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Preliminary Study on the Arsenic Remediation by Microbially Induced Calcium Carbonate Precipitation (MICP)

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Table of contents

List of Tables

List of Figures

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Abstract

Rapid urbanization and industrialization by the growing populations is a reason to contribute to environmental pollution by toxic metal. Arsenic (As) distribution and toxicology in the environment is a major problem, with millions of individuals worldwide being affected by arsenic toxicosis. Sources of arsenic contamination are both natural and anthropogenic and the scale of contamination ranges from local to regional. It is well known that arsenic contamination in soil and water sources poses a serious risk to both human health and the environment. Therefore, remediation/treatment methods are essential to reduce environmental contamination. Microbially Induced Calcium Carbonate Precipitation (MICP) method is being popular and effectively immobilizes the heavy metals. Therefore, this paper aims to investigate the potential of arsenic immobilization by using B. pasteurii. It was selected by its high urease activity. The ammonium-YE medium and ammonium sulfate ($(NH_4)_2SO_4$) were used as a culture medium. HPLC, AAS and ICP-MS were used to determine the concentration of ammonium, calcium, and arsenic. According to the results of our experiments, arsenic can be immobilized from 5% to 15% after one week at 30° C. That evidence was showing that has good potential to use B. pasteurii in MICP method for the remediation of arsenic contamination.

Keyword: B. pasteurii, Contamination, Environment, Immobilized, Urease

အာဆင်းနစ်လျှော့ချရေးအတွက် အဏုဇီဝပိုးမွှားများကို အသုံးပြု၍ ကယ်လ်ဆီယမ် ကာဗွန်နိတ် အနည်ကျစေခြင်း နည်းစဉ်အား ကနဦး လေ့လာဆန်းစစ်ခြင်း

သန်းထိုက်ဦး၊ ဘူမိဗေဒအရာရှိ

သတ္တုတွင်းဦးစီးဌာန

စာတမ်းအကျဉ်း

ကမ္ဘာ့လူဦးရေတိုးပွားလာမှုနှင့်အညီ မြို့ပြလူနေမှုစနစ်နှင့် စက်မှုလုပ်ငန်းများဖွံ့ဖြိုး တိုးတက်လာခြင်းကြောင့် သဘာဝပတ်ဝန်းကျင်အတွင်း အဆိပ်အတောက်ဖြစ်စေသော သတ္တု များ ညစ်ညမ်းမှုဖြစ်ပေါ်စေသည့် အကြောင်းအရင်းတစ်ခုဖြစ်ပေသည်။ တစ်ကမ္ဘာလုံး အတိုင်း အတာအားဖြင့် သဘာဝပတ်ဝန်းကျင်တွင် အာဆင်းနစ်အဆိပ်ပျံ့နှံ့မှုအန္တရာယ်သည် သန်းပေါင်း များစွာအတွက် အဓိကပြဿနာတစ်ခုအဖြစ် သက်ရောက်လျက်ရှိပါသည်။ အာဆင်းနစ် ညစ်ညမ်းမှု ဖြစ်ပေါ်စေသော အဓိကအကြောင်းအရင်းများမှာ သဘာဝအတိုင်း ဖြစ်ပေါ်ခြင်းနှင့် လူသားတို့၏ လုပ်ကိုင်ဆောင်ရွက်ခြင်းများရှိပြီး နေရာဒေသငယ်များမှသည် နေရာဒေသကြီး များအထိပါ ပျံ့နှံ့လျက်ရှိပါသည်။ ရေနှင့်မြေဆီလွှာများအတွင်း အာဆင်းနစ်ညစ်ညမ်းမှု ဖြစ်ပေါ် ခြင်းသည် သဘာဝပတ်ဝန်းကျင်နှင့် လူသားတို့၏ ကျန်းမာရေးအတွက် ကြီးမားသော ခြိမ်း ခြောက်မှု တစ်ခုပင်ဖြစ်ပါသည်။ ထို့ကြောင့် သဘာဝပတ်ဝန်းကျင်ထိခိုက်မှု လျှော့ချနိုင်ရေး အတွက် ကုစားနိုင်သည့် နည်းလမ်းများသည် မရှိမဖြစ် အရေးပါသော အရာများဖြစ်ပါသည်။ အဏုဇီဝပိုးမွှားများကို အသုံးပြု၍ ကယ်လ်ဆီယမ်ကာဗွန်နိတ် အနည်ကျစေခြင်း နည်းစဉ်သည် ထင်ရှားလျက်ရှိပြီး Heavy Metals များ ပျံ့နှံ့မှုမရှိစေရန်အတွက် ထိရောက်စွာ ထိန်းချုပ်နိုင်သည် ကိုတွေ့ရှိရပါသည်။ ထို့ကြောင့် ယခုသုတေသန စာတမ်းသည် *B. pasteurii* ဘက်တီးရီးယားကို အသုံးပြု၍ အာဆင်းနစ်ပျံ့နှံ့မှု အားထိန်းချုပ်နိုင်မှု အလားအလာ ရှိ/မရှိ စူးစမ်းလေ့လာခြင်း ဖြစ်ပါသည်။ အဆိုပါ ဘက်တီးရီးယားကို စမ်းသပ်ရန်ရွေးချယ်ရခြင်းမှာUrease activity မြင့်မား သောကြောင့် ဖြစ်ပါသည်။ ဘက်တီးရီးယား မွေးမြူရေးကြားခံပစ္စည်းအဖြစ် Ammonium-YE medium and Ammonium Sulfate တို့အား အသုံးပြုပါသည်။ အမိုနီယမ်၊ ကယ်လ်ဆီယမ်နှင့် အာဆင်းနစ် ပါဝင်နှုန်းများ စမ်းသပ်တိုင်းတာရန်အတွက် HPLC, AAS နှင့် ICP-MS စက်ပစ္စည်း

