Degradation of Oil in co-culture system of Alcanivorax borkumensis SK2 and Rhod coccus opacus B4

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Abstract. This study explores the bioremediation potential of petroleum contamination using a co-culture of Alcanivorax borkumensis SK2 and Rhod coccus opacus B4. The research investigates the effectiveness of these bacteria in degrading petroleum hydrocarbons, focusing on their growth and oil degradation capabilities in diesel and gasoline environments. Experimental results demonstrated that diesel, due to its long-chain hydrocarbons, supported more significant bacterial growth, especially for Rhod coccus opacus B4, than gasoline. The findings highlight the potential of using this co-culture for oil spill remediation, with tailored approaches for different oil compositions.

Keywords: bioremediation, co-culture, hydrocarbon, bacterial growth.

1. Introduction

Petroleum is a product in high demand because it generates electricity and brings energy to power facilities in cities for human beings. However, shipping petroleum is a high-risk task. Hence, there are many accidents where oil spills into the sea. It would lead to the seafood suffering from contamination. For example, fish with oil stuck in their gill would die out due to being unable to breathe. It would not only significantly affect the ecological environment in the ocean. For human society, negative influences will finally reach because seafood is also a nutrient for people to take in. In addition, some sea birds are also trapped, as their feathers and plumage are covered with oil, so they lose the ability to fly and maintain the balance of their body temperature. The data shows that "around three million tons of oil enters the sea each year, of which about 20% originates from oil-pumping operations, transport and refining activities and 25% from non-tanker shipping and natural seepages. More than half (55%) originates from illegal activities that include the dumping of ballast water and oil residues as well as accidents" [1]. The oil spill is more common than we suspect. People tend to think only the big issue posted on the news is the phenomenon of spilling. However, actually, during the process of cargo being refilled with oil, a small amount of oil drops into the ocean. Even though its result will not lead to extreme negative soon, the sea is sensitive. Therefore, the oil will eventually flow into the open sea and cause irreversible consequences.

Currently, three different methods are most often used to deal with oil spills. The first is the physical method, in which people use some specific type of adsorbent material to strip the oil from the sea's surface. Then, the oil is relocated to other places. The second is the chemical way, in which chemicals are sprayed on the surface of the spilled oil. It prevents the dispersion of the oil over the sea platform. According to the different dispersion states of oil, gelling agents, dispersants, or demulsifiers can diffuse or remove it based on different situations. However, the toxic elements in the chemicals would lead to side effects that further negatively influence the ocean. So far, the broad application would be burning the oil on the sea platform directly as it costs less. Nevertheless, it would also bring pollution to the ocean as sediment and remaining elements would perpetuate for a long while.

According to the situation, a practical solution for petroleum spills becomes essential. An environment-friendly technique, bioremediation, uses some microbes and bacteria to convert toxic elements of petroleum into non-toxic compounds. Some bacteria are found to decompose the oil and convert it to their energy for metabolic pathways. Therefore, this study aims to use oil-eating bacteria as an environmentally friendly solution to oil spill remediation. Thus, Alcanivorax borkumensis SK2 and Rhod coccus opacus B4 will be the primary bioremediation bacteria.

2. Properties

Alcanivorax borkumensis SK2 is aerobic, which means it uses oxygen as a part of energy production, and it is halophilic, which means it tends to thrive in a salty environment. It is a marine gram-negative bacterium isolated from seawater/sediment samples that almost exclusively uses alkanes as carbon and energy sources [2]. This bacterium can digest oil by breaking down petroleum hydrocarbons with special enzymes. The genus Rhod coccus opacus B4 is one of the most versatile concerning hydrocarbon degradations, being able to metabolize different types of hydrocarbons – from alkanes with C6–C36 to complex polycyclic aromatic compounds present in gasoline, diesel, engine, and crude oil. Rhod coccus opacus B4 is suitable for bioremediation under a diverse range of environments. Their ability to undergo microaerophilic respiration allows them to survive in environments containing low oxygen concentrations, and their ability to undergo aerobic respiration also allows them to survive in oxygenated environments [3]. The co-culture of multiple microorganisms is a standard method to expand the biodegradation of petroleum hydrocarbons, which is considered a promising treatment technology for petroleum-contaminated ecosystems [4]. Therefore, for experimental purposes, the main goal is to evaluate whether Rhod coccus opacus B4 would generate a positive relationship with the growth of Alcanivorax borkumensis SK2.

