International Journal of Chemical Sciences and Applications
ISSN 0976-2590, Online ISSN 2278 – 6015
Vol 3, Issue 2, 2012, pp 314-322
http://www.bipublication.com

KINETICS OF DEGRADATION OF ANTHRACENE BY THE ACTIVITY OF CORYNEBACTERIA SP AND PSEUDOMONAS PUTIDA IN CONTAMINATED WATER

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[Received-14/06/2012, Accepted-23/06/2012]

ABSTRACT:

The environmental threat of anthracene to humans on exposure through industrial effluent discharged and other combustion activities into the environment with expensive nature of the physical and chemical remediation techniques initiates this research. The kinetics of degradation of anthracene by the activity of *Corynebacterium sp* and *Pseudomonas putida* for the liberation of the anthracene contaminated water was aim to be investigated. The enriched inoculums of *Corynebacterium sp* and *Pseudomonas putida* was separately inoculated into anthracene contaminated water at room temperature of 28^0C under an optimum pH of 7.2 for 96hours, thereby decreased the anthracene content in the water. About 95.2% of anthracene was degraded by *Corynebacterium sp* while about 93.5w/w% of anthracene was degraded by *Pseudomonas putida*. The biodegradation kinetics parameter evaluated indicates that both *Corynebacterium sp* and *Pseudomonas putida* were favourable for bioremediation of anthracene contaminated water but *Corynebacterium sp* was preferred due to higher proportion of anthracene utilized.

Keywords: Biodegradation, Anthracene, *Corynebacterium sp*, *Pseudomonas putida*, Monod's kinetics.

[I] INTRODUCTION

The persistence, bioaccumulation and toxicological effect of anthracene in the environment had been a major concern in the world as a result of industrial developments through effluent discharged and oil spillage, thereby increased the level of anthracene as contaminants in soil, sediments and aquatic systems [1, 8, 13, 17, 18]. Anthracene consists of three fused benzene rings that persisted in

nature like other polycyclic aromatic hydrocarbon (PAHs) and it has been recently included in the Substances of Very High Concern list (SVHC) by the European Chemicals Agency (ECHA) due to toxicity nature on discharged the environment [6, 10, 17]. Anthracene generates from combustion processes and industrial effluent discharges, and its exposure to human occurs through tobacco smoke and ingestion of food contaminated with combustion of petroleum products [1, 7, 12]. Thereby, causes damage to the skin, itching and edema, headaches, nausea, loss of appetite, inflammation or swelling of the stomach and intestines [9]. Physical and chemical treatments as remediation techniques are quite widespread such as disposal in a landfill, incineration of the wastes and direct injection of chemical oxidants into contaminated soil, sediment and groundwater altered the nature of contaminated environments [4]. Phytoremediation is another growing technology that uses plant for clean up by establishment of vegetation in soils contaminated with hazardous organic and inorganic compounds, vegetation of roots of some plants such as mycorrhizospheric soil using ryegrass, populus nigra L. Loenen, have been studied to mineralized anthracene with cost effectiveness, aesthetic advantages, long-term applicability, minimizes land disturbance, eliminates transportation, and liability costs associated with offsite treatment and disposal compared with the physical and chemical techniques but adverse effect on the valuable plants and increase biomass in the environment are the major concerned of the phytoremediation [3, 12]. Biodegradation as a bioremediation technique has been considered as promising cleanup method due to its ecofriendly, bio-based treatment, cost-effectiveness and ecologically adaptable method. Biodegradation influenced by several factors such as nutrients, oxygen, pH value, concentration, and bioavailability of the contaminants, chemical and physical characteristics of the contaminants, and the pollution history of the contaminated environment [4, 5]. Many researchers employed some microorganisms like *Escherichia coli, Mycobacterium, Nocardioides, pseudomonas, Rhodococcus, Sphingomonas species* for removal of anthracene from environments as a source carbon and energy without quantify the biodegradation kinetics [1, 13, 17, 19, 21, 22]. The objective of this research was to quantified kinetics parameters on biodegradation of anthracene as a bioremediation technique using *Corynebacterium sp and Pseudomonas putida* naturally present in the contaminated water under an optimum pH so as to liberate environment contaminated with anthracene as a major of products of crude oil from danger that might be posed to humans and determine suitability of the microbes.

