ISOLATION AND IDENTIFICATION OF SELENATE RESISTANT BACTERIA (SeO_4^{2-}) FROM SEDIMENT MANGROVE BANYUGLUGUR BEACH OF SITUBONDO DISTRICT, EAST JAVA

THESIS

By:
MALIK NURIS SU'AIDI
NIM. 15620077

BIOLOGY DEPARTMENT
FACULTY OF SCIENCE AND TECHNOLOGY
STATE ISLAMIC UNIVERSITIES MAULANA MALIK IBRAHIM
MALANG
2019

ISOLATION AND IDENTIFICATION OF SELENATE RESISTANT BACTERIA (SeO_4^{2-}) FROM SEDIMENT MANGROVE BANYUGLUGUR BEACH OF SITUBONDO DISTRICT, EAST JAVA

THESIS

By:
MALIK NURIS SU'AIDI
NIM 15620077

Presented to:
Faculty of Science and Technology
State Islamic University Maulana Malik Ibrahim Malang
in partial to fulfillment of the requirements for degree of Sarjana Sains (S.Si)

BIOLOGY DEPARTMENT
FACULTY OF SCIENCE AND TECHNOLOGY
ISLAMIC STATE UNIVERSITY MAULANA MALIK IBRAHIM
MALANG
2019

ISOLATION AND IDENTIFICATION OF SELENATE RESISTANT BACTERIA (SeO_4^{2-}) FROM SEDIMENT MANGROVE BANYUGLUGUR BEACH OF SITUBONDO DISTRICT, EAST JAVA

THESIS

By:
MALIK NURIS SU'AIDI
NIM: 15620077

Has been examined and approved by thesis advisor for testing:
Date: December 12, 2019

Advisor,	Religious Advisor,
Romaidi, M.Si., D.Sc	**M. Mukhlis Fahruddin, M.S.I**
NIP.19810201 200901 1 019	**NIPT.20142011409**

Acknowledged,
The Head of Biology Department

Romaidi, M.Si., D.Sc
NIP.19810201 200901 1 019

ISOLATION AND IDENTIFICATION OF SELENATE RESISTANT BACTERIA (SeO_4^{2-}) FROM SEDIMENT MANGROVE BANYUGLUGUR BEACH OF SITUBONDO DISTRICT, EAST JAVA

THESIS

By:
MALIK NURIS SU'AIDI
NIM: 15620077

has been maintained
in front of the Thesis Examination Board and declared accepted as one of the requirements to obtain a Sarjana Sains (S.Si) degree

Date: December 12, 2019

Main Examiner	Dr. Hj. Ulfah Utami, M.Si NIP.19650509 199903 2 002	
Chief examiner	Bayu Agung Prahardika, M.Si NIP.19900807 201903 1 011	
Secretary of examiners	Romaidi, M.Si., D.Sc NIP.19810201 200901 1 019	
Member of examiner	M. Mukhlis Fahruddin, M.S.I NIPT.20142011409	

Acknowledged,
The Head of Biology Department

Romaidi, M.Si., D.Sc
NIP.19810201 200901 1 019

STATEMENT OF AUTHENTITY

The undersigned:

Name	:	Malik Nuris Su'aidi
NIM	:	15620077
Fakulty/Department	:	Science and Technology / Biology
Research Title	:	Isolation and Identification of Selenate Resistant Bacteria (SeO_4^{2-}) from Sediment Mangrove Banyuglugur Beach of Situbondo District, East Java

Certify that the thesis I wrote to fulfill the requitment for the degree of sarjan sains in Biology Department Faculty of Science and Technology, Universitas Islam Negeri Maulana Malik Ibrahim Malang, is truly my original work. It does not contain any thaterial previously written or published by other person, except that indicated theory, quotation and bibliography. Due to this fact, I am the only person responsible for the thesis if any objection or claim for others.

Malang, December 26, 2019
Author,

METERAI TEMPEL
71921AHF126170240
6000
ENAM RIBU RUPIAH

Malik Nuris Su'aidi
NIM 15620077

THESIS USAGE GUIDELINES

DEDICATION

My deepest gratitude only goes to King of the Kings, Almighty God, for giving me a chance to complete this thesis. I proudly dedicate this thesis to my beloved parents, Abah H. Munairi Ikhsan dan Umi Hj. Yus Anna which is endlessly encouraging, praying, giving advice and material to me. Prayers in every prostration of Abah Umi prayers, who don't forget to mention the names of your three children, may Allah SWT reward your children for success and may Allah SWT give Jannah to Abah and Umi later. Thank you to brother and sister, who also always provide support to his last younger brother.

FOREWORD

Assalamu'alaikum Wr. Wb.

My deepest gratitude only goes to King of the Kings, Almighty God, for giving me, can complete this thesis entitled "**Isolation and Identification of Resistant Selenate Bacteria (SeO_4^{2-}) of the Mangrove Sediment of Banyuglugur Beach, Situbondo Distric, East Java**". Prayers and greetings do not forget to pray to the great Prophet Muhammad who guided the path of *rahmatal lil alamin*.

The preparation of this thesis is certainly not able to be completed if there is no guidance, advice, advice, support and prayers from various parties. Speech Thank you for the writer to convey to:

1. Prof. Dr. H. Abd. Haris, M. Ag. as Chancellor of Maulana Malik Ibrahim State Islamic University of Malang.
2. My beloved parents Abah H. Munairi Iksan and Umi Hj. Yus Anna that always supported me and never stopped praying for my success.
3. Dr. Sri Harini, M.Si as Dean of the Faculty of Science and Technology of the State Islamic University of Maulana Malik Ibrahim Malang.
4. Romaidi, M.Sc., D.Sc., as Chair of the Department of Biology, Faculty of Science and Technology of the State Islamic University of Maulana Malik Ibrahim Malang as well as a supervisor who has been patient to provide guidance and direction to the author in the process of preparing this thesis.
5. Mrs. Dr. Hj Ulfah Utami, M.Si., as the first lecturer examiners thesis and Mr. Bayu Agung Prahardika, M.Si. as the second lecturer examiners who have given many directions, criticisms, and suggestions to the author in this thesis research.
6. Mr. M. Mukhlis Fahruddin, M.S.I as a religious examiner who has patiently provided advice, advice and criticism for the smooth preparation of this thesis.
7. All lecturers, Laboratory assistant, staff in the department of biology that helps provide ease, thanks on the science that has been given for less over 4 years of this.
8. All my friends, especially for Genetist (Biology 2015) who support me when I do my thesis. Thanks for being a new family in the city of Malang.
9. My Friends Biology C class 2015, a team of Microbiology (Atik, Devi, Septian, Ina, Anita), Reshma, Mas Hari, all my friends from physiology of animals Laboratory, thank you so have to accompany, support, entertain, and help me in the process of writing a thesis. We wish success to be the best gift for you later.
10. Biology members 2016, 2017 and 2018. thank you for your support, I hope, this research can provide benefits for you all.
11. Muhammad Zakaria Alwi, Farah Nahdia Noor, Sakhou Shofi, and Nuri Thobibatus Shofia Alfaruqi, which always remind and accompany eat up the process of writing the thesis of this.

12. All friends of writers who are not able to mention one by one, hopefully all the support you guys and prayer become intermediaries succeed in the world and the hereafter.

The author realizes that in the preparation of the thesis is still there is a shortage and the authors hope hopefully thesis is biased provide benefit to the reader in particular to the author in private. *Amiin ya Rabbal Alamiin*...

Wassalamu'alaikum Wr. Wb.

Malang, December 12, 2019

Author

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLE

LIST OF APPENDIX

Isolation and Identification of Selenate Resistant Bacteria (SeO_4^{2-}) from Mangrove Sediment of Banyuglugur Beach, Situbondo Distric, East Java.

Malik Nuris Su'aidi, Romaidi, M. Mukhlis Fahruddin

ABSTRACT

Pollution is a serious problem that must be faced by the global community. In addition to land pollution, water pollution also needs to be a serious concern. This is because the waters that have a very wide area and a variety of residual activities may be wasted in the waters. Among the pollution of the aquatic environment is the existence of mining and disposal of waste to the sea. Selenium is a metal element that is one source of pollution due to mining. Selenium can be toxic to Selenate (VI) and can accumulate so that it has the potential to threaten human health. Therefore, bacteria are used as bioremediation agents against selenate contamination. The media used were yeast agar extract (YMEA) with the addition of sodium selenate (Na_2SeO_4) concentration of 0 mM; 1 mM, 2 mM, 5 mM; and 10 mM. The methods in this study include bacterial isolation, isolate resistance testing, bacterial DNA isolation, and sequencing. Bacterial isolates which have the ability in selenate resistance were analyzed molecularly based on 16S rRNA genes and observed growth characteristics. The results found 2 bacteria that can be resistant to selenate by showing red in the media. The results of sequencing analyzed with phylogenic trees obtained isolate A-5-1 had genetic proximity of 57% with isolate B-10-1 and the distance between them was 0.201.

Keywords: Isolation, Resistant Bacteria, Selenate, Mangrove Sediment.

Isolasi dan Identifikasi Bakteri Resisten Selenate (SeO_4^{2-}) dari Sedimen Mangrove Pantai Banyuglugur Kabupaten Situbondo, Jawa Timur.

Malik Nuris Su'aidi, Romaidi, M. Mukhlis Fahruddin

ABSTRAK

Pencemaran menjadi masalah serius yang harus dihadapi oleh masyarakat global. Selain pencemaran di darat, pencemaran di perairan juga perlu menjadi perhatian serius. Hal ini dikarenakan perairan yang memiliki Kawasan yang sangat luas dan berbagai residu kegiatan memungkinkan akan terbuang di dalam perairan. Diantara pencemaran terhadap lingkungan perairan adalah adanya penambangan dan pembuangan limbah terhadap laut. Selenium merupakan unsur logam yang menjadi salah satu sumber cemaran akibat adanya penambangan. Selenium dapat bersifat toksik menjadi Selenate (VI), dan dapat terakumulasi sehingga berpeluang mengancam kesehatan manusia. Oleh karena itu, digunakan bakteri sebagai agen bioremidiasi terhadap kontaminasi selenate. Media yang digunakan adalah *yeast media ekstrak agar* (YMEA) dengan penambahan natrium selenate (Na_2SeO_4) konsentrasi 0 mM; 1 mM, 2 mM, 5 mM; dan 10 mM. Metode dalam penelitian ini meliputi isolasi bakteri, uji resistensi isolat, isolasi DNA bakteri, dan sekuensing. Isolat bakteri yang memiliki kemampuan dalam resistensi selenate dianalisis secara molekuler berdasarkan gen 16S rRNA dan pengamatan karakteristik pertumbuhan. Hasil penelitian didapatkan 2 bakteri yang dapat resisten selenate dengan menunjukkan warna merah pada media. Hasil *squencing* yang di analisis dengan pohon filogenik diperoleh isolat A-5-1 memilik jarak kedekatan genetik 57% dengan isolat B-10-1 dan jarak kekerabatan antar keduanya sebesar 0,201.

Kata kunci: Isolasi, Bakteri Resisten, Selenate, Sedimen Mangrove

العزلة وتحديد البكتيريا لمقاوم السيلينات (SeO_4^{2-}) من رواسب المانغروف لشاطئ بانيولوغور سيتوبوندو

مالك نور السعيدي، رميدي، مخلص فخر الدين

ملخص البحث

التلوث هو مشكلة جدا التى تجب أن تواجهها لمجتمع العالمي. بالإضافة إلى تلوث الأرض، يحتاج تلوث المياه أيضًا لأن يكون اهتمام جاد. وذلك لأن المياه التي لديها مساحة واسعة جدا ومجموعة متنوعة من الأنشطة المتبقية قد تضيع في المياه. واحد من انواع تلوث البيئة المائية هو وجود التعدين والتخلص من النفايات في البحر. السيلينيوم عنصر معدني الذى يعد أحد مصادر التلوث بسبب التعدين. يمكن أن يكون السيلينيوم سامًا الى السيلينات (VI) ، ويمكن أن يتراكم حتى يتسنى ان يهدد صحة الإنسان. لذلك، استخدم البكتيريا كعوامل المعالجة الحيوية على تلوث سيلينات. الوسائط هي استخراج أجار (YMEA) مع إضافة نتريوم سيلينات (Na_2SeO_4) لتركيز 0 مم ؛ 1 مم، 2 مم، 5 مم؛ و 10 مم. الطرق هذا البحث العزلة البكتيرية ، اختبار العزلة المقاومة ، العزلة الحمض النووي البكتيريا، والتسلسل. حللت العزلة البكتيرية التي لها القدرة على مقاومة سيلينيت جزيئيًا بناء على جينات rRNA 16S وملاحظة الخصائص النمو. وجدت النتائج 2 بكتيريا يمكنان أن يتقاوما السيلينات من خلال إظهار اللون الأحمر في الوسط. كانت نتائج التسلسل التي حللتها باستخدام الأشجار التطورية حصلت عليها العزلة A-5-1 لها مسافة القرب الوراثية بنسبة 57٪ مع العزلة B-10-1 ومسافة القرب بينها هي 0.201.

الكلمات الرئيسية: العزلة، البكتيريا المقاوم، سيلينات ، رواسب المانغروف

CHAPTER I
INTRODUCTION

1.1 Background of Research

Environmental pollution continues to be a global concern and is one of the major challenges faced by global society. Pollution can be in the form of natural compounds which when in contact with the environment cause adverse changes. There are various types of pollution, namely inorganic, organic, and biological (Masindi, 2018). Regardless of the type of pollution, it receives considerable attention due to the impact they have on the environment. Among all environmental contaminants, heavy metals receive important attention by environmental chemists because of their toxic nature. Heavy metals usually exist in small amounts in natural waters but many of them are poisonous even at very low concentrations. Metals such as arsenic, lead, cadmium, nickel, mercury, chromium, cobalt, zinc, and selenium are highly toxic even in small amounts (Salomons, et al., 1995). Heavy metals become toxic when not metabolized by the body and accumulate in body tissues (Herawati, et al., 2000; He, et al., 2005).

Heavy metals can come from natural and anthropogenic processes and end up in different environmental compartments (earth, water, air). Many studies have reported various natural sources of heavy metals, including volcanic eruptions, sea salt sprays, forest fires, rock weathering, biogenic sources, and wind-borne soil particles. The natural weathering process can cause the release of metals from their endemic spheres to different environmental compartments. Heavy metals can be found in the form of hydroxides, oxides, sulfides, sulfates, phosphates, silicates, and organic compounds. The most common heavy metals are lead (Pb),

nickel (Ni), chromium (Cr), cadmium (Cd), arsenic (As), mercury (Hg), zinc (Zn) and copper (Cu) (Herawati, et al 2000).

Selenium (Se) is an element that has semi-metallic properties and has a variety of forms that are naturally present and are widespread, such as in rocks and soil (Yunita, 2018). Selenium is found in two forms, namely inorganic form, and organic form. The inorganic form of selenium is selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}), and selenium in its organic form is selenomethionine and selenocysteine (Sunde, 2006). According to Nancharaiah and Lens, (2015) Selenate (VI) is a form of selenium that has a higher oxidation number than selenite (IV). Jorge et al., (2008) added that the higher oxidation number in selenium causes a high level of toxicity so that when in the environment it can be more dangerous than Selenite (IV).

Conditions with higher toxicity than selenium cause selenate (VI) to be one source of pollution to the environment. Several things cause the source of selenium pollution in nature. Risher et al., (2001) explain Se in the environment can be sourced from natural geological processes such as volcanic eruptions which cause the release of selenium into the air and can accumulate in the soil and also on the surface of the water. According to Goldhaber (2003), Mishra et al., (2011), and Combs (1998) Selenium originates mostly from activities in the electronics field. Selenium can also be found in the glass, plastic, paint, ink, and rubber industries and the manufacture of medicines (nutritional feed additives for poultry and livestock). Ike et al., (2000) and Javed, et al., (2016) explain, Se is also used in the production of pigments, pesticides, and stainless steel and is found in the results of waste tanning production and is no exception to power

plants contributing to the presence of metals selenium in aquatic environments. This can increase the amount of selenium which, if accumulated, will hurt the environment and marine life.

Relation to pollution in the environment, the actual damage or pollution that occurs is caused by human actions. They are more concerned with personal matters than the benefit of the people or care for the environment. In Al-Quran surah Ar-Rum verse 41, Allah says:

ظَهَرَ الْفَسَادُ فِي الْبَرِّ وَالْبَحْرِ بِمَا كَسَبَتْ أَيْدِي النَّاسِ لِيُذِيقَهُمْ بَعْضَ الَّذِي عَمِلُوا لَعَلَّهُمْ يَرْجِعُونَ

Meaning: "It has been seen that damage on land and at sea is caused by the deeds of human hands so that Allah feels to them part of their actions so that they return (to the right path)"

The position of humans as khalifah on earth has a mandate that is very important to prevent damage on earth. The word (الْفَسَادُ) *al-fasad* according to *al-Ashfahani* is the release of something from the balance, either a little or a lot. The word used shows anything, whether physical, spiritual, or other things. The verse also implies that the damage caused is an imbalance and the reduced value of benefits from land or water. The sea has been polluted, the land feels increasingly hot due to prolonged drought is one example of damage caused by human activity. Allah has created the universe in a very harmonious system and accordance with human life. But they do bad activities that interfere with damage so that the natural imbalance occurs in the natural work system (Shihab, 2002).

An element can be harmful to the human body if the levels exceed the normally acceptable threshold. According to Notodarmojo (2005), Se can also be toxic in the human body if it is accumulated or consumed excessively. Some problems that can arise because of excessive consumption of Se, for example,

disorders of the respiratory system because of inhaling Se, as well as disorders of the skin, and can also cause the cardiovascular symptom. Lemly (2004), explains that Se in high levels can be toxic and very toxic to the body of animals and plants when Se in the form of dissolved oxyanion. The chronic influence of selenium exposure will also impact selenosis, neurotoxicity, and can also disrupt the endocrine function of processed thyroid hormone synthesis (Vincenti, et al., 2014).

The emergence of selenium heavy metals in the environment of human life is one example of human attitudes that utilize natural resources on earth by not thinking about things that will then occur in the long run. According to Kozdroj and van Elsas (2001), human activities have polluted the environment with toxic heavy metals and metalloids during the last 200 years and, have caused severe disruption of the ecological balance in most ecosystems, including in the coastal environment. One beach is likely to be affected by selenium heavy metal pollution at a level of +6 (selenate) is Banyuglugur Beach, Situbondo Regency. The condition of the coast directly adjacent to unit III Paiton Steam Power Plant (PLTU) is an indicator of the likelihood of potential contamination of particles suspended in the waters. In addition to this, the existence of coal barges transporting the results of mining around the coast is also possible to be a source of selenate waste pollution in waters and coastal sediments.

