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Kinetic Study of Banana Stalk Ash Assisted Bioremediation of Crude-oil Contaminated Soil Based on Biomass Growth

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Abstract:
This work studies the kinetics of bioremediation of Crude Oil contaminated soil using banana stalk ash as a bio-stimulant, by studying the kinetics of biomass growth and nature of yield. Twelve samples of soil contaminated with 20g to 60g of Crude oil and with 0g to 60g of banana stalk ash added as bio-stimulant were studied based on the total petroleum hydrocarbon. The experimental data obtained were fit to four models based on biomass growth kinetics and yield using the Curve-fitting toolbox of MATLAB software and compared based on their adjusted R-square. The analysis of the results reveal that the biomass growth follows the logistic growth model with varying yield for all twelve experiments, with or without addition of bio-stimulant, to an accuracy of more than 99% thus making it the model of choice for explaining banana talk ash assisted bioremediation.
Keywords — bioremediation, banana stalk ash, biomass growth, yield, kinetics, bio-stimulant

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I. INTRODUCTION

Petroleum hydrocarbons represent a complex mixture of organic compounds mainly grouped into four fractions: alkenes, aromatics, resins and asphaltenes [1]. Hydrocarbon pollution of the environment has remained a major challenge for man over the years and has been escalating in proportions with increase in industrial activities. Such pollutions are usually occasioned by human error, equipment failure, vandalism, wars and natural disasters. Prominent among the deleterious effects of such pollutions on land is the destruction of natural flora and fauna thereby ultimately reducing the capacity of the ecosystem to support life. Several techniques have been developed over the years to combat this menace. These techniques are grouped broadly into two namely; In-situ methods (such as leaching or washing, isolation and containment, volatilization, bioremediation and passive bioremediation) and Ex-situ methods (such as incineration, solidification and stabilization, soil washing, and land farming) [2]. Bioremediation is the use of living microorganisms to breakdown or degrade petroleum hydrocarbon into harmless products such as CO_2 and H_2O. Microorganisms have been known to degrade hazardous compounds considered recalcitrant and resistant to biodegradation. Bioremediation has several advantages which include: cost effectiveness, environmental friendliness, simplicity in technology, conservation of soil texture and properties and its ability to produce harmless end products. This is contrary to other physical and chemical treatment methods whose limitations include; transfer of pollutants from one place/phase to another and being a complex technology and expensive to implement at full scale [3]. Due to the limitations of the physiochemical technologies stated above, great deal of literature has reported bioremediation as an alternative and/or supplement to these methods. Bioremediation of crude oil-contaminated soil can be carried out naturally (natural attenuation), or by the use of nutrients (organic or inorganic fertilizers); by the use of chemicals; or through mechanical means. Literature is rife with research works in the use of organic or inorganic fertilizers to enhance bioremediation [4]-[12].This study incorporates kinetic studies of laboratory experiments on crude oil contaminated soil by testing the effectiveness of bioremediation on crude-oil contaminated soil (based on Total Petroleum Hydrocarbon) using banana stalk ash (BSA) as substrate/bio-stimulant under aerobic conditions.

II MATERIALS AND METHODS

Materials: Crude oil, Banana stalk Ash (NPK 2.34/49/0.4), Soil sample, Chloroform, Distilled water.

Apparatus: Jenway UV-VIS Spectrophotometer (AAS), Sieve (mesh size: 0.3mm), Electronic weighing balance (ZL 200630014473.3), Digital thermometer, Measuring cylinder, Beaker, Conical flask, Oven (4824213), Spatula, Plastic bucket, PH meter, Stirrer, Stove, Sample bottles.

Preparation of Banana Stalk Ash: Banana stalk Ash collected from Jimeta (Yola North L. G. A., Nigeria) was crushed, sieved and dried in an oven for ninety minutes at a temperature of 200^oC.

