MICROBIAL COMMUNITY PROFILING OF ARSENIC-RICH MINE TAILING AND ARSENIC BIOADSORPTION BY INDIGENOUS BACTERIA

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UNIVERSITI TEKNOLOGI MALAYSIA

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ABSTRACT

Arsenic is a common contaminant in gold mine soil and tailings. Moreover, the contamination of water with arsenic is a serious health issue. Microbes present an opportunity to remove arsenic from wastewater via adsorption process, which is distinguished by its low cost and easy technique in comparison with conventional techniques include oxidation, coagulation-flocculation, and membrane techniques. However, the development of existing bio-treatment approaches depends on isolation of arsenic-resistant microbes from arsenic contaminated samples. In this study, a culture-independent approach using Illumina sequencing technology was used to profile the microbial community *in situ*. This was coupled with a culture-dependent technique to analyse the microbial population in arsenic-laden tailing dam sludge based on the culture-independent sequencing approach. Based on the culture-independent sequencing approach, 4 phyla and 8 genera were identified in a sample from the arsenic-rich goldmine. Firmicutes (92.23%) was the dominant phylum, followed by Proteobacteria (3.21%), Actinobacteria (2.41%), and Bacteroidetes (1.49%). The identified genera included *Staphylococcus* (89%), *Pseudomonas* (1.25%), *Corynebacterium* (0.82%), *Prevotella* (0.54%), *Pseudonocardia* (0.39%), *Megamonas* (0.38%) and S*phingomonas* (0.36%). The culture dependent method exposed significant similarities with culture independent methods at the phylum level with Firmicutes, Proteobacteria and Actinobacteria, being common, and Firmicutes was the dominant phylum whereas, at the genus level, only *Pseudomonas* was presented by both methods. Considering the advantage of the different structures of these bacterial cell walls in adsorption, attempts were made to use individual dried biomass of *Bacillus thuringiensis* strain WS3 (IDB) and mixed dried biomass of three species *B. thuringiensis* strain WS3, *Pseudomonas stutzeri* strain WS9 and *Micrococcus yunnanensis* strain WS11 (MDB) to achieve highest As (III) and As (V) removal under different conditions. Successively, MDB were found to be efficient in the removal of As (III) and As (V) up to 95 % and 98 %, respectively. The maximum adsorption capacity of As (III) and As (V) increased from 95 mg/g and 145 mg/g for IDB to 217 mg/g and 333 mg/g for MDB as obtained from the Langmuir isotherm. The pattern of adsorption fitted well with the Langmuir isotherm model and kinetic data followed a pseudo-second-order model for both IDB and MDB. The thermodynamic parameters $\Delta G°$, $\Delta H°$ and $\Delta S°$ revealed that the adsorptions of both As (III) and As (V) were spontaneous, feasible and endothermic in nature. FESEM-EDX analysis established diverse cell morphological changes with significant amounts of arsenic adsorbed onto biomass compared to original biomass. Results from FTIR have shown the involvement of mainly hydroxyl, thiol, amide and amino functional groups in the arsenic adsorption. Batch experimental data were taken into account to create an artificial neural network (ANN) model that mimicked the human brain function. 5-7-1 neurons were in the input, hidden and output layers respectively. The batch data was reserved for training (75%), testing (10%) and validation process (15%). The predicted output of the proposed model showed a good agreement with the batch experiments with reasonable accuracy. This study has demonstrated the potential for using mixed dried non-living biomass as a new biosorbent for arsenic removal.