Dense middle sea mining activities and shipping activities of coal carriers and natural geological activities are also likely to have an impact on the mangrove area around the coast. Mangrove area is a border between waters and land that allows all forms of residues or residual results of industrial activities and mining

carried by sea currents will be left around mangroves, both on plants and mangrove sediments. The residues can take various forms, including the selenium heavy metal waste originating from the activities of the community around the coast carried by the waves and factory effluents in various areas which are also responsible for selenium contamination in the water.

The presence of suspended particles of heavy metals in coastal waters causes toxic contamination, both in the environment and in biota. Efforts to reduce the effects of Se contamination is to do remediation to reduce adverse effects on organisms. According to Liu et al., (2015) several remediation methods include physical, chemical and biological (bioremediation). Of the three methods, bioremediation is the safest method and does not cause negative effects to be used in remediating polluted environmental conditions. Also, in terms of cost, this method is more efficient because it uses living things as agents. Bioremediation is generally used as a method to reduce toxicity to heavy metals, including selenium. Bioremediation of selenium can be done by using intermediaries of various types of macro-organisms (plants), and also microorganisms, such as fungi and bacteria (Srinivas, 2008).

Research on bacteria as a remediation agent for selenium is not widely studied. Research on bacteria that can be resistant and can reduce the level of selenium toxicity has been found in soils and freshwater. Some examples of bacteria found include *Ralstonia metallidurans* CH-34 in zinc plant sediments in Belgium (Roux et al., 2001), *Shewanella oneidensis* found in river sediments (Klonowska and Vermeglio, 2005), *Pseudomonas stutzeri* and P. *flourecens* found in Japanese river waters (Ike et al., 2000), P. *putida* KT2440 isolated from

selenium-contaminated drainage water in San Joaquin Valley, California (Avendano, 2016), *Desulfovibrio dessulfuricans* (Tomei et al., 1995). While bacteria isolated from marine waters are not commonly found, especially those that can reduce and accumulate high selenium levels or selenate (SeO_4^{2-}). While some bacteria in the mangrove environment are still very limited. Therefore, this research is focused to find bacteria that can be resistant and is thought to be able to accumulate selenium at a high level of toxicity (SeO_4^{2-}) from the mangrove sediments of Banyuglugur Beach, Situbondo Regency, which was obtained from macroscopic observation up to the molecular stage.

1.2 Problem of Research

Based on the background above, this study has the following problem formulation:

1. What types of bacteria can resist selenate in mangrove sediments of Banyuglugur Beach, Situbondo Regency based on morphological and molecular characteristics?
2. What are the characteristics of the growth of resistant selenate bacteria found in the mangrove sediments of Banyuglugur Beach, Situbondo Regency in the selenate media?

1.3 Research Objectives

The aim of this research is:

1. Determine the types of bacteria that can resist selenate in mangrove sediments in Banyuglugur Beach, Situbondo Regency based on morphological and molecular characteristics.
2. Knowing the growth characteristics of selenate resistant bacteria found in the mangrove sediments of Banyuglugur Beach, Situbondo Regency in the media selenate.

1.4 Limitation Problems

Limitation problems from this research are:

1. The location of sampling is conducted in the mangrove sediment area of Banyuglugur Beach, Situbondo Regency.
2. Samples are taken from the surface of the sediment around the mangrove location.
3. Determination of sediment sampling stations obtained from 3 points.
4. The concentration of sodium selenate used in this test is 0 mM, 1 mM, 2 mM, 5 mM, and 10 mM.
5. Bacterial growth media using YMEA (Yeast Malt Extract Agar), YMEB (Yeast Malt Extract Broth) containing sodium selenate (Na_2SeO^4).
6. The technique used in isolating bacteria by multilevel dilution is inoculated on the YMEA solid media using a streak plate.

7. Techniques in analyzing bacteria using macroscopic identification, gram paint, and molecular identification of 16s rRNA and phylogenic identification.

1.5 Benefits of Research

The benefits of this research are:

1. Provide information about the types of mangrove sedimentation of Banyuglugur Beach, Situbondo Regency which can be resistant to selenate metal.
2. Provide information about the role of resistant bacteria and accumulators in overcoming selenium contamination in marine ecosystems.
3. To inspire further research on resistant bacteria and accumulators from marine waters, especially in mangrove sediments.

CHAPTER II
REVIEW OF RELATED LITERATURE

2.1 Selenium

Selenium is a chemical element that has an atomic number 34 and has an atomic mass of 78.96 g/mol. Selenium is located in group 16, between the elements sulfur and tellurium and between arsenic and bromine in Period 4. Devi *et al*., (2017) describes the elements of selenium, also called the chalcogen group. Apart from being in the group to VIA, selenium can form sulfide (H_2S). Hydrogen sulfide has a colourless, highly toxic, and odiferous gas.

Selenium has four natural oxidation states, -2, 0, +4, and +6 (Butter *et al*., 2012). In oxidation (0) consists of selenium elemental (Se 0); oxidation (-2) consists of sodium selenide (Na_2Se) and hydrogen selenide (H_2Se); oxidation number (+4) consists of sodium selenite (Na_2SeO^3), selenium dioxide (SeO_2) and selenious acid (H_2SeO_3); oxidation number (+6) consists of sodium selenate (Na_2-SeO_4), and selenate acid (H_2SeO_4) (Dummont, 2006). Stadtman (1990) and Stolz *et al*., (2006) explain that the element selenium is a metalloid element which is a trait between metals and non-metals.

In general, selenium is divided into 2 forms, namely in organic form and inorganic form. The organic form of selenium binds to protein as an amino acid that can be found in the body in the form of selenocysteine and selenomethionine (Dilaga, 1992; Budiyanto, 2014). Meanwhile, according to Johanson *et al*., (2005) explains the inorganic form, selenium consists of selenide (Se^{2-}) and selenite (SeO_3^{2-}). The organic element selenium is generally found in the form of selenoprotein in the form of L-selenomethionine which is methionine whose

sulfur group is replaced by selenium. According to Nuttal (2006), some forms of organic selenium found in nature include selenocysteine, selenosis, selenosistin, selenomethionine, and selenodiglutation. According to Vincenti, *et al*., (2014) selenium in the form of selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) is the most dangerous and very toxic element for humans due to its oxidation number.

Selenium can be very easily absorbed indigestion. Selenium enters the body through foods containing selenium, for example, beans, meat and eggs. According to Sunde *et al*., (2012), seafood and meat organs are the richest food sources of selenium. While other food sources of selenium also include fibrous meat, cereals and grains, and can also be found in dairy products. Risher *et al*., (2001), the human body easily absorbs organic selenium compounds (for example, selenoamino acid).

Selenium can also enter the body through drinking water in the form of sodium selenate and inorganic sodium. However, the amount of selenium in drinking water is not nutritionally significant. Based on 2009-2010 data analysis conducted by the National Health and Nutrition Examination Survey (NHANES), the average daily selenium intake in the United States aged 2 years and over is 108.5 mcg obtained from food and 120.8 mcg originating from supplements (US Department of Agriculture). Adult men have a higher daily intake (134 mcg from food and 151 mcg from supplements) than adult women (93 mcg from food and 108 mcg from and supplements). About 18% to 19% of adults and children in America use dietary supplements containing selenium (Bailey *et al*., 2011).

2.2 Selenium Sources

Selenium is part of the element of chalcogen (VI A) which is not abundant in the earth's crust as other calcogenic elements, such as sulfur and Tellurium (Staicu, 2017). Naturally, as an element, selenium cannot be made or destroyed, although it can change shape in the environment. The appearance of selenium is divided into 2 namely naturally occurring and anthropogenic emergence. Selenium naturally arises from volcanic activity, rock weathering and soil sediments, and biogeochemical activities. While anthropogenically, industrial activities are the biggest contributor to the emergence of selenium in the environment, including water. Some industrial activities that are sources of the presence of selenium in the environment include the process of making rubber, glass, manufacturing electrical components, and fuel use. Selenium also arises from activities in agriculture, such as the use of pesticides, soil fertilizers, and phosphate mixtures (Eswayah, 2018: Triana, 2010; Haygart, 1994; Gebreyessus, 2019). According to Budiyanto (2014), the main source of the emergence of selenium in nature is derived from rocks that are rich in carbon and organic materials. Besides coal, gasoline also has a high amount of selenium (Luoma and Rainbow, 2008).

The largest selenium reservoir on Earth is sulfide ore, pyrite and high-sulfur coal. Geological and anthropogenic sources release selenium as SeO_4^{2-} into the environment (Figure 2.1). Selenium, an important element, is assimilated from selenate or selenite by microbes and plants at the bottom of the food web and subsequently by animals. Selenium is then assimilated into organoselenides (Selenoprotein) in living organisms. Decomposition of dead organisms releases

selenium back to the environment. Mining activities, burning of fossil fuels, agriculture, volcanic eruptions, and nuclear fuel activities release selenium into the atmosphere, soil and water-insoluble forms (SeO_4^{2-} and SeO_3^{2-}). Microorganisms play an important role in the cycle of selenium compounds in nature (Nancharaiah and Lens, 2015).

Selenium particles, such as ash, can settle to the ground or surface water. Disposal of selenium in commercial products and waste can also increase the amount of selenium in the soil. The form of selenium in soils is very dependent on the acidity of the environment and its interaction with oxygen. Areas with locations near volcanic mountains become very high concentrations of selenium, starting from 50-90 µg / kg and even higher biases (Gebreyassus, 2019).

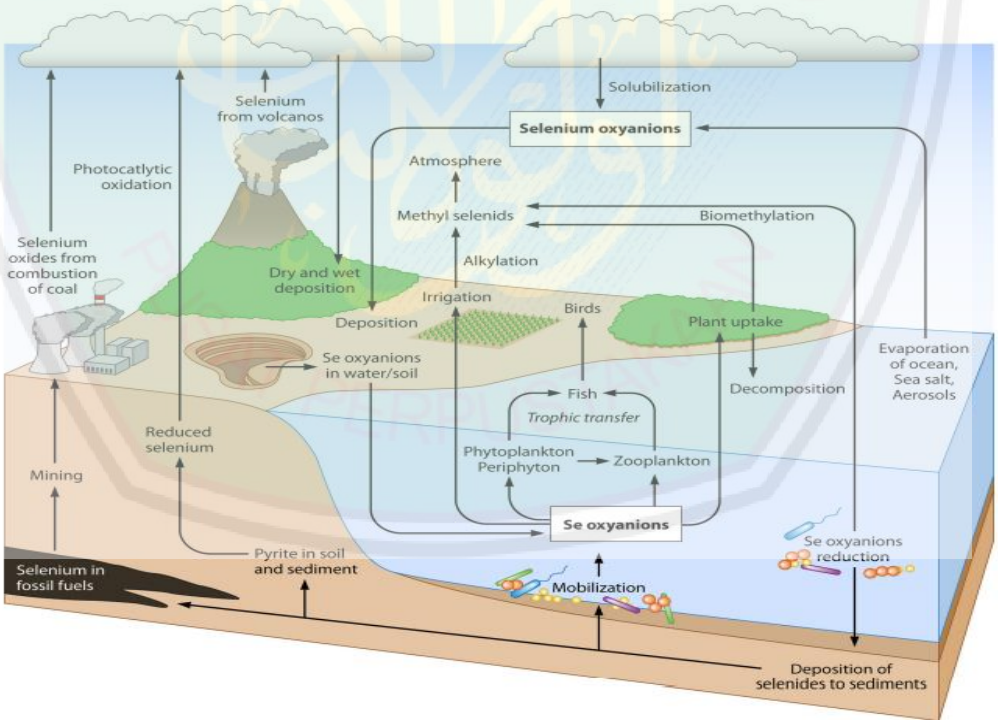


Figure 2.1 The selenium cycle globally in nature. (Nancharaiah and Lens, 2015).

The presence of selenium in the environment is strongly influenced by the degree of oxidation and differences due to the behaviour of different chemical compounds (EPA, 1979; NAS, 1976). The state of selenium oxidation in the environment depends also on the surrounding conditions, especially on pH, pE, and biological activity (Maier *et al*., 1988). In addition to being present in the environment resulting from anthropogenic activities and residues from industrial activities, the element selenium can also be found in natural food sources, such as in fruits, nuts and milk (Table 2.1).

Table 2.1 Concentrations of Selenium in Several Food Sources in the United States (mg selenium/kg, wet weight) (Schubert *et al*., 1987; Secor and Lisk, 1989).

Food Types	Average	Min	Max
Fruits and vegetables			
Apple, raw	0.004	0.003	0.006
Carrots, raw	0.017	0.006	0.029
Orange	0.015	0.013	0.018
Potato	0.013	0.004	0.023
Grains, nuts and cereals			
Bread, white	0.32	0.23	0.54
Bread, whole wheat	0.44	0.28	0.67
Corn cereal	0.063	0.026	0.12
Egg noodles, dry	0.66	0.43	1.35
Egg noodles, cooked	0.19	0.14	0.42
Brazil nut	14.7	0.20	253
Dairy products	0.016	0.011	0.025
Milk	0.083	0.062	0.10
Swiss cheese	0.060	0.052	0.068
Meat	0.21	0.17	0.26
Chicken, cooked	0.26	0.15	0.52
Beef, cooked	0.33	0.19	0.51
Seafood	0.75	0.31	1.49
Salmon, canned	0.64	0.21	1.61
Shrimp, canned/cooked	2.84	2.54	3.44

Selenium is photovoltaic, the ability to convert light into electricity. Selenium pollution on the surface of the water comes from several sources,

including from adjacent waters that may contain selenium, and from underwater drainage. Waste treatment plants are another source of release of selenium into water. Waste from sewage treatment plants and oil refineries is the main source of selenium in the San Francisco estuary system (Cutter 1989). Selenium was found to be released during coal mining because of the oxidation of selenium-bearing pyrite (Dreher and Finkelman 1992). WHO data (1971), noted that without the role of anthropogenic factors, the concentration of selenium in nature is very low and not even more than the ng-µg / L range. the spread of selenium contamination. According to Luoma and Rainbow (2008), selenium contamination is local and cannot be distributed as in the type of mercury (Hg).

2.3 Pollution and Toxicity Se

Contamination of heavy metals is a very important problem to be overcome. Although some regulations or policies govern and bind the government with the issuance of PP No. 82 of 2001 and LH Regulation No. 13 of 2010, it seems that there are still weak practices and supervision in the field, causing water quality and water ecosystems to decline. In PP No. 82 of 2001 has been regulated regarding water quality management and water pollution control, where the threshold of selenium is 0.01 mg/L for class 1 and 0.05 mg/L for classes 2.3 and 4 (Priadie, 2012).

Regarding selenium, Nuttal (2006) explains that the most important problem is that selenium is determined as its total concentration, even though various chemical forms of selenium have different toxic potentials on the environment and those living in them. In several recorded reports, serum selenium

concentrations cover the following ranges: 400-30,000 μg/L associated with acute toxicity, 500–1400 μg/L associated with chronic toxicity, and <1400 μg/L free of toxicity; the category is determined by the signs and symptoms in the patient. Most reports that describe acute selenium poisoning involve ingestion of inorganic compounds such as selenious acid, which is found in gun-turning agents, and deaths that occur on the first day are associated with postmortem blood selenium levels> 1400 μg/L. Tissue selenium levels show complex patterns and significant increases in organs such as the kidneys do not always indicate toxicity.

Dumont (2006) states that the element of selenium is one of 18 important trace elements in the body, especially in the metabolic process. Mertz (1981) explains that each trace element in an element can be toxic when the amount is high and the concentration exceeds the threshold. Several parameters determine the optimum value of a mineral; selenium is no exception.

A direct disposal study from an oil refinery in San Francisco found the average concentration of selenium in waste was 0.067 mg/L with a range of 0.0066-0.156 mg/L (Barceloux 1999; Cutter 1989). About 50-76% of the total in selenium waste is selenite (Cutter 1989). Around 150,000 - 460,000-tons of selenium per year are stored in the form of coal ash (Andren and Klein 1975; Doran 1982). Lemly (1985) notes that concentrations of 0.10-0.25 mg/L are found in sewage deposits from coal ash in North Carolina. The overflow from the ash bath from coal-fired power generation facilities to Lake Belews also produces selenium at the surface of the water with a concentration of 0.005-0.020 mg/L around the lake basin area. The peak Selenium concentration occurred in 1996,

around <0.001 mg/L (Lemly 1997). Selenium concentrations recorded as high as 0.28 mg/L for raw waste, 0.045 mg/L for primary waste, and 0.050 mg/L for secondary waste (Baird *et al*., 1972).

In addition to water, pollution and toxicity of selenium are also found in soils. The main factor controlling the concentration of selenium in the soil is the selenium content of the host rock material which releases selenium through weathering and washing (NAS 1976). The natural weathering process is estimated to release about 100,000-200,000 metric tons of selenium per year (Andren and Klein 1975). Selenium deposition in the atmosphere also contributes to selenium in the soil. In the past, selenium was used in pesticide products, but because of its stability in the soil and subsequent contamination of food crops, its use in pesticide products is now limited. The release of selenium into the soil from floating ash settling ponds and hazardous waste sites has not been widely recorded.

Gupta (2017) states that natural geological activity is a major factor in the case of poisoning of selenium in water. According to Grutzmacher, (2013), the impact of selenium pollution on the environment has an impact on organisms that live around it. Selenium poisoning generally occurs when ions in certain selenium in the soil or surface of the water are absorbed by living organisms and accumulate in the body of living things. Islam has provided such a detailed and explicit explanation through its two main sources (al-Quran and the hadith of the Prophet) regarding the phenomena or calamities that have befallen humans on earth.

Relation to the calamity that occurred on this earth, Allah SWT. It has been said in surah Ibrahim verse 7:

وَإِذْ تَأَذَّنَ رَبُّكُمْ لَئِنْ شَكَرْتُمْ لَأَزِيدَنَّكُمْ ۖ وَلَئِنْ كَفَرْتُمْ إِنَّ عَذَابِي لَشَدِيدٌ

It means: "And (remember), when your Lord informs:" If you are grateful, surely We will increase (your favors), and if you deny (my favors), verily my punishment will be very painful."

Disasters or disasters are punishments and curses from Allah to those who disbelieve in the favor of Allah SWT in a nature that is often destroyed without seeing its usefulness for life. According to Malik Madani (1997), it is the disobedience or human disbelief of the favor of Allah that has brought down the torment on the earth. The purpose of it all is to make people aware of their actions so that they will eventually return to God, the right path, a path that is always grateful for the enjoyment they receive from Allah Almighty. He emphasized that the damage referred to in the verse is the damage caused by the act of human hands, as well as acts of evil and other moral damages.