CRUDE OIL CONTAMINATED SOIL SAMPLES PREPARATION

Twelve 2.0-liter plastic buckets were labeled M to X and 1000g of soil was weighed and added to each of the twelve buckets. Crude oil was weighed and added to each of the soil

samples as follows: 20g of the sample was added to M,P,S,V; 40g was added to N,Q,T,W and 60g was added to O,R,U,X.
Oil was added to the content of the buckets and were mixed properly and kept in a room, away from rain, sunlight, and direct climatic influence. Ten days after the pollution of the soil samples, the samples were tilled for one minute each to allow aeration. The crude oil used was procured from Kaduna refinery, Kaduna, Nigeria. The sample from each bucket was allowed for 2 weeks after which each set of contaminated soil sample were collected in sample bottles for analysis of total petroleum hydrocarbon (TPH) before ash was added.
Banana stalk ash was added to samples as follows: 0g of (BSA) was added to (M,N,O), 20g of the samples was added to (P,Q,R), 40g was added to (S,T,U) and 60g of the sample was added to (V,W,X). Each sample was tilled for one minute every 24 hours and analyzed every two weeks for eight weeks to determine the total petroleum hydrocarbon (TPH). This method was adopted from [6] and presented in Table 1.

TABLE 1: QUANTITIES OF CRUDE OIL CONTAMINATION AND BANANA STALK ASH IN SOIL SAMPLES M TO X

Quantity of Ash	Quantity of Crude Oil		
	20g	40g	60g
0g	M	N	O
20g	P	Q	R
40g	S	T	U
60g	V	W	X

Measurement of Total Petroleum Hydrocarbon (TPH)
3g of each sample was taken into a conical flask and 40 ml of measured chloroform was added to sample bottles labeled M to X and the sample was tightly closed and shaken vigorously for proper mixing of the content and allowed to stand for seven hours to enable complete extraction of oil by the chloroform. After 24 hours each of the samples was decanted and a clear liquid was obtained and transferred into a fresh sample bottles and the volume made up to 50 ml utilizing chloroform. The clear liquid was poured gently into the beaker and placed on the heating mantle for evaporation at 40^0C. The beaker was weighed after cooking to determine the oil content [13]. The UV- vis spectrophotometer was standardized using chloroform blank with wavelength at 290nm and the results obtained were measured in g/kg.
Models Based on Biomass Growth
Reference [4] based on certain assumptions were able to develop some models which were fitted to experimental data from NPK fertilizer enhanced bioremediation. The models include:

If Microbial growth is exponential and yield is constant:

$$S = S_o + \frac{x_0}{Y_G}(1 - e^{\mu t}) \quad (1)$$

If Microbial growth is exponential and yield is not constant:

$$S = S_o\,(e^{\mu t})^{\frac{1}{Y_G}} \quad (2)$$

If microbial growth is Logistic growth with constant yield:

$$S = S_O + \frac{x_0}{Y_G}\left(1 - \frac{e^{\mu t}}{1-\gamma x_0(1-e^{\mu t})}\right) \quad (3)$$

If microbial growth is Logistic growth with yield not constant:

$$S = S_O\,\left(\frac{e^{\mu t}}{1-\gamma x_0(1-e^{\mu t})}\right)^{\frac{1}{Y_G}} \quad (4)$$

Where, S = substrate concentration TPH, (g/kg), So = Initial substrate concentration (initial TPH), x_0 = Initial microbial concentration, Y_G = Yield coefficient, μ = Specific growth rate of the microbes, γ = Inverse of the maximum microbial concentration, t = Time (weeks)
Equations 1, 2, 3 and 4 will be used to fit the experimental data and the model with the best fit (based on the adjusted R-square) will be considered the model that best describes the biomass growth rate and associated yield, with respect to the bioremediation process.

