POTENTIAL OF HYDROCARBONOCLASTIC BACTERIA FOR DEGRADING DIESEL OIL IN THE PORT OF GRESIK

THESIS

BY:

MOHAMED ABDIRAHMAN OMAR

NIM: H44219019

UNIVERSITAS ISLAM NEGERI SUNAN AMPEL

FAKULTAS SAINS DAN TEKNOLOGI

PROGRAM STUDI ILMU KELAUTAN

SURABAYA

2023

STATEMENT OF AUTHENTICITY

The undersigned

Name: Mohamed Abdirahman Omar

Nim: H44219019

Study Program: Marine Science

Class: 2019

Declaring that I have not committed plagiarism in writing my thesis, Potential of hydrocarbonoclastic bacteria for degrading diesel oil in the port of Gresik. If one day it is proven that I have committed plagiarism, then I will accept the sanctions that have been determined. Thus, I make this statement of authenticity honestly.

Surabaya 05 April 2023

Mohamed A. Omar

H44219019

VALIDATION SHEET

Thesis By:

Name: Mohamed Abdirahman Omar

Nim: H44219019

Title: Potential of hydrocarbonoclastic bacteria for degrading diesel oil in the port of Gresik

Checked and approved by the supervisor

Date: April 03, 2023

Supervisor I	Supervisor II
Mauludiyah, MT	Wiga Alif Violando, M. P
NUP.201409003	NIP. 199203292019031012

Approval Sheet of the Thesis Team

Thesis by: Mohamed Abdirahman Omar

Student number: H44219019

Thesis title: Potential of hydrocarbonoclastic bacteria for degrading diesel oil in the port of Gresik.

Study program: marine science

Examiner I

Maulidiyah, MT

NUP: 201409003

Examiner II

Wiga Alif Violando M.P., M. Si

NIP: 199203292019031012

Examiner III

Dian Sari Maisaroh, M. Si

NIP: 198908242018012001

Examiner IV

ANDIK DWI MUTTAQIN

NIP: 198204102014031001

Dean of faculty of science and technology

Dr. As Saepul Hamdani, M. Pd

Nip. 196507312000031002

KEMENTERIAN AGAMA
UNIVERSITAS ISLAM NEGERI SUNAN AMPEL SURABAYA
PERPUSTAKAAN
Jl. Jend. A. Yani 117 Surabaya 60237 Telp. 031-8431972 Fax.031-8413300
E-Mail: perpus@uinsby.ac.id

LEMBAR PERNYATAAN PERSETUJUAN PUBLIKASI
KARYA ILMIAH UNTUK KEPENTINGAN AKADEMIS

Sebagai sivitas akademika UIN Sunan Ampel Surabaya, yang bertanda tangan di bawah ini, saya:

Nama : Mohamed Abdirahman Omar
NIM : H44219019
Fakultas/Jurusan : Sains dan Teknologi/Ilmu Kelautan
E-mail address : mohaomar.info@gmail.com

Demi pengembangan ilmu pengetahuan, menyetujui untuk memberikan kepada Perpustakaan UIN Sunan Ampel Surabaya, Hak Bebas Royalti Non-Eksklusif atas karya ilmiah :
☒ Sekripsi ☐ Tesis ☐ Desertasi ☐ Lain-lain (.....)
Yang berjudul :
Potential of hydrocarbonoclastic bacteria for degrading diesel oil in the port of Gresik
Rob di Kabupaten Gresik, Jawa Timur

beserta perangkat yang diperlukan (bila ada). Dengan Hak Bebas Royalti Non-Ekslusif ini Perpustakaan UIN Sunan Ampel Surabaya berhak menyimpan, mengalih-media/format-kan, mengelolanya dalam bentuk pangkalan data (database), mendistribusikannya, dan menampilkan/mempublikasikannya di Internet atau media lain secara ***fulltext*** untuk kepentingan akademis tanpa perlu meminta ijin dari saya selama tetap mencantumkan nama saya sebagai penulis/pencipta dan atau penerbit yang bersangkutan.

Saya bersedia untuk menanggung secara pribadi, tanpa melibatkan pihak Perpustakaan UIN Sunan Ampel Surabaya, segala bentuk tuntutan hukum yang timbul atas pelanggaran Hak Cipta dalam karya ilmiah saya ini.

Demikian pernyataan ini yang saya buat dengan sebenarnya.

Surabaya, 17 April 2023

Penulis

(Mohamed A. Omar)

ABSTRACT

The high density of traffic at the port of Gresik, located in the Madura Strait, raises a new problem, namely the increasing number of ships, which increases the capacity of refueling diesel oil on ships, resulting in a volume of waste containing oil that tends to increase. In conditions of overcapacity and proneness to accidents, shipping will be at risk of an oil spill disaster from the ship's cargo. Alternative countermeasures are appropriate and do not disturb the environment by using hydrocarbonoclastic bacteria that can degrade diesel oil which is called bioremediation method. The purpose of this research is to find out which bacteria have the potential to degrade diesel oil in Gresik Port and to determine the ability of these bacterial isolates to degrade diesel oil. In laboratory experiments, a diesel oil-degrading bacterial consortium was used to biodegrade diesel oil. The bacterial consortium was prepared in liquid medium for laboratory experiments, and the water sample was taken at the port of Gresik. This study uses a purposive sampling method in seawater sampling and uses experimental methods in the laboratory for bacterial isolation, oil degradation, characterization of bacterial observations, and biodegradation tests on diesel oil. The results of research on the waters of the Port of Gresik were obtained 4 isolates of diesel oil degrading bacteria, where isolates are found bacteria, namely the genus *Marinobacter xestospongiae*. The results of the identification of bacteria capable of degrading diesel oil significantly are a consortium (mixed isolates in the genus *Marinobacter xestospongiae*) with a biodegradation percentage of 92.25%.

Keywords: Bioremediation, Hydrocarbonoclastic bacteria, Diesel oil, Oil spill.

TABLE OF CONTENT

Contents

LIST OF FIGURES

LIST OF TABLES

CHAPTER I
INTRODUCTION

1.1 Research background

Indonesia is an archipelagic country with two-thirds of its territory is waters. Sea transportation becomes the dominant means has a function to facilitate inter-island and inter-island relations Country. Located in strategic conditions, the Archipelago State as a stopover on the world route. Ports in Indonesia have a very vital function for sea transportation and trade activities. The function of the port is as a transportation infrastructure for layover, refueling, transport, and unloading passengers, loading and unloading of goods, and disposal of ballast water in boat. Ships entering the port to carry out routines tend to increase every year, both from within the country as well as from abroad.

The port of Gresik is one of the important ports in Gresik used by ships carrying goods, fishing boat from Gresik and outside of Gresik, as well as ship arrivals and departures are quite high so that loading and unloading of goods from ships, that are very important process for the running of the economy. Port terminal operations, such as loading/unloading time, refueling to the ship can cause oil spill in the ocean. As a result of terrestrial and freshwater runoff, waste from nearby oil refineries, offshore oil production, shipping activities, and unintentional accidents, petroleum hydrocarbons are major contaminants of marine habitats. (Arulazhagan et al., 2010).

Diesel oil is a distillate type of fuel containing fractions of heavy fraction or is a mixture of light fraction distillate and heavy fraction (residual fuel oil) and is dark black in color, but remains liquid at low temperatures. Use This diesel oil is generally used as fuel for diesel engines with high rpm medium or slow (300–1,000 RPM) or can also be used as a direct combustion fuel in industrial kitchens. This diesel oil usually called Industrial Diesel Oil (IDO) or Marine Diesel Fuel (MDF). Composition of Diesel oil is a compound of hydrocarbons and non-hydrocarbons. Compound the hydrocarbons in diesel oil are naphthenic, paraffinic and aromatic. As for non-hydrocarbon compounds in the form of non-metallic elements and metal. Non-metal elements such as nitrogen, sulfur and oxygen, while metals such as nickel, iron and vanadium (PERTAMINA 2005).

This research aims to know the characteristics of successful bacteria isolated from the seawater sample of the port of Gresik, to determine the ability to degrade diesel Oil pollutants.

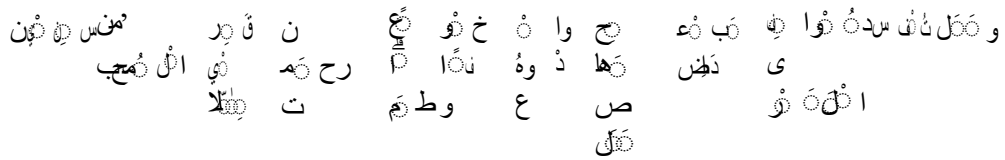
Seawater that has been polluted by oil spills will become difficult to be cleaned so as to block the sunlight that will enter and make the dissolved oxygen level decrease so that it can have an impact on the total destruction (disaster) of marine biota (Nugroho, 2007). Some of the oil components resulting from the impact of pollution will sink and accumulates in sediments as a black deposit on sand and rocks on the coast. While the oil component that is not soluble in water will float on the surface of the sea water and can cause cloudy sea water (Mukhtasor, 2006).

Some sources of marine pollution include: oil spills, waste products from the process use of ships, waste products from industry at sea, the occurrence of the drilling process oil in the sea, the results of organic and inorganic waste disposal carried out by land transportation from river flows, emissions from sea transportation and pesticide product discharge from agriculture. However, the main factor of pollution in the sea, which is sourced from oil spills that occur in the sea, either from ship processes, offshore drilling results or as a result of ship accidents (Sulistyono, 2012).

Oil spills at sea due to accidents and the increased volume of oil waste that is carried out every day will cause pollution effects on the waters of the port as a whole. The impact of this pollution will damage the quality of the water and the biota that exist in water bodies. As explained by Allah SWT in the Al-Qur'an letter AR-Rum verse 41, which reads:

Evil (sins and disobedience of Allah, etc.) has appeared on land and sea because of what the hands of men have earned (by oppression and evil deeds, etc.), that Allah may make them taste a part of that which they have done, in order that they may return (by repenting to Allah, and begging His Pardon). (Tafsir: MUHSIN KHAN).

According to Ali (2009), in Tafsir Yusuf Ali, the sentence "La'allahum Yarji'un" means that the ultimate goal is God's justice, and His punishment is that humans are ordered to repair any damage and return it to its origin as it was when He created it. The verse shows that humans are highly recommended in every deed or work to do good, including in protecting the earth, which is human habitation. Humans should control themselves not to make mischief on earth, either on land or in the sea. Allah SWT says in the Qur'an's Surah al-A'raaf verse 56, which reads:



Tafsir

"And do not do mischief on earth after it was created well. Pray to Him with fear so that you are more reverent and compelled to obey Him, and full of hope for His grace and the answer to your prayer. Indeed, Allah's mercy is very close to those who do well."

In this verse, Allah forbids humans from causing damage to the earth. The prohibition on making damage covers all areas, such as damaging the associations, physical and spiritual, of other people, their lives and sources of livelihood (agriculture, trade, etc.), the environment, and so on. This earth has been created by Allah with all its features, such as mountains, valleys, rivers, oceans, land, forests, and others, all of which are intended for human needs, so that they can be processed and utilized as well as possible for their welfare. Therefore, humans are prohibited from causing damage to the earth. In addition, Allah also sent down religion and sent messengers to give instructions so that humans can live in happiness, security, and peace. As the end of his prophethood approached, Allah sent Rasulullah SAW, who brought Islamic teachings as a mercy to the universe. If people follow Islamic teachings correctly, then everything will be good, people will be good, the nation will be good, and the country will be good too. Islam has taught about environmental preservation in accordance with the concept of returning to nature, namely the awareness that man's duty as a servant of God is to recognize that the universe and everything in it belong to Allah. Therefore, humans are prohibited from doing damage as a form of submission and obedience to Him (Suriyani and Kotijah, 2013). Oil spills may generally be cleaned up using a variety of techniques, including physical, chemical, and biological ones. One of the simplest ways to clean up an oil spill is biological, such as bioremediation.

Bioremediation of oil spills requires the identification of microbes which have the ability to degrade hydrocarbons present in the soil or water, so that in case of a large spill these can be stimulated further in order to clean-up the area. Identification of such strains can ensure better efficiency of remediation as these strains will be well adapted to grow in the respective environment. Numerous bacteria have been discovered to be capable of degrading the hydrocarbons left over from oil spills. These comprise several bacterial, fungal, yeast, and algal strains. Biodegradation is a natural process carried out by microorganisms to break down organic compounds into biomass and simpler form of water, carbon dioxide, and methane (Kazuya, 2001).

According to Nugroho (2007), hydrocarbonoclastic bacteria are bacteria that have the ability to produce hydrocarbon compounds for purposes of metabolism and development. Bacteria group Hydrocarbonoclastics are an easy group of bacteria to adapt to the environment, so it can use the residue of petroleum as a source of carbon and energy (Harayama et al., 1999:67).