ABSTRAK

Arsenik adalah pencemar biasa di tanah dan amang lombong emas. Selain itu, pencemaran air dengan arsenik merupakan masalah kesihatan yang serius. Mikrob menyediakan peluang menyingkirkan arsenik dari air sisa melalui proses penjerapan, yang dibezakan oleh kos rendah dan teknik mudahnya berbanding dengan teknik konvensional termasuk teknik pengoksidaan, koagulasi-flokulasi, dan membran. Walau bagaimanapun, perkembangan pendekatan bio-rawatan yang sedia ada bergantung kepada pemencilan mikrob rintang arsenik daripada sampel yang tercemar arsenik. Dalam kajian ini, pendekatan bebas kultur menggunakan teknologi penjujukan Illumina digunakan untuk memprofilkan komuniti mikrob in situ. Pendekatan ini digandingkan dengan teknik bergantung kultur, iaitu pemencilan menggunakan dua media pertumbuhan berbeza, LB dan CDM untuk menganalisis populasi mikrob dalam enap cemar empangan amang muatan arsenik berdasarkan pendekatan penjujukan bebas kultur. Berdasarkan pendekatan penjujukan bebas kultur, 4 fila dan 8 genus dikenal pasti dalam sampel dari lombong emas yang kaya dengan arsenik. Firmicutes (92.23%) merupakan filum dominan, diikuti oleh Proteobakteria (3.21%), Aktinobakteria (2.41%), dan Bakteroidetes (1.49%). Genus yang dikenal pasti termasuk *Staphylococcus* (89%), *Pseudomonas* (1.25%), *Corynebacterium* (0.82%), *Prevotella* (0.54%), *Pseudonocardia* (0.39%), *Megamonas* (0.38%) dan *Sphingomonas* (0.36%). Kaedah bergantung kultur mendedahkan bahawa terdapat persamaan yang signifikan dengan kaedah bebas kultur pada tahap filum dengan Firmikutes, Proteobakteria dan Aktinobakteria sebagai yang biasa, dan Firmikutes sebagai filum dominan manakala pada tahap genus, hanya *Pseudomonas* yang ditunjukkan oleh kedua-dua kaedah. Mempertimbangkan kelebihan struktur yang berbeza pada dinding sel bakteria-bakteria ini dalam penjerapan, percubaan dibuat untuk menggunakan biojisim kering *Bacillus thuringiensis* strain WS3 (IDB) dan campuran biojisim kering tiga spesies iaitu *Bacillus thuringiensis* strain WS3, *Pseudomonas stutzeri* strain WS9 dan *Micrococcus yunnanensis* strain WS11 (MDB) untuk mencapai penyingkiran tertinggi As (III) dan As (V) pada keadaan yang berbeza. Seterusnya, MDB didapati berkesan dalam penyingkiran As (III) dan As (V) masing-masing sebanyak 95% dan 98%. Kapasiti penjerapan maksimum As (III) dan As (V) meningkat daripada 95 mg/g dan 145 mg/g bagi IDB kepada 217 mg/g dan 333 mg/g untuk MDB seperti yang diperoleh daripada isoterm Langmuir. Corak penjerapan menepati dengan baik model isoterm Langmuir dan data kinetik mengikut model tertib pseudo-kedua bagi IDB dan MDB. Parameter termodinamik $\Delta G°$, $\Delta H°$ dan $\Delta S°$ mendedahkan bahawa penjerapan kedua-dua As (III) dan As (V) adalah spontan, boleh dilaksanakan dan endotermik. Analisis FESEM-EDX menunjukkan perubahan morfologi sel pelbagai dengan jumlah arsenik yang signifikan diserap ke biojisim berbanding dengan biojisim asal. Keputusan FTIR menunjukkan perubahan spektral utama pada kawasan jalur 400 cm^{-1} hingga 4000 cm^{-1} menunjukkan penglibatan kumpulan-kumpulan berfungsi terutamanya hidroksil, tiol, amida dan amino dalam penjerapan arsenik. Data eksperimen berkelompok diambil kira untuk membina model rangkaian neural buatan (ANN) yang meniru fungsi otak manusia. Neuron 5-7-1 masing-masing berada dalam lapisan input, lapisan tersembunyi dan lapisan output. Data berkelompok disimpan untuk latihan (75%), ujian (10%) dan proses pengesahan (15%). Output ramalan model yang dicadangkan menunjukkan persetujuan yang baik dengan eksperimen berkelompok dengan ketepatan yang munasabah. Kajian ini menunjukkan potensi untuk menggunakan biojisim kering tidak hidup sebagai biopanjerap baharu untuk penyingkiran arsenik.