2.4 Mangrove

Mangrove or so-called mangrove forests are a coastal biosphere that covers about a quarter of the world's tropical coastline but is endangered. Mangrove has many ecological benefits, such as being the main source of terrestrial organic matter to the oceans, and being the focal point of biodiversity. Simões *et al.*, (2015) microbes are a key component of the biodiversity found in mangrove ecosystems, with 91% being bacteria and fungi of the total biomass that lives around mangrove.

Mangrove is a unique and ecologically important coastal habitat in the tropics and subtropics and is often regarded as a pioneer vegetation in coastal areas. Specific areas where mangroves grow are referred to as "mangrove forests" occupying several million hectares of coastline worldwide and distributed in more than 112 countries and territories (Alongi, 2002). Mangroves also survive in some climatic zones but there is a rapid decline in the number of species with increasing latitude (Bandaranayake, 1998). Several static and dynamic biologicals, physical, and chemical factors are known to affect the development and stability of mangrove communities. These factors and their interactions play an important role in the nutrient flow of the system and become necessary to understand the various processes that interact (Ravilkumar *et al*., 2002).

Research on biography, botany, zoology, environmental pollution and the economic impact of mangrove forests has been extensively conducted. But little is known about microbial activity in mangrove waters and sediments. Studies on microbial diversity in mangrove waters and sediments are needed to clarify the biochemical cycle and pollutant removal. All forms of microbes including bacteria, fungi, cyanobacteria as well as macrophages and microbes are found in this ecosystem (Dias *et al*., 2010). Comprehensive studies of Sen and Naskar (2003) have reported that the common group of mangrove bacteria include: sulfate reduction (*Desulfococcus, Desulfosarcina, Desulfotomaculum, Desulfovibrio* spp.), N2 (*Azospirillum, Azotobacter, Clostridium, Klebsiella, Rhizobium sphizobium*).), phosphate solvents (*Bacillus, Chryseomonas, Enterobacter, Kluyvera, Pseudomonas, Xanthobacter* spp.), photosynthetic

anoxygen (*Beggiatoa, Chloronema, Chromatium, Leucothiobacteria, Thiopedia* spp.) and metanogenic (*Methanoccoides bacteria*).

Other sources also suggest that various fungi such as ligninolytic, cellulolytic, pectinolytic, amylolytic and proteolytic fungal and actinomycetes are present in the mangrove ecosystem. It should be noted that microorganisms play an important role in the enzymatic production process. This is due to its high production capacity, low cost and vulnerability to genetic manipulation. (Quecine *et al*., 2011; Ferreira *et al*., 2012). Not much information is available about the biotechnological potential of mangrove microbiota. According to Thatoi *et al*., (2012), mangrove rhizosphere soil accommodates a population of microorganisms benefiting from large-scale agricultural applications. They can fix nitrogen, dissolve phosphate, produce ammonia and produce indole acetic acid.

Kathiresan and Selvan (2006) have shown that bacteria isolated from the mangrove environment are good candidates for increasing arid soil and reclamation soil. The real challenge to the benefit of halophilic species prevailing in the mangrove saline ecosystem, in addition to their biotechnological importance, is how far these salt-tolerant members can be used as an inoculum for field crops cultivated in salt-affected soils.

2.5 Bioremediation

The use of bioremediation in overcoming heavy metal contamination and lowering pollutant levels is nothing new. Bioremediation will address environmental concerns as a result of mining or industrial activities (Priadie, 2012). Bioremediation is the degradation of soil and groundwater that is

biologically controlled by the soil microbes to produce stable and harmless end products, such as CO^2 and H_2O (Srinivas, 2008). Bioremediation is an appropriate technique as a way to neutralize environmental conditions, by using biological agents as mediators (Waluyo, 2009).

The Indonesian government already has regulations in carrying out bioremediation to overcome and reduce pollution from mining and petroleum activities and also as a result of the use of pesticides. The Government through the Decree of the Minister of Environment No.128 of 2003, which regulates the procedures and technical requirements and management of petroleum and soil waste contaminated by petroleum biologically or also called Bioremediation (Priadie, 2012).

Soil and water conditions will form an important basis for bacteria (microscopic creatures) to develop. However, microbes need adequate nutrition which is possible to be lacking in waste as well as in the soil because the soil has varying fertility levels (maybe very fertile or completely infertile). Of all waste treatment processes, biological treatment is cheaper than physical and chemical processes as long as the waste can be biodegradable, more soluble in water, and non-toxic to microbes (Srinivas, 2008).

Wastewater from sewage stabilization ponds and leakage of gasoline from underground fuel storage tanks contribute to soil and groundwater pollution. Also, the explosion of chemical containers, accidental spills, agricultural waste rich in pesticides, oils, and cleaning solvents from garages are also responsible for soil and water contamination. Each industry contributes to industrial waste which adds to soil and groundwater pollution. Paint and varnish waste (from sanitary

landfills) contribute to nervous system disorders and heavy metal poisoning in addition to being carcinogenic, inseparable from the presence of heavy metal selenium in it.

Bioremediation is used as a human effort to treat all gifts from Allah SWT. In Surah Al-Baqarah verse 30 Allah SWT says:

وَإِذْ قَالَ رَبُّكَ لِلْمَلَائِكَةِ إِنِّي جَاعِلٌ فِي الْأَرْضِ خَلِيفَةً ۖ قَالُوا أَتَجْعَلُ فِيهَا مَنْ يُفْسِدُ فِيهَا وَيَسْفِكُ الدِّمَاءَ وَنَحْنُ نُسَبِّحُ بِحَمْدِكَ وَنُقَدِّسُ لَكَ ۖ قَالَ إِنِّي أَعْلَمُ مَا لَا تَعْلَمُونَ

It means: Remember the compilation of your Lord said to the angels, "Verily, I want to take the caliph on the earth" by praising You and purifying You! "The Lord said" Surely I know what you do not understand."

Allah SWT pays homage to His creatures, namely humans before angels before they were created. The word *Khilafah* (خليفة) originally had a substitute or substitute meaning. Some interpret this word as the caliph's meaning that Allah intends with the appointment as a test for humans and gives humans respect. This verse shows that the Caliphate consists of authority from Allah, the creature entrusted with the task, namely Adam as. and his children and grandchildren, and the area of their assignment, namely the spread of the earth (Shihab, 2002).

Relation to nature, humans are given the mandate by Allah SWT to preserve nature and care for the earth in its entirety so that in life there is a balance, between abiotic factors and biotic factors. Caring for nature by utilizing all that is there to make nature better is an attempt to carry out the task of being the most perfect creature.

Bioremediation is the conversion of potential pollutants from organic and natural inorganic soil and water into substances that are harmless by microorganisms. Because it depends on the type of bacteria as the agent responsible for degradation, bioremediation is classified as aerobic or anaerobic because of the presence of oxygen or free oxygen in the combined state during the process. Most biodegradation is aerobic because the anaerobic process is relatively slow and difficult to maintain for the bioremediation process. But it is preferred if the reduction is preferred over oxidation as in the chlorinated compound. Bioremediation, namely the decomposition of waste by soil bacteria that has been used successfully for the degradation of petroleum mud is now used for biodegradation of Benzene, Alcohol, Chlorophenol, pesticides and other hydrocarbons that were once considered resistant to biodegradation.

2.6 Utilization of Bacteria in Bioremediation

About the role of bacteria in the process of bioremediation, Allah has explained in Surah Yunus verse 101:

قُلِ انْظُرُوْا مَاذَا فِى السَّمٰوٰتِ وَ الْاَرْضِ ۗ وَ مَا تُغْنِى الْاٰيٰتُ وَ النُّذُرُ عَنْ قَوْمٍ لَّا يُؤْمِنُوْنَ

It means: "Say: 'See what is in the heavens and the earth! ' But neither signs nor warnings help the unbelievers."

Shihab (2002) explains this verse explaining for humans to pay close attention to and contemplate everything related to the earth and all things that are evidence of the majesty and the Oneness of God. In this verse also encourages humans to develop science through contemplation, experimentation and

observation. Also, it means to share knowledge related to the universe with its contents.

Bioremediation generally involves microscopic living things or called microorganisms, both fungi or bacteria as a tool to reduce the level of pollutants or the level of pollution at certain levels. The purpose of biological agents is that the structure of pollutants that can be toxic can be decomposed by involving enzymes produced (synthesized) by distributing microorganisms (Priade, 2012). The use of bacteria as a repair solution to cope with environmental pollution for the existence of heavy metal contamination. According to Neuhirl *et al.*, (2005), the use of microorganisms that can restore environmental conditions polluted from heavy metals, such as lead (Pb), copper (Cu), and mercury (Hg). While the selenium metal is still more mediated by hyperaccumulator plants.

The difference from bioremediation in environments polluted by plants and by bacteria located on the detoxification side, according to Staicu (2017), detoxification by plants on heavy metals using assimilators, while by bacteria using dissimilatory. The difference lies in the transfer process. In the process of dissimilation, there was an overhaul between SeO_4^{2-} or selenate composition to SeO_3^{2-} or selenite. In that process the ions that occur in the cell wall. While assimilation occurs in cells by involving enzymes to produce protein or selenoprotein and produce gas.

The degree of freedom of heavy metals depends on the natural heavy metal content of the living substance is special, even the thickness is 2-3 times that of normal. According to Yajid (2007), bacteria that can survive against metals have a

good response and support among, among others, the processes of bioaccumulation, bio-preticipation, methylation, and bioreduction.

2.7 Resistance and Accumulation Planning in Bacteria

Bacteria survive under metal pressure. Develop several types of relationships to tolerate. These mechanisms include removal of metal ions outside the cell, assembled and complex metal ions inside the cell, and reduction of heavy metal ions in an unavoidable direction (figure 2.2). In high concentrations, heavy metal ions make up the toxic composition in cells (Nies, 1999). Spain (2003) explains, to have a toxic effect, heavy metal ions must first enter the cell. Because some heavy metals are needed for enzymatic function and bacterial growth, there is protection that allows the entry of metal ions into the cell. According to Staicu and Barton (2017), bacterial metabolism in reducing selenium is passed as a dissimilation or decomposition reaction. The reaction in this is the selenate compound (SeO_4^{2-}) is broken down into elemental selenium (Se0) with selenium which is not suitable as an electron receiver and bacteria as an electron donor agent.

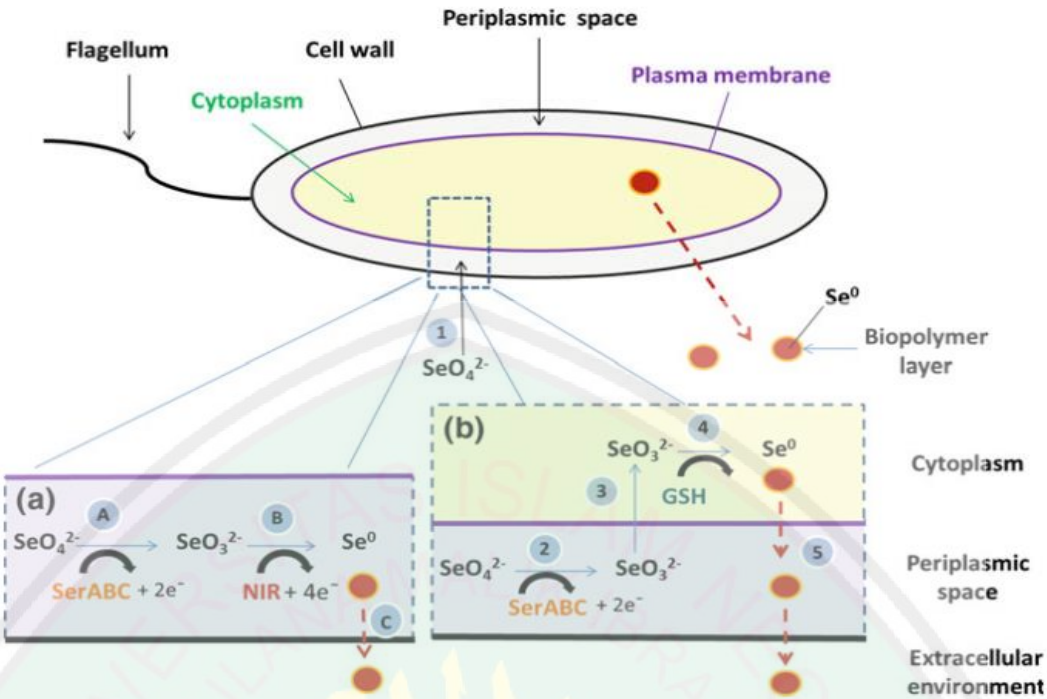


Figure 2.2 Selenium transformations in Thauera selenatis.a(1) Selenate enters the cell through thecell wall; (A) reduction of selenate to selenite in the periplasmic space, catalyzed by selenatereductase, SerABC; (B) reduction of selenite to elemental selenium in the periplasmic space,putatively catalyzed by nitrite reductase, NIR; (C) extrusion of elemental selenium coated with abiopolymer layer to the extracellular environment (Model according to Macy et al.1993);b(1)selenate enters the cell through the cell wall; (2) reduction of selenate to selenite in the periplasmicspace, catalyzed by selenate reductase, SerABC; (3) selenite transportation inside the cytoplasm;(4) reduction of selenite to elemental selenium mediated by thiols (Glutathione, GSH); (5)extrusion of elemental selenium coated with a biopolymer layer to the extracellular environment(Model according to Debieux et al.2011).

In general, the selenium reduction reaction is initiated by changing the selenate (SeO_4^{2-}) to selenite (SeO_3^{2-}) with the SerABC intermediate which is an enzyme that results from the secretion of the periplasmic membrane of the bacteria, namely selenite reductase with its role to help provide electrons for donation that occur in the membrane. periplasmic (Figure 2.2 (a)). While selenite compounds that have been formed will be reduced to selenium. SerABC acts as a

regulator of metabolism heme (type-b), elements of iron (Fe), sulfur (S), and molybdenum (Mo) that enter the cell (Dridge *et al*., 2007).

2.8 Molecular and Phylogenetic Identification Techniques

2.8.1 Isolation of DNA

DNA isolation is done to separate DNA from other materials such as protein, fat, and carbohydrates. The principles in DNA isolation are three: destructions (lysis), extraction or separation of DNA from solid materials such as cellulose and protein, and DNA purification. DNA isolation is the right step to study DNA. There are two principles, namely centrifugation and precipitation. Centrifugation is a technique for separating mixtures based on molecular weight. Molecules that have large molecular weight will be at the bottom of the tube and light molecules will be at the top of the tube (Abinawanto, 2011).

Faatih (2009) Cell lysis works by damaging cell walls and membranes aimed at removing cell contents. Fat is the main structure-forming membrane and cell wall. Detergent or table salt given to cells can make holes and damage cells so that the contents of the cell nucleus (DNA) can come out. The process after cell lysis is precipitation. Precipitation is done with ethanol, chloroform, isopropanol or phenol which aims to separate DNA from the residue. Precipitated DNA will separate from residue in the form of RNA and remaining protein. When the ethanol process is discarded and the pellet is dried in a tube, the dry pellet inside the tube is concentrated DNA (Bettelheim and Landesberg, 2007). Ethanol or alcohol does not dissolve DNA and the specific gravity of alcohol which is lighter than water makes DNA rise on the surface (Faatih, 2009).

Precipitation aims to eliminate residues from the extraction stage (Yuwono, 2006). Washing of pellets precipitated using ethanol aims to remove residual salt residues (Keller and Mark, 1989). Salts involved during the extraction process have insoluble properties in isopropanol so that they can precipitate with DNA, therefore re-precipitation is needed with ethanol as the final stage to remove salt residues (Ausubel *et al*., 2003) After the precipitation process (separation) next is the purification process. Purification was carried out by re-washing ethanol which was then discarded and the pellet dried. The purpose of the treatment is to remove ethanol residues from DNA pellets. Removal of ethanol residues is done by evaporation because of the nature of ethanol is volatile in the air (Surzycki, 2000).

2.8.2 DNA Amplification

The next stage of DNA isolation is DNA amplification by Polymerase Chain Reaction (PCR). This technique can be useful in increasing the amount of DNA so that it can be utilized more optimally because there are more numbers (Handoyo, 2011). PCR (Polymerase Chain Reaction) is an in vitro technique for multiplication (amplification) of DNA fragments in specific areas bounded by two oligonucleotide primers (Faatih, 2009). PCR can be influenced by the composition of the buffer solution or the number of reaction cycles performed (Yuwono, 2006). Added that the PCR process contained an annealing cycle where the temperature regulation of the cycle was very influential in the primary adhesion process. a temperature difference of one degree can cause the primer to fail to cling (Langga, 2012).

2.8.3 Electrophoresis of Agarose Gels

The results of the PCR can be seen in gel electrophoresis to determine the visualization of the previous steps and determine the molecular weight of the target DNA band (Handoyo, 2011). The basic principle of electrophoresis is to separate DNA molecules based on differences in size. Electrophoresis uses an electric charge with a voltage (voltage) in the process of its work and the separation of DNA is carried out with agarose gel (Firdausi and Kusumawati, 2008).

2.8.4 Sequencing DNA

The Sanger method is a method used for sequencing. This method is also known as the dideoxy chain termination method which produces a collection of nucleotide base fragments. Sequencing is done to determine the order of nucleotides arginine (A), cytosine (C), guanine (G), thymine (T) on bacterial DNA molecules. Sanger sequencing method uses a solution in the form of dNTPs (Deoxynucleotides Triphosphates) to synthesize new bacterial DNA molecules and ddNTPs (Dideoxynucleotides Triphosphates) which will stop the lengthening of bacterial DNA molecules in certain bases (Hunkapiller, 1992).

The discovery of the Polymerase Chain Reaction (PCR) technique by Sanger was very beneficial in the development of molecular biology. The process for DNA sequencing had to go through a long and tiring series of processes. With the method developed by Sanger and Maxam-Gilbert, sequencing can be done very easily and quickly so that this technology is increasingly developing (Muladno, 2002)

2.9 Phylogenic Analysis

Phylogenetics is the science of relationships between organisms based on kinship with each other, evolutionary relationships, and also the life history of a species (Brown, 2002). Phylogenetic trees can describe the relationship between species with the last ancestor that is closest to the comparison species. So that the proximity of one species to another species can be analyzed (Kocher *et al*., 1989).

CHAPTER III
METHODS

3.1 Research Design

This research is a quantitative and experimental descriptive study. The experimental descriptive type is because the data presented include bacteria resistant to Selenate, macroscopic characteristics, and identification of bacterial species with 16S rRNA molecular techniques and phylogenetic tree reconstruction. An experimental type was conducted to determine the ability of selected isolates to accumulate Selenate metal at certain selenate concentrations.

3.2 Time and Place

This research was conducted in August - December 2019. Located in the Biochemical Laboratory, Genetic and Molecular Laboratory and Microbiology Laboratory of the Department of Biology, Faculty of Science and Technology of UIN Maulana Malik Ibrahim Malang. PCR (sequencing) results analysis test was conducted by PT. Bioneer in South Korea.