Bacteria hydrocarbonoclastic are a group of bacteria that can easily adapt to the environment by using petroleum residue one of them is diesel oil as a source of carbon and energy. Bacteria hydrocarbonoclastic can carry out the emulsification process, namely when bacteria attached to the hydrocarbon compounds in diesel oil, the bacteria will produce biosurfactants which function to make diesel oil which hard to dissolve again to be soluble. Biosurfactants are excreted in oil compounds diesel fuel to help release the hydrocarbon bonds in diesel oil. This Process can reduce the surface tension of a liquid and the interfacial tension interface between the two different phases as well as increasing the stability of the emulsion. After in this process, hydrocarbonoclastic bacteria will carry out the biodegradation process by breaking down organic compounds into biomass and simpler form of water, carbon dioxide, and methane which are safe for environment (Gouma, 2014).

Ex situ research was conducted by Nasikhin (2013) with samples of seawater taken at a port to carry out research entitled Isolation and Characterization of Degrading Bacteria Diesel and Gasoline from Gresik Port Waters, identifying capable microbes that degrade diesel and gasoline in the port waters (Bacillus sp). This is the basis for the need to carry out research to test the potential of bacteria that have the ability to degrade diesel oil and identify the type of bacteria in the Port of Gresik.

1.2 Research question

The questions in this research are:

1. What are the hydrocarbonoclastic bacterial isolates found in seawater?
2. How is the effectiveness of oil spill degradation by hydrocarbonoclastic bacteria?

1.3 Research purpose

1. To analyze the hydrocarbonoclastic bacterial isolates found in seawater.
2. To analyze the effectiveness of oil spill degradation by hydrocarbonoclastic bacteria.

1.4 Research benefits

This research report is expected to provide information about the potential for hydrocarbonoclastic bacteria to degrade diesel oil.

1.4 Scope of problem

Based on the explanation mentioned above, then Limitations of problems that can be put forward in research are:

1. Bioremediation research on diesel oil pollution at ports Gresik was carried out ex situ, namely on a laboratory scale.
2. The bacteria used are indigenous bacteria (original bacteria) that originating from Port Gresik which has been bred.

CHAPTR II
LITERATURE REVIEW

2.1 Environmental pollution

Environmental pollution usually uses bacteria for biodegradation. The science of molecular ecology explains that less than 1% of microorganisms in the environment can be overcome by known culture techniques. Hydrocarbonoclastic microbes are a group of microbes that are able to use carbon sources derived from hydrocarbon compounds. This microbe has a hydrocarbon oxidizing enzyme in the form of an -hydroxylase enzyme. Natural hydrocarbon sources in the form of petroleum under atmospheric pressure and temperature conditions in the form of a liquid/solid phase obtained from the mining process. Hydrocarbons are compounds that are difficult to decompose because they can evaporate, be washed away by rainwater or enter the soil and precipitate as much as toxic substances. environmental pollution, namely the entry or inclusion of living things, substances, energy or other components into the environment and changes in the environmental order by human activities or natural processes, so that the quality of the environment drops to a certain level which causes the environment to become less or no longer functioning in accordance with allotment. (Munfarida, 2017).

2.2 Petroleum

Petroleum is the main energy source used in households, industry and transportation. This has led to increased exploration, exploitation, processing and transportation of petroleum production activities to meet human needs, thereby increasing the tendency to pollute the environment, especially in coastal areas. The pollution comes from oil refinery waste, by-products from the production, distribution and transportation processes. Waste generated from oil refineries in the form of liquid waste and solid waste. Petroleum mud (drilling mud) waste has both fatal (deadly) and sublethal effects on coral reefs, mangroves, and aquatic biota in coastal habitats (inhibiting growth, reproduction and other physiological processes). This is due to the complex hydrocarbon chemicals found in crude oil, such as the modest levels of aromatic compounds found in BTEX (benzene, toluene, ethyl benzene, and xylene isomer, or BTEX), which are hydrocarbons. But have a very large effect on pollution, water. In the case that occurred, oil in the Guilt of Eilat (Red Sea) had damaged the gonads of Stylophora pistillata, reduced the survival rate of coral colonies and reduced the amount of planula production and the spill of diesel oil and "Bunker C" Witwater oil in the Panama area 1968 caused seeds Avicennia and Rhizophora sp. and various

invertebrates, turtles, birds and algae that live in intertidal mangrove areas died, as well as many other cases such as fuel oil spills for steam gas power plants (PLTGU) sourced from oil barges transporting oil (Kompas, February 21, 2004).

2.2.1 Petroleum waste treatment

Petroleum waste treatment is carried out physically, chemically and biologically. Physical processing is carried out for initial treatment, namely by localizing the oil spill using oil booms, which will then be transferred by pumping devices (oil skimmers) to a "reservoir" receiving facility in the form of tanks or balloons and proceed with further processing. Chemical, but the cost is expensive and can cause new contaminants. Biological waste treatment is an alternative that is cost effective and safe for the environment. Processing with biological methods is also called bioremediation, namely biotechnology that utilizes living things, especially microorganisms to reduce the concentration or toxicity of pollutants (Ministry of Environment Decree No. 128, 2003).

2.2.2 Hydrocarbon components

Petroleum is mostly a hydrocarbon compound. According to Budiarto (1999), petroleum hydrocarbon compounds which are degraded by microorganisms can be classified based on the above 3 groups, namely:

1. *Pariffinic (aliphatic) Hydrocarbons*

   Pariffin hydrocarbon compounds, often called hydrocarbons Alkanes are saturated hydrocarbon compounds consisting of Normal paraffin is in the form of long, straight carbon chains, while isoparaffins have branched carbon atoms. Hydrocarbon alkanes (paraffin series) have the formula CnH_2n+_2, and they don't. It has double bonds between its carbon atoms. This compound is the largest fraction in the composition of petroleum.

2. *Naphthene (alicyclic) Hydrocarbons*

   Naphthene (alicyclic) compounds are characterized by the presence of simple closed rings of the constituent carbon atoms. In general, the chemical formula is CnH2n and has no double bonds between carbon atoms. As well as hydrocarbon

compounds, naphthenes (alicyclics) are insoluble in water and are a fraction of the second largest in the composition of petroleum.

3. *Aromatic Hydrocarbons*

Aromatic hydrocarbon compounds are characterized by the presence of a ring containing six carbon atoms, with bonds doubling between every other carbon atom, so there are three double bonds in the ring. This compound has the formula CnH2n-6 for a single-ring molecule and CnH_2n-12 for a double-ring molecule, and it smells (flavoured). Benzene is the simplest aromatic compound. There are fewer aromatic hydrocarbons in petroleum compared to paraffinic (aliphatic) hydrocarbons.

2.2.3 Non-hydrocarbon components

In addition to being composed of components derived from hydrocarbons, petroleum also contains small amounts of non-hydrocarbon components. Non-hydrocarbon components can be metal elements or those with metal-like properties, as well as other organic components. According to Atlas and Bartha (1992), the non-hydrocarbon components in petroleum are, namely:

1. Sulfur

   Sulfur compounds are the largest non-hydrocarbon component in petroleum. Sulfur has a compound form like sulfide, thiophene.

2. Oxygen

   Oxygen compounds in petroleum have a low concentration. Oxygen compounds can be in the form of naphthenic acids, phenols,and fatty acids.

3. Nitrogens

   In general, nitrogen compounds are present in small amounts.crude oil. Compounds found in nitrogen are quinoline,pyridine, isoquinoline, pyrrole, carbazole, and indone.

4. Metal

   Metal compounds are found in petroleum, among others, in the form of compounds,organic metal complexes and inorganic salts. Complex compounds

Organic metals in petroleum contain metals such as iron (Fe), cobalt (Co), vanadium (Vo), and nickel (Ni).

2.3 Diesel oil

Diesel oil is a product of the petroleum distillation process used specifically as fuel for compression ignition engines. Compression ignition is a compressed air engine so that it can cause a high pressure and heat and can burn the diesel that has been sprayed on the injector. Diesel oil starts from gas oil with a boiling point range between 250 °C to 350 °C which is commonly referred to as middle distillate. Composition of Diesel oil is a compound of hydrocarbons and non-hydrocarbons. Compound the hydrocarbons in diesel oil are naphthenic, paraffinic and aromatic. As for non-hydrocarbon compounds in the form of non-metallic elements and metal. Non-metal elements such as nitrogen, sulfur and oxygen, while metals in the form of nickel, iron and vanadium (Pertamina, 2005).

2.4 Oil pollution sources

According to Turiman Mijaya (2004:4) mentioned in his book which includes sources of pollution, namely:

1. Oil spill due to an accident

Oil spills caused by accidents are relatively large and the impact is large, but this is rare. For example, the ship ran aground, sank or collided with tankers or goods carrying oil or fuel.

2. Oil spill due to operational activities

Oil spills that occur are relatively small in number and their direct impact is also small, but this is that often occur so that it is very harmful to the environment.

a. From oil fields under the seabed, either through seepage or drilling errors in offshore operations.
b. From tanker operations where oil is wasted at sea as a result of tank cleaning, ballast water disposal and etc.
c. From ships other than tankers through bilge water disposal (got)
d. From port terminal operations, where oil can spill on loading/unloading time, refueling to the ship.
e. From refinery disposal waste.
f. From land sources, as in the use of lubricating oil or liquids containing hydrocarbon.

g. From hydrocarbons that fall from the atmosphere, for example factory chimneys, ship chimneys, airplanes and so on. The number of hydrocarbons that fall to Earth reaches 9% of the causes of pollution.

2.5 Diesel oil's impact on marine pollution

Marine pollution can be characterized by changes in marine environment that has a negative impact so that it can cause the destruction of marine resources, namely marine living resources, adversely affect human health, causing changes in activity in the sea, the emergence of a decrease in fishery products, a decrease in the quality of sea water and quality of utilization (Misran, 2002)

Some sources of marine pollution include: oil spills, waste products from the process use of ships, waste products from industry at sea, the occurrence of the drilling process oil in the sea, the results of organic and inorganic waste disposal carried out by land transportation from river flows, emissions from sea transportation and pesticide product discharge from agriculture. However, the main factor of pollution in the sea, which is sourced from oil spills that occur in the sea, either from ship processes, offshore drilling results or as a result of ship accidents (Sulistyono, 2012).

2.5.1 Oil spill and their effect on marine biota

Oil pollution causes reproductive and developmental problems, as well as, damage to the brain, liver, and kidneys of fish, marine mammals, and terrestrial species. Turtle at risk, because they tend not to remove themselves from areas contaminated with oil where a lot of oil accumulates. Therefore, Oil spills can pose a threat to water, swamps and ecosystems coast that is often affected by oil. For example, an oil spill Exxon Valdez causes contamination of fish along with embryos and larvae juveniles, and chronic effects on seabird foraging sediments resulting in decrease in their abundance (Peterson et al., 2003). About 10% of the total input from a devastating oil spill, which caused both damage ecological and economical. According to Hinchee et al. (1995),

• Oil spills kill marine biota and damage the ecosystem, which can last for generations, by forcing changes in reproduction and compromising complex food webs. Oil spills can cause damage to the waterways of birds and animals, destroy their immune systems, interrupt breeding, and foul breeding grounds. Besides, they thin bird and turtle egg shells and also damage the fish larvae, causing deformities. And also cause damage to sea grass beds and other shelter and feeding areas

through the tainting of algae, which perform a vital role in waterway ecosystems. [Web Ref. 7, 8].

• Due to oil spill on soil the insects and worms living in it are killed due to hydrocarbon toxicity, lack of oxygen supply and it reduce the pH of the soil. This affects the fertility of soil and its productivity in terms of the growth of plants [Wokocha, 2011].

As a result, the soil ecosystem is disturbed, which has an impact on plant growth. Additionally contaminating groundwater, soil oil makes people and animals sick when consumed.

2.6 Oil spill cleaning techniques

Oil is currently a nonrenewable energy source in everyday life. This fossil fuel is used for a variety of purposes, including transportation and manufacturing. Unfortunately, oil spills sometimes occur within the environment due to accidents and unavoidable actions such as weather and earthquakes or through intentional spills from war and dumping. Several methods can be used to clean up oil spills and to prevent further destruction by this hazardous constituent. The cleanup and recovery of spilled oil is difficult and dependent on a variety of factors, including the type of oil spilled, the climatic conditions of spilled site which includes temperature of the water, tidal intensity and the types of shorelines and beaches involved. In general, spilled oil can be cleaned in three methods namely physical, chemical and Natural methods.

2.6.1 Biodegradation of diesel oil

Biodegradation is a natural process carried out by microorganisms to break down organic compounds into biomass and simpler form of water, carbon dioxide, and methane (Oetomo, 2015).

