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Crude Oil Polluted Soil Bioremediation through Microbe Activity Utilization

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PREFACE

The 1st International Conference on Environment, Green Technology, and Digital Society 2023
December 13, Universitas Muhammadiyah Magelang, Indonesia

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Greetings,

We extend a warm welcome to the 1st International Conference on Environment, Green Technology, and Digital Society 2023.

The ongoing discourse revolves around sustainable development and the pivotal role of technology. Green technology is dedicated to the creation and implementation of eco-friendly solutions aimed at fostering a sustainable society. This encompasses initiatives in renewable energy, waste management, water conservation, sustainable transportation, and green manufacturing. On the other hand, Industry 4.0 incorporates cutting-edge technologies such as the Internet of Things (IoT), artificial intelligence (AI), big data analysis, robotics, and automation into industrial processes. Simultaneously, a digital society has emerged, utilizing tools, platforms, and networks for communication, commerce, governance, healthcare, education, and entertainment.

Recognizing the potential synergy between these domains, our present conference anticipates valuable contributions from esteemed keynote speakers and presenters to explore the combined impact of green technology and digital society on sustainable development. This marks the inaugural occasion where The 1st International Conference on Environment, Green Technology, and Digital Society, are being held in conjunction with The 5th Borobudur International Symposium 2023.

To guide the discussions, we are honored to have world-class keynote speakers, namely:
1. Prof. Thomas Kivevele, PhD., from The Nelson Mandela African Institution of Science and Technology, Tanzania.
2. Prof. Madihah M. Saudi, PhD., from Universiti Sains Islam Malaysia.
3. Prof. Hamit Solmaz, PhD., from Gazi University, Turkey.
4. Prof. Mustafa Mat Deris, from Universiti Muhammadiyah Malaysia.

In our records, this symposium has garnered participation from 400 presenters representing eight countries, namely Indonesia, Malaysia, Thailand, Taiwan, Hungary, Turkey, Tanzania, and the Philippines. These presenters hail from 64 institutions. We express our gratitude to all the co-hosts and sincerely hope for the continuation of this collaborative effort in the coming years.

We encourage you to immerse yourselves in the discussions and trust that this event will provide you with valuable insights.

Crude Oil Polluted Soil Bioremediation through Microbe Activity Utilization

Sharfina Nadhilah[1], *Astri* Rinanti[1*], *Riana Ayu* Kusumadewi[1], *Melati Ferianita* Fachrul[1], *Astari* Minarti[1], *Sarah* Aphirta[1], *Lutfia* Rahmiyati[1], *Sheilla Megagupita Putri* Marendra[1], and *Thalia* Sunaryo[1]

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Abstract. Environmental pollution by crude oil has become a serious problem all over the world with high level of oil spillage or leaks comes from damaged crude oil piping, tankers storage, offshore drilling, and illegal oil waste dumping cases. The objective of this research is to study crude oil polluted land recovery process with biotechnological approach. Technology to safely remove oil pollutant in the environment is bioremediation due to its low cost, high efficiency level, environmentally friendly, and sustainability. Three isolate bacteria namely *Pseudomonas* sp., *Pseudomonas xanthomarina*, and *Arthrobacter nitroguajacolicus* were utilized as bioremediation agents to perform land remediation with biostimulation-bioaugmentation (BS-BA) approach. After 25 days, 31,000 mg/kg of Total Petroleum Hydrocarbon (TPH) was decreased into 90-10000 mg/kg or equal to 67.7% to 99.70%. We also detected 14,000 mg/kg decrease of Polycyclic Aromatic Hydrocarbon (PAH) into 8 to 40 mg/kg with detection limit of 99.94% to % in just 5-10 days. TPH removal kinetic calculation by using *Pseudomonas xanthomarina* bacteria resulted Y_T, Kd, Yobs, and Ks respectively at 0.002/hour, 0.001/hour, 0.0361/hour, and 0.0002/hour, by using order 2 formula with regression value of 0.9482. We recommend conducting land farming processing which consists of 2 beds with volumes of 55 m3/bed in order to remediate 159 ton of crude oil polluted soil by utilizing 9.6 L of hydrocarbonoclastic bacteria for 3723 hours. Based on the findings, we concluded that bioremediation is available on crude oil polluted soil.

