



BIOKINETIC STUDY OF MICROBIAL DECONTAMINATION OF OILFIELD PRODUCED WATER

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ABSTRACT

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biokinetics.

This work was aimed at identifying microbials and determining the biokinetic parameters for bio-decontamination of oilfield produced water. An extant physical treatment unit for produced water was re-engineered and retrofit with a discontinuous aerobic bioreactor (Bio-Unit) system. The Bio-Unit was operated in a fill-and-draw sequence and the rates of total organic carbon (TOC) removal and biomass growth were monitored. The isolated microbial strains were identified as *Bacillus*, *Pseudomonas* and *Chryseobacterium spp.* The biokinetic parameters of the bio-detoxification process were determined by fitting experimental data into the Monod Equation. The maximum specific substrate utilization rate (k_m), maximum specific growth rate ($\mu_{m,h}$), substrate half saturation coefficient ($K_{S_{TOC}}$), yield (Y), and endogenous decay rate (b_h), were found to be 0.20 day^{-1} , 0.31 day^{-1} , 2.7 mg TOC/l , $1.6 \text{ mg MLSS/mg TOC}$, and 0.23 day^{-1} , respectively. These values are within the range published in literatures for oilfield produced water, thus will suffice for designing, modeling and control of biological treatment systems, since biokinetic parameters for real oilfield produced water treatment using discontinuous biological configuration is scanty.

Contribution/Originality: This study contributes to the biotreatability of oilfield produced water for reinjection into oil reservoir or ecofriendly disposal. The biokinetic coefficients obtained from this study are useful for similar operations.

1. INTRODUCTION

The traditional physicochemical techniques for treating oil and gas Produced Water (PW) are primarily designed to remove particulate or dispersed oil, but inept to remove dissolved petroleum hydrocarbons, significantly. Also, most of these physical treatment facilities can no longer withstand the increasing Water-to-Oil ratio due to aged crude oil and gas reservoirs.

These challenges are exacerbating environmental impacts, dislocating source of livelihood of oil-bearing communities, attracting stringent environmental regulations and sanctions, deferring production, instigating reservoir abandonment, depleting business profits, causing high economic losses, and portraying the image and reputation of oil and gas operators in a bad light. Other emerging trend is the keen focus on the treatment of dissolved

components in PW which were not considered in past years. Biological treatment methods are considered as cost-effective and eco-friendly for detoxifying oilfield PW [1, 2].

Biological decontamination of target pollutants by microbial catalytic activities is well articulated in several research and in PW treatment [3-9]. It has also been shown that indigenous microbial strains and consortium can biodegrade petroleum hydrocarbons in oilfield produced water [10-13]. In all these, only few works (namely, Tellez, et al. [10] and Kardena, et al. [12] have determined the biokinetic parameters for activated sludge bioreactor system (ASBS).

Also, due to equipment sizing, the use of ASBS for oilfield PW treatment is constrained by space availability, especially in offshore operations. Therefore, this work is aimed at decontaminating a real oilfield PW using indigenous microbial consortium in compact fill-and-draw aerobic bioreactor (Bio-Unit) system and determining the biokinetic parameters for the biotreatment process.

2. MATERIALS AND METHODS

2.1. Determination of Total Organic Carbon in the Oilfield Produced Water

The oilfield produced water (OPW) was sampled from a crude oil flow station located in Nigerian South-East State. The sample was preserved and analysed using standard test methods. The total organic carbon (TOC) was determined using high temperature catalytic oxidation method (ASTM D7573-18).

2.2. Isolation and Biodegradability Test for Indigenous Microbial Consortium

Indigenous microbial consortium was isolated from the crude oil saver pit crude oil production facility and enriched in mineral salt medium (MSM) (pH = 7.0 ± 0.2) supplemented with raw oilfield produced water 2% (v/v), which served as a sole carbon and energy source.

The content was incubated at 30°C and stirred at 150 rpm, and after 7 days, 10% of the enriched culture was further inoculated in a fresh mineral salt medium supplemented with raw oilfield produced water 2% (v/v) for three consecutive time. Isolation and identification of the isolates were performed using morphological and biochemical examinations [14, 15].

The ability of the isolated indigenous microbial consortium to degrade organic carbon in the oilfield PW was carried out by inoculating 1.0 ml of the enriched microbial consortium into 100.0 ml MSM with 1%v/v of raw oilfield PW as sole carbon source, in 250 ml Erlenmeyer flask.