2.6.2 Bioremediation

Bioremediation is a method used to change the environment toxic to harmless using microorganisms in particular microbes. Generally, the application of bioremediation uses bacteria or fungi originating from the polluted area (indigenous). Use Microorganisms can also be isolated from other polluted areas and applied in the destination area (Vidali, 2011). Bioremediation is the optimization of the process biodegradation, which starts with conditioning certain areas so that they can be overcome the factors that can be a barrier to the rate of biodegradation of petroleum components (Nugroho, 2007).

Microorganisms, especially bacteria that are able to degrade the compounds contained in petroleum hydrocarbons are called hydrocarbonoclastic bacteria. These bacteria are able to degrade hydrocarbon compounds by utilizing these compounds as a source of carbon and energy needed for growth. These microorganisms are able to decompose petroleum components because of their ability to oxidize hydrocarbons and make hydrocarbons as electron donors. These microorganisms participate in cleaning up oil spills by oxidizing petroleum into carbon dioxide gas (CO_2), petroleum-degrading bacteria will produce bioproducts such as fatty acids, gases, surfactants, and biopolymers that can increase the porosity and permeability of reservoir rocks of clastic and carbonate formations. When these bacteria decompose petroleum. The following is the degradation reaction of aromatic fraction hydrocarbon compounds by bacteria which begins with the formation of Pro-to-ca-techua-te or catechol or compounds that are structurally related to these compounds. These two compounds are further degraded into compounds that can enter the Krebs cycle (citric acid cycle), namely succinate, acetyl CoA, and pyruvate. Hydrocarbonoclastic bacteria include Pseudomonas, Arthrobacter, Alcaligenes, Brevibacterium, Brevibacillus, and Bacillus. These bacteria are widely distributed in nature, including in waters or sediments polluted by petroleum or hydrocarbons. We only need to isolate the hydrocarbonoclastic bacteria from nature and culture it, then we can use it as an effective and efficient petroleum waste treatment plant, and is environmentally friendly. Bioremediation is the use of microorganisms, in particular microbes to degrade a hazardous (toxic) environment harmless form. In its application, the process Bioremediation uses bacteria, fungi, and plants to degrade substances that are harmful, both to human health and environment. Microorganisms used to degrade can come directly from contaminated areas (indigenous bacteria) or can also be microorganisms isolated from places another is then applied to the contaminated area (bacterial endogenous) (Vidali, 2001).

Bioremediation is an optimization of the biodegradation process. In this matter, certain environmental conditioning and treatments are specifically required to address limiting environmental factors affecting the rate of microbial degradation of petroleum (Nugroho, 2006). Bacterial activity in degrading petroleum in water depends on the physiology of the bacteria and the condition of several parameters in the local environment, such as pH, temperature, and the availability of nutrients. The election of the appropriate inoculum can create optimal conditions for the rate at which bacteria accelerate the degradation process so that it is a possible occurrence

and a reduction in the concentration of petroleum hydrocarbons is optimal (Udiharto, 1996). These factors can be described as follows:

1. Physical State of Crude Oil

   Hydrocarbons used by bacteria are those that have a liquid form, whereas solid hydrocarbons can only be degraded when dissolved in liquid hydrocarbons (Nugroho, 2006). The surface area of the oil is available for bacterial colonization Degradation of oil is important because microbes work on the water-oil interface (Andina, 2014).

2. Nutrition

   Microbes rely heavily on nutrients to survive. Whether or not the microbes survive will be seen from nutritional adequacy. According to Gordon (1994) it is explained that Nutrients are supports for life, development multiply and produce enzymes to degrade hydrocarbons. The nutrients needed by microbes vary according to the type of microbe, but all microbes require nitrogen, phosphorus, and carbon. Carbon is the most basic element for all forms Life and carbon are needed in greater quantities than other elements, carbon is usually bonded to the element H. (Hydrogen), O (Oxygen), and N (Nitrogen). These four elements composing nearly 95% of the weight of living things (Nugroho, 2006). In addition, there are several other minerals needed small amounts such as potassium, manganese, calcium, iron, copper, cobalt and zinc. These compounds are usually in the form of inorganic salts and are usually present in quantity enough in the aquatic and soil environment so that it does not require special attention in the planning of bioremediation processes (Nugroho, 2006).

3. Temperature

   Temperature is an important factor in the biodegradation of compounds, especially in the processes of metabolism and bacterial growth. According to Nugroho (2006) in Andina (2014), at low temperatures, only certain fractions of the hydrocarbons are degraded, whereas at warm temperatures, various fractions are degraded at the same rate.

4. Oxygen

   Oxygen is needed by microorganisms, good oxygen dissolved in water or in the form of free oxygen obtained by air. Oxygen is the most important requirement in the process petroleum biodegradation. At the time of the biodegradation process, oxygen used for the

process of oxidation reactions and respiration by microorganisms (Andina, 2014).

Most petroleum degrading microorganisms belonging to aerobic microorganisms. According to Baker and Herson (2000) describes the amount of oxygen supply needed to aerobic degradation, because in principle the reaction is oxidation-reduction with oxygen as the electron acceptor. Oxygen is an important component that affects microbial growth in a hydrocarbon environment. According to According to Sharpley (1966), oxygen is used as a pathway to activate enzymes oxygenases in degrading hydrocarbon compounds. When limited oxygen, then the growth of bacteria will be inhibited, for The demand for oxygen can be met with aeration, viz by shaking with a shaker (Silvia and Jusfah, 2010).

5. Acidity

Mostly to support microbial growth on The biodegradation process occurs at a neutral degree of acidity (pH). Mark Extreme pH will negatively affect the rate of hydrocarbon degradation by bacteria (Andiana, 2014). Research done by Dibble and Bartha (1979) on biodegradation of oil deposits indicates that a pH value of 7-8 yields biodegradation, which is close to the optimal value (Nugroho, 2006).

2.7 Bacteria

Bacteria are a large group of single-celled phylogenetically related prokaryotes distinct from Archaea. Bacteria have a wide range of shapes, ranging from spheres to rods and spirals. They are ubiquitous, growing in soil, water, extreme environments, and deep in the Earth's crust (Vreeland et al. 2000; Wanger et al. 2008). Bacteria that have the potential to degrade hydrocarbons and are isolated from areas that have long been contaminated with hydrocarbon compounds, such as waters in the area around petroleum storage tanks. Isolation this can be done by spreading (spread-plate), pour (pour-plate) or scratch (streak-plate) (Cappuccino and Sherman, 2002).

2.7.1 Hydrocarbonoclastic bacteria

Hydrocarbonoclastic bacteria are a group of bacteria that can easily adapt to the environment by using oil residue one of them is diesel oil as a source of carbon and energy. Bacteria Hydrocarbonoclastics can produce biosurfactants compounds so that the oil the earth which was originally difficult to dissolve becomes soluble and blends with water

(emulsification). This process occurs because the biosurfactants released by the battery can lowers the surface tension of a liquid and the interfacial tension between two different phases and improve emulsion stability. After that process hydrocarbonoclastic bacteria will carry out the biodegradation process by releases lipase enzymes which have the ability to hydrolyze fat into simple compound and is used as a carbon source by bacteria hydrocarbonoclastic. The end result of the bacterial metabolic process in the form of H2O and CO2 that is safe for the environment (Gouma, 2014).

Microorganisms that can live and have a role in breaking down hydrocarbons are bacteria, while the presence of other microorganisms is sufficient role that is fungi, molds, algae, and actinomycetes (Nugroho, 2007). Bacteria hydrocarbonoclastic are bacteria that have the ability to degrade hydrocarbon compounds. Naturally, hydrocarbonoclastic bacteria have ability to bind, emulsify, transport, and degrade hydrocarbons (Zam, 2010).

Bacteria that have the potential to degrade hydrocarbons and are isolated from areas that have long been contaminated with hydrocarbon compounds, such as waters in the area around petroleum storage tanks. Isolation this can be done by spreading (spread-plate), pour (pour-plate) or scratch (streak-plate) (Cappuccino and Sherman, 2002).

Isolation and initial selection will determine which bacteria actually play a role and have the potential to developed and used specifically in handling oil or fat pollution (Sugiato et al al., 2003).

Table 1: some examples of bacteria hydrocarbonoclastic

Bacterial Isolate	**Type of Hydrocarbon**	**Reference**
Moraxellaceae Xanthomonadaceae and Pseudomonadaceae	Petroleum	Mahjoubi, M. et al., 2013
Pseudomonas mendocina and Ochrobactrum sp.	Bilge oil	C. Sivaraman et al.
	Crude Oil	Nadia et al, 2018

According to Purwoko (2007), there are four phases of bacterial growth, namely the adaptation phase (lag phase), the exponential phase, the static phase (stationary phase), and the death phase (death phase).

1. Adaptation Phase (Lag Phase)

   In the adaptation phase, there is no population growth bacteria. The adaptation process involves the synthesis of a new, suitable enzyme with the median and recovery of metabolites that are toxic (alcohol, acids, bases) in long media. In phase adaptation is not found in an increase in the number of cells, so avoid from the exponential phase unless the cells are in the media old in the propagation phase and transferred to new media with the same nutritional composition (Purwoko, 2007).

2. Propagation Phase (Exponential Lag)

   In the exponential phase, the multiplication of bacteria occurs going fast. In this phase, the bacteria acquire conditions ideal in growth so that cell division occurs. Cell division is an exponential equation, so the phase is also called the exponential phase (Purwoko, 2007). In phase Cell multiplication will consume nutrients in the media, and under these circumstances, the number of cells will increase to some extent, under the circumstances will remain constant until a change in the composition of the media occurs. which is quite significant (Purwoko, 2007).

3. Static Phase (Constant)

   In the static phase, there is a reduction in nutrients and buildup toxic product. In this static phase, there are several cells that grow and dividing with the number of living cells being fixed, and on the otherThere are dead cells (Pelczar, 2008; Rohmah, 2017). According to Dwidjoseputro (1989) This phase shows an almost straight line. horizontal, because the number of bacteria that live is equal to the numberdead bacteria. Bacteria in the static phase do not carry out cell division because various factors, such as nutrients in the media depleted, accumulation of toxic metabolites (acids, bases, and alcohols), decrease in oxygen levels and water availability in the media so that In this phase, the cells usually experience adaptation to unfavorable conditions (Purwoko, 2007).

3. Death Phase

In the death phase, the cells will die faster in comparison with the formation of new cells. Death rate in bacteria accelerates exponentially depending on the species. bacteria, all the cells will die within a few days, or several months (Pelczar, 2008; Rohmah, 2017). According to Purwoko (2007), bacteria will be able to survive several hours in the static phase and finally enters the phase on the other hand, there are bacteria that are only able to survive for days or weeks in a static phase before finally enteringdeath phase. There are also bacteria that can survive for tens of years before entering the death phase, that is, by changing cells into spores (Purwoko, 2007).

2.8 Previous research

The previous research on the isolation and testing of hydrocarbonoclastic bacteria and the degradation of diesel oil is presented in the table below.

Table 2: Previous research on oil-degrading bacteria

Title	**Researcher name, year**	**Result**	**Difference**
Hydrocarbonoclastic bacteria isolated from petroleum contaminated sites in Tunisia: isolation, identification and characterization of the biotechnological potential	Mouna Mahjoubi, Atef Jaouani, 2013, New Biotechnology	The highest emulsification activity was detected in Pseudomonas geniculata with 52.77% of emulsification. Our overall results suggest that the obtained bacterial isolates may constitute potential candidates for bioremediation and can be useful for biotechnological applications.	In this study used diesel oil while the previous one used crude oil. Also, this research used only sea water while the previous one used sediment and sea water.

Potential Consortium of Water Samples Kamal Harbor and Bittern in Degrading Diesel Fuel	Harfatia Chandra Puspita Sari, Haryo Triajie 2021 Jurnal Kelautan Tropis	The highest %total petroleum hydrocarbons (TPH) reduction by the indigenous consortium was in the 3% diesel treatment with a value of 3.87, while in the bittern consortium there was 2.5% diesel treatment with a value of 3.34.	The previous one used for crude fuel while this research will use diesel oil. And main different is the previous one doesn't make any identification bacteria while this will make.
Alternative Spill Management Crude Oil Using Bacillus subtilis and Pseudomonas putida	Widhowati Kesoema, 2020	It is estimated that the use of these two bacteria can be effective put aside crude oil spills by applying chemical physical handling as initial treatment, as well as some factors and procedures that must be carefully considered When the application of bioremediation is carried out.	The previous study was used chemical and physical cleanup; thus, this study will used biological only.
Potential for Oil Degradation by the Bacterial Consortium of Bintan Mangrove Sediments	Nur Fitriah Afianti, 2020	Descending order the highest oil concentrations were shown by the treatment of S2, S1, K1 and K2 with the percentage of TPH reduction was 52.9%,	The previous study was used mangrove and this study will used sea water.