1 Introduction

Soil is part of the atmospheric layer of the earth's crust and is located at the top position which is part of the life of organisms or microbes. Soil pollution by crude oil is a great problem because Indonesia is a country that produces large amounts of oil along with its waste [1]. In between 1967 and 2010, there are at least 1,200 oil spillage cases [2] and around 1,245,200 ton of crude oil has polluted the sea and coast areas [3]. If we refer to

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[4], crude oil is categorized as dangerous and toxic waste. Procedures and Technical Requirements for Treatment of Petroleum Waste and Biologically Contaminated Soil It is explained that the concentration of Total Petroleum Hydrocarbon (TPH) before the biological treatment process is not more than 15% [15%]. There are numerous methods available to control crude oil contamination such as physical, chemical, and biological methods. The safest technology for the environment is to remove crude oil pollutant by utilizing bacteria enzymatic activity to degrade crude oil [6]. Bioremediation is a safe technique to overcome crude oil pollution because it comes with a low cost and it is considered as the most environmentally friendly method to recover crude oil polluted soil and water to its original condition [7,8]. The process of bioremediation by microbes occurs because it is influenced by several factors including temperature, soil characteristics, soil porosity, pH, availability of O2, nutrients and the concentration of contaminants [9,10].

2 Method

2.1 Crude oil sample and bacteria cultivation preparation

Secondary data was obtained from two main literatures which are [11] and [12]. According to both literatures, Crude Oil Tank Bottom Sludge (COTBS) sample obtained from Azzawiya refinery in Libya. Crude oil from COTBS polluted soil was extracted with dichloromethane solvent and then dried. Bacteria were cultivated in a Bushnell & Hass growth media, nutrient agar, and crude oil nutrient with Hamada crude oil addition. Bacteria were incubated for 48 hours, on 30°C temperature, placed on an incubator shaker with rotation speed of 120 rpm. Pure bacteria culture was put on a dormant condition in a 50% glycerol on -80°C temperature.

2.2 Microcosms research

Crude oil slurry was produced with aquadest: COTBS ratio of 5 to 1, Carbon : Nitrogen : Phosphor ratio of 100 to 10 to 1, isolate bacteria of 1 x 10^4 cell/mL, placed on a shaker incubator with continued 150 rpm rotation for 35 days, on 30°C temperature. Total Petroleum Hydrocarbon (TPH) and PAH were tested by utilizing Gas Chromatography Mass Spectrophotometry (GCMS).

2.3 Bacteria growth kinetics

Bacteria growth kinetics was calculated with the following Eq. (1).

$$\frac{dX}{dt}=\mu X \quad (1)$$

X = cell concentration (g/L); t = time (hours); μ = specific growth rate (/hour). Eq. (2) shows q parameter as specific substrate utilization rate (crude oil).

$$\Delta S = qx.\Delta t$$
$$\frac{ds}{dt} = qx$$
$$q = \frac{(ds/dt)\ u}{x} \quad (2)$$

3 Result and discussion

3.1 Temperature optimization

Based on research by [11], the best temperature for bacteria to degrade crude oil is between 30 to 40 °C. **Fig. 1** shows result of research by [12], which represents optimum crude oil degradation performance on 30°C temperatures.

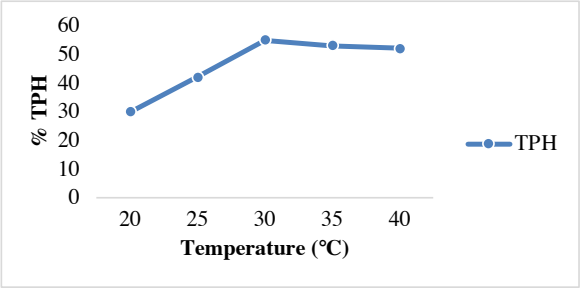

Fig. 1. Temperature optimization graphic [10]

3.2 Contact time optimization

Fig. 2 explains the results of a research by [12], which explained that the highest degradation level occurred on the 12th week. According to [13], COTBS contains high level of TPH which takes longer to degrade up until the expected level.