3.3 Research Tools and Materials

3.3.1 Tools

The tools used in this study are Petri dishes, Erlenmeyer glasses, measuring cups, analytical balance, micropipets, Laminar Air Flow (LAF), Autoclaves, hot plates, reckoning balls, glass pipettes, blue tips, yellow tips, stirrers, tools digestion, Bunsen, ose, L glass spreader, incubator, Eppendorf tube, beaker glass, stirrer, test tube, colony counter, plastic, mouthpiece, rubber band, aluminium foil,

bottle, spatula, PCR Thermal Cyler, refrigerator -20°C, electrophoresis with power supply, Gel-Doc Trans UV illuminator, microwave, and shaker incubator.

3.3.2 Ingredients

Ingredients needed in this study include Yeast Malt Extract Agar (YMEA) which contains 3 grams of yeast extract, 3 grams of malt extract, 5 grams of peptone, 5 grams of sucrose, 20 grams of bacterial agar in 1000 ml of distilled water, NA_2SeO_4 (sodium selenate), Banyuglugur Beach Mangrove Sediment, Situbondo, Sterile Aquades, NaCl, 70% alcohol, plastic wrap, tissue, methylated cotton, cotton, gauze, label paper, plastic, crystal violet, safranin, 96% alcohol, nuclease-free water, sucrose, loading dye, 2.5 Taq polymerase, sodium selenate (Na2SeO4), PCR mix, dNTP, Gene Ruler TM 1 Kb Ladder (Intron), ethidium bromide (EtBr), agarose gel 1% and using Primer 306 forward {5 'CCA GAC TCC TAC GGG AGG CAG C 3'} and Primer 935 reverse {5 'CGA ATT AAA CCA CAT GCT CCA C 3'}.

3.4 Work Procedures

3.4.1 Sterilization of Tools and Materials

Some tools such as Petri dishes are wrapped and some other tools are wrapped using aluminium foil. For the material in the form of a medium that is put into a 500 ml Erlenmeyer glass and then closed using cotton and gauze, while the physiological salt solution is put into a 20 ml test tube, each of which is covered using aluminium foil. All tools and materials are put in different plastic bags which are then tied using a rubber band. Sterilization of glassware and

materials other than media to be carried out using an autoclave at 121° C at a pressure of 1 atm for 15 minutes. Whereas the media should be sterilized twice using the same tools.

3.4.2 Sampling

Samples were taken from mangrove sediments in Banyuglugur Beach, Situbondo Regency at 3 stations. The first station is a location close to residents' activities. The second station is a location that is rarely used by residents' activities. While the third location is a location that is far from the activities of the population. Sampling is done by taking the surface part of the sediment from the mangrove. Sediment samples are taken using a small shovel, then put in plastic and labelled, then stored in an icebox. The sample obtained is then taken to a laboratory for isolation and bacterial identification.

3.4.3 Media Making

a) Growth Media

The media used as a bacterial growth medium from mangrove sediments is the Yeast Media Extract Agar (YMEA) media. Krutzman and Fell (1998) in 1000 ml consisting of 3 grams of yeast extract, 3 grams of malt extract, 5 grams of peptone, 5 grams of sucrose, 20 grams of agar bacteria dissolved in 1000 ml of distilled water. After that, the material is put into a 1 L Erlenmeyer tube and then covered with aluminium foil. The media is then cooked or heated on a hot plate until the media looks boiling and homogeneous. The media was then sterilized using an autoclave for 2x15 minutes at 121° C 1 atm.

The pouring of the media is done sterically in the Laminar Air Flow (LAF). The media was put into a sterile petri dish as much as 19.8 ml of hot media using a glass pipette and a sterile rational ball. Addition of Na_2SeO_4 (sodium selenate) with mM concentration was carried out simultaneously when pouring YMEA media on a petri dish. Petri dishes containing media were allowed to stand for 1x24 hours at 37° C incubator temperature to determine bacterial contaminants.

b) Isolation Media

Bacterial isolation media used for the serial dilution method are 1 ml of sterile equates, then put in a sterile 1.5 ml tube.

3.4.4 Bacterial Isolation

Bacterial isolation was carried out by taking 0.5 gr of mangrove sediment using a sterile spatula. The sediment is then dissolved in 1 ml of sterile distilled water in a 1.5 ml tube, then homogenized for 10 seconds. After that, 100 µl is taken from the first tube using a micropipette and transferred into the next tube. The same steps were carried out on the 3rd tube. The process of bacterial isolation on YMEA media was carried out by the streak plate method. A total of 30 microliters of equates containing sediment in a tube were then taken using a micropipette and inoculated into a petri dish containing YMEA media with different concentrations. Streaking was done using a sterile spreader in the LAF cupboard. The cup containing the sample was incubated using an incubator at 27° C for 3x24 hours until a bacterial colony was seen on the Petrie dish.

3.4.5 Bacterial Resistance Test

The research was continued with a resistance test or it could also be called a screening test as a step to find out how much bacterial isolates were able to grow against exposure to selenate heavy metals and the ability of bacteria isolates to reduce the metal selenate given. According to Amalia (2017), this test was carried out by pouring 10 µL isolates in a glycerol solution into a sterile YMEA medium containing 10 mM sodium selenate (Na_2SeO_4) which had been incubated for 24 hours. According to Avendano et al., (2016), bacteria are stated to positively reduce and accumulate when they are red and are said to be negative if they are white.

3.4.6 Identification of Bacteria

3.4.6.1 Macroscopic Observation

Bacterial characteristics from the results of isolation on YMA media were observed for their growth characteristics. The properties observed according to Dwijoseputro (1994) are:

a. Formation of the colonies that appear above includes: round (circular), filamentous, irregular, resembling roots (rhizoid), and similar to the coil (spindle).
b. Colony or elevation surfaces that are seen from the side include, among others, flat, raised, convex, and hilly or umbonate.
c. The edge of the colony without the top which includes: whole (whole), wavy (lobate), serrated, filamentous, and curly.

3.4.6.2 Microscopic Observations

Microscopic observations were made by looking at the cell shape and colour of the bacteria after gram staining. Gram staining of bacteria refers to the procedure carried out by Cappucino (2013), which is taken one ose of bacteria from the media and placed on a sterile glass object that has been dropped with distilled water on top. The preparations are then fixed to dryness and after drying the violet crystal solution is dropped. The preparation is then allowed to stand for 1 minute then washed with flowing distilled water. The next step drops with iodine solution and allowed to stand for 1 minute. After that, it was given flowing equates which were then given 96% alcohol. Then the preparation drops with safranin and allowed to stand for 1 minute which is then washed with running water and dried using a tissue. The preparations are then observed using a microscope, if it looks purple then it is gram-positive if it looks red it is included in gram-negative bacteria.

3.4.6.3 Molecular Observation

a. Amplification with Direct PCR

The process of amplification of 16S rRNA encoding genes from resistant bacteria and selenate accumulation was carried out using the Direct PCR Amplification method, which took a single bacterial colony on a petri dish using a sterile toothpick tip, then transferred it to the bottom of the PCR tube and added material including a 12 µL PCR mix, Primary forward 1 µL 10 pmol, reverse primer 1 µL 10 pmol, nucleus free water 9.5 µL. The primers used in this study

were 306F (5′-CCA GAC TCC TAC GGG AGG CAG C-3) as the primary forward and 935R (5′-CGA ATT AAA CCA CAT GCT CCA C-3 ′) as the reverse primer.

This process uses an initial denaturation phase at 94 ° C for 2 minutes, denaturation at 94° C for 30 seconds, annealing at 50° C 40 seconds, elongation at 72° C for 40 seconds and stabilization 72° C for 2 minutes. The process of denaturation, annealing and polymerization of 30 cycles.

b. DNA Quality Test

This test is carried out using 250 ml 1x TBE buffer solution mixed with 5 mL 50x TBE in 245 mL equates. 1% agarose gel was made with 0.3 grams of agarose dissolved in 1x TBE buffer up to 40 mL volume. A total of 10 µL DNA samples and 2 µL loading dye 6x were put into the gel well. Electrophoresis was performed at 100 V voltage and 30 minutes running time. Then the results of the electrophoresis are seen by using the Gel doc tool.

c. Sequencing of 16S rRNA Encoding Genes

The results of the amplification were then sequenced using the services of the South Korean Bioneer.

3.4.7 Bacterial Growth Curve

Preparation of bacterial growth curve by measuring optical density (OD) every 2 hours from 3 ml of broth YMEB media that has been inoculated with 30 µl of bacterial culture using a spectrophotometer at a wavelength (λ) of 600 nm.

The standard solution used is YM Breath. This test is carried out until there are 4 phases of bacterial growth.

3.4.8 Data Analysis

Molecular results were analyzed descriptively and qualitatively based on DNA quality of the 16S rRNA gene using electrophoresis with primers 306F and 935R. The sequencing results were then analyzed using the NCBI website (www.ncbi.nlm.nih.gov/BLAST/) online, the BLAST (Basic Local Alignment Search Tools) program, and the MEGA 6 program (Haddad *et al*., 2014). Analysis of the integration of Science and Islam was also carried out using spiritual reasoning and Islamic studies relating to research guided by the Qur'an and Al-Hadith.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Types of Selenate Resistant Bacteria in Banyuglugur Coastal Mangrove Sediments in Situbondo Regency based on Morphological and Molecular Characteristics

4.1.1 Isolation of Selenate Resistant Bacteria

Sampling was carried out in the coastal area of Banyuglugur Situbondo. In taking samples, bacteria were obtained from the top of mangrove sediments which were then isolated using Yeast Media Extract Agar (YMEA). The media is used as a bacterial isolation material based on Kashiwa *et al*., (2001) YMEA media contains ingredients as bacterial growth factors, such as casamino acid and vitamins. Other than that, there is glucose 20 gr/L which acts as a source of carbon used for bacterial cell propagation.

Bacterial samples were isolated and grown in 15 ml of YMEA media by giving sodium selenate 0 mM, 1 mM, 2 mM, 5 mM and 10 mM using the spread plate method. Bacteria that have been inoculated into the media are then incubated for 3 days at 27° C aerobically. Based on the incubation of bacterial isolation samples for 3 days, the results obtained as in table 4.1. Bacteria with a red indicator indicate the alleged ability to reduce the metal selenate (Se VI) to selenium elemental (Se 0). In these results, seen in the media with the treatment of selenate concentrations of 10 mM and 5 mM, 2 red colonies of bacteria were each obtained. Whereas in the 2 mM treatment there were 4 red colonies. Tomei *et al*., (1995) red colour changes due to metabolic processes of bacterial cell walls that are resistant to metal selenate.

Table 4.1 Bacterial Isolation Results

No.	Sample Dilution	Selenate Concentration in the Media	Colonies Total	
			White Colonies	Red Colonies
1.	10^{-2}	0 mM	8	-
		1 mM	3	-
		2 mM	10	-
		5 mM	-	-
		10 mM	-	-
2.	10^{-3}	0 mM	15	-
		1 mM	4	1
		2 mM	5	4
		5 mM	1	1
		10 mM	-	2

Note: Bacteria are the focus of research.

The reason for selecting bacterial isolates that grew at concentrations of 5 mM and 10 mM to be the focus of the study due to the rejuvenation using the 10 mM selenate YMEA media, five out of eight bacterial isolates found to be red (Table 4.1) did not show red colour nor did the features be red showed the presence of bacterial activity against selenate on 10 mM media. Rejuvenation treatment on 10 mM media is intended to look for bacteria that are most resistant to selenate metal in the highest concentration. The results of rejuvenation obtained on YMEA media, the five bacterial isolates showed white colour, so that obtained at this stage three bacterial isolates, namely 1 isolate from 5 mM and 2 isolates from 10 mM (Table 4.2). Khalilian, *et al*., (2015) the colour change of bacterial isolates to red on media that were given selenate with a concentration of 10 mM showed that the bacteria occurred in the process of reducing selenate and showed the existence of resistance and ability to accumulate metal selenate by bacteria against metals in the media.

4.1.2 Macroscopic Identification

Macroscopic observations were made of bacteria that gave a red response to testing resistance at high concentrations in solid media. The bacteria observed were bacteria that showed red on the YMEA rejuvenation media with the highest concentration of 10 mM. Based on the previous table (table 4.1), it was found that the isolates that were the focus of this study were bacteria with 3 isolates from 5 mM and 10 mM. This is because, in the rejuvenation of bacterial isolates on the selenate media concentration of 10 mM, only 3 isolates showed a red colour change in observations 3x24 hours at room temperature.

Macroscopic observation of bacteria was carried out by direct observation of Petri dishes. The macroscopic examination was assessed from the colour of the colony, the colour of the edge of the colony, the shape of the edge of the colony, and the surface of the colony. Observations obtained from macroscopic observations can be seen as in table 4.2. Bacteria that were observed macroscopically were then observed using gram paint to find out which bacteria were gram-positive or gram-negative bacteria.

Based on observations it was found that in the three observed on YMEA media with a concentration of 10 mM, all three isolates had a red colour in the middle part and also on the edges. The isolate surfaces of A-5-1 and isolate B-10-1 has a convex surface formation, while in isolate B-10-2, the surface isolate form is raised flat (raised). According to Sousa, *et al*., (2013) differences in the appearance of colonies can be made characteristic to distinguish microorganisms at the level of various taxonomic groups, including strains of one species. But the most important thing, all the characteristics of microorganisms depend on the

conditions of growth or rejuvenation, especially on the composition of the growing media, temperature, and also the time of growth. However, according to Puchkov (2016), explaining that the appearance of a colony cannot be the only parameter used to identify a microorganism.

Table 4.2 Characteristics of Selenate Resistant Bacteria at 10 mM

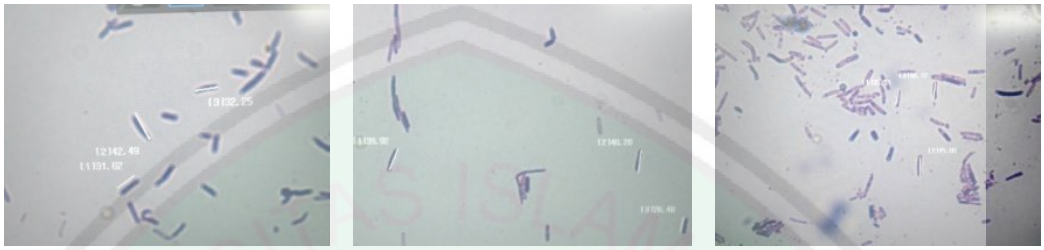
Bacterial Colony	Morphology of the Colonies			
	Colony Colour	Edge Colour	Edge of Colony	Colony Surface
Isolate A-5-1	Red	Red	*entire*	*convex*
Isolate B-10-1	Red	Red	*entire*	*convex*
Isolate B-10-2	Red	Red	*entire*	*raised*

4.1.3 Identification of Gram Bacteria

Gram identification in bacteria was carried out using Gram dye. Gram staining in bacteria aims to distinguish between Gram-positive or Gram-negative types of bacteria that are based on cell wall structure (Waluyo, 2005). The results of the observation of Gram stain bacterial paint at magnification 1000x, it was found that all three isolates have a positive Gram characteristic with a marked appearance of purple when observed (table 4.3 and figure 4.1). Also, the three isolates have the form of bacilli or stem with different lengths. Dwidjoseputro (1994) explains that bacteria with bacilli or stem formations can be observed as a single stem or can also be coupled with other rods. In the plural, the bacillus is called bacilli and some bacilli will resemble cocci or called coccobacilli.

Table 4.3 Results of Gram staining in Bacteria

Bacterial Colony	Size	Cell shape	Gram type
Isolate A-5-1	4,1 mm	bacilli	Positive
Isolate B-10-1	3 mm	bacilli	Positive
Isolate B-10-2	2,4 mm	bacilli	Positive



(a) **(b)** **(c)**

Figure 4.1 Gram staining; **(a)** Isolate A-5-1; **(b)** Isolate B-10-1; **(c)** Isolate B-10-2.

Based on research conducted by Javed, *et al*., (2016) it is known that bacteria with Gram-positive produce spores in conditions that are unfavourable in their growth. Nasrazadani, *et al*., (2011) explained that bacteria with Gram-positive have the highest resistance to heavy metals. That is because of its high ability to absorb and hold metals on the surface of the bacterial cell wall. Gram-positive bacteria have simpler and unspecified cell walls at the entrance and the presence of external material. Long-term contact of bacteria with heavy metals can cause more resistance and this is the main reason for the high tolerance level of bacteria to heavy metals.

4.1.4 Molecular Identification

Molecular identification by DNA isolation which is then followed by PCR. Both bacterial isolates were extracted DNA and proceeded to PCR using Primer 306 forward {5 'CCA GAC TCC TAC GGG AGG CAG C 3') and Primer 935 reverse {5 'CGA ATT AAA CCA CAT GCT CCA C 3'}. The bacterial isolates

used were two bacteria that showed colour changes when tested for resistance in a broth medium of 10 mM concentration (figure 4.6). B-10-2 bacterial isolates produced clots in broth media and did not show red colour changes as in isolates A-5-1 and B-10-1 in broth media. That is because according to Khalilian, *et al*., (2014) the change in colour to reddish or reddish yellow indicates the process of reducing levels of toxic heavy metal selenium to elemental selenium by bacteria.

Based on the results of DNA fragment amplification of 16S rRNA gene, it gives good results at the primary attachment temperature at 50° C with the appearance of DNA bands in isolate samples A-5-1 and isolates samples B-10-1, and on the left side so, visible bands of marker along with the 500 bp. As shown in Figure 4.2, DNA bands isolate A-5-1 and isolate B-10-1 are in the range of 600-500 bp amplicon in the marker. The results of DNA amplification using PCR are then purified and sequenced to obtain the sequence of the nucleotide bases.

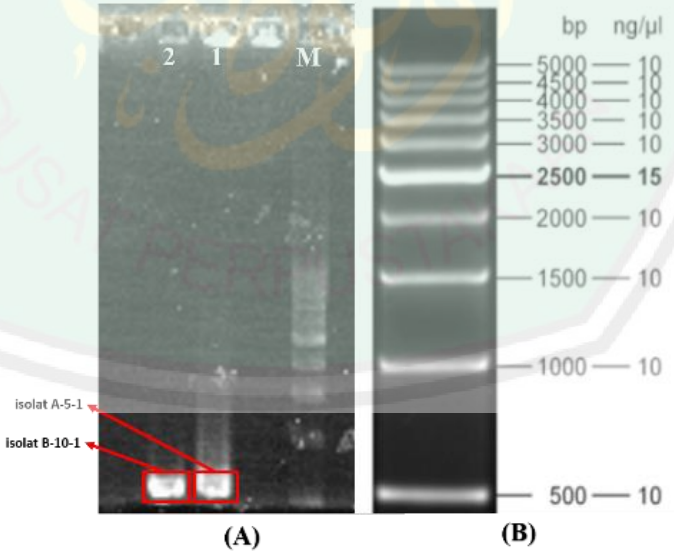


Figure 4.2 **(A).** Gene Ladder Jane B Base Pair Marker Length; **(B).** Electrophotogram of PCR products on agarose gel 1%; M = 500bp Marker; 1 = isolate A-5-1; 2 = isolate B-10-1. (Personal Doc, 2019).

