Arsenic removal from the contaminated soil by bio-based washing agent in a combined mechanical agitation and ultrasonication process

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สารหนูจัดเป็นมลพิษที่มีความสำคัญและเป็นปัญหาที่พบได้ทั่วโลก โดยสารหนูสามารถส่งผลกระทบต่อสุขภาพได้ในหลายระดับ การชะล้างดินเป็นการนำกระบวนการทางเคมีและกายภาพมาผสมผสานกันเพื่อลดต้นทุนสำหรับการแยกโลหะหนักที่ปนเปื้อนในดิน งานวิจัยนี้มุ่งศึกษาการประยุกต์ใช้สารลดแรงตึงชีวภาพชนิดลิโปเปปไทด์ จากแบคทีเรีย *Bacillus subtilis* GY19 สำหรับเป็นสารชะล้างเพื่อกำจัดสารหนูที่ปนเปื้อนในดิน โดยวิธีการเพิ่มประสิทธิภาพของสารลดแรงตึงผิวชีวภาพในการกำจัดโลหะหนัก คือ การเติมโซเดียมคาร์บอเนต และกรดแอทธิลีนไดอามีนเตตระอะซิติก (EDTA) ซึ่งจัดเป็นสารลดความกระด้างโดยจะช่วยลดการแลกเปลี่ยนไอออนบวกในดิน การศึกษานี้นำดินจากเกษตรกรรมมาเติมสารหนูให้มีความเข้มข้น 418 มิลลิกรัมต่อกิโลกรัมดิน และหาความเข้มข้นที่เหมาะสมของสารแรงตึงผิวชีวภาพชนิดลิโปเปปไทด์ พบว่าสารชะล้างที่ประกอบด้วยสารลดแรงตึงผิวชีวภาพชนิดลิโปเปปไทด์ที่มีความเข้มข้นเป็นจำนวน 5 เท่าของค่าการก่อเกิดไมเซลล์ และกรดแอทธิลีนไดอามีนเตตระอะซิติกที่มีค่าความเข้มข้น 0.01 โมล มีประสิทธิภาพสูงสุดในการชะล้างสารหนูในดิน ที่ร้อยละ 46.1 ± 0.9 ในขณะที่เมื่อใช้กรดแอทธิลีนไดอามีนเตตระอะซิติก หรือ น้ำกลั่นเพียงอย่างชนิดเดียวเป็นสารชะล้าง ประสิทธิภาพการชะล้างสารหนูในดินคือ ร้อยละ 34.1 ± 1.4 และ 16.8 ± 0.4 ตามลำดับ การเพิ่มประสิทธิภาพการกำจัดสารหนูของสูตรผสมสารลดแรงตึงผิวชีวภาพชนิดลิโปเปปไทด์และกรดแอทธิลีนไดอามีนเตตระอะซิติก ทำโดยใช้กระบวนการชะล้างที่ทำงานร่วมกันระหว่างการปั่นกวนและเทคนิคอัลตร้าโซนิค จากการศึกษาพบว่าประสิทธิภาพสูงสุดของการกำจัดสารหนูเมื่อใช้การปั่นกวนร่วมกับเทคนิคอัลตร้าโซนิค คือ ร้อยละ 71.45 ± 3.6% โดยใช้สารลดแรงตึงผิวชีวภาพที่มีค่าความเข้มข้นเป็นหนึ่งเท่าของค่าการก่อเกิดไมเซลล์ผสมกับกรดแอทธิลีนไดอามีนเตตระอะซิติกที่มีความเข้มข้น 0.01 โมล ซึ่งพบว่ามีประสิทธิภาพสูงกว่าการใช้สารลดแรงตึงผิวสังเคราะห์ที่เป็นชุดควบคุม นอกจากนี้ค่าความเป็นกรดด่างของดินหลังการชะล้างอยู่ในช่วงที่ยอมรับได้ตามมาตรฐานดินเพื่อการเกษตรกรรม เมื่อหาสภาวะที่เหมาะสมของการชะล้างโดยใช้วิธี Design of experiment (DOE) ผ่านทาง Box-Behnken design และแปรผันปัจจัยที่ใช้ศึกษา ได้แก่ ค่าความเป็นกรดด่างของสารชะล้าง เวลาในการชะล้าง และอัตราส่วนระหว่างของแข็งและของเหลว โดยทดสอบดิน 2 ตัวอย่าง ที่มีสารหนูความเข้มข้นต่ำ (434 มก./กก.) และความเข้มข้นสูง (2,572 มก./กก.) ผลการศึกษาพบว่าประสิทธิภาพในการชะล้างสูงสุดของดินที่มีสารหนูความเข้มข้นต่ำ คือ ร้อยละ 80 โดยมีค่า pH ของสารชะล้างเท่ากับ 4.0 อัตราส่วนของของแข็งและของเหลว มีค่า 1.0 และเวลาที่ใช้ในการชะล้าง 40 นาที สำหรับดินที่มีสารหนูความเข้มข้นสูงมีประสิทธิภาพในการชะล้างสูงสุด คือ ร้อยละ 86 โดยมีค่า pH ของสารชะล้างเท่ากับ 7.1 อัตราส่วนของของแข็งและของเหลว มีค่า 0.1 และเวลาที่ใช้ในการชะล้าง 20 นาที ดังนั้นการประยุกต์ใช้สารชะล้างโดยการผสมสารลดแรงตึงผิวชีวภาพชนิดลิโปเปปไทด์และสารลดความกระด้างเป็นอีกแนวหนึ่งที่ความสามารถช่วยกำจัดสารหนูที่ปนเปื้อนในดิน

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KEYWORD: Arsenic lipopeptide Bacillus subtilis GY19 combined soil washing remediation

Polwatte Arachchillaya Buddhika Prabath Abeyrathne : Arsenic removal from the contaminated soil by bio-based washing agent in a combined mechanical agitation and ultrasonication process. Advisor: Assoc. Prof. Prof. EKAWAN LUEPROMCHAI, Ph.D. Co-advisor: NICHAKORN KHONDEE, Ph.D.

A significant amount of arsenic (As) pollution has been found globally, with several health consequences. Soil washing is a cost-effective mix of chemical and physical procedures that have been used to remove heavy metals from polluted soil. This study investigated the possibility of using a lipopeptide biosurfactant from *Bacillus subtilis* GY19 as a washing agent to remove As from contaminated soil. To improve the effectiveness of biosurfactants, sodium carbonate and ethylenediaminetetraacetic acid (EDTA) were employed as builders to minimize the influence of exchangeable cations in the soil. The agricultural soil utilized in the study was spiked with As at an initial concentration of 418.7 mg/kg. Lipopeptide solutions were made in a series based on their critical micelle concentration (CMC). The maximal As removal capability of 5x CMC lipopeptide with 0.01 M EDTA solution was 46.1±0.9 %, while 0.01 M EDTA alone and DI water removed only 34.1±1.4 % and 16.8±0.4% As, respectively. The As removing efficiency of lipopeptide-EDTA formulation was increased with the implementation of the combined soil washing process with mechanical agitation and ultrasonication. The highest arsenic removal efficiency of 71.45±3.6% was recorded when the 1x CMC lipopeptide + 0.01 M EDTA washing agent was applied to the combination of mechanical agitation and ultrasonication washing process. The As removing efficiency was higher than the control synthetic surfactant and the soil pH of the washed soil was in the acceptable range for the agricultural standards. To optimize the soil washing condition, the Design of Experiment (DOE) was conducted through Box-Behnken design by varying the washing pH, washing time, and the solid: liquid ratio. Two soil samples with the initial concentrations of 434 mg/kg as low As concentrated soil and 2,572 mg/kg as high As concentrated soil were investigated. The maximum As removal efficiency for lower As concentrated soil was 80%, which was obtained by using pH 4.0 washing agent, 1.0 solid: liquid ratio, and 40 minutes washing time. For the higher As concentrated soil, the maximum washing efficiency of 86% was obtained from pH 7.1 washing agent, 0.1 solid: liquid ratio, and 20 minutes washing time. The results indicated that the mixture of lipopeptide and EDTA could be applied to wash As contaminated soil by the combination of mechanical agitation and ultrasonication process. However, the scale-up experiments should be investigated to confirm the efficiency of lipopeptide+EDTA washing agent and the combined washing process for As remediation in the soil. Also, the toxicity of the washing agent as well as recycling of the washed leachate must be studied.

Field of Study:	Hazardous Substance and Environmental Management	Student's Signature ....
Academic Year:	2021	Advisor's Signature ....
		Co-advisor's Signature ....

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CHAPTER 1

INTRODUCTION

1.1 Statement of the problem

Arsenic (As) is a toxic metalloid that acts as a carcinogen to human beings. This metalloid is omnipresent in nature and occurs due to the geological cycles and anthropogenic sources. Arsenic exists mainly in three valency states (–3, +3 and +5). The trivalent arsenic (As^{3+}) and the pentavalent arsenic (As^{5+}) occur widely in natural water sources and are soluble over a wide range of pH and Eh conditions (Liu et al., 2010). In oxidizing environmental conditions, As^{5+} is more predominant, whereas in reducing environmental conditions As^{3+} is more dominant (Duker et al., 2005). A more general classification can be; arsenic containing minerals, arsenic oxyanions and arsenic gases present in the geological cycles. Toxicity of arsenic differ in the order of Arsine > Arsenite > Arsenoxide > Arsenates > Arsenic (Duker et al., 2005). This abundant prevalence of this material in nature has led to the chronic arsenic poisoning, concentrated to the regions of South and Southeast Asia. Humans experience arsenic exposure mainly through drinking water and food. Arsenic is rendered toxic through the interaction between the sulfhydryl group in the proteins and enzymes. It denatures the proteins and enzymes from their structure. The first symptoms of acute arsenic poisoning include vomiting, abdominal pain, and diarrhea and, in long term, leading to skin lesions, skin cancers, neurological problems, cardiovascular diseases, and chronic kidney diseases (Duker et al., 2005).

In response to the worldwide arsenic contamination, the removal of arsenic from soil and water has become a dire necessity. Various in-situ and ex-situ remediation techniques have been identified for heavy metal remediation from the soil such as soil flushing, soil washing, surface capping, encapsulation, electro-kinetic extraction, stabilization, landfilling, solidification, vitrification, phytoremediation, and bioremediation (Liu et al., 2018). Soil washing is a combination of chemical and physical processes that are employed to remove heavy metals from contaminated soil by washing the soil ex-situ with uniquely developed solutions. Soil washing is regarded to be a cost-efficient remediation technique (Rodriguez et al., 2014) and the washing solutions mobilize heavy metals by altering solution ionic strength, soil acidity, redox potential, or complexation (Liu et al., 2018). Many synthetic surfactants have been suggested to eliminate arsenic from the soil such as Tween 80, Surfacpol 14014, and Polafix CAPB. The employment of chemicals for soil washing may cause dissolution of metals and lead to pH variations in the soil. These characteristics can affect other soil properties such as humic substances, living organisms and nutrient levels (Torres et al., 2011).

Biological removal of heavy metals has been studied via the use of biosurfactants. A biosurfactant is made up of a hydrophobic and hydrophilic component. Biosurfactants or natural surfactants can be produced extracellularly by a wide range of microorganisms including bacteria, fungi, yeast, and plant materials. Glycolipids, lipopeptides, polymeric biosurfactants and fatty acids are extensively used in natural soil remediation techniques (Usman et al., 2016). Studies indicate that biosurfactants reflect numerous advantages beyond the synthetic surfactants especially, biodegradability and biocompatibility with minimal harm to the environment. Cheap raw materials can be used to synthesize the biosurfactants. They have been employed in environmental control due to the high efficiency in industrial emulsions managing, oil spillage controlling, detoxification and biodegradation of industrial effluents and polluted soil bioremediation (Usman et al., 2016). Possible mechanisms for extraction of heavy metals by biosurfactants include ion exchange, precipitation-dissolution, and association with the counter-ion (Luna et al., 2016). Especially the anionic nature of the biosurfactant from microorganisms can bind with heavy metals and facilitate their desorption from soils. In this study, the biosurfactant from *Bacillus subtilis* GY19, a local bacterium isolated from soil, was applied to remove arsenic from soil. Lipopeptide biosurfactant obtained *Bacillus subtilis* GY19 shows the higher capacity in anionic ability (Rongsayamanont et al., 2017). It was expected that arsenic would be removed by forming complexes with this anionic surfactant on the soil surface, being detached into the soil solution due to the lowering of the interfacial tension, and hence associating with surfactant micelles.

To enhance the activity of biosurfactant, this study also investigated the effect of a combined mechanical agitation and ultrasonication process and the addition of builder in washing solutions. Application of ultrasonic soil washing with mechanical soil washing leads to a higher efficiency in metal removal from the bonded soil surface (Kim et al., 2016). The mechanical mixing causes macroscale contact between the soil particles and the washing liquid and induces the removal of pollutants weakly bonded on the surface of soil particles for the overall range of the slurry. The ultrasonication shows the microscale sonophysical effects caused by ultrasound, which can remove pollutants relatively strongly bonded on the surface and trenched in the pores of soil particles for the very limited cavitational zone (Kim et al., 2016). Moreover, the addition of builders such as sodium carbonate (Na_2CO_3) and ethylenediaminetetraacetic acid (EDTA), as a hardness reducing agent in the washing solution can increase the heavy metal removal capability of lipopeptide biosurfactant (Yangxin et al., 2008).

In retrospect, this research was divided into 3 phases: namely, 1) the formulation of the washing agent by optimizing the biosurfactant concentrations with the builder, 2) the development of the integrated soil washing process with ultrasonication and mechanical agitation, and 3) the optimization of the operation conditions for efficient arsenic removal from the soil using design of experiment. The application of bio-based washing agent and optimized washing process should lead to the final arsenic concentration in the soil below the Thai standards for habitable and agricultural sites 27.0 mg kg^{-1} (Henke, 2009). The acquired results will be used in an environmentally friendly manner to remediate the agricultural soil with high arsenic concentration.

1.2 Objectives

The goal of this research is to apply biosurfactant-based washing agent for removal of arsenic from the contaminated soil in an effective and environmentally friendly way. Several objectives were established as follows:

1. To formulate biosurfactant-based washing agent for arsenic removal from soil using lipopeptide biosurfactant and builder.
2. To enhance arsenic removal from soil using biosurfactant-based washing agent by the combined mechanical agitation and ultrasonication.
3. To optimize operation condition of arsenic soil treatment by biosurfactant-based washing agent and combined soil washing process using factorial design.

1.3 Hypothesis

1. Lipopeptide biosurfactant obtained from *Bacillus subtilis* GY19 along with builder can be used to wash arsenic from contaminated soil with higher efficiency than chemical surfactant-based washing agent.
2. The combined mechanical agitation and ultrasonic can enhance arsenic removal from soil by biosurfactant-based washing agent.
3. The optimized operation condition can enhance the efficiency of washing solution and combined soil washing process to meet up the final arsenic concentration in the soil below the Thai standards.

1.4 Scope of study

1. Lipopeptide biosurfactant obtained from *Bacillus subtilis* GY19 was used as the main component in the washing agent. This biosurfactant is classified as an anionic surfactant and has relatively hydrophobic property.
2. Agricultural soil from the Suphanburi area was used as a modeled arsenic-contaminated soil for the washing purpose. The sample collected from a corn and sugar cane plantation, which has recorded the concentrations of 4.8-1,070.4 mg/kg arsenic (Tiankao & Chotpantarat, 2018).
3. For the washing lipopeptide was taken as a series in concentration of 0.25xCMC, 0.5xCMC, 1.0xCMC, 5.0xCMC and 10.0xCMC for the amount reduction of biosurfactant
4. Ultrasonic soil washing was carried out using the Elmasonic E 30H ultrasonic device with 37 kHz with an optimal removing capacity of 80 W. Mechanical agitation was done through IKA KS orbital shaker in 200 rpm.
5. After the initial arsenic concentration analysis of the natural soil, artificial spiking of arsenic was carried out for obtaining the high arsenic concentrations in the soil samples.
6. For the optimization of washing condition, Box-Behnken Design was applied for analyzing the relationship between influenced factors and the arsenic removal efficiency.
7. All experiments in this research were performed on the laboratory scale.

CHAPTER 2

BACKGROUND AND LITERATURE REVIEWS

2.1 Arsenic and its contamination

2.1.1 Arsenic and toxicity

Arsenic (As) is known as an extremely toxic element and non-threshold carcinogenic element that can be identified all around the globe and the environment (Jayasumana et al., 2014). Arsenic is steel with the physical properties of gray, fragile, and crystalline. It is broadly known as three allotropic shapes in yellow, dark, and gray (Adriano, 2013). In the air, it appears as tarnish, which quickly oxidizes into arsenous oxide (As_20_3) with a garlic odor when heated. It belongs to Group V-A in the periodic table and has an atomic weight of 74.92, chemically similar to phosphorus. Gray As has a density of 5.73 g cm^{-3}, a melting point of 817°C, and a sublime temperature of 613°C. Arsenic's most common oxidation states are -3, 0, +3, and +5. The reduction of arsenic oxides produces elemental arsenic (Adriano, 2011). Trivalent arsenic (As^{3+}) and pentavalent arsenic (As^{5+}) are both abundant in natural waters and are soluble over a wide pH and Eh range (Liu et al., 2010). Although As^{5+} is more prevalent in oxidizing environments, As^{3+} is in a stronger position in reducing environments. (Duker et al., 2005). In general, As^{3+} is more poisonous than As^{5+} (Duker et al., 2005). Arsenic compounds compete for chemical binding sites with their phosphorous analogs. In both its trivalent and pentavalent phases, arsenic produces stable organic compounds by covalently bonding with most nonmetals and metals.

As^{3+}, for example, is an oxyanion found in the environment. Arsenic is found in abundance in the natural world.

Environmental contamination with As has been observed all around the world (Wang et al., 2011). Arsenic levels in the continental crust of the earth range from 1.5 to 2 parts per million. However, geological activities changed the concentration from 126 parts per million to 8000 parts per million in various parts of the planet. In uncontaminated, untreated soils, arsenite levels rarely surpass 10 ppm. Anthropogenic sources of As, on the other hand, have raised background concentrations (Figure 2.1). As a result, As residues can build up to dangerous amounts in agricultural areas where As pesticides or defoliants have been applied often. As a result, arsenical use in agriculture has resulted in 600 ppm or higher of As in the surface soil (Adriano, 2011). Large amounts of poisonous substances have been released into the biosphere as a result of global industrialisation, conflict, and nuclear processes. Heavy metals produced as effluent from various businesses, nuclear radiation, and heavy metals released by another activity in the environment may pollute the soil (Akhtar et al., 2021). Contamination with As has become a serious problem among them. Since the middle Ages, the toxicological significance of arsenic as a suicide and homicidal toxin has been widely documented. The element's deadly property has been used by civilization into the contemporary age as arsenicals, which are commonly employed in agriculture and forestry to control pests and noxious weeds, as well as defoliants. Arsenic compounds are mostly utilized as pesticides, herbicides, and silvicides in agriculture and forestry (Figure 2.1). Because of its threat to human health and detrimental impacts on plant and animal growth, As poisoning in the environment has become a red hotspot in recent years (Liu et al., 2010). Mining, smelting, and agriculture are the most common anthropogenic sources of arsenic contamination in water and soil. Contamination with As has become a big problem all over the world, particularly in India, Bangladesh, Sri Lanka, Thailand, Cambodia, and China (Figure 2.2). In most countries followed by the agricultural industries and mining constructions for the root causes in arsenic contamination. During the last two decades, from 1990 to 2010, the Rajarata area of Sri Lanka reported a significant number of people with chronic kidney disease (CKD). According to government sources, 14,000 people died as a result of arsenic poisoning and CKD during this time. This is not a tragedy that is unique to Sri Lanka. Field samples of soil, water, rice, vegetable, fish, human hair, and urine were gathered in an abandoned tungsten mine in Shantou City, southern China, as part of an extended environmental research. Soil As levels ranged from 3.5 to 935 mg kg^{-1}, with a mean value of 129 mg kg^{-1}, As concentrations in groundwater reached up to 325 g L^{-1}, and As concentrations in hair and urine samples reached up to 2.92 mg kg^{-1} and 164 g L^{-1}, respectively, indicating a possible health concern among surrounding populations (Liu et al., 2010). Bangladesh is currently facing a serious public health issue, with research indicating that 85 million people are at risk of arsenic poisoning via drinking water and soil (Hossain, 2006).

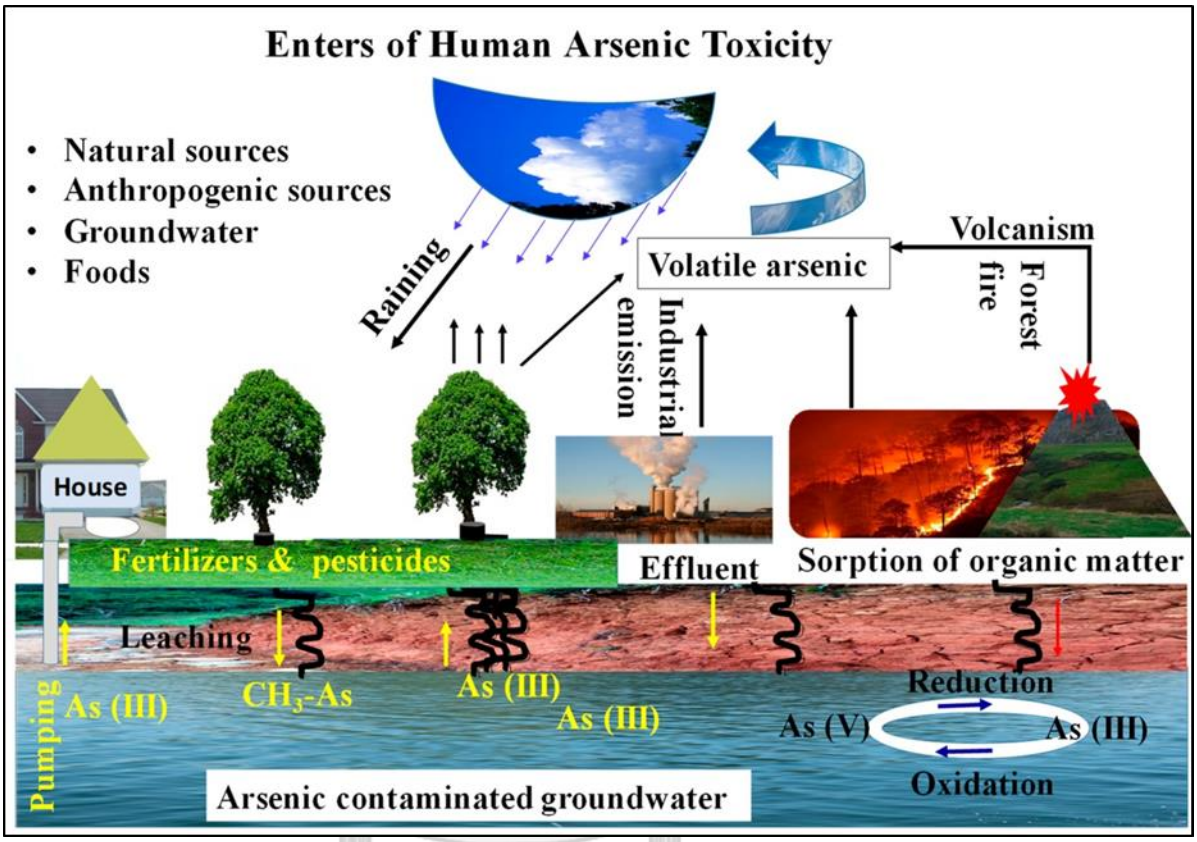


Figure 2.1 Graphical representation of human exposure to arsenic and arsenic cycle (Kolya et al., 2021)

Arsenic can be absorbed by ingestion, inhalation, or through permeation of the skin or mucous membrane. Arsenic can be toxic through the interaction between the sulfhydryl group in the proteins and enzymes. It breaks down the structure of proteins and enzymes. Arsenic has the ability to interact with important enzyme processes and gene transcriptional events. Arsenic's depletion activity can stop DNA transcription from taking place inside the cell system. Arsenite can inhibit more than 200 enzymes in the body because arsenate has a similar structure as phosphate, it can substitute for phosphorus in the body, which can lead to the replacement of phosphorus in the bone for many years. Because arsenate is quickly hydrolyzed in the cell, it prevents phosphate from being transferred to adenosine diphosphate (ADP) to produce adenosine triphosphate (ATP), depleting the cell's energy (Duker et al., 2005).

