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APLIKASI *RESPONSE SURFACE METHODOLOGY* UNTUK OPTIMASI PROSES BIODEGRADASI PASIR PANTAI TERKONTAMINASI MINYAK BUMI MENGGUNAKAN *BIO-OIL SPILL DISPERSANT (OSD)* DAN *Bacillus subtilis* CYA27

APPLICATION OF RESPONSE SURFACE METHODOLOGY FOR OPTIMIZATION OF THE BIODEGRADATION PROCESS OF PETROLEUM-CONTAMINATED BEACH SAND USING BIO-OIL SPILL DISPERSANT (OSD) AND Bacillus subtilis CYA27

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ABSTRACT

In coastal ecosystems, oil pollution from recurrent spills is a crucial environmental challenge, particularly in regions such as the Malacca Strait and the northern coast of West Java. Crude oil is rich in persistent organic pollutants (POPs) that are resistant to natural degradation, leading to long-term ecological risks and threats to coastal communities. To mitigate the impacts, urgent and effective remediation strategies are needed. Bioremediation, by utilizing microbial activity to degrade hydrocarbons, provides an environmentally sustainable alternative to conventional clean-up methods. Therefore, this study investigated the bio-degradation efficiency of crude oil-contaminated sand using a combination of Bio-OSD dispersant and Bacillus subtilis CYA27 under controlled laboratory conditions. Response Surface Methodology (RSM) through Design Expert software (DX13) was adopted to optimize key variables, including the dispersant-to-oil ratio (DOR) and bacteria dosage. Experimental trials evaluated response parameters such as Total Petroleum Hydrocarbon (TPH) degradation, Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS) reduction, pH, and bacteria population. The optimized conditions recommended by the RSM model were a DOR of 0.77:1 and a 5% bacteria dosage. The results showed that the parameters achieved 50.25% TPH degradation, COD reduction to 895.41 mg/L, pH adjustment to 8.21, and bacteria population of 6.39 Log CFU/mL. Furthermore, the model featured a desirability score of 0.64, reflecting satisfactory optimization, though further refinement could enhance efficiency. The study shows the potential of integrating dispersants with microbial agents as a viable method for bioremediation of oil-polluted coastal environments, contributing valuable insights for sustainable pollution management.

KATA KUNCI:

Bakteri Pendegradasi Minyak Bumi, Biodegradasi, Minyak Bumi, Total Hidrokarbon Minyak Bumi

ABSTRAK

Pencemaran minyak di wilayah pantai akibat tumpahan minyak merupakan masalah lingkungan yang harus diantisipasi. Beberapa tumpahan minyak telah terjadi berulang kali di seluruh dunia, seperti di Selat Malaka dan Pantai Utara Pulau Jawa Barat. Minyak bumi yang mengandung polutan organik persisten (POP) sulit terdegradasi. Penelitian ini bertujuan mengevaluasi efisiensi biodegradasi pasir terkontaminasi minyak bumi menggunakan Bio-OSD dan *Bacillus subtilis* CYA27. Optimasi bioremediasi pasir pantai terkontaminasi minyak bumi dilakukan dengan menggunakan *software design expert* (DX13) untuk menentukan kombinasi optimum dari variabel *dispersant to oil ratio* (DOR) dan dosis bakteri *B. subtilis* CYA27. Berdasarkan hasil proses pencucian pasir terkontaminasi didapatkan nilai tengah (0) pemberian OSD dengan DOR 1.25:1, nilai batas atas 1.75:1, nilai batas bawah 0.75:1, nilai alfa (+) dan alfa (-) yaitu 1.95:1 dan 0.54:1. Variabel bebas termasuk konsentrasi OSD dengan DOR dan dosis bakteri dengan respon parameter yaitu degradasi *Total Petroleum Hydrocarbon* (TPH), *Chemical Oxygen Demand* (COD), penurunan *Total Dissolve Solid* (TDS), pH dan populasi bakteri. Hasil optimum yang direkomendasikan oleh model RSM adalah DOR 0.77:1 dan dosis bakteri *B. subtilis* sebanyak 5%, nilai optimum degradasi TPH (50.25%), nilai COD (895.41 mg/L), persentase penurunan nilai TDS (57.83%), pH (8.21), jumlah populasi bakteri yaitu 6.39 Log CFU/mL. Hasil *Desirability* dari rekomendasi yaitu sebesar 0.64. Titik optimum yang baik memiliki *desirability* yang tinggi atau mendekati 1.