The set up was kept at 30 ± 5 °C on a mechanical shaker at 160 rpm for the content to mix and homogenize, adequately. The set up was observed for 5 days. 1.0 ml of samples were collected, every 12 hours, to determine the microbial growth, by measuring optical density at 600 nm (OD_{600}). The measured optical density of the microbial consortium was plotted against the observed time to get the microbial growth curve. The initial and final total organic carbon (TOC) concentration was measured using standard method.

2.3. Treatment of the Oilfield Produced Water using the Pilot-Scale Bio-Unit

A simplified scheme of the oilfield PW gathering, and treatment facility is shown in Figure 1. The operating parameters of the pilot-scale Bio-Unit is given in Table 1. The enriched indigenous microbial was acclimated in the Bio-Unit by stepwise increase of PW concentration until minimum TOC concentration and maximum microbial growth were attained.

The Bio-Unit was operated as a fill-and-draw system at various biosolid retention time (BRT) to enable the determination of biokinetic parameters.

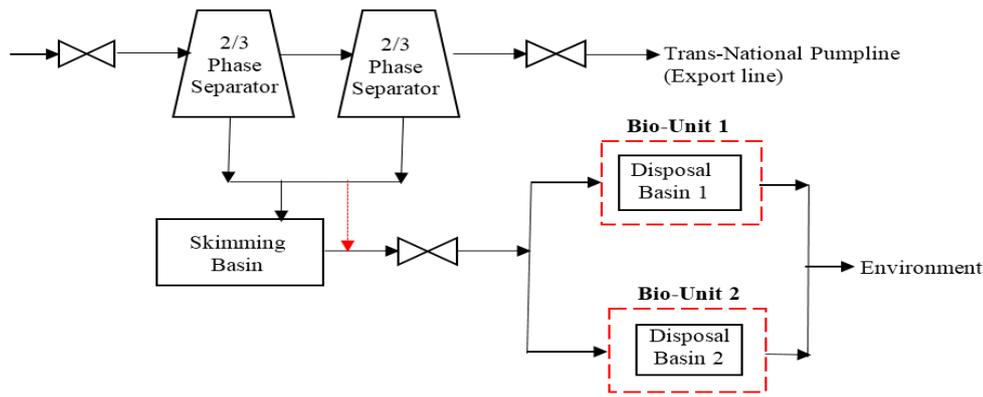


Figure 1. Schematic of the oilfield produce water treatment.

Table 1. Operational parameters of the bio-unit.

Parameter	Value
Temperature (°C)	30.5
pH (-)	7.70
Dissolved Oxygen (mg/l)	3.80 ± 2
Influent TOC ($S_{TOC(0)}$) (mg/l)	466.1
Initial MLSS ($X_{h(0)}$) (mg/l)	1000.0
Hydraulic Retention Time, HRT, (θ) (day)	1.00
Biosolid Retention Time, BRT, (θ_c) (day)	21.0
Influent Flow rate (q) (L/h)	625.0
Volume (L)	15000
Cycle fraction for Fill and React Period (β) (-)	0.94

2.4. Determination of Biokinetic Parameters from Experimental Result

The Monod equation was used to determine the maximum specific growth rate ($\mu_{m,h}$), decay rate (b_h), yield coefficient (Y), half-saturation coefficient ($K_{S_{TOC}}$). The Monod equation relates specific substrate-TOC utilization rate to biomass concentration in the bioreactor and substrate-TOC concentration around the biomass as:

$$r_{S_{TOC}} = \frac{k_m X_h S_{TOC}}{K_{S_{TOC}} + S_{TOC}} \quad (1)$$

Where:

k_m = maximum specific organic carbon utilization rate, (mg S_{TOC} /mg X_h).