Arsenic exposure mostly affects the gastrointestinal tract, circulatory system, liver, kidney, skin, and tissues, as these live tissues are extremely vulnerable to metalloid exposure. Hair loss, hyperkeratosis, desquamation, dermal lesions, peripheral neuropathy, skin cancer, and peripheral vascular disease are all symptoms of dermal arsenic exposure. Because it contains more sulfhydryl groups connected to keratin protein, skin is well recognized for its arsenic localization. The worst-case scenario is skin cancer, which includes in situ cell carcinoma or Bowen's disease, invasive cell carcinoma, and numerous basal cell carcinomas, which have all been linked to chronic arsenic exposure with carcinogenic effects following long-term exposure (Duker et al., 2005). The symptoms can be followed in the following order:

Initial stage: dermatitis, keratosis, conjunctivitis, bronchitis, and gastroenteritis

Second stage: peripheral neuropathy, hepatopathy, melanosis, depigmentation and hyperkeratosis

Last stage: gangrene in the limbs and malignant neoplas

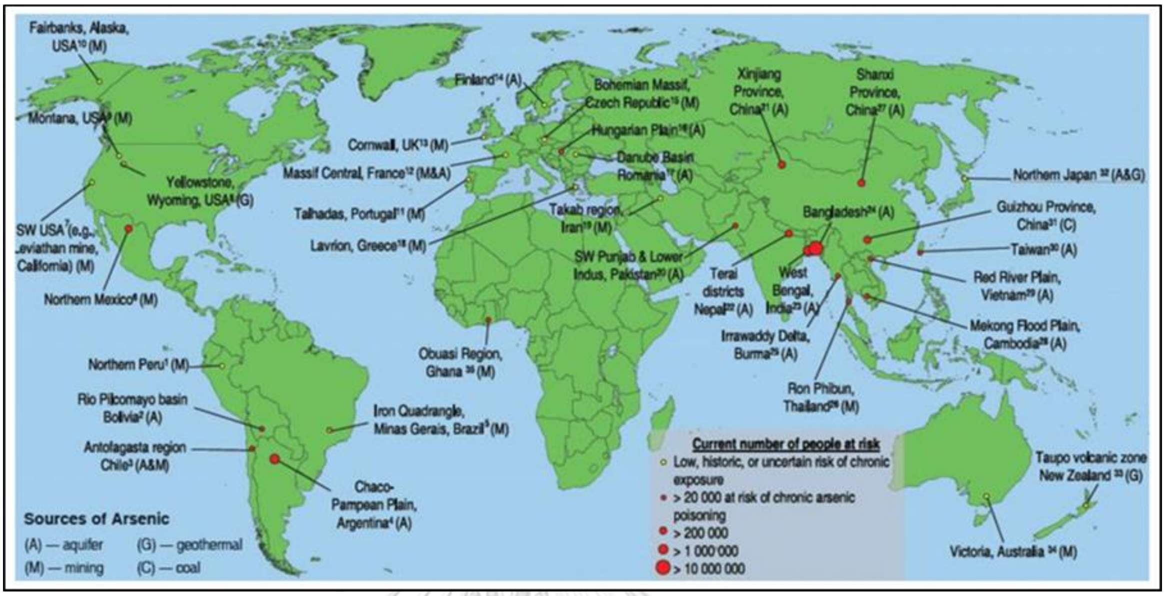


Figure 2.2 Worldwide distribution of arsenic contaminated regions, showing source of arsenic and numbers of people at risk of chronic exposure (Thakur et al., 2010)

Due to the seriousness and the higher contamination governments in worldwide have stated various standards to the As regulation (Table 2.1). Regulations related to arsenic in drinking water, solid and liquid wastes, food, commercial products, and sediment and soil are often very complex (sometimes industry- and site-specific), vary from nation to nation, and frequently change over time.

Table 2.1 Regulation and limits of the As contamination standards in various countries in drinking water, solid and liquid wastes, and sediment and soil (Henke, 2009).

Country	Drinking Water (μg/l)	Surface & Ground Water (μg/l)	Solid & Liquid Waste (μg/l)	Sediment & agricultural Soil (mg/kg)
Australia	7.0	50.0	-	20.0
Canada	10.0	18.0	5.0	13.6
Japan	10.0	10.0	100.0	15.0
USA	10.0	150.0	-	22.0
India	50.0	50.0	-	10.0
Thailand	50.0	10.0	-	27.0
EU Union	10.0	10.0	-	10.0

2.1.2 Arsenic mobility and fractionation

Arsenic mobility and bioavailability in the environment are affected by a number of factors. The chemical specificity of arsenic, soil pH, manganese and iron oxides, soil texture and clay minerals, redox potential, and competing ions are all factors to consider. Chemical species have a significant impact on As sorption affinity. At pH below 6, for example, As adsorption on alumina decreased in the following order: As^{+5} > monomethyl arsenic = dimethyl arsenic > As+3, whereas at pH above 6, it was As^{+5} > monomethyl arsenic = dimethyl arsenic > As^{+3} (Wang et al., 2014). In general, As^{+5} has a stronger affinity for soils and sediments than As^{+3} does. In well-drained acidic soils, arsenic is found as $H_2As0_4^-$, while in well-drained alkaline soils, it is found as $HAsO_4^{2-}$. For solids, the effect of pH on arsenic absorption varies significantly. It is mostly determined by the mineral surface's nature. Increased pH had low effect on the amount of As^{5+} adsorbed in low-oxide soils, whereas increased pH lowered adsorption of As^{5+} in highly oxidized soils (Smith et al., 2000). Adsorption of As^{5+} reduces with rising pH in general. In contrast to As^{5+}, As^{3+} adsorption increases as pH rises. The increase in negatively charged As^{5+} species in the soil solution was attributable to two interacting factors: a rising negative surface potential on the plane of adsorption and an increasing amount of negatively charged As^{5+} species in the soil solution. Sorption of As^{5+} and As^{3+} differs between soils and appears to be linked to the soil's oxide level. Arsenic absorption has been aided by amorphous Fe oxide and amorphous Al oxide. Furthermore, the amounts of ammonium oxalate-extractable Fe and Mn were found to be strongly linked with soil arsenic sorption ability. Arsenic mobility and bioavailability are higher in sandy soils than in clayey soils when it comes to soil and texture. Arsenic phytotoxicity to horticultural crops is highest on a loamy sand soil and lowest on a silty clay loam (Adriano, 2011). The competition for sorption arsenic in the soil is also influenced by redox potentials and chelating compounds. Arsenic's sorption behavior is mostly determined by its oxidation state, as well as the medium's pH and redox potential. In general, As^{3+} is less firmly sorbed to a range of sorbents than As^{5+}; it is more mobile in the +3 oxidation state than in the +5 oxidation state; and the oxidation of As^{3+} to As^{5+} enhances As immobilization.

Kaewlongloi et al. (2020) investigated the contamination of As in agricultural soil, which has a big influence on arsenic. Soil properties and the distance from the source of arsenic to the arsenic form in the soil were investigated as a consequence. Twelve top and bottom soil samples were obtained at a depth of 100 cm at 0.5–6.0 km from the arsenic source, and soil properties and arsenic form were evaluated using hierarchical extraction. In comparison to the recognized limits in Thai agricultural soil (27.0 mg/kg), both the upper and lower soils exhibited high total arsenic levels (365.87 and 192.87 mg/kg, respectively) (Kaewlongloi et al., 2020). Arsenic connected to iron and amorphous aluminum oxide makes up the majority of arsenic in the top soil. Non-specific form of arsenic decreased with increasing distance from the source. There was a link discovered between non-specific and particular adsorbed arsenic and thin soil qualities.

As speciation and fractionation in the soil impact anions' potential to influence As mobility in subsurface habitats. The impact of different anions on As mobility has been evaluated by analyzing its extractability by various salts such as Na_3PO_4, Na_2CO_3, Na_2SO_4, and NaCl, according to Goh and Lim (2005). A selective sequential extraction (SSE) strategy was designed to fractionate As in the soil into 6 fractions. The bulk of the As was extensively adsorbed in the fine soil via surface complexation, according to the SSE data. A large quantity of As was found in the exchangeable fraction, amorphous Fe oxyhydroxides, and the residual fraction. The anions' abilities to mobilize As bound in fine soil were in the following order: PO_4^{3-} $>>CO_3^{2-}>SO_4^{3-}=Cl^{-}$. The ligand exchange process in which the securely bound As was replaced by the phosphate anion was assumed to be the primary cause of phosphate-mediated arsenic mobilization (Goh and Lim, 2005). The As fractionation through the soil reveals that the property of the soil has a major influence on As mobility in the soil.

2.2 Biosurfactant and its application for heavy metal removal

2.2.1 Properties of biosurfactants

Biosurfactants are surface-active agents that are generated by a variety of microorganisms including bacteria, fungi, and yeast. A biosurfactant is made up of a hydrophobic and hydrophilic component. The polar part of biosurfactant can be any amino acid, a carbohydrate, and/or a phosphate group, while a long-chain fatty acid constitutes the nonpolar portion (Usman et al., 2016). Biosurfactants have the characteristic property of reducing the surface and interfacial tensions using the components which consist of a hydrophilic head and hydrophobic tail. These natural surfactants are mainly classified by their chemical structure and their microbial origin (Banat et al., 2010). Biosurfactants are classified based on the chemical composition, such as fatty acids, antibiotics, peptides, glycolipids, phospholipids, lipopeptides.

Moreover, they can be classified into two main categories based on the molecular weight they have, high-molecular-weight polymers or bioemulsans and low-molecular-weight molecules called biosurfactants. The low molecular weight biosurfactants with a lower surface and interfacial tension include groups of macromolecules such as proteins, lipopeptides, glycolipids, and phospholipids (Usman et al., 2016). The most frequently used types of biosurfactants are glycolipids. Glycolipids consist of monosaccharides, disaccharides, trisaccharides, and tetrasaccharides including glucuronic acid, galactose sulfate, galactose, mannose, glucose, and rhamnose. The fatty acid constituent usually has a composition similar to that of the phospholipids of the same microorganism. The glycolipids can be categorized as trehalose lipids, sophorolipids (produced by different strains of the yeast), and rhamnolipids (Sanchez et al.,2013). High molecular weight biosurfactants are generally polyanionic heteropolysaccharides containing proteins and polysaccharides. As an example, extracellular lipopolysaccharide biosurfactant produced by *Acinetobacter calcoaceticus* and *Acinetobacter radioresistance* is a high-molecular-weight bioemulsifier (Alizadeh-Sani et al., 2018).

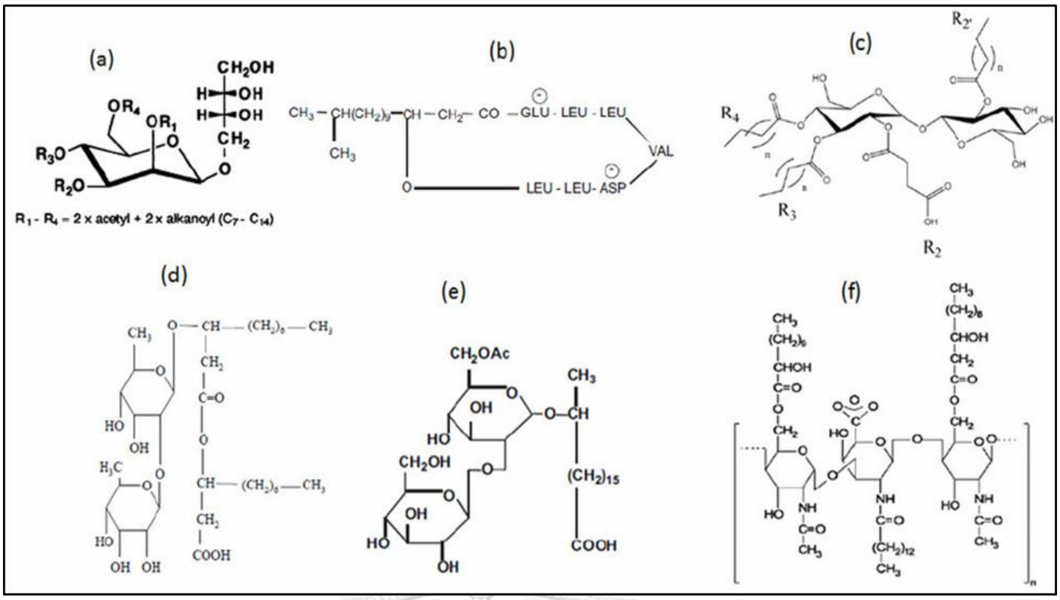


Figure 2.3 Chemical structures of some common biosurfactants (a) Mannosylerythritol lipid (b) Surfactin (c) trehalose lipid (d) Sophorolipid (e) Rhamnolipid and (f) Emulsan. (Salihu et al., 2009)

When the concentration of surfactants in aqueous phase exceeds a certain value, the surfactant monomer molecules form organized aggregates of a large number of molecules called ‘micelles’, and this specific concentration is called as critical micelle concentration (CMC). The dominant mechanisms of surfactant enhanced remediation based on mobilization and solubilization. The mobilization mechanism occurs below the CMC value and the solubilization mechanism occurs above the CMC value. Below the concentration of CMC value, surfactant monomers tend to decrease the surface and interfacial tension in between air-water and soil-water systems, while, above the CMC value surfactant monomers aggregate to form micelles to increase the solubility (Uram and Pekdimir, 2004 & Nguyen et al., 2008). When the surfactant present in a heterogeneous system like water-soil system, they could adsorb onto the surface of soil particles and make interactions. In the situation, hydrophilic head group enters to the aqueous phase and liphophilic tail groups tend to combine with the soil particles. Therefore, surfactants at a low concentration mainly accumulate at solid–liquid or liquid–liquid interface in the form of monomers. With the increase in monomer concentrations, surfactant molecules gradually replace the interfacial solvent like water, and then resulting in a lower polarity of the aqueous-phase and decreased surface tension. Furthermore, the increase in the concentration forms the micelles and increase in the solubility in aqueous phase to promote the decontamination of the pollutants from the soil. The dissolved contaminants in aqueous phase have better mobility, being conducive to the subsequent removal of contaminants via either biotic route or abiotic route (Mao et al., 2015).

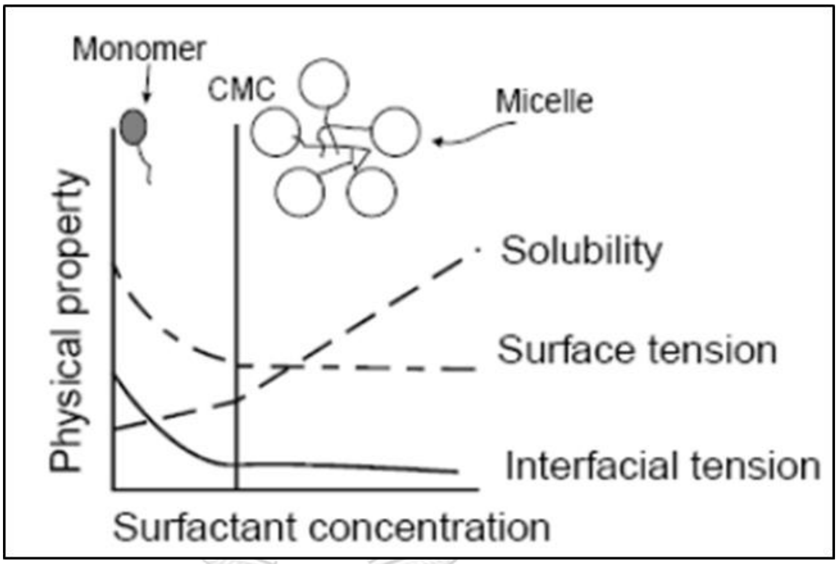


Figure 2.4 Surface tension, interfacial tension and solubilization as a function of surfactant concentration (Mulligan et al., 2001)

2.2.2 Lipopeptide biosurfactant

Lipopeptide biosurfactants are one of the five major classes of biosurfactants produced by microorganisms, and they have gotten a lot of attention in the scientific community and in industry because of their powerful interfacial and biological activities, as well as their environmentally friendly properties. In the last two decades, 90 distinct lipopeptide species have been discovered in 26 different microbial families (Usman et al., 2016). Currently, many lipopeptide biosurfactant-producing microorganisms have been isolated and identified as belonging to *Bacillus* sp. Most lipopeptide biosurfactants have been shown to have a structure similar to surfactin (Figure 2.5), the biosurfactant produced by *Bacillus subtilis*. These are cyclic structured biosurfactants, defined as those with a cyclic component created by a carboxyl group in the C-terminal of a peptide chain bonded to an amino group in the peptide chain or a hydroxyl group in the fatty acid chain. Linear lipopeptides, on the other hand, have a linear amino acid group coupled one at a time, with the fatty acid bound to the -amino group or another hydroxyl group.

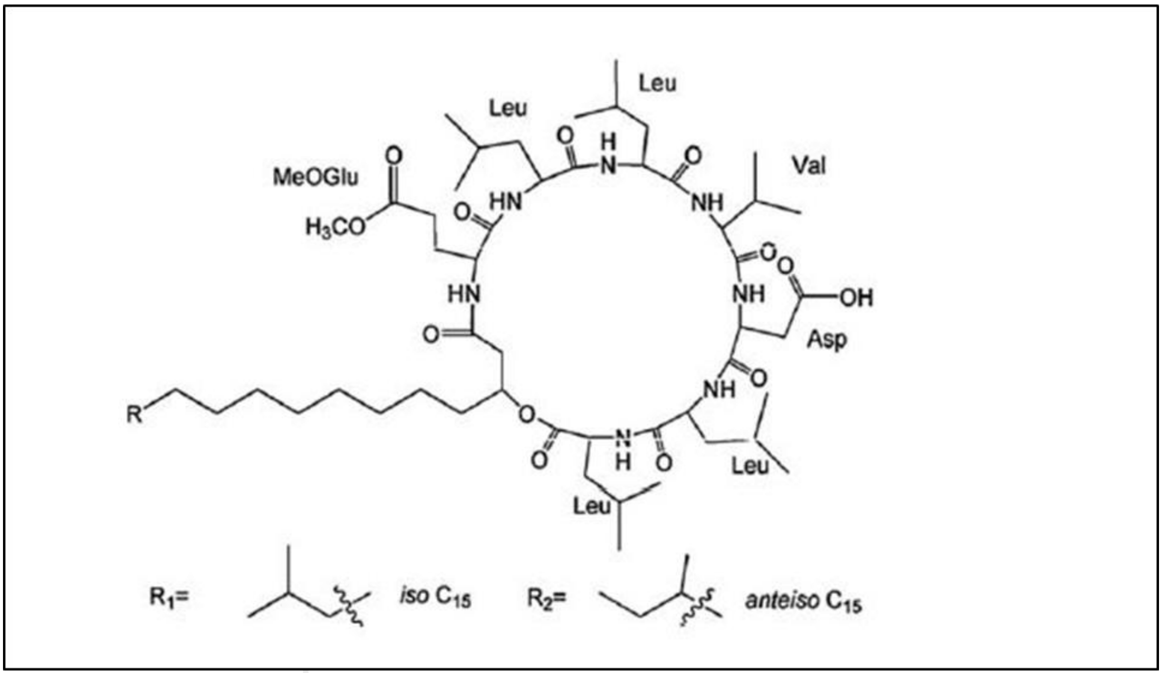


Figure 2.5 Basic chemical structure of C15-surfactin-O-methyl. (Liu et al., 2015)

So, the activity of the lipopeptide biosurfactant depends on the structural behaviors of the amino acids and the fatty acid chain placements. Lipopeptide surfactants have more anionic capabilities with the amino acid groups in the hydrophilic head, and that reflects the more suitable capabilities to remove contaminated cations from the soil. Consequently, this research focused on the application of lipopeptide biosurfactant as the main component in washing agent.

2.2.3 Application of biosurfactant for heavy metal removal

For organic compounds such as heavy oil and diesel oil, the main role of biosurfactants in the remediation is increasing bioavailability or mobilizing and removing the contaminants by solubilization and emulsification mechanisms. For inorganic compounds, such as heavy metals, the application of biosurfactants in the remediation is targeted at chelating and removal of such ions during a washing step facilitated by the ionic interactions between the biosurfactants and the metal ions (Banat et al., 2010). Possible mechanisms for extraction of heavy metals by biosurfactants include ion exchange, precipitation-dissolution, and association with the counter-ion. It is contemplated that the metals are removed by forming complexes with the surfactant at the soil surface, being separated from the soil by reducing interfacial tension and associating, consequently, to the surfactant micelles. Anionic surfactants provide satisfactory results since the metal cations have an affinity for negatively charged surfactants. Studies indicate the possibility that the biosurfactant allows greater removal efficiency due to its ability to decrease interfacial tension (Luna et al.,2016).

The metal removal mechanism through biosurfactant is as follows, the first approach is through the complexation of the free form of metal ions residing in solution. This would decrease the solution-phase activity of the metal and, therefore, promotes desorption according to Le Chatelier's principle. The second approach is through the accumulation of biosurfactants at the solid-solution interface under the condition of reduced interfacial tension. This would enable direct contact between the biosurfactant and the sorbed metal (Figure 2.6). The efficiency of the biosurfactant is based on its characterization. Biosurfactant structure, size and charges influence the movement of biosurfactant-metal complexes through the soil. The access of biosurfactants through soil pores impacts the interaction of biosurfactants with sorbed metals (Usman et al., 2016). In the industrial scale, heavy metal removal through the biosurfactant has three crucial steps. They are sorption and binding of the biosurfactant to the soil surface and also to the metal; separation of the metal from the soil to the solution; and lastly association of the heavy metal with micelles. Heavy metals are entrapped within the micelles through electrostatic interactions and can be conveniently recovered through precipitation or membrane separation techniques (Figure 2.7). For the industrial scale, the efficient washing time may increase, heavy metal removing efficiency may decrease with compared to the pilot scale work due to the bulk of soil structure and with the experimental errors (Usman et al., 2016).

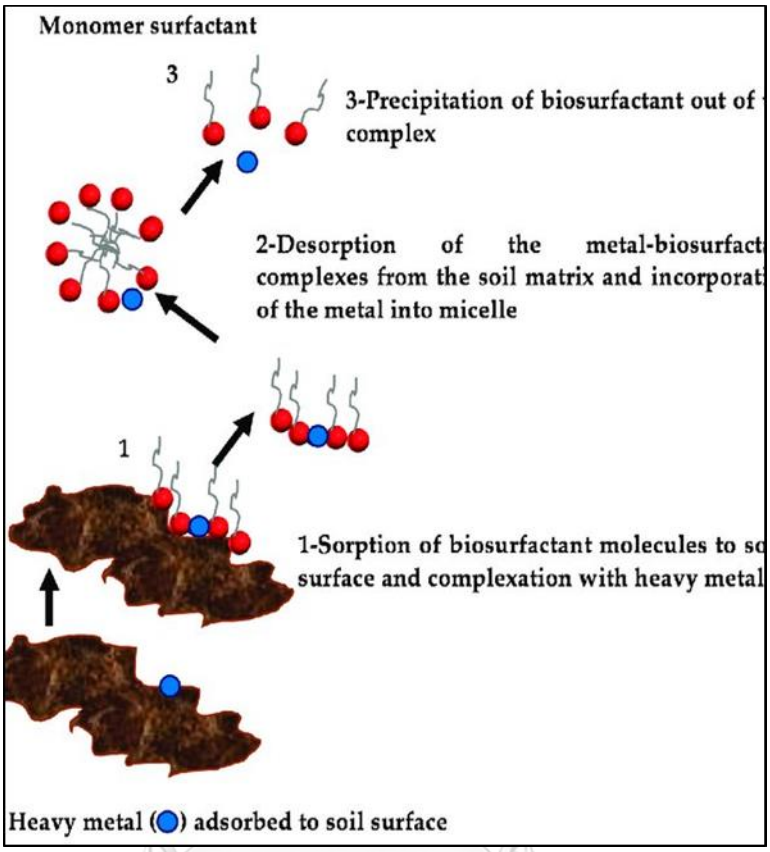


Figure 6 Mechanism of removal of heavy metals from contaminated soil by biosurfactants (Santos et al., 2016)

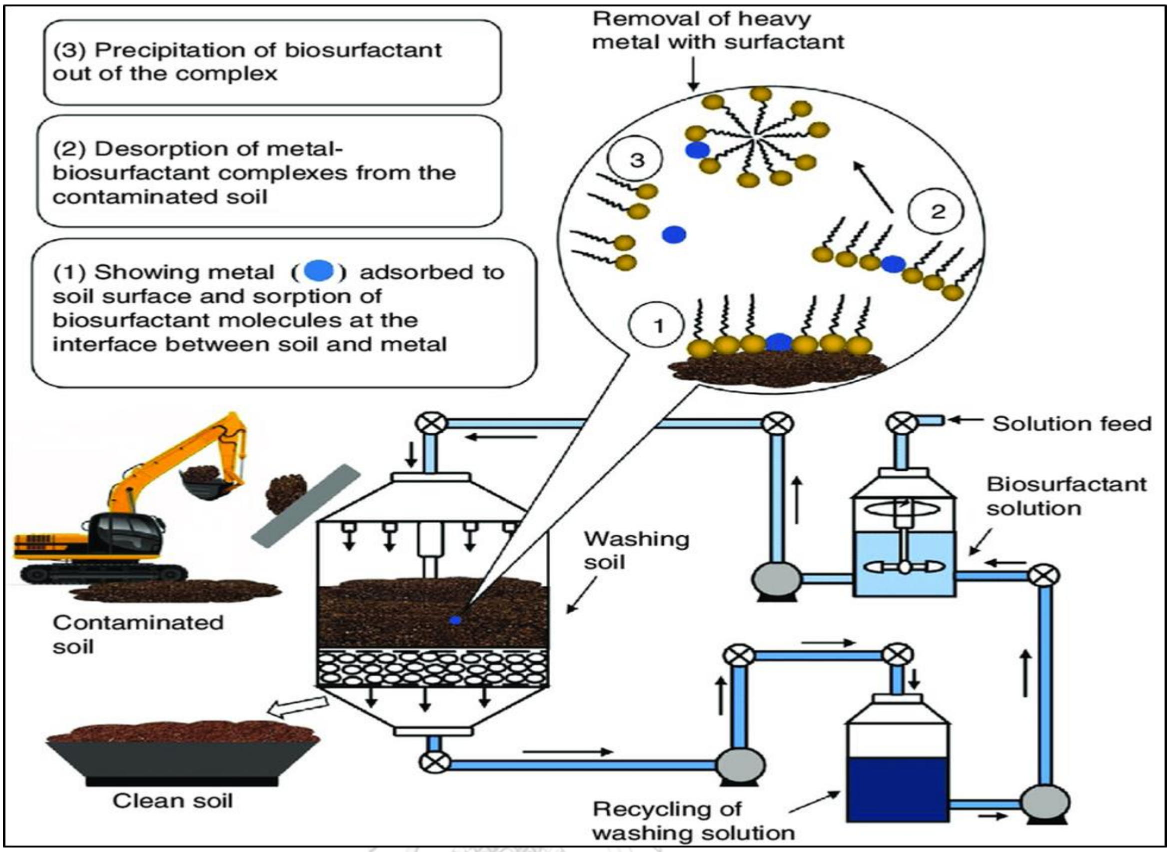


Figure 7 Mechanism of heavy metal contaminated soil remediation using biosurfactant in industrial scale (Akbari et al.,2018)

Different types of biosurfactants have been used to remediate contaminated soil compared with the synthetic surfactants (Table 2.2). Studies have indicated that biosurfactants have marginal advantages than synthetic washing agents. Firstly, biosurfactants have high biodegradability as they could be effortlessly degraded by bacteria and other microbes; therefore, they produce lesser harm to the environment. Secondly, biosurfactants contain lower toxicity compared to chemically synthesize surfactants, and productions from extremophiles have high efficiency at critical pH and temperature values. Also, cheap raw materials can be employed to synthesize biosurfactants, and that can be accessed in huge quantities easily. For instance, cheaper agro-industrial waste material like bagasse, molasses, and plant material residues can be utilized to produce biosurfactants that are much more economically and show better environmental compatibility.