III RESULTS AND DISCUSSIONS

Experimental results from the study of the twelve samples were fit to the four kinetic models based on biomass growth rate and yield kinetics (equation 1 to equation 4), to determine the kinetics of the biomass growth and yield and hence the kinetics of the bioremediation process. The graphical fit results (Figure 1) for the experiment on soil contaminated with 20g of Crude Oil, without any bio-stimulant added (sample M) shows that the bioremediation fits well to logistic growth with constant yield and logistic growth with varying yield. The numerical fit results of the same experimental data (Table 2) reveal that logistic growth with varying yield has the overall best fit with adjusted r-squared of 0.9894 (r-squared of 0.9947) as against adjusted r-squared of 0.9729 (r-squared of 0.9865) for logistic growth with constant yield. The model for logistic growth with varying yield thus explains more that 99% of the experimental results performed for sample M, as model with the best fit.
The graphical fit results (Figure 2) for the experiment on soil contaminated with 20g of Crude Oil, with 20g of bio-stimulant added (sample P) shows that the bioremediation fits well to logistic growth with constant yield and logistic growth with varying yield. The numerical fit results of the same experimental data (Table 3) reveal that logistic growth with varying yield has the overall best fit with adjusted r-squared of 0.9897 (r-squared of 0.9948) as against adjusted r-squared of 0.9707 (r-squared of 0.9854) for logistic growth with constant yield. The model for logistic growth with varying yield thus explains more that 99% of the results from experiment for sample P, as model with the best fit.

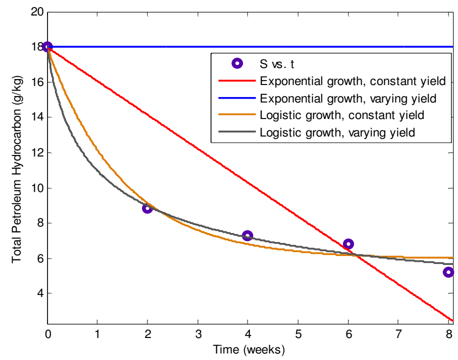

Fig. 1: Fit for 0g ash and 20g Crude oil (M)

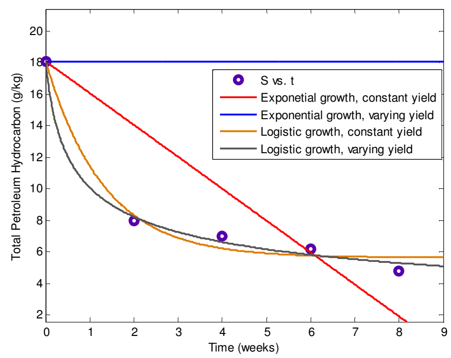

Fig. 2: Fit for 20g ash and 20g Crude oil (P)

TABLE 2: FIT DATA FOR 0g ASH AND 20g CRUDE OIL (M)

Model	R^2	Adj-R^2
Exponential growth, constant yield	0.5712	0.4283
Exponential growth, varying yield	-3.738	-3.738
Logistic growth, constant yield	0.9865	0.9729
Logistic growth, varying yield	0.9947	0.9894

The graphical fit results (Figure 3) for the experiment on soil contaminated with 20g of Crude Oil, with 40g of bio-stimulant added (sample S) shows that the bioremediation fits well to logistic growth with constant yield and logistic growth with varying yield.

The numerical fit results of the same experimental data (Table 4) reveal that logistic growth with varying yield has the overall best fit with adjusted r-squared of 0.9801 (r-squared of 0.9900) as against adjusted r-squared of 0.9578 (r-squared of 0.9789) for logistic growth with constant yield. The model for logistic growth with varying yield thus explains more that 99% of the results from the experiment for sample S, as model with the best fit.

TABLE 3: FIT DATA FOR 20g ASH AND 20g CRUDE OIL

Model	R^2	Adj-R^2
Exponential growth, constant yield	0.5214	0.3619
Exponential growth, varying yield	-3.806	-3.806
Logistic growth, constant yield	0.9854	0.9707
Logistic growth, varying yield	0.9948	0.9897

The graphical fit results (Figure 4) for the experiment on soil contaminated with 20g of Crude Oil, with 60g of bio-stimulant added (sample V) shows that the bioremediation fits well to logistic growth with constant yield and logistic growth with varying yield. The numerical fit results of the same experimental data (Table 5) reveal that logistic growth with varying yield has the overall best fit with adjusted r-squared of 0.9932 (r-squared of 0.9966) as against adjusted r-squared of 0.9815 (r-squared of 0.9908) for logistic growth with constant yield. The model for logistic growth with varying yield thus explains more that 99.6% of the results from the experiment for sample V, as model with the best fit.

