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Kinetics of crude oil degradation by an indigenous mixed culture isolated from palm oil mill effluent

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Abstract

Objectives: To investigate the microbial growth kinetics and degradation of crude oil (CO) by mixed cultures isolated from palm oil mill effluent (POME). **Methods/Statistical analysis:** The scanning electron microscope was employed to examine the surface morphology of POME and the CO contaminated soil. Microbial count using the total viable count (TVC), mold, and coliform counts were investigated at different levels of CO contamination. **Findings:** The TVCs at 20, 40, and 60 mg/L of CO were 7.3×10^7 CFU/g, 6.8×10^6 CFU/g and 7.2×10^6 CFU/g, respectively. The concentration of the CO was utilized at 20 mg/L indicating that this concentration did not inhibit microbial growth. Based on the correlation coefficient (R^2) values obtained for the four growth kinetic models, the Haldane model with R^2 of 0.97 gave the best fit. **Application/Improvements:** Finally, the one way ANOVA and Tukey's honest significant difference (HSD) indicated the statistically significant effect ($P < 0.05$) of the initial CO concentration on microbial growth.

Keywords: Kinetics; Degradation; Mixed culture; Crude oil; Palm oil mill effluent

1 Introduction

The incident of the spill from leakage pipes, storage tanks and lack of safety measures during the drilling of crude oil (CO) are some of the many ways by which crude oil gets in contact with the soil and causes environmental pollution[1]. CO and other xenobiotic contaminants are generally referred to as toxic hydrocarbon compounds and are toxic to soil microorganisms and plants[2]. Thus, the elimination of CO from the soil is necessary to preserve environmental quality[3]. However, biological treatment of CO has been proven to be economical and an easily manage approach towards CO degradation from the environment[3]. Also, the microbial population could have a considerable prospect to remedy hydrocarbon contaminated soil. Previous works on the isolation of hydrocarbon degrading microbial culture indicated that a consortium of hydrocarbon degraders with better degrading properties was more beneficial[4]. However,[5] reported that the mixed cultures of *Pseudomonas aeruginosa* and *Bacilius subtilis* were

able to degrade 250mg/L of phenol and wattle completely within 36 and 48hours, respectively. In this present study,M we investigated the growth kinetics and degradation capabilities of mixed microbial culture isolated from palm oil mill effluent (POME) on crude oil degradation. Microbial counts in the palm oil mill effluent (POME) and CO contaminated soil were estimated using the total viable count (TVC), mold, and coliform counts. The one-way analysis of variance (ANOVA) and Tukey's post-hoc multiple comparison tests was used to ascertain the statistical significance ($P<0.05$) effect of crude oil concentration on microbial growth. Furthermore, the surface morphologies of the raw samples of POME and crude oil contaminated soil were studied using the scanning electron microscope (SEM).

2 Materials and Methods

2.1 Samples collection and preparation

The crude oil (CO) was obtained from the Nigerian National Petroleum Cooperation (NNPC) located in Port Harcourt Rivers State Nigeria. The palm oil mill effluent was obtained from a palm oil milling factory located in Ogwofia Ozom Mgbabu-owa Enugu State Nigeria. Prior to the soil contamination with CO, the soil was obtained from the botanical garden of the University Of Nigeria Nsukka Enugu State.

2.2 Microbial count (total viable count, mold and coliform counts)

1 g of the sample was placed into 9 ml of Ringer solution containing (g/mL); 6.5 NaCl, 0.25 CaCl, and 0.2 NaHCO$_3$. The mixtures were mixed thoroughly on a rotary shaker at 150 rev min^{-1}. The mixture was further diluted by transferring 1 mL into three test tubes containing 9 mL Ringer solution using a sterile pipette. Then 0.1 mL of the diluted solution was transferred from each dilution test tube into three sterile Petri dishes using a sterile pipette. For Total viable count, (TVC) mold count, and coliform count, 15 ml each of sterile nutrient agar, Sabouraud Dextrose Agar (SDA) and sterile violet red bile agar, respectively was poured into each of the Petri dishes and gently rotated on the table to mix. The nutrient agar, SDA and sterile violet red bile agar in the Petri dishes were allowed to gel for 10 min before sealing and was incubated in an inverted position at 37°C for 24 hours. The colonies formed were counted using Quebec colony counter and expressed in colony forming unit per gram (CFU/g).