ကိရိယာတို့ကို အသုံးပြုပါသည်။ ယခုစမ်းသပ်ချက် ရလဒ်များအရ အပူချိန် ၃၀ဒီဂရီစင်တီဂရိတ်တွင် တစ်ပတ်ကြာ ထားရှိပြီးနောက် အာဆင်းနစ် ၅% မှ ၁၅ %ထိ ထိန်းချုပ်ထားနိုင်သည်ကို တွေ့ရှိရပါသည်။ အဆိုပါတွေ့ရှိချက်အရ *B. pasteurii* ကို အသုံးပြု၍ ကယ်လ်ဆီယမ် ကာဗွန်နိတ် အနည်ကျစေခြင်းဖြင့် အာဆင်းနစ်ညစ်ညမ်းမှု လျှော့ချရေးအတွက် အသုံးပြုနိုင်ရန် အလားအလာကောင်းများ ရှိနိုင်သည်ကို တွေ့ရှိရပါသည်။

Preliminary Study on the Arsenic Remediation by Microbially Induced Calcium Carbonate Precipitation (MICP)

1. Introduction

Rapid urbanization and industrialization by the growing populations is a reason to contribute to environmental pollution by toxic metal. The production of goods, services, and infrastructure depends mainly on mining, which also improves the standard of living of society. On the other hand, mining and smelting activities, the burning of fossil fuels, agricultural practices, industrial operations, and waste disposal are the main sources of metal contaminants in soils. Moreover, after mineral beneficiation, small and uniform-sized particles called "tailings" are remained. Tailings have a high moisture content but poor cohesion and shear strength. They typically liquefy easily as well. Due to these factors, a tailings-based dam is vulnerable to failure, which could cause severe losses for the downstream part. Moreover, leaching of heavy metals leads to contamination of nearby soil and water because many metallic elements are present in tailings. The estimated annual waste production was varied from 5-7 billion tones to up to 10 billion tones at the global level (Martins et al., 2021). The properties and quantities of such tailings might vary substantially depending on the characteristics of the mined materials and the extraction methods utilized (Vidal, 2018). For example, the Rio Pilcomayo basin in Bolivia near the Porco mine tailings dam was discovered to contain high concentrations of arsenic (As), antimony (Sb), and cadmium (Cd) (Âo Pilcomayo et al., 2001). It is well known that arsenic contamination in soil and water sources poses a serious risk to both human health and the environment (Bhattacharya et al., 2007; Choong et al., 2007). Arsenite (As (V)) and arsenate (As (III)) are the two main oxidation states of inorganic arsenic, both of which are hazardous to both people and plants. Inorganic arsenic is always threatened to human carcinogen, associated with increased risk for skin, lungs, urinary bladder, liver, and kidney cancers (National Academy of Sciences, Washington DC, 1999). Heavy metals like arsenic, cadmium, chromium, lead, copper, and zinc have been identified at hazardous quantities (above guidelines) over the past few decades as a result of urbanization and excessive anthropogenic activities (Anbu et al., 2016; Yao et al., 2017; Zou et al., 2015) .