The graphical fit results (Figure 5) for the experiment on soil contaminated with 40g of Crude Oil, without any bio-stimulant added (sample N) shows that the bioremediation fits well to logistic growth with constant yield and logistic growth with varying yield. The numerical fit results of the same experimental data (Table 6) reveal that logistic growth with varying yield has the overall best fit with adjusted r-squared of 0.9971 (r-squared of 0.9986) as against adjusted r-squared of 0.9914 (r-squared of 0.9957) for logistic growth with constant yield. The model for logistic growth with varying yield thus explains more that 99.8% of the results for sample N, as model with the best fit.

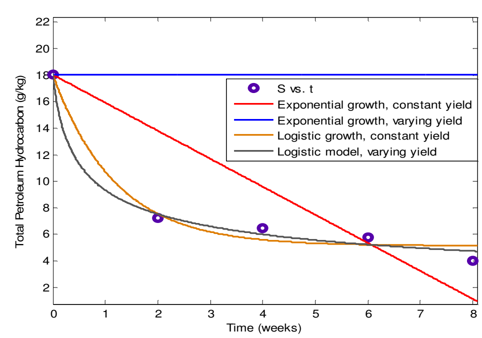

Fig. 3: Fit for 40g ash and 20g Crude oil (S)

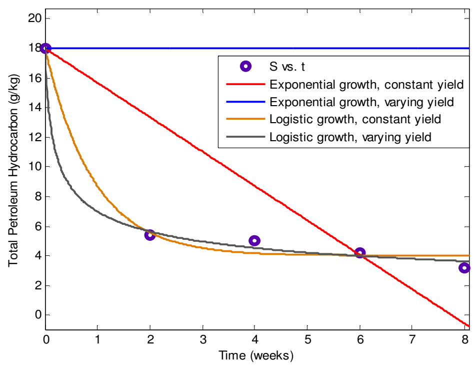

Fig. 4: Fit for 60g ash and 20g Crude oil (V)

TABLE 4: FIT DATA FOR 40g ASH AND 20g CRUDE OIL (S)

Model	R^2	Adj-R^2
Exponential growth, constant yield	0.5020	0.3360
Exponential growth, varying yield	-3.818	-3.818
Logistic growth, constant yield	0.9789	0.9578
Logistic growth, varying yield	0.9900	0.9801

TABLE 5: FIT DATA FOR 60g ASH AND 20g CRUDE OIL (V)

Model	R^2	Adj-R^2
Exponential growth, constant yield	0.3892	0.1856
Exponential growth, varying yield	-3.924	-3.924
Logistic growth, constant yield	0.9908	0.9815
Logistic growth, varying yield	0.9966	0.9932

The graphical fit results (Figure 6) for the experiment on soil contaminated with 40g of Crude Oil, with 20g of bio-stimulant added (sample Q) shows that the bioremediation fits well to logistic growth with constant yield and logistic growth with varying yield. The numerical fit results of the same experimental data (Table 7) reveal that logistic growth with varying yield has the overall best fit with adjusted r-squared of 0.9969 (r-squared of 0.9984) as against adjusted r-squared of 0.9919 (r-squared of 0.9959) for logistic growth with constant yield. The model for logistic growth with varying yield thus explains more that 99.8% of the results from the experiment for sample Q, as model with the best fit.

The graphical fit results (Figure 7) for the experiment on soil contaminated with 40g of Crude Oil, with 40g of bio-stimulant added (sample T) shows that the bioremediation fits well to logistic growth with constant yield and logistic growth with varying yield. The numerical fit results of the same experimental data (Table 8) reveal that logistic growth with varying yield has the overall best fit with adjusted r-squared of 0.9968 (r-squared of 0.9984) as against adjusted r-squared of 0.9916 (r-squared of 0.9958) for logistic growth with constant yield. The model for logistic growth with varying yield thus explains more that 99.8% of the results from the experiment for sample T, as model with the best fit.