Also, the TOC utilization rate at steady state is given as:

$$r_{S_{TOC}} = \frac{1}{\theta} (S_{TOC,in} - S_{TOC}) \quad (2)$$

It therefore implies, from Equations 1 and 2, that:

$$\frac{(S_{TOC,in} - S_{TOC})}{\theta} = \frac{k_m X_h S_{TOC}}{K_{S_{TOC}} + S_{TOC}} \quad (3)$$

Dividing Equation 3 across by X_h and taking the inverse will give:

$$\frac{1}{r_{S_{TOC}}} = \frac{\theta X_h}{(S_{TOC,in} - S_{TOC})} = \frac{K_{S_{TOC}} + S_{TOC}}{k_m S_{TOC}} \quad (4)$$

Recasting Equation 4 in a linearized form using Lineweaver-Burke technique (i.e., the form of the equation of a straight line): $y = mx + c$ as:

$$\frac{1}{r_{STOC}} = \frac{\theta X_h}{(S_{TOC,in} - S_{TOC})} = \left(\frac{K_{STOC}}{k_m}\right) \frac{1}{S_{TOC}} + \frac{1}{k_m} \quad (5)$$

Equation 5 relates the rate of substrate-total organic carbon utilization (r_{STOC}) to substrate-TOC concentration, half saturation coefficient of total organic carbon (K_{STOC}) and maximum specific substrate-TOC utilization rate (k_m). Plotting the term $\frac{1}{r_{STOC}}$ against $\frac{1}{S_{TOC}}$, will give the values of K_{STOC} and k_m , where the slope of the equation, m corresponds to $\left(\frac{K_{STOC}}{k_m}\right)$ and the intercept, c corresponds to $\frac{1}{k_m}$.

In the same manner, the values of endogenous decay rate (b_h), and yield coefficient (Y) is determined through their relationship with effective biosolid retention time BRT (θ_c), and the substrate utilization rate (r_{STOC}), which is expressed as:

$$\frac{1}{\theta_c} = \left[\mu_{m,h} \left(\frac{S_{TOC}}{K_{STOC} + S_{TOC}} \right) - b_h \right] \quad (6)$$

The maximum specific growth rate is related to yield and maximum specific substrate utilization by the expression:

$$\mu_{m,h} = k_m Y \quad (7)$$

Therefore, substituting Equation 7 into Equation 6 will give:

$$\frac{1}{\theta_c} = Y \left(\frac{k_m S_{TOC}}{K_{STOC} + S_{TOC}} \right) - b_h \quad (8)$$

Substituting Equation 4 into Equation 8 will give:

$$\frac{1}{\theta_c} = Y r_{STOC} - b_h \quad (9)$$

Equation 9 shows the relationship between the biosolids retention time (θ_c) biomass yield (Y), endogenous decay rate (b_h) and substrate-total organic carbon utilization rate (r_{STOC}). Plotting the term $\frac{1}{\theta_c}$ against r_{STOC} , gives the values of Y and b_h , where the slope of the equation (m) corresponds to Y and the intercept (c) corresponds to b_h . Thereafter, the maximum specific growth rate, $\mu_{m,h}$ is obtained from Equation 7.

3. RESULTS AND DISCUSSION

3.1. Result of Isolation and Identification of Organic Carbon Degrading Microbials

Five microbial strains were isolated using phenotypic differences and counted as colony forming units/ml (CFU/ml). The result narrates different colonial structures, shapes and colours in the indigenous microbial consortium. The biochemical test carried out to identify the five strains showed that they belonging to the *Bacillus species*, *Pseudomonas species* and *Chryseobacterium Species*. The result agrees with other studies that reported the ability of these species in degrading petroleum hydrocarbons [12, 16, 17].

3.2. Result of Organic Carbon Degradability by Indigenous Microbial Consortium

The result of the biodegradability of organic carbon in the oilfield PW by the isolated indigenous microbial consortium is shown in Table 2.

The result as depicted in Figure 2 shows a typical microbial growth trend of a lag phase where the microbials adjust to their new environment. The lag phase was from 0 to 12 hours (i.e., 12 hours lag phase). After 12 hours, the accelerated growth phase commenced from 12 to 36 hours (i.e., 24 hours lag phase) whereby the microbials density gradually increased. It was followed by the log growth phase, from 36 to 60 hours, where the microbials grew exponentially. Then from 60 to 96 hours, the microbial growth ceased and there was a stationary growth phase. From

96 to 120 hours, the microbial growth declined into the death phase, may be because of depletion of substrate-TOC or build-up of toxic byproducts.

Table 2. Result of TOC biodegradability by isolated indigenous microbials.