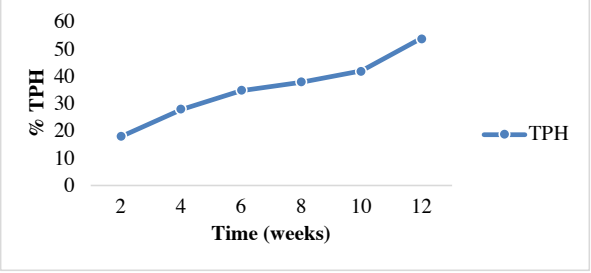

Fig. 2. Contact time optimization graphic [10]

3.3 TPH and PAH degradation on microcosms

The efficiency of TPH and PAH degradation can be seen in **Fig. 3** and **Fig. 4**. TPH degradation efficiency on 25 days of inoculum with initial concentration of 31,000 mg/kg to 90 to 10,000 mg/kg or equal with 67.7 to 99.70%, and reached the highest level in just the first 15 days at 99.67% for *Pseudomonas* sp., *Pseudomonas xanthomarina* bacteria (**Fig. 3**). PAH degradation in slurry phase form by the same bacteria occurred on the 30th day (**Fig. 4**). PAH concentration degradation of 14,000 mg/kg into 8 to 40 mg/kg with detection level of 99.94% to 100% happened in just 5 to 10 days meanwhile NA shows PAH concentration degradation of 67.85% in just 20 days. Based on the presented data in **Fig. 1** to **Fig. 4**, we were able to calculate the kinetics of TPH removal rate on crude oil by utilizing *Pseudomonas xanthomarina* bacteria.

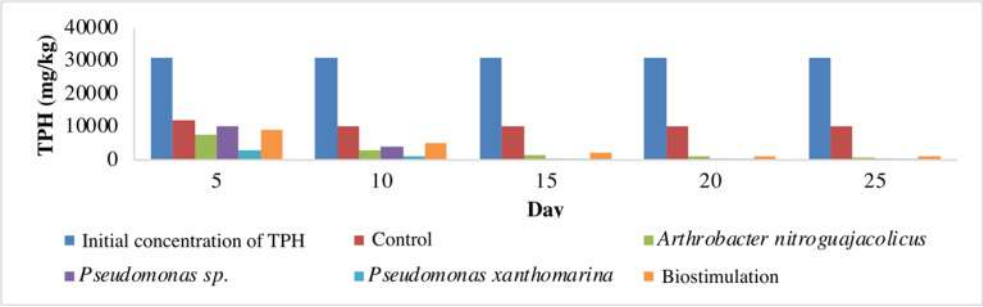

Fig. 3. TPH degradation in microcosms [12]

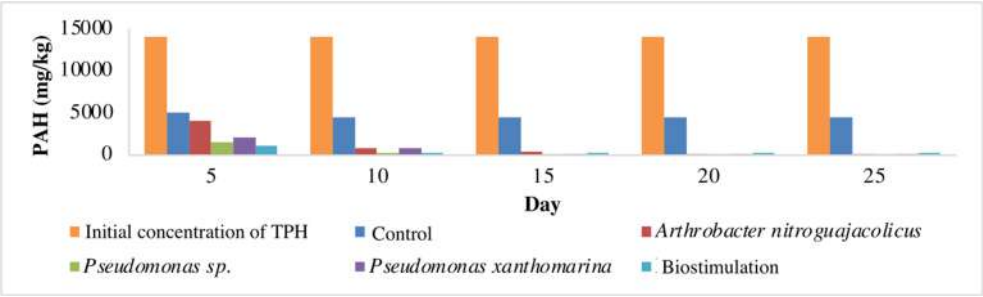

Fig. 4. TPH degradation in microcosms [12]

The specific growth rate (μ) and specific substrate utilization rate (q) of *Pseudomonas xanthomarina* bacteria are consecutively at 0.0017-0.0048/hour and 0.0025-0.0167/hour. **Fig. 5** explains the relationships between μ and q. **Fig. 6** shows relationships between bacteria growth rate on limited culture (X_m) with specific substrate utilization (S) to produce linear line that forms a slope (S). The obtained Yobs result is at 0.0361/hour.