1. INTRODUCTION

Indonesia is the world's largest archipelago state, consisting of 17,504 islands with a coastline of 99,093 km and an area of approximately 6.32 million km^2 of waters (KKP 2018). The marine waters of the country are among the most vulnerable in the world to oil pollution. This is because of the global adoption of water as the primary oil transportation route. Pollution of seawater by petroleum is caused by spills during processing, production, distribution, or usage (Kim et al. 2019).

Oil pollution in coastal areas due to spills is an environmental problem that needs to be anticipated. Several oil spill accidents in Indonesian waters have repeatedly occurred in the Malacca Strait, Riau Islands, East Kalimantan, and the North Java Sea (Sari 2008; Setiawan 2018; Gatra 2019; Maulana 2020, IOJI 2023). The incident on Karawang Beach in 2019 caused pollution of around 100 km, which spread to the North Coast of West Java Island (Gatra 2019). Based on observation, oil pollution in the ocean has far-reaching consequences (Yang et al. 2020). It also has long-term impacts on the surrounding environment, such as changes in the characteristics of marine and coastal populations and ecological systems. Oil spills in marine environments result in significant contamination of coastal ecosystems. This occurs because coastal zones, located at the land–sea boundary, are shaped by oceanic processes including tidal fluctuations, sea breezes, and seawater infiltration. These factors facilitate the contamination of coastal sand and rock substrates (Munawar et al. 2007). The transported spills by tidal currents can infiltrate and accumulate within beach sands, adhering to the surfaces and leading to persistent pollution. Consequently, oil spills classified as hazardous waste are threats to both ecosystem integrity and the hydrological cycle. Petroleum-degrading bacteria possess the metabolic capability to use hydrocarbon compounds as sources of carbon and energy essential for their growth, enabling the survival and proliferation of the organisms in petroleum-contaminated environments. These hydrocarbon-degrading microorganisms are capable of breaking down petroleum constituents through oxidation processes, with hydrocarbons as electron donors. Therefore, the microbes play a crucial role in the bioremediation of oil spills by converting petroleum hydrocarbons into carbon dioxide (CO_2), thereby reducing adverse environmental impact in soils (Yolantika et al. 2015; Manalu et al. 2016).

Biological pollution treatment is an effective alternative because it is non-carcinogenic, non-flammable, low-cost, and environmentally friendly compared to other conventional methods. Biodegradation removes petroleum hydrocarbons from the marine environment and restores oil-contaminated ecosystems (Sakthipriya et al. 2015; Fu et al. 2021). Indigenous and preadapted bacteria are efficient hydrocarbon-degrading agents (Moghimi et al. 2017; Uribe et al. 2021) for petroleum pollutants in the marine environment. The characteristics of these microorganisms include the ability to use hydrocarbon compounds as a source of carbon and energy needed for growth. Bacteria can decompose petroleum components by oxidizing hydrocarbons to CO_2 and H_2O. Furthermore, the process is environmentally friendly (Fu et al. 2020), and hydrocarbons are used as electron donors (Yalaoui-Guellal et al. 2021). The result is the reduction or removal of petroleum pollutants (Yang et al. 2020). To optimize the bioremediation process, oil-contaminated sand should be dispersed in water to facilitate microbial degradation. The addition of surfactants to contaminated water can break the oil into smaller droplets and reduce the interfacial tension, thereby enhancing biodegradability (Liu dan Callies 2019; Mohajer et al. 2020). Investigations that systematically determine the optimal combination of palm oil-based oil spill dispersants (OSD) and indigenous *Bacillus subtilis* isolates for the remediation of contaminated coastal areas in Indonesia remain limited. Therefore, this study aims to determine the effectiveness of the combination of Bio-OSD and *B. subtilis* CYA27 of indigenous bacteria in optimizing the bioremediation process of oil-polluted coastal areas through the RSM model method.

2. MATERIAL AND METHODS

2.1 Preparation of Petroleum-Contaminated Beach Sand

Beach sand samples were collected in December 2020 from an area affected by an oil spill along the northern coast of Bekasi, Indonesia. The site was previously contaminated in 2019 due to an incident caused by a petroleum company. A randomized sampling method was adopted to ensure a representative sample collection. The application was conducted at the Soil Biotechnology Laboratory, IPB University.

2.2 Preparation of Bio-Based Oil Spill Dispersant (Bio-OSD)

The OSD used in this study was Bio-OSD, a biodegradable formulation derived from palm oil. The composition was based on the formulation developed by Leo (2021), consisting of an anionic and non-ionic surfactant, namely 7.5% methyl ester sulfonate (MES) and 5% diethanolamide (DEA), respectively. These components were mixed in a ratio of 3:7 MES:DEA to create the final product.