The PCR tape visible on agarose 1% gel shows that the concentration of DNA from the isolation results is high enough to be visualized on the gel using trans-UV (gel doc). According to Langden (2017), the plasmid band looks thick and clearly shows that the concentration of the isolated plasmid is high.

4.1.5 Phylogenetic Analysis

a. Identity of Species

The sequencing of bacterial sequences is done using Blast on the NCBI online page. The alignment results showed that Isolate A-5-1 had similarities with 3 types of bacteria namely *Bacillus cucumis* strain PK15, *Bacillus fumarioli* strain PF1, and *Bacillus aestuarii* strain IAE10 which both had a similarity percentage of 98.83%. Whereas B-10-1 isolates found 3 different types of bacteria, namely *Klebsiella oxytoca* strain Rizhao 567.2; *Klebsiella oxytoca* strain Rizhao 615.1; and *Klebsiella oxytoca* strain Rizhao 615.1 with a similarity of 89.69%.

Table 4.4 Bacterial isolate results from the NCBI website

No	Samples	Types of Identical Bacteria	NCBI Accession	% Similarity
1.	Isolate A-5-1 (A-16S F)	*Bacillus cucumis* strain PK15	MK519225.1	98.83%
		Bacillus fumarioli strain PF1	MK909907.1	98.83%
		Bacillus aestuarii strain IAE10	MK414790.1	98.83%
2	Isolate B-10-1 (B-16S F)	*Klebsiella oxytoca* strain Rizhao 567.2	MN249586.1	89,69%
		Klebsiella oxytoca strain Rizhao 615.1	MN249590.1	89,69%
		Klebsiella oxytoca strain Rizhao 614.1	MN249589.1	89,69%

The percentage of isolate A (98.83%) was based on the similarity of the nucleotide sequences in each species with the nucleotides in isolate A-5-1. This

means that there is 1.17% which causes homology to isolate A to be different from the other three species. Sequences were analyzed using the Multalin v.5.4.1 (multalin.toulouse.inra.fr/multalin/) and the results showed a difference in the nucleotide sequence. The difference is seen at each end of the nucleotide sequence (positions 1, 4, 5, 6, 8, 9, 590, 591, 592, and 605) (figure 4.3). While the isolate B-10-1 had a difference of 10.31% with the three bacteria produced by Blast. The difference in nucleotides in isolate B-10-1 appears to be 41 points of difference which are spread at the ends of the sequence and in the middle of the sequence (figure 4.4).

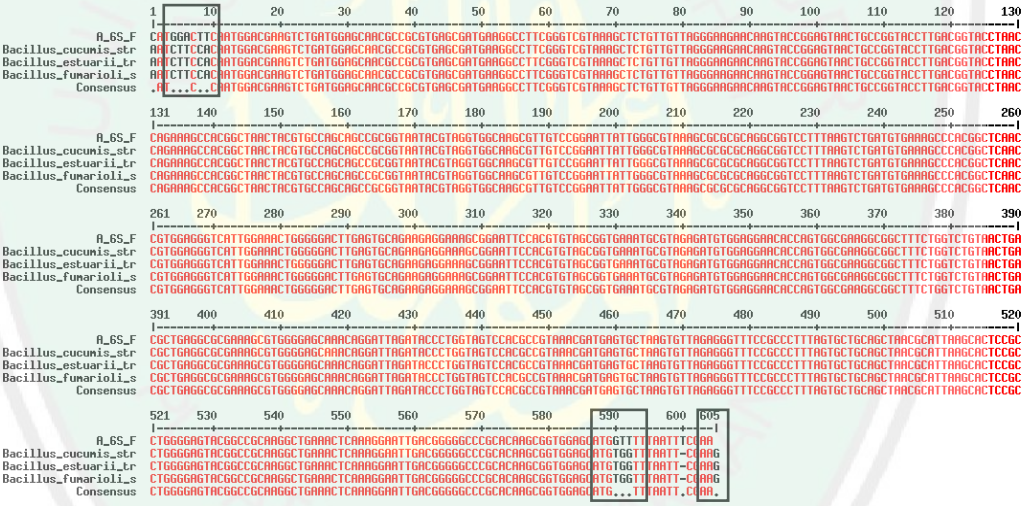


Figure 4.3 Alignment of isolate A-5-1 sequence BLAST results using the Multalin v5.4.1

Figure 4.4 Alignment of isolate B-10-1 sequence BLAST results using the Multalin v5.4.1

Jhonson, (1984) explained that species are said to be the same when the DNA homology of a species has a homology percentage (similarity) ranging from 60-100%. Whereas a species is said to be closely related to other species is when it has a DNA homology of 20-60%, and if the DNA homology of a species has a homology percentage of less than 20% then the species is considered a different species. Whereas in Kwaśna, *et al* (2008) explain that a difference of 3% in the similarity percentage shows differences at the species level.

The outgroup of selected bacterial species has a character that is quite different so that it is selected from a different class from the results of BLASS Isolate A-5-1 and Isolate B-10-1, namely *Salmonella enterica* from the Gammaproteobacteria class. Mount (2008) Selection of outgroup sequences that are too far away will cause phylogenetic tree analysis to be incorrect and inaccurate due to random and too many differences between outgroup sequences and in-group sequences. Meanwhile, according to Muzzayinah (2012) outgroups are selected from those who have a close kinship with the in-group but not as

close as the in-group group. The outgroup is needed in phylogenetic analysis because it aims to determine the primitive (plesiomorph) and derivative (apomorph) characters of the in-group group and also determine the starting point of formation of a phylogenetic tree.

b. Genetic Distance of Bacterial Isolates

Genetic distance from bacterial sequences was carried out using MEGA 6. Fasta documents from the nine sequences were first merged with the data using the BioEdit application and then the documents were saved with the Mega (**.mega**) file type. The document is then opened in the MEGA 6 application and then in the sequence bases in both ends of the sequence the cuts are made to the limit of the base end of the sample sequence (Isolate A-5-1 and Isolate B-10-1). The alignment process is done with ClustalW Multiple alignments. According to Tamura *et al*., (2011) ClustalW is a system that is widely used in aligning nucleotide sequences with progressive methods. Homologue sequences with the best value are aligned first, followed by sequences that are further resemblance until global alignment is obtained.

Files that have been saved with the type **.mega** then open and do modelling to find the right method in determining the genetic distance of a sample or species. Based on the results of the modelling obtained 24 models that can be used in analyzing the genetic distance from the bacterial sequence, and the best model using the HKY model (Hasegawa-Kishino-Yano). The best model used is seen from the BIC (Bayesian Information Criterion) score. Nei and Kumar (2000)

explain the smaller the BIC score in the analysis of the model, the more suggested it is to carry out the genetic distance and phylogenetic tree analysis.

Based on the results of the analysis using the MEGA6 application, the results of genetic distance from bacteria in table 4.5. According to Dwiko (2018), the results of calculations from genetic distances show the value of the close relationship between species. Afryani (2014) explains that if the genetic distance value approaches 0 then it can be interpreted that there is no significant genetic variation. Tallei *et al*., (2016) also explained that the less genetic distance between organisms, the closer the kinship between these species.

Based on the analysis of genetic distance using the Tamura 3-Parameter method, the distance between Isolate A-5-1 and Isolate B-10-1 has genetic proximity of 0.287 (table 4.5). The sequence of isolate A-5-1 has the same distance as 3 other species which have 98.83% similarity when analyzed using the NCBI online website which is within 0.0099. As for Isolate B-10-1, it has a genetic distance with Bacillus estuarii IAE10 strain, Bacillus cucumis strain PK15, and Bacillus fumarioli strain PF1 with genetic proximity of 0.1173. For species produced from the A-5-1 sequence blast (genus Bacillus) have a genetic distance of 0.2674 with species produced from the B-10-1 sequence blast (genus Klebsiella). Outgroup species used are *Salmonella enterica* strain SC681 because they have different gram characteristics from the two isolates observed. The genetic distance of strain A-5-1 with outgroup species is 1.1041 and genetic distance for strain B-10-1 is 1.1382.

Table 4.5 Genetic Distance of Isolates

	1	2	3	4	5	6	7	8	9
1. A_6S_F									
2. B_6S_F	0.2879								
3. Bacillus_cucumis_strain_PK15	0.0099	0.2808							
4. Bacillus_estuarii_trai_IAE10	0.0099	0.2808	0.0000						
5. Bacillus_fumarioli_strain_PF1	0.0099	0.2808	0.0000	0.0000					
6. Klebsiella_oxytoca_strain_Rizhao_567_2	0.2780	0.1173	0.2674	0.2674	0.2674				
7. Klebsiella_oxytoca_strain_Rizhao_615_1	0.2780	0.1173	0.2674	0.2674	0.2674	0.0000			
8. Klebsiella_oxytoca_strain_Rizhao_614_1	0.2780	0.1173	0.2674	0.2674	0.2674	0.0000	0.0000		
9. Salmonella_enterica_strain_SC681	1.1041	1.1382	1.1041	1.1041	1.1041	1.0784	1.0784	1.0784	

Kimura (1980), the measurement of genetic distance functions to compare a DNA sequence of one nucleotide with another nucleotide using the nucleotide substitution model. Humairah (2015) measures of genetic distance will be relatively close if there were no significant quantitative differences in numbers when crossed because the nature of diversity is quite the same. Whereas genetic distance will be relatively far if when crossed there are significant quantitative differences in the number of figures due to the different nature of diversity.

c. Phylogenic Tree

The results of the phylogenetic tree obtained showed that Isolate A-5-1 (A-16S F) had a close relationship with bacteria with the genus Bacillus and Isolate B-10-1 (B-16S F) had a close kinship with the genus Klebsiella (figure 4.5). That is because both bacteria Isolate A-5-1 or Isolate B-10-1 are in the same clade with a bootstrap value of 92 for A and 98 for B. While the *Salmonella enterica* species which are outgroup from this bacterial sample form new branches which shows the primitive species in the phylogenetic tree. The bootstrap value is indicated by a number located in the phylogenetic tree branches (arrows).

Phylogenetic trees can be accepted in biosystematics analysis when they are monophyletic, dichotomous, no polyatomic, have high bootstrap values and clones that form is consistent and sturdy. Monophyletic groups only have one ancestor and all of their offspring originate from that ancestor. This causes members in monophyletic groups to be considered to have a very close relationship and is assumed to carry the same genetic and biochemical traits or patterns (Hidayat and Pancoro, 2008; Rahayu and Nugroho, 2015).

The phylogenic tree is a graph used to illustrate the familial relationship between taxa consisting of several nodes and branches with only one branch connecting the two nodes most close together. Branching patterns formed from phylogenetic trees are called typologies (Lie and Graur, 1991). Phylogenetic analysis is inseparable from biological evolution that studies variations and genetic differences between populations, while genetic distances can be calculated based on differences in the polymorphic language of a gene locus for each DNA sequence (Cavalli-Sforza, 1997).

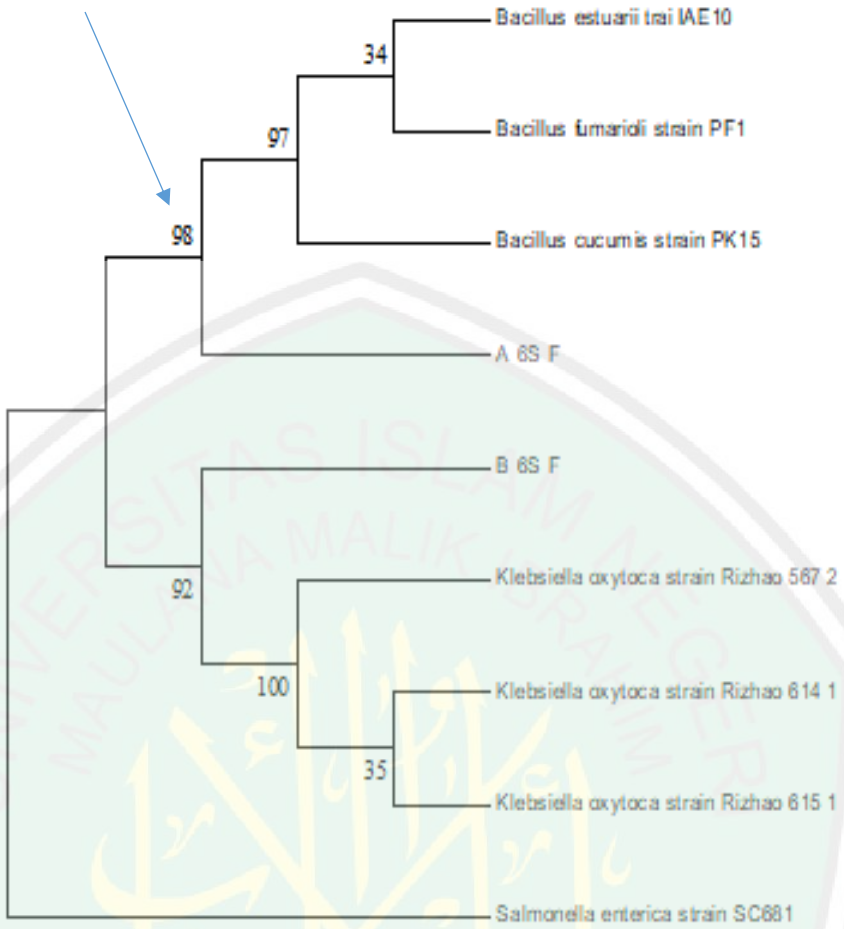


Figure 4.5 MEGA6 Phylogenetic Tree

Files from the nine edited sequences in the BioEdit application are opened with the MEGA6 application. Then do the phylogenetic data analysis using the Phylogenetic Analysis menu and choose Construct / Test Maximum Likelihood Tree. Yang and Rannala, (2012) explained that the Maximum Likelihood method is a statistical method that has a character base by comparing all sequences in alignment in calculating the likelihood value of each tree. This method considers all possible number of changes/mutations in the sequence for each tree. Dharmayanti, (2011) added that the Maximum Likelihood (ML) method was carried out in the form of a bootstrap method that did repeated sampling of

sequences to see the validity of the arrangement of phylogenetic trees, with a total replication of 500x to shorten the construction time. This study bootstrap was used with 1000 replications.

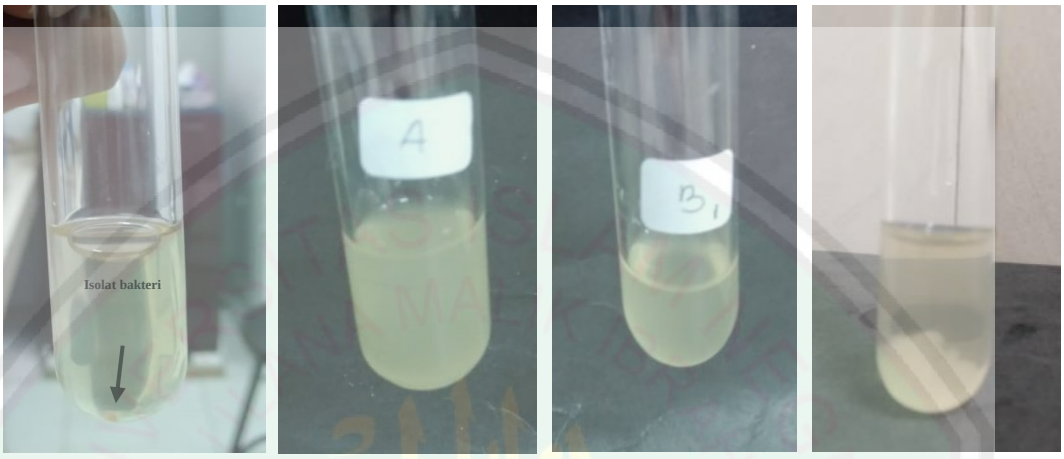
4.2 Characteristics of Growth of Selenate Resistant Bacteria Discovered in the Mangrove Sediment of Banyuglugur Beach, Situbondo Regency in Media containing Selenate

4.2.1 Bacteria Resistance Ability Test

The isolates grown in broth media will also be measured for absorbance using a spectrophotometer with a wavelength of 600 nm to determine the growth curve of bacterial isolates. Khalilian, *et al*., (2015) tests on broth media were carried out to ensure red discolouration that occurred in solid media did not occur due to pigmentation in bacterial isolate colonies. The three bacterial isolates were cultured in broth media with a concentration of 10 mM which was then grown in 1x24 hours. According to Mas'ud (2013), the purpose of rejuvenation of bacteria is to get new and young cultures, so that they can multiply well and can be used by their functions. This was also explained by Dwidjoseputro (1994) that bacterial incubation was carried out for 24 hours because at that time the bacteria might have been in the log-phase. In this phase, the bacteria make a constant division and the number of cells increases. According to Pleczar and Chan, (2008), 24-hour time is harvest time, which time is in the logarithmic or exponential phase with the highest number of cells, reaching 10 to 15 billion bacterial cells per millimeter.

The results of growing bacterial isolates on culture media for 24 hours can be seen in Figure 4.6. From these results obtained isolates A-5-1 and isolates B-

10-1 that was rejuvenated in culture media showed a more turbid colour change compared to isolates B-10-2 which showed a lump in the bottom of the broth media.



(a) (b) (c) (d)

Figure 4.6 YM broth media (a) 0 hour media (b) isolates A-5-1 24 hours; (c) isolate B-10-1 24 hours; (d) isolate B-10-2 24 hours

Bacterial isolates A-5-1 and isolates B-10-1 were chosen because they produced bacteria that grew with marked changes in the colour of the broth media. The two isolates were then inoculated in broth media with different concentrations of 1 mM, 2 mM, 5 mM, and 10 mM. The results obtained in the media change colour to red which indicates a bacterial reaction to the media containing selenate. Figure 4.7 and Figure 4.8 show a change in red in all concentrations. Figure 4.7 is a condition of the first 2 hours after inoculation of 30 microlites of bacteria from culture media. Media on isolate A-5-1 with a concentration of 10 mM appear darker in colour than other concentrations. Whereas at the same concentration B-10-1 isolate media did not show a change in colour that was significantly different from the other concentrations. Based on these results indicate that the isolate A-5-

1 has a response to the accumulation of metal selenate very quickly compared to isolate B-10-1 in the first 2 hours after inoculation of bacterial culture media. However, in subsequent observations, the colour of the media with isolate B-10-1 showed a redder colour. This shows the difference in the time of accumulation of bacteria on the metal selenate in the media. The media is red with increasing growth time in the growing media. In Figure 4.8 the media colour changes to red. Khalilian, *et al*., (2014) explained that the change in colour to reddish or reddish yellow indicates the process of reducing the levels of toxic heavy metal selenium to elemental selenium by bacteria.