[II] MATERIALS AND METHODS

Anthracene, dichloromethane and hexane (Analytical grade Chemicals) were purchased from Patanne Chemicals, a renowned laboratory chemicals and equipments dealer in Benin City.

2.1. Collection of Soil Samples

The microorganisms *Corynebacterium sp* and *Pseudomonas putida* for the experiment were isolated from the subsurface soil of about 0-15cm depth obtained from an uncultivated land in the Nigerian Institute for oil palm research (NIFOR), Benin City in Nigeria. The soil material used has been described by Salami [20]. The soil was sieved using 2mm mesh screen for uniform particle size and stored in sterilized polyethylene bag at room temperature covered with aluminium foil for further use.

2.2. Mineral Salt Medium Preparation

Mineral salt medium (MSM) was used to avoid drastic fluctuation of pH which may be detrimental to the viability of the microbes in the batch medium and it was carbon free before anthracene was added after autoclaved at 121^0C for 15 minutes. The MSM was prepared with Analytical grade chemicals composition: KH_2PO_4 (9.0g/l), K_2HPO_4 (1.5g/l), NH_4Cl (1.5g/l), $CaCl_2$ (20mg/l), and $MgSO_4$ (0.2g/l).

The pH of the medium was standardized to 7.2 using 0.1N NaOH. The MSM was sterilized in an autoclaved at 121^0C for 15 minutes and then stored in a secured corner in the laboratory until the experiment was set up.

2.3. Microbial Isolation

0.5 g of soil samples were added into 100 ml MSM. The medium containing the soil and 0.1% w/v anthracene was incubated at 28ºC on a rotary incubator shaker at 150 revolutions per minute for 24 h. The pure culture of colonies of *Corynebacterium sp* and *Pseudomonas putida* were maintained on nutrient agar plates for 72hours at 28^0C temperature for production of the microbes' enmasse, reduction of the lag phase and suitability of the inoculums in anthracene contaminated environment before the biodegradation.

2.4. Biodegradation of Anthracene

0.15 g of anthracene was dissolved in 10% dichloromethane solution and make up to 2 liters of water. The applied anthracene concentration was 75 mg/l of water. The solvent was volatized from anthracene solution under fume-hood. 250 ml of each of the anthracene solution measured as A, B and C in 500 ml cotton-plugged Erlenmeyer flasks wrapped with aluminium foil to prevent contamination and light effect. 5 ml of inoculums was transferred from each agar plate of *Corynebacterium sp* and *Pseudomonas putida* into anthracene contaminated water A and B respectively in 500 ml cotton-plugged Erlenmeyer flasks wrapped with aluminium foil and incubated at 28^0C on a rotary incubator shaker at 150 revolutions per minute for 96 h except only when a flask was withdrawn for the aliquots to be taken for analysis. Non-inoculated C sample was the control of the experiment.

2.5. Biodegradation Analysis

10 ml of aliquots were taken from A and B at every 12 hours for the analysis of microbial mass concentration of *Corynebacterium sp* and *Pseudomonas putida* respectively, and utilized anthracene concentration. The biomass concentration was determined using the procedure described by Azeez [2]. The supernatant was centrifuged, decanted and *Corynebacterium sp* and *Pseudomonas putida* cells that settled down at the bottom of the centrifuge tube were scooped and dried in an oven at a temperature of 60^0C for 8 hours to a constant weight and recorded. The weight obtained was taken as the dry weight of *Corynebacterium sp* and *Pseudomonas putida* in the sample analyzed. The method of Kumar [14] was employed using UV visible spectrophotometer to measure absorbance of the anthracene in aliquot. The absorbance of the anthracene was recorded at a wavelength of 267 nm in the UV region after isolation of the microbes by centrifuge 10 ml aliquots of rotating at 10,000 revolutions per minute for 20 minutes and allowed to settle for 30minutes to get a clear supernatant. 5 ml of the clear supernatant was extracted with 5ml of hexane for 10 minutes in a separating funnel. The top solution in a separating funnel at the end of the extraction was a solution of the anthracene in hexane and poured into the corvettes of the spectrophotometer and absorbance readings at a wavelengths of 267 nm was recorded. The procedure was repeated in twelve hourly intervals immediately after inoculation with *Corynebacteria sp and Pseudomonas putida* for 96 hours of incubation and the kinetics parameters were determined from the data obtained.