2.3 Isolation of CO degrading microorganism from POME

Nutrient agar and broth purchased from Oxoid, Basingstoke UK were used for plating and culturing the microbial strain, respectively. The mixed culture was prepared by growing them on a malt extract agar plates at 25 °C for 30 days in the dark. In order to investigate the ability of the mixed microbial culture to degrade CO, the culture was grown on a 250 mL Erlenmeyer flask containing 50 mL of mineral salt medium (MSM). The MSM had the following compositions (g/L): 0.01 (NH$_4$)$_2$SO$_4$, 0.2 NH$_4$Cl, 0.25 K$_2$HPO$_4$, 0.02 MnSO$_4$, 0.5 MgSO$_4$.7H$_2$O, and 0.01 CaCl$_2$. The pH was maintained at 7.0±0.2. The MSM containing CO as a sole carbon source [6] was incubated for 30 days on a rotary shaker (VXR, Vibrax shaker, 220V, 50/60Hz) set at 150 rpm and 37 °C. This was in order to obtain cultures with a stable CO degrading ability.

2.4 Crude oil degradation procedure

The batch degradation of crude oil (CO) was conducted in a 500mL Erlenmeyer flask containing 200g of the CO contaminated soil with 100ml of sterile MSM. The Erlenmeyer flasks contained different initial CO concentrations (20, 40, and 60 mg/L). Prior to the inoculation of the isolated culture from POME into the flasks containing the contaminated soil, the culture was transferred into 100mL of the MSM containing CO and grown at 27°C in an incubator while the shaking speed was maintained at 150rev/min.

2.5 Extraction of residual CO

Soxhlet extraction process was used to extract 5mL of the liquid culture using acetone/dichloromethane (1:1v/v) as the solvent. After extraction, the solvent was left to evaporate and the remaining residue was dissolved in 5mL of dichloromethane. The CO concentration was estimated using a gas chromatography coupled with a mass spectrometer (GC-MS Shimadzu QP-500). The carrier gas was helium (purity > 96.9%) at a constant flow rate of 1.5 mL min^{-1} with column pressure at 100 kpa. The injection volume and temperature were 1 μL and 80 °C, respectively. The concentrations of CO were determined after calibrations of the method with standard chrysene samples at different concentrations. CO degradation was calculated using Eqn. (1).

$$\%CO\ degradation = \frac{initial\ CO\ concentration\ -\ final\ CO\ concentration}{initial\ CO\ concentration} X\ 100 \tag{1}$$

2.6 Statistical Analysis

One way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) was processed using IBM SPSS statistical package (version 22). Tukey's HSD test predicts the honest significant difference that must exist in distance between two groups mean. The statistical significance of the data analysis was set at $P < 0.05$. The analysis was used to investigate the statistical significance of CO initial concentration on microbial growth.
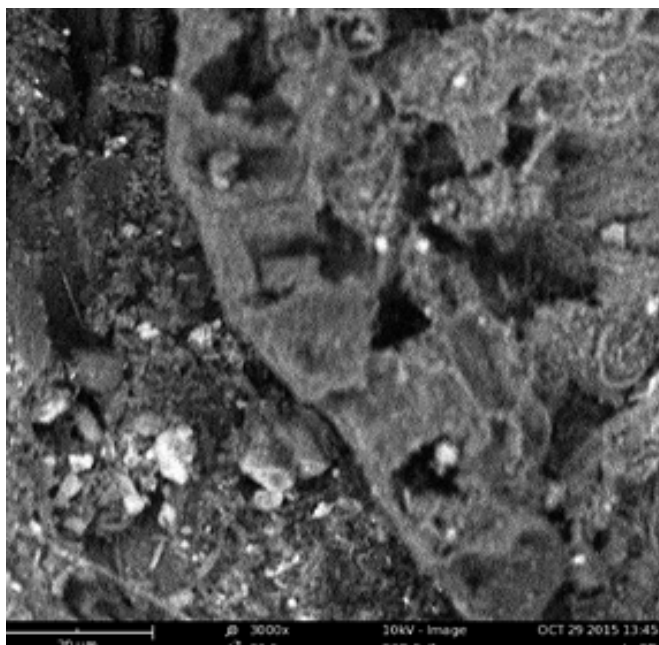
2.7 SEM Analysis