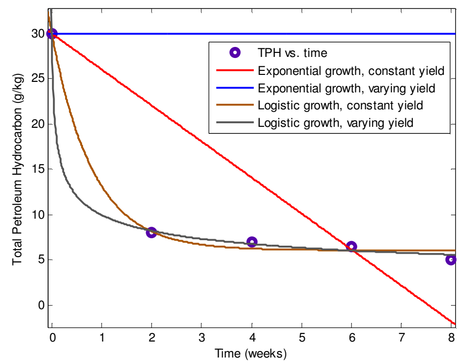

Fig. 5: Fit for 0g ash and 40g Crude oil (N)

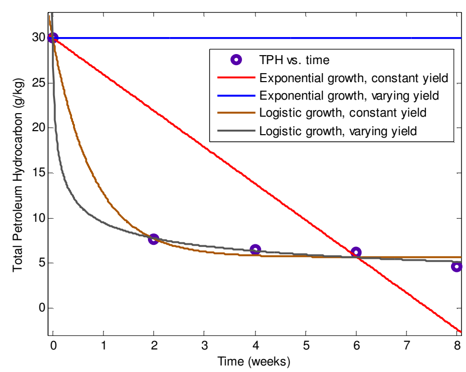

Fig. 6: Fit for 20g ash and 40g Crude oil (Q)

TABLE 6: FIT DATA FOR 0g ASH AND 40g CRUDE OIL (N)

Model	R^2	Adj-R^2
Exponential growth, constant yield	0.3333	0.1111
Exponential growth, varying yield	-3.958	-3.958
Logistic growth, constant yield	0.9957	0.9914
Logistic growth, varying yield	0.9986	0.9971

TABLE 7: FIT DATA FOR 20g ASH AND 4g CRUDE OIL (Q)

Model	R^2	Adj-R^2
Exponential growth, constant yield	0.3270	0.1027
Exponential growth, varying yield	-3.960	-3.960
Logistic growth, constant yield	0.9959	0.9919
Logistic growth, varying yield	0.9984	0.9969

The graphical fit results (Figure 8) for the experiment on soil contaminated with 40g of Crude Oil, with 60g of bio-stimulant added (sample W) shows that the bioremediation fits well to logistic growth with constant yield and logistic growth with varying yield. The numerical fit results of the same experimental data (Table 9) reveal that logistic growth with varying yield has the overall best fit with adjusted r-squared of 0.9989 (r-squared of 0.9994) as against adjusted r-squared of 0.9944 (r-squared of 0.9972) for logistic growth with constant yield. The model for logistic growth with varying yield thus explains more that 99.9% of the experimental results for sample W, as model with the best fit.

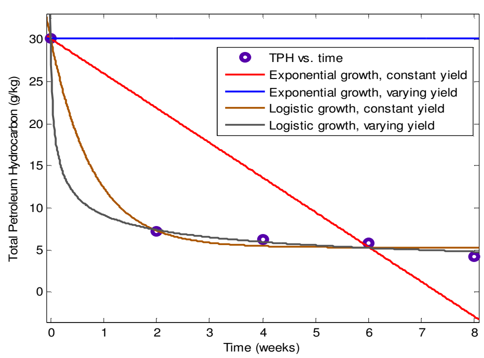

Fig. 7: Fit for 40g ash and 40g Crude oil (T)

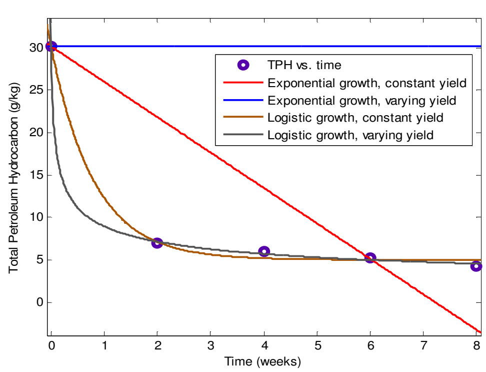

Fig. 8: Fit for 60g ash and 40g Crude oil (W)