Time (Hours)	OD (600nm)	TOC (mg/l)
0	0.00	1.00
12	0.02	0.96
24	0.13	0.94
36	0.28	0.92
48	0.72	0.75
60	1.15	0.50
72	1.20	0.15
84	1.20	0.10
96	1.15	0.10
108	0.85	0.13
120	0.55	0.32

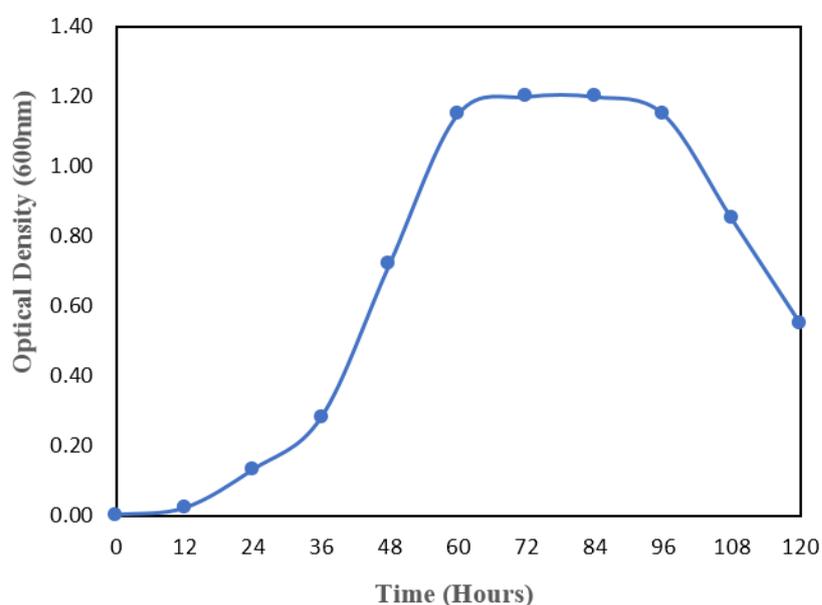


Figure 2. Plot of microbial growth with time.

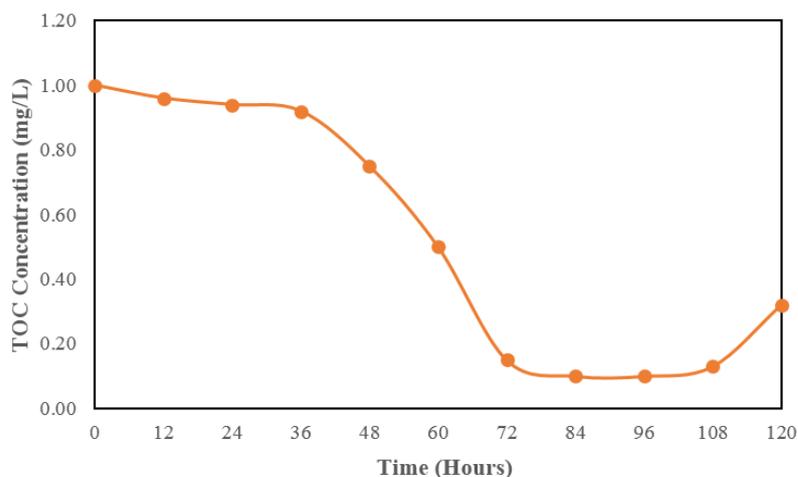


Figure 3. Plot of TOC degradation with time.

Figure 3 shows that the reduction of total organic carbon (TOC) concentration in the oilfield produced water was closely related to microbial growth. The TOC concentration decreased rapidly during the exponential phase and

attained 98.5% reduction. Thereafter, removal of TOC stagnated and declined, which indicates inactivity and deadness of microbials. The high biodegradability of organic carbon from PW could be attributed to the synergistic effects of the different microbial strains than monocultures.

3.3. Result of Acclimation of Microbial Consortium in the Bio-Unit

The result of the acclimation of the enriched indigenous microbial consortium in the aerobic biological treatment unit (Bio-Unit) is depicted in Figure 4, which illustrates that the biomass (MLSS) concentration increased from 1680 mg/l to 2577.2 mg/l, while the TOC concentration decreased from 466.1 mg/l to 9.8 mg/l, with acclimation time. This signifies that the indigenous microbials were well acclimated to the Bio-Unit to actualize removal of organic carbon from the oilfield PW.