		48.4%, 45.7% and 35.7%. Hydrocarbonoclastic Bacteria Consortium Xylocarpus granatum mangrove sediments from the Lagoi area (S2) produced the most percentage of TPH degradation Tall. In this study, there was no visible effect of mangrove species on the rate of oil degradation.	
Exploration of Indigenous Bacteria Degrading Oil Waste Earth in PT Pertamina UBEP Limau Muara Enim Area	Bambang Yudono, 2013	based on the results of identification: 4 bacterial isolates belong to the genera Pseudomonas, 4 isolates belong to the Bacillus genera, 1 isolate belongs to the Micrococcus genus and 1 isolate belongs to the genus Flavobacterium	The previous study was used only 4 isolate identifications thus this study will used 8-12 bacterial isolation.

CHAPTER III

RESEARCH METHODOLOGY

3.1 Place and time of research implementation

The sampling location of this study was carried out from Port of Gresik. Seawater samples were taken with purposive sampling method in pier II section (-7.146630, 112.657550). The method used in this study is the observation method in the Microbiology Laboratory of UIN Sunan Ampel Surabaya from November 2022 to April 2023.

3.2 Research tools and materials

3.2.1 Tools

The tools used in the research are: GPS, camera, sample bottles, analytical scales, digital scales, glasses watches, horn spoon bars, measuring cups, beakers, flasks Erlenmeyer, hot plate, glass stir bar (spatula), autoclave, pipettes, Laminar Air Flow (LAF), Bunsen, incubator shakers, petri dishes, L glass, test tube, tube rack, loop needle, funnel, separating funnel, pH meter, thermometer, incubator cabinet, slide glass, microscope, refrigerator, plastic wrap, aluminum foil, heat-resistant plastic, cotton, filter paper, brown wrapping paper, vacuum filter and fume hood biosafety cabinet.

3.2.2 Materials

The materials used in the study include Sample sea water that was once polluted by diesel oil in the port of Gresik, Diesel oil from PERTAMINA, distilled water, 70% alcohol, NA dan NB. Isolate growth media used is *Stone Mineral Salt Solution Extract Yeast* (SMSSe) which consist of 0.5 g $CaCO_3$; 0.25 g NH_4NO_3; 0.1 g $Na_2HPO_4.7H_2O$; 0.05 g KH_2PO_4; 0.05 g $MgSO_4.7H_2O$; and 0.02 g $MnCl_2$.7 H2O dissolved in 200 ml of sea water. Yeast extract as much as 0.02 g. added to the SMSS medium as a nitrogen source in the form of amino acids and growth factor additionally, 2% Diesel oil was added to the medium as a carbon source, and pH this medium is 6.8-7.

3.3 Research variable

This study uses the types of variables including the independent variables in the form of types of hydrocarbonoclastic bacterial isolates. The dependent variable is the result of the calculation oil contents using the gravimetric test. The control variable in the form of time

used by bacteria in degrading and the large number of bacteria degrading diesel oil in the sample.

3.4 Research methods

This research method uses the experimental method on laboratory. In the flow of research that begins with preparing tools and materials, sterilize tools, perform water sampling sea to get indigenous bacteria in Gresik port, which has experienced diesel oil spills as well as the volume of waste disposal containing oil from activities the next ships the sample to be brought to the Laboratory Microbiology, Sunan Ampel State Islamic University, Surabaya.

The next step is the preparation of bacterial growth media. Furthermore, isolation of bacteria that can degrade oil is carried out until pure culture is obtained and observation is made characteristics of bacteria and identify the type with using biochemical tests, the next step is testing the ability of bacteria to biodegrade diesel oil.

Figure 3.1 in the flow of research that begins with secondary data, which journals, and a thesis about the test of bacterial potential oil degradation Bioremediation book Petroleum Hydrocarbons, Earth Berge's classification book Manual of Determinative Bacteriology, Eight Editions. primary data which is preparing tools and materials, sterilize tools, perform water sampling sea to get indigenous bacteria in Gresik port, which has experienced diesel oil spills as well as the volume of waste disposal containing oil from activities the next ships the sample to be brought to the Laboratory Microbiology, Sunan Ampel State Islamic University, Surabaya.

3.4.1 Research procedure

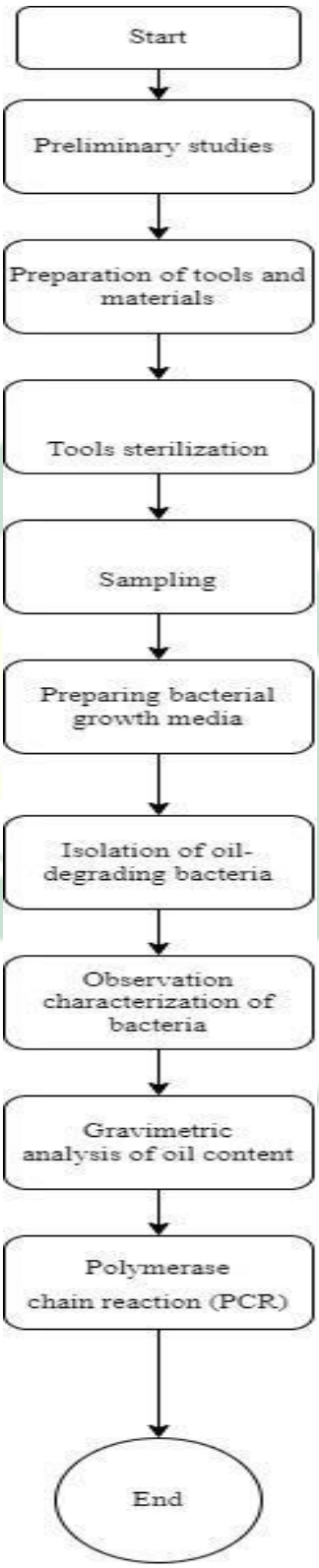


Figure 1: 3.1 Research procedure

3.5. Preliminary studies

Preliminary studies were conducted to collect initial information regarding the discussion and study of previous research obtained from literature studies of journals, theses, news, and other related scientific literatures in accordance with the research topic and research location.

3.5.1 Sampling

Water samples were taken at the port of Gresik, using purposive sampling method, Seawater samples were taken by holding a plastic bottle on the bottom, with pre-sterilized bottles. Then the bottle is immersed as deep as 20 cm below sea level in a position with the bottle upside down against the direction of the water flow. Seawater samples that have been taken in sterile bottles are then labeled and stored in the cool box so that there is no degradation of the number of bacteria or bacterial death in the samples. The measurements of water quality carried out are temperature and ph. Seawater samples were treated with a grace period of no more than 24 hours for isolation. (Nadia, 2018).

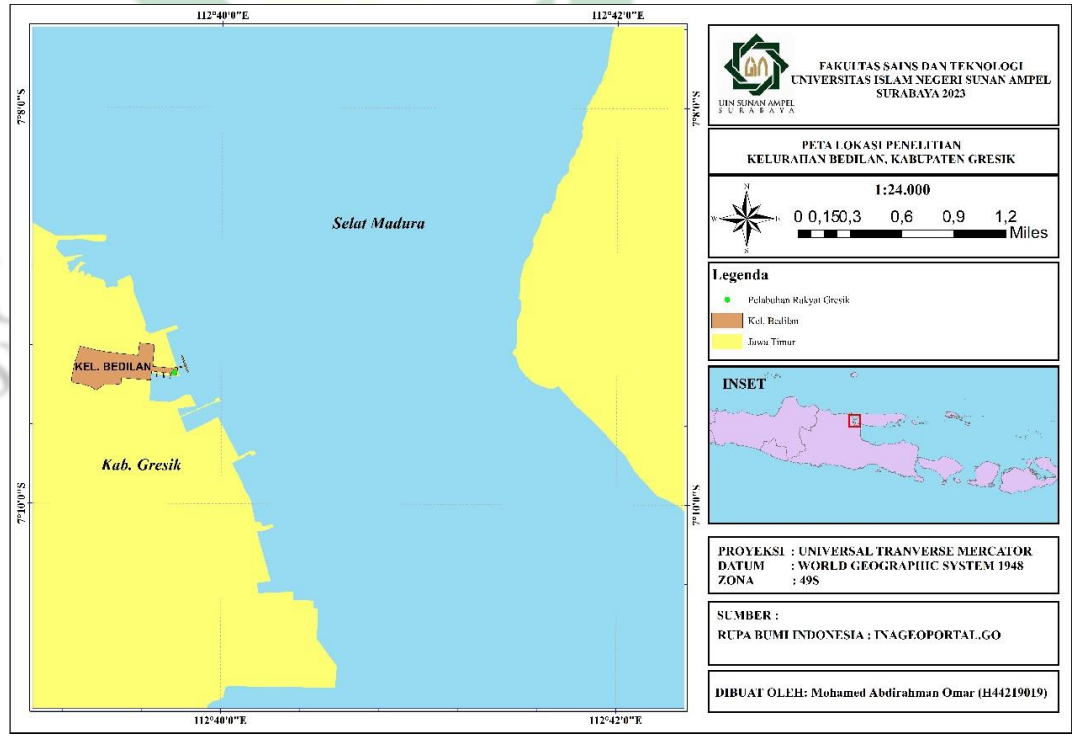


Figure 2: 3.2 Sampling Location at Gresik Port

3.5.2 Preparation of tools and materials

Preparation of research tools and materials with certainty regarding the condition, presence, and cleanliness of the tools and materials.

3.5.3 Tools sterilization

The tools used in this research were first washed and dried. Clean tools are then wrapped in wrapping paper and put inside a steamer. The next step is steaming sterilization on the tool using an autoclave with a temperature of 120°**C**.

3.5.4 Preparing bacterial growth media

Media used in isolation and degradability tests diesel oil using media Stone Mineral Salt Solution Extract Yeast (SMSSe). Creating SMSSe media on Bacterial growth consists of $CaCO_3$, NH_4NO_3, $Na_2HPO_4.7H_2O$, KH_2PO_4, $MgSO_4.7H_2O$, $MnCl_2.7\ H_2O$, with added extract yeast 0.01% (w/v) (Sharpley, 1966; Andina, 2014). In the manufacture of SMSSe liquid media, it consists of 0.5 grams of $CaCO_3$; 0.25 grams of NH_4NO_3; 0.1-gram $Na_2HPO_4.7H_2O$; 0.05 grams KH_2PO_4; 0.05-gram $MgSO_4.7H_2O$; 0.02-gram $MnCl_2.7\ H_2O$ with added yeast extract as much as 0.01% (w/v) or equivalent with 0.02 gram dissolved in 200 ml of sterile distilled water. In that medium added with diesel oil as a selective medium as much as 2% (w/v) as well as a carbon source and the pH of the media until it reaches a value of 6.8–7 (Pikoli et al., 2000 in Nababan, 2008). Each media that has been prepared, then sterilized using an autoclave at 120 °**C**.

3.5.5 Isolation of oil-degrading bacteria

In the isolation stage, the sample is diluted in isolation by adding a seawater sample of 2% (w/v) into liquid SMSSe media containing 2% diesel oil (b/v) and incubating at room temperature using an incubator shaker at 120 rpm for 14 days (Nababan, 2008).

Samples that have been shaken for isolation purposes are taken in as much as 1 ml for dilution and put in a test tube that has been filled with 9 ml of sterile distilled water, then vortex briefly to make it homogeneous. Furthermore, as much as 1 ml taken from the first test tube, then put into in the second diluent tube containing 9 ml of sterile distilled water, then the second diluent tube was vortexes, and 1 ml was taken from the second tube to be inserted into the diluent tube third, and so on up to the fifth dilution ($10^{-}5$). Each of the last three dilutions (10.3, $10^{-}4$, and $10^{-}5$) with sterile sea water was taken in as much as 0.1 ml

and grown on a nutrient agar media plate containing 2% diesel oil (w/v), then incubated at 30°**C** for 48 hours (Andina, 2014). Bacteria that grow are purified again based on the same morphological characteristics to obtain a single colony.

3.5.6 Observation characterization of bacteria

The isolates that have been obtained are then identified using macroscopic and microscopic observations.

1. **Macroscopic observations**

According to Dwidjoseputro (1989), microscopic observation conducted to observe the characteristics of the colony's bacteria are as follows:

a. Colony color: whitish or yellowish almost clear and gray
b. Colony shape (viewed from above): round with edges (circular), irregular edges, such as roots and spread (rhzoid)
c. Colony edges or margins (viewed from above): even or intact (entire), wavy or grooved (lobate), jagged (serrate), thread (filamentous), curly (undulate)
d. Colony surface/elevation (viewed from the side): flat or almost medium (flat), embossed-curved (convex), raised-flat (raised), hilly (umbonate).