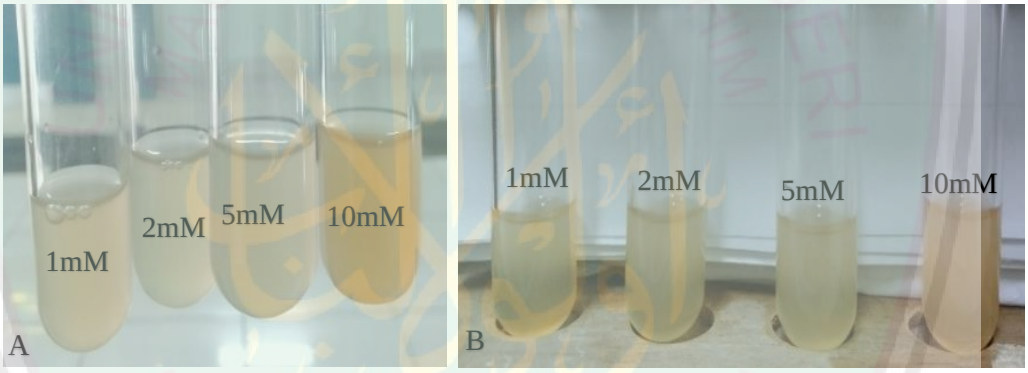


Figure 4.7 Results of Media Colour Change 2 hours after inoculation of culture bacteria

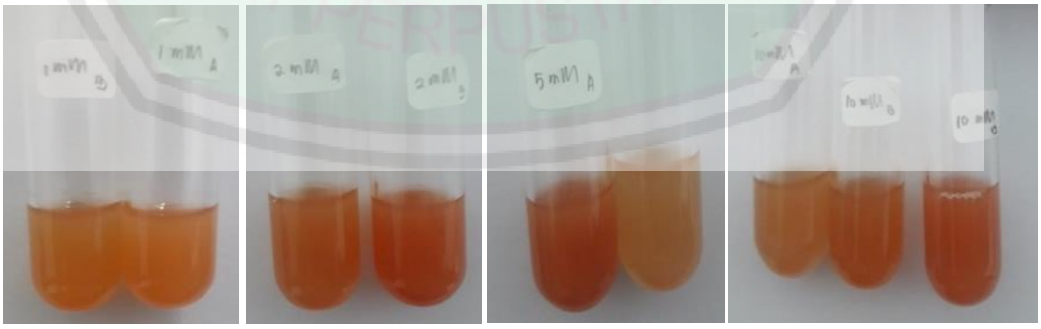


Figure 4.8 Results of Colour Changes in Media for 26 hours

4.2.2 Bacterial Growth Curve

Bacterial growth curve to find out the optimum time the bacteria can grow. Purwoko (2007), bacterial growth in growth media has phases that show their activity on growing media. These phases include the adaptation phase (lag-phase), the multiplication or enhancement phase (log-phase or exponential-phase), the static phase (stationary-phase) and finally the death phase. Measurement of bacterial growth curve on YMEB media was carried out for 26 hours with time intervals of 2 hours to obtain a decrease in OD from the sample. In this observation, a wavelength of 600 nm was used and the standard medium used was YMEB media with different concentrations. Bacterial samples taken 50 µl were put into cuvettes and 650 µl standard media were added. Bacterial isolates measured for growth curves were two bacterial isolates that observed resistance on broth media showing turbid discolouration (figure 4.6). The results of the growth curve of Isolate A-5-1 and Isolate B-10-1 can be seen in figures 4.9 and 4.10.

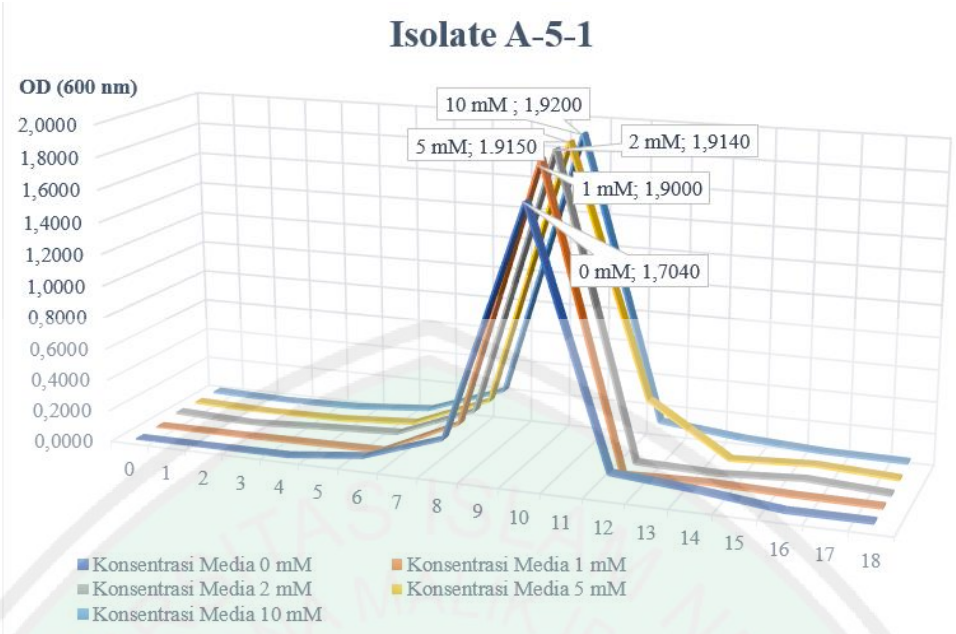


Figure 4.9 Growth curve of bacterial isolate A-5-1.

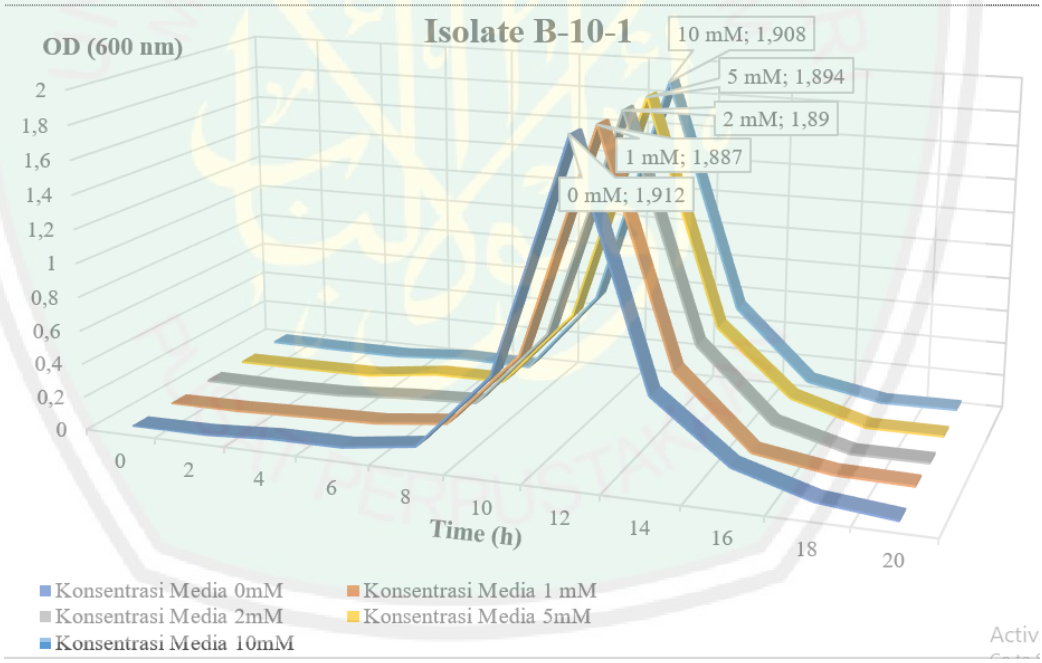


Figure 4.10 Growth curve of bacterial isolate B-10-1

Based on the growth curve of isolate A-5-1 (Fig. 4.9), an exponential phase is obtained at the 5 (8 hours) and 6 (10 hours) measurements marked by increasing the absorbance rate to the maximum point. Whereas the B-10-1 isolate

curve (figure 4.10), growth increased was obtained at the 6th measurement (10 hours) to the 7th measurement (12 hours). In this study, it was found that the peak increase in the number of A-5-1 isolate cells at different concentrations had the same time, namely at 8-10 hours after inoculation of culture bacteria. This also happened to media with isolate B-10-1 which showed an increase in the number of bacterial cells at the same time ie at 10-12 hours after culture. In both isolates, the presence of selenate in broth media caused an increase in the number of bacterial cells as an increase in concentration. Media with higher concentrations have higher absorbance values than media with lower concentrations. This is possible because the addition of metal selenate concentration causes an increase in the growth of the number of bacterial cells in the media. Also, the isolated bacteria may have been exposed to metal selenate (Se VI) directly in the environment.

Based on research conducted by Hindersah *et al*., (2014) on Azotobacter, it was found that bacterial growth increased in line with the increase in lead nitrate levels in the culture so that microorganism resistance had a positive effect on bacterial growth. Hindersah *et al*., (2009) the addition of heavy metals in the culture media may have a positive effect on bacterial growth. Research conducted by Zheng (2007) shows that heavy metals at low concentrations have a stimulating effect on carbon microbial biomass of the soil. Wyszkowska and Wyszkowski (2002), an increase in the number of certain microorganisms in heavy metal contamination at a relatively low amount due to the presence of available nutrients derived from the degradation of bacterial cells that are intolerant of heavy metals.

The first phase (lag-phase) of the two bacteria did not get an additional population. Pelczar (2008), in the first phase, the bacteria do not experience an increase in population but have changes in chemical composition, an increase in cell size, and increase intracellular survival. Purwoko (2017) said that the process of adaptation of bacteria to the media includes the process of synthesizing new enzymes following the given media and recovery of toxic metabolite elements, such as alcohol, acids, and bases on the origin media (old media). Bacteria will divide the cells in the media after an ideal condition is found in their growth. This phase will enter the log-phase or phase of improvement.

The increment phase or log-phase occurs in all concentrations of treatment media at the same time. This happens because the bacteria suspended on the test media are bacteria that originate from the same culture growth media, which are derived from culture bacteria on 24-hour YMEB media at a broth concentration of 10 mM selenate. This is thought to cause changes in the growth phase to occur almost simultaneously. In this phase, the bacteria have an increase in the number of cells which is characterized by OD values at a wavelength of 600 nm. Obtained on Isolate A-5-1, the 5th measurement (8 hours) was 0.246 at 1 mM; 0.25 at 2 mM; 0.24 at 5 mM; and 0.234 at 10 mM. Whereas in the 6th measurement (10 hours) the peak of the exponential phase was obtained with 1,900 at 1 mM; 1,914 at 2 mM, and 1,915 at 5 mM; and 1,920 at 10mM.

Increased growth was also found in isolate B-10-1 with increasing OD values at observations of 6 (10 hours) and 7 (12 hours). At a 10-hour observation, an OD value of 0.540 was obtained at 1 mM; 0.539 at 2 mM; 0.538 at 5 mM; and 0.530 at 10 mM. Whereas in the 7th measurement (12 hours) the peak of the

exponential phase was 1.887 at 1 mM; 1,890 at 2 mM, and 1,894 at 5 mM; and 1,908 at 10mM. Pelczar (2008) explains that in this phase bacteria consume nutrients in the media. Also, the bacteria will carry out physiological processes so that there is an increase in the number to a certain boundary mark marked by a decrease in OD.

The highest OD value can also be called the static phase or stationary phase. because in that phase there is a halt in the division of bacterial cells so that the absorbance value does not increase. Dwidjoseputro (1994) explains that static conditions occur when there is a buildup of toxic products obtained from the accumulation of toxic metabolites and the unavailability of nutrients for survival. Also, there is no sufficient oxygen content in a bacterial growth medium.

The decline in OD values initiates the death phase. Pelczar (2008) explains that in this phase bacteria experience rapid death compared to the formation of new cells. Purwoko (2007) explains that the existence of bacterial cell death is due to a decrease in energy in the cell so that autolysis occurs in the cell. The ability of bacteria to survive the gripping conditions varies and to overcome this can be done by re-isolating the bacteria into new media.

4.3 Dialogue on Research and Islamic Results

The pollution in the form of pollution in the location around the *Pembangkit Listrik Tenaga Uap* (PLTU) Paiton is caused by human actions, one of which is coal mining in the middle of the sea and coal shipping activities that cause heavy metal residues to accumulate and accumulate in waters and coastal sediments. Al-Qur'an explicitly explains in Surah Ar-Rum verse 41. In that verse, it is man as

God's creation who must take responsibility while bearing the various kinds of adverse effects of things done on earth. Allah SWT has also forbidden humans from doing actions that can damage lives.

Allah SWT says in Surah Al-A'raf verse 56:

وَلَا تُفْسِدُوا فِي الْأَرْضِ بَعْدَ إِصْلَاحِهَا وَادْعُوهُ خَوْفًا وَطَمَعًا ۚ إِنَّ رَحْمَتَ اللَّهِ قَرِيبٌ مِنَ الْمُحْسِنِينَ

It means: "And do not make mischief on the earth, after (Allah) has repaired it and pray to Him with fear (will not be accepted) and hope (will be granted). Surely Allah's grace is very close to those who do good."

Tafsir Ibn Katsir explained that Allah SWT forbids humans to do acts that cause damage on earth and also things that endanger their preservation after being repaired. In this research, activities that are included in the activity of "causing damage" are conducting mining without offsetting the activities of the filtration of metals remaining from mining which causes the carrying of metals that are harmful to the environment and marine biota.

Everything has gone according to its preservation, and if there is damage to it, it will endanger not only the human impact but all the servants of Allah SWT including microorganisms. So Allah Almighty commands humans to worship Him and pray to Him and to be humble and ask for His mercy. That is explained at the end of His verse which means "*And pray to Him with fear (will not be accepted) and hope (will be granted)*".

Relation to the presence of bacteria that grow in polluted conditions as well as recovering polluted environments, in this study obtained 2 bacterial isolates, namely isolate A-5-1 and isolate B-10-1 which can resist in conditions polluted selenate environment. In addition to this, the isolates obtained were also thought

to have the ability to reduce or reduce the contamination of heavy metal selenate (Se VI) to selenium (Se 0) found in mangrove sediments. In this case, the role of humans as caliphs is very important. The *Khalifah* is the role of a man that Allah has determined not only to be the ruler of the earth but also as the prosperity of the earth. The Caliph has the meaning of replacing anyone who came before to enforce His will (Shihab, 2011). The caliph was assigned to be able to balance what is on earth so that damage does not occur that can result in an imbalance of natural resources.

Allah SWT has said in the Qur'an Surah Al-Baqarah verse 30 which reads:

وَإِذْ قَالَ رَبُّكَ لِلْمَلَائِكَةِ إِنِّي جَاعِلٌ فِي الْأَرْضِ خَلِيفَةً ۖ قَالُوا أَتَجْعَلُ فِيهَا مَنْ يُفْسِدُ فِيهَا وَيَسْفِكُ الدِّمَاءَ وَنَحْنُ نُسَبِّحُ بِحَمْدِكَ وَنُقَدِّسُ لَكَ ۖ قَالَ إِنِّي أَعْلَمُ مَا لَا تَعْلَمُونَ

It means: "And (mention, o Muhammad), when your Lord said to the angels, "Indeed, I will make upon the earth a successive authority." They said, "Will you place upon it one who causes corruption therein and sheds blood, while we declare Your praise and sanctify You?" God said, "Indeed, I know that which you don't know."

Humans were created to be *Khalifah* on this earth not to do vandalism and bloodshed. But to build a life that is peaceful, prosperous and full of justice. Ridwan (2005), environmental destroyers can be classified as ecological infidels (*kufr al-bi'ah*). That is because among the signs of the greatness of Allah SWT is the existence of the earth and everything in it. Doing damage to the environment is the same as denying (infidels) the greatness of Allah SWT. As in the Qur'an surah Shad verse 27:

وَمَا خَلَقْنَا السَّمَاءَ وَالأَرْضَ وَمَا بَيْنَهُمَا بَاطِلا ذَلِكَ ظَنُّ الَّذِينَ كَفَرُوا فَوَيْلٌ لِلَّذِينَ كَفَرُوا مِنَ النَّارِ

It means: "And We did not create heaven and earth and what is in between without wisdom. Such is the assumption of unbelievers, so woe to unbelievers because they will go to hell."

This verse explains about us who talked about infidels before they came to destroy. The discovery of bacterial isolates (Isolate A-5-1 and isolate B-10-1) which can resist and accumulate heavy metal selenate at the Banyuglugur beach location becomes one of the reserves for all that Allah SWT created, all of which is beneficial to take advantage of great benefits. Bacterial isolates obtained can be used by residents or even by mining companies to prevent natural damage as coastal pollution, before on metal selenate. The use of bacteria that utilize is the use of bioremediation. Bioremediation can be done through the following five agreements: bioreactor, solid-phase handling, composting, land farming, and in situ processing.

Many things humans can do in their lives today. In a simple discussion, the attitude or way of humans as caliphs in the natural agreement has plenty of room for science, including in the space for social sciences, space for natural sciences, and also space for jurisprudence. The scope of social science includes human activities consisting of hablum minan naas wa hablum minal ‘alam. The point is human activities that involve social activities that are agreed with nature. As one example is to become an environmental activist or by carrying out social activities that improve the environment in a better direction. The discussion room in legal science can make rules or regulations regarding natural resource management that can be done to prevent damage to nature. Meanwhile, within the scope of natural science or also referred to as the scientific dressing room, human activities on the

environment reviewed by humans examine the events of government ties and formulate them in a study to use the wisdom of the signs of the transfer of His power in-universe, one of the only research is finding alternative sources to prevent and reduce the level of heavy metal pollution to the environment.

Allah SWT in Surah Al-Maidah verse 32:

مِنْ أَجْلِ ذَلِكَ كَتَبْنَا عَلَى بَنِي إِسْرَائِيلَ أَنَّهُ مَنْ قَتَلَ نَفْسًا بِغَيْرِ نَفْسٍ أَوْ فَسَادٍ فِي الأَرْضِ فَكَأَنَّمَا قَتَلَ النَّاسَ جَمِيعًا وَمَنْ أَحْيَاهَا فَكَأَنَّمَا أَحْيَا النَّاسَ جَمِيعًا وَلَقَدْ جَاءَتْهُمْ رُسُلُنَا بِالْبَيِّنَاتِ ثُمَّ إِنَّ كَثِيرًا مِنْهُمْ بَعْدَ ذَلِكَ فِي الأَرْضِ لَمُسْرِفُونَ

It means: "Because of that, we decreed upon the Children of Israel that whoever kills a soul unless for a soul or corruption (done) in the land - it is as if he had slain mankind entirely. And whoever saves one - it is as if he had saved mankind entirely. And our messengers had certainly come to them with clear proofs. Then indeed many of them, (even) after that, throughout the land, were transgressors."

