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# Marine Bioremediation Using *Alcanivorax Borkumensis* SK2 as A Waste Prevention Oil Industry on the Tunda Island to the Impact of Flood in Banten Region

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**ABSTRACT**

Marine pollution in the water triggers many problems, one of them is in the area of Tunda Island, Banten, Indonesia. Waste pollution that occurs in the area occurs due to oil drilling activities conducted by Pertamina. As result, such drilling waste pollutes the environment of Tunda Island. The distance between residential areas and oil rigs is about 18 to 20 miles, which will take 2 hours to complete. As a result of the waste, many octopuses on Tunda Island were found drunk and dead. It will be more dangerous if the waste sticks to mangrove seedlings, in mangrove trunks and in seagrass beds, when exposed to sunlight it will melt because the gravel waste is pitch black and can burn. As the world's largest island nation, 60% of Indonesia's 265 million people depend on one-system waters. Toxic chemicals enter the oceans every day from industrial waste. This problem can lead to the risk of flood impacts for the environment affected by industrial waste pollution. Marine cleanliness plays an important role in realizing the prosperity of land and marine ecosystems. Efforts can be made with bioremediation that is recovery with the help of live bacteria such as *Alcanivorax borkumensis SK2* which is γ-proteobacterium of the sea that can be used to clean contaminated environments. This research aims to prevent industrial waste pollution and prevent the risk of flood impacts in Indonesia, especially in Tunda Island, Banten. Various literature explains that *A. borkumensis SK2* is able to digest linear and branched alkanes commonly found in crude oil. Bacteria grow naturally in polluted areas, when added with inorganic nitrogen and phosphorus, they can accelerate the cleaning process to 2 -5 years faster than natural processes for 5 - 10 years. Therefore, the application of the use of *A. borkumensis SK2* has the potential to be applied in Indonesia to overcome the problem of marine pollution.

**Keywords:** *Alcanivorax borkumensis SK2*; Marine ecosystems; Bioremediation; pollution

## 1. INTRODUCTION

Banten Island is an important fishing area in the Northern Region of Banten Province. Because this island is a source of livelihood of fishermen who come from seven sub-districts in Coastal Regency and Serang City. In addition, Banten Island is also a fairly crowded water area in addition to the activities of fishermen, this area is also a place for trade ships originating from various islands and ships with a cargo of raw materials to support industrial activities along Banten Island.

For the past four decades, although most people's economies have relied on marine ecosystems. Every day, toxic chemicals enter the oceans. This is due to weak pollution controls that allow the industry to dump toxic waste into the ocean almost without penalty. For example, cases of pollution on Tunda Island have occurred since 2019. Although Law No. 32 of 2009 on Environmental Protection and Management regulates aspects of management and sanctions

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for marine pollution actors. However, the government seems difficult to find evidence and bring it to court so that until now there has been no follow-up of the violation (Sudrajad, 2006; Malison, 2017).

Inadequate waste disposal infrastructure and weak policies make the sea a dumping ground for local industries and communities. In addition to petroleum, chemicals are one of the biggest causes of marine pollution. Chemicals are usually sourced from industrial disposal and naturally flow from the land and then rivers, and eventually end up in the ocean (Utami et al., 2020).

There are various kinds of losses obtained from worsening marine pollution. Poor water quality can worsen health conditions, reduce food production, and hinder economic growth, thereby exacerbating poverty in many countries. This is due to the direct impact of water pollution on health, agriculture, and ecosystems. It is known that improvements in water quality and sanitation contribute at least 80% to overall economic benefits (Aldosari, 2017). Marine pollutants can also accumulate in marine organisms such as fish or invertebrates such as corals1.2 Formulaic Problems (bioaccumulation) and cause a variety of physiological disorders that vary from subcellular changes (Utami et al., 2020).