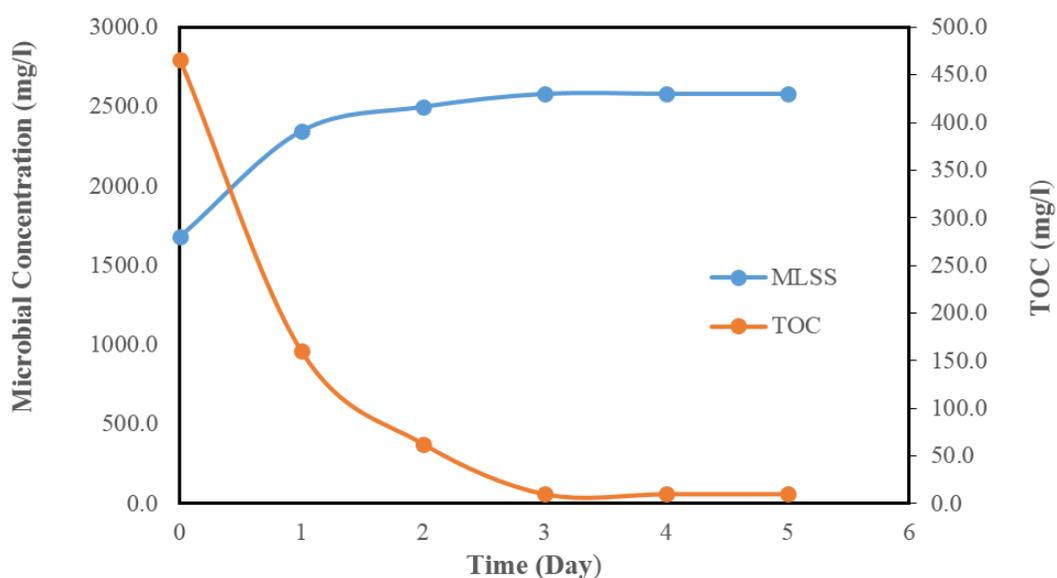


Figure 4. Microbial and TOC vs acclimation time.

3.4. Determination of Biokinetic Parameters from Experimental Data

The biosolid retention time (BRT), as a key process parameter in the designing and control of biological treatment systems, is the experimental variable in this study, especially in determining biokinetic coefficients, the total organic carbon (TOC) of the treated PW at various BRT was measured as and computed to determining the biokinetic parameters as shown Table 3.

Table 3. Determination of biokinetic parameters from experimental data.

HRT (θ) (day)	BRT (θ_c) (day)	Effluent MLSS (X) (mg/l)	Effluent TOC (S) (mg/l)	$X\theta$	$\frac{X\theta}{S_0 - S}$ ($1/r_{STOC}$)	$\frac{1}{S}$	$\frac{S_0 - S}{X\theta}$ (r_{STOC})	$\frac{1}{\theta_c}$
1	3	1320.6	35.95	1320.6	3.0701	0.0278	0.3257	0.3333
1	6	1702.8	32.91	1702.8	3.9308	0.0304	0.2544	0.1667
1	9	1915.3	29.53	1915.3	4.3872	0.0339	0.2279	0.1111
1	12	2112.9	27.27	2112.9	4.8149	0.0367	0.2077	0.0833
1	15	2334.2	25.58	2334.2	5.2987	0.0391	0.1887	0.0667
1	18	2510.1	13.72	2510.1	5.5487	0.0729	0.18022	0.0556
1	21	2900.7	6.81	2900.7	6.3156	0.1468	0.15834	0.0476
1	24	2905.4	6.85	2905.4	6.3264	0.1460	0.15807	0.0417
1	27	2910.6	6.85	2910.6	6.3377	0.1460	0.15779	0.0370
1	30	2911.1	6.85	2911.1	6.3388	0.1460	0.15776	0.0333

Figure 5 describes the plot of $\left(\frac{1}{r_{STOC}}\right)$ against $\left(\frac{1}{S_{eTOC}}\right)$, where the slope of the equation, m corresponds to $\left(\frac{K_{STOC}}{k_m}\right)$ and the intercept, c corresponds to $\left(\frac{1}{k_m}\right)$, which are established from the equation of the line, $y = 13.856x + 5.1022$, with the value of the best fit given as $R^2 = 0.9302$, revealing reliability of 93.02% of the result. From the equation of the line, the value for maximum substrate utilization rate (k_m) is obtained as 0.20 day^{-1} and the value for half saturation constant, (K_{STOC}) is 2.7 mg/l . The K_{STOC} value epitomizes the affinity of the biomass to the substrate, thus the low K_{STOC} value shows that indigenous microbial consortium have affinity for target pollutants in the oilfield PW.