Application of biosurfactants instead of synthetic surfactants for the contaminated soil remediation is an emerging technology in soil remediation. This shows a novel approach for the heavy metal removing from the contaminated soil that shifting artificial surfactants into ecofriendly and biodegradable chemical compositions (Table 2.2). For example; Singh et al., 2013 reported the usage of lipopeptide biosurfactant obtained from *Bacillus subtilis* A21, which was consisted of surfactin and fengycin. Soil washing with biosurfactant solution have removed significant amount of petroleum hydrocarbon (64.5 %) and metals namely cadmium (44.2 %), cobalt (35.4 %), lead (40.3 %), nickel (32.2 %), copper (26.2 %) and zinc (32.07 %) (Singh and Cameotra, 2013). Finally, the investigation has proved the lack of toxicity condition in the soil through the 100 % mustard seed germination test for the further usage of soil under normal environmental conditions. The maximum efficiency of the heavy metal removal occurred in the higher CMC value and higher pH (pH 9) condition in the washing solution. At the lower pH conditions such as pH 5 and 7, the biosurfactant precipitation was reported (Singh and Cameotra, 2013). pH has a significant effect on the morphology and the lipopeptide activity of micelle formation. Mulligan (2009) reported the pH increasing in the solution creates small micelles with a higher surface area. Chen et al. (2007) showed that the usage of lipopeptide biosurfactant to remove Ni and Cd from spiked clay and proved higher in efficiency on removing metals from the soil in 10 x CMC value rather than synthetic surfactants SDS and Tween 80. Yang et al., 2016 reported an environment-friendly bioleaching technique with biosurfactants related to *Burkholderia* sp. Z-90. This lipopeptide anionic surfactant has withdrawn heavy metals from the soil 44.0% for Zn, 32.5% for Pb, 52.2% for Mn,37.7% for Cd, 24.1% for Cu, and 31.6% for As, respectively. The bioleaching efficiency of heavy metals through the anionic surfactants has depended on the specific chemical form of the metals present in the soil. Heavy metals in acid-soluble, reducible and oxidizable fractions are considered to be more mobile and leachable. The residual fractions are considered to be more stable and non-leachable (Yang et al., 2016). Also, they have reported the heavy metal removal were efficient in soils under alkaline condition. With the study, removal efficiencies of heavy metals by strain Z-90 broth leaching were higher than that by free-cell broth leaching in which the removal percentages were 1.9% for Zn, 7.7% for Pb,14.3% for Mn, 4.5% for Cd, 8.4% for Cu, and 3.0% for As. The study performed the ATR-FTIR analysis to determine the interaction between metals in soil surface properties and the biosurfactant. The results have shown the changes in the symmetric stretching carboxyl group O-C=O bond. It can be seen that the symmetric stretching carboxyl group O-C=O bond shifted from 1456.38 to 1411.64 cm^{-1} after bioleaching. This was due to the complexation of heavy metals with the carboxyl group in the strain Z-90 broth. ATR–FTIR spectra have confirmed that the bonding between carboxylic groups and metal ions was mainly ionic bonding through the lipopeptide biosurfactants (Yang et al., 2016).

Table 2 Types of surfactants and their heavy metal removal efficiencies

Surfactant	**Surfactant producing agent**	**Type of soil**	**Targeted heavy metal**	**Initial heavy metal concentration (mg/kg)**	**Heavy metal removal efficiency (%)**	**Reference**
Rhamnolipid	*Pseudomonas aeruginosa*	Agricultural soil	Arsenic	2180	6.79	Wang & Mulligan, 2009
Sophorolipid	*Candida bombicola*	Agricultural soil	Arsenic	2567	11.7	Arab & Mulligan, 2018
Glycolipid	*Burkholderia*	Agricultural	Cadmium	450	37.7	Yang et

Surfactant	Surfactant producing agent	Type of soil	Targeted heavy metal	Initial heavy metal concentration (mg/kg)	Heavy metal removal efficiency (%)	Reference
	sp. Z 90	soil	Copper Arsenic	710 560	24.1 31.6	al., 2016
Rhamnolipd	*Pseudomonas aeruginosa*	Agricultural soil	Nickel Cadmium	2010 1706	68.1 73.2	Mulligan & Wang, 2006
Sophorolipid	*Starmerella bombicola* CGMCC 1576	Artificial spiked soil	Cadmium Lead	142 265	95 52	Qi et al., 2018
Lipopeptide	*Bacillus subtilis* A21	Industrial soil	Lead Nickel Cadmium Cobalt	143.7 227.9 989.9 166.8	40.3 32.2 44.2 35.4	Singh & Cameotra, 2013
Polafix CAPB (Zwitterionic)	Chemically synthetic	Industrial soil	Arsenic	4019	49.7	Torres et al., 2012

Surfactant	Surfactant producing agent	Type of soil	Targeted heavy metal	Initial heavy metal concentration (mg/kg)	Heavy metal removal efficiency (%)	Reference
	surfactant					
Tween 80	Chemically synthetic surfactant	Industrial soil	Cadmium Zink Copper	35582 261 70	85.9 85.4 81.5	Torres et al., 2012

2.3 Soil washing process

2.3.1 Soil remediation techniques, ultrasonication and conventional mechanical agitation

Various remediation techniques have been developed for heavy metal contaminated soil. In-situ and ex-situ remediation techniques among them to rectify the heavy metal-contaminated sites, including surface capping, encapsulation, landfilling, soil flushing, soil washing, electrokinetic extraction, stabilization, solidification, vitrification, phytoremediation, and bioremediation (Liu et al., 2018). Soil washing is a mixed physical and chemical process to remove heavy metals from contaminated soil by washing the soil ex-situ with special-formulated solutions. Soil washing relies on washing solutions to mobilize heavy metals by altering soil acidity, solution ionic strength, redox potential, or complexation (Liu et al., 2018). The metal extraction efficiency of a washing solution also varied with the metal species and was further influenced by the soil pH, texture, and organic matter content (Liu et al., 2018). Soil washing can effectively remove the contaminated particles from the soil with effectively with less cost and energy consumption.

Ultrasonic soil washing is a powerful soil washing technique that consists of the shock wave, micro jet, microstreaming, and micro bubbling. But in most cases, the ultrasonic soil washing method has a problem for scale-up experiments. A combination of both mechanical agitation and ultrasonic soil washing method has shown higher efficiency rather than single method. The mechanical mixing caused macroscale contact between the soil particles and the washing liquid and induced the removal of pollutants weakly bonded on the surface of soil particles for the overall range of the slurry. On the other hand, microscale sonophysical effects caused by ultrasound could remove pollutants relatively strongly bonded on the surface and trenched in the pores of soil particles for the very limited cavitational zone. The integrated system of mechanical and ultrasound systems showed higher efficiency in the soil washing experiment (Kim et al., 2016). However, this approach has not been used in a prominent remedial technique for the contaminated soil along with a biosurfactant. We therefore investigated the ability of lipopeptide-based washing agent on arsenic removal from soil through comparison of soil washing with mechanical, ultrasonication and the combined approach.

2.3.2 Washing conditions, mechanical agitation and ultrasonication for the soil washing experiment

The efficiency of soil washing with aqueous solutions can be influenced by a variety of factors. Washing time, agitation rate, pH of the washing agent, and washing agent concentration are just a few of them. Singh and Cameotra (2013) used a lipopeptide biosurfactant produced from *Bacillus subtilis* A21 to investigate the parameters that influence heavy metal removal including Cd, Co, Pb, Ni, Cu, and Zn from oil-contaminated soil. The duration of the agitation was changed between 0, 12, 24, and 48 hours. As part of the research, the amount of pollutant removed by biosurfactant solution was steadily raised, but after 24 hours, it reached its peak. The agitation rates were varied between 0, 50, 100, 150, and 200 rpm. The best agitation rates were found at 150 and 200 rpm. Higher agitation rates resulted in excessive foaming and increased the frequency of interactions between biosurfactant solution and soil particles harboring contaminants, according to the study (Singh & Cameotra, 2013). At pH 9, the lipopeptide biosurfactant had the best soil washing performance, while lower (pH 5) and higher (pH 11) pH levels reduced heavy metal removal efficiency. The study also demonstrated that lower pH conditions generated biosurfactant precipitation, which was evident as a white precipitate on the soil following centrifugation (Singh & Cameotra., 2013).

The solubilization of heavy metals in washing agents is affected by pH. Arsenic, for example, is tightly bonded to the soil at roughly neutral pH, and mobilization of arsenic requires either a very high (>12) or extremely low (2) pH (Dalgren et al., 2009). The study also examined the concentration variation of the lipopeptide biosurfactant with CMC values of 0.5x CMC, 1x CMC, 25x CMC, and 50x CMC. The heavy metal removal effectiveness gradually rose when the concentrations were increased, and the optimal condition was reached at the 50x CMC value. Chen et al. (2017) investigated the effects of different washing times on the removal of Cu, Cd, Pb, and Cr using rhamnolipid solution. Cu and Cd removal efficiency peaked after 5 hours, while Pb and Cr removal efficiency peaked after 10 hours, according to the study. With increased washing time, weakly bound Cu and Cd were eliminated first, followed by firmly bound Pb and Cr (Chen et al., 2017). Cu and Cr efficiencies increased dramatically as the pH of the washing solution was raised, but Cr and Pb efficiencies grew more slowly (Chen et al., 2017). They discovered that as the pH of the rhamnolipid was increased, it converted from giant vesicles to small vesicles to micelles. It was discovered that as pH climbed, rhamnolipid size decreased, which was good because smaller rhamnolipid micelles were much simpler to diffuse among sediment particles and reach heavy metals (Chen et al., 2017).

Mechanical agitation has taken the major ex-situ soil washing technique in industrial scale and scientific experiments. Within the last 10 years, ultrasonic washing techniques have developed in a massive range of soil and water remediation. The use of ultrasound power has been investigated to speed up sequential extraction methods because it has long been recognized that the cavitational effect created by ultrasound waves can break down the particle size, exposing a fresh surface and aggressively agitating the solution system (Hwang et al., 2007). Chen et al. (2016) successfully implemented ultrasound-enhanced soil washing that has evaluated at 20 kHz and 50 W (equal to about 0.5 W/cm^2), to enhance the desorption of pollutants while avoiding too much damage on the indigenous soil microflora. Mainly, they targeted PAH and seven heavy metals including Pb, Cd, Zn, Cr, Cu, As, and Ni (Figure 2.8).

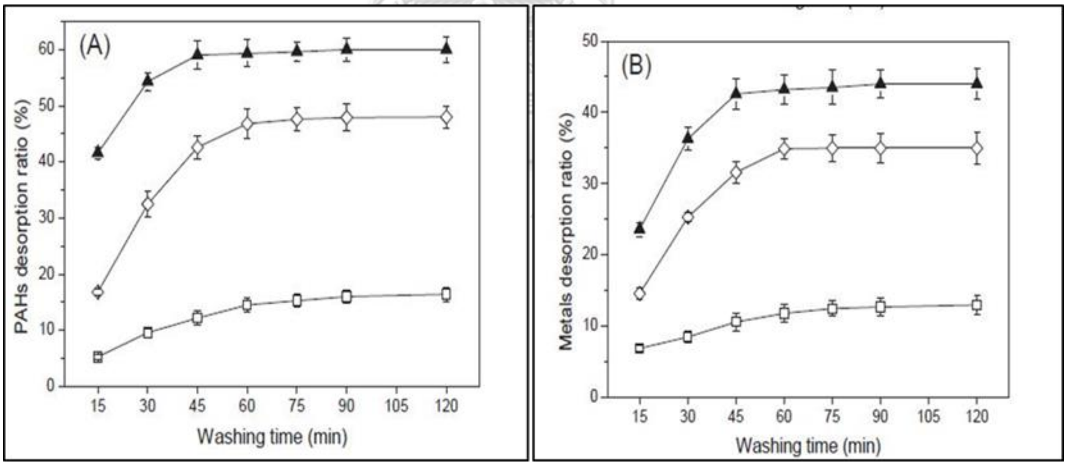


Figure 8 Variations of desorption efficiency of (A) 16 PAHs and (B) 7 heavy metals under different soil washing conditions. (▲ Ultrasonic & mechanical agitation; ◊ mechanical agitation; ☐ ultrasonic). The mechanical agitation frequency was 150

rpm. Washing solution: 75 g/L MCD and 25 g/L EDDS. Soil: solution ratio: 1:5. (Chen et al., 2016)

Kim et al. (2016) presented the microscale effect of the ultrasonic soil washing application along with mechanical agitation. It has powerful sonophysical effects including shock wave, microjet, and microstreaming. The mechanical mixing has caused macroscale contact between the soil particles and the washing liquid and induced the removal of pollutants weakly bonded on the surface of soil particles for the overall range of the slurry. Although, microscale sonophysical effects caused by ultrasound could remove pollutants relatively strongly bonded on the surface and trenched in the pores of soil particles for the very limited cavitational zone (Kim et al., 2016). The cavitational active zone was discovered indirectly using an aluminum foil erosion test (Figure 2.9).

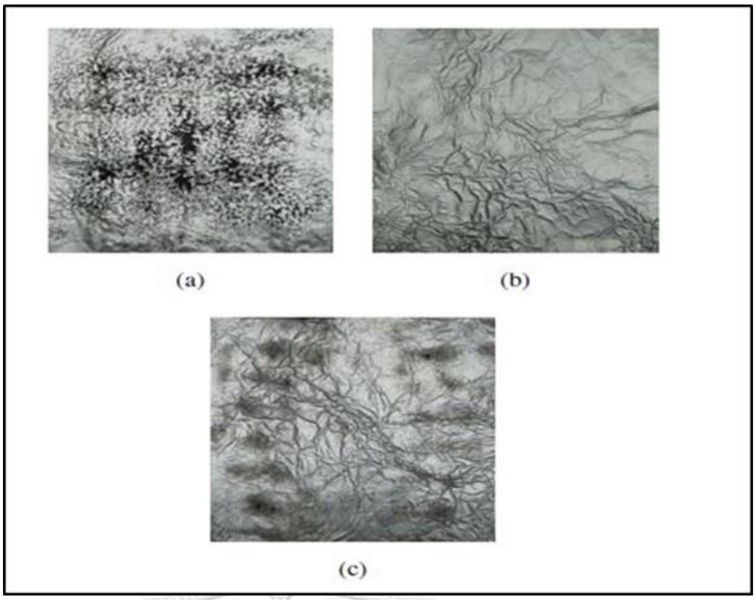


Figure 9 Images of damaged aluminum foil caused by cavitational action: (a) a foil placed on the bottom without soil; (b) a foil placed on the soil; (c) a foil placed on the bottom and covered with soil (Kim et al., 2016)

While sonophysical effects are engaged in mixing and cleaning employing micro-jet, micro-streaming, and shock waves, in heterogeneous systems including solid and liquid phases, sonophysical effects can be more violent (Son et al., 2012). They investigated the removal efficiency of diesel and heavy metals using mechanical soil washing, ultrasonic soil washing, and a combination soil washing procedure. According to the findings, ultrasonic soil washing had a removal efficiency of 58 percent, mechanical soil washing had a removal efficiency of 76 percent, and combined soil washing had a removal efficiency of 85 percent (Son et al., 2012). Mechanical mixing did not cause any noticeable damage to the surface of the soil particles after the SEM pictures of the washed soil were taken. As a result, it was discovered that sonophysical effects caused by ultrasonication mostly affected the soil particle surface, potentially increasing contaminant removal from the soil.

On the basis of conventional mechanical soil washing, Park and Son, 2017 reported full-scale heavy metal removal from contaminated soil using high-power ultrasound. In the study they used transducer sonoreacter with 28 kHz ultrasound frequency with the 200-rpm mechanical stirring. A study varies the solid liquid ratios in 1:2 and 1:3 in 300 g of copper, zink and lead contaminated soil. As 1M HCl used as the washing agent, mechanical soil washing reported 39.4%, 27.3% and 42.2% heavy metal removal efficiency in Cu, Pb and Zn respectively in 1:2 solid liquid ratio (Park & Son, 2017). Furthermore, combined soil washing process reported 66.8%, 65.8% and 65% heavy metal removal efficiency in Cu, Pb and Zn respectively. In the 1:3 solid liquid ratios, mechanical soil washing reported 47.2% Cu, 46.9% Pb and 46.3% Zn. With the combined soil washing experiment, the efficiencies were 76.2% Cu, 75.4 % Pb and 72.0% Zn with more than 50% removal enhancement. Higher heavy metal removal efficiencies were reported in lower solid/liquid ratio, which means the increased contact between soil and the washing agent and relatively low concentrations of extracted metals in the liquid (Park & Son, 2017). Also, the study showed that soil particles moved in ultrasound cavitation activity zone by the mechanical agitation and heavy metals were exposure through the powerful sonophysical effects in the strong acidic condition. As a result of this macroscale effects the strongly attached heavy metals can detached easily. The study showed that, the reason for higher removal efficiencies in the case of 1:3 ratios might be due to less attenuation of ultrasound in less particulate condition. Ultrasound can drastically attenuate as it travels in the porous media due to the boundary layer loss (Park &Son, 2017). The scale-up experiment was done through this study with 0.5 M HCl as the washing agent in 1:3 ratio, which resulted 64.2% Cu, 55.3% Pb and 66.3% Zn in combined soil washing process. However, no significant removal efficiency was studied in this experiment of ultrasonic washing without mechanical mixing (Park & Son, 2017). It stated that sonophysical effect was in a limited range of area and suggested that mechanical mixing should combined with ultrasonic system for better cavitational desorption of heavy metal from the soil particles (Park & Son, 2017).

2.3.3 Addition of builders to enhance soil washing

In hard water, biosurfactant efficiency is considerably diminished because of the interactions between biosurfactant and Ca^{2+} and Mg^{2+} ions in hard water and soils. Detergency builders are frequently used in conjunction with surfactants to reduce the amount of surfactants in detergent compositions (Yangxin et al., 2008). Sodium tripolyphosphate (STPP) fits all of a builder's requirements, it was once the most extensively used builder because of its great capacity to remove the Ca^{2+} and Mg^{2+} ions presented in hard water and soils. Phosphates, on the other hand, are a good fertilizer for algae, bacteria, and other flora and fauna in rivers, lakes, and oceans, causing rapid algal blooms and draining oxygen supplies in both the surface and bottom layers of water bodies, leading to eutrophication (Yangxin et al., 2008). Instead of using phosphate containing builders, sodium carbonate, sodium silicate, and ethylenediaminetetraacetic acid (EDTA) were used as a substitute. The performance of sodium silicate-based detergents is nearly identical to that of leading phosphate formulations. They are also in charge of creating bacteria breeding sites (Yangxin et al., 2008). Mukhopadhyay et al. (2015) used a soapnut surfactant in combination with a phosphate system to remove arsenic from soil with a 70% removal efficiency in pH ranges of 4-5. With two active carboxylic groups, EDTA serves as an organic builder as well as a chelating agent. With the addition of EDTA, the anionic surfactant, sodium dodecyl sulfate (SDS) was able to remove 90% of arsenic (Gitipour et al., 2016). Alkyl glucoside surfactant along with methylglycinediacetic acid trisodium (MGDA) as a phosphate-free builder and achieved 85.7% arsenic removal efficiency at pH 12 (Dalgreen et al., 2009). In the wide range application of builders along with surfactants, organic builders like EDTA and MGDA showed higher pollutant removal ability than inorganic builders like phosphate systems, silicate systems and carbonate systems (Table 2.3). After attaching the exchangeable cations along with the GY 19 biosurfactant monomers, this study expected an efficiency loss with As remediation. The applying of organic and inorganic builders would increase the As removal efficiency. In this study, EDTA was used as an organic builder, and the Sodium carbonate (Na_2CO_3) used as an inorganic builder. EDTA acts as a chelating agent with two acting carboxylic groups. It may facilitate exchanging the H^+ ions with the exchangeable cations. Na_2CO_3 also provides the room for exchangeable cations with interchange Na^+ ions.

Table 3 Addition of builder along with surfactants and heavy metal removal efficiency

Washing agent with builder	**Type of soil**	**Targeted heavy metal**	**Initial heavy metal concentration (mg/kg)**	**Heavy metal removal efficiency (%)**	**Reference**
Dithionite+EDTA	Agricultural soil	Arsenic	165.5	90	Wang et al., 2017
Soapnut + Phosphate system	Aquifer soil	Arsenic	88.2	70	Mukhopadhyay et al., 2015
Alkyl glucoside	Industrial	Arsenic	105.4	85.7	Dalgren et al.,

Washing agent with builder	Type of soil	Targeted heavy metal	Initial heavy metal concentration (mg/kg)	Heavy metal removal efficiency (%)	Reference
surfactant+MGDA	soil				2009
Triton X100+EDTA	Industrial soil	Lead Nickel Zink	9.9 29.5 42.4	75 85 90	Saeedi et al., 2019
Rhamnolipid+Citric acid	Artificial spiked soil	Cadmium Lead	554.3 581.1	76.4 26.1	Wan et al., 2015

2.4 Design of Experiment (DOE)

The design of experiment (DOE) is a sophisticated approach for planning an experiment and obtaining efficient findings. In general, the analytical experiment has been carried out on an experimental response by trial and error or one factor at a time. The experimental conditions for these traditional approaches are usually established by conjugating univariate with response. As a result, if the response or optimization was influenced by the other dependent variables, the univariate approach might result in a lot of mistakes. Furthermore, the number of experimental conditions generated by traditional designs may result in a variety of experimental numbers, increasing the time and cost of studying the inquiry. Currently, the advance mathematical and statistical techniques are applied for analyzing the chemical experiment such as Box–Behnken design. This is an experimental design for Response Surface Methodology that was devised by George E. P. Box and Donald Behnken in 1960. The multivariate experimental designs were used to optimize the chemical variables since these approaches may reduce the number of experiments, as well as the time and expense of research (Bezerra et al., 2008)

The Box-Behnken design, which determines the first and second-order coefficients of a mathematical model based on three-level factorial designs, is characterized as rotatable or nearly rotatable. In the Box-Behnken design, the experimental point is equidistant from the center point on a hypersphere. This experimental design is commonly used to examine the relationship between three independent factors and to optimize the relationship between independent factors and response (Bezerra *et al.*, 2008). Box-Behnken design for 3 factors can be divided in 2 types i.e. three-variable factorial design (N = 3k) and Box-behnken design (N = 2k(k-1) +Cp), where k is the number of factors, Cp is the number of the central points as shown in Figure 3.4.

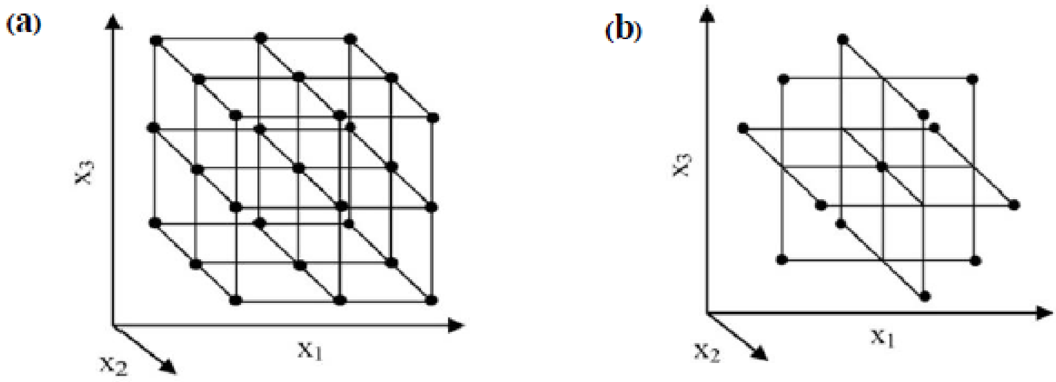


Figure 10 Experimental design based on Box-Behnken design (BBD) in three-level (a) three variables and (b) Box-Behnken design for the optimization of three variables (Bezerra et al., 2008)

Finally, the correlation equation expressed in the form of a polynomial function, and the fit of the model have assessed using analysis of variance (ANOVA) to check the precision of the model before it is used in real-world applications. Theoretically, an ANOVA study may compare the variance of experimental data with the variance of an equation that has an effect on the response (dependent variable). Surface response profiles have used to interpret the data (Figure 3.5).