2.3 Preparation of Petroleum-Degrading Bacteria

The bacterial isolate used in this study was Bacillus subtilis CYA27, known for its biosurfactant-producing ability and paraffin emulsification activity. This strain can degrade petroleum and persistent organic pollutants (POPs), including phenanthrene and fluorene, which serve as the sole sources of carbon and energy (Cahyani et al. 2022).

To prepare the bacterial inoculum, a single colony of B. subtilis CYA27 was cultured in an oil broth medium supplemented with 0.001% yeast extract. The culture was incubated on a rotary shaker at 120 rpm at ambient temperature until the bacterial population reached approximately 10^7 CFU/mL. The resulting suspension was then used for application in the bioremediation treatments.

2.4 Experimental Design: Bioremediation Optimization of Crude Oil Contaminated Sand

Bioremediation trials were conducted in 500 mL Erlenmeyer flasks containing 400 mL of working volume. The setup included 40% (w/v) petroleum-contaminated sand, supplemented with Bio-OSD and B. subtilis CYA27. Experimental combinations of dispersant and bacteria dosages are detailed in Table 1. The bioremediation process was conducted on a shaker at 180 rpm for 14 days at room temperature.

Optimization was performed using Response Surface Methodology (RSM) with the Central Composite Design (CCD) method, implemented through the Design Expert® 13.00 (DX13) software. The objective was to identify the optimal combination of OSD concentration (expressed as the Dispersant-to-Oil Ratio, DOR) and bacterial dosage that maximized bioremediation efficacy, as presented in Table 1. This was done to ensure that the range of values and points covered the appropriate area for determining the optimum conditions.

After obtaining the upper and lower limits of each variable, the values were entered into the DX13 program to calculate the number of treatments and the respective combinations. The number of treatments in this study was 11, with different combinations of variables.

Table 1. Bioremediation process design using CCD (RSM)

Treatment	OSD Concentration (DOR) (X_1)	Bacteria Dosage (mL) (X_2)
1	1.25:1	7
2	1.95:1	7
3	1.75:1	9
4	1.75:1	5
5	1.25:1	7

6	0.75:1	5
7	0.75:1	9
8	0.54:1	7
9	1.25:1	9.82
10	1.25:1	7
11	1.25:1	4.17

The selected central point for DOR was 1.25:1, with an upper limit of 1.75:1, a lower limit of 0.75:1, as well as axial (α) values of 1.95:1 and 0.54:1. For bacteria dosage, the upper limit was set at 10% based on previous studies (Chanif et al. 2017; Aziz et al. 2020), which showed effective degradation of petroleum. Each level of the variable of OSD and bacteria dose is presented in Table 1 and entered in the DX13 program to obtain the number of treatments.

Several variables were measured including Total Petroleum Hydrocarbon (TPH), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS), pH, and total bacteria population, using methods adapted from previous studies and standardized guidelines (Behera et al. 2021; Li et al. 2021; Mostafa et al. 2021; SNI 6989.2:2019), as well as dedicated meters and the plate count method. Analysis of Variance (ANOVA) was used to analyze the experimental responses. This process, guided by the Design Expert software, identified a statistically significant effect of variable components on the responses and selected the most appropriate model based on the highest. The CCD method was applied to optimize the model based on the input variable and response measurement data. The output of the optimization phase included several recommended formulations that were deemed optimal by the software. Optimization was conducted by defining goal constraints for the desired response criteria within achievable ranges. The most suitable formulation was identified with the highest desirability value. The desirability value represents the objective function used for optimization and reflects the ability of the model to meet target criteria for the final product. A desirability value approaching 1 signified an increasingly ideal match between model prediction and desired outcome (Ramadhani et al. 2017).