The samples were analyzed using SEM (ASPEX 3020) at 10 Kv to determine their morphological characteristics. The samples were centrifuged, dried at 110°C for 4 hours and coated with a gold film in a sputter coater before the examination.

3 Results and Discussion

3.1 SEM Analysis for POME and CO contaminated soil.

The SEM micrographs for the crude oil contaminated soil and POME is shown in Figures 1 and 2, respectively.

Fig 1. SEM image for CO contaminated soil

The micrographs of POME in Figure 2 showed a dotted surface morphology, which was attributed to the oily nature of the POME used for the SEM analysis. However, the surfaces of the CO contaminated soil and POME was rough and porous, respectively. The levels of porosity observed the CO contaminated soil and POME showed that the CO contaminated soil was more porous (Figure 1). The rough surface of the CO contaminated soil indicated that soil topography and texture may have been altered after the CO contamination. The CO may have penetrated the closely packed inner layer of the soil thereby enlarging the CO contaminated soil (Figure 1).

3.2 Microbial Count

Microbial populations in POME and the CO contaminated soils were estimated using the Total viable count (TVC), mold, and coliform count (Table 1). The TVC is the total viable microbial population while the coliform and mold were the active bacteria and filamentous fungi present in the samples, respectively.

The colony forming unit per gram (CFU/g) of molds in POME was in the magnitude of 6.3×10^7, indicating a higher level of molds in POME in comparison with the TVC (4.1×10^6 CFU/g) and coliform counts (3.1×10^4 CFU/g) (Table 1). These results
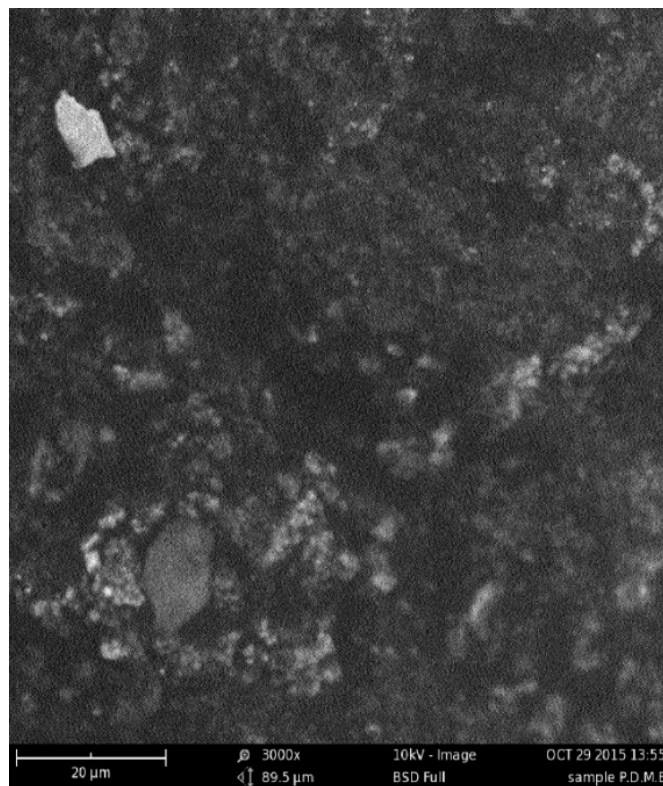
Fig 2. SEM image for POME