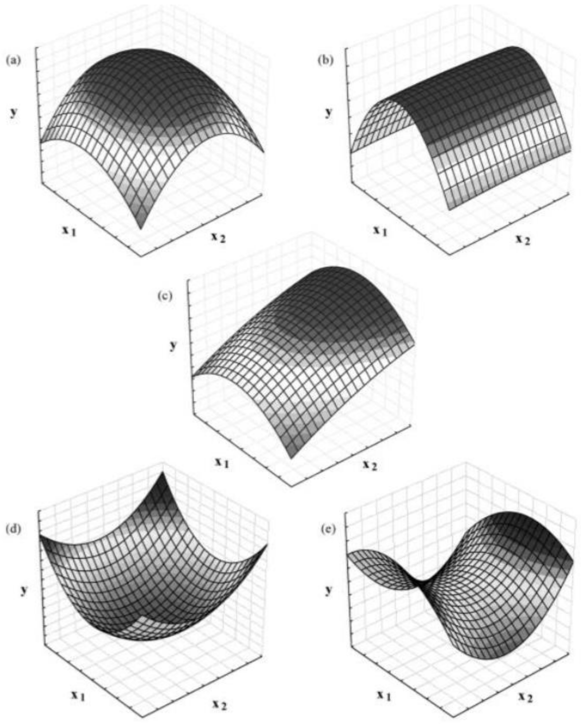


Figure 2.11 Response surface profile types (a) maximum point inside the experimental region, (b) plateau: selected factors not impact to the response, (c) maximum outside the experimental region, (d) minimum point inside the experimental region, and (e) saddle surfaces: maximum and minimum point inside the experimental region (Bezerra et al., 2008)

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Materials

3.1.1 Microorganisms

The laboratory strain, *Bacillus subtilis* GY19 (MSCU0789) for biosurfactant production was isolated from soil in Thailand. The biosurfactants from this bacterium were classified as lipopeptides (Khondee et al., 2015).

3.1.2 Chemicals

i. Sodium carbonate was purchased from BioTech Thailand for use as a builder for the soil washing experiment.

ii. EDTA was purchased from BioTech Thailand for use as a builder for the soil washing experiment

iii. Sodium dodecyl sulfate was purchased from Smart Science Thailand for use as a synthetic surfactant, for the negative control.

iv. Productive media used for biosurfactant production consists of 1 g glucose, 0.5 g beef extract, 3.3 g K_2HPO_4, 0.14 g KH_2PO_4, 3.3 g $NaNO_3$, 0.04 g NaCl, 0.1 g $FeSO_4.7H_2O$, and 1 L of distilled water (Nawawi et al., 2010).

v. LB (Luria-Bertani) was used as a rich medium for inoculum preparation, immobilization process, and bacterial number determination.

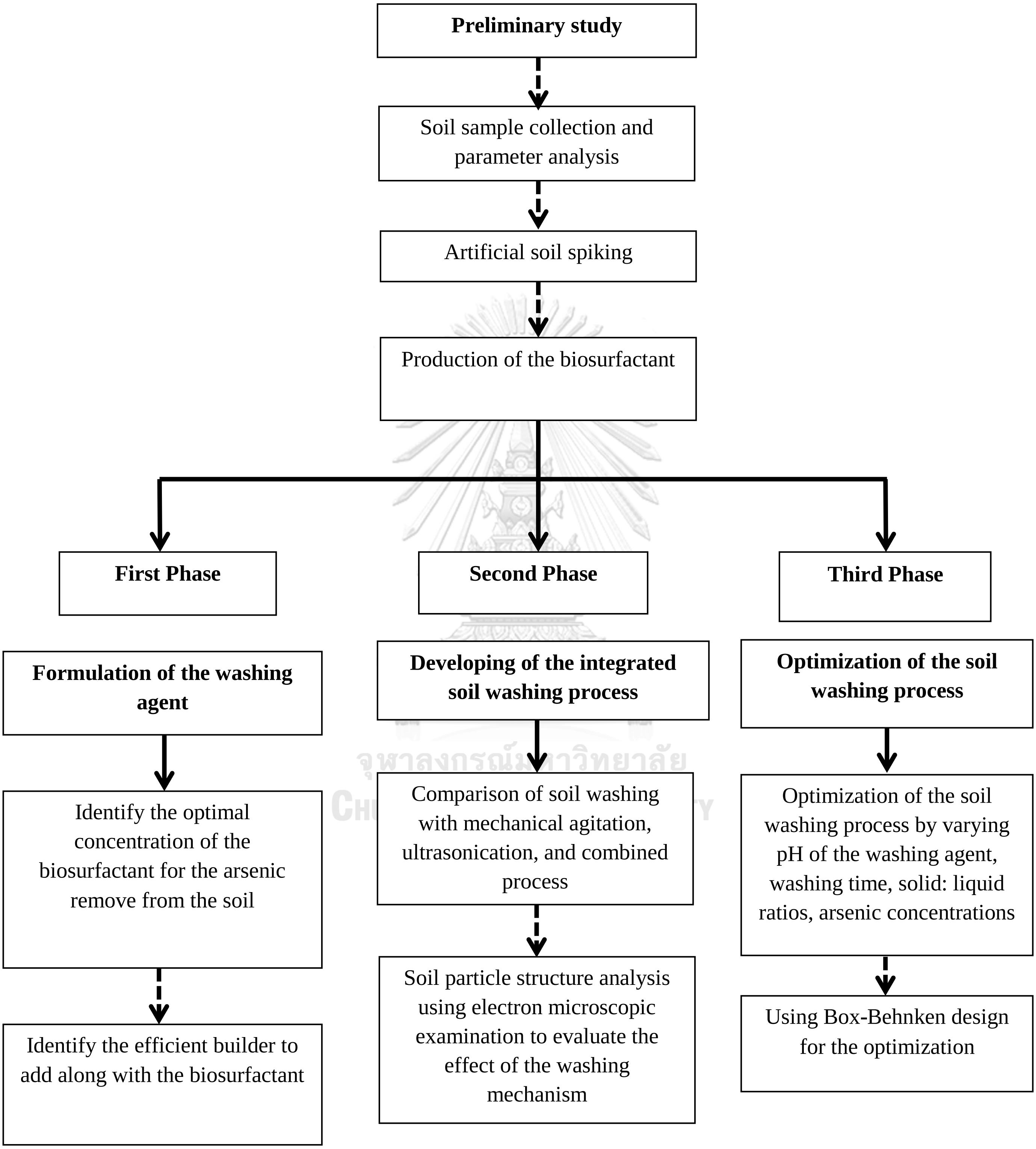
3.1.3 Instruments

i. Arsenic concentration analysis was done through the ICE-OES method with Plasmaquant PQ 9000 elite with detection limit up to 10 µg/L of As.

ii. Ultrasonication was done through the Elmasonic 30 Hz sonicator made in Germany.

iii. Mechanical agitation was carried out using IKA KS 260 orbital shaker.

3.2 Work flow of the experiment



3.3 Soil sample collection and preparation

Surface soil samples (0-15 cm in depth) at 60 kg each was collected in the Suphan Buri area (14.940801, 99.553045) consisting with agricultural fields (Figure 3.1). There were two sites (Site No: 10&17) which occupy high and moderate arsenic concentrations, respectively.

In higher arsenic contaminated agricultural areas in the world, there are >160 mg/kg arsenic concentration in the soil (Liu et al., 2010; Hossain, 2006). To gain more arsenic concentration in the contaminated agricultural soil, the soil was spiked with arsenic in 2:3 (v/w) with 2000 mg/L $Na_2HAsO_4.7H_2O$ solution, because arsenic salts have higher affinity to bind with iron particles in the soil and Mukhopadhyay et al. (2014) reported that nearly 25% of arsenic absorbed to the soil from the prepared liquid arsenic solution. For further investigations with different arsenic concentrations in the soil, liquid arsenic salt solution was prepared according to the expected arsenic concentration in the soil. Then, the spiked soil was dried at room temperature for 14 days as in Mukhopadhyay et al. (2014).

Figure 12 Soil sample collection in the As contaminated area in Suphan Buri, Thailand

3.4 Soil parameter analysis

For the soil parameter analysis, these parameters were analyzed for further soil specifications.

- Total arsenic concentration
- Total nitrogen concentration in the soil
- Total phosphorus concentration in the soil
- Total potassium concentration in the soil
- Total Organic matter content in the soil
- Soil texture
- Soil pH level

- The electrical conductivity of the soil
- Cation exchange capacity
- C/N ration of the soil

The soil was sent to identify these properties at the Department of Agriculture, Kasetsart University, and Bangkok Thailand.

3.5 Production of biosurfactant

Lipopeptide biosurfactant in this study was produced by *Bacillus subtilis* GY19 using crude palm oil and waste glycerol as carbon sources as in Khondee et al. (2015).

3.5.1 Inoculum preparation

Bacterial colony on LB agar plates were picked and transferred to 100 mL of 25% LB broth 250 mL flask and was shaken at room temperature, 200 rpm for 1 day. Then, cell suspension was centrifuged at 8000 rpm, 4 ^{0}C for 20 minutes. Cell pellets were washed with 0.85% NaCl solution twice, and resuspension in 0.85% NaCl solution until on OD600 equal to 1.

3.5.2 Production step

Bacterial inoculum at 10% (v/v) was transferred on Erlenmeyer flask that contains productive media and shaken at room temperature, 200 rpm for 5 days. The concentration of the substrate was 2% (w/v). After the cultivation, the medium was centrifuged at 8000 rpm, 4^{0}C for 20 minutes, and collect the cell-free broth to further investigations, then, biosurfactant products were lyophilized and stocked in powder form (Khondee et al., 2015).

3.5.3 Surface tension analysis

For the CMC analysis and the surface tension determination the data from Rongsayamanont et al., 2017 was used. For further investigation of the property changes of the CMC value of biosurfactant was measured through the surface tension analysis (Adamson & Gast, 1967) through the automatic tensiometer. After preparing the relevant graph of the concentration vs surface tension of the GY 19 biosurfactant, the CMC value was determined as concentration value lipopeptide grams per liter.

3.6 Formulation of the washing agent

3.6.1 Selection of the optimal biosurfactant concentration

The soil was sieved up to the size of ≤ 4 mm for the homogenization. Biosurfactant solution series were prepared as, 0.25x CMC, 0.5x CMC, 1x CMC, 5x CMC, and 10xCMC. Control experiments were conducted using DI water. These numbers reflected the concentrations below and the above CMC value of the biosurfactant, which corresponded to the removal of arsenic in the mobilization phase or the solubilization phase, respectively. Ten g of agricultural soil and 100 mL of washing solution were added to the 250 ml Erlenmeyer flask. The mechanical soil agitation was carried out using a rotary shaker at 200 rpm for 20 minutes within room temperature. Finally, the supernatant was collected by centrifuging at 8000 rpm for

10 min in 4 ^{0}C, and the supernatant was filtered through 0.45 μm nitrocellulose. All experiments were done in triplicates.

The arsenic concentration of the supernatant was measured by ICP – OES following Luna et al. (2016). The supernatant sample analysis was conducted by Environment Research Institute of Chulalongkorn University (ERIC). Before the ICP-OES analysis, all the washed leachate samples were gone through the acid digestion process. The percentage of arsenic removal (%removal) was calculated as follows: (Ci-Ce) x100/Ci (Peligro et al., 2016). The Ci and Ce are the initial and equilibrium soil As concentration, respectively. The soil As concentration was obtained from the reverse calculation through the results from the As concentration in the washed leachate. Surface tension of the washed leachate was measured by a digital tensiometer (Kruss, K10ST, Germany) at 25^{0}C using the plate method, to identify the surfactant monomer reduction whether it can use for another washing cycle or not. The pH of washed soil was measured with an electrode pH meter. Soil pH was measured after the soil washing because of find out the chemical character changes in the soil structure.

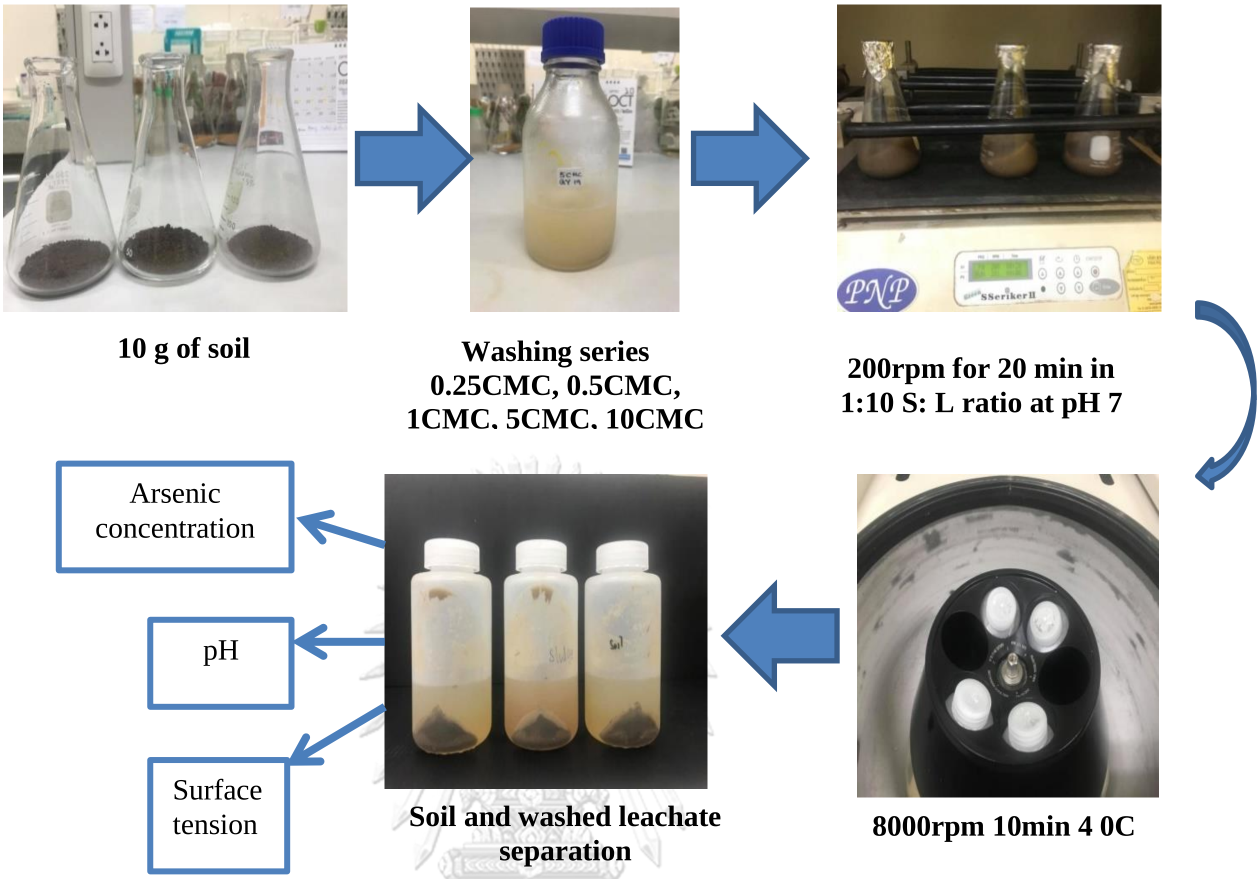


Figure 13 Graphical representation of the phase I experiment in identification of the optimal biosurfactant concentration

3.6.2 Optimization of the washing agent with builders

In this experiment, the optimal biosurfactant concentration from the previous experiment was tested along with the builders at 0.01 M concentration (EDTA and Na_2CO_3). The optimal concentration of the biosurfactant was determined using the highest arsenic concentration in washed solution and calculation of the arsenic removal efficiency in each biosurfactant concentrations. The washing solutions were prepared as follows.

Washing solutions

- Optimal biosurfactant concentration from the previous experiment alone
- Optimal biosurfactant concentration + 0.01 M EDTA
- Optimal biosurafactant concentration + 0.01 M Na_2CO_3
- 0.01M EDTA alone
- 0.01M Na_2CO_3 alone
- DI water

The soil washing experiment was carried out as in the above experiment. All experiments were done in triplicate. The efficient washing solution was determined from the highest arsenic concentration in the washed biosurfactant + builder solution. Moreover, the arsenic removal efficiency was justified with mass balancing with the initial arsenic concentration in the soil, final arsenic concentration in the soil and arsenic concentration in the washed leachate.

3.7 Developing of the integrated soil washing process

The efficient washing solution was chosen from the previous experiment. For justification of the ultrasonic reaction in the soil, the cavitational activity test was initially conducted with aluminium foil on agricultural soil sample as described in Kim et al. (2016). Briefly, aluminum foil was cut into round shape in 3 cm radius and then three experiments and one control experiment were conducted as follows. Experiment (a) was kept as the control without experimenting any cavitational test. For the cavitational activity test, 100 g of agricultural soil was filled to 250 mL beaker. The aluminum foil was placed in the bottom of the beaker in experiment (b), while the experiments (c) and (d) placed the aluminum foil in the middle and top of the soil structure, respectively. Then, 200 mL of DI water poured into the beakers and placed in the sonicator bath for 10 minutes of ultrasonication with 37 kHz in 80 W. After the experiments the aluminum foils were cleared from the scrubbed soil and photographed the cavitations on the foil with Canon 80D with 100 mm macro lens.

DI water and SDS was used as the control for soil washing activity. Ten g of agricultural soil and 100 mL of washing solution was added to the 250 mL Erlenmeyer flask. For the comparison of mechanical agitation, ultrasonication, and a combination of both activities, the soil washing experiment was conducted to compare the arsenic removing efficiency. The mechanical soil washing was carried out using a rotary shaker at 200 rpm in 20 min within room temperature. Ultrasonication was conducted by placing the flasks in sonicator bath (Elmasonic sonicator, Germany) for 20 minutes at 37 kHz in 80 W at room temperature as in Son et al. (2012). The combined soil washing was conducted by placing the flasks in sonicator bath for 10 minutes of ultrasonication with 37 kHz in 80 W and then removing the flasks for agitation washing with 200 rpm for 10 minutes. The entire washing time was designed for 20 minutes and the combined process conducted in 10 minutes for each activity. It was expected that arsenic would be removed from the soil using sonophysical and cavitation activity first and then macroscale bubbling would accelerate the arsenic removal from the soil structure. The sample was centrifuged at 8000 rpm for 10 min at 4 ^{0}C, and the supernatant was filtered through 0.45 μm nitrocellulose filter paper, to collect the washed solution. That was conducted to collect maximum washed soil from the washing solution. After the supernatant collection, the washed soil was rinsed twice using DI water (Arpornpong et al., 2020). This was done to remove the residual biosurfactants on the soil surface which might combine with the heavy metal part. The washed soil samples were analyzed through Scanning Electron Microscopic (SEM) to identify the effect from agitation soil washing, ultrasonic soil washing and combined application. To conduct the SEM sample analysis the soil was dried under the normal room temperature condition and crushed using mortar and pestle. Arsenic concentration was analyzed with ICP-OES from the collected washed soil instead of supernatant in section 3.6 because the process of soil washing included the rinsing with water. All experiments were done in triplicates and the analysis was similar to the above experiment in section 3.6.1.

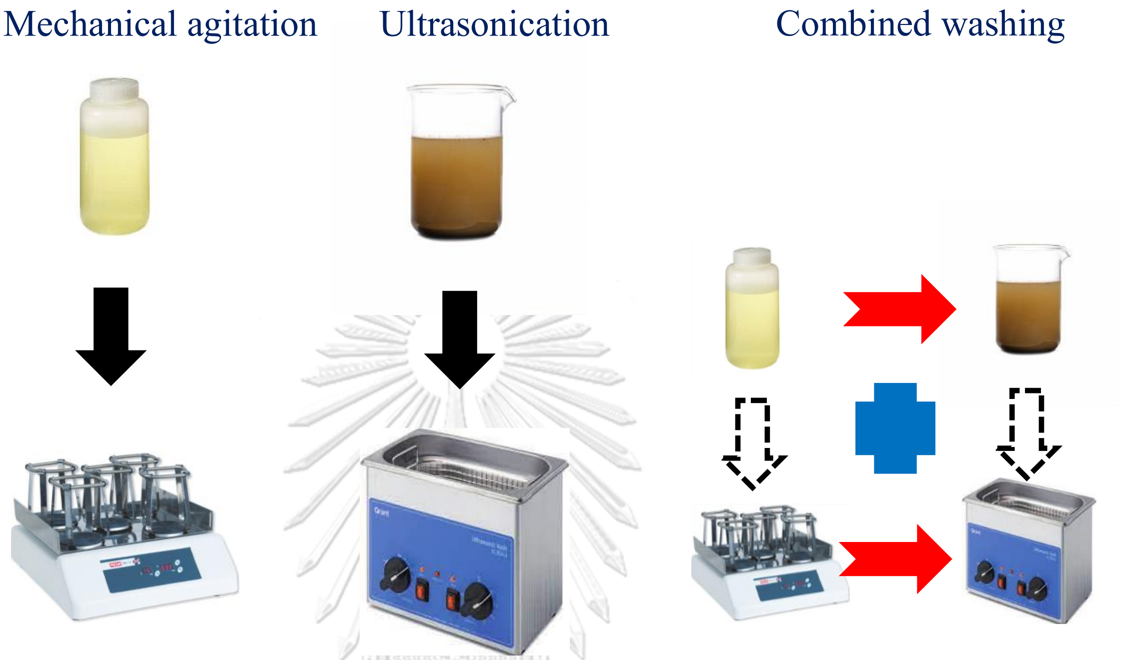
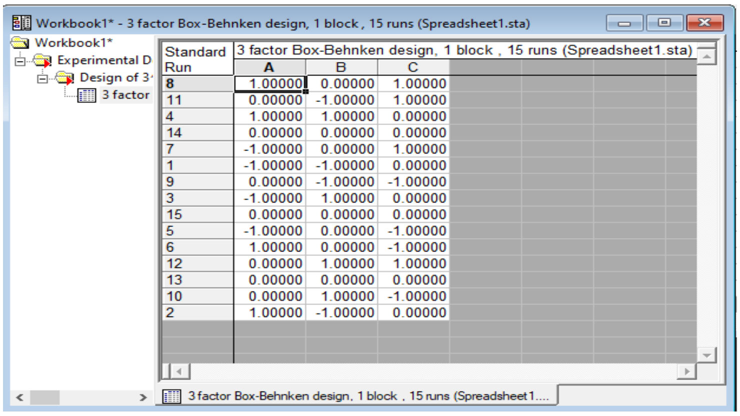


Figure 14 Graphical representation of the three types of soil washing process

3.8 Optimization of the soil washing process

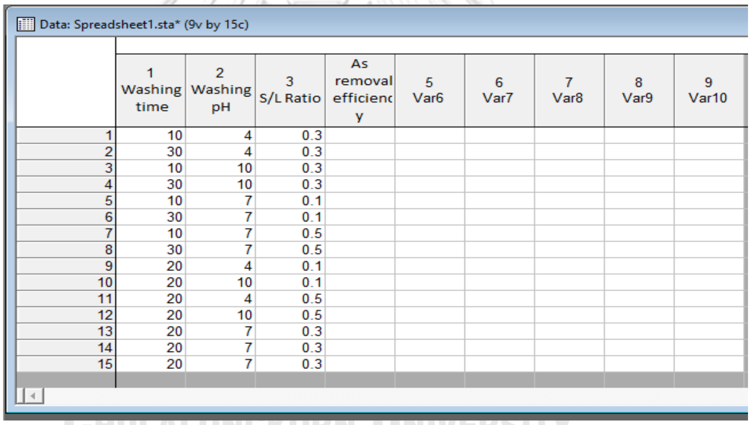
The comparison of soil washing activities from section 3.7 showed the efficient soil washing methodology, as combined soil washing, thus it was chosen for further optimization. For the identification of washing time, the washing experiment was conducted for 10, 20, and 30 minutes. To identify the sufficient solid-liquid ratio, the washing performance was conducted at 1:2, 1:3, and 1:10 ratio. To identify the efficient pH condition, the pH of washing agent was varied at 4, 7, and 10. Different factors can affect to the efficiency of the soil washing using aqueous solution. Washing time can affect the pollutant removing using liquid solution, because compound structure and its bonding strength would require more time for mobilization. Chen et al. (2017) have reported trivalent cations take higher removal time than divalent cations. pH can affect the solubilization of heavy metals in washing agents. Arsenic is strongly bound to the soil at around neutral pH, thus it is necessary to have lower or higher pH condition to mobilize arsenic (Dalgren et al., 2009). The solid:liquid ratio can influence the desorption of the contaminants from the soil due to the contact in between soil particle and washing liquid agent (Park & Son, 2016). These experiments was done with two different arsenic concentrations in the ranges between 400-600 mg/kg and >1000 mg/kg. These ranges were the most frequent As contamination rates all around the world. It may reflect that different arsenic concentrations need different operation conditions for the maximum removal efficiency. The experiment in this phase was designed by STATISTICA10 program (StatSoft Tulsa, OK, USA) using Box-Behnken design analysis (Figure 3.6 & 3.7). The soil washing process, As concentration in the washed soil and As removal efficiency was carried out and analyzed as the section 3.7.