3. RESULT AND DISCUSSION

3.1 TPH Degradation

Using Response Surface Methodology (RSM), a cubic polynomial equation was used to model TPH degradation efficiency. The model showed a lack-of-fit value of 0.32, which was not statistically significant ($p>0.05$), confirming its suitability for the data (Nur et al. 2022). The coefficient of determination (R^2) was 0.70, implying that 70% of the variability in TPH degradation (Y) could be explained by dispersant-to-oil ratio (DOR, X_1) and the dose of *Bacillus subtilis* CYA27 (X_2). R-squared (R^2) was used to evaluate the quality and accuracy of the model produced from the experiment. The TPH degradation efficiency was discovered to be between 30% and 65% through a hexane extraction and gravimetric analysis. As shown in the response surface graph in Figure 1, higher degradation efficiencies were represented by red areas, while lower efficiencies were signified by blue areas. The model's p-value ($p>0.05$) showed that neither the OSD concentration nor the bacteria dosage had a statistically significant effect on TPH degradation. The following represents the polynomial regression equation:

$$Y = 46.78 - 3.35 X_1 - 6.23 X_2 + 5.53 X_1X_2 + 3.10 X_1^2 + 2.43 X_2^2 + 16.51 X_1^2X_2 + 1.69 X_1X_2^2$$
$$(R^2 = 0.6963)$$

Information: Y: TPH degradation response, X_1: *Dispersant to oil ratio* (DOR), X_2: Dose of *B. subtilis* bacteria (mL)

The optimum TPH degradation (50%) was achieved at a DOR of 0.77:1 and a bacteria dose of 5%. The combination of OSD and petroleum-degrading bacteria showed promise for crude oil bioremediation. This was supported by Chanif et al. (2017) and Aziz et al. (2020), who reported TPH reductions of 90% and 54%, respectively, using similar strategies. Chanif et al. (2017) recorded a higher rate of degradation, attributed to the addition of nutrients essential for bacterial growth, specifically carbon (C), nitrogen (N), and phosphorus (P). A favorable environmental condition for bacterial activity enhanced the degradation rate, while extreme environmental conditions slowed down the process. The activity of the microorganisms was affected by temperature, oxygen, nutrients, and pH. Nutrients are essential for effective contaminants biodegradation, particularly nitrogen, iron, and phosphorus. Some can become a limiting factor, thereby impacting the processes of biodegradation (Al-Hawash et al. 2018). The presence of OSD enhanced bioavailability by dispersing hydrophobic oil components into smaller droplets in aqueous media, improving bacteria access and degradation efficiency (Khanpour-Alikelayeh et al. 2020). Based on observations, *B. subtilis* is known to use hydrocarbons as a sole carbon and energy source.

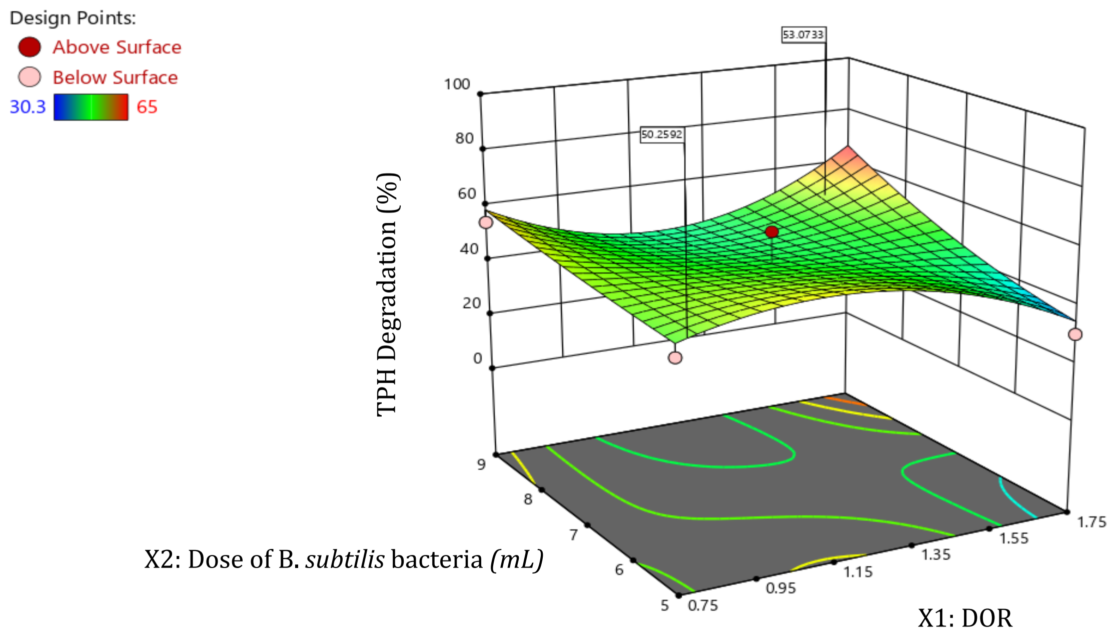

Figure 1. 3D surface graph of TPH degradation response

3.2 COD

COD was determined using the dichromate oxidation method ($K_2Cr_2O_7$) and measured spectrophotometrically at 600 nm. Its response was modeled using a cubic polynomial, with a significant lack-of-fit ($p < 0.05$) but a high R^2 value of 0.89, signifying that 89% of the variation in values was explained by the model. Figure 2 provides a surface graph of the COD value response model. The COD values were observed to be in the range from 442.67 to 1359.33 mg/L. Although the model p-value was > 0.05, reflecting non-significance at the 5% level, the high R^2 suggests its provision of valuable insights. The following represents polynomial regression for the COD response:

$$Y = 660.44 + 324.09X_1 - 49.50X_2 + 110.84X_1X_2 + 73.20X_1^2 + 127.36X_2^2 + 108.66X_1^2X_2 - 308.25X_1X_2^2$$
$(R^2 = 0.89)$

Information: Y: COD Response, X1: *Dispersant to oil ratio* (DOR), X2: Dose of *B. subtilis* bacteria (mL)

The optimum COD value of 895.41 mg/L was observed under the same conditions of 0.77:1 DOR and 5% *B. subtilis* dose. OSD increases oil solubility by forming micro-droplets, which, being derived from palm oil, can contribute to an increase in COD due to the release of organic matter (Aziz et al. 2020). The dispersant also enhanced the solubility or emulsification of hydrocarbon compounds in water. This results in a greater amount of TPH entering the aqueous phase, which was previously undetectable. The increased degradation of TPH consequently elevates the COD values, due to the oxidation of a larger quantity of hydrocarbons. According to Choudhury et al. (2021), the early increase in oxygen demand during bioremediation was associated with enzymatic activity, such as oxygenases and peroxidases, that broke down complex organic matter. Bacteria can potentially use contaminants as a carbon source to obtain energy for growth and cellular activity (Jenifer et al. 2020). Organic compounds are converted into metabolites, leading to increased biomass growth and solubility.