TABLE 8: FIT DATA FOR 40g ASH AND 40g CRUDE OIL (T)

Model	R^2	Adj-R^2
Exponential growth, constant yield	0.3258	0.1011
Exponential growth, varying yield	-3.961	-3.961
Logistic growth, constant yield	0.9958	0.9916
Logistic growth, varying yield	0.9984	0.9968

TABLE 9: FIT DATA FOR 60g ASH AND 40g CRUDE OIL (W)

Model	R^2	Adj-R^2
Exponential growth, constant yield	0.3203	0.09368
Exponential growth, varying yield	-3.965	-3.965
Logistic growth, constant yield	0.9972	0.9944
Logistic growth, varying yield	0.9994	0.9989

The graphical fit results (Figure 9) for the experiment on soil contaminated with 60g of Crude Oil, without any bio-stimulant added (sample O) shows that the bioremediation fits well to logistic growth with constant yield and logistic growth with varying yield. The numerical fit results of the same experimental data (Table 10) reveal that logistic growth with varying yield has the overall best fit with adjusted r-squared of 0.9964 (r-squared of 0.9982) as against adjusted r-squared of 0.9910 (r-squared of 0.9955) for logistic growth with constant yield. The model for logistic growth with varying yield thus explains more that 99.8% of the results from the experiment for sample O, as model with the best fit.

The graphical fit results (Figure 10) for the experiment on soil contaminated with 60g of Crude Oil, with 20g of bio-stimulant added (sample R) shows that the bioremediation fits well to logistic growth with constant yield and logistic growth with varying yield. The numerical fit results of the same experimental data (Table 11) reveal that logistic growth with varying yield has the overall best fit with adjusted r-squared of 0.9964 (r-squared of 0.9982) as against adjusted r-squared of 0.9924 (r-squared of 0.9962) for logistic growth with constant yield. The model for logistic growth with varying yield thus explains more that 99.8% of the results from the experiment for sample R, as model with the best fit.

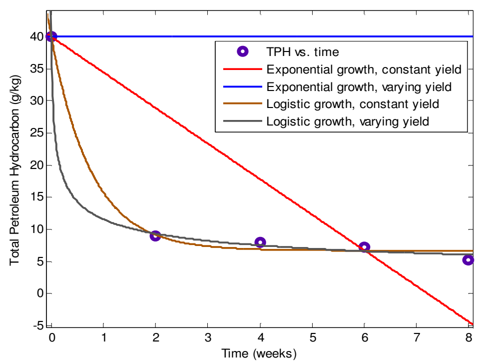

Fig. 9: Fit for 0g ash and 60g Crude oil (O)

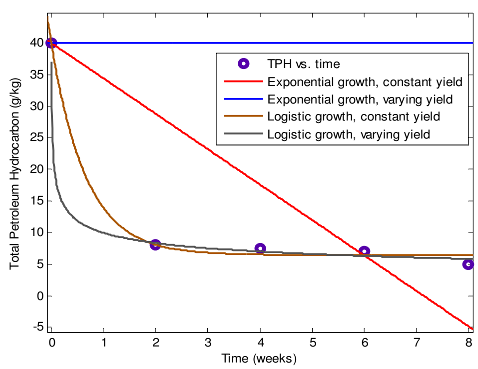

Fig. 10: Fit for 20g ash and 60g Crude oil (R)

TABLE 10: FIT DATA FOR 0g ASH AND 60g CRUDE OIL (O)

Model	R^2	Adj-R^2
Exponential growth, constant yield	0.3187	0.09165
Exponential growth, varying yield	-3.964	-3.964
Logistic growth, constant yield	0.9955	0.9910
Logistic growth, varying yield	0.9982	0.9964

TABLE 11: FIT DATA FOR 20g ASH AND 60g CRUDE OIL (R)

Model	R^2	Adj-R^2
Exponential growth, constant yield	0.2843	0.04516
Exponential growth, varying yield	-3.977	-3.977
Logistic growth, constant yield	0.9962	0.9924
Logistic growth, varying yield	0.9982	0.9964