Table 1. Microbial count in POME and CO contaminated soil

Parameters	TVC (CFU/g)	Mold (CFU/g)	Coliform (CFU/g)
POME	$4.1x10^6$	$6.3x10^7$	$3.1x10^4$
CO contaminated soil	$7.3x\ 10^6$	$4.3x10$	$4.1x10^5$

suggest that the predominant microbial population in POME was mold. The pH is another important factor that affects soil microbial population[7]. The slightly acidic pH (4 to 5), of POME as reported previously[8–10] could be contributing to the increased CFU/g of molds, as molds generally grow well in acidic condition[11].

The naturally occurring microbial population in the soil prior to CO contamination in terms of the TVC coliform and mold count showed that the soil contained a higher level of TVC ($7.3x\ 10^6$ CFU/g). The mold and coliform counts were relatively close in the order of 4.3x10 CFU/g and $4.1x10^5$ CFU/g, respectively. These results indicated how rapidly the microbial population adapted to the soil (Table 1).

However, one of CO's indirect effects on soil was attributed to the changes in the microbial population[12]. However, the changes in the microbial counts in the CO contaminated soil could be stimulatory or inhibitory to soil microorganisms. The effects of CO concentrations on soil microbial count (TVC, mold and coliform count), is presented in Table 2. It was observed that the addition of CO to the soil increased the TVC of the microbial population as the TVCs at 20, 40, and 60 mg/L of CO were $7.3x10^7$ CFU/g, $6.8x10^6$ CFU/g and 7.2 x 10^6 CFU/g, respectively (Table 2). These observations suggest that the CO within the concentrations used in this study could be stimulatory and also be acting as a source of carbon for the growth of the indigenous microbial population[5]. However, the raw CO used in this study may be less volatile to atmospheric losses thereby increasing their bioavailability as a carbon source for the soil microbial population.

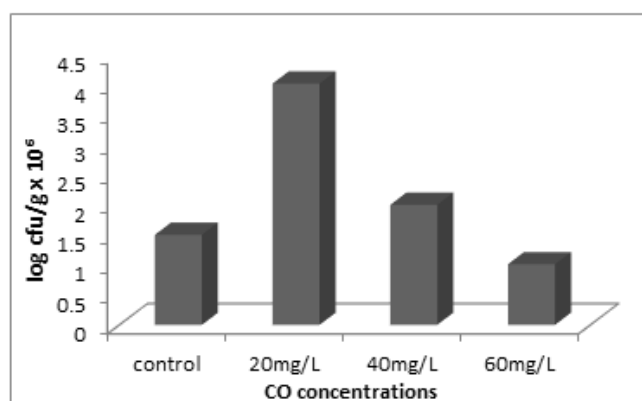### 3.3 Effects of CO initial concentration on microbial growth

The results from the effect of initial CO concentration on microbial growth are shown in Figure 3 Figure 3 . The log CFU/g of soil sample was observed to be high at 20 mg/L initial CO concentration. The high log CFU/g (4.3 x 10^6 CFU/g) observed at 20

Table 2. Microbial counts at different CO concentration in CO contaminated soil

Parameters	CO concentrations (mg/L)		
	20	40	60
TVC (CFU/g)	7.3×10^7	6.8×10^6	7.2×10^6
Coliform (CFU/g)	5.2×10^3	2.0×10^3	1.6×10^3
Mold count (CFU/g)	7.0×10^5	5.2×10^4	6.2×10^4

TVC; Total viable count of microorganism, CFU/g; colony forming unit per gram.

mg/L could be due to the acclimatization period (one month), which was favorable at 20 mg/L. The CO could serve as a carbon source, but at extreme concentration can be toxic to microbial growth. However, previous studies reported the importance of carbon to microbial growth and synthesis [13]. The CO degradation could be successful at 20 mg/L as high log colony forming unit (CFU) of microorganism per gram of soil was obtained. Consequently, the available carbon at 20 mg/L might be supplying the needed carbon and energy for microbial growth [6,13]. However, the log CFU/g at 40 mg/L (2.12×10^6 CFU/g) was observed to be low indicating a reduced CO degradation at this concentration [14,15]. The low log CFU/g in the control sample indicated that the presence of the indigenous microorganism in the uncontaminated soil. The available microorganism in the control sample but might not be suitable for CO degradation as the microorganisms were not acclimatized in the presence of CO (Figure 3).

