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Research Paper

Performance and Kinetics of Bioaugmentation, Biostimulation, and Natural Attenuation Processes for Bioremediation of Glyphosate-Contaminated Soils in Bauchi Metropolis.

Adukwu Amedu[1,2] *, Surajudeen Abdulsalam[1] , Idris Muhammad Misau[1] and Jibril Mohammed[1].

1. Department of Chemical Engineering, Faculty of Engineering, Abubakar Tafawa Balewa University; PMB 0248, Bauchi, Bauchi State, Nigeria.
2. Environmental Studies Division National Institute For Freshwater Fisheries Resesrch; PMB 6006, New Bussa, Niger State.

Abstract
This study was conducted to demonstrate the potential use of bioremediation in polycyclic aromatic hydrocarbons (PAHS) contaminated soil using glyphosate as a model pollutant, a laboratory study with the objectives of investigating, evaluating and comparing the methods of natural attenuation, biostimulation, bioaugmentation, and combined biostimulation and bioaugmentation was performed on the hydrocarbon degradation efficiency. The study dealt with glyphosate biodegradation in soil using fortified cattle dung with KH_2PO_4 (Organic Manure) and mixed culture of Pseudomonas aeruognosia and Bacillus subtilis as source of biostimulation and bioaugmentation, respectively. Each treatment strategy contained 5% (w/w) glyphosate in soil as a sole source of carbon and energy. Four (4) different microcosms with different treatment option containing glyphosate- contaminated soil were tested. Reactor (A1) was operated as the natural attenuation process. Then, a microbial inoculums and nutrients were added to Reactor (A4) to promote combined biostimulation and bioaugmentation. In Reactor (A3), only BA process was adopted, whereas in Reactor (A2), only the BS process was adopted. After 6 weeks of remediation, the result revealed that natural attenuation, biostimulation, bioaugmentation, and combined biostimulation and bioaugmentation exhibited 50 000 mg/kg to 29 000 mg/kg, 20 300 mg/kg, 16 400 mg/kg, 14 000 mg/kg and 6 500 mg/kg in six(6) weeks of remediation and corresponding to 42%, 59.4%, 67.2% and 87% glyphosate reduction was achieved under A1, A3, A2 and A4 respectively. Reaction rate data were fitted with a first-order reaction rate model. The Monod kinetic constants, maximum specific growth rate (μ_{max}), and substrate concentration at half-velocity constant (Ks) were also estimated. This study showed that the glyphosate removal efficiency in the combined BA and BS process was higher than in other processes tested. The populations of Organophosphorus degrading microorganisms in soil tanks were positively related to organophosate pesticide removal efficiency during bioremediation of glyphosate -contaminated soils.

Keywords: *bioaugmentation; bioremediation; biostimulation; glyphosate; first-order reaction rate model; Monod model; natural attenuation*

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I. Introduction

Glyphosate [N-(phosphonomethyl) glycine] is one of the most extensively used broad-spectrum organophosphorus herbicides (Gill *et al.*, 2017). It is a widely used herbicide in agriculture against perennial and annual weeds and in silviculture, domestic gardens, and urban areas (Zhang *et al.*, 2015). It is an essential component of non-selective and post-emergent herbicides used to protect the crop from grasses, annual broad-leaved weeds, woody plants, etc. (Conrad *et al.*, 2017). The parent compound was firstly sold in 1974 under the trade name "Roundup" by Monsanto (Singh *et al.*,2020). This compound tends to be a zwitterion, in which phosphonic hydrogen detaches and joins the amine group. Glyphosate was first synthesized by Henri Martin

while working at Cilag (a Swiss pharmaceutical company), but J.E. Franzo in 1970 conducted the herbicidal test on this compound and commercialized it in 1974 (Duke and Powles, 2008; Singh *et al.,* 2020). The potential mode of action of glyphosate makes it an herbicide of interest. The global glyphosate market was $23.97 billion in 2016, and at a growth rate of 6.05% for the forecasting period, it is estimated to reach $34.10 billion in 2022 (Dill, (2005); Singh *et al.,* 2020). Glyphosate is the only herbicide that targets 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) without any available analog and obstructs the aromatic amino acid biosynthesis in the shikimate pathway (Haslam, (2014)). Inhibition of EPSPS by glyphosate retards the synthesis of essential secondary metabolites and proteins; additionally, it curbs the vital energy pathways in soil microbes and plants (Sviridov *et al.,* 2015). A study reveals that glyphosate alters the soil texture and microbial diversity by reducing the microbial richness and increasing the population of phytopathogenic fungi (Hadi *et al.,* 2013). This herbicide is considered safer than others, but its overuse imposes chronic effects on the environment and humans (Wang *et al.,* 2016)

The International Agency for Research on Cancer (IARC) classified glyphosate as "Category 2a," which specifies probable carcinogenic to humans (IARC (2017). The United States Environmental Protection Agency (USEPA) classifies this herbicide as "Group E carcinogen," which means non-cancerous for humans (Singh *et al.,* 2020). In contrast to the European Food Safety Authority, which determines glyphosate as a potent carcinogen for humans, but the experimental evidence does not support this determination (Singh *et al.,* 2020), even though traces of glyphosate have been detected in human urine samples, highlighting its persistence, bioaccumulation, and potential health risk (Niemann *et al.,*2015). Although glyphosate residual concentrations have never crossed over the threshold level, its harmful effects cannot be ignored (Mesnage *et al.,*2015). Degradation of glyphosate can be achieved using abiotic and biotic means, e.g., absorption, photolysis, thermolysis, and biodegradation with catabolic enzymes. Lately, a blend of photocatalyst with UV light has come in the limelight for their ability to treat pollutants like pesticides. An eco-friendly strategy like bioremediation would be another promising alternative to overcome the environmental and health risks derived from glyphosate and its residues. Therefore, it has become essential to study glyphosate biodegradation driven by microbial degraders. Therefore, the objectives of this study were to examine, evaluate, and compare the methods of natural attenuation, biostimulation, bioaugmentation, and combined biostimulation and bioaugmentation in the bioremediation of soil contaminated with organophosphate pesticide. The kinetics of glyphosate biodegradation process was modeled as well as estimation of the biodegradation half-life time was carried out.

II. Materials and Methods

2.1 Materials

Fresh uncontaminated soil, with no prior history of oil contamination was excavated from Abubakar Tafawa Balewa University School Farm, Bauchi-Nigeria between 0-30cm from the soil surface. The soil was sieved on 2mm sieve to enhance proper mixing and extract consisting mainly of stones and dead plant debris discarded. The sieved soil was contaminated artificially organophosphate pesticide (Round up) to a pollutant level of 50 000 ppm. Cow dung (stimulant) was sun dried, grinded and homogenized; the stimulant was package in polythene bags prior to their application for bioremediation experiment. Physicochemical and microbiological analyses were performed on the soil and the organic stimulant (cow dung). Initial quantity of water in kilograms was applied to all the treatment options in the microcosm's base on the calculation of the experimental design. In the subsequent weeks, percentage moistures were determined and used to adjust the water lost and what need to be added. Moisture content level in each microcosm was increased from 3.5% to 12% using distilled water. The soil matrix was properly mixed at ambient temperature (25^0C- 30^0C). Each of the contaminated soil (5 kg) were stacked into eleven wooden boxes lagged internally with formica material to prevent absorption of organophosphate pesticide and moisture reacting with the wooden surface. Each box had dimension of 15cm height × 40cm length × 40cm width with soil layer $0.024m^3$ deep. Various treatment options were prepared according to Table 1

2.2 Soil Sampling

A clean soil sample was obtained from a site where there was no previous history of contamination. Before loading the wooden boxes (Reactor), the soil sample was grinded and large particles were removed. Then, it was air-dried at room temperature for 48 h. The volume of clean soil sample for each process, varied according to the experimental design of the different treatment options. Reactor 1 (NA), Reactor 2 (BA), Reactor 3 (BS fortified with KH_2PO_4), Reactor 4 (BA+ BS fortified with KH_2PO_4).