The graphical fit results (Figure 11) for the experiment on soil contaminated with 60g of Crude Oil, with 40g of bio-stimulant added (sample U) shows that the bioremediation fits well to logistic growth with constant yield and logistic growth with varying yield. The numerical fit results of the same experimental data (Table 12) reveal that logistic growth with varying yield has the overall best fit with adjusted r-squared of 0.9990 (r-squared of 0.9995) as against adjusted r-squared of 0.9979 (r-squared of 0.9989) for logistic growth with constant yield. The model for logistic growth with varying yield thus explains more that 99.9% of the results from our experiment for sample U, as model with the best fit.

The graphical fit results (Figure 12) for the experiment on soil contaminated with 60g of Crude Oil, with 60g of bio-stimulant added (sample X) shows that the bioremediation fits well to logistic growth with constant yield and logistic growth with varying yield. The numerical fit results of the same experimental data (Table 13) reveal that logistic growth with varying yield has the overall best fit with adjusted r-squared of 0.9995 (r-squared of 0.9998) as against adjusted r-squared of 0.9984 (r-squared of 0.9992) for logistic growth with constant yield. The model for logistic growth with varying yield thus explains more that 99.9% of the results from the experiment for sample X, as model with the best fit.

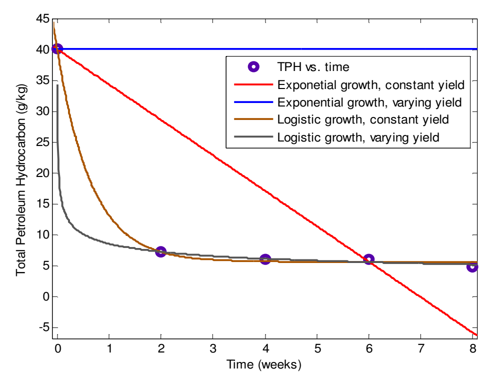

Fig. 11: Fit for 0g ash and 60g Crude oil (U)

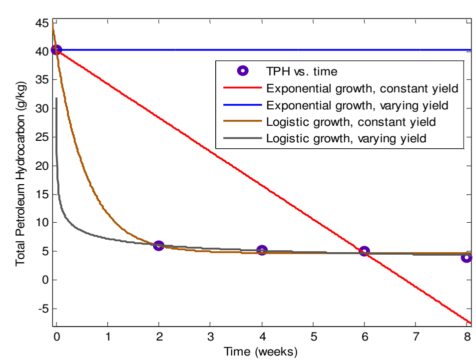

Fig. 12: Fit for 20g ash and 60g Crude oil (X)

TABLE 12: FIT DATA FOR 0g ASH AND 60g CRUDE OIL (U)

Model	R^2	Adj-R^2
Exponential growth, constant yield	0.2522	0.00289
Exponential growth, varying yield	-3.988	-3.988
Logistic growth, constant yield	0.9989	0.9979
Logistic growth, varying yield	0.9995	0.9990

TABLE 13: FIT DATA FOR 20g ASH AND 60g CRUDE OIL (X)

Model	R^2	Adj-R^2
Exponential growth, constant yield	0.2388	-0.01488
Exponential growth, varying yield	-3.992	-3.992
Logistic growth, constant yield	0.9992	0.9984
Logistic growth, varying yield	0.9998	0.9995

IV CONCLUSION

The kinetics of banana stalk ash assisted bioremediation has been studied using four kinetic models based on biomass growth kinetics and the nature of yield. The analysis of the results reveal that the biomass growth follows the mechanism explained by the logistic growth model and the yield varies based on the model of Oyoh and Osoka (2007). The model for logistic growth with varying yield, $S = S_o\left(\frac{e^{\mu t}}{1-\gamma x_0(1-e^{\mu t})}\right)^{\frac{1}{Y_G}}$, gave best fit to data from all twelve experiments, with or without addition of bio-stimulant, to accuracy of more than 99% and is therefore the model of choice for explaining banana talk ash assisted bioremediation.

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