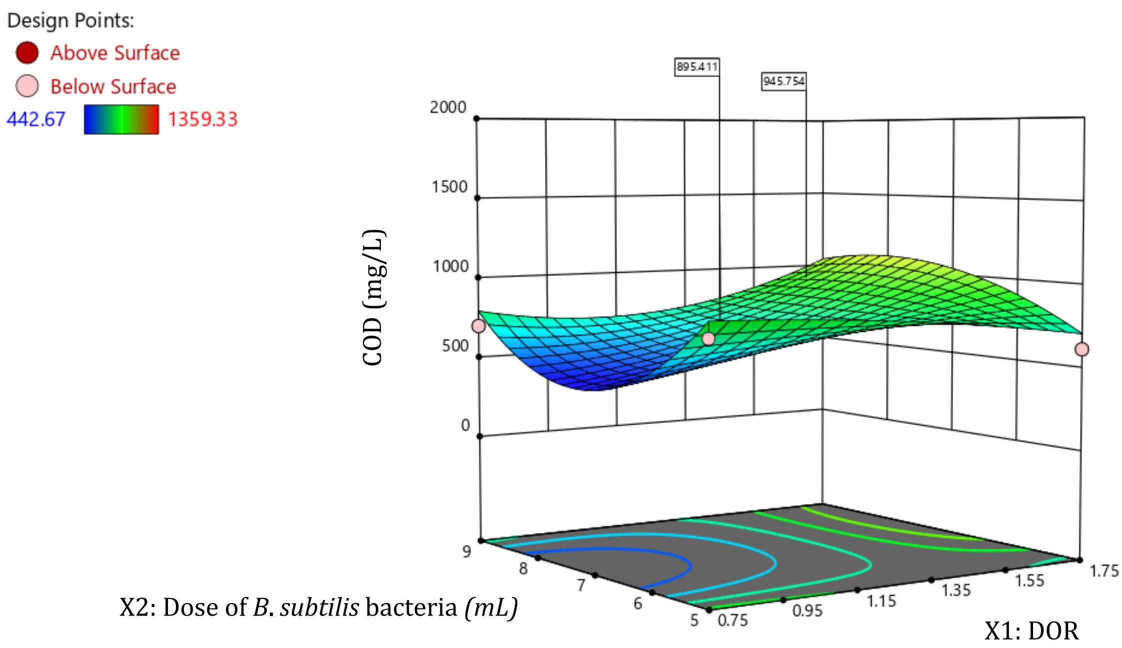

Figure 2. 3D surface graph of COD response

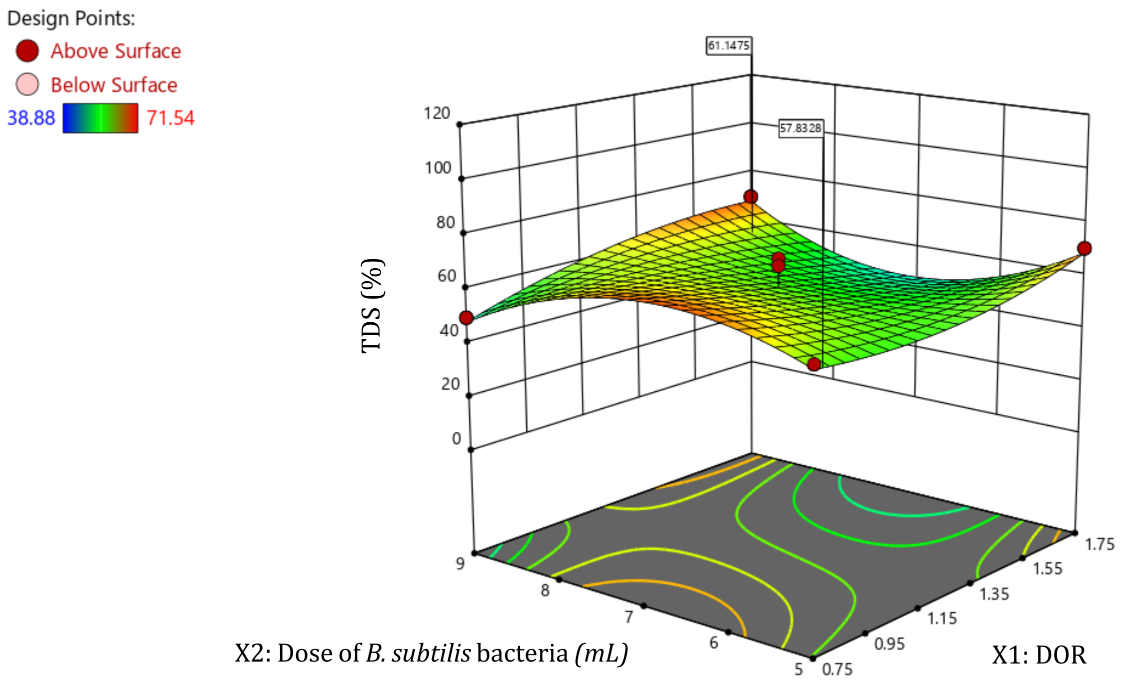

Figure 3. 3D surface graph of TDS response

3.3 TDS

The TDS response model followed a cubic equation, with an insignificant lack-of-fit (p = > 0.05) and a moderate R^2 of 0.57. This signified that approximately 57% of the variability was explained by the model. TDS was measured using a conductivity-based meter, with observed values ranging from 40% to 72%. The model's p-value at > 0.05 confirmed its non-significance at the 5% level. Figure 3 provides a surface graph of the response model, while the following represents polynomial regression for the TDS response:

$Y = 58.10 - 10.16\ X_1 + 3.07\ X_2 + 2.40\ X_1X_2 + 0.39\ X_1^2 + 2.27\ X_2^2 - 5.74\ X_1^2X_2 + 17.81\ X_1X_2^2$ $(R^2 = 0.57)$

Information: Y: TDS Response, X1: *Dispersant to oil ratio* (DOR), X2: Dose of *B. subtilis* bacteria (mL)

The optimum TDS reduction of 58% occurred under the same treatment conditions. When OSD disperses petroleum into degradation products such as dissolved compounds and ions, the TDS level increases. It then decreased as the degradation products were assimilated by bacteria. The metabolism of bacteria is believed to reduce TDS by degrading complex organic solids into simpler molecules such as CO_2 and water (Jenifer et al. 2020; Mazhar et al. 2019).