2.3 Loading Of the Microcosm

Clean soil samples were placed in the microcosm and mixed thoroughly by a PVC stirrer for homogenization. Afterwards, the soil samples were contaminated by adding 50000ppm of organophosphate pesticide (round- up

herbicide) to each reactor. Distill water was added to the reactors to sustain minimum of 20% water content. Then, the soil samples in all reactors were mixed again for homogenization

2.4 Experimental Design for the Bioremediation of Glyphosate Spiked Soil

Four wooden boxes used as bioreactors were prepared for each treatment as shown in Figure1, designated as bioattenuation (treatment A_1), biostimulation (treatment A_2), bioaugumentation (treatment A3), and combined biostimulation and bioaugumentation (treatment A4). Each bioreactor contained 5 kg of soil contaminated and well mixed with 100 ml of 50 g of Organophosphate pesticide (5% w/v) dissolved in water. The bioreactor under treatment (A_2) was amended with 8.0 kg of organic manure (cow dung) fortified with 0.04088kg of KH_2PO_4 (biostimulation), and the bioreactor under treatment (A_3) were amended with 50 ml of inoculum $2.6\times10^5\ CFU/g$ (combine *Bacillus subtilis & Pseudomonas aeruogonisa)*) (bioaugumentation). The bioreactor under treatment (A_4) was amended with both 50ml of inoculums (hybrid) and 8.0 kg of cow dung, fortified with 0.04088kg of KH_2PO_4 (combined biostimulation and bioaugumentation). The bioreactor under treatment A_1 was not amended with either Organic manure or inoculum (natural bioattenuation). The bioreactor or microcosm under organic manure treatment had a C: N: P ratio of 100:10:1(Beolchin *et al.*, 2010; Agarry *et al.*, 2015). Soil in the bioreactor used for some experimental optional design was sterilized by autoclaving at 121^0C for 15 min; all the bioreactors with its contents were incubated at a room temperature (28^0C±2^0C) for Six weeks. The water (moisture) content of soil in each bioreactor was adjusted every week by addition of distilled water in order to make up for water losses used by microbes to enhance their activities. In order to avoid anaerobic conditions, the samples in the microcosms were tilled and aerated daily to enhance oxygenation and kept moist during the experimental period. Samples were taken weekly and analyzed for residual pesticide

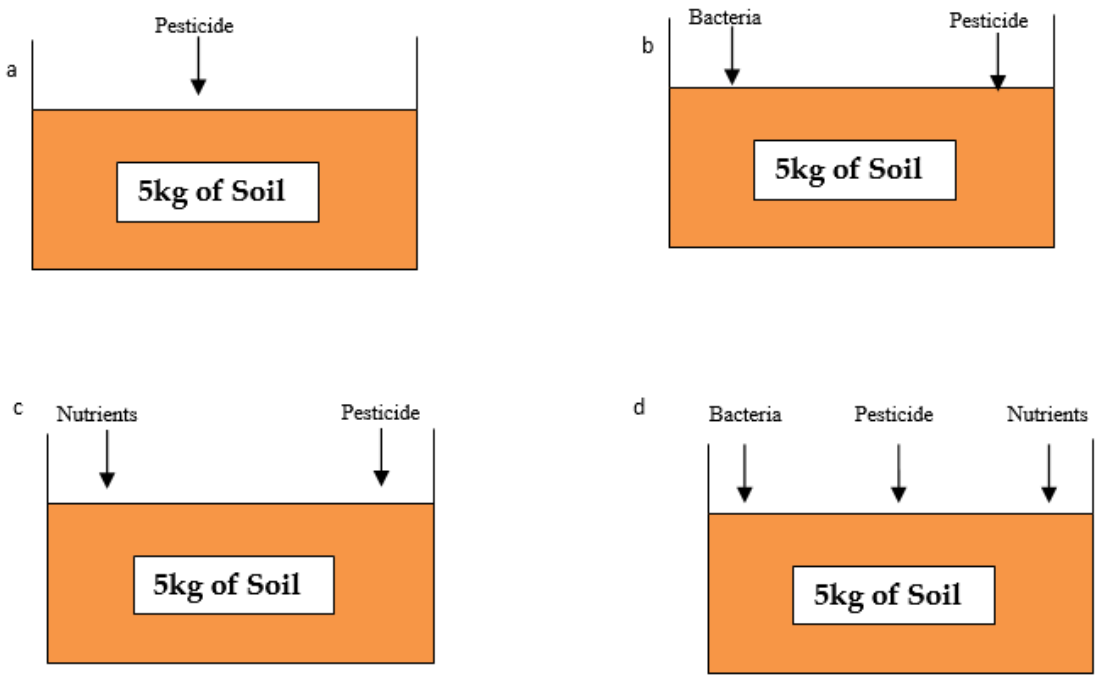

Figure 1: Schematics of Soil Treatment Reactors: (a) Natural Attenuations (NA), (b) Bioaugmentation (BA), (c) Biostimulation (BS) and (d) Biostimulation + Bioaugmentation (BS+BA).

Table 1: Configuration of the Treatment Microcosm

Reactor	Microbial Inoculum Addition (Bioaugumentation)	Nutrient Addition (Biostimulation)	Pesticide Addition
A1(NA)	**NO**	**NO**	**YES**
A2 (BA)	**YES**	**NO**	**YES**
A3 (BS)	**NO**	**YES**	**YES**
A4 (BA)	**YES**	**YES**	**YES**

2.5 Microbial Analysis

The pure isolate (*Bacillus subtlis and Pseudomonas aerugonisa*) in form of slants, was obtain from veterinary research institute in Vom, Jos, Plateau State. The slant was sub-culture using the streaking method on the nutrient agar in disposable petri dishes for 24 h in an incubator at 37 ^{0}C to monitor its cell growth. A

loopful of each stock culture of *Bacillus subtlis* and *Pseudomonas aerugonisa* were respectively inoculated into 3 different 250 ml Erlenmeyer flasks containing 100 ml of freshly prepared sterile nutrient broth medium (0.8 %) made up of yeast extract 2.0 g/L, peptone 5.0 g/L, NaCl 5.0 g/L, and agar 15.0 g/L, incubated at 37 ^{0}C for 48 hrs to monitor a turbid suspension. 1 mls of the stock solution was serially diluted into 9 mls of sterile nutrient broth(same constituents above) in a serial dilution in triplicate for each stock solution to get a viable counts of 30-300 cfu/ml. 200 milliters ($2.3\times10^5\ CFU/g$ *Bacillus subtilis*, $2.5\times10^5\ CFU/g$ *Pseudomonas aeruogonisa* and $2.6\times10^5\ CFU/g$ Combine *Bacillus subtilis & Pseudomonas aeruogonisa)* of the inoculums was inoculated on the surface of the microcosm and was also used for the bioaugumentation experimental design option to study the biodegradation of glyphosate in soil. All the microcosms were mixed three times every week for aeration for the (6) weeks. After mixing, these microcosms were kept away from sunlight at room temperature in order to prevent rate of dehydration.

Colony forming unit (CFU) was determined by using the formula; CFU/g = $\frac{number\ of\ colonies \times dilution\ factor}{volume\ of\ culture\ plate}$

2.6 Kinetic Model Analysis

A mass balance in the experimental microcosm was used to find a kinetic model for degradation of total hydrocarbon. The kinetic model can be defined as shown in Equation (1) (Komilis *et al.,* 2009; Yaman, 2020)

$$-r = -\frac{dc}{dt} = kC^n \cdots\cdots\cdots (1)$$

Where; r: reaction rate, k: biodegradation rate, C: concentration, t: time, n: reaction order.