The primary purpose of this experiment is to research the bioremediation potential of petroleum contaminants by Alcanivorax borkumensis SK2 and Rhod coccus opacus B4. Based on the success of the co-culture of Bacillus subtilis SL and Pseudomonas aeruginosa WJ-1 [5], we hypothesize that their co-culture can enhance oil degradation.

3. Experiment Setup

3.1. Cultivation of Alcanivorax borkumensis SK2 and Rhodococcus opacus B4

Alcanivorax borkumensis SK2 (DSM 11573T) and Rhod coccus opacus B4 (NBRC 108011) were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany), and the National Institute of Technology and Evaluation, Biological Resource Center, Japan (NBRC). Maintenance and growth of the bacterial cultures were performed using 809 media for Alcanivorax borkumensis SK2 [4] and 802 media for Rhod coccus opacus B4 [4]. Cultures were incubated at 30°C in a rotary shaker (120 rpm). Making the noncarbon medium for experiment phase II used.

3.2. Monitor the Bacterial Growth

Before starting the degradation, acknowledging the growth of bacteria is essential. First, take a certain number of active bacteria from the previous culture and inoculate them into a fresh and new culture medium. Ensure the inoculum is sufficient to support bacterial growth but avoid over-inoculation. Second, place them in the appropriate conditions—30°C- in a rotary shaker (120 rpm). Third, regularly sample the bacteria to monitor their growth, using a spectrometer to count the number of cells. Take 0.1ml bacteria medium out of the flask and put it into the sample container of the spectrometer. Under the wavelength 600nm, the result will show the abs of the sample. If it is lower than one abs, put the flask back in the incubator and grow one more night. If it is larger than one abs, pour the medium back into the flask and add water to make it one abs. As both samples are from two bacteria, flasks have close enough to 1 abs. Assuming that the concentration of bacteria in the different flasks is equal, the research will move to the next step.

3.3. Impact of different hydrocarbon oil

The objective of Phase III is to investigate the behavior of Alcanivorax borkumensis SK2 and Rhod coccus opacus B4 in environments with oils of varying densities. To commence this phase, two types of oils with different densities will be selected: diesel (high concentration in hydrocarbon) and gasoline (moderate concentration in hydrocarbon).

The goal was to evaluate how the co-culture of these two oil-degrading bacteria could impact their growth and oil degradation abilities. The hypothesis was that their combination would enhance biodegradation, especially in a diesel-rich environment. This aligns with previous research on the biodegradation potential of co-cultured microorganisms and their ability to utilize hydrocarbons in petroleum spills. The impact of these diverse oil densities on Alcanivorax borkumensis SK2 and Rhod coccus opacus B4 will be evaluated using a Spectrophotometer to test the adaptability and efficiency of Alcanivorax borkumensis SK2 and Rhod coccus opacus B4 under these varied environments by measuring the change of the concentration of bacteria.

To prepare and inoculate the culture medium, start by transferring the old bacteria to a new agar plate. Prepare two types of media: nutrient media for the bacteria (Alcanivorax borkumensis SK2, Rhod coccus opacus B4) and a degradation medium without carbon. For the experimental setup, create a total of 12.3 ml of medium for 14 groups: 10 ml of degradation medium, 2 ml of gasoline or diesel, and 0.3 ml of bacterial medium, which includes two experimental groups, one bacterial control group for each, and two oil control groups. Incubate these mixtures at 30°C on a shaker set to 120 rpm for seven days, allowing the microorganisms to utilize and degrade petroleum hydrocarbons as a carbon source for their growth and reproduction. After incubation, measure the absorbance of the extracted solution at 600 nm using a UV spectrophotometer to assess bacterial growth under different petroleum concentrations.