So that it is expected that by behaving or acting to protect the environment with some spare scope of science, humans can increase one's faith in the Creator of the universe. Also, with the behavior of protecting the environment, it is expected that humans will be able to improve themselves not to do things that are contrary and prohibited by religion about managing and utilizing the universe and everything in it.

CHAPTER V
CONCLUSION AND SUGGESTION

5.1 Conclusions

Based on the results of the research conducted, the following conclusions are obtained:

1. The results of isolation of selenate resistant bacteria in the mangrove sediments of Banyuglugur Beach, Situbondo Regency, obtained 2 bacterial isolates, namely isolates A-5-1 and B-10-1 which were shown with red colonies. Molecular test results obtained Isolate A-5-1 has a similarity percentage of 98.83% with *Bacillus cucumis* strain PK15 species, *Bacillus fumarioli* strain PF1, and *Bacillus aestuarii* strain IAE10. Isolate B-10-1 has a similarity percentage of 89.86% with the *Klebsiella oxytoca* strain Rizhao 567.2; *Klebsiella oxytoca* strain Rizhao 615.1; and *Klebsiella oxytoca* strain Rizhao 615.1.
2. Characteristics of bacterial isolates A-5-1 and isolate B-10-1 show colour changes in liquid media in various concentrations. Increasing the growth of bacterial isolates A-5-1 faster than bacterial isolates B-10-1.

5.2 Suggestions

Suggestions for further research should be to test the accumulation of Selenate in mangrove sediments and to compare the ability of mangrove plants as remediator plants.

BIBLIOGRAPHY

Al-Albani, M.S. (2006). *Shahih Sunan Tirmidzi* (Seleksi Hadits Shahih Dari Kitab Sunan Tirmidzi Buku: 2). Jakarta: Pustaka Azzam.

Alongi, D. M. (2002). Present state and future of the world's mangrove forests. *Environmental conservation*, *29*(3), 331-349.

Amalia, W. (2017). *Bioakumulasi selenium oleh bakteri resisten selenium yang diisolasi dari Pantai Utara Desa Campurejo Kecamatan Panceng Gresik* (Doctoral dissertation, Universitas Islam Negeri Maulana Malik Ibrahim).

Andren, A. W., Klein, D. H., & Talmi, Y. (1975). Selenium in coal-fired steam plant emissions. *Environmental Science & Technology*, *9*(9), 856-858.

Avendaño, R., Chaves, N., Fuentes, P., Sánchez, E., Jiménez, J. I., & Chavarría, M. (2016). Production of selenium nanoparticles in Pseudomonas putida KT2440. *Scientific reports*, *6*, 37155.

Bailey, R. L., Gahche, J. J., Lentino, C. V., Dwyer, J. T., Engel, J. S., Thomas, P. R., ... & Picciano, M. F. (2010). Dietary supplement use in the United States, 2003–2006. *The Journal of nutrition*, *141*(2), 261-266.

Baird, R. B., Pourian, S., & Gabrielian, S. M. (1972). Determination of trace amounts of selenium in wastewaters by carbon rod atomization. *Analytical chemistry*, *44*(11), 1887-1889.

Bandaranayake, W. M. (1998). Traditional and medicinal uses of mangroves. *Mangroves and salt marshes*, *2*(3), 133-148.

Barceloux, D. G., & Barceloux, D. (1999). Selenium. *Journal of Toxicology: Clinical Toxicology*, *37*(2), 265-278.

Budiyanto, F. (2014). Distribution of Metals in Cisanggarung Estuary Sediment, West Java, Indonesia. *Marine Research in Indonesia*, *39*(1), 23-30.

Combs Jr, G. F., & Gray, W. P. (1998). Chemopreventive agents: selenium. *Pharmacology & therapeutics*, *79*(3), 179-192.

Cutter, G. A. (1989). The estuarine behaviour of selenium in San Francisco Bay. *Estuarine, Coastal and Shelf Science*, *28*(1), 13-34.

Debieux, C. M., Dridge, E. J., Mueller, C. M., Splatt, P., Paszkiewicz, K., Knight, I., ... & Richardson, D. J. (2011). A bacterial process for selenium nanosphere assembly. *Proceedings of the National Academy of Sciences*, *108*(33), 13480-13485.

Devi, P., Jain, R., Thakur, A., Kumar, M., Labhsetwar, N. K., Nayak, M., & Kumar, P. (2017). A systematic review and meta-analysis of voltammetric and optical techniques for inorganic selenium determination in water. *TrAC Trends in Analytical Chemistry*, *95*, 69-85.

Dias, A. C., Andreote, F. D., Rigonato, J., Fiore, M. F., Melo, I. S., & Araújo, W. L. (2010). The bacterial diversity in a Brazilian non-disturbed mangrove sediment. *Antonie Van Leeuwenhoek*, *98*(4), 541-551.

Dilaga, S. H. (1992). Nutrisi Mineral pada Ternak-Kajian Khusus Unsur Selenium. *Akademia Presindo. Jakarta*.

Doran, J. W. (1982). Microorganisms and the biological cycling of selenium. In *Advances in microbial ecology* (pp. 1-32). Springer, Boston, MA.

Dreher, G. B., & Finkelman, R. B. (1992). Selenium mobilization in a surface coal mine, Powder River Basin, Wyoming, USA. *Environmental Geology and Water Sciences*, *19*(3), 155-167.

Dridge, E. J., Watts, C. A., Jepson, B. J., Line, K., Santini, J. M., Richardson, D. J., & Butler, C. S. (2007). Investigation of the redox centres of periplasmic selenate reductase from Thauera selenatis by EPR spectroscopy. *Biochemical Journal*, *408*(1), 19-28.

Dumont, E. (2006). *Hyphenated techniques for speciation of Se in biological matrices* (Doctoral dissertation, Ghent University).

Dwijoseputro. 1994. *Dasar – Dasar Mikrobiologi*. Djembata: Jakarta.

Ekawati, Evy Ratnasari, Ni'matuzahroh, Tini Surtiningsih, dan Agus Supriyanto. 2012. Eksplorasi dan Identifikasi Bakteri Selulolitik pada Limbah Daduk Tebu (*Saccharum officinarum* L). *Berk Penel Hayati*. Vol. 18: 31–34.

EPA. 1979. Water-related Environmental Fate of 129 Priority Pollutants. Washington, DC: U.S. Environmental Protection Agency, Office of Water Planning and Standards. EPA 440/4-29-029.

Eswyah, A. S. (2018). *Bioremediation of selenium species in solution by methanotrophic bacteria* (Doctoral dissertation, Sheffield Hallam University).

Ferreira Filho, A. S., Quecine, M. C., Bogas, A. C., de Barros Rossetto, P., de Souza Lima, A. O., Lacava, P. T., ... & Araújo, W. L. (2012). Endophytic Methylobacterium extorquens expresses a heterologous β-1, 4-endoglucanase A (EglA) in Catharanthus roseus seedlings, a model host plant for Xylella fastidiosa. *World Journal of Microbiology and Biotechnology*, *28*(4), 1475-1481.

Ganther, H. E. (1968). Selenotrisulfides. Formation by the reaction of thiols with selenious acid. *Biochemistry*, *7*(8), 2898-2905.

Gebreeyessus, G. D., & Zewge, F. (2019). A review on environmental selenium issues. *SN Applied Sciences*, *1*(1), 55.

Goldhaber, S. B. (2003). Trace element risk assessment: essentiality vs. toxicity. *Regulatory toxicology and pharmacology*, *38*(2), 232-242.

Grützmacher, G., Kumar, P. S., Rustler, M., Hannappel, S., & Sauer, U. (2013). Geogenic groundwater contamination—definition, occurrence and relevance for drinking water production. *Zbl Geol Paläont Teil I*, *1*, 69-75.

Gupta, M., & Gupta, S. (2017). An overview of selenium uptake, metabolism, and toxicity in plants. *Frontiers in Plant Science*, *7*, 2074.

Haygarth, P. M. (1994). Global importance and global cycling of selenium. *Selenium in the Environment*, 1-27.

He, Z. L., Yang, X. E., & Stoffella, P. J. (2005). Trace elements in agroecosystems and impacts on the environment. *Journal of Trace elements in Medicine and Biology*, *19*(2-3), 125-140.

Herawati, N., Suzuki, S., Hayashi, K., Rivai, I. F., & Koyama, H. (2000). Cadmium, copper, and zinc levels in rice and soil of Japan, Indonesia, and China by soil type. *Bulletin of environmental contamination and toxicology*, *64*(1), 33-39

.

Hindersah, R., Arief, D. H., Soemitro, S., & Gunarto, L. (2009). Pengaruh CdCl2 terhadap Produksi Eksopolisakarida dan Daya Hidup Azotobacter. *Jurnal Natur Indonesia*, *12*(01), 34-37.

Hindersah, R., & Kamaluddin, N. N. (2014). Pengaruh Timbal terhadap Kepadatan Sel dan kadar Eksopolisakarida Kultur Cair Azotobacter. *Bionatura*, *16*(1).

Ike, M., Takahashi, K., Fujita, T., Kashiwa, M., & Fujita, M. (2000). Selenate reduction by bacteria isolated from aquatic environment free from selenium contamination. *Water Research*, *34*(11), 3019-3025.

Javed, S., Sarwar, A., Tassawar, M., & Faisal, M. (2015). Conversion of selenite to elemental selenium by indigenous bacteria isolated from polluted areas. *Chemical Speciation & Bioavailability*, *27*(4), 162-168.

Jhonson, J. L. (1984). Nucleic Acid in Bacterial Classification. *Bergey's Manul for Systematic Bacteriology*. Vol. *1*, 8-11.

Johansson, L., Gafvelin, G., & Arnér, E. S. (2005). Selenocysteine in proteins—properties and biotechnological use. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1726*(1), 1-13.

Kathiresan, K., & Selvam, M. M. (2006). Evaluation of beneficial bacteria from mangrove soil. *Botanica Marina*, *49*(1), 86-88.

Khalilian, M., Zolfaghari, M. R., & Soleimani, M. (2015). High potential application in bioremediation of selenate by Proteus hauseri strain QW4. *Iranian journal of microbiology*, *7*(2), 94.

Khalilian, M., Zolfaghari, M. R., Soleimani, M., & Zand Monfared, M. R. (2014). Bacillus sp. strain QW90, a bacterial strain with a high potential application in bioremediation of selenite. *Report of Health Care*, *1*(1), 7-11.

Klonowska, A., Heulin, T., & Vermeglio, A. (2005). Selenite and tellurite reduction by Shewanella oneidensis. *Appl. Environ. Microbiol.*, *71*(9), 5607-5609.

Kozdroj, J., & van Elsas, J. D. (2001). Structural diversity of microorganisms in chemically perturbed soil assessed by molecular and cytochemical approaches. *Journal of Microbiological Methods*, *43*(3), 197-212.

Kwaśna, H., Bateman, G. L., & Ward, E. (2008). Determining species diversity of microfungal communities in forest tree roots by pure-culture isolation and DNA sequencing. *Applied Soil Ecology*, *40*(1), 44-56.

Lemly, A. D. (2004). Aquatic selenium pollution is a global environmental safety issue. *Ecotoxicology and environmental safety*, *59*(1), 44-56.

Lemly, A. D. (1985). Toxicology of selenium in a freshwater reservoir: Implications for environmental hazard evaluation and safety. *Ecotoxicology and Environmental Safety*, *10*(3), 314-338.

Lemly, A. D. (1997). Environmental hazard of selenium in the Animas La Plata water development project. *Ecotoxicology and Environmental Safety*, *37*(1), 92-96.

Liu, Y. T., Chen, T. Y., Mackebee, W. G., Ruhl, L., Vengosh, A., & Hsu-Kim, H. (2013). Selenium speciation in coal ash spilled at the Tennessee Valley Authority Kingston site. *Environmental science & technology*, *47*(24), 14001-14009.

Luoma, S. N., & Rainbow, P. S. (2008). *Metal contamination in aquatic environments: science and lateral management*. Cambridge university press.

Macy, J. M., Rech, S., Auling, G., Dorsch, M., Stackebrandt, E., & Sly, L. I. (1993). Thauera selenatis gen. nov., sp. nov., a member of the beta subclass

of Proteobacteria with a novel type of anaerobic respiration. *International Journal of Systematic and Evolutionary Microbiology*, *43*(1), 135-142.

Maier KJ, Foe C, Ogle RS, *et al.*, 1988. The Dynamics of Selenium in Aquatic Ecosystems. In: Hemphill dd, Ed. Trace Substances in Environmental Health. *XXI Proceedings*. Columbia, MO: University of Missouri, 361-408.

Madani, M. (1997). Memahami Musibah dan Amanah: Kajian atas Surah alAnfal. *dalam Moh. Mahfud MD dkk. (Ed.). Spiritualitas Alquran dalam Membangun Kearifan Umat. Yogyakarta: LPPAI UII.*

Masindi, Vhahangwele dan Khathutshelo L. Muedi. 2018. Environmental Contamination by Heavy Metals. *IntechOpen*. Chapter 7

Mertz, W. (1981). The essential trace elements. *Science*, *213*(4514), 1332-1338.

Mishra, R. R., Prajapati, S., Das, J., Dangar, T. K., Das, N., & Thatoi, H. (2011). Reduction of selenite to red elemental selenium by moderately halotolerant Bacillus megaterium strains isolated from Bhitarkanika mangrove soil and characterization of reduced product. *Chemosphere*, *84*(9), 1231-1237.

Nancharaiah, Y. V., & Lens, P. N. L. (2015). Ecology and biotechnology of selenium-respiring bacteria. *Microbiol. Mol. Biol. Rev.*, *79*(1), 61-80.

NAS. 1976a. Selenium. *Comm Med Biol Effects Environ Pollut Subcomm - Selenium*. Washington, DC: National Academy of Sciences.

Nasrazadani, A., Tahmourespour, A., & Hoodaji, M. (2011). Determination of bacteria resistance threshold to lead, zinc and cadmium in three industrial wastewater samples.

Nei, M., & Kumar, S. (2000). *Molecular evolution and phylogenetics*. Oxford university press.

Nies, D. H. (2000). Microbial heavy-metal resistance. *Appl. Microbiol. Biotechnol.*, *51*, 451-460.

Notodarmojo, S. 2005. *Pencemaan Tanah dan Air Tanah*. Bandung: ITB Press.

Nuttall, K. L. (2006). Evaluating selenium poisoning. *Annals of Clinical & Laboratory Science*, *36*(4), 409-420.

Pelczar., Michael, J., and Chan, E. C. S. 2008. *Dasar-Dasar Mikrobiologi Jilid I*. Jakarta: UI Press.

Priadie, Bambang. 2012. Teknik Bioremediasi sebagai Alternatif dalam Upaya Pengendalian Pencemaran Air. *Jurnal Ilmu Lingkungan*. Volume 10, Issue 1: 38-48.

Pelczar, M. J., & Chan, E. C. S. (2008). Dasar-Dasar Mikrobiologi Dasar Jilid 1.

Priadie, B. (2012). Teknik bioremediasi sebagai alternatif dalam upaya pengendalian pencemaran air. *Jurnal ilmu lingkungan*, *10*(1), 38-48.

Puchkov, E. 2016. Image Analysis in Microbiology: A Review. *Journal of Computer and Communications*, 4, 8 -32.

Quecine, M. C., Lacava, P. T., Magro, S. R., Parra, J. R. P., Araújo, W. L., Azevedo, J. L., & Pizzirani-Kleiner, A. A. (2011). Partial characterization of chitinolytic extract from endophytic Streptomyces sp. and its effects on the boll weevil. *Nong Ye Ke Xue Yu Ji Shu*, *5*(4).

Ravikumar, S., Ramanathan, G., Suba, N., & Jeyaseeli, L. (2002). Quantification of halophilic Azospirillum from mangroves.

Risher, John, A. Rosa McDonald, Mario J. Citra, Stephen Bosch, B.S. dan Richard J. Amata, M.S. 2001. *A Toxicological Profile for Selenium.* Georgia: Division of Toxicology.

Roux, M., Sarret, G., Pignot-Paintrand, I., Fontecave, M., & Coves, J. (2001). Mobilization of selenite by Ralstonia metallidurans CH34. *Appl. Environ. Microbiol.*, *67*(2), 769-773.

Salomons, W., Förstner, U., & Mader, P. (Eds.). (2012). *Heavy metals: problems and solutions*. Springer Science & Business Media.

Schubert, A., Holden, J. M., & Wolf, W. R. (1987). Selenium content of a core group of foods based on a critical evaluation of published analytical data. *Journal of the American Dietetic Association*, *87*(3), 285-299.

Secor, C. L., & Lisk, D. J. (1989). Variation in the selenium content of individual Brazil nuts. *Journal of food safety*, *9*(4), 279-281.

Sen, N., & Naskar, K. (2003). *Algal flora of Sundarbans mangals*. Daya Books.

Shihab, M. Q. (2002). Tafsir al-misbah. *Jakarta: Lentera Hati*, *2*.

Simões, M. F., Antunes, A., Ottoni, C. A., Amini, M. S., Alam, I., Alzubaidy, H., ... & Bajic, V. B. (2015). Soil and rhizosphere associated fungi in gray mangroves (Avicennia marina) from the Red Sea—a metagenomic approach. *Genomics, proteomics & bioinformatics*, *13*(5), 310-320.

Sousa, A. M., Machado, I., Nicolau, A., & Pereira, M. O. (2013). Improvements on colony morphology identification towards bacterial profiling. *Journal of microbiological methods*, *95*(3), 327-335.

Spain, A., & Alm, E. (2003). Implications of microbial heavy metal tolerance in the environment.

Srinivas. T. (2008). *Environmental Biotechnology*. New Delhi: New Age International Publishers.

Stadtman, T. C. (1990). Selenium biochemistry. *Annual review of biochemistry*, *59*(1), 111-127.

Staicu, L. C., Morin-Crini, N., & Crini, G. (2017). Desulfurization: Critical step towards enhanced selenium removal from industrial effluents. *Chemosphere*, *172*, 111-119.

Shils, M. E., & Shike, M. (Eds.). (2006). *Modern nutrition in health and disease*. Lippincott Williams & Wilkins.

Sunde, R. A. (2006). Selenium. Present Knowledge in Nutrition, Bowman BA, Russell RM eds.