Eq. (1) is a typical first-order model. The use of first order kinetics in the description of biodegradation rates in environmental fate models is common because mathematically the expression can be easily incorporated into the model (Coulon *et al.,* 2012). The following relation of substrate concentration to time can be obtained as given in Eq. (2):

$$\ln C = a + k_1 t \cdots\cdots\cdots (2)$$

The constants, k and n, are found by plotting concentration vs. time and determining the best suitable line. The half time ($t_{1/2}$) can be calculated as follows (Equation (3)) (Tellez *et al.,* 1995; Chemlal *et al.,* 2012)

$$t_{\frac{1}{2}} = \frac{\ln 2}{k} \cdots\cdots\cdots (3)$$

Moreover, the growth of microorganisms can be determined by using the Monod equation as shown in Equation (4)

$$\mu = \mu_{max} \frac{C}{K_s + C} \cdots\cdots\cdots (4)$$

Where; μ: specific growth rate, μ max: maximum specific growth rate, Ks: THC value at half-time. Then,

$$r = \frac{\mu X}{Y} = \frac{\mu_{max}}{Y} \frac{C}{K_s + C} X \cdots\cdots\cdots (5)$$

Where X shows the microorganism concentrations and Y is yield that is expressed as biomass formed per mass of substrate used. The rate of reaction determined numerically was used to obtain μmax and Ks as defined in Equation (6):

$$\frac{1}{\mu} = \frac{K_s}{\mu_{max}} \left(\frac{1}{C}\right) + \frac{1}{\mu_{max}} \cdots\cdots\cdots (6)$$

III. Results and Discussion

3.1 Microbial Analysis

The results obtained from this study showed that microorganisms can successfully biodegrade TPH, particularly when combined BS and BA processes are used. Results of this study also showed that BA has a higher effect on biodegradation efficiency than BS process. Adding nitrogen and phosphorus, along with microbial inoculation and aeration can create an optimum condition for microorganisms to degrade TPH. Analysis of the contaminated soil indicated that the C: N: P ratio was 100:10:1, which corresponds to the nutrients needed for microorganisms (Alexander *et al*., 1999; Li *et al*., 2007; Agarry and Oghenejoboh, 2015; Yaman, 2020). In addition, the initial soil contained low numbers of TPH biodegraders. Therefore, adding optimum amount of nutrients and introducing TPH degraders (Addition of consortium of *Pseudomonas aerugonisa* and *Bacillus subtilis)* on pesticide polluted soil, apparently increasing the microbial populations in the contaminated soil an offers interesting possibility of enhancing more efficient TPH biodegradation of the soil after few weeks. Counts at the end of the investigation revealed that sample A4 in the organophosphate pesticide contaminated soil treated with combined biostimulation and bioaugumentation strategies supported more bacteria growth. Figure 1 shows the variation of total heterotrophic bacteria counts (THBC) against bioremediation time, the counts are in order of A4>A2>A3>A1 with maximum count (A4) obtained at week 9. This indicated that in the soil, the addition of *Pseudomonas aerugonisa* and *Bacillus subtilis*

(bioaugumentation) and cow dung fortified with KH_2PO_4 (biostimulation) resulted in the highest number of bacteria at the beginning of remediation period as compared to natural attenuation (or bioattenuation). The lower bacteria population in natural attenuation treatment may be due to the presence of low nutrients (limiting nutrient) and the distribution of nutrient ratio especially of C:N:P that is needed in the correct ratio for bacteria growth

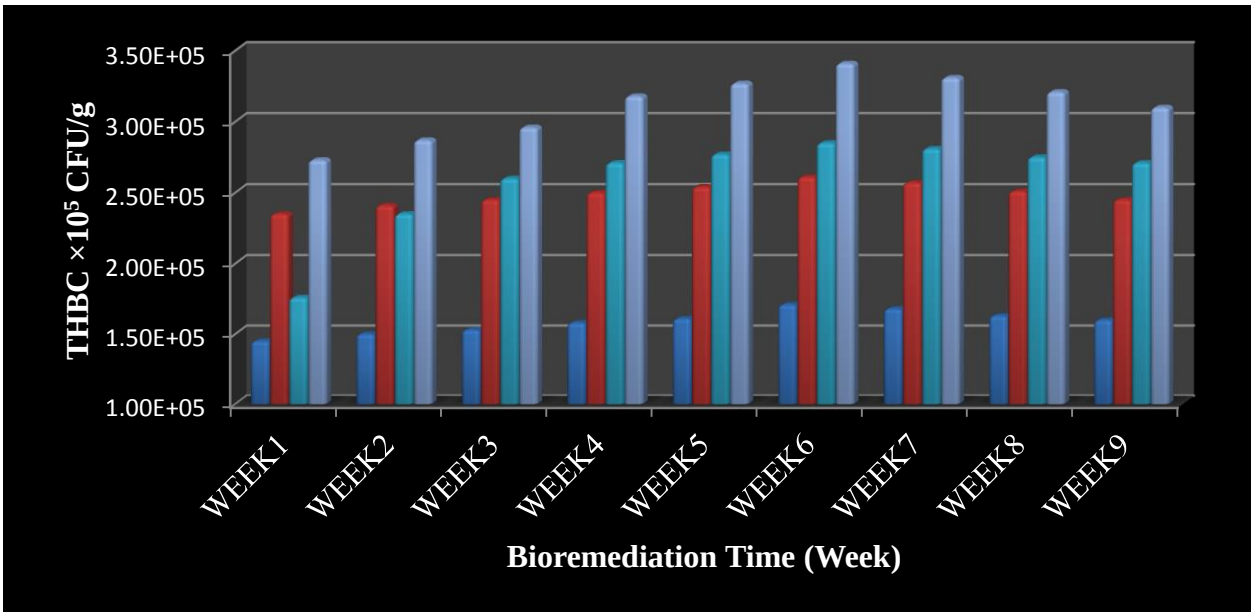

Figure 1: Variation of THBC with Bioremediation Time

Natural Attenuation (A1)

Bioaugumentation (A2)

Biostimulation (A3)

Combined Bioaugmention and Biostimulation (A4)

The variation in concentrations of pesticide residual content (mm/kg and %) against bioremediation time is shown in Figures 2 and 3 respectively. Biodegradation of the organophosphate pesticide started very fast during the first week of remediation in all the treatments and slowly continued up to the 9th week (day 63). The concentration of glyphosate was reduced from the initial concentration of 50 000 mg/kg to 29 000 mg/kg, 20 300 mg/kg, 16 400 mg/kg, and 6 500 mg/kg in six(6) weeks of remediation and corresponding to 42%, 59.4%, 67.2%, and 87% glyphosate reduction was achieved under A1, A3, A2 and A4 respectively. This observation revealed that during the glyphosate biodegradation in soil, addition of organic manure (Cow Dung fortified with KH_2PO_4) and bacterial inoculums individually resulted in a more effective bioremediation response than the natural attenuation. Similar observations have been reported for naphthalene (Agarry and Oghenejoboh, 2015), kerosene (Shabir *et al.,* 2008; Agarry *et al.,* 2013 and Agarry and Oghenejoboh, 2015) and spent engine oil (Abdulsalam and Omale; 2009 and Abdulsalam *et al.,* 2011). Nevertheless, bioaugmentation strategy elicited a higher biodegradation of glyphosate than the biostimulation treatment. This is in agreement with the observation of Bento *et al.,* (2003) and Agarry and Oghenejoboh, 2015) who reported that among the individual methods of natural attenuation, biostimulation, and bioaugmentation that were used for the remediation of a soil contaminated by diesel oil, bioaugmentation method elicited higher diesel oil degradation than others. In contrast, other workers have shown that biostimulation strategy enhanced the bioremediation of kerosene contaminated soil (Shabir *et al.*, 2008; Agarry *et al.*, 2013; Agarry and Oghenejoboh, 2015), crude oil contaminated soil (Lu *et al.,* 2010), spent engine oil contaminated soil (Abdulsalam *et al.,*2011) and lubricating oil contaminated soil (Agarry *et al.*, 2013) than bioaugmentation. Generally, in this work, the combination of biostimulation (Cow Dung fortified with KH_2PO_4) and bioaugmentation treatment strategy (which has not been reported for glyphosate removal) showed relatively greater glyphosate reduction than other treatments during the whole period of remediation.