3.4 pH of the Environment

The model for pH response was statistically significant ($p < 0.05$), with a high R^2 of 0.96 and an insignificant lack-of-fit ($p < 0.05$), signifying a strong fit and reliable prediction. Based on observations, water pH ranged from 7.41 to 8.32. The following represents polynomial regression for the response:

$Y = 7.81 - 0.1662\ X_1 - 0.2051\ X_2 + 0.042\ 5X_1X_2 + 0.08291\ X_1^2 + 0.0804\ X_2^2 + 0.3376\ X_1^2X_2 - 0.1563\ X_1X_2^2$ $(R^2 = 0.96)$

Information: Y: pH response, X1: *Dispersant to oil ratio* (DOR), X2: Dose of *B. subtilis* bacteria (mL)

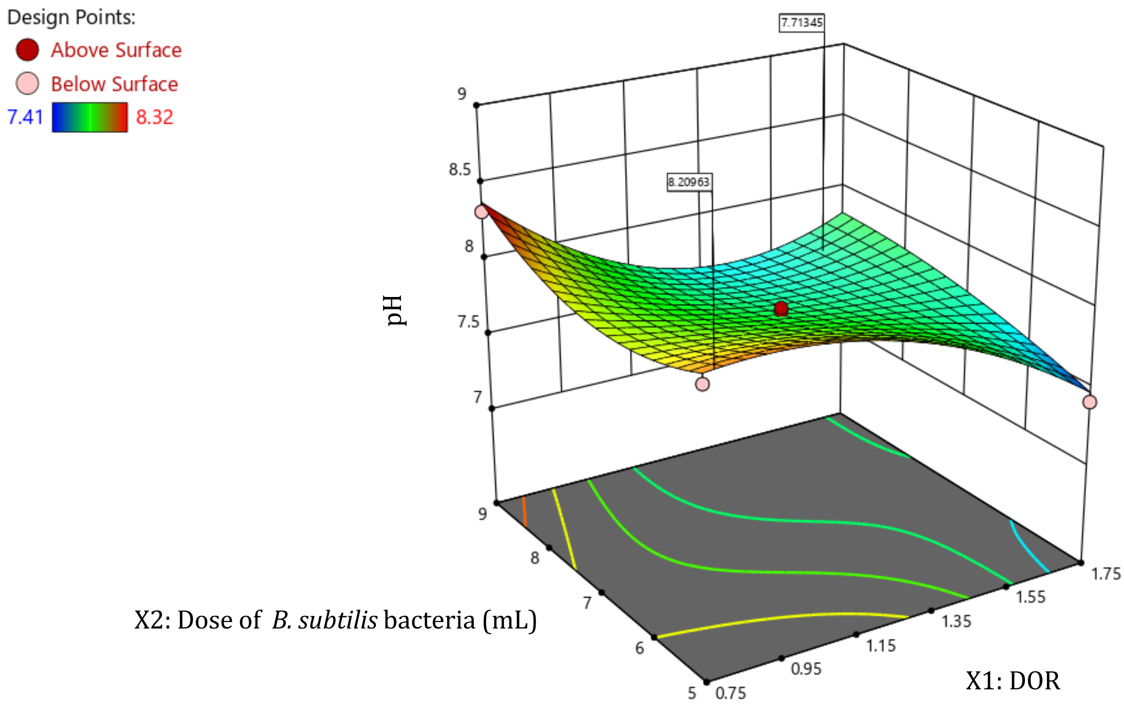

Figure 4. 3D surface graph of pH response

The optimum pH of 8.21 was achieved at the standard treatment condition. pH is a crucial factor in the degradation of petroleum hydrocarbons, as it influences bacterial activity, nutrient availability, and enzyme stability. This parameter can be highly variable and should be carefully considered when optimizing biological treatment methods. Environmental pH affects key cellular processes such as membrane transport and the balance of catalytic reactions, including enzyme function (Al-Hawash et al., 2018). Hydrocarbon-degrading bacteria typically show optimal activity under neutral to slightly alkaline conditions, specifically within the range of 6.5 to 8.0. Based on observations, pH changes are associated with microbial degradation of hydrocarbons and organic acids. *B. subtilis* shows tolerance to fluctuations through proton-pumping mechanisms and ion exchange processes that help maintain internal pH homeostasis (Nugroho 2006; Chanif et al. 2017; Holifah 2018).

3.5 Bacteria Population Growth

The bacteria population model was assessed based on Total Plate Count (TPC) data. It showed a significant lack of fit ($p < 0.05$) but a good R^2 of 0.76, and the values ranged from 5.00 to 6.76 Log CFU/mL. Although the overall model was not statistically significant ($p > 0.05$), key trends were identified. The following represents polynomial regression for population response:

$$Y = 6.38 + 0.2616\ X_1 + 0.2758\ X_2 - 0.1300\ X_1X_2 - 0.1992\ X_1^2 - 0.3392\ X_2^2 - 0.3392\ X_1^2X_2 - 0.7966X_1X_2^2;\ (R^2 = 0.7576)$$