2. **Microscopic observation**

Microscopic observation is done to be able to see cell shape and bacterial properties. Microscopic observations consisted from gram staining and biochemical tests (Holt et al., 1994 in Rohmah, 2017).

a. Gram stain

one of the most common differential coloring techniques what is important is the gram stain, which can differentiate bacteria that are gram positive and gram negative. In terms of staining, there are differences between gram-positive and observable gram-negative bacteria (Pelczar, 2008). Here are the steps for coloring grams of bacteria:

1. Preparation of bacterial stains

Pure cultures to be identified on solid media are taken using a needle that has been sterilized, then placed on the object glass that has previously been given sterile distilled water and has been leveled. Then the culture leveled using a needle loop on a glass object. Then it is dried.

2. The glass object containing the culture is fixed three times through the fire

3. Smeared bacteria that have stuck to the position have dried, then been dripped with crystal violet and flattened on the glass object, then let stand for 1 minute. Then the excess dye is removed by rinsing with running water.
4. Second staining by dripping with iodine solution, lugol and flattened on a glass object, and allowing it to stand for 1-2 minutes.
5. Then wash with running water, then in decolonization (given bleach solution) with 96% alcohol until the purple color disappears or for 20 seconds (overuse may result in an error).
6. At the last stage, apply safranin solution, which is then flattened on the glass object and left for 1-2 minutes. Finally, the smear from the bacteria is rinsed with running water and dried.
7. Then make observations using a microscope. Observations below Microscopy was done by adding 1 drop of immersion oil to the object glass to avoid refractive index on observations.
8. Next, observe the shape and color of the cell's bacteria. The color of bacterial cells on staining gram consists of two, namely positive gram staining and negative gram staining.

b. Observation of cell shape

On observing the shape of the bacterial cell on a microscope consists of round (coccus), rod (bacilli) and spiral (spiralia).

3.6 Polymerase chain reaction (PCR)

PCR, or polymerase chain reaction is a DNA amplification technique performed in vitro. PCR is a sensitive, selective, and very fast way to reproduce desired DNA sequence.

Methods

1. Genomic DNA extraction with Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005).

The Quick-DNA™ Fungal/Bacterial Miniprep Kit is designed for the simple, rapid isolation of DNA from tough-to-lyse fungi, including *A. fumigatus, C. albicans, N. crassa, S. cerevisiae, S. pombe*, as well as from mycelium and Gram positive and Gram-negative bacteria. The procedure is easy and can be completed in as little as 15 minutes: fungal and/or bacterial samples are added directly to a ZR Bashing Bead™ Lysis Tube (0.1 & 0.5 mm) and rapidly and efficiently lysed by bead beating without using organic denaturants or proteinases. The DNA is then isolated and purified using our Zymo-Spin™ Technology and is ideal for downstream molecular-based applications including PCR, array, etc.

DNA isolated from *Saccharomyces cerevisiae (spores) and E. coli* using the Quick-DNA™ Fungal/Bacteria Kit was high-quality and structurally intact. Equivalent amounts of yeast and bacteria were processed using the Quick-DNA™ Fungal/Bacterial Kit or the Supplier a kit. Equal volumes of eluted DNA were analyzed on a 0.8% (w/v) agarose gel stained with EtBr. (Stackebrandt and Goebel, 1994).

2. PCR amplification with (2x) My Taq HS Red Mix (Bioline, BIO-25048)

3. Bi-directional Sequencing.

3.7 Ability assay of bacterial isolates in degrading diesel oil

Each bacterial isolate that will be purified in the form of a suspension is taken as much as one ml and then added to the liquid SMSSe medium (100 ml) containing 1% (1 ml), 2% (2 ml) diesel oil and 3% (3 ml) separately and also without bacteria (control). The culture was incubated at room temperature and stirred at on a shaker with a speed of 120 rpm, then the remaining oil content analysis will carry out on the 7th day. (Nadia, 2018).

3.7.1 Gravimetric analysis of oil content

Gravimetric analysis of petroleum content was carried out by means of liquid SMSSe media containing diesel oil containing 2% (w/v) of pure bacterial ability.
In degrading diesel oil put into a separator funnel. The separator funnel was then filled with liquid media, which had a pH of 2, along with 5 ml of 3 N HCl (Greenberg, 1992). Then 30 ml of chloroform were added. Then the separating funnel was shaken vigorously for 2 minutes (with occasional opening of the lid on the separating funnel to expel the gas formed). After 2 minutes, let the funnel stand until 2 separate, stable layers are formed, and then remove the water layer (SNI, 06-6989.10-2004). Layers of chloroform and diesel oil from a separator funnel were removed and filtered using filter paper that smeared 0.5 grams of Na_2SO_4 into the beaker that had been previously weighed. Then the beaker glass contains chloroform and oil, which are heated to 60 oC (according to the boiling point of chloroform) until the chloroform runs out, the water runs out and evaporates, and what remains is only oil (APHA, 1981).

Furthermore, the beaker glass containing the oil removed and left to cool, and then weighed record the weight. Based on the SNI (Standard National Indonesia) 06 6989.10-2004 formula Oil content can be calculated as follows:

$$\text{Oil Content (mg/L)} = \left(\frac{\text{A}-\text{B}}{\text{mL of test sample}} \text{x } 1000\right) \quad (3.1)$$

Note: A = Weight of flask + extract (mg)

B = Weight of empty flask (mg)

According to Herdiyantoro (2005), the formula for determine the percentage of biodegradable petroleum occur can be known by:

$$\%B = \left(\frac{Bmo-Bmn}{Bmo} \text{x } 100\right) \quad (3.2)$$

Note: %B = Biodegradation Percentage

Bmo = initial Diesel oil weight (g)

Bmn= final Diesel oil weight (g)

CHAPTER IV
RESULTS AND DISCUSSION

Use of hydrocarbonoclastic bacteria from the waters around Port Gresik to degrade oil in the polluted environment of the region is better because hydrocarbonoclastic bacteria originate from the location where the pollution occurs. These bacteria will take advantage of the oil that is widespread in the environment as a source of nutrition.

4.1 Isolation and Purification of Bacteria hydrocarbonoclastic from the Port of Gresik

Bacteria from the waters of the Port of Gresik were found in a mixed microbial population. Bacteria that potentially degrade oil can be cultured using selective media, which is media used to grow certain bacteria while inhibiting the growth of other bacteria (non-target bacteria). Selective media used for growing oil-degrading bacteria on bacterial media (hydrocarbonoclastic bacteria) using stone media and Mineral Salt Solution Extract Yeast (SMSSe).

On the four isolation of hydrocarbonoclastic bacteria using media SMSSe is enriched with the nutrients needed, namely in the form of $CaCO_3$, NH_4NO_3, $Na_2HPO_4.7H_2O$: these compounds are the main nutrient needed for life, multiply and produce enzymes for degrade hydrocarbons. Apart from that, the SMSSe media is also available minerals, namely KH2PO4, $MgSO_4.7H_2O$, and $MnCl_2.7H_2O$, these minerals are needed in small amounts as nutrition, then also add yeast extract as a source of nitrogen in the form of amino acids and additional growth factors (Nababan, 2008). On the SMS media also added diesel oil as much as 2% (w/v) as carbon source for microbes.

Bacterial isolation is accomplished by adding the sample water from Gresik Port, which was polluted by oil at 2% (w/v) or 4 ml, into the SMSSe liquid medium containing 200 ml and incubating at room temperature with an incubator shaker at a speed of 120 rpm (Figure 4.1).

Figure 3:4.1 incubated medium SMSSe

After the bacteria were incubated for 14 days in SMSSe medium, there was a change in the media, namely a change in color and turbidity, which indicates that the bacterial population degraders have grown and can be isolated from the congested SMSSe medium. If performed in artificial media in the laboratory, there will be a change in the color of the medium solution; the Color formed on the oil medium is between yellow and dark orange. Change the color of the media in bacterial culture from seawater samples (Figure 4.2).

Figure 4: 4.2 media in bacterial culture

Table 3: samples 1, 7 and 14.

Day	**Bacterial isolate code**				**Figure**
Day 1	NDOUW	NDOPW	SDOPW	SDOUW	
Day 7	NDOUW	NDOPW	SDOPW	SDOUW	
Day 14	NDOUW	NDOPW	SDOPW	SDOUW	

This isolate code stands for: new diesel oil unpolluted water (NDOUW), new diesel oil polluted (NDOPW), second diesel oil polluted water (SDOPW), and second diesel oil unpolluted water (SDOUW).

All isolates of oil-degrading bacteria were able to degrade diesel oil according to the concentration determined. The ability of bacterial isolates to degrade diesel oil was tested; the bacterial isolates tested were NDOUW, NDOPW, SDOPW and SDOUW. The highest degradation was shown in SDOUW isolates, which also had the highest degradation.

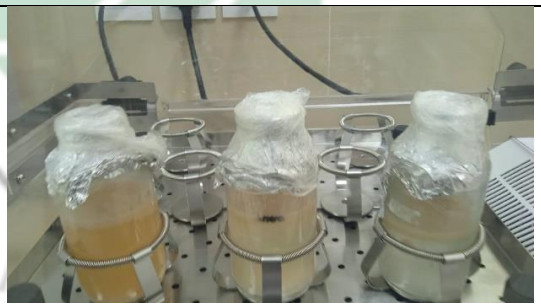
4.1.1 Results of Visual Observation of Diesel Oil Biodegradation

Observation of biodegradation of diesel oil on Marine bacteria was shown visually during the observation media color change on the first day. Diesel oil on the surface of the SMSSe media the first day started at degradation by Marine bacteria.

4.1.1.1 Observation of biodegradation of diesel oil

Table 4: 4.1 observation

Incubation Time	Medium Color	Figure	Diesel Oil Conditions
24 hours	clear yellow and starting to get cloudy		Diesel oil viscosity started to reduce and spread across the surface partially attached to the uc bottle wall.
48 hours	Yellow and murky		Less diesel oil, and most are on the margins and attached to the wall uc bottle with white granules attached
72 hours	Cloudy yellow		Diesel oil is reduced as well there are small details at the center of the surface and uc bottle wall

96 hours	Dark yellow		Diesel oil is reduced as well there are small details at the center of the surface and uc bottle wall
120 hours	Dark yellow and murky		On the surface, diesel oil starting to decrease. There are details – small grains are on the uc bottle walls.
144 hours	Dark yellow		Reduced diesel oil, granules – small grains on the edge and uc bottle wall
168 hours	Dark yellow		Some small details large on the uc bottle wall

Observation of diesel oil biodegradation in Marinobacter were shown visually presented during the observation began to experience media color change on the third day. First day observation, diesel oil on the surface of the SMSSe media been degraded by Marinobacter.

Figure 5: 4.3 before inoculation

Figure 6: 4.4 after inoculation

Populations of hydrocarbonoclastic bacteria that have grown in SMSSe liquid medium are then inoculated on the media nutrient agar solid in a petri dish with the pour plate method (dish spread). Furthermore, the bacteria were incubated for 2x24 hours, growing and differing in morphological characteristics (color, elevation, edge size, and shape). To obtain a pure bacteria culture, it must be purified once more using the streak plate method (scratch method), which involves placing the culture on media. Solid SMSSe using a sterile loop needle and regrown on sterile solid SMSSe medium, respectively. Different colonies were purified again on the same medium to get a single colony. After the colonies on the media, similar petri dishes were used, indicating that the bacterial colonies on the cup were pure.

Figure 7: 4.5 media nutrient agar

4.1.1.2 Gravimetric result

Gravimetric analysis of petroleum content was carried out by means of liquid SMSSe media containing diesel oil containing 2% (w/v) of pure bacterial ability.

In degrading diesel oil put into a separator funnel. The separator funnel was then filled with liquid media, which had a pH of 2, along with 5 ml of 3 N HCl (Greenberg, 1992). Then 30 ml of chloroform were added. Then the separating funnel was shaken vigorously for 2 minutes (with occasional opening of the lid on the separating funnel to expel the gas formed). After 2 minutes, let the funnel stand until 2 separate, stable layers are formed, and then remove the water layer (SNI, 06-6989.10-2004). Layers of chloroform and diesel oil from a separator funnel were removed and filtered using filter paper that smeared 0.5 grams of Na2SO4 into the beaker that had been previously weighed. Then the beaker glass contains chloroform and oil, which are heated to 60 °C (according to the boiling point of chloroform) until the chloroform runs out, the water runs out and evaporates, and what remains is only oil (APHA, 1981).