Research in the search for efficient processes to clean and minimize water pollution has been conducted over the past few years especially in the use of bioremediation processes using microorganisms such as *Alcanivorax borkumensis SK2* to remove toxic heavy metals from petroleum and industrial waste (Golyshin et al., 2003). These bacteria are found in marine ecosystems and can absorb, and digest linear and branched alkanes found in crude oil and its products. Based on this study, with the addition of proper nutrients, oil spills can be cleaned within 2-5 years faster than natural processes that take from 5-10 years (Rojo, 2009).

The potential of the results of the above research is very likely to improve the quality of seawater and benefit people's lives. This paper is a literature study to analyze the potential use of *A borkumensis SK2* to repair damage to the aquatic environment in Banten Tunda Island.

## 2. LITERATURE REVIEW

### 2.1. Current Marine Protection

Located at the Western End of Java Island positions Banten as the gateway of Java and Sumatra Island and is directly adjacent to the DKI Jakarta area as the Capital of the Country. This geostrategic position certainly causes Banten as the main link of the Sumatra-Java trade route even as part of the Asian and International trade circulation as well as the location of economic agglomeration and potential settlements. The northern border borders the Java Sea, west of the Sunda Strait, and in the southern border the Indian Ocean, so that this region has potential marine resources.

But unfortunately, the protection of the sea is currently fairly vulnerable. Because many factors cause the sea in Banten province to be affected by pollution. Although there has been a Regional Regulation of Banten Province No. 3 of 2018 that regulates the protection and empowerment of coastal communities, the impact of pollution is inevitable. So, it needs action from the government and local communities to keep protecting the sea, especially in Banten Province**.**

### 2.2. Risks of Marine Pollution and Its Impact on the Environment

Environmental pollution caused by petroleum is of great concern because the hydrocarbon characteristics of petroleum are toxic to all forms of life and endanger aquatic and terrestrial ecosystems. Statistics estimate that 3.2 million tons of petroleum per year are released into the environment (Saadoun, 2015). This pollution is considered the most common organic pollutant in aquatic ecosystems.

Petroleum contains many volatile compounds that are emitted as gases from oil. The buildup of toxins in the water transported by the wind can worsen asthma attacks and chronic obstructive pulmonary disease among residents of coastal areas (Lenes et al., 2013). One of the main effects of oil is narcosis, a reversible anesthetic effect caused by the partition of petroleum into cell membranes and nerve tissues that can cause central nervous system dysfunction (Fingas, 2011). Oil components that sink and accumulate in sediment as black deposits on sand and rocks on the beach cause the body of the black marine biota to be enveloped in oil sludge making it impossible to move anymore. This affects the reproduction, development, growth, and behavior of marine life, especially in plankton, can even kill fish thus decreasing fish production (Kuncowati, 2010).

In addition, water that experiences "heavy" pollution usually has a high content of heavy metals in the water and the organisms that live in it. The absorbed metals will be distributed quickly throughout the body so that there is an accumulation of metals in the body of aquatic organisms. This will block the work of enzymes until the body's metabolism is disrupted so that it can cause cancer and mutations. If water organisms containing heavy metals are consumed by humans, it will have a detrimental impact on human health such as strep throat, headache, dermatitis, allergies, anemia, kidney failure, pneumonia, and so on (Pratiwi, 2020).

### 2.3. Materials or Agents That Serve to Improve the Environment

Oil spills are harmful to a country's economy and environment. Therefore, a number of oil spill cleaning methods to improve the environment have been developed. These methods can be grouped into four main categories: biodegradation, mechanical methods, chemical dispersants, and in situ combustion (Obi et al., 2014).

The four methods given above are aimed at removing oil from the surface of the water, but only mechanical methods can remove oil completely from the marine environment. Mechanical methods can only be implemented in calm ocean conditions without large ocean waves, high wind speeds, and high waves. Chemical dispersants are more effectively used within an hour or two of the initial spill time. However, dispersants are not suitable for use in most oils and locations. The use of chemical dispersants can also influence marine organisms to accumulate temporary oils. In situ combustion can be an effective method of cleaning up oil pollution that occurs in open bodies of water, but this method is often limited by wave and wind conditions and the proximity of spills to populated areas (Obi et al., 2014).