3 factor Box-Behnken design, 1 block , 15 runs (Spreadsheet1.sta)

Standard Run	A	B	C
8	1.00000	0.00000	1.00000
11	0.00000	-1.00000	1.00000
4	1.00000	1.00000	0.00000
14	0.00000	0.00000	0.00000
7	-1.00000	0.00000	1.00000
1	-1.00000	-1.00000	0.00000
9	0.00000	-1.00000	-1.00000
3	-1.00000	1.00000	0.00000
15	0.00000	0.00000	0.00000
5	-1.00000	0.00000	-1.00000
6	1.00000	0.00000	-1.00000
12	0.00000	1.00000	1.00000
13	0.00000	0.00000	0.00000
10	0.00000	1.00000	-1.00000
2	1.00000	-1.00000	0.00000

Figure 15 Predicted three factors Box-Behnken design using Statistical analysis



	1 Washing time	2 Washing pH	3 S/L Ratio	As removal efficienc y	5 Var6	6 Var7	7 Var8	8 Var9	9 Var10
1	10	4	0.3						
2	30	4	0.3						
3	10	10	0.3						
4	30	10	0.3						
5	10	7	0.1						
6	30	7	0.1						
7	10	7	0.5						
8	30	7	0.5						
9	20	4	0.1						
10	20	10	0.1						
11	20	4	0.5						
12	20	10	0.5						
13	20	7	0.3						
14	20	7	0.3						
15	20	7	0.3						

Figure 16 Spreadsheet of the data input with varying the washing time, washing pH and the solid/liquid ratio

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characterization of soil and lipopeptide solution

The As concentration in Suphan Buri soil was 51 mg/kg (Table 4.1). When the soil was spiked, the As concentration was increased to 418.7 mg/kg (Table 4.1), which was within the range of As contamination elsewhere. According to previous investigations, high As concentrations in the soil have been found in South Asia and Southeast Asia, ranging from 40 to 2500 mg/kg (Wang et al., 2014). The pH of the soil was 7.0 and the organic matter level was 2.5% (Table 4.1). Higher pH values in the soil allow metals to be released from organic matter, including heavy metals, which have been known to combine with organic matter in addition to oxides (Violante et al., 2010). Also, arsenic is firmly linked to the soil structure at neutral pH, and mobilization requires either a very high (>12) or extremely low (2) pH (Dalgren et al., 2009). Low organic content mineral soil is defined as soil with less than 3% organic matter (Huang et al., 2016). The research region has dark loam soil with an electrical conductivity of 88.7 dS/m (Table 4.1). According to the USDA soil quality index, this soil falls into the very saline soil category, with a criterion of 16 dS/m. In general, loam soil has 15-24 meq/100 g soil of exchangeable cations. However, because of the high salinity, Suphan Buri soil may include greater cation levels (Ca^{2+}, Mg^{2+}, K^+, Na^+, and NH_4^+), which can impair anionic biosurfactant efficiency. According to the

Sarkar et al., 2007 within a short period of time after the soil spiking with As salts, 60% of water soluble As fractions were recorded in the agricultural soil rather As bound to Fe/Al (NaOH- extractable) or bound to Ca/Mg phases (H_2SO_4-extractable). They have suggested that lower amounts of potential As sorbents like Fe, Al, Ca, and Mg tend to decrease the As sorption into the soil micropore and quick saturation with binding sites in the soil structure. With the present of higher electrical conductivity in the Suphan Buri soil, it contains higher exchange cations and may facilitated higher As sorption to the soil micropore and tends to decrease the As mobility after the soil spiking (Sarkar et al., 2007). Soil pH, as well as other parameters such as competing ligands, soil ionic strength, and other competing metals in the soil, are known to influence soil sorption activity and heavy metal leaching (Luna et al., 2016).

Table 4 Chemical and physical properties of the arsenic-contaminated soil

Characteristics	**Value**
Soil type	Loam soil
Soil pH	7.2
Moisture content (75 ^{0}C in 20 hr %)	1.1
Organic Matter (%)	2.5
Electrical Conductivity	88.7 dS/m
Organic Carbon (%)	1.5
Total Phosphorus (%)	<0.3
Total Nitrogen (%)	<0.3
Total arsenic in the soil	51 mg/kg
Arsenic concentration in spiked soil	418.7 mg/kg

Lipopeptide solutions were prepared from lyophilized GY 19 biosurfactant and analyzed for the critical micelle concentration (CMC) value. According to the Figure 4.1, the CMC value of lipopeptide solution from this study was 0.4 g/L with the surface tension of 34.95 mN/m. According to the Rongsayamanont et al. (2017), the CMC of the lyophilized GY19 biosurfactant was 0.5 g lipopeptide/L, with a surface tension of 29.81 mN/m. Our result reflected that the CMC of the biosurfactant had reduced 20% with the previous value recorded by Rongsayamanont et al. (2017). However, the surface tension at the CMC value increased from 29.81 mN/m to 34.95 mN/m with showing the activity changes after storage. Due to the monomer reduction of the surfactant concentration, the As removal efficiency might be effected, so the lipopeptide washing agent series were prepared based on the CMC value of Rongsayamanont et al. (2017).

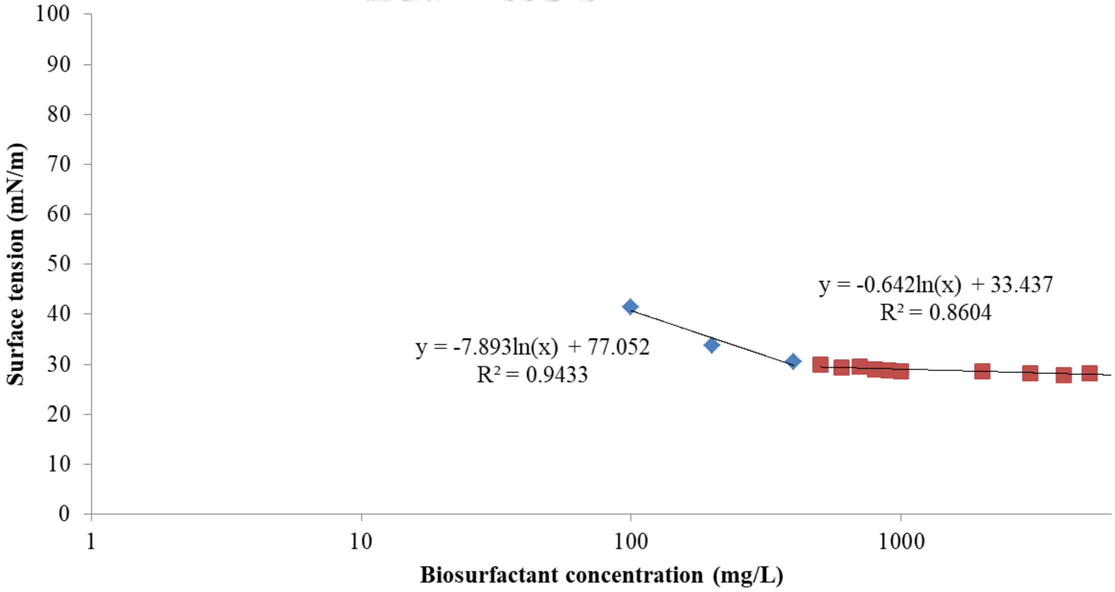


Figure 17 Surface tension variation along with the surfactant concentration change in the lyophilized GY 19 biosurfactant

GY19 lipopeptide biosurfactant resembles surfactin more than iturin or fengycin in structure (Rongsayamanont et al., 2017). It may help remove arsenic cations from the soil structure because of the big anionic amino acid head groups.

The heavy metal binding of amino acids in the anionic head, such as leucin, aspartic acid, valine, and glutamic acid, may be facilitated by the GY19 surfactant monomer.

4.2 Identification of optimal biosurfactant concentration for arsenic removal from the soil

Contaminated soils were combined with a washing solution and stirred to remove pollutants during soil washing. After the soil washing with all solutions including control (DI water), there was not any significant color difference between washed and contaminated soil samples. It reflected that the organic matter loss was negligible with the biosurfactant-based soil washing activity (Figure 4.2). Soil organic matter significantly improves the soil's capacity to store essential nutrients such as nitrogen, phosphorus, and potassium. It enables the soil to deal with fluctuations in soil acidity and speeds up the decomposition of soil minerals. With the application of such soil remediation techniques, there should be a thorough revision on the soil organic content, because if there is a huge loss of the organic matter in the remediated soil may affect further tree and crop productions with a lack of nutrients and fertilizing needs (Gul et al., 2015).

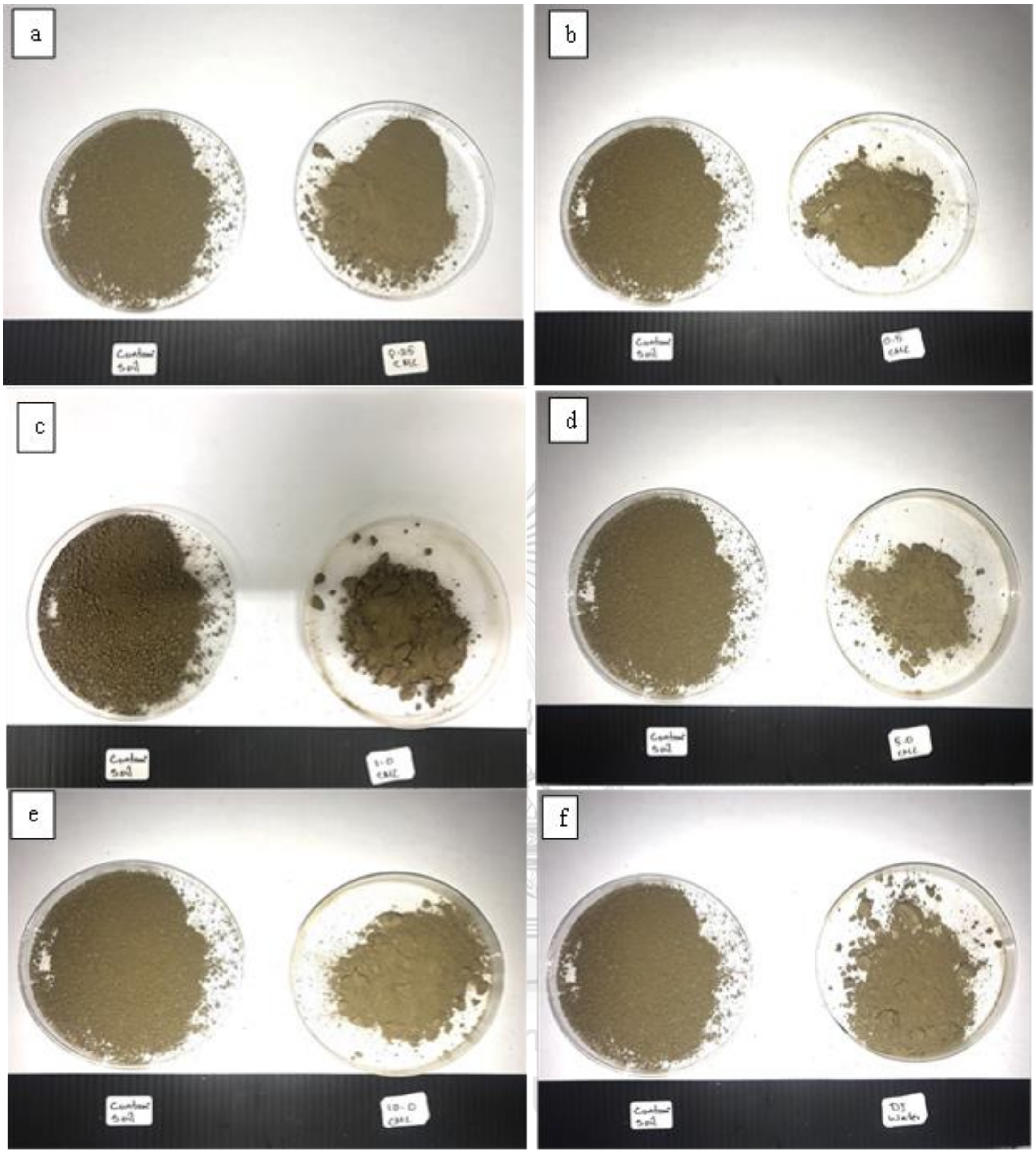


Figure 18 Soil color variation after washing with (a) 0.25x CMC (b) 0.5x CMC (c) 1x CMC (d) 5x CMC and (e) 10x CMC biosurfactant solutions or (f) DI water.

4.2.1 Arsenic removal efficiency along with the different GY 19 lipopeptide concentrations

The As removal efficiencies of biosurfactant solutions with the different CMC values were showed in the Figure 4.3. The results indicated that increment of the monomer concentrations affected on the As removal efficiency. The biosurfactant solution at 10x CMC had the highest As removal effectiveness from the soil, at 29.4±0.8% (Figure 4.3). Mobilization and solubilization are the mechanisms involved in heavy metal washing (Bai et al., 1997). The mobilization phase takes place below the CMC value, whereas the solubilization phase takes place above it. Surfactant monomers tend to reduce surface and interfacial tension in air-water and soil-water systems below the CMC value, but above the CMC value, surfactant monomers combine to form micelles, increasing the solubility (Urum et al., 2003 & Nguyen et al., 2008). Surfactant micelles trap heavy metals in different chemical relationships, such as electrostatic interactions (Akbari et al., 2018). GY19 lipopeptide biosurfactant washed arsenic from the soil by increasing solubilization, according to the findings. Carboxyl groups in the biosurfactants play an important role to bind the heavy metals into the structure. For example, 10x CMC biosurfactant obtained from *Flavobacterium* sp. had asymmetric stretching carboxyl group that shifted FT-IR spectroscopy from 1706 cm^{-1} to 1656 cm^{-1} due to the addition of lead into the biosurfactant solution (Kim et al., 2016). Qi et al. (2018) stated that a significant appearance of asymmetrical stretching and symmetrical stretching vibration band of

O=C-O group at 1625 cm^{-1} and 1407 cm^{-1}, respectively in FT-IR spectrum. That was proved carboxylic end of the fatty acid in acidic sophorolipids produced by *Starmerella bombicola* formed complexes with Cd in the solution. The bonds of Cd—acidic sophorolipids complexes were stronger than the bonds between Cd and the soil (Qi et al., 2018). The efficiency of other biosurfactants on As removal has been reported. For example, glycolipid extracted from *Burkholderia* sp. Z 90 exhibited 31.6% soil removal from the initial 56.0 mg/kg As concentration in the soil (Yang et al., 2016) whereas sophorolipid extracted from *Candida bombicola* showed 11.7% soil removal, but along with this washing solution the remediation efficiency decreased up to 6.2% with the temperature increasing from 15 °C to 35 °C (Arab & Mulligan, 2018). Wang and Mulligan (2009) stated that 6.79% As removal using rhamnolipid surfactant that extracted from *Pseudomonas aeruginosa*, with the initial As concentration 2,567 mg/kg. Compared to these results, the application of the GY 19 biosurfactant showed higher efficiency to remove As from the soil with less amount of surfactant concentration and through a single washing cycle.

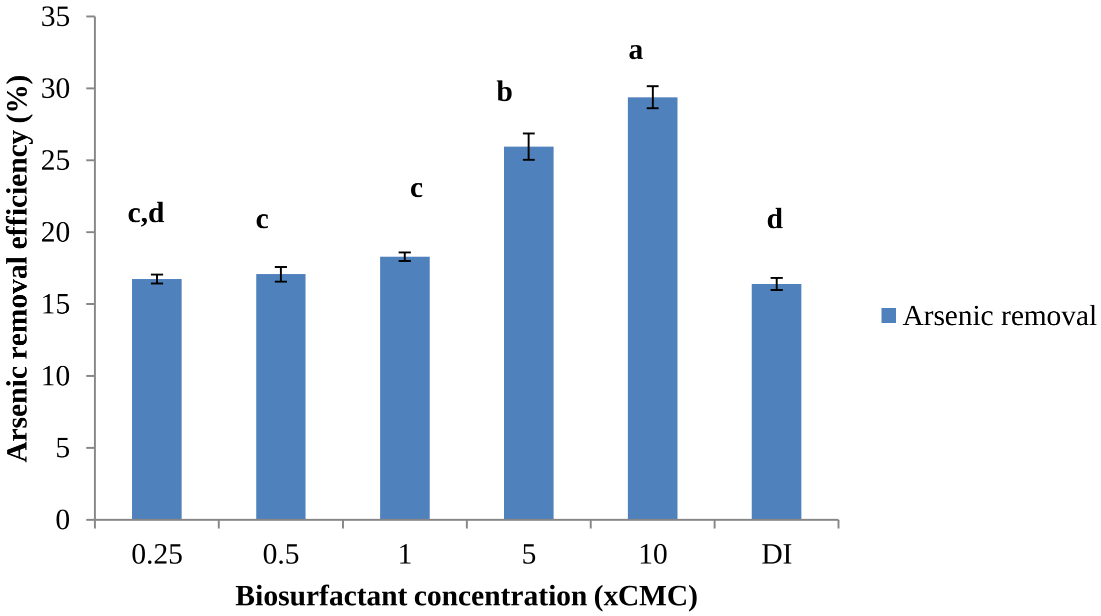


Figure 19 Arsenic removal efficiency of the GY19 biosurfactant solutions in different CMC values. The error bars represent the mean ± standard deviation of the mean (n=3). Means with the same letters are not significantly different among each arsenic removal efficiency ($p>0.05$) values. The error bars represent the mean ± standard deviation of the mean (n=3). Means with the same letters are not significantly different among each arsenic removal efficiency ($p>0.05$) values

4.2.2 Surface tension variation in the lipopeptide biosurfactant concentration series

The surface tension in the washed leachate was compared with the initial surface tension values of the washing agents. When the biosurfactant solution was applied at below the CMC values, there was a significant rise in surface tension in the washed leachates after the soil washing (Figure 4.4). The greater adhesion and precipitation of surfactant monomers to the soil surface might be the cause of the problem. Many processes can connect biosurfactant molecules to soil particles, including ionic bonding between surfactant polar heads and ions in the soil, and attachment of surfactant non-polar tail and hydrophobic material in the soil (Akhbari et al.,2018). To lower the number of biosurfactant samples, 1x CMC and 5x CMC biosurfactant solutions were employed instead of 10x CMC in the following phase. The 10x CMC biosurfactant solution was eliminated due to its relatively high cost.

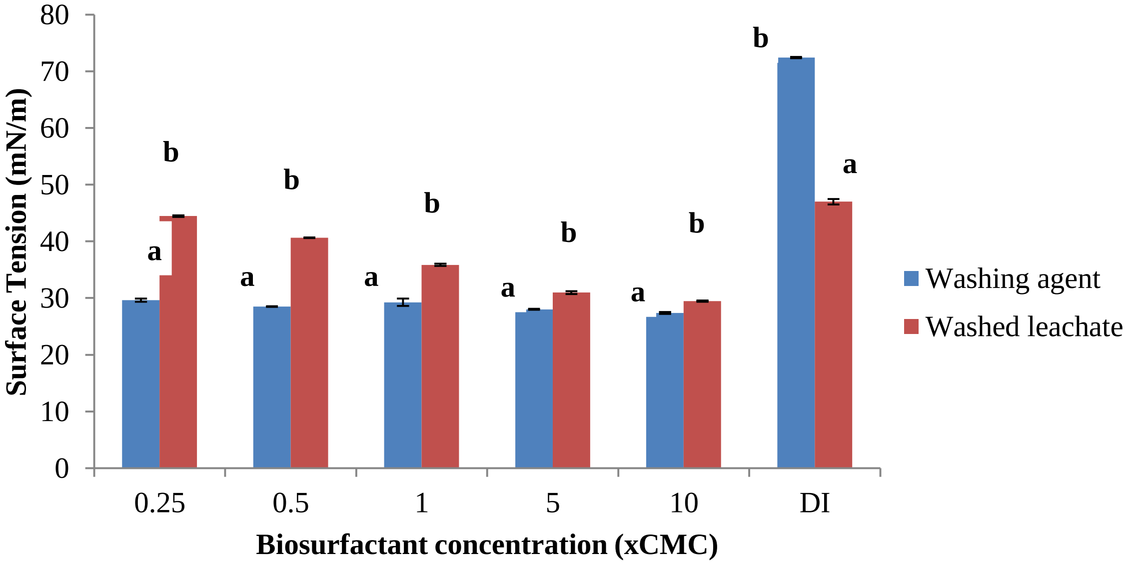


Figure 20 Surface tension comparison of the GY19 biosurfactant solutions (washing agent) and the washed leachate. The error bars represent the mean ± standard deviation of the mean (n=3). Means with the same letters are not significantly different among each surface tension value of washing agents and the washed leachates ($p>0.05$).

4.2.3 Soil pH variation after the soil washing along with the different GY 19 lipopeptide concentrations

After the washing, the soil pH was slightly decreased. The washed soil pH reduction has occurred with the increasing of biosurfactant concentrations. The least pH variation was recorded from the use of 0.25x CMC biosurfactant solution as 7.13±0.02 and the highest pH reductions were recorded from the use of the 5x CMC and 10x CMC biosurfactant solutions as 6.92±0.13 and 6.94±0.07, respectively (Figure 4.5). The results confirmed that moderate concentration of biosurfactant should be applied as washing agent.

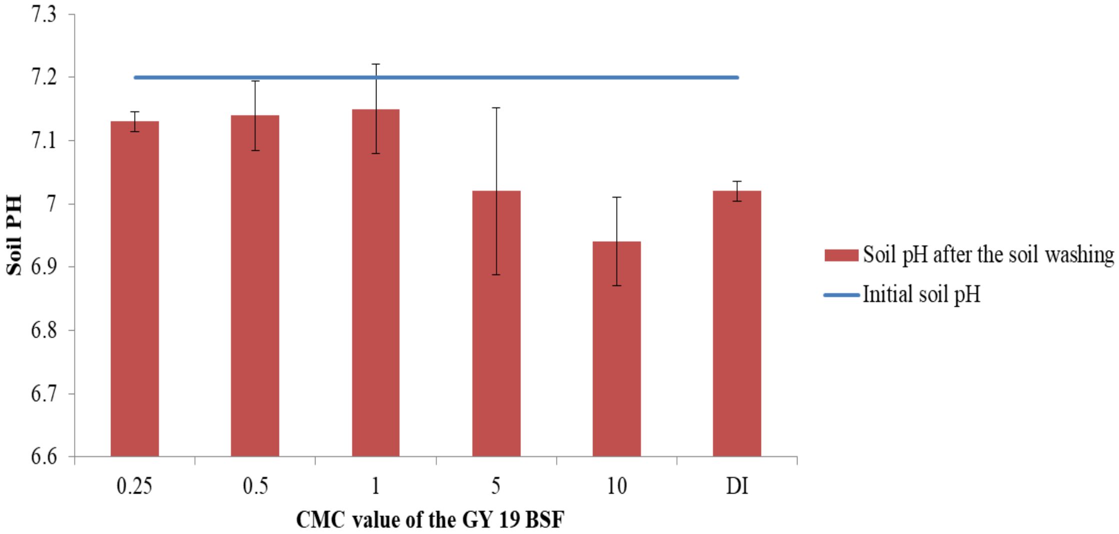


Figure 21 Soil pH variation after washing with the GY 19 biosurfactant solutions.

4.3 Efficiency of arsenic removal from soil by the washing solution containing biosurfactant and builder

4.3.1 Arsenic removal efficiency with the formulation of lipopeptide biosurfactant along with the builder

Builders, in general, provide alkalinity to the washing solution to avoid cation precipitation. Calcium and magnesium ions could impair sorption activity when utilizing the anionic lipopeptide biosurfactants. The electrical conductivity of the soil sample was found to be 88.7 dS/m in this investigation (Table 4.1), with increased exchangeable cation capacity such as K^+, Ca^{2+}, and Mg^{2+}. The study compared 2 builders including EDTA and Na_2CO_3 on enhancing the efficiency of biosurfactant solution on As removal from soil, and the initial As concentration in the soil was 418.7 mg/kg. The maximum As removal effectiveness was 46.1±0.8% when EDTA was combined with the 5x CMC lipopeptide biosurfactant, whereas the lowest As removal efficiency was 21.2±0.8% when Na_2CO_3 was combined with the 5x CMC biosurfactant (Figure 4.6). The bio-based washing agent containing biosurfactant and EDTA had a greater As removal efficiency than the biosurfactant alone (Figure 4.4). In addition, EDTA demonstrated greater arsenic removal ability than Na_2CO_3. EDTA is an aminopolycarboxylic acid-containing carboxylic acid functional group that binds to metal ions as a hexadentate agent. The results found that 0.01 M EDTA alone had a 34.1±1.4% As elimination capacity, thus EDTA had the potential to chelate As metalloids in the soil as well as inhibit cation precipitation of lipopeptides. EDTA may shift the metal sorption and precipitation equilibrium to increase the dissolution of metals by creating strong complexes with two amines and four carboxylic groups (Yangxin et al., 2008). Based on statistical analysis, there were no significant differences in As removal abilities between washing agents containing 1x CMC biosurfactant +0.01 M EDTA and 5x CMC biosurfactant +0.01 M EDTA (Figure 4.6). For the next investigations, a washing agent consisting of 1x CMC biosurfactant +0.01 M EDTA was utilized. Furthermore, numerous soil washing processes were explored since they might be efficient in removing larger levels of As from the soil. Also EDTA showed an synergistic effect on heavy metal removing along with the lipopeptide biosurfactant with providing more binding sites and creating complexes with As cations in the soil.