Fig 3. Effect of CO concentration on microbial growth (values are three replicates $\pm$ standard deviation)

3.4 One way ANOVA for the effect of initial CO concentration on microbial growth

Considering the independent variable of initial CO concentration, one way ANOVA was used to judge the statistical significance (P<0.05) of this variable on microbial growth. The null hypothesis under investigation suggests that the mean number of microbial growth is the same at all initial CO concentration. The P-value, in Table 3 was used to decide the conclusion of the investigation. It was evident from Table 3 the mean effect of the initial CO concentration on microbial growth was statistically significant with F statistics of 721 and P < 0.0001. However, based on the one way ANOVA results in Table 3, the null hypothesis was rejected.

Table 3. ANOVA for the effect of initial CO concentration microbial growth

	Sum of Squares	df	Mean Square	F	P-value
Between Groups	240.333	2	120.167	721	.000
Within Groups	0.500	3	0.167		
Total	240.833	5			

The Tukey's honest significant difference (HSD) test explores the statistical significance among the group means in order to

provide specific information on which means differed. From the Tukey's HSD test in Table 4, statistically, significant means were identified with asterisks (*). However, these means correspond to statistically significant P-values (P < 0.0001). Accordingly, the HSD test in Table 4 shows that the mean difference (I-J) between the group means at 20 mg/L and 60 mg/L were statistically significant (P < 0.0001) whereas, the mean difference (I-J) at 40 mg/L was not statistically significant (P < 0.05). Also, a nonzero difference in mean was probably unlikely due to the non-zero 95% confidence level (Table 4).

Table 4. Tukey post-hoc (HSD) analysis for the effect of initial CO concentration

(I) concentration	(J) concentration	Mean Difference (I-J)	Std. Error	P-values	95% Confidence Interval	
					Lower Bound	Upper Bound
20 mg/L	40 mg/L	-15.500*	0.408	0.000	-17.21	-13.79
	60 mg/L	-8.000*	0.408	0.001	-9.71	-6.29
40 mg/L	20 mg/L	15.500	0.408	0.802	13.79	17.21
	60 mg/L	7.500	0.408	0.550	5.79	9.21
60 mg/L	20 mg/L	8.000*	0.408	0.001	6.29	9.71
	40 mg/L	-7.500*	0.408	0.001	-9.21	-5.79

* The mean difference is significant at P-values less than 0.05

3.5 Kinetics of microbial growth

Microbial growth kinetic models in Table 5 were fitted to the experimental data at varying initial CO concentration. In order to determine the microbial growth parameters, experimental values of the specific growth rate (μ) were estimated using the expression in Eq. (2) where X_t and X_o are the final and initial microbial concentration, respectively, t is the time (hrs)[16].

$$X_t = X_0 e^{\mu t} \tag{2}$$

The obtained values of μ were used to estimate the kinetic parameters for Monod, Haldane, and Verhulst models using the nonlinear regressions according to[16]. From Tables 5 and 6, μ_{max} (hr^{-1}) is the maximum specific growth rate, K_i (mg/L) is the CO inhibition constant, K_s is the CO affinity constant, X_m (mg/L) is the maximum microbial cell concentration and S (mg/L) is the CO concentration[17].