One biological effort that can be used to overcome oil pollution is to use bioremediation technology (Hadrianto, 2018). Bioremediation technique is the utilization of microorganisms to reduce the polluted environment, one of which is *Alcanivorax sp bacteria*. The use of these bacteria in degrading oils is considered safe, cheap, and easy. *Alcanivorax sp*. It is usually closely related to the presence of compounds from oil spill contaminants. This is said because according to Evitasari (2020), contaminants are one of the sources of carbon and energy in the life process for a large number of microbes including *Bacillus sp., Pseudomonas aeruginosa, and Aerococcus sp*. For example, chromium (Cr) and zinc (Zn) act importantly as micronutrients for bacterial metabolic and redox processes, so heavy metals can be degraded by microbes naturally. Indonesia needs to optimize this field considering people's dependence economically on coastal and marine areas (Umroh, 2011).

Currently, efforts to control and combat water pollution are generally carried out through technology that considers the characteristics of wastewater and its effluent quality

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standards. The selected technology is expected to be able to change the quality of effluent-standard so that it can meet the quality standards of the receiving water body (stream standard) that can be applied optimally to protect the environment and provide tolerance for industrial development (Catania et al., 2015).

## 3. METHOD

### 3.1. Data collection techniques and Processing

The data used in this study is secondary data from articles written by the researchers earlier. The articles collected are those published in reputable international journals (Q1, Q2, and Q3), sinta 1 or 2 accredited national journals, proceeding seminars (Local, National, and International), and books of legality published by trusted publishers. Data collection techniques begin by searching and collecting information from journals with a period of 2011-2021. Journal searches are conducted using databases PubMed, ScienceDirect, Scopus, Sinta. The keywords used are bioremediation, marine ecosystems, *Alcanivorax borkumensis SK2*, pollution.

Data processing methods describe data processing and analysis procedures by the article used. The articles obtained are classified based on the sub-topics discussed, namely various types of toxic waste in the waters, damage to ecosystems, water bioremediation efforts, and the activity of *Alcanivorax borkumensis SK2* in ecosystem repair. Data from the selected article will be extracted and displayed in the table and then discussed and concluded.

### 3.2. Data Analysis

The data analysis method used in this study is descriptive narrative. This is done to describe the object of research so that it can answer the formulations of problems that have been formulated before. This technique is performed by means (1) Data Reduction, (2) Presentation of data and (3) Withdrawal of conclusions.

1. Data reduction is carried out by summarizing and focusing on the things that are needed by the theme in the research conducted.
2. Presentation of data is usually in the form of a chart, a brief description, a relationship between categories and text that is narrative. This is done to make it easier for researchers to understand the object of the study so that researchers can determine and plan their next work by the findings that have been understood.
3. The initial conclusions presented are still temporary and will change if no strong and supporting evidence is found at the next stage of data collection. But if the conclusion is at an early stage, it is supported by valid and consistent evidence. The conclusions expressed are valid.
4. The data that has been obtained from various literature is collected in the unity of documents used to answer the problems that have been formulated.

### 3.3. Frame of Mind

This literature review is analyzed using narrative methods by grouping search results data according to the results measured to answer the purpose of research journals. The qualifying criteria are then collected and made a journal summary. Finally, the data that has been collected is sought equations, and differences are then discussed to be concluded. The process is can be seen in Figure 1.