Fig. 7 shows relationship between growth rate (μ) and TPH concentration (mg/kg). The maximum and minimum values of TPH utilization rate are respectively at 0.0048 and 0.0017 which enabled us to obtain saturation concentration (Ks) of 0.0024/hour. Order reaction determination was conducted by creating growth rate graphic of order 0, order 1, and order 2 as shown in **Fig. 8** to **Fig. 10**.

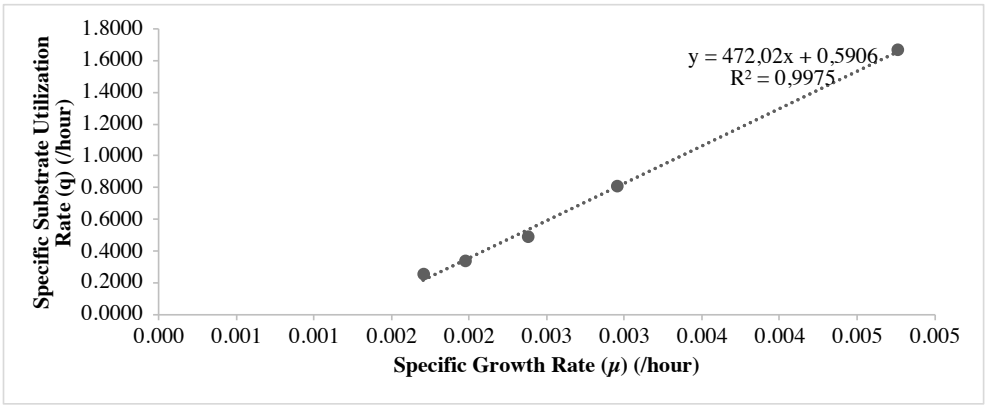

Fig. 5. Relationships between specific growth rate (μ) and specific substrate utilization rate (q)

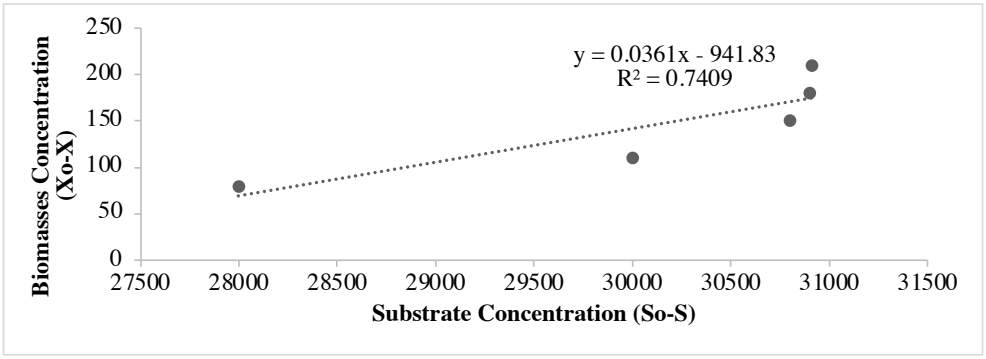

Fig. 6. Relationships between TPH limitation (S0-S) and bacteria growth (X-X0)

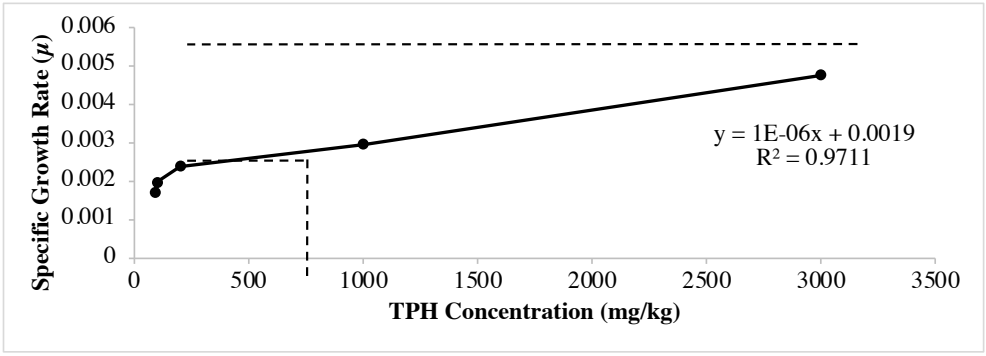

Fig. 7. Relationships between growth rate (μ) and TPH concentration (mg/kg)