It's crucial to create sustainable remediation methods based on natural processes to counter this threat. Various environmental cleaning technologies are already being used, such as conventional chemical and thermal methods. However, both methods are not only technically difficult but also expensive, and the soil's components can be degraded by these methods (Krishna & Philip, 2005). In recent years, it has been discovered that harmful metal contaminants can be effectively immobilized by a biomineralization process involving ureolytic microbes that results in calcium carbonate precipitation. Some researchers have used it to immobilize heavy metals, while others have used it to improve the mechanical properties of the soil.

2. Objective

Arsenic (As) distribution and toxicology in the environment is a major problem, with millions of individuals worldwide being affected by arsenic toxicosis. Sources of arsenic contamination are both natural and anthropogenic and the scale of contamination ranges from local to regional. Therefore, remediation/treatment methods are essential to reduce the environmental contamination. However, treatment cost and operational complexity of the technology are factors should be considered before treatment method selection. MICP method is being popular and effectively immobilize the heavy metals. Therefore, this paper aim to investigate the potential of arsenic immobilization by using *B. pasteurii*.

3. Material and Method

3.1. Bacteria

B. pasteurii was selected because of its high urease activity. The experiment was carried out by inoculating Yeast extract, ammonium sulfate $(NH_4)_2SO_4$ and Tris liquid medium in Erlenmeyer flask and autoclaved at 121° C for 15 minutes. After that, *B. Pasteurii* strain was cultured for three days at 30° C at an agitation speed of 200 revolutions per minute (rpm) in the medium.

3.2. OD_{600}

A Unico 2000 visible spectrophotometer was used to measure the optical density (OD_{600}) of the bacterial solution at a wavelength of 600 nm. To prepare the bacterial solution with the same concentration, the OD_{600} value of the solution with known cell concentration which was obtained from the serial dilution method was used later.

3.3. Urease activity

Urease activity was detected using a model LCR meter LCR 700. The urea solution 40 ml was adjusted to concentration of 1.5 mol/L and 10 ml of cell culture solution (if the preparation and 10 ml of distill water) are placed in a beaker. Then, measure the electrical conductivity every 2 minutes while stirring at 120 rpm. The measurement was carried out until 20 minutes and record the amount of increased electrical conductivity.

3.4. Culture medium and Nutrient solution

The ammonium–YE medium contained 20 g/l of YE and 10 g/l of ammonium sulfate ($(NH_4)_2SO_4$). The pH of the medium was adjusted to 9 with Tris (hydroxyethyl) aminomethane ($C_4H_{11}NO_3$). Three different nutrient solutions were used in the experiment deionized water (no nutrient substance), 1.5 mol/L urea solution and a mixed solution of 0·15 mol/L urea and 0·15 and 0.3 mol/L calcium chloride ($CaCl_2$).

3.5. Preparation of arsenic stock solution

For the preparation of 0.05mg/l of arsenic stock solutions the necessary quantities of Na 2HAsO_4 (Wako Pure Chemical Industries, Ltd, Osaka, Japan) were dissolved in Milli-Q water. The solutions were filtered through a 0.22µm filter and stored in the dark at 4 ° C.

Table 1. Experimental Schemes

Group	Number	Bacteria	Fixative	Temperature
1	Control	No bacteria	6 ml of filtrated Culture Medium 2 ml of Pure Water 2 ml of Arsenic (0.04 mg/L)	30 ° C
2	2-A 2-B 2-C	*B.Pasteurii*	6 ml of filtrated Culture Medium ($CaCl_2$ 0.3 mol/L) 2 ml of urea solution 2 ml of Arsenic (0.04 mg/L)	30 ° C
3	3-A 3-B 3-C	2 ml of *B.Pasteurii* solution	6 ml of filtrated Culture Medium ($CaCl_2$ 0. 15 mol/L) 2 ml of urea solution 2 ml of Arsenic (0.04 mg/L)	C

3.6. Liquid Test

Nine Specimens were prepared in 3 groups, as shown in Table 1. In group one 6 ml of filtrated culture medium, 2 ml of pure water and 2 ml of arsenic (0.04 mg/L) were used and store at 30° C for one week. In group two and three, 6 ml of filtrated culture medium, 2 ml of bacterial solution and 2 ml of arsenic (0.04 mg/L) were used and stored at 30° C for one week.