Table 5. Nonlinear microbial growth kinetics models

Growth models	Nonlinear	Plots	References
Monod	$\mu = \frac{\mu \max \cdot S}{Ks+S}$	μ vs S	[18]
Haldane	$\mu = \frac{\mu max. \ S}{Ks + S + \frac{S^2}{Ki}}$	μ vs S^2	[17]
Verhulst	$\mu = \mu max \left(1 - \frac{Xt}{Xm}\right)$	μ vs X$_t$	[18]

Figure 4, showed the nonlinear plots of specific growth rate (μ) versus CO concentrations. It could be observed that the μ of the indigenous microorganisms increased with an increase in the initial CO concentration as no lag phase was observed. This result indicated a good adaptation of the indigenous microbial population towards the CO contaminated soil[18,19].

According to Figure 4, the μ increased at 20 mg/L after which, a slight decline was observed. The increase in the μ was mainly during the exponential phase when the microbial cells are actively growing. Another reason for the observed increase in μ at low CO concentration could be that at 20 mg/L, the effect of CO inhibition on indigenous microbial population was negligible. However, at low contaminant concentrations,[20] reported that the effect of substrate toxicity was negligible in microbial metabolic activity.

From the K_s values presented in Table 6, It was observed that the K_s values increased with an increase in CO concentrations as the lowest K_s values were obtained at 20 mg/L. However,[21,22] noted that the low K_s value implies a higher affinity for the substrate while a high K_s value indicated a lower affinity for the substrate. This indicated that the indigenous microbial population had a high affinity at low CO concentration (20 mg/L).

The maximum microbial concentration (X_m) values were also calculated using the Verhulst model and presented in Table 6. The results showed that the X_m values at low CO concentration were high. For example, the X_m at 20 mg/L initial CO concentration was 12.17 mg/L, whereas; it was 3.0 mg/L at the initial CO concentration of 60 mg/L. However, the K_i values inherent
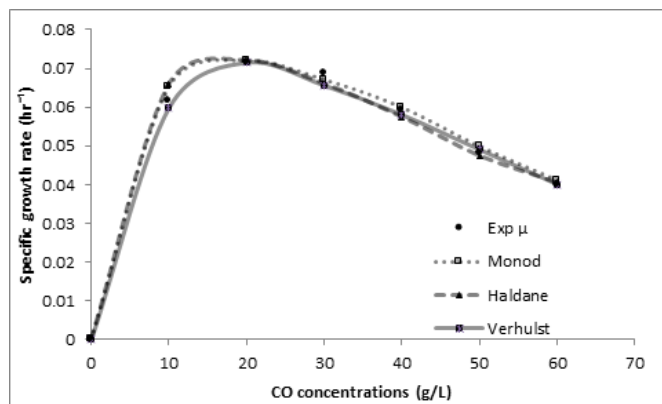
Fig 4. Non-linear plot of specific growth rate versus CO concentrations

Table 6. Microbial growth kinetic parameters

Initial CO conc. (mg/L)	Monod	Haldane	Verhulst
20	μ_{max}= 0.0481 K_s = 5.11 R^2 = 0.903	μ_{max} = 0.0401 K_i = 1.9 K_s = 28.9 R^2 = 0.989	μ_{max} = 0.0249 X_m = 12.17 R^2 = 0.831
40	μ_{max} = 0.0439 K_s = 15.2 R^2 = 0.892	μ_{max} = 0.0401 K_s = 35.39 K_i = 2.3 R^2 = 0.992	μ_{max} = 0.0320 X_m = 10.01 R^2 = 0.843
60	μ_{max} = 0.0362 Ks = 18.92 R^2 = 0.821	μ_{max} = 0.0332 Ks = 37.22 K_i = 5.3 R^2 = 0.988	μ_{max} = 0.0315 X_m = 6.2 R^2 = 0.832

to the Haldane model increases with an increase in CO concentration indicating that the effect of CO inhibition was negligible at low CO concentrations [23,24].

However, the R^2 was frequently used to judge whether the studied model represents correctly the experimental data [25–27]. Among the microbial growth models studied the Haldane inhibition growth model showed the best fit of the experimental data (R^2 greater than 0.95) at all initial CO concentration as compared to Monod and Verhulst models (R^2 less than 0.95) Table 6.