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LIST OF ABBREVIATIONS

As (III)	-	Arsenite
As (V)	-	Arsenate
IDB		Individual Dried Biomass
MDB		Mixed Dried Biomass
LB	-	Luria-Bertani medium
CDM	-	Chemically defined medium
IDM	-	Individual dried biomass
MDB	-	Mixed dried biomass
sp.	-	species
FESEM-EDX	-	Field Emission Scanning Electron Microscope – Energy Display x-ray
FTIR	-	Fourier-transform infrared spectroscopy
WHO	-	World Health Organization
Ppm	-	Part per million
Ppb	-	Part per billion
$AgNO_3$	-	Silver Nitrate
BLAST	-	Basic Local Alignment Search Tool
NCBI	-	National Centre of Biotechnology Information
HCl	-	Hydrochloric acid
PCR	-	Polymerase Chain Reaction
pKa	-	Acid dissociation constant
R^2	-	Coefficient of determination
Rpm	-	Rotation per minute
rRNA	-	Ribosomal ribonucleic acid
v/v	-	Volume per volume
ANN	-	Artificial Neural Network

LIST OF SYMBOLS

M	-	Micro
%	-	Percentage
°C	-	Degree Celcius
K	-	Kelvin
H	-	Hour
mL	-	Millilitre
mM	-	Millimolar
μM	-	Micromolar
G	-	Gram
mg	-	Milligram
V	-	Volume
M	-	Mass

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Metagenomics (culture-independent) is a relatively new, yet a rapidly developing technology to analyse and characterize microbial communities in the environment. Microorganisms are an important aspect of ecological processes; helping for biogeochemical cycling for important elements such as sulfur, nitrogen, phosphorous, and carbon, decay of organic substance and xenobiotics and formation of soil structure. Thus, bacteria play a significant role in regulating the biogeochemical cycles and affect life on Earth (McHardy and Rigoutsos, 2007; Smith *et al.*, 2015). Studies on bacterial dynamics and their interaction with the abiotic and biotic elements are essential to understanding their involvement in energy generation, mining, bio-treatment, and biotechnology (Rastogi and Sani, 2011; Santoyo *et al.*, 2017). A well-ordered microbial community with a firm level of diversity is stable (Wu *et al.*, 2018; Yannarell and Triplett, 2005). However, when presented with some kind of stress, the diversity of the community might change, thus collapsing its stability. Therefore, microbial diversity used to study the effect of perturbations in the environment. In the regular environment, microorganisms occur in great numbers despite the fact that there are millions of bacterial species that have not been identified. Therefore, pure seawater might contain 10^6 bacteria per millilitre and one gram of soil or sediment might contain approximately $l0^{10}$ bacteria as calculated via fluorescence microscopy after staining with a dye (Fakruddin and Mannan, 2013; Torsvik *et al.*, 1990).

Recently, new non-cultural approaches have been developed that can be used extensively in a microbial consortium for comprehensive analysis of various communities (Lugli *et al.*, 2019; Mahajan *et al.*, 2018). Metagenomics or genomic microorganism studies refer to a non-cultural approach in which the genomes of a mixed microbe population are collectively studied. Population genomics, environmental genomics and Community genomics are frequently used as metagenomics synonyms (Neelakanta and Sultana, 2013). Since the use of the metagenomics method in this research, several other studies have used this technique to study microbial communities in different environments such as acid-mine drainage (Tyson *et al.*, 2004), marine water and sediments (DeLong *et al.*, 2006; Yooseph *et al.*, 2010) and arsenic-contaminated soils (Layton *et al.*, 2014; Luo *et al.*, 2014).