Solutions of anthracene in the hexane were prepared to give a concentration of 0.3mg/ml. The absorbance of the solutions was read at the appropriate wavelengths 267nm for the anthracene solution. Calculated quantities of the solution of anthracene were taken and calculated quantities of hexane were added to give lower concentration of the anthracene in hexane 0.27, 0.24, 0.21, 0.18mg/ml etc. The concentration of anthracene (mg/ml) against the absorbance of the anthracene solutions were determined, recorded and then plotted as shown in the Fig. 1. A line of best fit plotted for the points obtained. The model of the standard plots was determined and used to convert the anthracene concentration readings from values in absorbance to µg/ml.

2.6. Biodegradation Kinetics Model

Biodegradation of anthracene was based on the growth of the microbial mass of Malthus correlation concept of first order reaction rate in anthracene contaminated area. The rate of formation of microbial mass on consumption of anthracene by Malthus correlation is given as:

$$\frac{\partial X}{\partial t} = \mu X \qquad (1)$$

$\frac{\partial X}{\partial t}$ is the rate of formation of biomass, X is the biomass concentration at any time t, μ is the specific growth rate of biomass and t is the degradation time. Integration of equation (1) at the boundary conditions: $X = X_0 = 0$ at $t = 0$ because there is no inoculation of the microbes in the anthracene broth medium and $X = X$ at $t = t$ gave

$$\frac{Ln(X)}{t} = \mu \qquad (2)$$

But Monod's model expressed the growth rate of the microbial cells as function of concentration of the anthracene and it is given as;

$$\mu = \frac{\mu_m C}{k_s + C} \qquad (3)$$

C is the mass concentration of Anthracene at any time t, k_s is the Monod's kinetics constant and $\mu_m$is the maximum specific growth rate of the microbes. Linearization of equation (3) gave

$$\frac{1}{\mu} = \frac{k_s}{\mu_m}\frac{1}{C} + \frac{1}{\mu_m} \qquad (4)$$

Combination of equation (2) and (4) gave

$$\frac{t}{Ln(X)} = \frac{k_s}{\mu_m}\frac{1}{C} + \frac{1}{\mu_m} \qquad (5)$$

Equation 4 is a linear model in which $\frac{k_s}{\mu_m}$ is the gradient of the curve of $\frac{t}{Ln(X)}$ against $1/C$ and $\frac{1}{\mu_m}$ is an intercept of the curve.

More so, the production rate of microbial mass is a function of rate of consumption of anthracene [2, 15]

$$\frac{dX}{dt} = -Y_{x/s}\frac{dC}{dt} \qquad (6)$$

Integration of equation 6 with the boundary conditions: $C = C_0, X = X_0$ at $t = 0$ and $C = C, X = X$ at $t = t$.

$$X - X_0 = Y_{x/s}(C_0 - C) \qquad (7)$$

C_0 and X_0 is the mass concentration of anthracene and biomass concentration of the microbe respectively before commencement of degradation at time zero and $Y_{x/s}$ is the microbial yield coefficient on contaminant as a substrate.