Furthermore, the beaker glass containing the oil removed and left to cool, and then weighed record the weight. Based on the SNI 06 6989.10-2004 formula Oil content can be calculated as follows:

$$\text{Oil Content (mg/L)} = \left(\frac{\text{A}-\text{B}}{\text{mL of test sample}} \text{x } 1000\right)$$

Note: A = Weight of flask + extract (mg)

B = Weight of empty flask (mg)

$$\text{Oil Content (mg/L)} = 4\left(\frac{131.61-1227.93}{200} \text{x } 1000\right)$$

According to Herdiyantoro (2005), the formula for determine the percentage of biodegradable petroleum occur can be known by:

$$\%\text{B} = \left(\frac{\text{Bmo} - \text{Bmn}}{\text{Bmo}} \text{x } 100\right)$$

Note: %B = Biodegradation Percentage

Bmo = initial Diesel oil weight (g)

Bmn= final Diesel oil weight (g)

$$\%4 = \left(\frac{4-0.32}{4} \text{x } 100\right) = 92.25$$

The result shows, the fractions of hydrocarbons diesel oil can be degraded by the consortium. The consortium will change the composition of the oil to light fraction in diesel oil so that it is thick (viscosity) will decrease. Based on the above formula shows that consortium can degrade diesel oil by 92.25%. The consortium is an oil degrader diesel fuel is the highest compared to isolate isolates other single. Zhu et.al (2001).

Figure 8: 4.18 separator funnel filled with SMSSe media

The process of biodegradation of hydrocarbon compounds by Bacterial isolates are highly dependent on composition community and adaptive response to presence hydrocarbons (Leahy and Colwell, 1990). The consortium is an oil degrader Diesel fuel is the most expensive when compared to isolate isolates. Other single. According to Zhu et.al (2001), biodegradation

Natural oil is not only degraded by one bacterial isolate, but involves several bacterial isolates (consortium). The presence of mixed isolates Thus, the degradation of hydrocarbons is more effective.

A sufficient number of bacteria will produce enzymes which varies in degrading oil in a manner faster than single bacterial isolates (Nugroho, 2006).

4.2 Morphology of hydrocarbonoclastic bacteria from Gresik Port

The results of the isolation that has been carried out are bacterial isolates from Gresik Port. Based on the isolation results, bacterial colonies were found to be hydrocarbonoclastic. Bacterial colony morphology from the outside looks the same, but is then identified by observation macroscopically for size, colour, shape, edges, and elevation, while microscopically, namely by doing a Gram stain, to identify the bacteria as either gram positive or gram negative. According to Andina (2014),

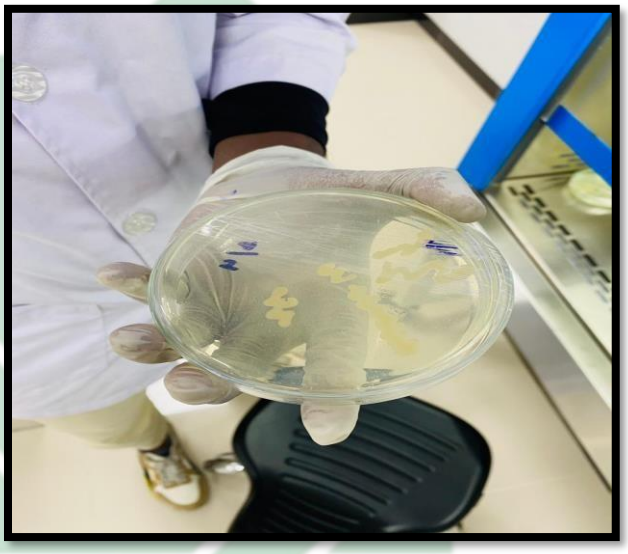


Figure 9: 4.6 Bacterial colony morphology

4.2.1 Gram staining process

The Gram stain procedure consists of smear preparation, Gram stain, and examination under a microscope. Smear Preparation How to prepare smears to be stained are: Sterilize the inoculating loop on a Bunsen flame until it turns red, then wait for it to cool for about 30 seconds. If the loop is still hot when the specimen is taken, the bacterial cells may be damaged Using a clean slide (slide), place the specimen in the centre of the slide. If the specimen is taken from an agar plate, add 1 drop of water to make a suspension first Using an inoculating loop, wipe the specimen over the slide until a thin layer is obtained, then air dry Heat the slide by passing it over a Bunsen flame 2-3 times to fix it.

4.2.2 Procedural

The Gram stain procedure consists of smear preparation, Gram stain, and examination under a microscope.

4.2.2.1 Smear Preparation

How to prepare smears to be stained are:

1. Sterilize the inoculating loop on a Bunsen flame until it turns red, then wait for it to cool for about 30 seconds. If the loop is still hot when the specimen is taken, the bacterial cells may be damaged.

2. Using a clean slide, place the specimen in the center of the slide. If the specimen is taken from an agar plate, add 1 drop of water to make a suspension first.

3. Using an inoculating loop, wipe the specimen over the slide until a thin layer is obtained, then air dry.

4. Heat the slide by passing it over a Bunsen flame 2-3 times to fix it.

Figure 10: 4.7 Heat the slide over a Bunsen flame

4.2.2.2 Gram stain

Gram staining is done by:

a) Pour the liquid crystal violet dye on the preparation evenly, wait for 1 minute

b) Tilt the preparation and rinse with a small amount of running water
c) Pour the mordant liquid on the preparation, wait for 1 minute
d) Tilt the preparation back and rinse with a little running water
e) Perform the decolorization by dripping the decolorizing liquid little by little on the preparation until no dye flows out of the preparation. Then rinse the preparation under running water

Figure 11: 4.8 preparation under running water

f) Pour the counterstain (safranin) on the preparation, wait for 30 seconds to 1 minute. Then rinse the preparations under running water, then dry the preparations.

Figure 12: 4.9 Pouring safranin

g) Observe the preparations using a microscope with a magnification of 100 times, 400 times, up to 1000 times.

Figure 13: 4.10 pouring the liquid crystal violet

Gram staining can see pure isolated bacteria down to the cellular level. Gram staining was carried out using bacterial isolates aged 24-48 hours. The new bacterial culture will reduce the occurrence of aberrations on Gram stain due to different cultures. Over time, many cells experience damage to their cell walls so that the Gram-positive bacteria can no longer maintain the colour of Lugol's violet-iodine crystal complex and become Gram negative (Waluyo,2010).

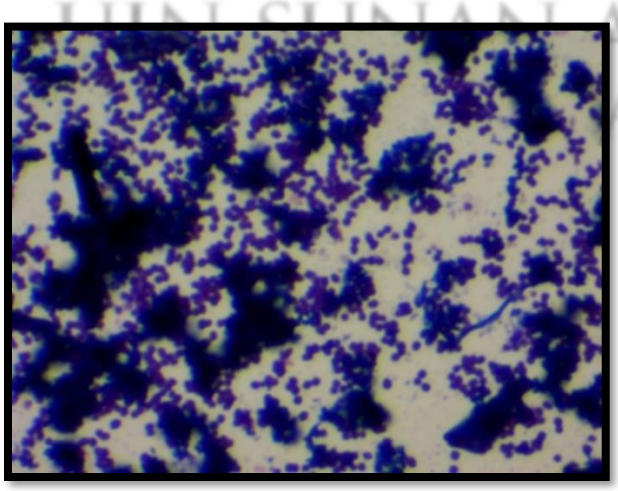


Figure 14: 4.11 Gram-positive

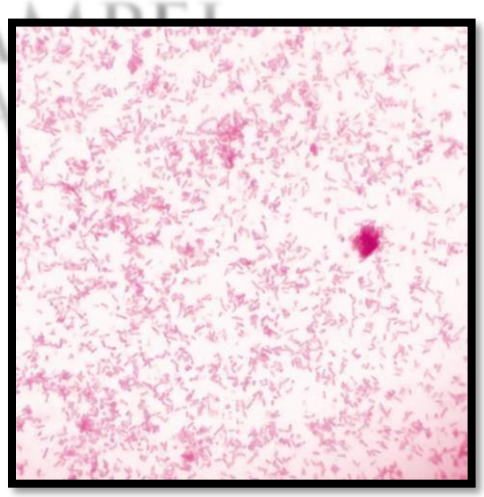


Figure 15: 4.12 Gram negative

Characteristic results and identification of bacterial isolation oil degradation from Gresik Port

Table 5: 4.2 identification of bacterial isolation

Sample code	Isolate samples	Color	Elevation	Shape	Gram
NDOUW	A 1,2,3,4,5	Yellow	Flat	Circle	+
NDOPW	B 1,2,3,4,5	Yellow	Flat	Circle	+
SDOPW	C 1,2,3,4,5	Yellow	Flat	Irregular	+
SDOUW	D 1,2,3,4,5	Yellow	Convex	Rod	-

Polymerase Chain Reaction (PCR)

PCR, or Polymerase Chain Reaction is a DNA amplification technique performed in vitro.

PCR is a sensitive, selective, and very fast way to reproduce desired DNA sequence. The basic principles of PCR are:

1. Denaturation

Initial denaturation is carried out before the Taq polymerase enzyme is added in a test tube. DNA denaturation is the process of opening double-stranded DNA into single-stranded DNA (Murray et al. 2006)

2. Annealing (primary attachment)

The criterion that is commonly used to design a good primer is that the primer should be 18–25 base sizes, contain 50–60% G+C, and for the second the primer should be the same. The DNA sequences in each primer itself as well should not complement each other, as this will result in formation. Secondary structure in the primer and reduce PCR efficiency. Annealing time, the usual time used in PCR is 30 to 45 seconds. (Handoyo and Rudiretna, 2000)

3. Primary Elongation (Extension)

During this stage, Taq polymerase begins its activity to elongate DNA primer from the third end. The rate at which nucleotides are assembled by the enzyme at temperature 72ºC estimated to be 35–100 nucleotides/sec, depending on buffer, pH, and salt concentration and target DNA molecules. Thus, for a PCR product with a length of 2000 pairs alkaline, 1 minute is more than

enough for this primary elongation step. Usually in the end of the PCR cycle, the time used for this step was extended to 5 minutes. So that all PCR products are expected to form double-stranded DNA. Those reactions Above are repeated again from 25-30 times (cycle) so that at the end of the cycle will be obtained new double-stranded DNA molecules that are the result of internal polymerization much greater than the amount of template DNA used.

4.3 DNA Barcoding

In order to identify or "barcode" an organism to distinguish it from other species, DNA barcoding entails the generation of PCR amplicons from specific regions to be sequenced (Lebonah et al., 2014).

Figure 16: 4.13 instagene matrix kit and micropipette

4.3.1 Molecular Identification Using 16S rRNA PCR

The primer used in this research is *16S (27F-1492R)*

Identification of more specific bacteria can be done by molecular identification using the 16S rRNA molecule, because this molecule is Subcutis and has an identical function in all organisms. There are many 16S rRNA sequencing analyzers used in microbiology. This molecular-based method is considered to be faster and more accurate in identifying bacteria and has a number of advantages over conventional microbiological methods. The 16S ribosomal RNA (16S rRNA) gene has a region that is conserved (sustainable) so that it is appropriate for use in the polymerase chain reaction (PCR) and sequencing analysis to determine taxonomy, phylogeny, and intercultural diversity species (Rinanda, 2011).

4.3.2 PCR Products

Species Barcoding Bacteria (~1400bp)

4.3.3Results

1. Nucleic Acid (Genomic DNA) Quantification (Nanodrop)

Table 6: 4.3 Nucleic Acid (Genomic DNA)

No.	Sample name	Sample Code	Conc. (ng/µl)	A260/280	A260/230	Volume (µl)
	SDOUW	2697	49.1	1.96	1.15	30