4. Data Analysis

Table 1. Gasoline Environment Group

	AB: 2RO	AB: RO	2AB:RO	AB	RO	No bacteria	AB no oil	RO no oil
Concentration of bacteria in 600OD	0.068	0.097	0.251	0.2	0.205	0.05	0.3	0.286
	0.16	0.13	0.177	0.295	0.16	0.05		

Table 2. Gasoline Environment Group

	AB: 2RO	AB: RO	2AB:RO	AB	RO	No bacteria	AB no oil	RO no oil
Concetration of bacteria in 600OD	1.066	0.939	0.963	0.7	1.179	0.176	0.264	0.212
	0.965	0.976	1.004	0.626	0.962	0.176		

At the start of the experiment, we measured the initial concentration of Alcanivorax borkumensis SK2 (AB) and Rhod coccus opacus B4 (RO) using a spectrophotometer at 600 nm. Both bacterial strains grew to an absorbance of 0.5, which served as the baseline concentration (C0). This baseline marked the initial condition for tracking bacterial growth over the 7-day experiment period under two types of oil—gasoline and diesel.

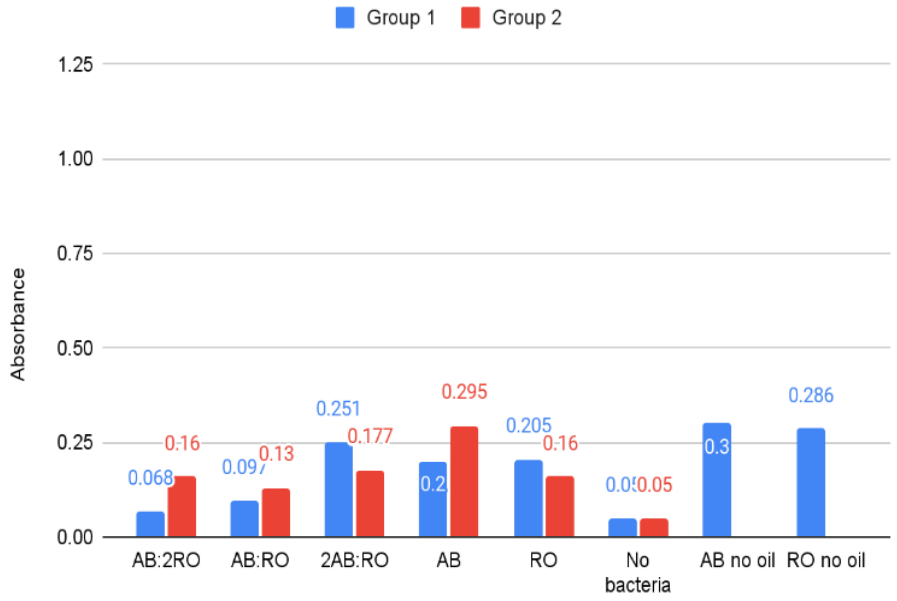


Figure 1. Concentration of bacterial growth under 600OD in gasoline environment

When we examined the results from the gasoline environment, it became evident that gasoline did not provide an optimal growth medium for the bacteria. The absorbances recorded after seven days showed a slightly higher bacterial concentration in groups 3 (2AB) and 4 (AB), with absorbances of 0.214 and 0.2475. Despite these two groups, the absorbances for all groups, including those with the highest values, were below the starting concentration (C0 = 0.5). This suggests that gasoline did not provide a sufficient number of hydrocarbons to support significant bacterial growth. Alcanivorax borkumensis SK2 and Rhod coccus opacus B4 rely on hydrocarbons for energy and growth. However, the composition of gasoline, which primarily contains shorter-chain hydrocarbons, may not be ideal for the metabolic pathways of these bacteria. Moreover, when comparing the experimental groups to the control group (which lacked gasoline but contained the same two types of bacteria), it becomes clear that gasoline as a nutrient was not effective enough in promoting bacterial growth. The control group, with no gasoline, showed similar or even slightly better growth, reinforcing the conclusion that gasoline, as a hydrocarbon source, did not provide the essential nutrients required for robust bacterial activity. This aligns with existing literature, which suggests that some bacteria prefer longer-chain hydrocarbons, typically found in diesel or crude oil, over shorter-chain compounds like those found in gasoline.