[III] RESULTS AND DISCUSSION

The mass concentration of anthracene against absorbance at 267nm obtained using spectrophotometer was illustrated as Fig. 1. The standard model for the conversion of absorbance to mass concentration of anthracene obtained as equation 8.

$$C = 63.96C_{abs} - 2.929 \qquad (8)$$

C_{abs} is the absorbance of anthracene at any time t.

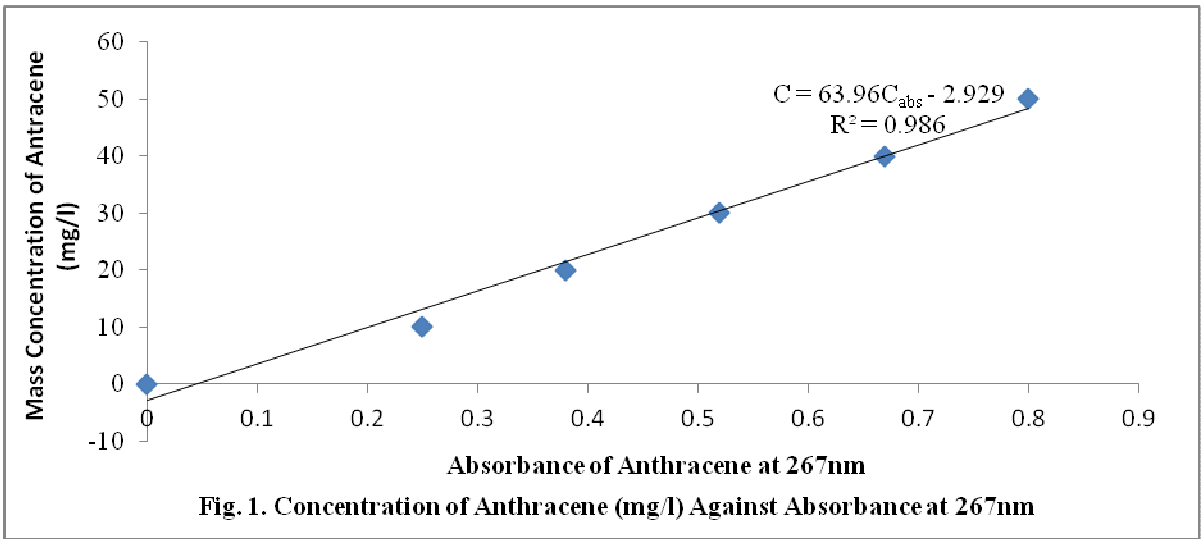

Fig. 1. Concentration of Anthracene (mg/l) Against Absorbance at 267nm

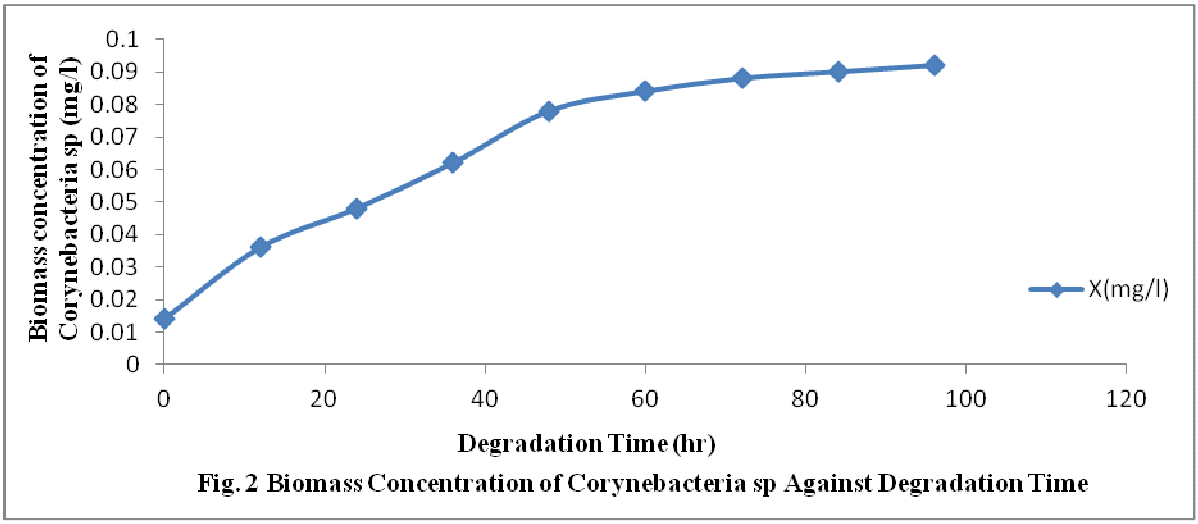