2. Gel Photo – PCR Products

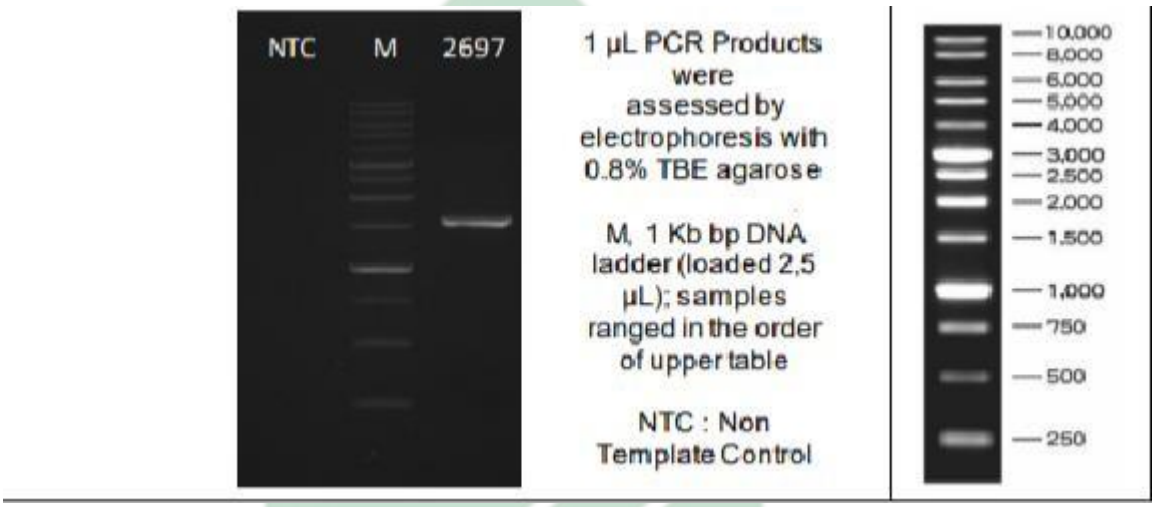


Figure: 4.14 Gel Photo – PCR Products

3. Sequence Assembly Result – PCR Products

No	Sample Name	Sequences
1	Second oil / unpolluted water	**Sequence of 1492 Reverse Primer 1008 bp** 1 TAGACTAACC ACTTCTGGTG CAATCCACTC CCATGGTGTG ACGGGCGGTG TGTACAAGGC 61 CCGGGAACGT ATTCACCGCG ACATTCTGAT TCGCGATTAC TAGCGATTCC GACTTCACGG 121 AGTCGAGTTG CAGACTCCGA TCCGGACTAC GATGCACTTT GTGGGATTAG CTCCCCCTCG 181 CGGGTTTGCA GCCCTCTGTA TGCACCATTG TAGCACGTGT GTAGCCCTGG TCGTAAGGGC 241 CATGATGACT TGACGTCATC CCCACCTTCC TCCGGTTTGT CACCGGCAGT CTCCCTAGAG 301 TTCCCCAACC CG AACTGCTAGC AACTAAGGAT AGGGGTTGCC CTCGTTACGG GACTTAACCC 361 AACATTTCCC AACACAAGCT GACCACGGCC ATGCAGCACC TGGACCTGCA TTCCCGAAGG 421 GACCAATCTA TCTCTAGAAA GTTCCCAGGA TGTCAAGGAC AGGTAAGGTT CTTCGCGTTG 481 CGTCTAATTA AACCACATGC TCCACCGCTT GTGCGGGCCC CCGTCAATTC ATTTGAGTTT 541 TAACCTTGCG GCCGTACTCC CCAGGCGGTC TACTTAATGC GTTAGCTGCG CAACTAACAC 601 TTCAAGAGTC CCAACGGCTA GTAGACATCG TTTACGGCGT GGACTACCAG GGTATCTAAT 661 CCTGTTTGCT CCCCACGCTT TCGCACCTCA GTGTCAGTGT TGGTCCAGGG GGCCGCCTTC 721 GCCACTGGTG TTCCTTCCTA TATCTACGCA TTTCACCGCT ACACAGGAAA TTCCACTACC 781 CTCTACCAAA CTCTAGCTTG CCAGTTTCAA ATGCCGTTCC CAGGTTAAGC CCGGGGCTTT 841 CACATCTGAC TTAACAAACC ACCTACCTGC GCTTTACGCC CAGTAATTCC GATTAACGCT 901 TGCACCCTCC GTATTACCGC GGCTGCTGGC ACGGAATTAG CCGGTGCTTC TTCTGTAAGT 961 AACGTCAAAG CTGGCCGATA TTAACGACAA CCTTTCCTCC CCACTGAA

Figure 17: : 4.15 Sequence Assembly Result – PCR Products

4. Top 10 Hit BLAST Results Against NCBI Database, Excluding Uncultured Sample Sequences

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☑	Marinobacter xestospongiae strain Rf-13 16S ribosomal RNA gene, partial sequence	1629	1629	100%	0.0	95.84%	KP843674.1
☑	Marinobacter sp. strain HNIBRBA833 16S ribosomal RNA gene, partial sequence	1629	1629	100%	0.0	95.84%	OL742686.1
☑	Marinobacter sp. L21-PYE-C23 16S ribosomal RNA gene, partial sequence	1629	1629	100%	0.0	95.84%	KJ188007.1
☑	Marinobacter xestospongiae strain WHER211 16S ribosomal RNA gene, partial sequence	1629	1629	100%	0.0	95.84%	OK083240.1
☑	Marinobacter xestospongiae strain WHER210 16S ribosomal RNA gene, partial sequence	1629	1629	100%	0.0	95.84%	OK083239.1
☑	Marinobacter sp. DQHS-13 16S ribosomal RNA gene, partial sequence	1629	1629	100%	0.0	95.84%	GQ200194.3
☑	Marinobacter mobilis strain HH2 16S ribosomal RNA gene, partial sequence	1629	1629	100%	0.0	95.84%	HQ284162.1
☑	Marinobacter xestospongiae strain UST090418-1611 16S ribosomal RNA, partial sequence	1629	1629	100%	0.0	95.84%	NR_109066.1
☑	Marinobacter sp. 2M46 gene for 16S rRNA, partial sequence	1629	1629	100%	0.0	95.84%	AB435646.1
☑	Alteromonadaceae bacterium LA50 16S ribosomal RNA gene, partial sequence	1629	1629	100%	0.0	95.84%	AF513454.1

Figure 18: 4.16 Hit BLAST Results

In the result link column, there is six information as follows:

1. Description is annotation information about the genus, species, strain, gen fragment
2. Max score is the alignment score between the query sequence and target sequence in the DNA database.
3. Total score is the total score of all alignment that exists between query and target sequence in the DNA database. The total score can be different from the max score if the query sequence matches more than one site with the target sequence
4. Query coverage is the percentage of the length of the query sequence with the target sequence. If the query sequence covers all of the target sequences, then the query coverage is 100%

5. E value is a number that describes how many times you would expect a match by chance in a database of that size. The lower the Evalue is, the more significant the match.
6. Percentage identity shows the similarity between the query sequence and target sequence. For microorganisms, using 16srRNA primers, similar to species level (above 97.5%), genus level (above 95%).

5. Phylogenetic Tree – Neighbor-Joining (Unrooted Tree) by NCBI Blast Tree Method.

Sequence Result of 27 Forward Primer

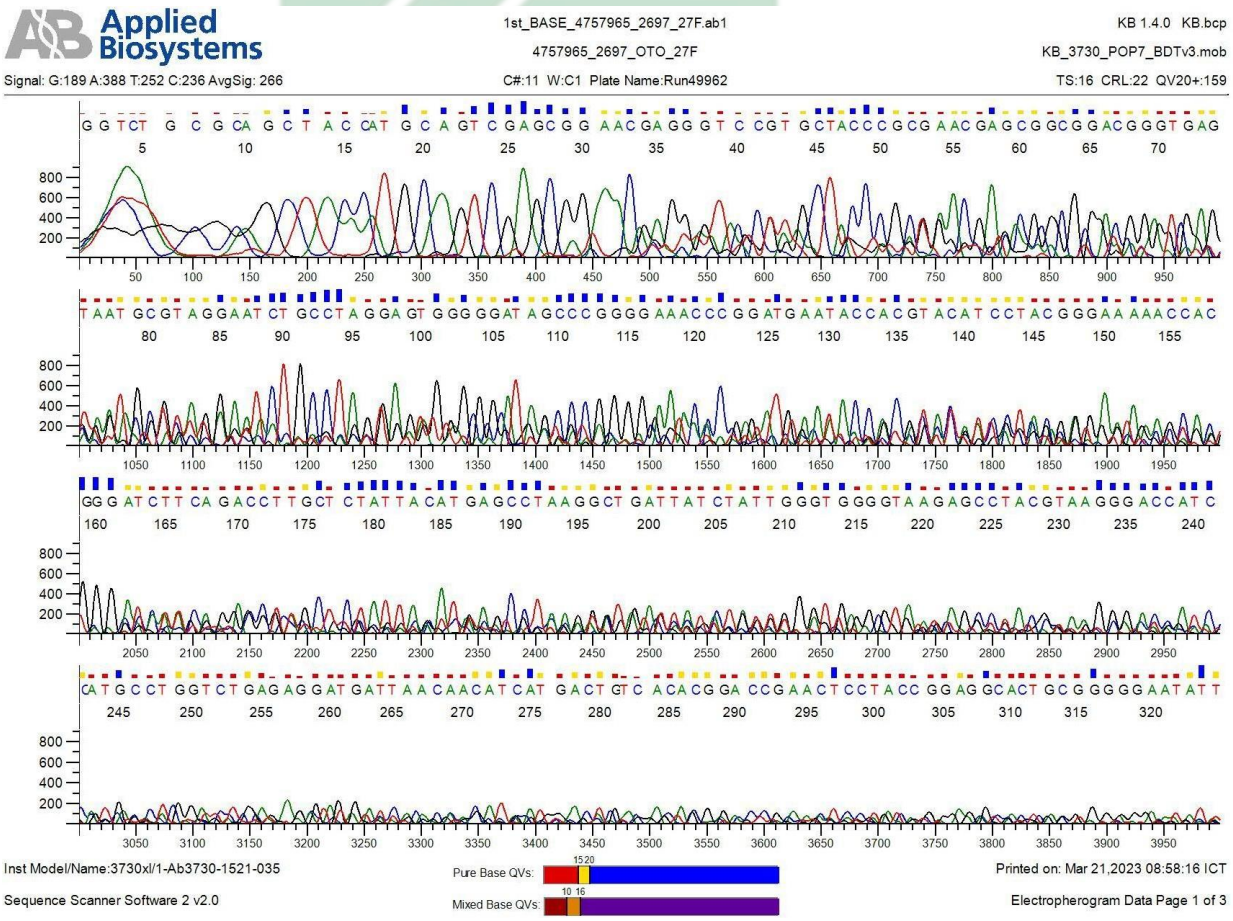


Figure 19: 4.17 Sequence Result of 27 Forward Primer

The BLAST results of this sample used a reverse primer. There are double peaks in the sequence of the sample.

4.4 Marinobacter xestospongiae strain Rf-13 16S ribosomal RNA gene, partial sequence

4.4.*1Marinobacter xestospongiae strain*

Domain: Bacteria

Phylum: Pseudomonadota

Class: Gammaproteobacteria

Order: Alteromonadale

Family: Alteromonadaceae

Genus: Marinobacter

Species: *Marinobacter xestospongiae*

Full scientific name: *Marinobacter xestospongiae* Lee et al. 2012

4.4.2 Hydrocarbonoclastic bacteria

Hydrocarbonoclastic bacteria are a group of bacteria that can easily adapt to the environment by using oil residue one of them is diesel oil as a source of carbon and energy. Bacteria hydrocarbonoclastics can produce biosurfactant compounds so that the oil the earth which was originally difficult to dissolve becomes soluble and blends with water (emulsification). This process occurs because the biosurfactants released by the battery can lowers the surface tension of a liquid and the interfacial tension between two different phases and improve emulsion stability. After that process hydrocarbonoclastic bacteria will carry out the biodegradation process by releases lipase enzymes which have the ability to hydrolyze fat into simple compound and is used as a carbon source by bacteria hydrocarbonoclastic. The end result of the bacterial metabolic process in the form of H2O and CO2 that is safe for the environment (Gouma, 2014).

Oil degradation bacteria can be found in various habitats in the marine environment including in the waters around the harbor

Microorganisms that can live and have a role in breaking down hydrocarbons are bacteria, while the presence of other microorganisms is sufficient role that is fungi, molds, algae, and

actinomycetes (Nugroho, 2007). Bacteria hydrocarbonoclastic are bacteria that have the ability to degrade hydrocarbon compounds. Naturally, hydrocarbonoclastic bacteria have ability to bind, emulsify, transport, and degrade hydrocarbons (Zam, 2010).

Bacteria that have the potential to degrade hydrocarbons and are isolated from areas that have long been contaminated with hydrocarbon compounds, such as waters in the area around petroleum storage tanks. Isolation this can be done by spreading (spread-plate), pour (pour-plate) or scratch (streak-plate) (Cappuccino and Sherman, 2002).

4.4.3 Pseudomonadota

Pseudomonadota (synonym: Proteobacteria) is a major phylum of Gram-negative bacteria.

1. **Characteristics**

All Pseudomonadota (Proteobacteria) are diverse. They are nominally gram-negative, although in practice some may actually stain gram-positive or gram-variable. Their outer membrane is mainly composed of lipopolysaccharides. Many moves about using flagella, but some are nonmotile or rely on bacterial gliding. Pseudomonadota have a wide variety of metabolism types. Most are facultatively or obligately anaerobic, chemolithoautotrophic, or heterotrophic, but numerous exceptions occur. A variety of genera, which are not closely related to each other, convert energy from light through conventional photosynthesis or anoxygenic photosynthesis.

2. **Taxonomy**

The group is defined primarily in terms of ribosomal RNA (rRNA) sequences. The Pseudomonadota are divided into several classes. These were previously regarded as subclasses of the phylum, but they are now treated as classes.

3. **Transformation**

Transformation, a process in which genetic material passes from one bacterium to another, has been reported in at least 30 species of Pseudomonadota distributed in the classes' alpha, beta, and gamma.

4.4.4 Gammaproteobacteria

Gammaproteobacteria are a class of bacteria in the phylum Pseudomonadota (synonym: Proteobacteria). It contains about 250 genera, which makes it the most genus-rich taxon of the Prokaryotes. Several medically, ecologically, and scientifically important groups of bacteria belong to this class.