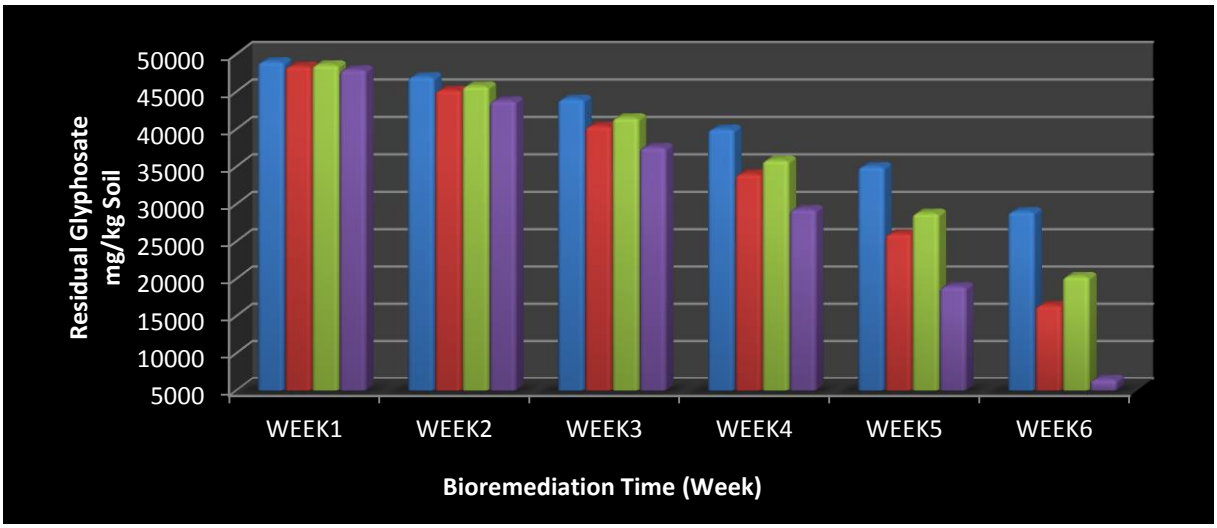

Figure 2: Variation of Residual Glyphosate content with Bioremediation Time.

Natural Attenuation (A1)

Bioaugmentation(A2)

Biostimulation(A3)

Combined Bioaugmention and Biostimulation (A4)

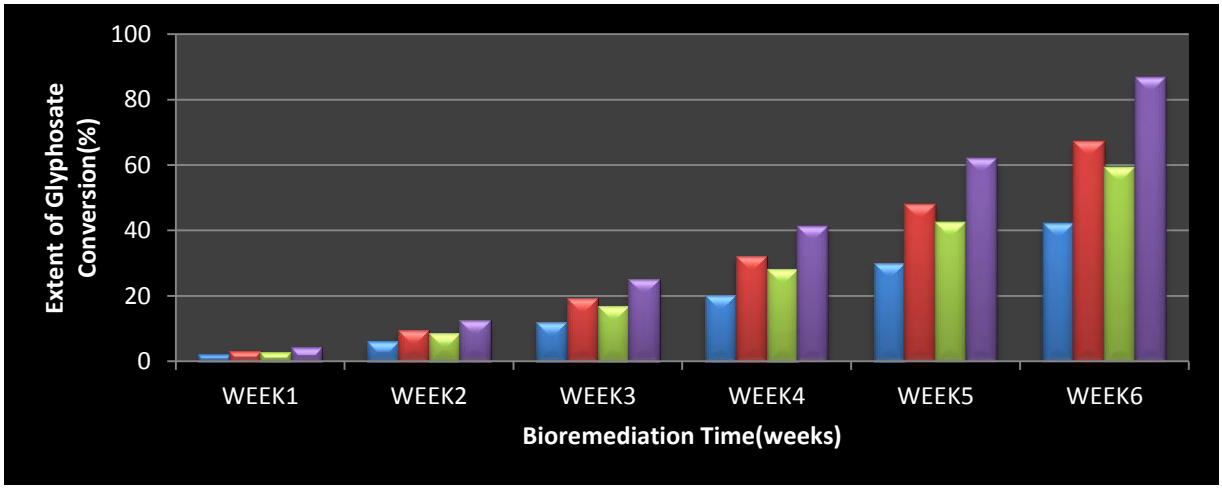

Figure 3: Variation of Percentage Conversion with Bioremediation Time

Combined Bioaugmention and Biostimulation (A4)

Biostimulation (A3)

Bioaugumentation (A2)

Natural Attenuation (A1)

3.2 Bioremediation Kinetics

The data obtained from the microcosm were applied to the first-order rate model and the Monod model. Several investigators have reported that first-order kinetics and Michaelis-Menten kinetic can be used for petroleum-hydrocarbon degradation (Brook *et al.*, 2001; Roncevi *et al.*, 2005; Shewfelt, 2005; Chemial *et al.*, 2012). The yield values (Y), which define the microorganism concentrations in CFU per mg of TPH biodegraded, were determined from the values of CFU and TPH. Figure 4-11 shows fits of the first-order and the Monod models to data from the NA, BS + BA, BS, and BA treatment processes. It is clearly seen that the Monod model represents the data better than the first-order reaction rate model, which is validated by the high correlation determination of the R^2 values indicates that the combination of BS and BA (A4) has the highest regression fitting of 0.95 as compared to other treatment options.,A2 (0.91), A3 (0.89), A2 (0.87) and A1 (0.86), when compared to the first order kinetic model in the observe trend of regression A4 (0.89), A2 (0.83), A3 (0.81), and A1 (0.76). The reaction rate coefficient (k), maximum specific growth rate (μmax), half-reaction time ($t_{1/2}$), and TPH value at half-time (Ks) were determined from the curves in Figure 4-11 and are

summarized in Table 2 for each treatment process, The coefficients calculated from the first-order reaction rate model clearly showed that the Monod model fits better for the BS+BA (A4) process than the other treatment processes. The first-order reaction rate (k) in the BS+BA (A4) process was higher compared to NA, BA, and BS processes. The half-reaction time determined from the reaction rate (k) in the BA+BS (A4) process was thus smaller than in the other processes. Table2 shows that the summary of results of the kinetic parameters for biodegradation of organophosphate pesticide kinetic parameters in soil under different treatment strategy; Natural Attenuation A1 (k=0.0238d^{-1}, ($t_{1/2}$) =29.07d), Biostimulation A3 (k=0.0274d^{-1}, ($t_{1/2}$) =25.2973d), Bioaugumentation A2; k=0.03016d^{-1}, ($t_{1/2}$) =22.9823d and Combined Biostimulation and Bioaugumentation (A7; k=0.06284d^{-1}, ($t_{1/2}$) =11.03d, A4 had the highest k (0.06284 day^{-1}) corresponding with the lowest ($t_{1/2}$) (11.03 days) as compared to other bioremediation treatment option strategies in the first-order reaction rate model, It is to be noted that the higher is the biodegradation rate constants, the higher or faster is the rate of biodegradation and consequently the lower is the half-life time or the faster the reaction rate. The maximum specific growth rate (μ_{max}) and TPH value at half-time (Ks) were determined from the curves in Figure 4-11 and are summarized in Table 2 for each treatment process. The results indicate maximum specific growth rate (μ_{max}) was higher and TPH value at half-time (Ks) was lower in the combination of BA+BS process (A4); μ_{max}=4.928 d^{-1}, Ks =13340mg/kg which indicates that BA+BS process had a faster reaction rate when compared to other treatment options in the observe trends; A2 (μ_{max}=2.5641d^{-1}, Ks =25340mg/kg), A3 (μ_{max}=2.4038 d^{-1}, Ks =27885mg/kg), and A1 (μ_{max}=1.85873 d^{-1}, Ks =33100mg/kg). The results of these both model applications indicated that BS and BA together achieved the best OPP removal efficiency respectively. Therefore, value of the kinetic parameter showed that the degree of effectiveness of these bioremediation strategies in the cleanup of soil contaminated with OPP is in the following order: bioattenuation < biostimulation < bioaugmentation < combined biostimulation and bioaugmentation