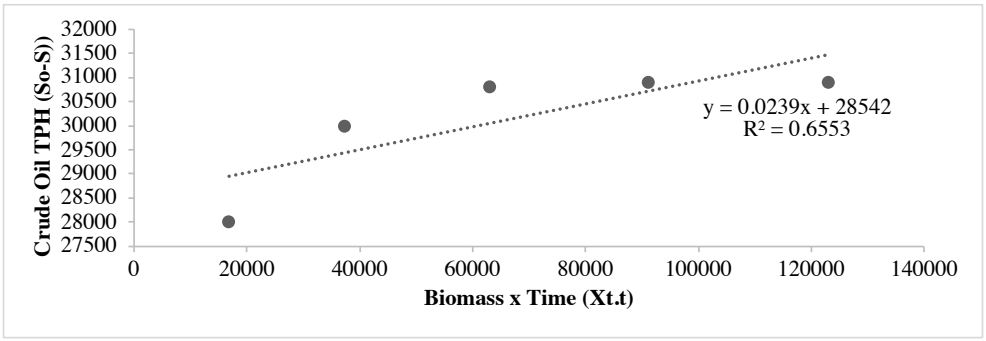

Fig. 8. Reaction 0 order

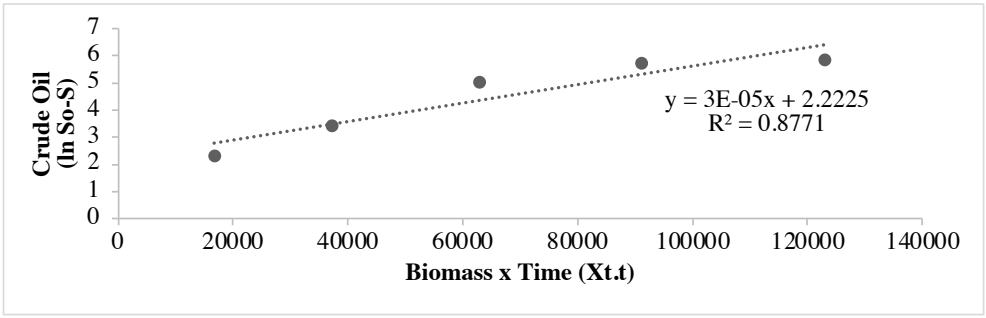

Fig. 9. Reaction 1 order

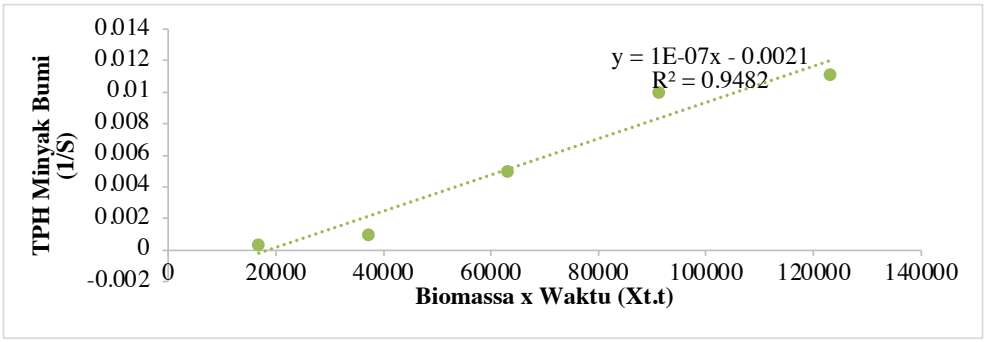

Fig. 10. Reaction 2 order

3.4 Crude oil removal implementation in the field

This research is recommended to be implemented on a larger scale by using land farming method. With assumption that the initial TPH concentration is at 31,000 mg/kg, the weight of the soil should be at 290,000 kg, and clay density of 2,900 kg/m^3, we can calculate the polluted soil volume which is at 100 m^3. Crude oil polluted soil processing can be implemented with 2 rectangle beds with ratio of length to width at 1:2. Processing capacity for 1 bed is at $\frac{100\ m^3}{2}$ or 50m^3. If the volume can be calculated with L x W x H (H = 1 m), the 50m^3 calculation result is at 2W x W x H or 2W^2 x 1 m; if the width is at 5 m and the length is at 10 m. Based on the calculation, the design used with additional