4.4.5 Alteromonadale

Alteromonadales is an order of bacteria. There are 422 species of Alteromonadales, in 49 genera and 8 families. It includes groups like Shewanellaceae, Moritellaceae, and Pseudoalteromonadaceae. The Alteromonadales are an order of Pseudomonadota. Although they have been treated as a single family, the Alteromonadaceae, they were divided into eight genera by Ivanova et al. in 2004. The cells have straight or curved rods. They are motile because they use a single flagellum. Most of the species are marine.

4.4.6 Alteromonadaceae

Alteromonadaceae is a Pseudomonadota family. They are now one of several families in the order Alteromonadales, which includes Alteromonas and its closest relatives. Species in this family are mostly rod-shaped and move by using a single polar flagellum.

4.4.7 Marinobacter

Marinobacter Hydrocarbonoclastics is a bacterial species found in seawater that can degrade hydrocarbons. The cells are rod-shaped and have a single polar flagellum that allows them to move.

Marinobacter is a bacterial genus found in seawater. They can also be found in a number of salt lakes. Hydrocarbons can be degraded by a variety of strains and species. *M. alkaliphilus, M. thermophilus, and M. thermophilus* are among the species involved in hydrocarbon degradation.

Many Marinobacter species degrade aliphatic and polycyclic aromatic hydrocarbons as well as acyclic isoprenoid compounds efficiently. They are frequently isolated as hydrocarbon-degrading organisms in a wide range of contaminated marine environments, implying that they play an important role in oil pollution mitigation. Studies on their physiology, biochemistry, and genomics have improved our understanding of Marinobacter

psychophysiological role in marine contaminated ecosystems and highlighted their biotechnological potential.

4.4.8 Marinobacter xestospongiae

Marinobacter xestospongiae is a Gram-negative, slightly halophilic and non-spore-forming bacterium from the genus of Marinobacter which has been isolated from the sponge *Xestospongia t*estudinaria.

CHAPTER V
CONCLUSION

5.1 Conclusion

In the discussion that has been described above can be taken conclusion as follows:

1. At Gresik Port, 4 bacterial isolates were obtained degrading diesel oil, where the 4 isolates were obtained bacterial isolates namely *Marinobacter xestospongiae.*
2. In testing the potential of bacteria to degrade diesel oil, all isolates able to degrade well. The results of the identification of bacteria that able to significantly degrade diesel oil *(Marinobacter xestospongiae)* with a biodegradation percentage of 92.25%.

5.2 suggestion

Based on the conclusions that have been described, the suggestions on future research are

1. Be able to conduct research on biosurfactants tests produced by oil degrading bacteria

2. For future researchers, they can carry out oil biodegradation tests diesel fuel by using temperature and pH conditions in each medium hydrocarbonoclastic bacterial isolates culture.

REFERENCE

Andina, Fika.2014. Biodegradasi Total Petroleum Hydrocarbon (TPH) dengan Bakteri Penghasil Biosurfaktan Asal Air Laut dan Sedimen Pantai Karangsong Kabupaten Indramayu Jawa Barat. Skripsi. Universitas Padjadjaran Fakultas Perikanan Dan Ilmu Kelautan Program Studi Ilmu Kelautan Jatinangor.

Arulazhagan Ph.D. 2010. Biodegradation of polycyclic aromatic hydrocarbon by a halotolerant bacterial consortium isolated from marine environment, International Journal of Environmental Science & Technology, (2010), 639-652, 7.

Ali, Abdullah Yusuf. 2009. Tafsir Yusuf Ali Teks, Terjemahan Dan Tafsir Qur'an 30 Juz. Bogor : Litera Antarnusa.

APHA. 1981. Standart and Methoda.7th Edition. California:Cumming Publishing Company Inc.

Aprilia, N. S., dan Oetomo, H. W. (2015). Perbandingan Kinerja Keuangan Sebelum dan Sesudah Akuisisi Pada Perusahaan Manufaktur. Jurnal Ilmu dan Riset Manajemen, Vol 4 (No.12).

Atlas ,R.M. dan Bartha, R. (1992) Microbial Ecology : Fundamentals and Application. Redwood City, California. Third Ed. The Benjamin/ Cummings Publishing Company, Inc. 393-399.

Cappucino, JG dan Sherman N. 1983. Microbiology a Laboratory Manual 4th ed Menlo Park : Addison-Wesley Publ. Company, Inc.

D. E. Lebonah, K. Chandrasekhar, 2014, DNA Barcoding on Bacteria, Hindawi Publishing Corporation Advances in Biology Volume 2014, Article ID 541787, 9 pages http://dx.doi.org/10.1155/2014/541787

Dwidjoseputro. 1990. Dasar-Dasar Mikrobiologi. Jakarta: Djambatan.

Facundo j. Biodegradation of diesel oil in soil by a microbial consortium. Centro de investigación científica y de educación superior de ensenada, ensenada, méxico. 11 october 1999;

Gouma, S., Fragoeiro, S., Bastos, A.C. and Magan, N. 2014. Bacterial and Fungal Bioremediation Strategies. Journal of Biodegradation and Bioremediation. Vol. 13.

Handoyo, Darmo and Rudiretna, Ari (2000) general principles and implementation of polymerase chain reaction (pcr) [General Principles and Implementation of Polymerase Chain Reaction]. Unity, 9 (1). Pp. 17-29. ISSN 0854-3097.

Harayama,S.,H.Kishira.,Y.Kasai &K.Shutsubo.1999.Petroleum Biodegradation In Marine Environments.Marine Biotechnology Institute l (1) :63-70.

Herdiyantoro, D. 2005. Biodegradasi Hidrokarbon Minyak Bumi oleh Bacillus sp. Galur ICBB 7859 dan ICBB 7865 dari Ekosistem Air Hitam Kalimantan Tengah dengan Penambahan Surfaktan.Tesis. Sekolah Pascasarjana Institut Pertanian Bogor.

Hinchee MA, Connor-Ward DV, Newell CA, mcdonell RE, Sato SJ, Gasser CS, Fishhoff DA, Re DB, Fraley RT, Horsch RB. Production of transgenic soybean plants using *Agrobacterium*-mediated DNA transfer. *Bio/Technol.* 1988;6:915–922.

Kazuya. 2001. Microorganisms relevant to bioremediation. Journal environmental biotechnology vol 12.

Mahjoubi, M. Et al., Hydrocarbonoclastic bacteria isolated from petroleum contaminated sites in Tunisia: [37_TD$DIFF]isolation, identification and characterization of the biotechnological potential, New Biotechnol. (2013), http://dx.doi.org/10.1016/j.nbt.2013.03.004.

Mijaya Turiman. 2004, Pencegahan dan Penanggulangan Pencemaran Lingkungan Laut, Semarang.

Ministry of Environment Decree No. 128, 2003.

Misran, Erni.2002. Aplikasi Teknologi Berbasiskan Membran dalam Bidang Bioteknologi Kelautan : Pengendalian Pencemaran. Program Studi Teknik Kimia. Universitas Sumatera Utara.

Munfarida, Ida. 2017. *Panduan Praktikum Bioteknologi*. Universitas Islam Negeri Sunan Ampel Surabaya. Surabaya.

Mukhtasor. 2006. Pencemaran Pesisir dan Laut. PT Paradnya Paramita, Jakarta.

Mukhtasor. 2006. Pencemaran Pesisir dan Laut. PT Paradnya Paramita, Jakarta.

Murray, R. K., Granner, D. K., & Rodwell, V. W. 2009. Biokimia Harper .Edisi 27. Jakarta: Buku Kedokteran EGC.

Nababan, B. 2008. Isolasi dan Uji Potensi Bakteri Pendegradasi Minyak Solar dari Laut Belawan. Tesis. Universitas Sumatera Utara, Medan.

Nadia Andhini, Syahril, 2018, Isolasi Bakteri Pendegradasi Minyak Dari Perairan Sekitar Pelabuhan Bengkalis Provinsi Riau, jurnal perikanan dan kelautan volume 23 no. 1, juni 2018: 15-20.

Nasikhin, Maya.2013. Isolasi dan Karakterisasi Bakteri Pendegradasi Solar dan Bensin dari Perairan Pelabuhan Gresik. Jurnal Sains Dan Seni Pomits Vol. 2, No.2, (2013) 2337-3520 (2301-928x Print). Jurusan Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Institut Teknologi Sepuluh Nopember (ITS).

Nugroho, Astri, Effendi, E. & Fiona, A. (2007). Pertumbuhan Konsorsium Isolat Bakteri Asal Benakat pada Media Minyak Bumi Bersalinitas Tinggi: Studi Kasus Biodegradasi Minyak Bumi Skala Laboratorium. Jurnal Ilmu Dasar. Vol 8. No. 2.

Nugroho, Astri. 2007. Dinamika Populasi Konsorsium Bakteri Hidrokarbonoklastik: Studi Kasus Biodegradasi Hidrokarbon Minyak Bumi Skala Laboraturium. Jurnal Ilmu Dasar. Vol.8 No.1.

Oetomo, Dwi. 2015. Biodegradasi Minyak Bumi oleh Mikroba pada Media Air Laut dan Air Tawar. Jurnal Bio-Pendagogi. Vol 4. No.1.

Pelczar, Michael J. ESC. Chan. 2008. Dasar-Dasar Mikrobiologi. Jakarta. UI Press.

Peterson et al. 2003. Uncertainty and the Management of Multistate Ecosystems: an apparently rationale route to collapse. Ecology 84: 1406-1411.

Peterson.2003. Long-term ecosystem response to the exxon valdes oil spill: institute of marine science, at chapel hill, university of north Caroline.

Pertamina, 2005. Industrial Diesel Oil (Minyak Diesel). Diakses tanggal 17 Oktober 2017. Http://www.pertamina.com/indonesia/headoffice/hilir-ppdn/product/prdsolar.html.

R H Vreeland. 2000. Isolation of a 250-million-year-old halotolerant bacterium from a primary salt crystal. Department of biology west Chester university Pennsylvania .

Rinanda, T. (2011). Analisis Sekuensing 16S rrna di Bidang Mikrobiologi Jurnal Kedokteran Syiah Kuala, 11(3), 172–177. Article.

Rohmah, Nita Shilfiani. 2017. Isolasi dan Identifikasi Bakteri yang Berpotensi Sebagai Agen Bioremediasi Timbal (Pb) dari Lumpur Lapindo. Skripsi . Jurusan Biologi Fakultas Sains dan Teknologi . Universitas Islam Negeri Maulana Malik Ibrahim. Malang.

Stackebrandt, E., Goebel, B.M., 1994. Taxonomic Note: A Place for DNA-DNA Reassociation and 16S rrna Sequence Analysis in the Present Species Definition in Bacteriology.

International Journal of Systematic and Evolutionary Microbiology 44, 846–849. Https://doi.org/10.1099/00207713-44-4-846

Sugianto, I., R. W. Kusumaningtyas, dan F. Illaningtyas. 2003. Uji sifat probiotik asam laktat sebagai kandidat bahan pangan fungsional. J. Teknol. Ind. Pangan. 18(2):89-95.

Sulistyono I. 2012. Dampak Tumpahan Minyak (Oil Spill) di Perairan Laut Pada Kegiatan Industri Migas Dan Metode Penanggulangannya. In Forum Teknologi. Vol.3 No.1.

Suriyani, Irma dan Kotijah, Siti. 2013. Kajian Islam Dalam Masalah Lingkungan Hidup Di Kota Samarinda. Risalah Hukum Fakultas Hukum Unmul. Vol. 9. No. 1. Kalimantan Timur: Hal. 71-78.

Udiharto. 1999. Penanganan Minyak Buangan Secara Bioteknologi. Makalah Umar, Faiqah. 2000. Biodegradasi Petroleum dan Hidrokarbon Eikosana Oleh Isolat Bakteri. Skripsi. Fakultas Matematika Ilmu Pengetahuan Alam. Universitas Hasanuddin. Makassar.

Vidali, M..2001. Bioremediation. An Overview. Pure Appl.Chem., Vol. 73, No. 7, hal 1163-1172.

Vreeland R, Rosenzweig W, Powers D (2000) Isolation of a 250-million-year-old halotolerant bacterium from a primary salt crystal. Nature 407:897–900. Doi:10.1038/35038060.

Wokocha, G.A., 2011. Impact of crude oil spillage on soil and food production in rivers state, Nigeria. Journal of Money, Investment and Banking, 9, 29-34.

Waluyo, L. 2010. Teknik Metode Dasar Mikrobiologi. Umm Press, Malang. 305 hlm

Zam, Syukira Ikhsan. 2011. Bioremediasi Tanah Yang Tercemar Limbah Pengilangan Minyak Bumi Secara In Vitro Pada Konsentrasi Ph Berbeda. Jurnal Agroteknologi. Vol 1. No.2.