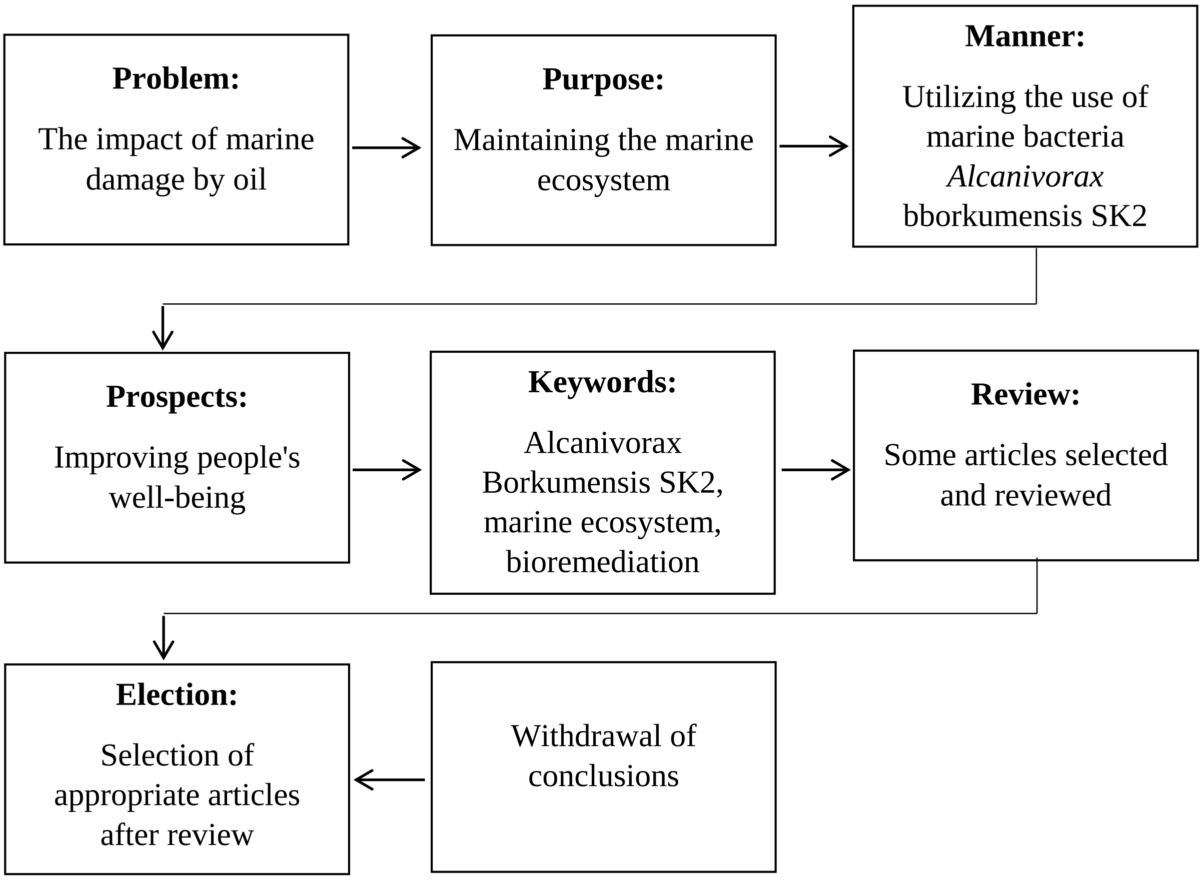


**Figure 1.** The process of literature review

## 4. RESULTS AND DISCUSSIONS

### 4.1. Impact of Damage to Waters by Oil

Oil spill events have a real impact on the marine and terrestrial environment as well as human health. Oil spills can affect the marine meteorological environment through dispersion, dissolution, emulsification, and evaporation of crude oil (Jenkins and Adams, 2011). In the event of an oil spill into the sea, the oil will spread and form a layer at sea level. Based on research by Xue et al. (2015), a ton of spilled oil can form 5 x 106 x $m^2$ of oil layers at sea level. This layer can block the direct exchange of oxygen and carbon dioxide and cause oxygen depletion and pH changes in seawater. This can form anaerobic conditions in seawater that lead to the death of flora and fauna. In addition, toxins contained in oil can poison or kill birds, mammals, fish, and other marine organisms and damage underwater ecosystems, adversely affecting the global food chain, and ultimately endanger human health. One example of the impact of an oil spill is on the Exxon Valdez accidents in the Gulf of Mexico in 2010 and "Penglai 19-3" in 2011. Both of these events have proven the impact of oil spills on environmental damage to the local area. The Exxon Valdez oil spill accident in the Gulf of Mexico is considered the most serious oil spill phenomenon that causes major financial losses as well as damages wetlands and beaches (Xue et al., 2015).