Information: Y: Bacteria population growth response, X1: *Dispersant to oil ratio* (DOR), X2: Dose of *B. subtilis* bacteria (mL)

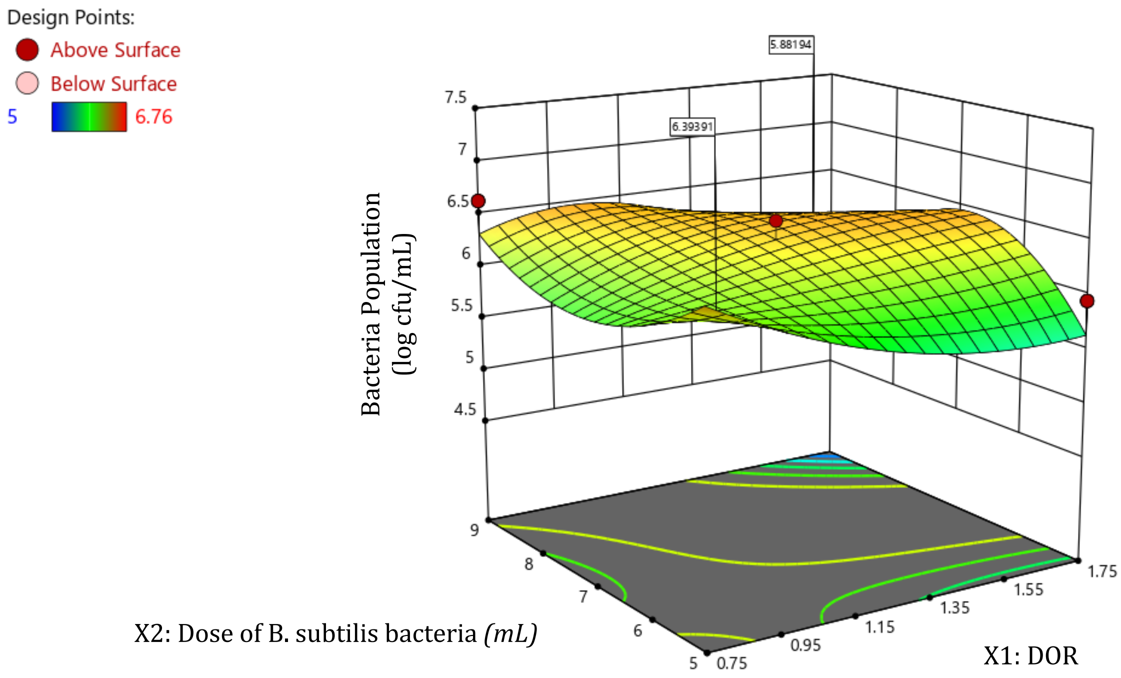

Figure 5. 3D surface graph of bacteria population response

The highest population (6.39 Log CFU/mL) was observed at DOR 0.77:1 and 5% bacteria dose. Growth was supported by the availability of macro- and micronutrients (Hu et al. 2013; Nayak et al. 2018). *B. subtilis* degrades hydrocarbons via direct contact with pollutants or through interactions with emulsified droplets, aided by OSD dispersal (Chanif et al. 2017).

3.6 Optimization of Bioremediation Conditions

Optimization was performed using Design Expert (DX13) software. The recommended optimal conditions were a DOR of 0.77:1 and a 5% bacteria dose, yielding the following:

The desirability value was 0.64, signifying a moderately optimized model. This value implied that the model achieved 64% of the desired optimization targets. Desirability reflects how closely a solution approaches the optimal target, with values nearer to 1 denoting a higher degree of optimization (Montgomery 2017). A desirability of 1 reflects a perfectly optimized system, while 0 suggests no utility (Hendrawan et al. 2016).

Table 2. The Optimization value of the RSM model recommendations

DOR	Bacteria Dose (%)	TPH Degradation (%)	COD Value (mg/L)	Percentage of TDS Value Decrease (%)	pH	Bacteria Population (Log CFU/mL)	*Desirability*
0.77:1	5	50.25	895.41	57.83	8.21	6.39	0.64

4. CONCLUSIONS

In conclusion, bioremediation of petroleum-contaminated beach sand was successfully optimized using *B. subtilis* CYA27 and OSD. The best performance was achieved at a DOR of 0.77:1 and a 5% bacteria dose, as suggested by TPH degradation efficiency, COD reduction, TDS reduction, pH balance, and bacteria growth. The model showed reasonable accuracy with a desirability of 0.64.

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