4 Conclusions

It can be concluded that mixed microbial culture isolated from palm oil mill effluent (POME) was able to degrade crude oil (CO) at 20 mg/L initial concentration. The growth kinetic parameters from the microbial growth models show the intrinsic behavior of the mixed cultures towards the different levels of CO. The statistically significant effect (p < 0.05) of the CO concentration on microbial growth was evident according to the one way ANOVA and Tukey's post-hoc HSD tests. However, the Haldane model gave the best fit for the experimental data on specific growth rate at all CO concentration (R^2 greater than 0.95). The capability of the mixed culture to degrade CO shows the benefits of mixed cultures in hydrocarbon degradation.

References

1) Sarma SJ, Pakshirajan K, Saamrat BG. Pyrene biodegradation by free and immobilized cells of mycobacterium frederiksbegense using solvent encapsulated system. *Indian Journal of Biotechnology*. 2011;10(4):495–501. doi:10.1080/10406638.2012.661828.

2) Saravanan P, Pakshirajan K, Saha P. Batch growth kinetics of an indigenous mixed microbial culture utilizing m-cresol as the sole carbon source. *Journal of Hazardous Materials*. 2009;162(1):476–481. doi:10.1016/j.jhazmat.2008.05.069.

3) Hadibarata T, Tachibana S, Itoh K. Biodegradation of chrysene, an aromatic hydrocarbon by Polyporus sp. S133 in liquid medium. *Journal of Hazardous Materials*. 2009;164(2-3):911–917. doi:10.1016/j.jhazmat.2008.08.081.

4) R A, N N, G A, J R, B U. Kinetics of biodegradation of phenol and a polyphenolic compound by a mixed culture containing Pseudomonas aeruginosa and Bacillus subtilis. *Journal of Applied environmental research*. 2014;12(3):615–625. doi:10.15666/aeer/1203_615625.

5) Bundy JG, Paton GI, Campbell CD. Microbial communities in different soil types do not converge after diesel contamination. *Journal of Applied Microbiology*. 2002;92(2):276–288. doi:10.1046/j.1365-2672.2002.01528.x.

6) Dahalan FA, Yunus I, Johari W, Shukor MY, Halmi M, Shamaan NA, et al. Growth kinetic of diesel degrading microbial strain from petroleum-contaminated soil. *Journal of Environmental Biology*. 2014;35:399–406.

7) Killham K. Soil Ecology. Cambridge University Press. 2006;p. 242–242.

8) Ma AN. Treatment of palm oil mill effluent. *Malaysian oil palm growers Council Kuala Lumpur*. 1999a;p. 113–126. doi:10.4236/tel.2018.811162.

9) Maila MP, Cloete TE. The use of biological activities to monitor the removal of fuel contaminants—perspective for monitoring hydrocarbon contamination: a review. *International Biodeterioration & Biodegradation*. 2005;55(1):1–8. doi:10.1016/j.ibiod.2004.10.003.

10) Yacob S, Hassan MA, Shirai Y, Wakisaka M, Subash S. Baseline study of methane emission from open digesting tanks of palm oil mill effluent treatment. *Chemosphere*. 2005;59(11):1575–1581. doi:10.1016/j.chemosphere.2004.11.040.

11) America Industrial Hygiene Association AIHA; Standard Methods for the Examination of mold count in Water and Wastewater. 2011.

12) Nicolotti G, Egli S. Soil contamination by crude oil: impact on the mycorrhizosphere and on the revegetation potential of forest trees. *Environmental Pollution*. 1998;99:37–43. doi:10.1016/s0269-7491(97)00179-6.

13) Alrumman SA, Standing DB, Paton GI. Effect of hydrocarbon contamination on soil microbial community and enzyme activity. *Journal of King Saudi University - science*. 2014;27:31–41. doi:10.1016/j.jksus.2014.10.001.