In addition, oil spill accidents can affect marine crops and agricultural production by blocking the exchange of light and gas. It is estimated that half of the total coastal wetlands loss is caused by oil spill accidents. Ultimately, oil pollution in the marine environment can cause significant economic losses in the tourism industry and marine resources, such as the

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coastal salt industry, marine chemical production, marine aquaculture, and the fishing industry (Xue et al., 2015).

### 4.2. Bioremediation Efforts of Polluted Waters

Bioremediation for hydrocarbon degradation is a widely used alternative to the recovery of contaminated sites. This is because compared to conventional physical and chemical methods, bioremediation technology is cheaper and very effective for marine ecological restoration. In addition, precise and accurate knowledge of the metabolic potential of microorganisms and the factors that make up degrading interactions, viability, and activity is needed to optimize bioremediation intervention strategies. In general, hydrocarbon-degrading marine microorganisms can be divided into general to specific according to the ability to grow on hydrocarbons or a wider set of carbon sources. **Table 1** shows and compares microorganisms strains of bacteria, archaea, and fungi that can metabolize different types of pollutants.

**Table 1**. The activity of microorganisms to metabolize the type of pollutants

| A/ AN | Micro-organisms | Phylogeny | Target Substrate | Habitat Ecology | Physiology |
|---|---|---|---|---|---|
| A | *Alcanivorax borkumensis* | $\gamma$-Proteobacteria, Alcanivoracaceae | n-Alkana | Seawater, sediment, beach sand, beach salt marsh | Biosurfactant manufacturer, BHKO |
| A | *Alcanivorax dieselolei* | $\gamma$-Proteobacteria, Alcanivoracaceae | n-Alkana | Seawater, sediment | Resistance to mild pressure increases, BHKO |
| A | *Marinobacter hydrocarbono-clasticus* | $\gamma$-Proteobacteria, Alteromonadaceae | n-Alkana, HAP | Seawater, sediment | Biofilm producer; oil surface invaders |
| A | *Cycloclasticus pugetii* | $\gamma$-Proteobacteria, Piscirickettsiaceae | HAP | Sediment | Highly efficient transport system for capturing nutrients and oligo elements |
| A | *Oleispira antarctica* | $\gamma$-Proteobacteria, Oceanospirillaceae | n-Alkana | Seawater | BHKO adapts to the cold |

A, A, aerobics; AN, anaerobic; BHKO, obligate hydrocarbonolastic bacteria; HAP, polycyclic aromatic hydrocarbons

(Mapelli et al., 2017)

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Among millions of microorganisms, bacteria from the hydrocarbonoclastic group are the most frequently studied bacteria from physiological, ecological, to biotechnological aspects due to their presence and prevalence. One of the most popular genera of this group and often isolated from hydrocarbon-contaminated environments is *Alcanivorax borkumensis* (Hassanshahian, 2015). *A. borkumensis* was first reported in 1998 as a biosurfactant-producing hydrocarbon degrading bacterium. These bacteria are found in high concentrations in the area around oil spills and produce glycolipid biosurfactants to help access hydrocarbons in emulsion droplets. The growth of *A borkumensis* relies heavily on nutrient concentrations in hydrocarbon regions, namely the amount of phosphorus and nitrogen, and is found to grow in both n-alkana-water and branched alkanes (Bookstaver et al, 2015).

High enzymatic production reached 145.71 U/mg for hydroxylase alkanes, 3628.57 U/mg for lipase, and 2200 U/mg for esterase causing significant degradation efficiency of different hydrocarbon petroleum substrate concentrations reaching 73.75% for 5000 ppm hexadexane, 82.80% for 1000 ppm motor oil, 64.70% for 70 ppm BTEX and 88.52% for 6000 ppm of polluted soil. The study showed that *A. borkumensis* is a potential hydrocarbon degrading bacterium with a higher enzymatic capacity for hydrocarbon-polluted environmental bioremediation than the genus of the same group (Kadri et al., 2018).