Fig. 2 Biomass Concentration of Corynebacteria sp Against Degradation Time

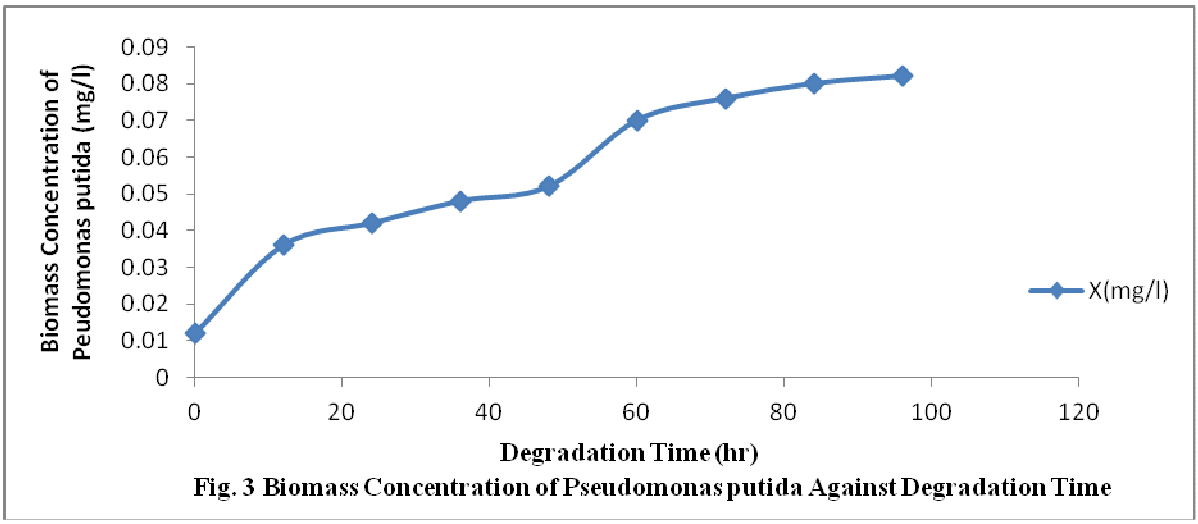

Fig. 3 Biomass Concentration of Pseudomonas putida Against Degradation Time

Fig. 2 and 3 shows the increased mass concentration of *Corynebacterium sp* and *Pseudomonas putida* with degradation time as the mass concentration of anthracene decreased in water as shown in the fig. 4 and 5 for *Corynebacterium sp* and *Pseudomonas putida*. This can be associated with anthracene as a source of food and energy for *Corynebacterium sp* and *Pseudomonas putida*.