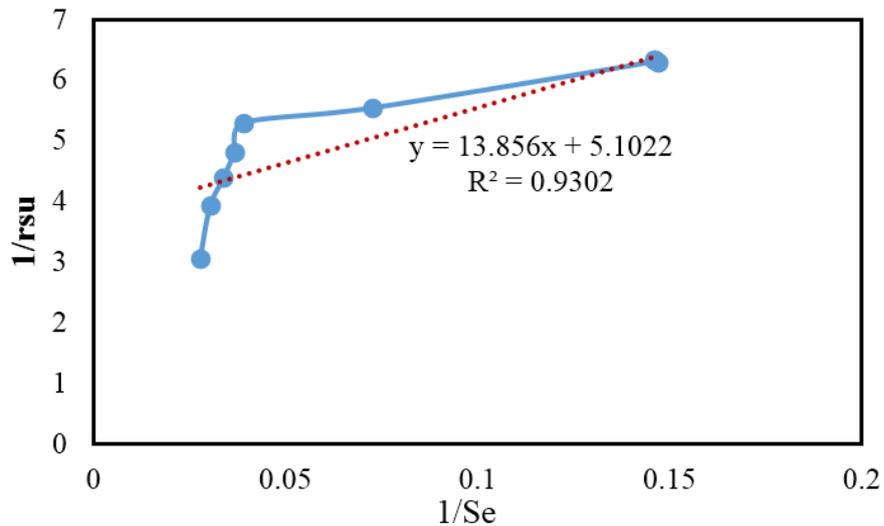


Figure 5. Plot of $1/rsu$ vs $1/Se$.

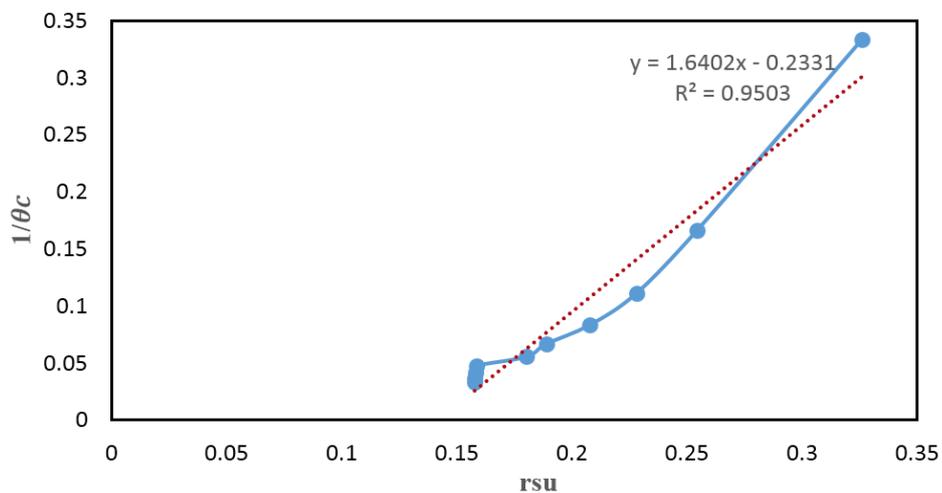


Figure 6. Plot of $1/\theta_c$ vs rsu .

Figure 6 is a plot of $\left(\frac{1}{\theta_c}\right)$ against $\left(\frac{1}{r_{STOC}}\right)$, where the slope of the equation (m) corresponds to the yield, Y and the intercept (c) corresponds to the decay rate, b_h . From Figure 6, the slope (yield, Y) and the intercept (endogenous decay rate) are established from the equation of the line, $y = 1.6402x - 0.2331$, with the value of the best fit given as $R^2 = 0.9503$, revealing reliability of 95.03% of the result. From the equation of the line, the slope (yield, Y) of the heterotrophic biomass is obtained as $1.6 \text{ mg MLSS/mg TOC}$, and the endogenous decay rate of the heterotrophic biomass is 0.23 day^{-1} . The maximum specific growth rate, $\mu_{m,h}$, which is related to yield and maximum specific

substrate utilization ($\mu_{m,h} = k_m Y$), is determined as 0.31 day^{-1} . Table 4 shows the biokinetic coefficients obtained from the Figure 5 and 6.