14) Dharni AM, Samad AF, Khan S, Abdul-Kaliq S, Luqma DD. Phylogeneic Analysis and biological activity of streptomeyces sp CIMAP-A2 isolated from industrially polluted soil. *Indian Journal of Biotechnology*. 2012;11:438–444.

15) Shukor MY, Dahalan FA, Jusoh AZ, Muse R, Shamaan NA, Mccormack WP, et al. Isolation and characterization of pseudomonas diesel degrading strain from Antarctica. *Journal of Environmental Biology*. 2009;p. 1–6.

16) Hossani AM, Ngo HH, Guo W. Introductory to Microsoft Excel SOLVER Function spreadsheet method for isotherm and kinetics Modeling of Metals Bio sorption in waste water. *Journal of Water Sustainability*. 2013;3:223–237.

17) Aravindhan R. Kinetics of biodegradation of phenol and a polyphenolic compound by a mixed culture containing Pseudomonas aeruginosa and Bacillus subtilis. *Applied Ecology and Environmental Research*. 2014;12(3):615–625. doi:10.15666/aeer/1203_615625.

18) Adestani F. Survey of the nutrient utilization and cell growth kinetics with Verhulst, Contios model for Penicillum brevicompactum in batch reactor. *World Applied science Journal*. 2012;16(1):135–140.

19) Deriase SF, Younis SA, El-Gendy NS. Kinetic evaluation and modeling for batch degradation of 2-hydroxybiphenyl and 2,2′-dihydroxybiphenyl by Corynebacterium variabilis Sh42. *Desalination and Water Treatment*. 2013;51:4719–4728. doi:10.1080/19443994.2012.744950.

20) Dutta K, Venkata D, Mahanty B, Anand PA. Substrate inhibition growth kinetics for cutinase producing Pesudomonas Cepacia using tomato-peel extracted Cutin. *Journal of Bio chemical Engineering*. 2015;29(3).

21) Tazdait D, Abdi N, Grib H, Lounici H, Pauss A, Mameri N. Comparison of different models of substrate inhibition in aerobic batch biodegradation of malathion. *Turkish Journal of Engineering And Environmental Sciences*. 2013;37:221–230. doi:10.3906/muh-1211-7.

22) Saidu H, Jamaluddin H, Mohamad SE. Nutrient Removal and Biokinetic Study of Freshwater Microalgae in Palm Oil Mill Effluent (POME). *Indian Journal of Science and Technology*. 2017;10(24). doi:10.17485/ijst/2017/v10i24/114584.

23) Pacheco-Pérez K, Baia-Olivares M, Meza-González D, González-Delgado AD. Exergy Analysis of Hydrogen Production from Palm Oil Solid Wastes using Indirect Gasification. *Indian Journal of Science and Technology*. 2018;11(2). doi:10.17485/ijst/2018/v11i2/120429.

24) Takriff MS, Zakaria MZ, Sajab MS, T YH. Pre-treatments Anaerobic Palm Oil Mill Effluent (POME) for Microalgae Treatment. . *Indian Journal of Science and Technology*. 2016;9(21). doi:10.17485/ijst/2016/v9i21/95248.

25) Annuar MSM, Tan IKP, Ibrahim S, Ramachandran KB. A kinetic model for growth and biosynthesis of medium-chain-length poly-(3-hydroxyalkanoates) in Pseudomonas putida. *Brazilian Journal of Chemical Engineering*. 2008;25(2):217–228. doi:10.1590/s0104-66322008000200001.

26) Don MM, Shoparwe NF. Kinetics of hyaluronic acid production by Streptococcus zooepidemicus considering the effect of glucose. *Biochemical Engineering Journal*. 2010;49(1):95–103. doi:10.1016/j.bej.2009.12.001.

27) Ani AK, Ezeugwu F, Ochin E. Growth and optimization process of mixed microbial population degrading chrysene. *Gazi University Journal of Science*. 2018;31(3):740–757.