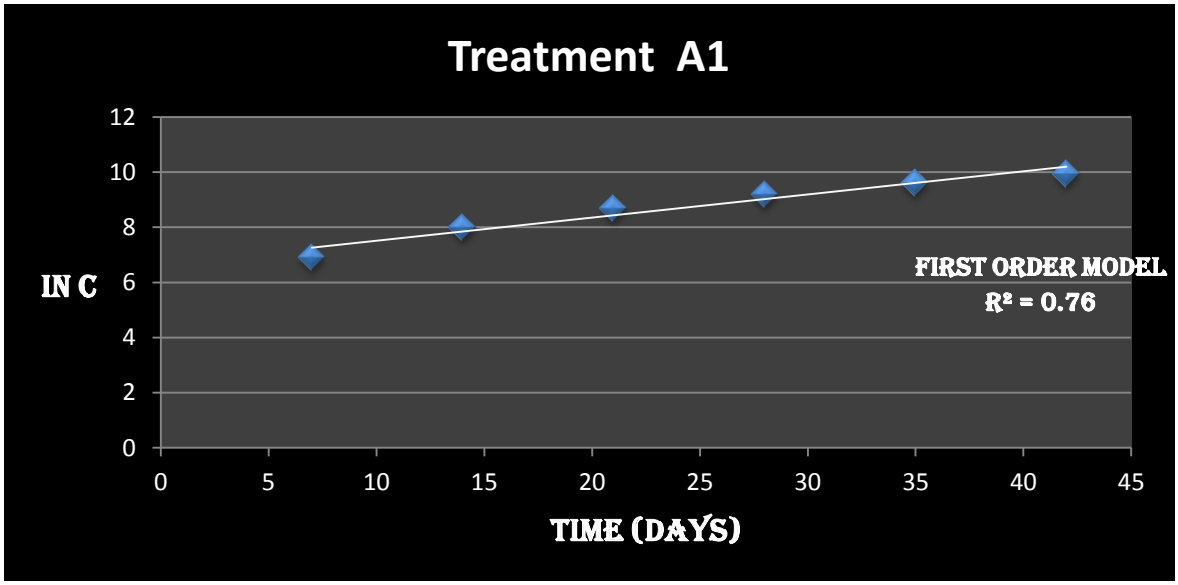

Figure 4: Reaction rate data of First-Order Model for Bioattenuation.

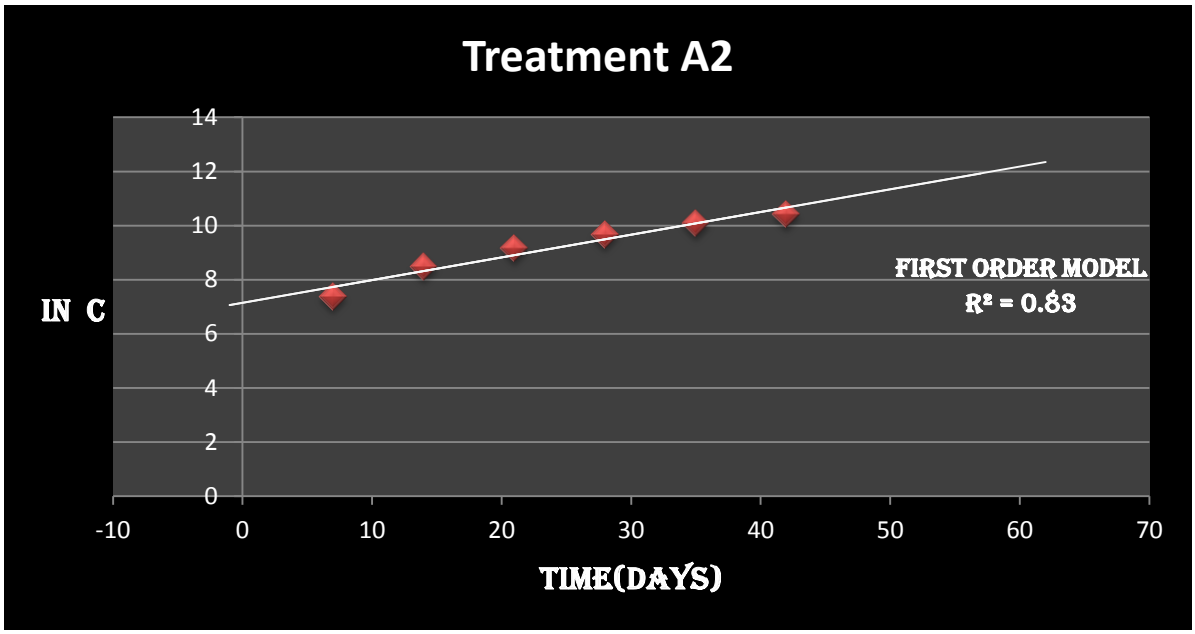

Figure 5: Reaction rate data of First-Order Model for Bioaugumentation.

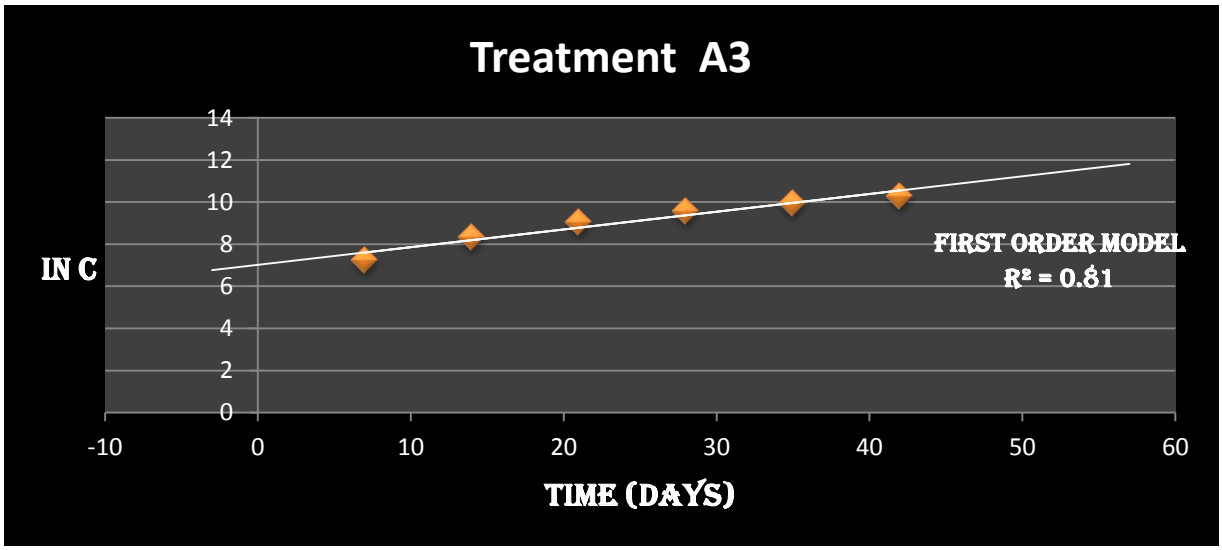

Figure 6: Reaction rate data of First-Order Model for Biostimulation.