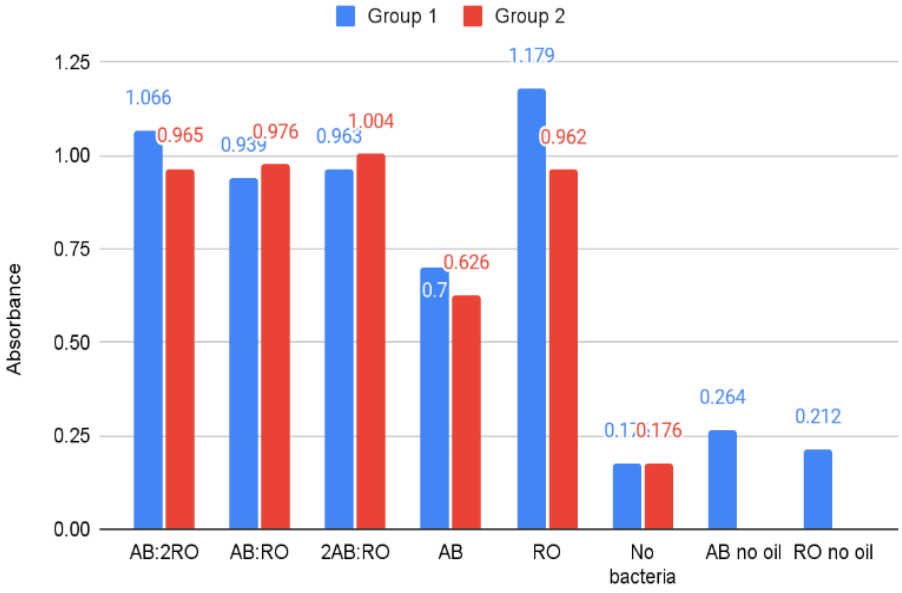


Figure 2. Concentration of bacterial growth under 600OD in disel environment

The results from the diesel environment tell a different story. Diesel, known to have longer-chain hydrocarbons compared to gasoline, provided a more suitable environment for bacterial growth. All experimental groups in the diesel environment showed higher absorbances than the baseline (C0), indicating that both Alcanivorax borkumensis SK2 and Rhod coccus opacus B4 could thrive. This supports the hypothesis that diesel serves as a more reliable hydrocarbon nutrient for oil-degrading bacteria. Additionally, the control group without diesel showed that bacteria would eventually decrease in concentration as nutrients were exhausted, confirming that diesel plays a crucial role as a nutrient source. Notably, the three combination groups (AB ratios) showed minimal differences in absorbance, indicating similar bacterial concentrations. However, group 5 (RO) exhibited the highest absorbance at 1.0705, suggesting that Rhod coccus opacus B4 (RO) adapted better to the diesel environment than Alcanivorax borkumensis SK2 (AB). While the combination groups showed no significant differences, group 1 (AB:2RO), which had a higher Rhod coccus opacus B4 ratio, displayed a relatively higher absorbance (1.0155). This further suggests that Rhod coccus opacus B4 might be more effective in utilizing diesel as a hydrocarbon nutrient compared to Alcanivorax borkumensis SK2.

5. Conclusion

In conclusion, the co-culture of Alcanivorax borkumensis SK2 and Rhod coccus opacus B4 demonstrated significant potential for bioremediation of petroleum hydrocarbons, particularly in diesel-rich environments. Experimental results indicated that all bacterial groups exhibited growth in diesel, with Rhod coccus opacus B4 achieving the highest absorbance (1.0705), suggesting greater adaptability to diesel as a nutrient source. In contrast, gasoline provided a less suitable environment, with bacterial growth remaining below the initial concentration across all groups. These findings underscore the effectiveness of Rhod coccus opacus B4 in degrading diesel and suggest that bioremediation strategies tailored to specific oil compositions can enhance the efficiency of oil spill remediation efforts.

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