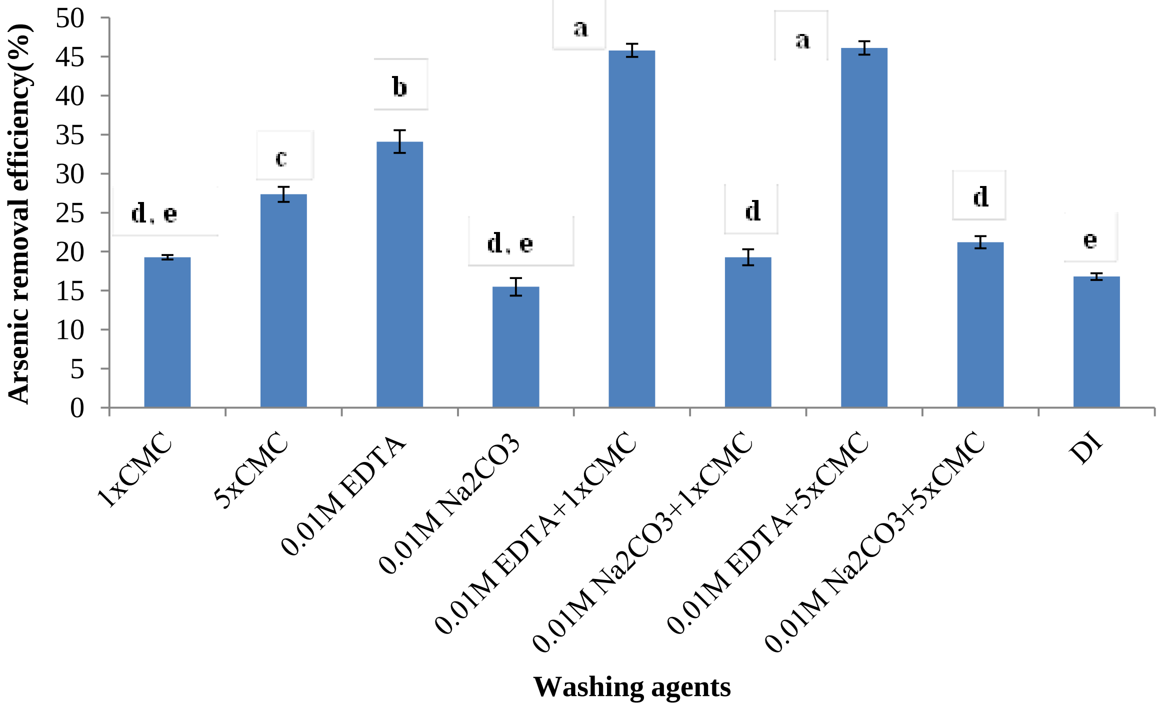


Figure 22 Arsenic removal efficiency after the washing by biosurfactant mixed with the builders. The error bars represent the mean ± standard deviation of the mean (n=3). Means with the same letters are not significantly different among each arsenic removal efficiency (p>0.05).

4.3.2 Surface tension variation of the washing agents and washed leachates

After washing the soil, the washed leachates from 1x CMC biosurfactant +0.01 M EDTA and 5x CMC biosurfactant +0.01M EDTA treatment showed a small increase in surface tension. Initially, 1x CMC biosurfactant +0.01M EDTA washing agent yielded 28.6±0.45 mN/m and 5x CMC biosurfactant +0.01 M EDTA washing agent yielded 27.90±0.09 mN/m, which are equivalent to the surface tension of GY19 lipopeptide at the CMC value (Figure 4.7). After soil washing, the surface tension of the 1x CMC biosurfactant +0.01 M EDTA washed leachate increased slightly to 30.28±0.35 mN/m, whereas the surface tension of the 5x CMC biosurfactant + 0.01 M EDTA washed leachate increased slightly to 29.28±0.21 mN/m (Figure 4.7). As a result, the washing agent had some reusability potential.

Figure 23 Surface tension variation of builder added washing agents and washed leachates. The error bars represent the mean ± standard deviation of the mean (n=3). Means with the same letters are not significantly different among each surface tension value of washing agents and the washed leachates (p>0.05).

4.3.3 Soil pH variation of the washed soil due to the influence of washing agent

After washing, Na_2CO_3 application tends to raise soil pH, with the maximum pH rise being 8.54±0.07. The use of EDTA, on the other hand, invariably reduced the soil pH following soil washing, with the lowest value of 6.00±0.18 when 5x CMC biosurfactant +0.01 M EDTA washing agent was used. EDTA is buffering weak acid. As a result, EDTA may resist dissolving in the washing solution and may precipitate in the soil structure (Bai et al., 1997). In addition, EDTA might lower the soil pH after soil washing. In comparison, sodium carbonate is an inorganic compound with moderately alkaline ability. The application of high GY19 biosurfactant concentrations tended to reduce the alkaline ability of the Na_2CO_3 as seen from the final soil pH of 6.78±0.09 after washing with 5x CMC biosurfactant +0.01M Na_2CO_3 washing agent (Figure 4.8).

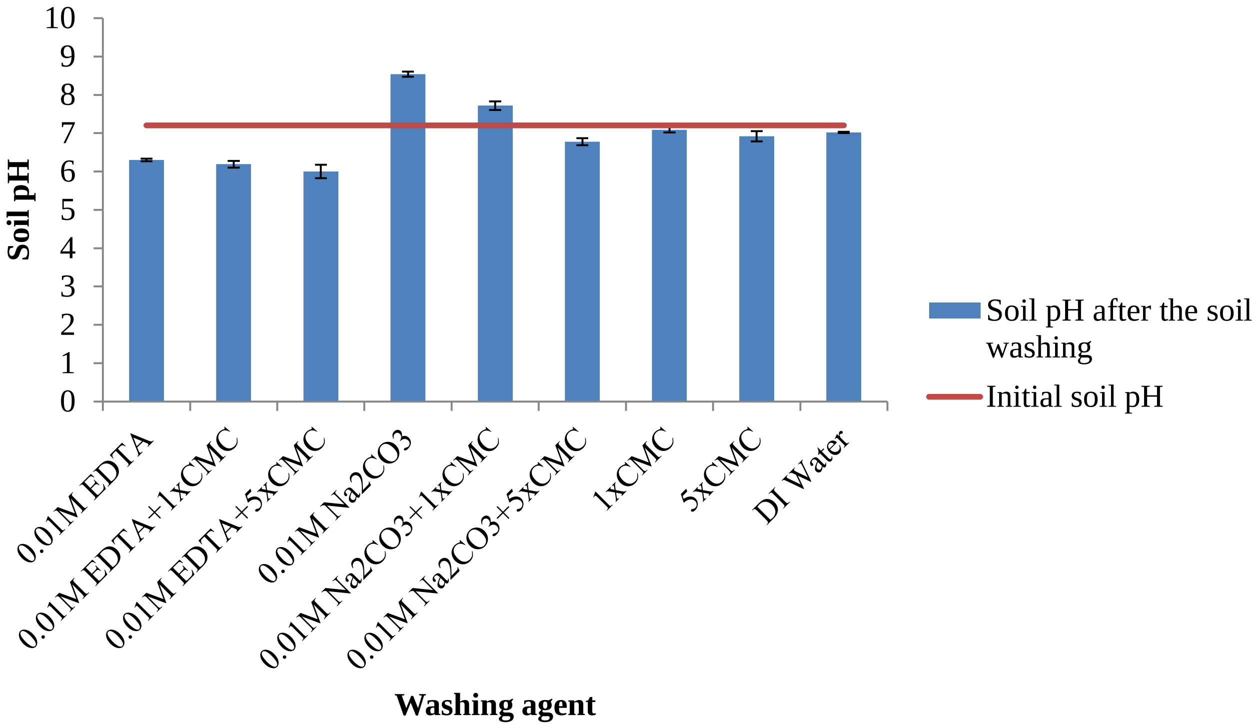


Figure 24 Soil pH variation after the soil washing along with the usage of builders along with the biosurfactant.

4.3.4 Potential sorption of biosurfactants on soil particles based on CMD series analysis of the washing agents

With the application of lipopeptide biosurfactant, the lipopeptide molecules might attach and sorp on to the soil and mineral particles during soil washing and reduce the As removal efficiency. However, lipopeptide biosurfactant mixed with EDTA worked well for As removal from soil. The critical micelle dilution (CMD) values in the lipopeptide biosurfactant, EDTA and the mixture solution were therefore analyzed to identify the role of EDTA. With the comparison of the CMD values before and after soil washing, the washed leachate of GY 19 biosurfactant showed the monomer loss after 2x dilution (Figure 4.9 a). The surface tension of the leachate at the second dilution point increased to 69.08 mN/m, which was similar to surface tension of deionized water. It showed that the application of the biosurfactant alone had huge surfactant monomer loss which corresponded with its less As removal efficiency compared to the biosurfactant+EDTA mixture. With this kind of situation it can predict that monomers might attach with the soil particles or they had attached with the heavy metals in the washed soil structure.

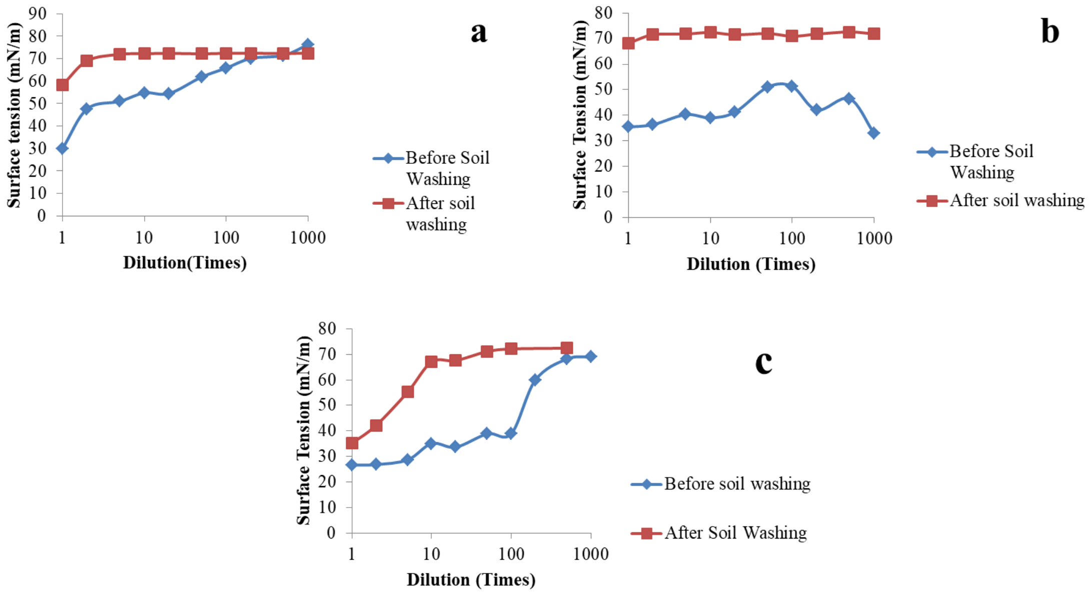


Figure 25 (a). Surface tension variation of the GY 19 biosurfactant along with the dilution time (b). Surface tension variation of the EDTA along with the dilution time (c). Surface tension variation of the GY 19 + EDTA along with the dilution time

Application of the EDTA alone showed high sorption ability with the soil washing. It also attained 71.45 mN/m at the second dilution point (Figure 4.9b). Application of 1x CMC biosurfactant +0.01 M EDTA solution showed the high efficiency with the arsenic removal. With the analysis of the surface tension variation along with the dilution time, 1x CMC biosurfactant +0.01 M EDTA solution had low sorption value that achieved the 67.03 mN/m at the 10th dilution value. It reflected that combination of the biosurfactant with the EDTA as builder has less sorption ability (Figure 4.9c). EDTA had the potential to chelate As metalloids in the soil as well as inhibit cation precipitation of lipopeptides, that facilitated the reduction in the sorption of lipopeptide biosurfactant.

4.3.5 Mass balance of the As during soil washing process

To calculate the mass balance of As, the washed soil and washed leachate from 0.01M EDTA+5x CMC biosurfactant and the 0.01M EDTA+1x CMC biosurfactant experiments were selected to represent the samples with the highest and the second highest As removal efficiencies, respectively. The samples with the DI water were used as the control experiment. In each experiment, there was a slight As mass reduction with compared to the initial As mass in the contaminated soil (Figure 4.10). The 0.01M EDTA+5x CMC biosurfactant experiment showed 0.47±0.11 mg As loss (Figure 4.10a), while the 0.01M EDTA+1x CMC biosurfactant experiments resulted in 0.91±0.11 mg As loss with compared to the initial mass (Figure 4.10b). The control DI water showed 1.06±0.01 mg As loss (Figure 4.10c). The imbalance of the mass of the As might be due to the experimental errors in various steps. The As particles can be undetectable, as an example undigested organic matter covering the As particles, machine errors on detection levels of the heavy metals, and etc. Moreover, the highest As loss by DI water might due to higher solubilization of organic particles that disrupted the metal detection by ICP-OES analysis. Nonetheless, the results indicated that the process for soil washing and As analysis was acceptable.

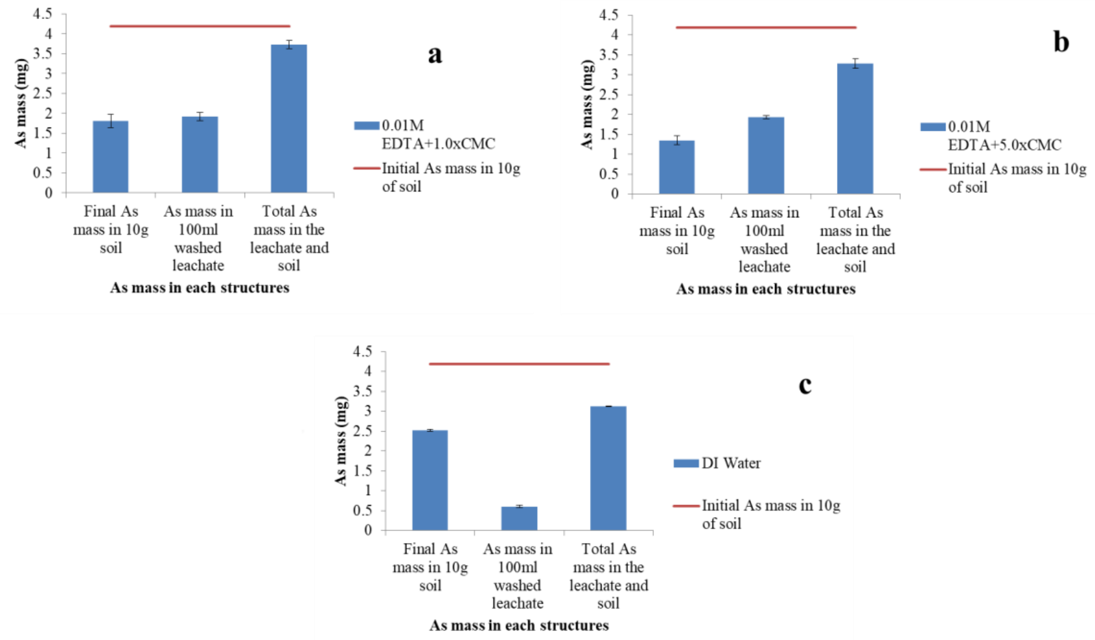


Figure 26 Mass balance of As in different soil washing system including (a) 0.01M EDTA+1x CMC GY 19 biosurfactant, (b) 0.01M EDTA+5x CMC biosurfactant and (c) DI water

4.4 Application of the ultrasonication for the combined soil washing

In the mechanical agitation process (section 4.3), the maximum As removal was 46.1±0.8%, from 418.7 mg/kg initial As concentration of the soil. In this phase, the main priority was to increase the As removal efficiency in the contaminated soil using the ultrasonic waves. There were 4 washing agents including biosurfactant, biosurfactant+EDTA, synthetic surfactant (SDS) and SDS+EDTA. All surfactants were prepared at 1x CMC solution i.e. 0.5 g/L lipopeptide and 2.4 g/L SDS. The concentration of EDTA was 0.01 M.

Initially, the cavitational activity of ultrasonication was tested using the aluminum foils in varying depths of beakers containing soil samples. The highest cavitational activity was observed when the aluminum foil was placed at the bottom of beaker, which showed large cavities (Figure 4.11). On the other hand, the aluminum foil at the middle and top of beaker showed slight changes (Figure 4.11). The results reflected that the sonication power was decrease with the soil depth and the sonication bath, Elmosonic sonicator had powerful cavitational activity at 37 kHz with 80W for the soil washing activity.

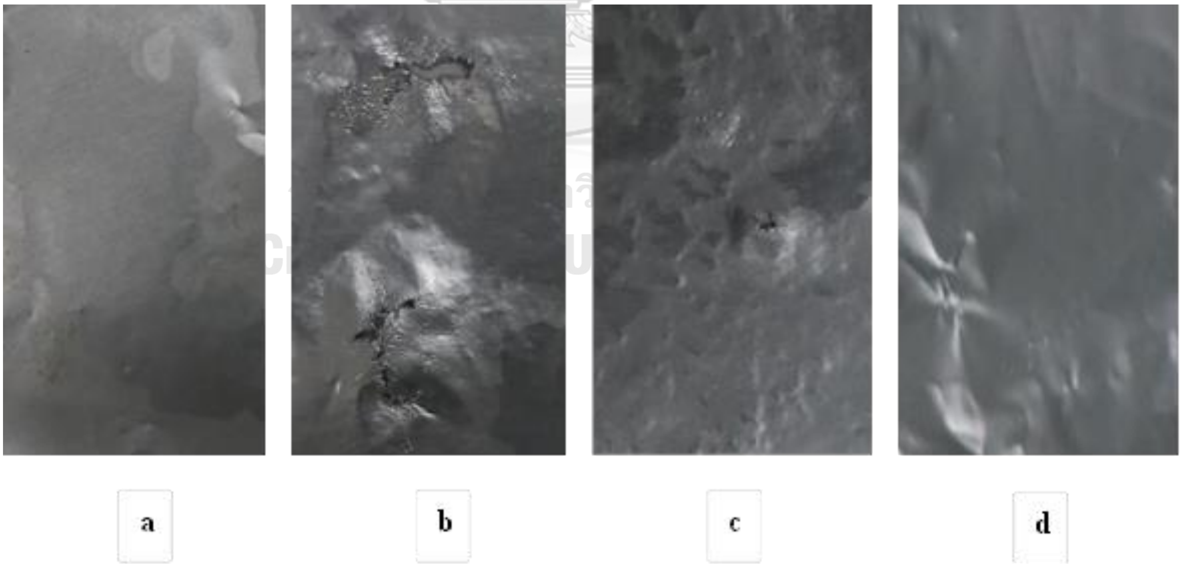
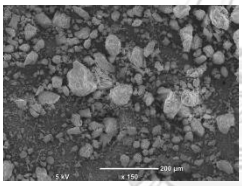
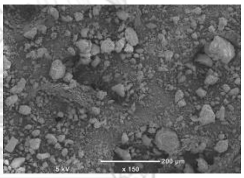
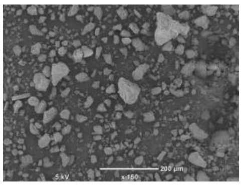
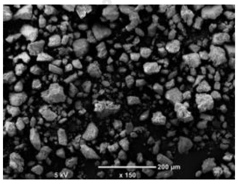
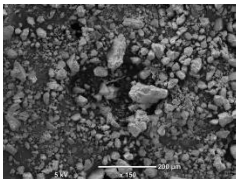
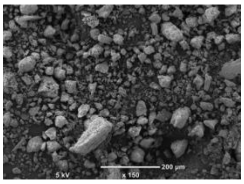
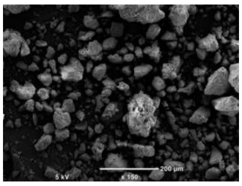
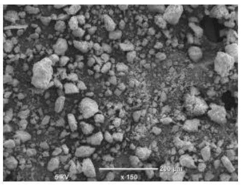
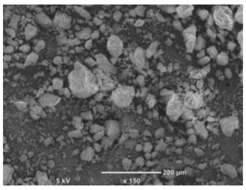


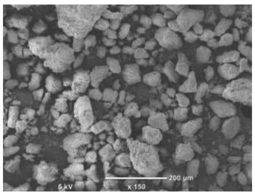
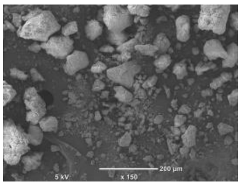
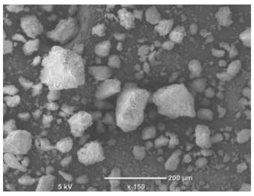
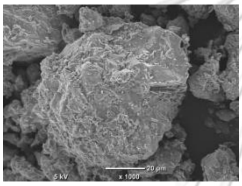
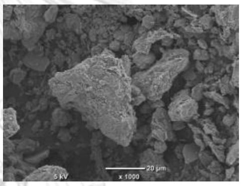
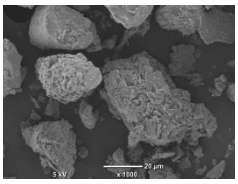
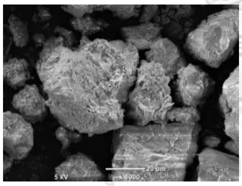
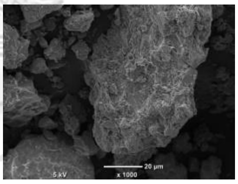
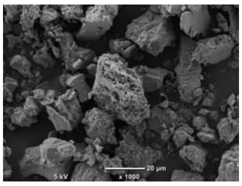
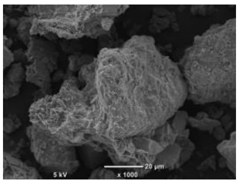
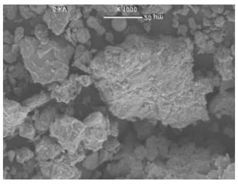
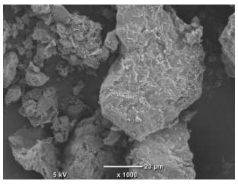
Figure 27 a) Control experiment with aluminum foil placed in an empty beaker b) Aluminum foil in the bottom of the beaker with soil, c) Aluminum foil middle of the

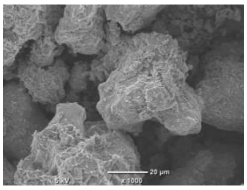
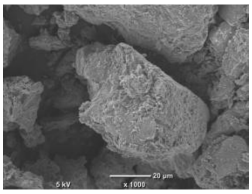
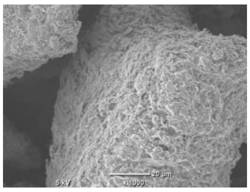
jar with soil, d) Aluminum foil placed in the top of the soil, sonicator bath (Elmasonic sonicator, Germany) for 20 minutes at 37 kHz in 80 W at room temperature

After the application of the washing processes, there was a significant difference in between soil structure fragmentation. The soil particle distribution was higher in the washed soil. According to the 150x magnification SEM pictures, the combined soil washing process led to more particle fragmentation than other two washing methods, that reflected agitation power and the ultra-waves had more structure breakable power (Table 4.2a). When compared the three washing processes, the cavitations on the soil particle surfaces gradually increased in the following manners; mechanical agitation, ultrasonication and the combined soil washing process (Table 4.2). In addition, the 1xCMC surfactant + 0.01M EDTA solution in the combined soil washing process created more ruptures and the deep holes in the surface of the soil particles. It reflected that combination of the mechanical soil washing and the ultrasonication had more sonophysical and microscale bubble effect on the soil structure rather than mechanical soil washing and the ultrasonication. According to Kim et al. (2016), microscale sonophysical effects caused by ultrasound could remove pollutants relatively strongly bonded on the surface and trenched in the pores of soil particles for the very limited cavitational zone. Consequently, the application of ultrasonication made the soil particles ruptured into small particles and allowed higher surface areas to contact with washing solution while the mechanical agitation.

Table 5 Scanning Electron Microscopic (SEM) analysis of the effect of washing methods on soil structure

Washing agent	**Mechanical washing**	**Ultrasonication**	**Combined soil washing**
(a) magnification 150x			
SDS 1xCMC			
BSF 1x CMC + 0.01M EDTA			
SDS 1x CMC + 0.01M EDTA			

Washing agent	Mechanical washing	Ultrasonication	Combined soil washing
DI Water			
		(b) magnification 1000x	
SDS 1x CMC			
BSF 1x CMC + 0.01M EDTA			
SDS 1x CMC + 0.01M EDTA			

Washing agent	Mechanical washing	Ultrasonication	Combined soil washing
DI Water			

After the application of ultrasonication along with the mechanical agitation, the arsenic removal efficiency was increased. The highest As removal efficiency was recorded at 71.45±3.62% from the 1x CMC GY 19 biosurfactant + 0.01M EDTA solution that used mechanical soil washing combined with the ultrasonication. In addition, the As removal efficiency in the combined soil washing process was higher than that of other soil washing methods. The other As removal efficiencies was recorded as 65.21±0.14 %, 53.34±10.68 %, 31.77±0.99 %, and 25.97±8.29 % in biosurfactant alone, SDS+EDTA, DI water and SDS alone, respectively (Figure 4.12), while the initial As concentration at 418.7 mg/kg. When compared to the other washing methods in ultrasonication process, biosurfactant + EDTA mixture had the highest As removing efficiency as 68.07±3.45 %. For the mechanical soil washing method, the highest As removal efficiency recorded at the biosurfactant + EDTA mixture as 55.83±6.28 %. In conclusion, biosurfactant + EDTA mixture had a synergistic effect on As removing from the contaminated soil and the removal efficiency was increased with the combined soil washing process at nearly two time increment of the washing efficiency.

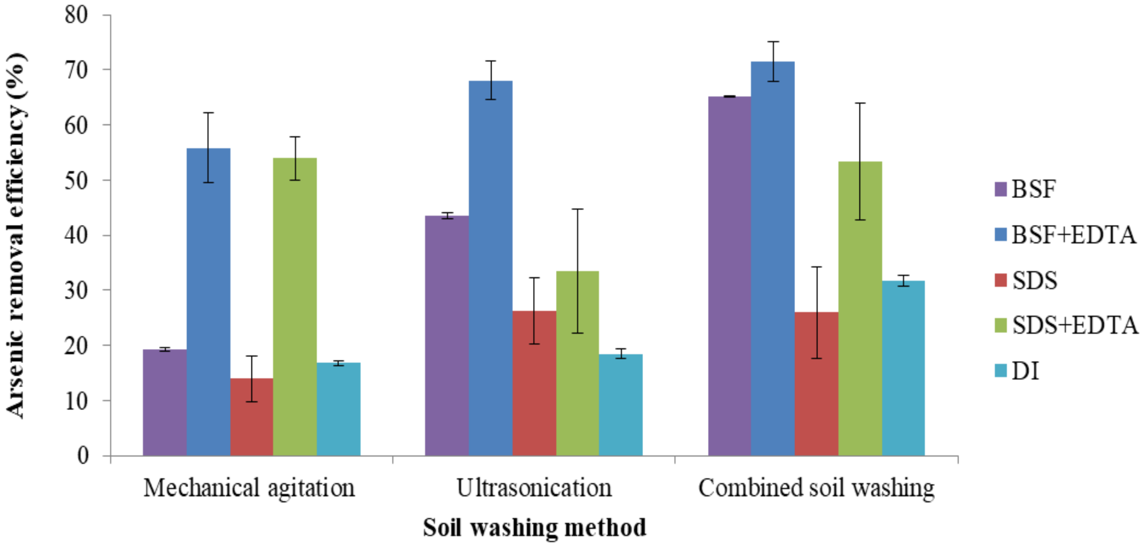


Figure 28 Arsenic removal efficiency in different soil washing methods, the Sodium dodecyl sulfate (SDS) was used as the positive control and the DI water used as the negative control

After the soil washing, the soil pH was measured. The highest pH value recorded as the 7.3±0.01 in the application of SDS at the combined soil washing process. It was slightly increased from the pH of SDS at the 1x CMC concentrations that was around 7.0-7.2. The lowest soil pH was recorded at the SDS + EDTA mixture at 6.3±0.11. The result was corresponded to the pH of EDTA solution at the 0.01M concentration of 5.0-6.0. According to the USA standards of agricultural soil, it should maintain the soil pH for good crop production between pH 6.5-7.5. Consequently, the washed soil had acceptable pH ranges for the crop production systems (Figure 4.13).