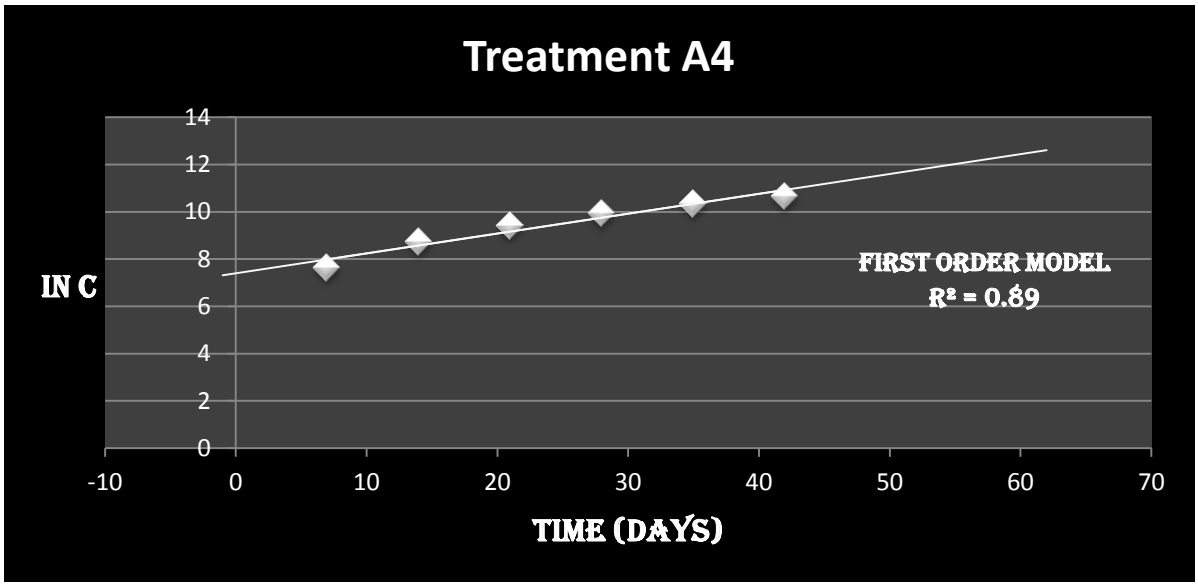

Figure 7: Reaction rate data of First-Order Model for Combined Biostimulation and Bioaugumentation.

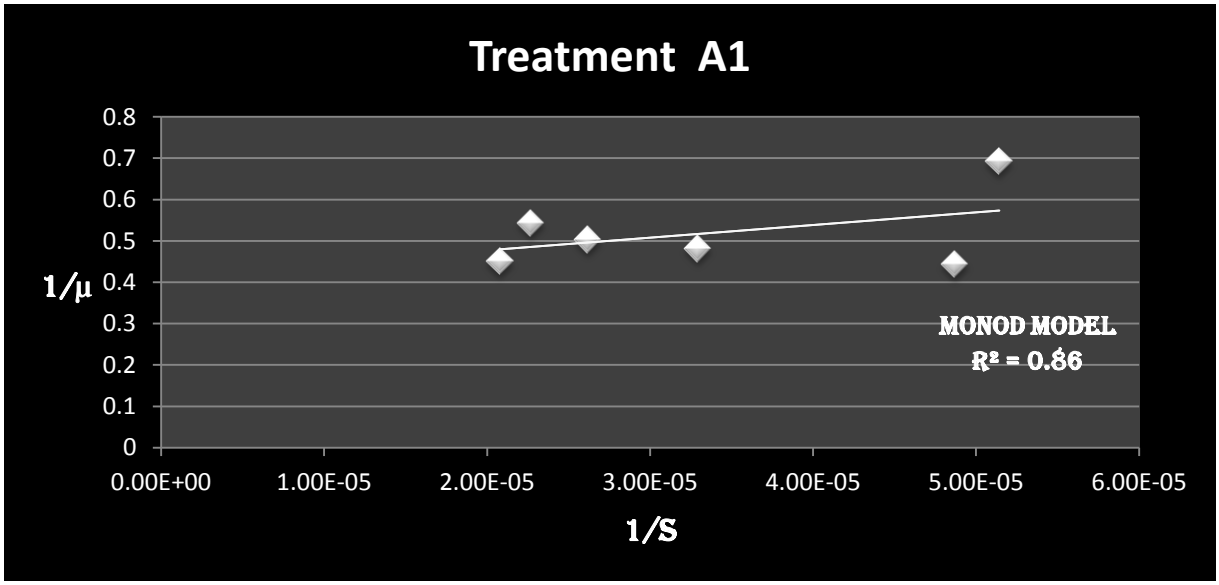

Figure 8: Reaction rate data of Monod Model for Bioattenuation.

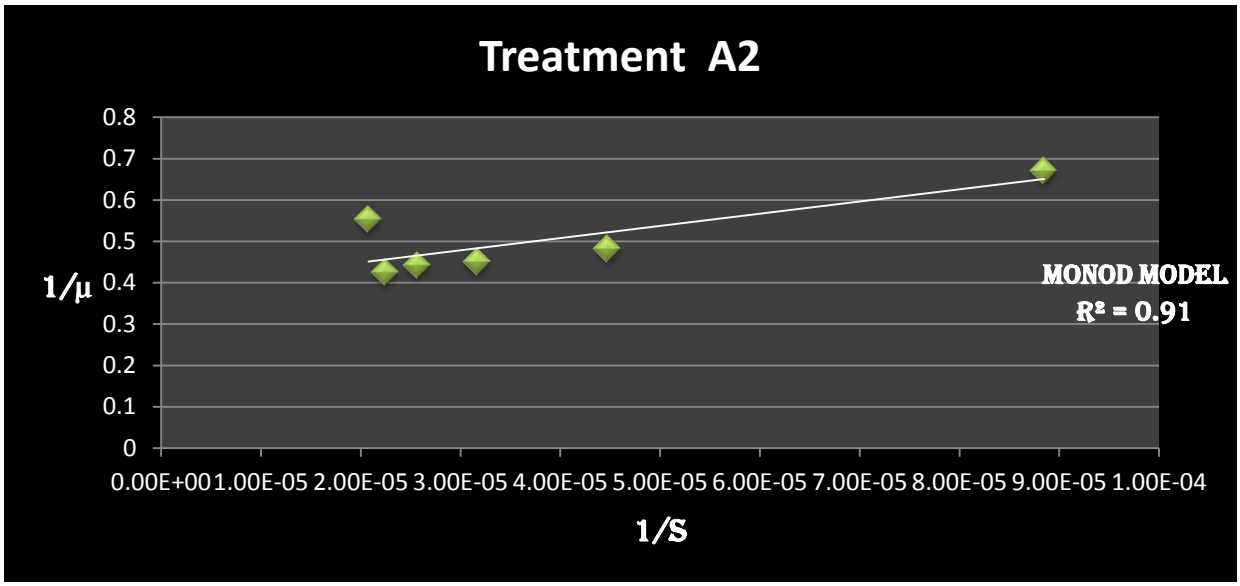

Figure 9: Reaction rate data of Monod Model for Bioaugumentation.