Although bioremediation proved to be a utility, natural and non-destructive character, bioremediation technology remains controversial in some aspects. The effectiveness of bioremediation practices depends on several factors, such as the presence of appropriate hydrocarbon-degrading microbial consortiums as well as favorable environmental conditions. In addition, progress on this topic is often difficult due to inadequate consistency between data obtained from laboratory experiments and those obtained in situ experiments. On the other hand, these experiments are difficult to do and the data obtained is not easy to interpret due to the complexity of the biological processes analyzed (Hassanshahian et al., 2014).

### 4.3. Activities and Potential of *Alcanivorax Borkumensis SK2* in Ecosystem Improvement

*Alcanivorax borkumensis SK2* is an unusual stem-shaped marine γ-proteobacterium bacterium that can grow on a very limited spectrum of substrates, especially alkanes. *A. Borkumensis SK2* has special physiology that is the ability to metabolize and grow efficiently and exclusively on alkanes (Sabirova et al., 2011). These bacteria are found in low amounts in unpolluted environments, but quickly become dominant in polluted seas, and can account for 80 – 90% of oil-decomposing microbes (Sabirova et al., 2011).

These "hydrocarbonoclastic" bacteria degrade vast alkane hydrocarbons. *A. Borkumensis SK2* is capable of degrading large amounts of alkanes to C32 and branched aliphatics, as well as isoprenoid hydrocarbons (e.g. phytoene), arena alkyl, and cycloalkane alkyl. Degradation of alkanes in *A. borkumensis SK2* occurs through several terminal oxidation routes involving AlkB hydroxylase, flavin-binding monooxygenase, and cytochrome P450 (Sabirova et al., 2011).

The bacterium *A. borkumensis SK2* is capable of producing glycolipid biosurfactants that can increase hydrocarbon biodiversity availability by increasing hydrocarbon solubility in the water phase or by expanding the contact surface area due to emulsification. The genome of *A. borkumensis SK2* includes 11 genes that encode lipase/esterase in contrast to unknown specificity. Two of these esterases are purified and functionalized. Esterase exhibits up to twice as much enzymatic activity as general esterase, has a large substrate spectrum, remarkable enantiosis selectivity, and superior chemical resistance to esterases from microorganisms and other enzymes for chicataly and mixture resolution in biocatalysts (Sabirova et al., 2011).

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The decrease in bacterial cells was caused by a mortality factor thought to be due to reduced nutrient content N and P in the sample. Nitrogen is a protein element that plays a role in cell growth, propagation, and cell wall formation. Phosphorus is a major component of nucleic acids and membrane cell fats that play a role in the process of biological energy transfer, the formation of amino acids. Elements N and P are mostly found in fertilizers (Umroh, 2011).

The availability of nutrients such as N, C, and P can make bacteria grow well. Nitrogen in ammonia is a nutrient that serves for the growth and reproduction of bacteria so that the provision of nutrients at a higher rate can increase bacterial growth. In addition, nitrate is one of the electron acceptors that can provide an anaerobic degradation stimulus, but in anaerobic conditions, nitrate compounds can be reduced (Umroh, 2011). This can decrease the process of degradation by the bacterial community.

Other studies tested the efficiency of *A. borkumensis* to address various sources of fatty acids and studied the growth rate and patterns of membrane fatty acids from these bacteria cultivated on diesel, biodiesel, and horseradish oil as sources of carbon and energy. The results obtained showed significant differences in both parameters depending on the growth substrate. The highest growth rates were observed with biodiesel, while the growth rates of horseradish and diesel oil were lower than in standard reference compounds (hexadecane). The most remarkable observation is that cells grown on horseradish oil, biodiesel, and diesel showed significant amounts of the two polyunsaturated fatty acid linoleic acids and linolenic acid in their membranes. By directly inserting these external fatty acids, bacteria conserve energy thus allowing them to degrade those pollutants more efficiently. Such rapid adaptations can increase the resilience of *A. borkumensis* and allow them to fight and sustain populations in more complex hydrocarbon-destroying microbial communities (Koniecz et al., 2018).