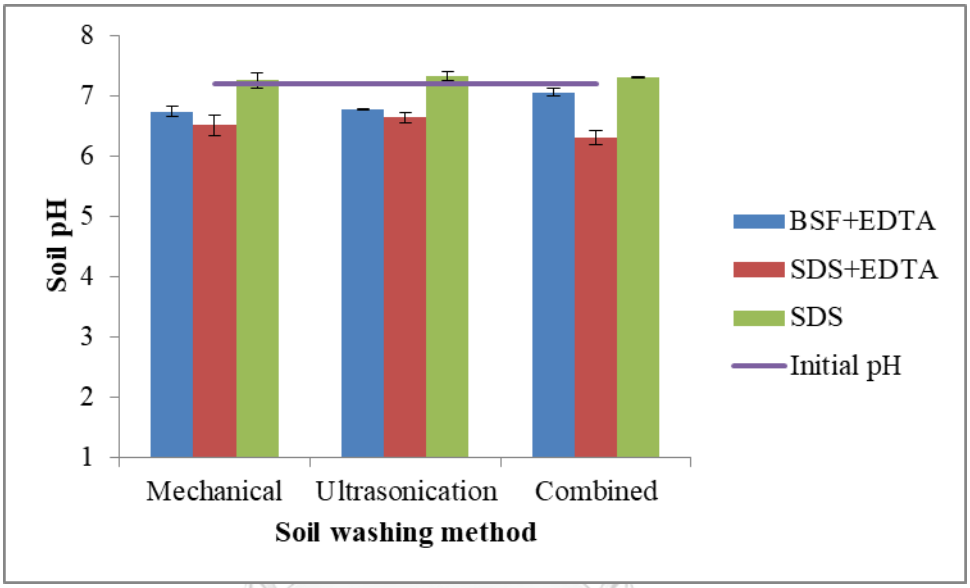


Figure 29 Soil pH variation after the soil washing in different soil washing methods.

Soil EC has been used as an indirect measure of the quantity of nutrients available for plant absorption and salt levels, although it has no direct effect on plant development. Salt content, organic matter, cation-exchange capacity, soil texture, soil thickness, nutrients (e.g., nitrate), water-holding capacity, and drainage conditions have all been measured using EC. Initially the soil EC was recorded as 88.7 dS/m, according to the class of the salinity it can stated as strongly saline soil (Table 4.4). After the soil washing, the soil electrical conductivity was marginally reduced with compared to the initial electrical conductivity value of the soil. It might result from the high organic matter reduction due to the soil washing.

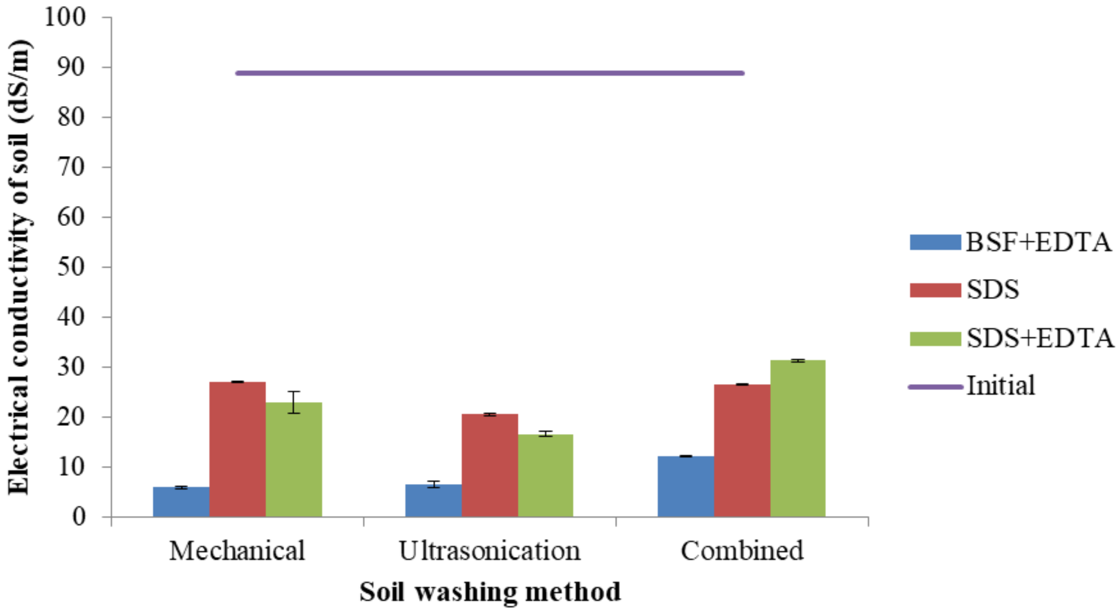


Figure 30 Soil electrical conductivity variation after the soil washing with different methods

The lowest EC was recorded at the mechanical agitation while using 1xCMC BSF+0.01M EDTA solution as 5.81±0.25 dS/m (Figure 4.14), while the combined soil washing process soil with a similar washing agent resulted the 12.13±0.15 dS/m. The highest electrical conductivity was recoded at 31.27±0.25 dS/m from the combined soil washing while using SDS+EDTA mixture. According to the USDA crop tolerance standards with the salinity and compared to the results indicated in the Figure 4.14, the washed soil had moderate salinity condition in level 8-16 dS/m (Table 4.3). High EC can indicate salt issues, which can hamper crop development, cause difficulty to absorb water even when it is there, and increase microbial activity. Soils with a high EC due to a high salt content have poor structure and drainage, and sodium can be hazardous to plants. Xiao et al. (2019) reported the influence on soil pH, soil organic matter and the electrical conductivity of the soil by various washing agents. The application of hydrochloric acid, oxalic acid, and citric acid had great influence on decreasing each soil properties considerably, while EDTA and the acetic acid had fewer influence on soil pH reduction with compared to other soil washing agents (Xiao et al., 2019). Yi and Sung (2015) reported 29% of the soil organic matter reduction that resulted the electrical conductivity reduction in the soil content after having high dissolved organic matter. Wang et al. (2014, 2016) described that each soil washing application may reduce the soil properties such as electrical conductivity, soil pH and the soil organic matter due to the adsorption of organic acid on soil particles. According to the reference, our results showed the pH and soil property variations after the soil washing, thus the augmentation of the soil nutrients and the electrical conductivity increasing agents such as high magnesium, phosphate, and potassium chemicals up to the standards should be conducted before the soil is reintroduced to the agricultural world.

Table 6 Salinity classes of the soil and the crop tolerance (USDA AG Handbook, 60,1954)

USDA Class	**Electrical conductivity (dS/m)**	**Salinity class**	**Osmotic Potential (kPa)**	**Crop Tolerance**
A	0<2	Non Saline	0 to -70	Sensitive
B	2<4	Very slightly saline	-70 to -140	Moderately sensitive
C	4<8	Slightly saline	-140 to -280	Moderately sensitive
D	8<16	Moderately saline	-280 to -560	Tolerant
E	≥16	Strongly saline	-	-

In comparison with other soil washing methods, the combined soil washing with lipopeptide and EDTA formulation showed higher efficiency. For example, Yang et al. (2016) showed 31.6% As removal efficiency using bioleaching, while Yan et al. (2017) showed 54.04% As removal efficiency using soil flushing and phytoremediation method. Yuan et al. (2008) achieved 44.8% As removal efficiency using electrokinetic system. The combined soil washing process showed 71.45±3.62% As removal efficiency with efficient and successful remediation compared to other As removing methods.

4.5 Optimization of the soil washing process with Design of experiment

Design of experiment (DOE) was used to plan and experiment the findings. The Box-Behnken design, which determines the first and second-order coefficients of a mathematical model based on three-level factorial designs, was used in the design of experiment process (Table 4.4). From section 4.4, the highest As removal was recorded as 74.92% from the washing condition of the 20 minutes washing time, pH 7 and the 1:10 solid-liquid ratio, in 418.7 mg/kg initial As concentration in the soil. The soil washing was conducted in two soil samples with the initial As concentrations 434 mg/kg as the lower As concentration soil and 2572 mg/kg as the higher As concentration.

4.5.1 Design of Experiment for the lower As concentrated Soil

Table 4.4 showed the predicted and observed As removal efficiency after the 45 runs for the lower As concentration contaminated soil. The experiment was triplicated as follows for the observations.

Table 7 Box-Behnken design for 15 number of runs in the lower As concentrated soil

Run	Washing time (Minutes)	Washing pH	S/L Ratio	Observed As removal (%)	Predicted As removal (%)
1	10	4	0.3	84.18	84.38
2	30	4	0.3	84.94	85.09
3	10	10	0.3	85.89	85.75
4	30	10	0.3	84.77	84.58
5	10	7	0.1	85.36	85.80
6	30	7	0.1	87.72	88.21
7	10	7	0.5	86.18	85.68
8	30	7	0.5	83.26	82.82
9	20	4	0.1	85.49	84.85
10	20	10	0.1	85.57	85.28
11	20	4	0.5	81.80	82.10
12	20	10	0.5	81.88	82.52
13	20	7	0.3	82.90	83.60
14	20	7	0.3	84.15	83.60
15	20	7	0.3	83.74	83.60

With the application of the suitable model for the analysis the data, two-way interaction with linear-linear model was chosen because it’s Coefficient of determination (R^2) value was 0.92496 which closed to 1, that regression predictions fit the data. With considering the lack of fit P value Lack of fit P value = 0.398 that was shown as > 0.05 proving the well fitted model for the lower As concentrated soil remediation. The conditions were calculated to the lower As concentrated soil (Table 4.5).

Table 8 Analysis of the variable that influence the As removal efficiency in the lower As concentrated soil from Box-Behnken design

Factor	P Value	Coefficient
Washing time(a)-Linear	0.658	-0.115
Washing time-Quadratic	0.038	-0.823
Washing pH(b)-Linear	0.442	0.213
Washing pH-Quadratic	0.466	0.147
S/L Ratio(c)-Linear	0.130	-0.993
S/L Ratio-Quadratic	0.363	-0.385
ab	0.276	-0.470

ac	0.053	-1.319
bc	0.999	0.001

The correlation of variables was analyzed from the data in regression table (Table 4.5). Correlation model was exhibited in term of polynomial equation as shown in Eq.4.1.

y=Arsenic removal efficiency in lower As concentrated soil

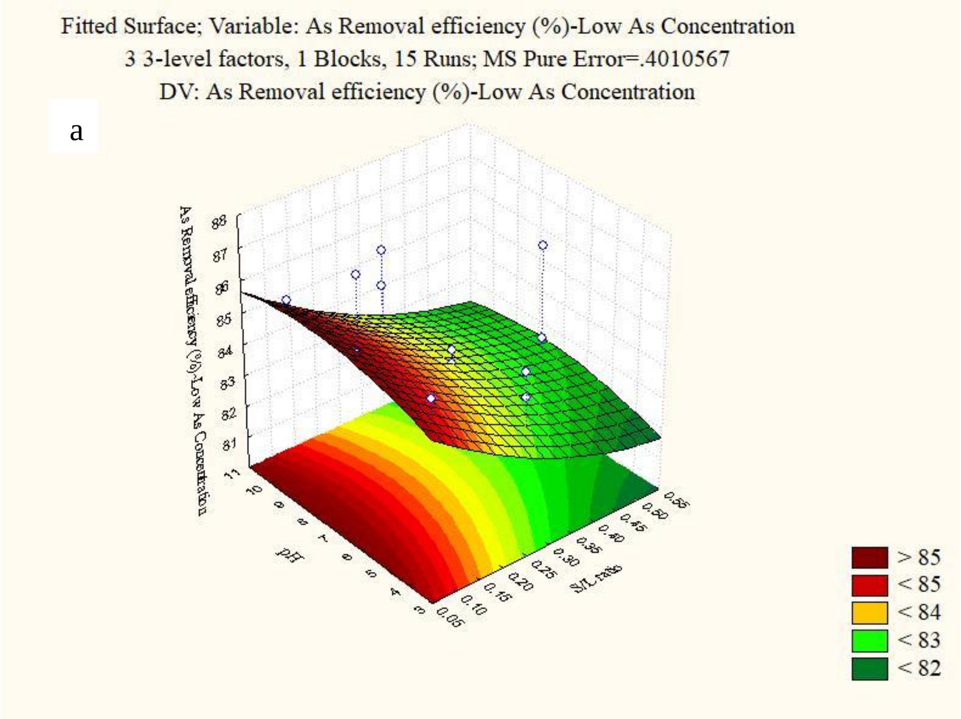
a=Washing time (Minutes)

b= Washing pH

c=S/L Ratio

$$\boxed{y = 85.012-0.115a-0.823a^2+0.213b+0.147b^2-0.993c-0.385c^2-0.470ab-1.319ac+0.001bc \quad \text{(Eq 4.1)}}$$

The variable y in this equation was As removal efficiency (%). The variable a, b and c was washing time (minutes), washing pH and S/L ratio, respectively. This equation indicated the correlation between three factors with R^2 was 0.92496.


Fitted Surface; Variable: As Removal efficiency (%)-Low As Concentration
3 3-level factors, 1 Blocks, 15 Runs; MS Pure Error=.4010567
DV: As Removal efficiency (%)-Low As Concentration
a
As Removal efficiency (%)-Low As Concentration
88
87
86
85
84
83
82
81
pH
11
10
9
8
7
6
5
4
3
S/L ratio
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
> 85
< 85
< 84
< 83
< 82

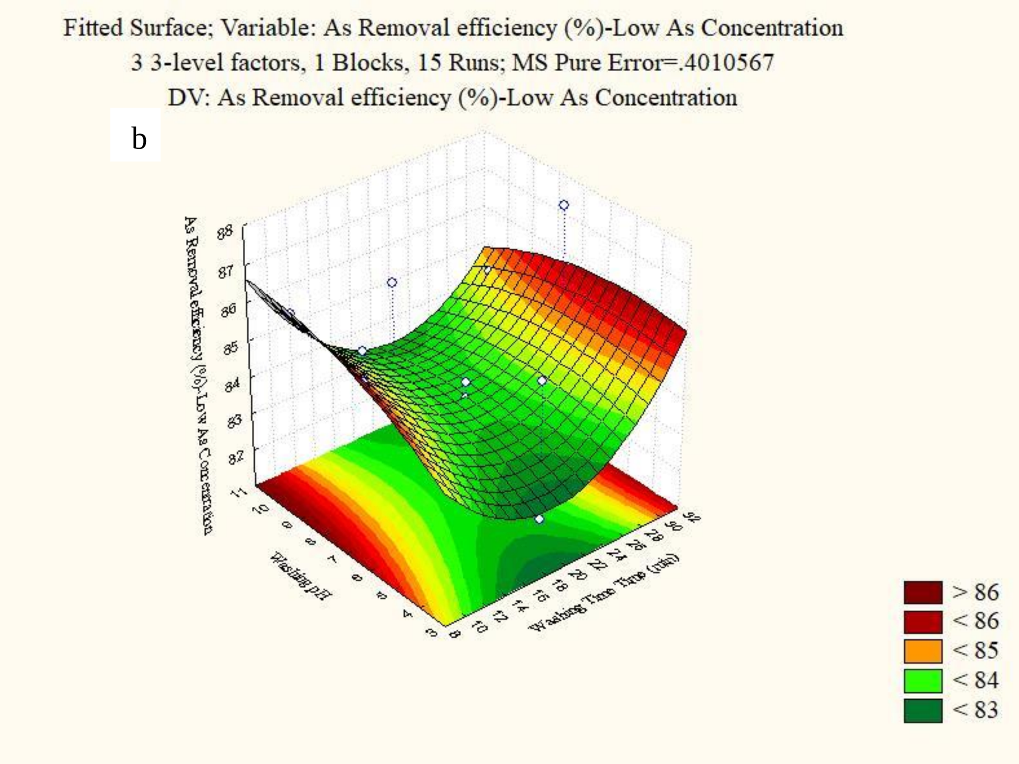

Fitted Surface; Variable: As Removal efficiency (%)-Low As Concentration
3 3-level factors, 1 Blocks, 15 Runs; MS Pure Error=.4010567
DV: As Removal efficiency (%)-Low As Concentration
b
As Removal efficiency (%)-Low As Concentration
88
87
86
85
84
83
82
Washing pH
11
10
9
8
7
6
5
4
3
Washing Time Time (min)
8
10
12
14
16
18
20
22
24
26
28
30
32
> 86
< 86
< 85
< 84
< 83

Figure 31 Surface response profiles of correlation between influenced factors to As removal efficiency in lower As concentrated soil a) Washing pH-S/L ratio, (b) Washing pH-Washing time

The As removal efficiency in different washing pH, washing time and solid: liquid ratio was plot and the results were shown in the Figure 4.15. According to the predicted values, the critical conditions for As removal efficiency of 79.91% could be obtained from pH 4, 1.0 solid:liquid ratio and 40 minutes washing time.

4.5.2 Design of Experiment for the higher As concentrated Soil

According to the Table 4.6 the experiment was conducted to observe the As removal efficiencies in As contaminated soil in higher As contaminated level reported as 2,572 mg/kg. In the Table 4.6 the observed and the predicted As removal efficiencies were compared after the experiment.

Table 9 Box-Behnken design for 15 number of runs in the higher As concentrated soil

Run	Washing time (Minutes)	Washing pH	S/L Ratio	Observed As removal (%)	Predicted As removal (%)

1	10	4	0.3	80.99	81.05
2	30	4	0.3	81.21	82.13
3	10	10	0.3	82.43	81.50
4	30	10	0.3	81.92	81.86
5	10	7	0.1	80.87	81.58
6	30	7	0.1	81.19	81.04
7	10	7	0.5	73.00	73.16
8	30	7	0.5	75.86	75.14
9	20	4	0.1	88.78	88.00
10	20	10	0.1	87.45	87.67
11	20	4	0.5	80.64	80.42
12	20	10	0.5	80.16	80.93
13	20	7	0.3	82.94	84.74
14	20	7	0.3	84.75	84.74
15	20	7	0.3	86.53	84.74

With the application of the suitable model for the analysis the data, two-way interaction with linear-linear model was chosen because its Coefficient of determination (R^2) value was 0.95473 which closed to 1, that regression predictions fit the data.

With considering the analysis of the influence, lack of fit P value was 0.759 that was higher than > 0.05, proved the model was well suitable for the higher As concentrated soil remediation. The conditions were calculated to the higher As concentrated soil (Table 4.7).

Table 10 Analysis of the variable that influence the As removal efficiency in the higher As concentrated soil from Box-Behnken design

Factor	P Value	Coefficient
Washing time(a)-Linear	0.627	0.361
Washing time-Quadratic	0.036	2.408
Washing pH(b)-Linear	0.951	0.044
Washing pH-Quadratic	0.209	-0.855
S/L Ratio(c)-Linear	0.036	-5.775
S/L Ratio-Quadratic	0.143	2.195
ab	0.858	-0.181
ac	0.554	0.632
bc	0.836	0.211

The correlation of variables was analyzed from the data in regression table (Table 4.7). Correlation model was exhibited in term of polynomial equation as shown in Eq.4.2.

y=Arsenic removal efficiency in higher As concentrated soil

a=Washing time (Minutes)

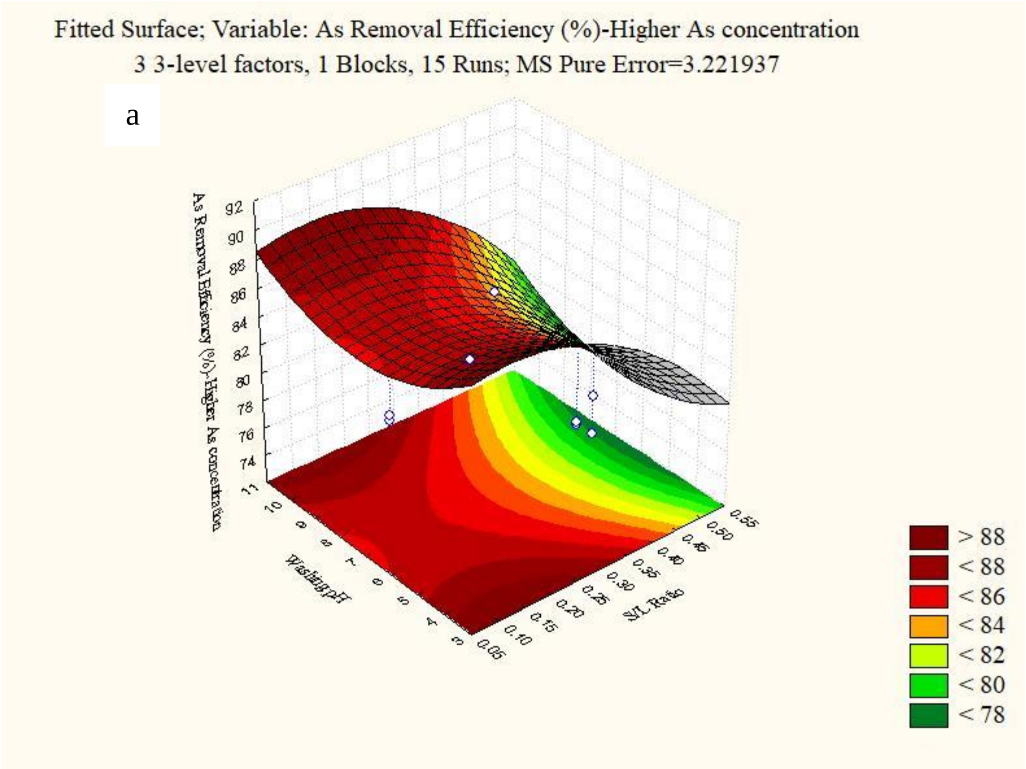
b= Washing pH

c=S/L Ratio

$$y = 79.745+0.361a+2.408a^2+0.044b-0.855b^2-5.775c+2.195c^2-0.181ab+0.632ac+0.211bc$$

Eq 4.2

The variable y in this equation was As removal efficiency (%). The variable a, b and c was washing time (minutes), washing pH and S/L ratio, respectively. This equation indicated the correlation between three factors with R^2 was 0.95473.



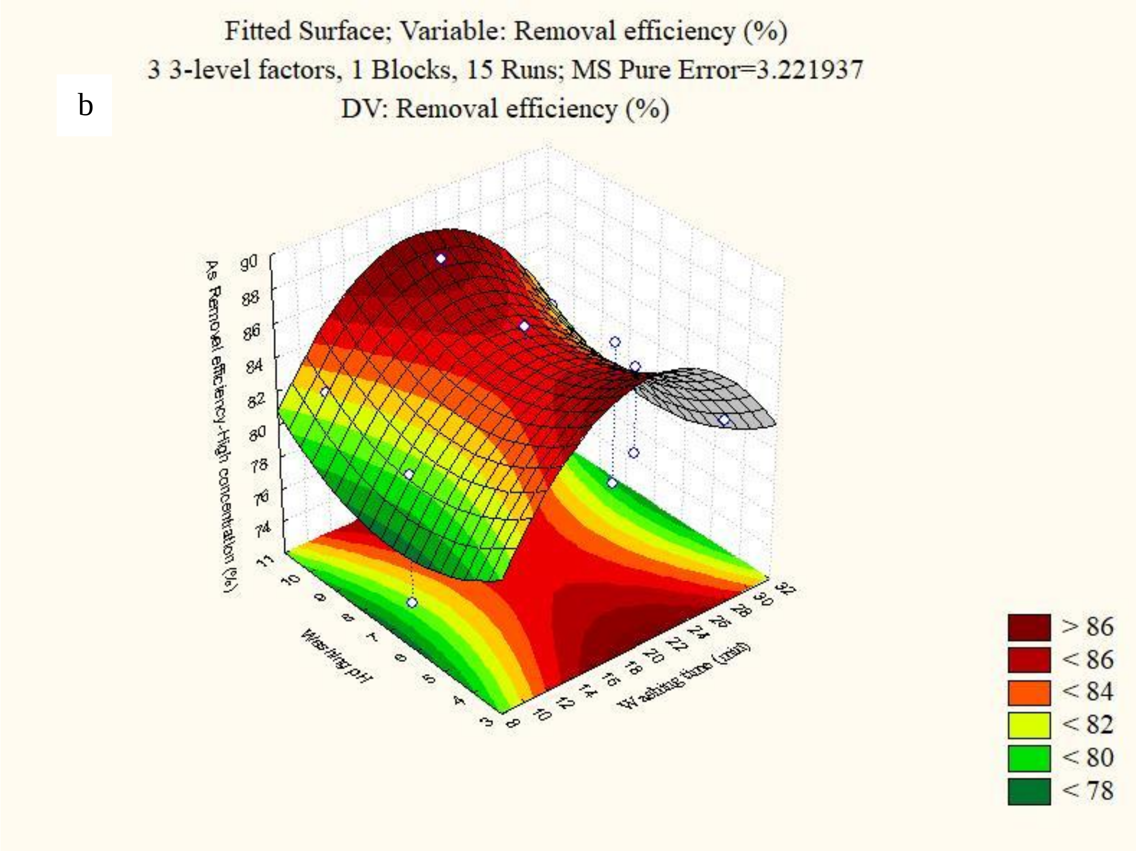


Figure 32 Surface response profiles of correlation between influenced factors to As removal efficiency in higher As concentrated soil a) Washing pH-S/L ratio, (b) Washing pH-Washing time

According to the correlation responses with washing time, washing pH and the solid: liquid ratio the critical values predicted As removal efficiency was 86.21% at the washing time at 20 minutes, washing pH at the 7.1 and the solid: liquid ratio was at 0.1.