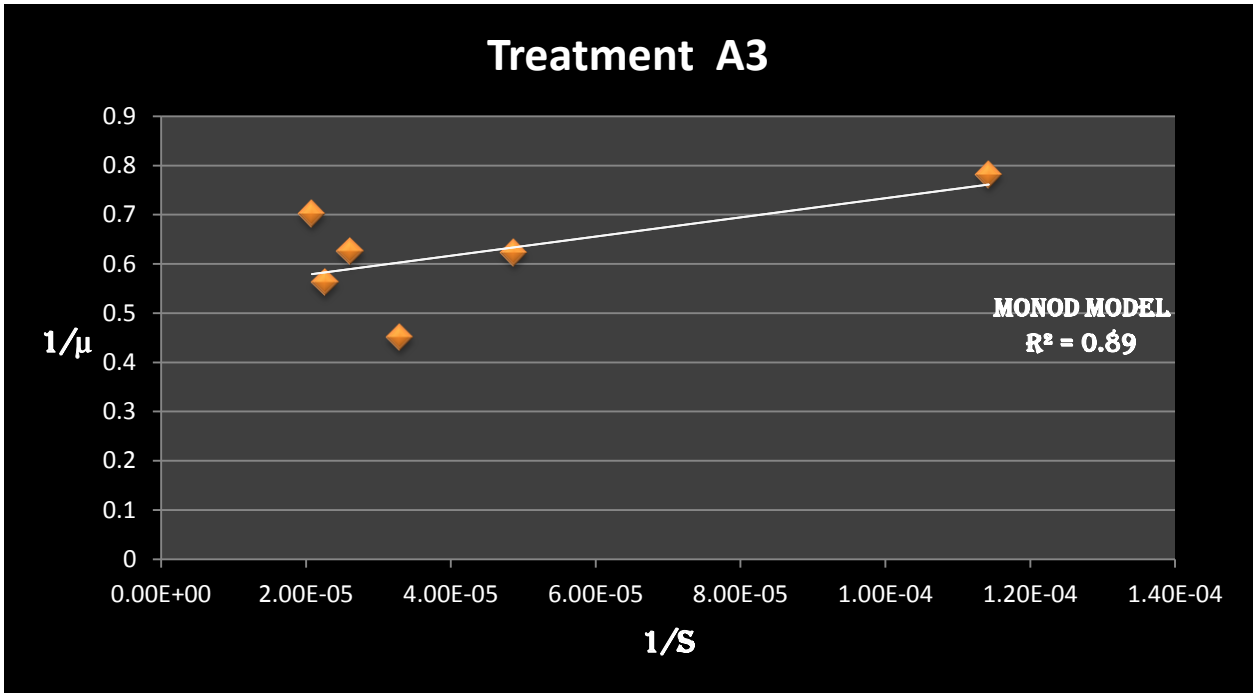

Figure 10: Reaction rate data of Monod Model for Biostimulation.

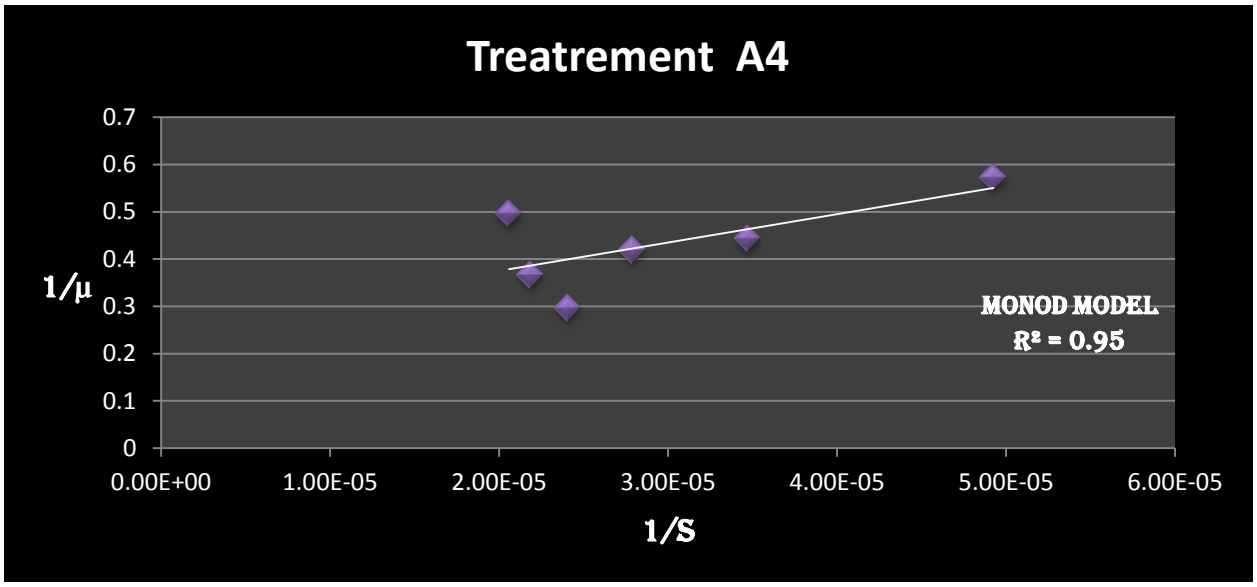

Figure 11: Reaction rate data of Monod Model for Combined Biostimulation and Bioaugumentation.

Table 2: Calculated reaction rate factors for the first-order and the Monod models.

S/No	Treatment Options	First Order Model	Monod Model
1	A1	K= 0.02384 d^{-1} $t_{1/2}$ =29.07d R^2= 0.76	μ_{max} = 1.858736d^{-1} K_s= 33100mg/kg R^2= 0.86
2	A3	K= 0.03016 d^{-1} $t_{1/2}$=22.9823d R^2= 0.83	μ_{max} =2.5641d^{-1} K_s= 25340mg/kg R^2= 0.91
3	A2	K= 0.0274d^{-1} $t_{1/2}$=25.2973d R^2= 0.81	μ_{max} =2.4038d^{-1} K_s= 27885.71mg/kg R^2= 0.89
4	A4	K= 0.06284d^{-1} $t_{1/2}$=11.03d R^2= 0.89	μ_{max} = 4.928d^{-1} K_s= 13340mg/kg R^2= 0.95

IV. Conclusion

The hybrid of bioaugumentation and biostimulation (A4) has the highest conversion rate of the Organophosphate Pesticide (87.5%) at the 6th week of investigation, followed by, A2 (67.2%), A3 (59.4%) and A1 (42.0%). The total heterotrophic bacteria count (THBC) analysis was carried out using the serial dilution technique and also revealed that bioremediation actually took place. The result obtained at the end of the 6th week corresponds to the highest rate of the hydrocarbon removal from the contaminated soil with A4 had the highest maximum bacteria growth (3.41E+05 CFU/g), followed by A2 (2.96 E+05 CFU/g), A3 (2.85 E+05 CFU/g), and A1 (1.71E+05 CFU/g). The concentration of glyphosate was reduced from the initial concentration of 50 000 mg/kg to 29 000 mg/kg, 20 300 mg/kg, 16 400 mg/kg, and 6 500 mg/kg in six(6) weeks of remediation and corresponding to 42%, , 59.4%, 67.2%, and 87% glyphosate reduction was achieved under A1, A3, A2 and A4 respectively Therefore A4 was the best in cleaning OPP contaminated soil artificially. The soil treatment under combined biostimulation and bioaugmentation exhibited the highest degree of biodegradation with the highest biodegradation rate constant (A4; k=0.06284d^{-1}, ks=13340mg/kg, μ_{max} =4.928 d^{-1}) and lowest half-life time ($t_{1/2}$) =11.03d and the soil treatment under natural attenuation the least degradation with the lowest biodegradation rate constant (A1 (k=0.0238d^{-1}, ks=33100mg/kg, μ_{max} = 1.85873 d^{-1}) and highest half-life time ($t_{1/2}$ =29.07d). Thus, value of the kinetic parameter showed that the degree of effectiveness of these bioremediation strategies to enhance OPP biodegradation in the soil could be one of the severally sought bioremediation strategies of remediating natural ecosystem (environment) contaminated with Aromatic hydrocarbons

References

[1]. Abdulsalam, S., Bugaje, I. M., Adefila, S. S., Ibrahim, S. (2011) Comparison of biostimulation and bioaugmentation for remediation of soil contaminated with spent motor oil. International Journal of Environmental Science and Technology 8(1), 187-194, 2011

[2]. Abdulsalam, S., Omale, A.B (2009).. "Comparison of biostimulation and bioaugmentation techniques for the remediation of used motor oil contaminated soil". Brazilian Archives of Biology and Technology 52, 747-754

[3]. Agarry, S. E., Oghenejoboh, K. M.(2015) "Enhanced Aerobic Biodegradation of Naphthalene in Soil: Kinetic Modelling and Half-Life Study." International Journal of Environmental Bioremediation & Biodegradation, vol. 3, no. 2 (2015): 48-53. doi: 10.12691/ijebb-3-2-2.