Table 4. Biokinetic parameters obtained from experiment.

Coefficient	Value
Maximum Specific Substrate utilization rate, k_m (day^{-1})	0.20
Maximum Specific growth rate, $\mu_{m,h}$ (day^{-1})	0.31
Half saturation constant, $K_{S_{COD}}$ (mg TOC/l)	2.72
Endogenous Decay Rate, b_h (day^{-1})	0.23
Yield, Y (mg MLSS/mg TOC)	1.64

The values of yield, Y and endogenous decay coefficient, b_h denote the biomass production and decay during biocatalytic processes, respectively. Thus, the high value of Y and low b_h indicate high net biomass production [18]. The Y and b_h values are vital parameters for determining the volume of the reactor of the sludge handling facilities during design of a wastewater treatment facility. The values for half saturation coefficient ($K_{S_{TOC}}$), maximum specific substrate utilization constant (k_m), heterotrophic yield (Y), endogenous decay rate (b_h) and maximum specific growth rate ($\mu_{m,h}$) obtained in the present study differ slightly from that of other related studies [10-12].

Tellez, et al. [10]; Tellez, et al. [11] used activated sludge system for oilfield produced water treatment and obtained the values of heterotrophic yield as $0.44 \text{ mg MLSS/mg TPH}$ and $0.69 \text{ mg MLSS/mg TPH}$, respectively and decay rate as 0.04 day^{-1} and 0.01 day^{-1} , respectively, maximum specific growth rate as 0.27 day^{-1} , maximum specific substrate utilization as 0.44 and $3.28 \text{ mg TPH/mg MLSS day}$ respectively, half saturation coefficient as 1.36 mg/l and 2.00 mg/l . Kardena, et al. [12] using activated sludge system for synthetic oilfield produced water treatment, obtained the heterotrophic yield of $0.533 \text{ mg MLVSS/mg COD}$ and decay rate of 0.167 day^{-1} , maximum specific growth rate of 0.985 and half saturation constant of 255.46 mg/l . Talaiekhosani, et al. [16] reported a yield of 0.8896 gg^{-1} , decay rate of 0.1284 day^{-1} , and the maximum specific growth rate of 8.28 day^{-1} for microbial degradation of crude oil.

The differences in the biokinetic coefficients may be attributed to the produced water characteristics, espousing of discontinuous configuration, operational conditions for the bioactivities and the parameter used. Tellez, et al. [10]; Tellez, et al. [11] expressed their biokinetics coefficients in mg TPH , while Kardena, et al. [12] reported their biokinetic coefficients in mg COD . However, in this work, the biokinetic coefficients are expressed in mg TOC . Biokinetic coefficients need not be the same since systems operate at conditions dissimilar from one another and microbial adaptability to changing conditions differs. Conducting batch tests with the actual wastewater could describe that system more accurately or verify the default parameters. Review of biokinetic parameters has shown variances and value ranges for these parameters [19]. The biokinetic coefficients obtained from this study are within these ranges.

4. CONCLUSION

The determination of biokinetic coefficients of the microbial biodegradation of the oilfield produced water in the pilot scale biological treatment unit (Bio-Unit) was achieved in this work. The biokinetic coefficients for the heterotrophs; yield (Y), endogenous decay rate (b_h), maximum specific substrate utilization rate (k_m), maximum specific growth rate ($\mu_{m,h}$), and substrate half saturation coefficient ($K_{S_{TOC}}$), were found to be $1.6 \text{ mg MLSS/mg TOC}$, 0.23 day^{-1} , 0.20 day^{-1} , 0.31 day^{-1} , and 2.71 mg TOC/l , respectively. These values which are within the range published in literatures for oilfield produced water, infer that, the biokinetics coefficients ($\mu_{m,h}$, k_m , $K_{S_{TOC}}$, Y , b_h) are relative, for specific wastewater, microbial consortium, and set of environmental conditions. These biokinetic coefficients will be useful for designing of biological treatment systems, since studies on biokinetic parameters on oilfield produced water treatment using biological method are not readily available.

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Authors' Contributions: All authors contributed equally to the conception and design of the study.

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