Bioremediation techniques are innovative technologies for processing contaminants by utilizing microbes. This technology is an alternative introduced to reduce the impact of oil contamination in Indonesia's marine and coastal waters. *Alcanivorax borkumensis SK2* can be utilized in bioremediation techniques because, in addition to producing coarse enzymes that show high efficiency in removing hydrocarbons, compounds contained in the oil. The use of *A. borkumensis SK2* in degrading oil is quite safe, cheap, and easy so that these bacteria have good prospects as an alternative to cleaning the polluted environment of compounds from industrial waste in the aquatic environment.

Although little is known about the exact mechanism used by A. Borkumensis to reduce oil, the hypotheses encapsulate the method with the following steps:

1) Oil leakage into the aquatic environment leads to increased concentrations of phosphorus and nitrogen.
2) Increased availability of nutrients causes A. Borkumensis to metabolize and grow faster so that the population increases
3) *A. borkumensis* attaches and forms biofilms around oil droplets. Biofilms help recruit additional bacteria to contamination sites
4) The enzymes AlkB1 and AlkB2 are synthesized and used to oxidize C-alkanes, thus catalyzing oil degradation
5) Biosurfactants are produced and the rest of the oil and water form an emulsion, making the oil more readily available to A. Borkumensis.

### 4.4. Implementation of Bioremediation *Alcanivorax Borkumensis* SK2 in Indonesia

Bioremediation *Alcanivorax Borkumensis* SK2 is very appropriate to be applied in Indonesia, where for the manufacture of bioremediation process using many tools and materials and easily accessible in Indonesia, as well as with the geographical conditions of Indonesia which is dominated by marine areas and has dozens of oil factories in each area that has the potential for oil waste that continues to pollute the marine environment. Therefore, prevention and recovery is necessary with the help of this bioremediation process and is feasible to apply in Indonesia.

But until now, on Tunda Island has not been implemented bioremediation *Alcanivorax Borkumensis* SK2 due to lack of innovation and awareness of the surrounding community. In Indonesia, currently only PT. Chevron Pacific Indonesia conducts bioremediation. PT CPI operates nine bioremediation facilities in our operations in Riau Province, Sumatra, covering an area of more than 10 acres, with a combination of capacity capable of remediating approximately 42,000 cubic meters of land each cycle. Therefore, the existence of this paper is expected to help contribute in the form of ideas for ecosystem improvements on Tunda Island, so that the Bioremediation method of *Alcanivorax Borkumensis* SK2 can be implemented to reduce and even eliminate oil that pollutes the sea.

## 5. CONCLUSION

Based on the results of the journal search, it can be concluded that:

1. Heavy metal contaminants can accumulate in the body of marine organisms and are harmful to humans who consume them. This problem-solving effort, using bioremediation technology, is an innovative, inexpensive, and effective technology for restoring marine ecology and processing contaminants by utilizing various biological agents including *Alcanivorax borkumensis SK2*.
2. *Alcanivorax borkumensis SK2* bacteria can interfere with oil spills. These bacteria degrade the oil safely, cheaply, and easily. Cleaning oil spills with *Alcanivorax borkumensis SK2* plus proper nutrition can speed up oil cleaning time by 2-5 years from natural processes that take from 5-10 years..
3. Biosurfactants of glycolipid produced by *Alcanivorax borkumensis SK2* can expand the contact surface area to increase hydrocarbon biodiversity availability. These bacteria have twice the enzymatic activity of general esterase, a large substrate spectrum, exceptional enantios selectivity, and superior chemical resistance.

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