Detailed insight into microbial communities in arsenic contaminated water in the natural environment is challenging owing to their extreme conditions and uncultivated status (Das *et al.*, 2017). However, a large variety of metagenomies of microbial communities in arsenic contaminated water have been reported include: *Helicobacter pylori* (0.01%), *Campylobacter jejuni* (0.01%), *Staphylococcus aureus* (0.02%), *Shigella flexneri* and *Shigella dysenteriae* (0.03%), *Cronobacter sakazakii* (0.03%), *Clostridium difficile* (0.03%), *Salmonella enterica* (0.07%), *Vibrio cholerae* (0.08%) and *Vibrio parahaemolyticus* (0.08%) (Layton, *et al.*, 2014). Furthermore, a great diversity of arsenic-resistant microbes have been stated, including *Bacillus* sp. and *Aneurinibacillus aneurinilyticus* (Dey *et al.*, 2016); *Acinetobacter calcoaceticus, A. baumannii, A. junii, A. venetianus, A. soli*, and *Microbacterium oleivorans* (Goswami *et al.*, 2015); *Enterobacter* sp. and *Klebsiella pneumoniae* (Abbas *et al.*, 2014); *Bacillus smithii*, *B. cereus*, *Pseudomonas maltophilia*, *Vibrio parahaemolyticus*, *Pseudomonas* sp, *Micrococcus varians*, *M. luteus*, and *M. roseus* (Shakya *et al.*, 2012); *Geobacillus kaustophilus* (Cuebas *et al.*, 2011), *Bacillus* sp., *Enterobacter* sp., *Stenotrophomonas* sp., and *Rhizobium* (Tiwari *et al.*, 2016); *P.* strain As-11(Jebelli *et al.*, 2017); *B. cereus* strain SZ2 (Bahari *et al.*, 2013), and *Microbacterium* sp. strain SZ (Bahari *et al.*, 2017). Furthermore, there are microbes that can adapt to diverse environmental conditions on earth and decay chemical components produced by living things (Fakruddin and Mannan, 2013).

Arsenic is generally distributed in Earth's crust. It is leaked by common phenomena such as mineral weathering or volcanic ash and human activities such as gold mining and various resources (Cullen and Reimer, 1989; Smedley and Kinniburgh, 2002; Tamaki and Frankenberger, 1992). Arsenic can be found in the environment as arsenious acids ($H_3AsO_3^{2+}$, H_3AsO_4, H_3AsO_3). Moreover, As (V) is like a soft acid and can form a complex with sulfides. On the other hand, As (III) is a firm acid which makes a compound with nitrogen and oxides (Mohan and Pittman, 2007). However, there are large numbers of arsenic contaminated areas with high concentrations of arsenic around the world, especially in Argentina, Chile, Mexico, China, Hungary, West Bengal (India), Bangladesh, Vietnam, and the USA (Herath *et al.*, 2016). There are generally 15 gold mines in Malaysia with large gold mines in Pahang (Penjom, Raub and Selinsing gold mines), Kelantan and Terengganu. Therefore, it is very likely that arsenic concentrations in these areas are high.

Microbes have co-habited with different metals from initial history. Thus, microorganisms have been effectively used to remove heavy metal such as Arsenic (As) from wastewater in a variety of patterns. Consequently, from a functional concept, metals divided into three groups: (i) non-toxic and essential such as Mg and Ca, (ii) harmful at high concentrations and essential in low concentration such as Zn, Mo, Cu, Ni, Co, Fe, and Mn, and (iii) toxic even in low concentration such as Cd, Hg and As. In addition, interaction with metals relies on specific metal and its chemical speciation (Valls and De Lorenzo, 2002). The basic mechanism of adsorption by biomass can be described as passive metal ions immobilization. Briefly, it essentially relies on the physicochemical interaction between metals and different functional groups of the cell wall. Microorganisms have been effectively used to remove arsenic from wastewater (Bahari, *et al.*, 2013; Haris *et al.*, 2018; Kao *et al.*, 2013; Prasad *et al.*, 2013). Likewise, the cell wall of bacteria generally comprises proteins, lipids and polysaccharides, which contain functional groups, such as amine groups, phosphate, hydroxyl and carboxylate, and these functional groups offer binding sites for metals (Mohan and Pittman, 2007).