4. Statistical Analysis

All experiments were repeated at least three times, and the data presented here are the means and standard deviations of three independent experiments. All statistical analyses were performed with 365 Microsoft Excel.

5. Result and Discussions

Multiple civil constructions have been targeted for strengthening and remediation using biocalcification, also known as MICP. The urease in the urea ($CO(NH_2)_2$) hydrolysis process comes from microorganisms. The main biochemical reaction can be seen in Eq (1) and (2) as an example of MICP (Murata & Hata, 2018; Tsesarsky et al., 2018).

$$CO(NH_2)_2 + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{2-} \quad (1)$$

$$CO_3^{2-} + Ca^{2+} \leftrightarrow CaCO_3\downarrow \quad (2)$$

To change free metal ions into carbonates or hydroxides or other types of precipitation by using Microbial metabolic process is the fundamental assumption. Calcium carbonate ($CaCO_3$) precipitates when the free metal ion is calcium (Ca^{2+}), and this phenomenon is typically referred to as microbially induced carbonate precipitation (MICP). This precipitation process is a complicated mechanism that is affected by cell concentration, ionic strength, and medium pH. Calcium source such as calcium chloride was supplemented to growth the microorganism, which is precipitated as calcium carbonate. The following equations (3), (4) and (5) was shown the complex set of reaction (Anbu et al., 2016; Kumari et al., 2016):

$$Ca^{2+} + Cell \rightarrow Cell\text{-}Ca^{2+} \quad (3)$$

$$Cl^- + HCO_3^- + NH_3 \rightarrow NH_4Cl + CO_3^{2-} \quad (4)$$

$$Cell\text{-}Ca^{2+} + Co_3^{2-} \rightarrow Cell\text{-}CaCO_3\downarrow \quad (5)$$

High-performance liquid chromatography (HPLC), Atomic Absorption Spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS) were used to determine the concentration of ammonium, calcium, and arsenic. Calcium ion utilization may be affected by the residual calcium ion concentration in the lateral solution. In order to determine how much calcium carbonate was deposited, studies were carried out using the solution that was left over after mineralization and a calcium ion titration test. The precipitation amount of calcium ions was shown in **Fig. 1**. This indicates that increasing the concentration of calcium ions in the solution is a viable method to increase the precipitation of calcium carbonate, but it should be controlled within a certain concentration, otherwise the rate of hydrolysis will decrease, leading to a decrease in the experimental efficiency. Urease is the main function for the calcite precipitation and is produced in considerable quantities in media containing urea and calcium sources (de Muynck et al., 2010). According to the results of our experiments, the removal of arsenic and the remediation potentials of the bacteria was observed to vary from from 5% to 15% after one week at 30° C **Fig.2**. The arsenic concentrations were reduced from 2.06 mg/L to 6.15 mg/L in group three and 9.89 mg/L to

12.23 mg/L in group two respectively **Fig.3**. As previous studies have shown that $CaCo_3$ may be the leading sorbent for a variety of metals in carbonate aquifers (Zachara et al., 1991). Moreover, heavy metal can be sorbed on the calcite surface, it is assumed that surface process irrespective of mechanism, adsorption, or precipitation (Garrison Sposito, 1984).

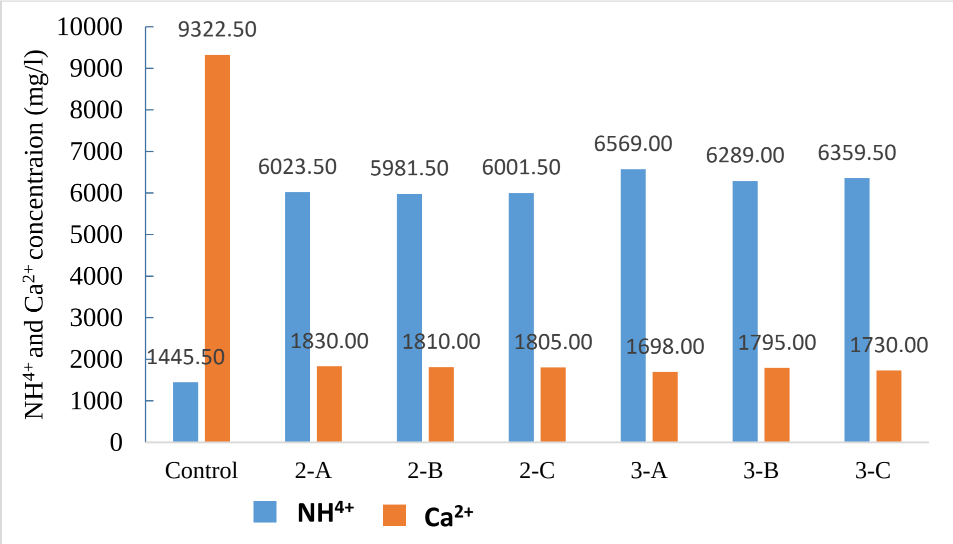

Figure 1. Comparison Amount of $CaCo_3$ Precipitation and NH^{4+} released rate after one week.