Tomei, F. A., Barton, L. L., Lemanski, C. L., Zocco, T. G., Fink, N. H., & Sillerud, L. O. (1995). Transformation of selenate and selenite to elemental selenium byDesulfovibrio desulfuricans. *Journal of Industrial Microbiology*, *14*(3-4), 329-336.

Triana, E., Nurhidayat, N., Yulinery, T., Kasim, E., & Dewi, R. M. (2010). IDENTIFIKASI GEN SELENOMETIL TRANSFERASE (smt) PADA ISOLAT Geobacillus sp. 20K YANG RESISTEN TERHADAP SELENIUM. *Berita Biologi*, *10*(3), 323-328.

U.S. Department of Agriculture, Agricultural Research Service. What We Eat in Americaexternal link disclaimer, 2009-2010

Vinceti, M., Mandrioli, J., Borella, P., Michalke, B., Tsatsakis, A., & Finkelstein, Y. (2014). Selenium neurotoxicity in humans: bridging laboratory and epidemiologic studies. *Toxicology letters*, *230*(2), 295-303.

Waluyo, L. (2005). Mikrobiologi Lingkungan. Malang.

WHO. 1971. International Standards for Drinking-water, Third Edition.

Wyszkowska, J., & Wyszkowski, M. (2002). Effect of cadmium and magnesium on microbiological activity in soil. *Polish Journal of Environmental Studies*, *11*(5), 585-592.

Yazid, M. (2007). Kajian Pemanfaatan Bakteri hasilIsolasi sebagai Agen Bioremediasi Radionuklida Uranium di Lingkungan. *Jurnal Prosiding PPI*.

Sumiwi, S. A. (2018). SELENIUM DAN MANFAATNYA UNTUK KESEHATAN: REVIEW JURNAL. *Farmaka*, *16*(2).

Zeng, L. S., Liao, M., Chen, C. L., & Huang, C. Y. (2007). Effects of lead contamination on soil enzymatic activities, microbial biomass, and rice physiological indices in soil–lead–rice (Oryza sativa L.) system. *Ecotoxicology and Environmental Safety*, *67*(1), 67-74.

ATTACHMENT

Attachment 1. Preparations solution of sodium selenate (Na_2SeO_4)

1. Pembuatan larutan Stok 100mM

Molar mass of Sodium Selenate = 188,85 gr/mol

1 Molar = 1000 mM

1000 mM = 188,85 gr/L

100 mM $= \frac{188,95 \text{ gram}}{10}$

= 18,9 gr/L

Making 100 Mm Selenate stock in 100 ml = $\frac{18,9 \text{ gram}}{10}$

= 1,89 gram

So, to make a 100 ml solution of sodium selenate at a concentration of 100 mM by dissolving 1,89 gram of Na_2SeO_4 powder into 100 ml of distilled water.

2. Making Solution for Treatment

In this study, using 5 different concentrations of sodium selenate on YMEA and YMEB media, including 0mM, 1mM, 2mM, 5mM, and 10mM. To make this concentration, it is carried out using a dilution formula which is:

$$M1 \; x \; V1 = M2 \; x \; V2$$

Description:

M1: Time / concentration of sodium Selenate stock solution

V1: Volume of solution of the stock sodium Selenate were taken

M2: Time / concentration for the required media solution

V2: The total volume of media to be given sodium selenite

Preparation of 1mM selenate media in 15 ml of YMEA agar medium

M1 x V1 = M2 x V2

100 mM x V1 = 1mM x 15 ml

V1 = $\frac{15}{100}$

V1 = 0,15 ml or 150 µl

So, to make a medium for YMEA agar with a concentration of 1 ml as much as 15 ml, take 150 µl sodium selenate from a stock of 100 mM and then add 14.85 ml of YMEA agar medium into a petri dish.

Concentration	Sodium Selenate	YMEA/YMEB Media	Total Volume
0 mM	0 ml	15 ml	15 ml
1 mM	0,15 ml	14,85 ml	15 ml
2 mM	0,3 ml	14,7 ml	15 ml
5 mM	0,75 ml	14,25 ml	15 ml
10 mM	1,5 ml	13,5 ml	15 ml

Appendix 2. Bacterial Growth Curve

The curve of growth of bacteria carried out measurements using a spectrophotometer long wave 600 nm every 2 hours once to found a decrease in the absorbance in the sample.

1. Absorbance results on isolate A-5-1

Time	Media Concentration				
	0 mM	1 mM	2 mM	5 mM	10 mM
0	0,002	0,003	0,021	0,016	0,014
2	0,002	0,004	0,002	0,001	0,001
4	0,005	0,009	0,029	0,008	0,013
6	0,059	0,015	0,051	0,04	0,055
8	0,228	0,246	0,250	0,241	0,234
10	1,704	1,906	1,910	1,910	1,899
12	0,135	0,039	0,036	0,355	0,139
14	0,093	0,053	0,022	0,034	0,087
16	0,034	0,038	0,052	0,058	0,058
18	0,033	0,033	0,022	0,032	0,052

2. Absorbance results on isolate B-10-1

Time	Media Concentration				
	0mM	1 mM	2mM	5mM	10mM
0	0,001	0,002	0,014	0,007	0,016
2	0,005	0,007	0,011	0,008	0,011
4	0,031	0,021	0,018	0,012	0,01
6	0,033	0,033	0,05	0,067	0,042
8	0,092	0,082	0,069	0,069	0,032
10	0,549	0,54	0,539	0,538	0,53
12	1,912	1,912	1,898	1,884	1,906
14	0,537	0,537	0,563	0,549	0,546
16	0,193	0,123	0,142	0,161	0,129
18	0,059	0,053	0,022	0,032	0,047
20	0,013	0,043	0,022	0,032	0,052

Appendix 3 . Nucleotide Base Order

1. Isolate A-5-1

>A-16S_F

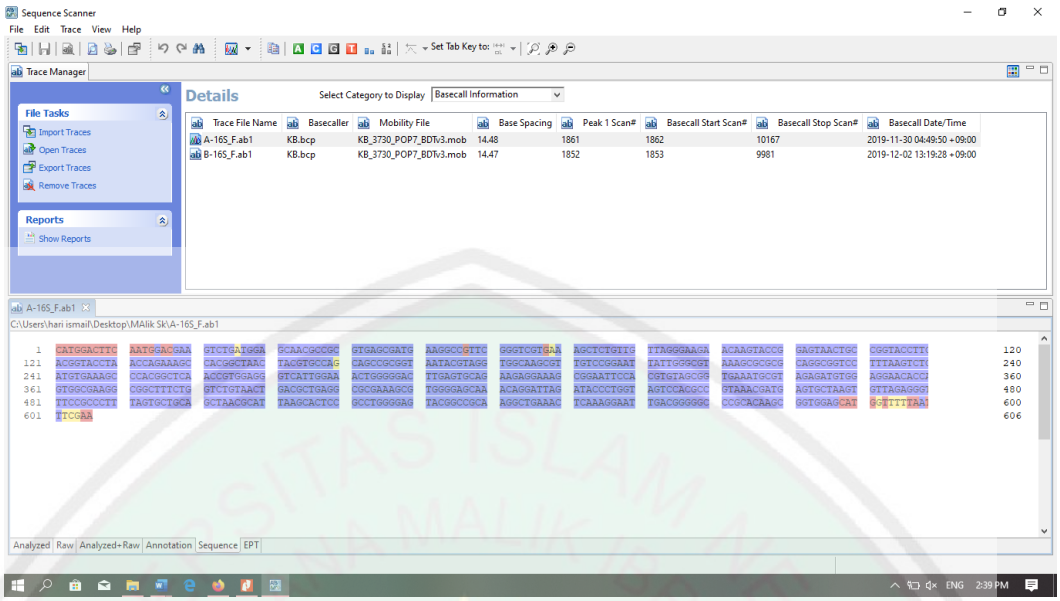
CATGGACTTCAATGGACGAAGTCTGATGGAGCAACGCCGCGTGAGCG
ATGAAGGCCGTTCGGGTCGTGAAAGCTCTGTTGTTAGGGAAGAACAA
GTACCGGAGTAACTGCCGGTACCTTGACGGTACCTAACCAGAAAGCC
ACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGC
GTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCCTTTAAG
TCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACT
GGGGGACTTGAGTGCAGAAGAGGAAAGCGGAATTCCACGTGTAGCGG
TGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTT
CTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAG
GATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGT
TAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCC
GCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGGTTTTTAATTTCGAA

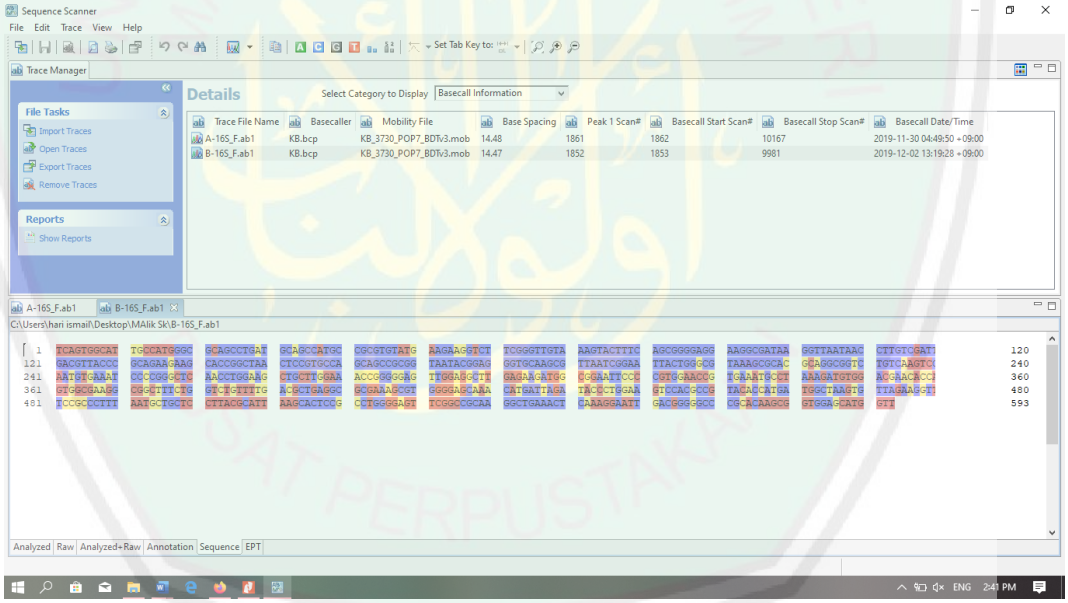
2. Isolate B-10-1

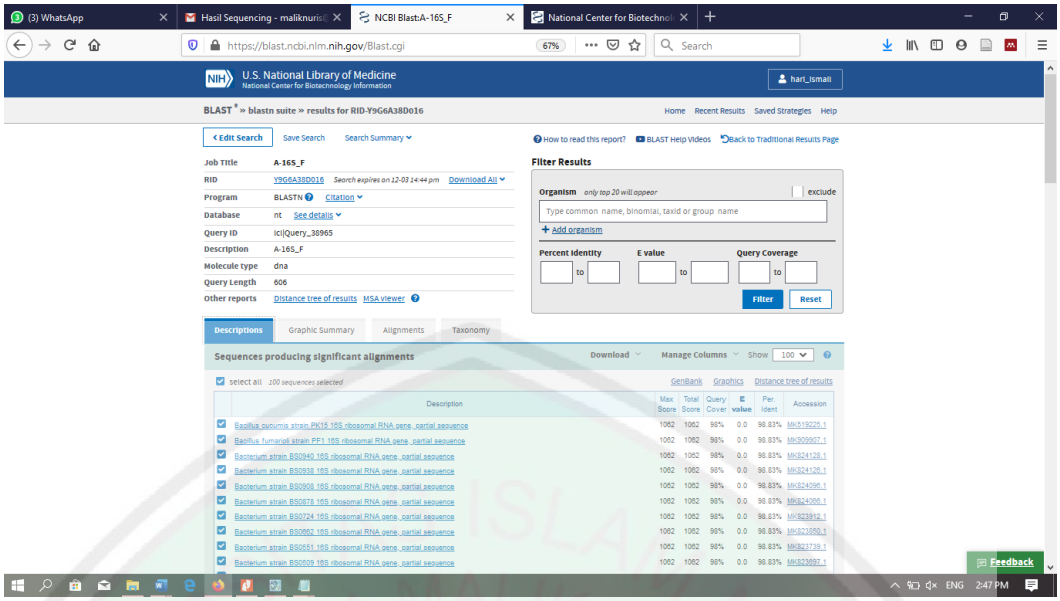
>B-16S_F

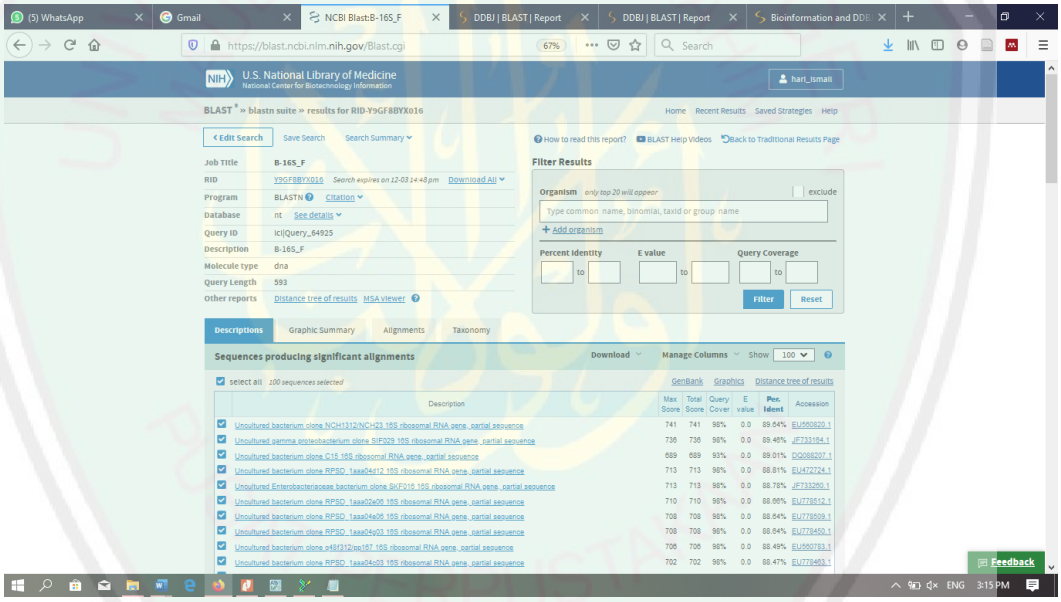
TCAGTGGCATTGCCATGGGCGCAGCCTGATGCAGCCATGCCGCGTGTA
TGAAGAAGGTCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGC
GATAAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCAC
CGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCG
TTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGT
CGAATGTGAAATCCCCGGGCTCAACCTGGAAGCTGCTTGGAAACCGGG
GGAGTTGGAGGCTTGAGAAGATGGCGGAATTCCCCGTGGAACCGTGA
AATGCCTAAAGATGTGGACGAACACCAGTGGCGAAGGCGGCTTTCTG
GTCTGTTTTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACATGATTA
GATACCCTGGAAGTCCACGCCGTACACCATGATGGCTAAGTGTTAGAA
GGTTTCCGCCCTTTAATGCTGCTCCTTACGCATTAAGCACTCCGCCTGG
GGAGTTCGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCG
CACAAGCGGTGGAGCATGGTT

Appendix 4 . Isolate Sequencing Results









Appendix 5. Sampling Locations

KEMENTERIAN AGAMA
UNIVERSITAS ISLAM NEGERI
MAULANA MALIK IBRAHIM MALANG
FAKULTAS SAINS DAN TEKNOLOGI
JURUSAN BIOLOGI
Jl. Gajayana No. 50 Malang 65144 Telp./ Faks. (0341) 558933
Website: http://biologi.uin-malang.ac.id Email: biologi@uin-malang.ac.id

KARTU KONSULTASI AGAMA SKRIPSI

Nama : Malik Nuris Su'aidi
NIM : 15620077
Program Studi : Biologi
Semester : Ganjil (IX)
Pembimbing : Romaidi, M.Si., D.Sc.
Judul Skripsi : Isolasai dan Identifikasi Bakteri Resisten Selenate (SeO_4^{2-}) dari sedimen Mangrove pantai Banyuglugur Kabupaten Situbondo, Jawa Timur.

NO.	TANGGAL	URAIAN KONSULTASI	TTD PEMBIMBING
1.	14-08-2019	Konsultasi BAB I, II, III	1.
2.	15-08-2019	ACC BAB I, II, III	2.
3.	03-12-2019	Konsultasi BAB IV	3.
4.	05-12-2019	ACC Skripsi	4.

Malang, 26 Desember 2019

Pembimbing Skripsi

M. Mukhlis Fahruddin, M.S.I
NIPT.20142011409

Ketua Jurusan,

Romaidi, M.Si, D.Sc
NIP. 19810201 200901 1 019

KEMENTERIAN AGAMA
UNIVERSITAS ISLAM NEGERI
MAULANA MALIK IBRAHIM MALANG
FAKULTAS SAINS DAN TEKNOLOGI
JURUSAN BIOLOGI
Jl. Gajayana No. 50 Malang 65144 Telp./ Faks. (0341) 558933
Website: http://biologi.uin-malang.ac.id Email: biologi@uin-malang.ac.id

KARTU KONSULTASI SKRIPSI

Nama : Malik Nuris Su'aidi
NIM : 15620077
Program Studi : Biologi
Semester : Ganjil (IX)
Pembimbing : Romaidi, M.Si., D.Sc.
Judul Skripsi : Isolasai dan Identifikasi Bakteri Resisten Selenate (SeO_4^{2-}) dari sedimen Mangrove pantai Banyuglugur Kabupaten Situbondo, Jawa Timur.

NO.	TANGGAL	URAIAN KONSULTASI	TTD PEMBIMBING
1.	09-01-2019	Konsultasi judul skripsi	1.
2.	14-01-2019	Revisi judul skripsi	2.
3.	15-01-2019	Konsultasi BAB I dan III	3.
4.	13-02-2019	Konsultasi BAB II	4.
5.	04-08-2019	Revisi BAB I II dan III	5.
6.	14-08-2019	ACC Proposal	6.
7.	20-11-2019	Konsultasi BAB IV	7.
8.	03-11-2019	Revisi BAB IV	8.
9.	05-11-2019	Konsultasi BAB IV V dan Daftar Pustaka	9.
10.	02-12-2019	Konsultasi analisis data dan lampiran	10.
11.	05-12-2019	ACC Skripsi	11.

Malang, 26 Desember 2019

Mengetahui, Pembimbing Skripsi

Romaidi, M.Si, D.Sc
NIP. 19810201 200901 1 019

Mengetahui, Ketua Jurusan,

Romaidi, M.Si, D.Sc
NIP. 19810201 200901 1 019