Artificial Neural Network (ANN) is classified as an artificial intelligence modelling technique because of its ability to recognize patterns and relationships in historical data and then to deduce new data (Aleboyeh *et al.*, 2008). The ANN uses a specified algorithm to analyse data cases or similarity patterns and then divides them into a defined class number. In addition, the ANN learns to accurately predict the output parameter value when data with adequate input parameters are given

(Yetilmezsoy and Demirel, 2008). Process models and model-based process monitoring are the main applications of ANN in the water treatment industry (Shetty and Chellam, 2003). Therefore, adsorption results can be predicted using the artificial neural network (ANN), as ANN can efficiently map inputs and outputs in complex situations (Aleboyeh, *et al.*, 2008; Annadurai *et al.*, 2007; Chu, 2003; Saha *et al.*, 2010; Texier *et al.*, 2002; Yetilmezsoy and Demirel, 2008).

1.2 Problem Statement

Globally, arsenic contamination in groundwater is presently a major problem, particularly in areas where people depend on groundwater. Poisonous arsenic has resulted in health disasters for over 100 million people universally, mainly in China, India, Bangladesh, Taiwan, Thailand, Chili and Romania (Miyatake and Hayashi, 2009; Shahid *et al.*, 2018; Singh *et al.*, 2007; Tabassum *et al.*, 2019). The Environmental Protection Agency of the United States declared that all forms of arsenic pose a serious health risk (Sarkar *et al.*, 2007). Accordingly, the recommended concentration of arsenic in drinking water has since been modified from 50 ppb (0.05 mg/L) to 10 ppb (0.01 mg/L) by the World Health Organization (WHO), while the standard concentration of arsenic in industrial effluents is restricted to 0.1 mg/L (0.1 ppm) (Wu *et al.*, 2010). Whereas, the Malaysian Environment Department reported in 1985 that drinking water quality standard for maximum arsenic concentration is 0.01 mg /L (Huang *et al.*, 2015).

Chronic arsenic poisoning causes skin lesions with hyperkeratosis, depigmentation, and hyperpigmentation (Sun, 2004; Yoshida *et al.*, 2004), vascular diseases, such as cardiovascular, arteriosclerosis and hypertension (Rahman *et al.*, 1999; Wang *et al.*, 2002; Yu *et al.*, 2002) and non-specific signs of the effect on the digestive system, such as dyspepsia, diarrhoea and abdominalgia (Sun *et al.*, 2001) and also has extensive and complex effects on developing infants, such as poor memory, mental slowing, cognitive delays and reduced intelligent quotient (IQ) (Chattopadhyay *et al.*, 2002). Therefore, arsenic contamination of water has become a serious problem for the community (Hao *et al.*, 2018; Nidheesh and Singh, 2017;

Zhang *et al.*, 2018). Currently, there is no medical treatment for arsenicosis and the only guaranteed way of preventing chronic arsenic poisoning is to stop the ingestion of arsenic (Sun *et al.*, 2006).

Several researchers identified biotreatment of arsenic ions from contaminated water by living organisms as a viable solution for the removal of these contaminants (Ike *et al.*, 2008; Lu *et al.*, 2018; Pandey and Bhatt, 2015), whereas the effective biotreatment depends on our ability to study microbes that are indigenous to polluted sites regardless of the approach taken (Stefani *et al.*, 2015). Therefore, in this study, culture independent was combined with culture-dependent methods to isolate indigenous microbes using soil samples harvested from tailing dam sludge because it contains the highest concentration of arsenic in the gold mining environment. Consequently, the removing of arsenic from contaminated water is necessary for confirming the safety of drinking water and protect public health (Nickson *et al.*, 2000; Zaini *et al.*, 2011). Some of the conventional solutions to remove As (III) and As (V) from wastewater are filtration, flotation, flocculation with sulfide or ferric hydroxide and ion exchange. However, these techniques require pre-treatment, the oxidation of As (III) to As (V) and involve a high cost (Valls and De Lorenzo, 2002).