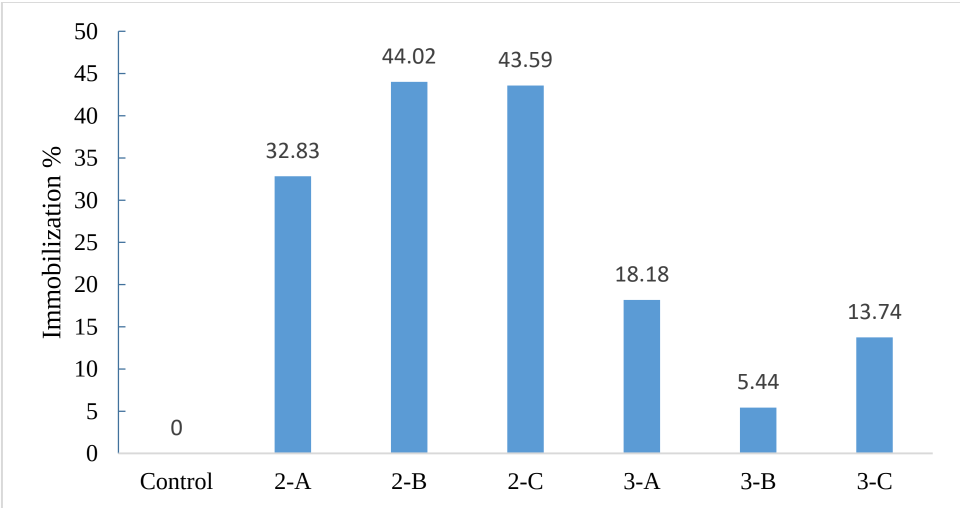

Figure 2. Removal Percentage of arsenic by the *B.Pasteurii* after one week.

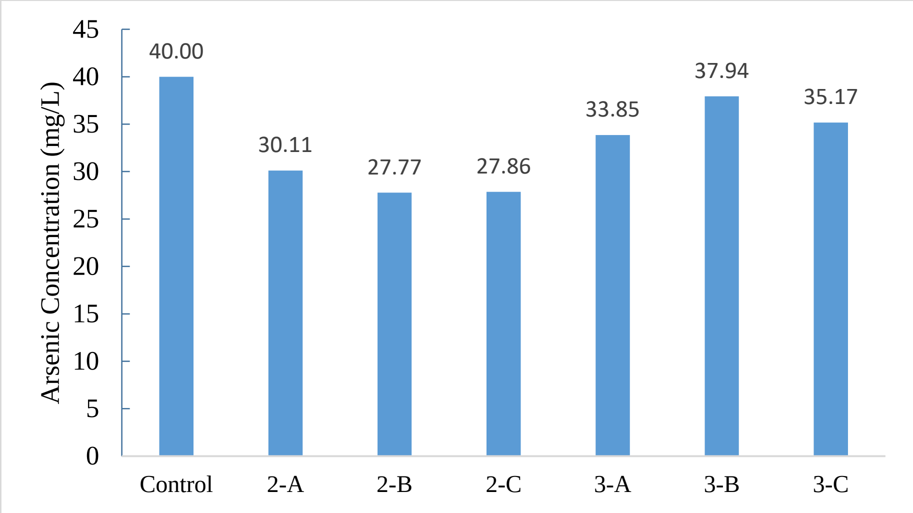

Figure 3. Removal of arsenic by the *B.Pasteurii* after one week.

6. Conclusion

In this study, this evidence was showing that has good potential to use *B. pasteurii* for the immobilization of arsenic. Also, bioremediation with bacterial mixture is an effective method for arsenic removal from contaminated environments. This may prove to be a good strategy for developing an effective, efficient, and economic method of arsenic bioremediation, and the introduction of these indigenous bacteria could be used for contaminated soil bioremediation without disturbing the target environment.

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