From the design of experiments, the washing pH showed a crucial influence on As mobilization in the contaminated soil. With the STATISTICA prediction, the lower As concentrations (400-600 mg/kg) well expressed their maximum As removal efficiency in high acidic level (pH 4) and the higher As concentration (>1000 mg/kg) showed the maximum As removal efficiency at neutral pH condition (pH 7.1). According to Gersztyn et al. (2013), As can be mobilized in extreme acidic conditions (< pH 3) or extreme basic conditions (> pH 10). With the study, As mobilization in strong acidic conditions may cause from the dissolution of the iron oxides that make the surfaces to attach As in the soil structure (Gersztyn et al., 2013). According to Wang and Mulligan (2009) the mobilization of As can be increased dramatically with the ionization of the carboxyl groups in the biosurfactants. The ionized carboxyl groups can enhance the water solubility of the biosurfactant and provide more binding sites to the metal cations (Wang and Mulligan, 2009). With the lower As concentration, the solid: liquid ratio for the soil washing was 1.0 showing a potential for reduction of the surfactant amount.

Nonetheless, the higher As concentrated soil were spiked within a short period of time (two weeks) and the As cations might be conjugated with the soil in loose bonds rather than attaching with the microspore of the soil particles. In this condition, the ionization of the carboxyl groups at the neutral pH conditions might be sufficient for the washing of loosely bonded As cations from the soil. To confirm its efficiency, the optimal condition from this study should be evaluated with soil samples from As contaminated sites. The solid: liquid ratio for the higher As contaminated soil was 0.1, which was a magnitude lower than that in the lower As contaminated soil. The results confirmed that more biosurfactant molecules were required to mobilize more As from soil.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Since heavy metal contamination is a major environmental issue, the application of biosurfactant remediation is playing a protagonist role in removing heavy metal from the contaminated sites. Arsenic (As) pollution has been widely recorded across the world as a result of anthropogenic activities such as ore mining, the use of arsenic-containing fertilizers and pesticides, and the use of fossil fuels. Soil washing is a mix of chemical and physical techniques used to remove heavy metals from polluted soil by ex-situ washing using specially designed solutions. The washing solutions mobilize heavy metals by varying solution ionic strength, soil acidity, redox potential, or complexation, whereas the mechanical mixing process creates macroscale contact between the soil particles and the washing liquid, resulting in the removal of pollutants from the soil particles' surfaces. The application of the ultrasonication process enhances the pollutant removing efficiency by creating a microscale contact with tidily bonded heavy metal particles in the soil. In this study, the utilization of lipopeptide biosurfactant from *Bacillus subtilis* GY19 as a washing solution was examined. The objectives of this work were to formulate biosurfactant-based washing agent for arsenic removal from soil using lipopeptide biosurfactant and builder, to enhance arsenic removal from soil using biosurfactant-based washing agent by the combined mechanical agitation and ultrasonication, and to optimize operation condition of arsenic soil treatment by biosurfactant-based washing agent and combined soil washing process using factorial design.

With anionic heads, the biosurfactant can form complexes with metals on the soil surface, and the metals can then be removed from the soil by lowering the interfacial tension and interacting with the surfactant micelles. The biosurfactant's capacity to remove metal was varied with application concentrations. The concentration increment tended to increase the As removal efficiency in the contaminated soil. The findings revealed that lipopeptide in combination with EDTA had better efficacy in removing As from the soil, that had 418.7 mg/kg initial As concentration from the soil. The 5x CMC lipopeptide + 0.01M EDTA solution was found to have the highest As removal (46.1±0.9%), while 0.01 M EDTA alone and DI water removed only 34.1±1.4 % and 16.8±0.4% As, respectively. In addition, it exhibited more than 20% As removal efficiency compared to the usage of biosurfactant alone in the same surfactant concentration. The objective of the adding builder along with the biosurfactant proved that it has a significant influence on working with exchangeable cations in the soil slurry and stopped the precipitation of the surfactant monomers to the soil structure.

As removal efficiency could successfully be increased through the application of ultrasound along with mechanical agitation. Through the ultrasonication process, it could create more cavities and ruptures on the soil surface than the conventional mechanical agitation. Also, with the sonophysical effect, the soil particles have broken down into small particles with facilitating a higher surface area to contact with the washing biosurfactant solution. With the combination of ultrasonication and the mechanical agitation, the As removal efficiency of the 1x CMC GY 19 biosurfactant + 0.01 M EDTA solution was increased by more than 30%, from 45.81±0.9% to 71.45±3.6% compared to the conventional mechanical soil washing method. The results also suggested that lower concentration of biosurfactant could be applied with the combined washing process. To apply the lipopeptide-EDTA formulation, response surface methodology (RSM) analysis was used to identify the factors influencing the remediation efficiency and to predict the maximum As removal efficiency depending on As concentrations. The varying washing conditions included washing pH, washing time, and the solid: liquid ratio. The results showed that the washing pH has a major influence in As removal efficiency that reflected high As removal in lower pH conditions. According to the predicted values, the maximum As removal efficiency in lower As concentration (400-600 mg/kg) of 80% could be obtained by using pH 4 washing agent, 1.0 solid: liquid ratio, and 40 minutes washing time. For the higher As concentrated soil (>1000 mg/kg), the maximum washing efficiency 86% can be obtained from pH 7.1 washing agent, 0.1 solid: liquid ratio, and 20 minutes washing time. The optimized operation condition enhanced the efficiency of washing agent and combined soil washing process. It was also suggested that the contaminated soil could be remediated through two or more washing cycles. Also, with the low monomer loss in the lipopeptide and EDTA formulation, it can be used for the rewashing as a cost effective way. In conclusion, the application of lipopeptide-EDTA formulation along with the combined soil washing process is an effective, environmentally friendly, and cost healthy soil remediation approach to eradicate the present and future threat of heavy metal contamination and poisoning in the earth. As recommendation for more efficient As removal, ultrasonication and the mechanical agitation can be applied simultaneously with a developing appropriate machine that can provide shaking and the ultrasonic waves in a same time. This may reduce the washing time and the usage of power for the soil remediation along with the usage of biosurfactant as a washing agent.

5.2 Recommendations for future study

The lipopeptide-EDTA formulation with the combination of ultrasonication and mechanical agitation showed high efficiency for remediating contaminated As in soil. To improve their activities, the recommendations were listed as follows,

1. Application of the Response Surface Methodology (RSM) with more trail experiments such as fractional factorial design to form the correlation in between the washing condition.
2. Conducting the Box-Behnken design to experiment with more number runs to reduce the experimental errors.

3. Conducting the soil washing experiment using the lipopeptide-EDTA formulation to investigate As contaminated soil with different mineral composition soil.
4. Comparing the As remediation efficiency of the lipopeptide-EDTA formulation with other anionic, cationic, non-anionic, and synthetic surfactants to evaluate the better output of the soil washing.
5. Application of the correlation equation to real soil condition to conduct the soil washing experiments to spike and non-spiked natural soil.
6. Soil washing experiments should be confirmed with the larger scale experiments to evaluate the As removal efficiency in real-time situations.
7. Application of the lipopeptide-EDTA formulation along with the combined soil washing process with different washing temperature conditions to see the influence of the heat to remediate As from the soil.
8. Conducting the FT-IR analysis to identify the heavy metal bonding to the washing agents
9. Conducting the seed germination experiment to the washed soil in a real application situation to identify the toxicity of the washing solution.
10. The nature of soil microbes and the soil nutrient condition should be investigated after the washing to investigate any fluctuation in the properties.

11. The shelf-life of the lipopeptide and lipopeptide-EDTA formulation and the storage condition should be investigated to ensure the effectiveness of the washing agent.
12. Recycling, reusing, and incinerating methods of the washed leachate should be identified before introducing it to the normal habitable environment.

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APPENDIX

APPENDIX A

MEDIA

Table A.1 LB broth (Luria-Bertani broth) (per 1 Liter)

Ingredient	**Amount (g)**
Tryptone	10
Yeast Extract	5
Sodium Chloride	10

Suspend/dissolve all in 1 L of purified water, and adjusted pH to 7.0

APPENDIX B

SUPPLEMENTARY DATA OF CHAPTER 4

Table B.1: Soil pH variation after the soil washing with biosurfactant

Initial soil pH	7.2					
CMC Value	0.25	0.5	1	5	10	DI Water
Soil pH	7.13	7.09	7.01	6.97	6.88	7.03

	7.14	7.2	7.1	6.77	7.02	7
	7.11	7.14	7.15	7.02	6.94	7.02
Average	7.126667	7.143333	7.086667	6.92	6.946667	7.016667
ST DEV	0.015275	0.055076	0.070946	0.132288	0.070238	0.015275
ST ERROR	0.008819	0.031797	0.04096	0.076374	0.040551	0.008819

Table B.2: Surface tension of the washing solutions

	Surface tension of the surfactant (mN/m)					
Concentration (CMC)	0.25	0.5	1	5	10	DI
Surface tension	29.963	28.508	29.34	27.909	27.515	72.351
	29.593	28.444	28.927	28.08	27.203	72.515
	29.42	28.549	29.444	28.03	27.377	72.394
Average	29.65867	28.50033	29.237	28.00633	27.365	72.42
ST Dev	0.277392	0.052918	0.273457	0.087922	0.156346	0.085035
ST Error	0.160148	0.030551	0.157876	0.050761	0.090264	0.049094

Table B.3: Surface tension of the washed leachate

	Surface tension of the washed leachate (mN/m)					
Concentration (CMC)	0.25	0.5	1	5	10	DI
Surface tension	44.457	40.565	35.739	30.794	29.358	46.595
	44.343	40.624	36.075	30.818	29.579	46.857
	44.614	40.689	35.758	31.262	29.393	47.52
Average	44.47133	40.626	35.85733	30.958	29.44333	46.99067
ST Dev	0.136067	0.062024189	0.188744	0.263545	0.118787	0.476767
ST Error	0.078556	0.035808665	0.108968	0.152153	0.06858	0.275253

Table B.4: Arsenic removal efficiency calculation

GY19 Biosurfactant						
(mg/L)	0.25 CMC	0.5 CMC	1.0 CMC	5.0 CMC	10.0 CMC	DI
Concentration	7.239	7.32	8.078	11.77	13.32	6.99
	7.5	7.775	8.189	11	12.65	6.874
	7.412	7.508	7.937	11.58	12.92	7.239

Arsenic in 100ml of Washing Solution						
(mg)	0.25				10.0	
	CMC	0.5 CMC	1.0 CMC	5.0 CMC	CMC	DI
Amount of As	0.7239	0.732	0.8078	1.177	1.332	0.699
	0.75	0.7775	0.8189	1.1	1.265	0.6874
	0.7412	0.7508	0.7937	1.158	1.292	0.7239
Remaning Arsenic in the 10g of soil (mg)						
Amount of arsenic	3.6861	3.678	3.6022	3.233	3.078	3.711
	3.66	3.6325	3.5911	3.31	3.145	3.7226
	3.6688	3.6592	3.6163	3.252	3.118	3.6861
Remaning arsenic concentration in the soil (mg/kg)						
Concentration	368.61	367.8	360.22	323.3	307.8	371.1
	366	363.25	359.11	331	314.5	372.26
	366.88	365.92	361.63	325.2	311.8	368.61
Mean	367.1633	365.6567	360.32	326.5	311.3667	370.6567
ST DEV	1.327868	2.286402	1.262973	4.011234	3.370954	1.864949
ST Error	0.766623	1.320017	0.729157	2.315821	1.946166	1.076698

Arsenic removing efficiency (%)						
GY19 BSF	16.41	16.6	18.32	26.69	30.2	15.85
	17.01	17.63	18.57	24.94	28.68	15.59
	16.81	17.02	18	26.26	29.3	16.41
Mean	16.74333	17.08333	18.29667	25.96333	29.39333	15.95
St DEV	0.305505	0.517912	0.285715	0.911939	0.764286	0.419047
ST Error	0.176378	0.299008	0.164953	0.526493	0.441248	0.24193

Table B.5: Soil pH variation after soil washing with builder and biosurfactant

Initial soil pH				7.2					
Solution concentrations	0.01 M EDTA	0.01M EDTA+ 1CMC	0.01M EDTA+ 5CMC	0.01 M Na2 CO3	0.01M Na2CO3 +1CMC	0.01M Na2CO3 +5CMC	1 CMC	5 CMC	DI Water
Soil pH	6.34	6.2	6.1	8.47	7.65	6.68	7.01	6.97	7.03
	6.3	6.1	5.8	8.55	7.66	6.86	7.1	6.77	7
	6.27	6.27	6.11	8.6	7.85	6.79	7.15	7.02	7.02

Average	6.303333	6.19	6.003333	8.54	7.72	6.776667	7.086667	6.92	7.016667
ST DEV	0.035119	0.08544	0.176163	0.065574	0.112694	0.090738	0.070946	0.132288	0.015275
ST ERROR	0.020275	0.049327	0.101705	0.037858	0.065062	0.052386	0.04096	0.076374	0.008819

Table B.6: Surface tension of the washing agent before the soil washing

	Surface tension of the washig agent with builder (mN/m)								
Washing agent	1 CMC	5 CMC	0.01 M EDTA	0.01M EDTA+ 1CMC	0.01M EDTA+ 5CMC	0.01 M Na2CO3	0.01M Na2CO3 +1CMC	0.01M Na2CO3 +5CMC	DI
Surface tension	29.34	27.909	57.001	28.039	27.836	34.98	30.192	28.718	72.351
	28.927	28.08	59.609	28.785	27.987	37.079	30.4	28.698	72.515

	29.444	28.03	61.356	28.861	27.822	38.92	30.899	28.544	72.394
Average	29.237	28.00633	59.322	28.56167	27.88167	36.993	30.497	28.65333	72.42
ST Dev	0.27345749	0.087922	2.191639	0.454235	0.09149	1.971407	0.363344	0.095212	0.085035
ST Error	0.15787627	0.050761	1.265308	0.262245	0.05282	1.13816	0.209771	0.054969	0.049094

Table B.7: Surface tension of the washed leachate

	Surface tension of the washed leachate (mN/m)								
Washing agent	1 CMC	5 CMC	0.01 M EDTA	0.01M EDTA+1 CMC	0.01M EDTA+5 CMC	0.01M Na2CO3	0.01M Na2CO3+1CMC	0.01M Na2CO3+5CMC	DI
Surfac	35.7	30.7	59.9	29.88	29.057	37.79	31.75	28.961	46.5

e	39	94	36			3			95
tension	36.075	30.818	68.433	30.384	29.484	38.488	31.895	29.391	46.857
	35.758	31.262	64.787	30.589	29.305	39.124	32.205	29.405	47.52
Average	35.85733	30.958	64.38533	30.28433	29.282	38.46833333	31.95	29.25233	46.99067
ST Dev	0.188744	0.263545	4.262717	0.364857	0.214427	0.665717908	0.232433	0.252399	0.476767
ST Error	0.108968	0.152153	2.461011	0.210644	0.123796	0.384341498	0.134191	0.145719	0.275253

Table B.8: Soil washing efficiency with builder and biosurfactant

			GY19 Biosurfactant + Builder				
(mg/L)	0.01M	0.01M	0.01M	0.01M	0.01 M	0.01M	DI

	EDTA	EDTA+1 CMC	EDTA+5 CMC	Na_2CO_3	Na_2CO_3+1CMC	Na_2CO_3+5CMC	
Concentration	13.68	19.87	19.64	7.015	7.668	9.132	6.99
	14.28	19.66	19.34	6.454	8.009	8.997	6.874
	14.88	18.01	18.92	6.11	8.533	8.507	7.239

Arsenic in 100ml of Washing Solution

	0.01M	0.01M	0.01M	0.01M	0.01 M	0.01M	
(mg)	EDTA	EDTA+1 CMC	EDTA+5 CMC	Na_2CO_3	Na_2CO_3+1CMC	Na_2CO_3+5CMC	DI
Amount of As	1.368	1.987	1.964	0.7015	0.7668	0.9132	0.699
	1.428	1.966	1.934	0.6454	0.8009	0.8997	0.6874
	1.488	1.801	1.892	0.611	0.8533	0.8507	0.7239

Remaning Arsenic in the 10g of soil (mg)

Amount of arsenic	2.819	2.2	2.223	3.4855	3.4202	3.2738	3.488
	2.759	2.221	2.253	3.5416	3.3861	3.2873	3.4996
	2.699	2.386	2.295	3.576	3.3337	3.3363	3.4631

Remaning arsenic concentration in the soil (mg/kg)

Concentration	281.9	220	222.3	348.55	342.02	327.38	348.8
	275.9	222.1	225.3	354.16	338.61	328.73	349.96
	269.9	238.6	229.5	357.6	333.37	333.63	346.31
Mean	275.9	226.9	225.7	353.4367	338	329.9133	348.3567
ST DEV	6	10.18676	3.616628	4.568154	4.357144	3.288743	1.864949

ST Error	3.464003	5.881159	2.088002	2.63735	2.515527	1.898703	1.076698
Arsenic removing efficiency (%)							
GY19 BSF	32.67	47.46	46.91	16.75	18.31	21.81	16.69
	34.11	46.95	46.19	15.14	19.13	21.49	16.42
	35.54	43.01	45.19	14.59	20.38	20.32	17.29
Mean	34.10667	45.80667	46.09667	15.49333	19.27333	21.20667	16.8
St DEV	1.435003	2.435371	0.86379	1.122512	1.042417	0.784368	0.445309
ST Error	0.828476	1.406022	0.498695	0.648064	0.601823	0.452842	0.257092

Table B.9: CMD series analysis in washing agents

	BSF 1xCMC-Surface tension of the surfactant (mN/m)									
Dilution times	1	2	5	10	20	50	100	200	500	1000
Before soil washing	29.928	47.667	51.027	54.811	54.519	61.855	65.844	70.147	71.493	76.29

After Soil Washing	58.297	69.078	71.967	72.242	72.388	72.204	72.416	72.436	72.372	72.451
		0.01M EDTA-Surface tension of the surfactant (mN/m)								
Dilution times	1	2	5	10	20	50	100	200	500	1000
Before soil washing	35.398	36.371	40.263	39.033	41.237	51.005	51.287	42.055	46.527	32.982
After Soil Washing	68.187	71.545	71.811	72.416	71.515	72.012	70.929	71.776	72.623	71.848
		1 xCMC GY 19 BSF+0.01M EDTA-Surface tension of the surfactant (mN/m)								
Dilution times	1	2	5	10	20	50	100	200	500	1000
Before soil washing	26.667	26.845	28.713	35.078	33.669	38.887	38.974	59.899	68.184	69.014
Dilutio	1	2	5	10	20	50	100	500	1000	

n times									
After Soil Washing	35.199	42.159	55.279	67.028	67.563	71.112	72.093	72.407	72.502

Table B.10: Soil pH variation of the washing agents in different soil washing mechanisms

	BSF+EDTA			SDS+EDTA			SDS		
Soil pH	Mechanical	Ultrasonication	Combined	Mechanical	Ultrasonication	Combined	Mechanical	Ultrasonication	Combined
	6.66	6.76	7.12	6.67	6.53	6.41	7.11	7.23	7.29
	6.83	6.78	7.01	6.52	6.71	6.31	7.32	7.37	7.31
	6.72	6.77	7.03	6.33	6.66	6.19	7.32	7.37	7.3
Average	6.736667	6.77	7.053333	6.506667	6.633333	6.303333	7.25	7.323333	7.3
ST Dev	0.086217	0.01	0.058595	0.170392	0.092916	0.110151	0.121244	0.080829	0.01

Table B.11: Soil electrical conductivity (EC) variation of the washing agents in different soil washing mechanisms

Soil Conductivity (dS/m)	BSF+EDTA			SDS+EDTA			SDS		
	Mechanical	Ultrasonication	Combined	Mechan	Ultra	Combin	Mechan	Ultra	Combined
	5.6	5.7	12.3	25.3	16.8	31.5	27	20.7	26.7
	6.1	6.8	12	21.8	16.8	31.3	27	20.7	26.4
	5.9	6.9	12.1	21.4	16	31	27.1	20.2	26.4
Average	5.866667	6.466667	12.13333	22.83333	16.53333	31.26667	27.03333	20.53333	26.5
ST DEV	0.251661	0.665833	0.152753	2.145538	0.46188	0.251661	0.057735	0.288675	0.173205

Table B.12: Soil washing efficiency of the washing agents in different soil washing mechanisms

Remaining arsenic concentration in the soil (mg/kg)															
Concentration	BSF+EDTA			BSF			SDS			SDS+EDTA			DI		
	Mech	Ultra	Com	Mech	Ultra	Com	Mech	Ultra	Com	Mech	Ultra	Com	Mech	Ultra	Com
	155.0268	130.2202	127.5923	337.92	239	145	346.0928	280.2435	290.3418	178.0685	248.5149	181.0084	348.8	343	289
	204.6178	149.557	102.0432	336.81	235	146	355.1029	326.0998	350.0498	210.1708	332.7377	245.4852	349.9	337	287
	195.1075	121.3344	128.9672	339.33	236	146	379.8805	320.3109	289.5522	190.4593	254.2507	159.6002	346.31	344	281
Mean	184.9174	133.7039	119.5342	338.02	236.6667	145.6667	360.3587	308.8848	309.9813	192.8995	278.5011	195.3646	348.3367	341.3333	285.6667

ST DEV	26.31915	14.4302	15.16324	1.262973	2.081666	0.57735	17.49627	24.97235	34.70257	16.18972	47.05779	44.70611	1.839302	3.785939	4.163332
ST Error	15.19494	8.331044	8.754254	0.729157	1.201816	0.333324	10.10119	14.41738	20.03497	9.346871	27.16805	25.81035	1.061892	2.185751	2.403633
Arsenic removing efficiency (%)															
GY19 BSF	62.97	68.9	69.53	19.29	42.91	65.37	17.34	33.07	30.66	57.47	40.65	56.77	16.69	18.08	30.98
	51.13	64.28	75.63	19.55	43.87	65.13	15.19	22.12	16.4	49.8	20.53	41.37	16.43	19.51	31.45
	53.4	71.02	69.2	18.95	43.64	65.13	9.27	23.49	30.85	54.51	39.28	61.88	17.29	17.84	32.89
Mean	55.83333	68.06667	71.45333	19.26333	43.47333	65.21	13.93333	26.22667	25.97	53.92667	33.48667	53.34	16.80333	18.47667	31.77333
St	6.2	3.4	3.6	0.3	0.5	0.1	4.1	5.9	8.2	3.8	11.	10.	0.4	0.9	0.9

DEV	83887	46409	20861	00888	01232	38564	79191	65956	88408	6813	24169	67655	41059	02903	95205
ST Error	3.627901	1.989729	2.090446	0.173713	0.289378	0.079998	2.412788	3.444349	4.785178	2.233203	6.490209	6.163933	0.254638	0.521276	0.574566

Table B.13: DOE Results for the lower As concentrated soil

Initial As concentration (mg/kg)		436	433		433	434
No	As con (mg/kg)					Efficiency (%)
	1	2	3	4	Mean	
1	73.3	73.56	73.46	58.9	68.64	84.18433
2	61.24	61.32	61.72	73.01	65.35	84.9424
3	61.43	59.96	60.71	62.98	61.21667	85.89478
4	64.38	63.22	64.4	70.62	66.08	84.77419

	5	60.81	61.69	62.13	66.8	63.54	85.35945
	6	44.38	45.73	46.19	67.99	53.30333	87.71813
	7	59.9	61.5	59.56	58.92	59.99333	86.17665
	8	79.29	80.31	81.12	56.53	72.65333	83.2596
	9	64.64	65.04	65.15	58.68	62.95667	85.49386
	10	56.91	56.91	56	74.92	62.61	85.57373
	11	87.86	89.89	88.4	58.68	78.99	81.79954
	12	86.89	87.89	87.48	60.53	78.63333	81.88172
	13	81.75	81.59	83.02	57.97	74.19333	82.90476
	14	75.47	73.01	74.47	58.92	68.8	84.14747
	15	70.28	71.96	69.62	70.15	70.57667	83.7381

Table B.14: DOE Results for the higher As concentrated soil

Initial As concentration	2543	2576	2597	2572

(mg/kg)

No	As con (mg/kg)				Efficiency
	1	2	3	Mean	
1	486	489	492	489	80.98756
2	478	485	487	483.3333	81.20788
3	453	451	452	452	82.42613
4	468	462	465	465	81.92068
5	487	492	497	492	80.87092
6	490	483	478	483.6667	81.19492
7	687	698	698	694.3333	73.00415
8	621	613	629	621	75.85537
9	289	291	286	288.6667	88.77657
10	323	324	321	322.6667	87.45464
11	497	503	494	498	80.63764
12	512	509	510	510.3333	80.15811
13	431	447	438	438.6667	82.94453
14	395	392	390	392.3333	84.74598
15	342	341	356	346.3333	86.53447

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