1.3 Contributions to Knowledge

This is the first study on biodiversity of microbes for Malaysian gold mining environment that use independent and dependent approaches. Moreover, employment of culture based approach with metagenomics analysis helps to isolate indigenous arsenic resistant microbes and their potential use in bioremediation of arsenic contaminated sites. Furthermore, individual and mixed culture dried biomass of indigenous arsenic resistant microbial (WS3, WS9, WS11) have been used after regeneration by acid washing to enhance the removing of As (III) and As (V). Mixed dried biomass of WS3, WS9 and WS11 was found to be efficient in removing As (III) and As (V) due to the benefits of the distinct structures of these bacterial cell walls in adsorption.

The experimental data was then taken into account to develop an artificial neural network (ANN) model; the ANN model mimicked the function of the human brain, to predict the removal of As (III) and As (V) from aqueous solution by adsorption process. Moreover, mixed dried biomass of three indigenous arsenic resistant bacteria (WS3, WS9, and WS11) is distinguished by its low cost and high capacities for bio-treatment of arsenic from wastewater. Consequently, the adsorptions of As (III) and As (V) ions using the above microbial mix have not been reported elsewhere. Hence it was considered the best choice to use these microbes for adsorption of these two ions. The novelty of this work is to use new indigenous arsenic resistant microbes for the removal of As (III) and As (V) and the results are compared with a model to find the validity of the experimental results.

1.4 Objectives

1) To investigate the microbial communities' in soil contaminated by arsenic using culture independent strategy (metagenomics approach) and isolate and characterize the indigenous arsenic resistant bacteria (culture dependent approach), according to the metagenomics profile obtained and assess the reusability of the bacterial biomass by acid washing (regeneration study).

2) To study the adsorption of As (III) and As (V) using individual and mix dried bacterial biomass, by varying the various process parameters e.g initial contact time, arsenic concentration, pH, temperature and adsorbent dose.

3) To evaluate the mechanism of As (III) and As (V) adsorption using various adsorption isotherms, kinetic and thermodynamic models and characterized the indigenous arsenic resistant bacteria biomass before and after adsorption.

4) To predict and compare the removal efficiency of As (III) and As (V) from aqueous solution by individual and mixed dried biomass of indigenous arsenic resistant bacteria using artificial neural network (ANN) model.

1.5 Scope of Study

In this study, we combined metagenomics with culture-dependent methods to isolate indigenous microbes from high arsenic contaminated soil samples harvested from a tailing dam sludge in one gold mine in Pahang (Selinsing Gold Mine) using two different culturing media (LB and CDM with 2mM As (III) or 5 mM As (V). Furthermore, this study was proposed a suitable conventional pre-treatment technology of indigenous arsenic resistant bacterial biomass to increase arsenic removal efficiency from wastewater and regenerate the biomass. Moreover, this study is determined the efficacy of individual and mixed dried biomass of three strains WS3, WS9 and WS11 in the removal of As (III) and As (V). The present study aimed to evaluate the isolated indigenous biomass in order to remove As (III) and As (V) through experimental and theoretical (mathematical modelling) studies.

