{nd}, the efficiency of As removal showed no significant difference between *E. cordifolius* inoculated with *A. creatinolyticus* conditions, *E. cordifolius* inoculated with *B. subtilis* conditions and non-inoculated *E. cordifolius* (Fig.1).

In cycle 3^{rd} of As removal was removed completely by *E. cordifolius* inoculated with *A. creatinolyticus*. However, the efficiency of arsenic removal by *E. cordifolius* inoculated with *B. subtilis* and non-inoculated *E. cordifolius* were not significantly different (Fig.1).

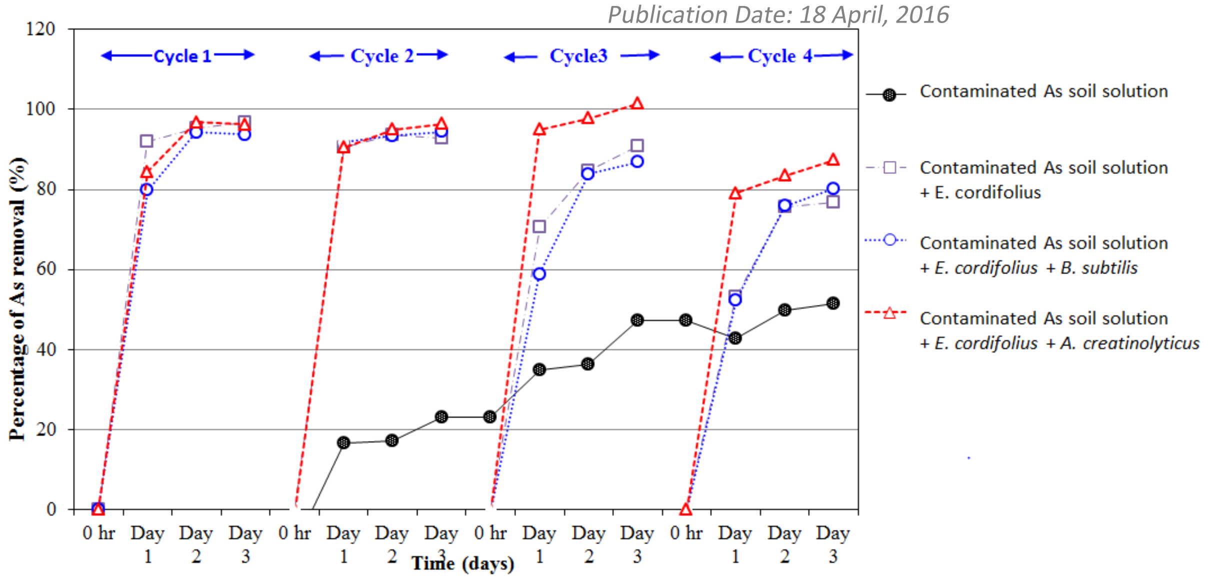

Fig. 1 Percentage of As removal by non-inoculated *E. cordifolius*, inoculated *E. cordifolius* with B. subtilis and A. creatinolyticus for 4 cycles

Moreover, the last cycle of the experiment, *E. cordifolius* inoculated with *A. creatinolyticus* (87.44%) have the highest efficiency of As removal, and the efficiency of As removal in *E. cordifolius* inoculated with *A. creatinolyticus* was still significantly different compared to other conditions (Fig.1). In this case, *A. creatinolyticus* tolerated to As concentration greater than *B. subtilis*. Additionally, *A. creatinolyticus* might due enhance plant ability for As removal from water by producing ammonium (Fig.2) and plant growth hormone (Indole-3-acetic acid) (Fig.3).

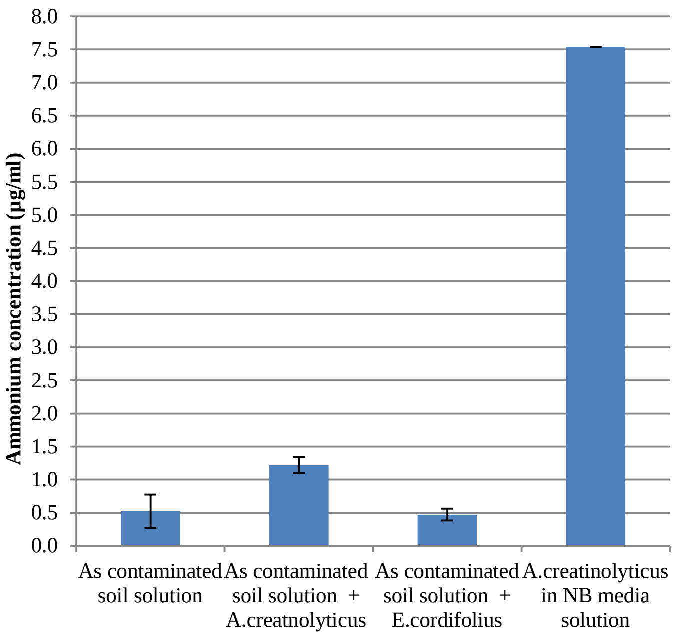

Fig. 2 Ammonium concentration produced from various conditions

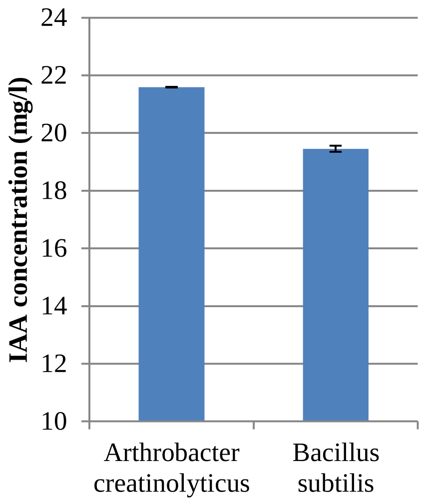

Fig.3 IAA concentration

Additionally, in control conditions, As contaminated soil solution was used through 4 cycles of experiment. It was found that natural microorganisms can grow and adsorb As at 2nd, 3rd, and 4th cycle of experiment.

IV. CONCLUSION

The screening plants of As removal from wastewater were compared the ability of plants. *E. cordifolius* showed the highest efficiency for As removal. The application of *A. creatinolyticus* to increase As phytoremediation can be an effective method for arsenic contaminated water treatment. *A. creatinolyticus* could enhance plant abilities for As removal by producing nitrogen source and Indole-3-acetic acid (IAA) for plant growth.

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VI. Reference

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