freeboard should be at 5.5m wide, 10m long, and 1m high which makes the volume of designed bed at 55 m^3 (**Fig. 11**).

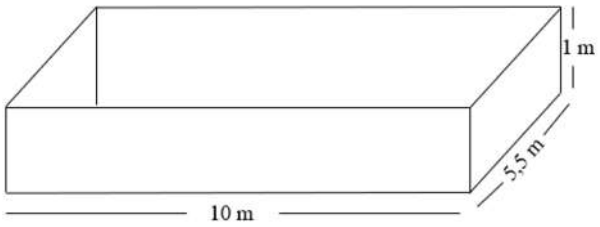

Fig. 11. Processing bed design

55m^3 Bed volume should be able to contain soil based on mass calculation formula of ρ x v; or 2,900 kg/m^3 x 55 m^3 or 159,500 kg. Based on the secondary data, *Pseudomonas xanthomarina* bacteria is able to remove 99.7% of TPH contained on 3% (b/b) of crude oil, on 25 days period on pH level of 7. Based on microcosms' research by [8], 30 g of polluted soil requires 1 mL of isolate bacteria on 30°C temperature and pH level of 7. If at similar condition the amount of polluted soil is at 290,000 kg, the mixed culture requirement used on land farming method can be calculated as follows:

$$\frac{\text{Research soil amount (gr)}}{\text{bacteria amount (ml)}} = \frac{\text{soil amount (gr)}}{\text{bacteria amount (ml)}} = \frac{30\text{ gr}}{1\text{ ml}} = \frac{29 \times 10^7 \text{gr}}{\text{mixed culture amount (ml)}} \quad (3)$$

The required bacteria volume to implement larger removal with a scale of 9,666,666 mL or 9,666.6 L per bed should be much higher. If 2 beds are available, the requirements should be at 19,333.2 L. The crude oil removal efficiency probability is different as the ones resulted from laboratory research because environmental factor in the field is not easily controlled as the ones in the laboratory.

Based on TPH removal rate kinetics calculation by using order 2 formula which is Y =1 x 10^{-7}x – 0.0021 and bacteria growth curve on exponential phase which is Y = 0.0009 x + 1.97 the result is:

$$Y = 1 \times 10^{-7}x - 0.0021; \frac{1}{S} = 1 \times 10^{-7}x - 0.0021; t = \frac{\frac{1}{s} + 0.0021}{10^{-7}} = \frac{10^7}{s} + 21000 \quad (3)$$

The maximum detention time required for *Pseudomonas xanthomarina* bacteria to degrade crude oil can be calculated as follows:

$$T = \frac{\frac{10^7}{s} + 21000}{0.0009\,t + 1{,}97} \text{ or } 0.009\,t^2 + 1.97t = \frac{10^7}{90} + 21000 \text{ or } 0.009\,t^2 + 1.97t - 132111 \quad (3)$$

Based on the calculation, the required detention time should be at 11070.5 hours. Based on that, we can say that *Pseudomonas xanthomarina* bacteria are able to degrade crude oil polluted soil in 11,071 hour or 461 days.

4 Conclusion

The most effective crude oil removal occurred on 30°C temperature and pH level of 7, with highest TPH removal level of 99% by 1% (v/v) enzymatic activity performed by *Pseudomonas* sp and *Pseudomonas xanthomarina* bacteria as hydrocarbonoclastic type bacteria. Land farming bioremediation on one $55m^3$ bed is expected to be able to process 159 ton of crude oil polluted soil by 9.6 L hydrocarbonoclastic bacteria in approximately 3,723 hours.

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