In the present study, the ANN model mimicked the function of the human brain, which has billions of neurons. These neurons are connected to each other through pathways that transmit electronic signals. These connections enable the neurons to send or receive electrical impulses, which in turn are responsible for the brain function. Likewise, ANN has the capability of mapping inputs and outputs professionally. The ANN consists of an input layer, a hidden middle layer, and an output layer. The hidden and output layers are composed of computational nodes called neurons, and one-layer neurons are connected to the neurons of the preceding layer by means of weights, which regulate the connection between two neurons. The neurons use differentiated activation functions to generate output by transferring weighted input from the previous layer (Prasenjit *et al.*, 2012). The inputs included contact time, arsenic concentration, pH, temperature and adsorbent dosage. On the contrary, the output of the model was the predicted removal of arsenic (ppm). MATLAB2017b function was utilized to create the model. The process of the model was categorized into three steps, which were training, testing, and validation. In addition, the model was also verified with experimental data to evaluate the outcome vector using statistics indicators such as mean square error and correlation coefficient. In addition, Isotherm, kinetic and thermodynamic studies were applied to analyse the mechanisms of arsenic adsorption. The biomass of indigenous arsenic resistant bacteria was characterized before and after arsenic adsorption by using FESEM–EDAX and FTIR analyses.

1.6 Significance of the Study

Currently, the metagenomics method is regarded as the most efficient, reliable, rapid and accurate way to reveal the entire microbial composition of a community under complex environment conditions. Also, culture dependent method is used to complement the microbial biodiversity and this extends our knowledge of microbial diversity in a gold mining environment. A deep and direct insight into the soil biodiversity and microbial community and its functions can be investigated by using culture independent and dependent methods. In addition, employment of culture based approach with metagenomics analysis helps to know the biodiversity of microbes from Malaysian gold mining environment and isolate indigenous arsenic resistant microbes and their potential use in bioremediation of arsenic contaminated sites.

Until now a few reports have been put forward studying water, sediment, contaminated environments with relatively different concentrations of arsenic by both methods (Luo, *et al.*, 2014). Therefore, an alternative technique to remove arsenic from wastewater is in demand. Considerable efforts have been devoted to overcoming this serious issue by using individual and mixed dried biomass of indigenous arsenic resistant microbial as an adsorbent to remove arsenic. The biomass is distinguished by its low cost and high capacities. Furthermore, a majority of previous research focused primarily on laboratory and experimental works and they suffer from a lack of modeling in order to accurately predict the experimental behaviour of As (III) and As (V) removal by biomass. Moreover, the experimental results were compared with a model to find the validity of the experimental adsorption results.

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LIST OF PUBLICATIONS

1) Altowayti, W.A.H., Algaifi, H.A., Bakar, S.A. and Shahir, S., 2019. **The adsorptive removal of As (III) using biomass of arsenic resistant *Bacillus thuringiensis* strain WS3: Characteristics and modelling studies.** *Ecotoxicology and environmental safety*, *172*, pp.176-185.
2) Altowayti, W.A.H and Shahir, S., 2018. **The Effect of Acid-Washing Pretreatment on Arsenic Removal by Dried Biomass of Indigenous Arsenic Resistant Bacteria**. THE INTERNATIONAL CONFERENCE ON GLOBAL & EMERGING TRENDS 2018 (ICGET) held on 2nd – 4th May 2018 at the Baze University, Abuja, Nigeria.
3) Altowayti, W.A.H, Dahawi, A.A., and Shahir, S., (**Significance of Bio-treatment by Acid Washing for Enlargement of Arsenic Desorption in Indigenous Arsenic-Resistant Bacteria from Gold Mine**) *Malaysian Journal of Fundamental and Applied Sciences* (Accepted)

JOURNAL UNDER REVIEW/COMMUNICATED:

1) Altowayti, W.A.H, Almoalemi, H., and Shahir, S., (Isolation And Identification Of Indigenous Arsenic Resistant Microbes From Arsenic-Rich Mine Tailings Using Culture-Independent And Dependent Approaches)
2) Altowayti, W.A.H, Haris, S. A., Shahir, S., Zakaria, Z., Ibrahim, S. (Removal of As (III) and As (V) By Using Mixed Dried Biomass of Three Indigenous Arsenic Resistant Microbes Isolated From Gold Mining Environment)