[4]. Agarry, SE, Aremu, MO., Aworanti, OA. (2013) Kinetic modelling and half-life study on bioremediation of soil co-contaminated with lubricating motor oil and lead using different bioremediation strategies. Soil and Sediment Contamination- An International Journal 22 (7), 800-816, 2013

[5]. Alexander, M.(1999). Biodegradation and Bioremediation; Academic Press: San Diego, CA, USA, 1999.

[6]. Bento, F. M., Camargo, F.A., Okeke, B., Frankenberger, Jr. T.W. (2003) Bioremediation of soil contaminated by diesel oil. Brazilian Journal of Microbiology 34(Suppl. 1), 65-68, 2003.

[7]. Brook, T.R.; Stiver, W.H.; Zytner, R.G. (2001) Biodegradation of diesel fuel in soil under various nitrogen addition regimes. Soil Sediment Contam. Int. J. 2001, 10, 539–553.

[8]. Cevat Y. (2020) Performance and Kinetics of Bioaugmentation, Biostimulation, and Natural Attenuation Processes for Bioremediation of Crude Oil-Contaminated Soils Environmental Engineering, College of Engineering, Imam Abdulrahman Bin Faisal University, Dammam 34212, Saudi Arabia; cyaman@iau.edu.sa;

[9]. Chemlal, R.; Tassist, A.; Drouiche, M.; Lounici, H.; Mameri, N.; Drouiche, N.(2012). Microbiological aspects study of bioremediation of diesel-contaminated soils by biopile technique. Int. Biodeterior. Biodegrad. 2012, 75, 201–206.

[10]. Conrad, A.; Schröter-Kermani, C.; Hoppe, H.W.; Rüther, M.; Pieper, S.; Kolossa-Gehring, M. (2017) Glyphosate in German adults—Time trend (2001 to 2015) of human exposure to a widely used herbicide. Int. J. Hyg. Environ. Health 2017, 220, 8–16.

[11]. Coulon, F.; Brassington, K.J.; Bazin, R.; Linnet, P.E.; Thomas, K.A.; Mitchell, T.R.; Lethbridge, G.; Smith, J.W.N.; Pollard, S.J.(2012). Effect of fertilizer formulation and bioaugmentation on biodegradation and leaching of crude oils and refined products in soils. Environ. Technol. 2012, 33, 1879–1893

[12]. Dill, G.M. (2005) Glyphosate-resistant crops: History, status and future. In Proceedings of the Pest Management Science; John Wiley & Sons, Ltd: Hoboken, NJ, USA, 2005; Volume 61, pp. 219–224.

[13]. Duke, S.O.; Powles, S.B. (2008) Glyphosate: A once-in-a-century herbicide. In Proceedings of the Pest Management Science; John Wiley & Sons, Ltd: Hoboken, NJ, USA, 2008; Volume 64, pp. 319–325.

[14]. Gill, J.P.K.; Sethi, N.; Mohan, A. (2017)Analysis of the glyphosate herbicide in water, soil and food using derivatising agents. Environ. Chem. Lett. 2017, 15, 85–100.

[15]. Hadi, F.; Mousavi, A.; Noghabi, K.A.; Tabar, H.G.; Salmanian, A.H. (2013) New bacterial strain of the genus Ochrobactrum with glyphosate-degrading activity. J. Environ. Sci. Health—Part B Pestic. Food Contam. Agric. Wastes 2013, 48, 208–213.

[16]. Haslam, E. (2014). The Shikimate Pathway: Biosynthesis of Natural Products Series; Butterworths: London, UK, 2014.

[17]. International Agency for Research on Cancer. Some Organophosphate Insecticides and Herbicides. IARC(2017). Monographs on the Evaluation of Carcinogenic Risks to Humans; International Agency for Research on Cancer: Lyon, France, 2017; Volume 112, pp. 1–452.

[18]. Komilis, D., Vrohidou, A.-E.K., Voudrias, E.A.(2009). Kinetics of aerobic bioremediation of a diesel-contaminated sandy soil: Effect of nitrogen addition. Water Air Soil Pollut. 2009, 208, 193–208.

[19]. Li CH, Wong YS, Tam NF. (2010). Anaerobic biodegradation of polycyclic aromatic hydrocarbons with amendment of iron (III) in mangrove sediment slurry. Bio resource Technology.vol; 101:8083-92.

[20]. Li, H.; Zhang, Y.; Kravchenko, I.; Xu, H.; Zhang, C.-G(2007). Dynamic changes in microbial activity and community structure during biodegradation of petroleum compounds: A Laboratory experiment. J. Environ. Sci. 2007, 19, 1003–1013

[21]. Mesnage, R.; Defarge, N.; Spiroux de Vendômois, J.; Séralini, G.E. (2015). Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. Food Chem. Toxicol. 2015, 84, 133–153

[22]. Niemann, L.; Sieke, C.; Pfeil, R.; Solecki, R. A (2015) critical review of glyphosate findings in human urine samples and comparison with the exposure of operators and consumers. J. Verbrauch. Leb. 2015, 10, 3–12.

[23]. Roncevi´c, S.. Dalmacija, B., Ivancev-Tumbas, I., Tri˘ckovi´c, J., Petrovic, O., Klasnja, M., Agbaba, J. (2005) Kinetics of degradation of hydrocarbons in the contaminated soil layer. Arch. Environ. Contam. Toxicol. 2005, 49, 27–36.

[24]. Shabir, G., Afzal, M., Anwar, F., Tahseen, R., Khalid, Z.M.(2008). "Biodegradation of kerosene in soil by a mixed bacterial culture under different nutrient conditions". International Journal of Biodeterioration and Biodegradation 61, 161-166, 2008

[25]. Shewfelt, K.; Lee, H.; Zytner, R.G.(2005) Optimization of Nitrogen for Bioventing of Gasoline Contaminated Soil. J. Environ. Eng. Sci. 2005, 4, 29–42.

[26]. Singh, S. ; Vijay, K ; Jatinder, P. G.; Shivika, D.; Satyender, S.; Vaishali, D.; Dhriti, K.; Abdul, B. W.; Daljeet, S. D.;, Manoj, K.; Harikumar, S. L.; Joginder, S.(2020). Herbicide Glyphosate: Toxicity and Microbial Degradation, *Int. J. Environmental Research and Public Health 2020, 17, 7519; doi:10.3390/ijerph17207519*

[27]. Singh, S.; Kumar, V.; Datta, S.; Wani, A.B.; Dhanjal, D.S.; Romero, R.; Singh, J.(2020). Glyphosate uptake, translocation, resistance emergence in crops, analytical monitoring, toxicity and degradation: A review. Environ. Chem. Lett. 2020, 18, 663–702

[28]. Sviridov, A.V.; Shushkova, T.V.; Ermakova, I.T.; Ivanova, E.V.; Epiktetov, D.O.; Leont'evskii, A.A. (2015). Microbial degradation of glyphosate herbicides (review). Prikl. Biokhim. Mikrobiol. 2015, 51, 183–190.

[29]. Tellez, G.T., Nirmalakhandan, N., Gardeatorresdey, J.L.(1995) Evaluation of biokinetic coefficients in degradation of oil-field produced water under varying salt concentrations. Water Res. 1995, 29, 1711–1718.

[30]. Wang, S.; Seiwert, B.; Kästner, M.; Miltner, A.; Schäffer, A.; Reemtsma, T.; Yang, Q.; Nowak, K.M. (2016) (Bio)degradation of glyphosate in water-sediment microcosms—A stable isotope co-labeling approach. Water Res. 2016, 99, 91–100

[31]. Zhang, C.; Hu, X.; Luo, J.; Wu, Z.; Wang, L.; Li, B.; Wang, Y.; Sun, G.(2015) Degradation dynamics of glyphosate in different types of citrus orchard soils in China. Molecules 2015, 20, 1161–1175.