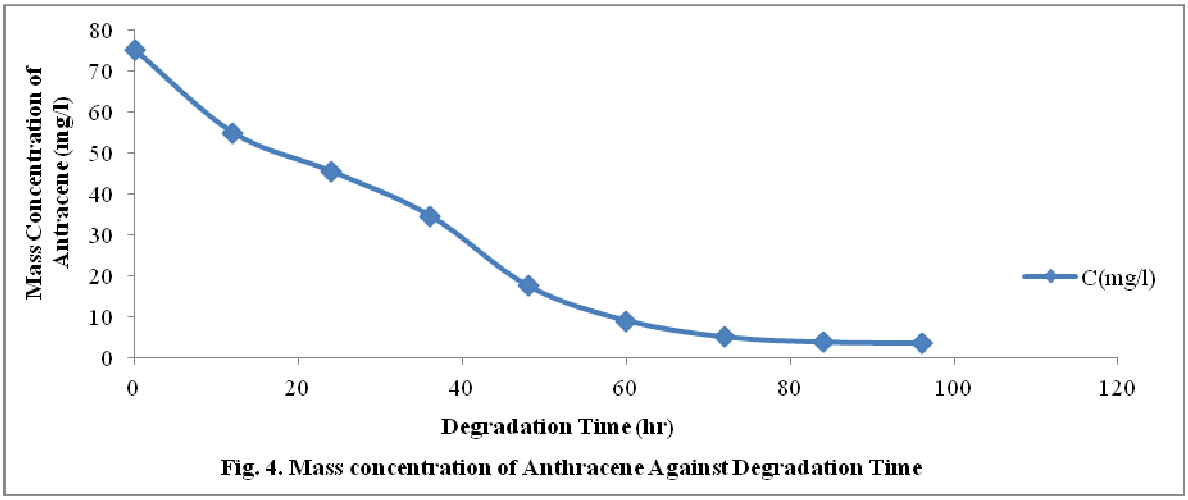

Fig. 4. Mass concentration of Anthracene Against Degradation Time

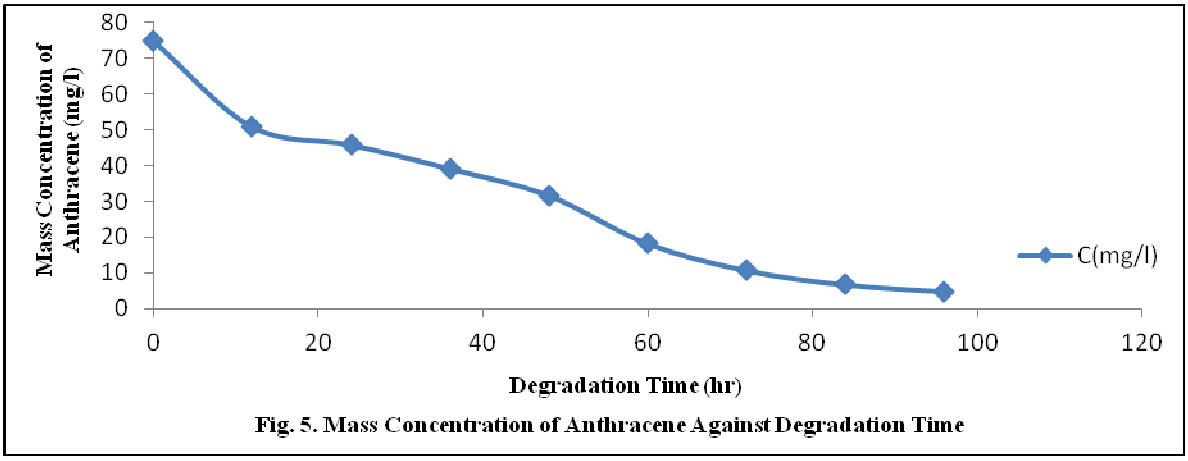

Fig. 5. Mass Concentration of Anthracene Against Degradation Time

The yield coefficients of *Corynebacterium sp* and *Pseudomonas putida* are the same with a magnitude of 0.001. This implies that there is no difference in the magnitude of the yield coefficient of *Corynebacterium sp* and *Pseudomonas putida* on degradation of anthracene as substrate for the microbes. More so, the correlation coefficient (R^2) value of 0.997 as shown in the fig. 7 for *Pseudomonas putida* on anthracene was higher compared with *Corynebacterium sp* with magnitude of 0.994 as shown in the fig. 6 but statistically, there is no significant difference between the fitness of two curves of the yield microbes used and this does not means that biodegradation kinetics was favourable for *Pseudomonas putida* compared with *Corynebacterium sp* but rather predicts degree of fitness of the data.

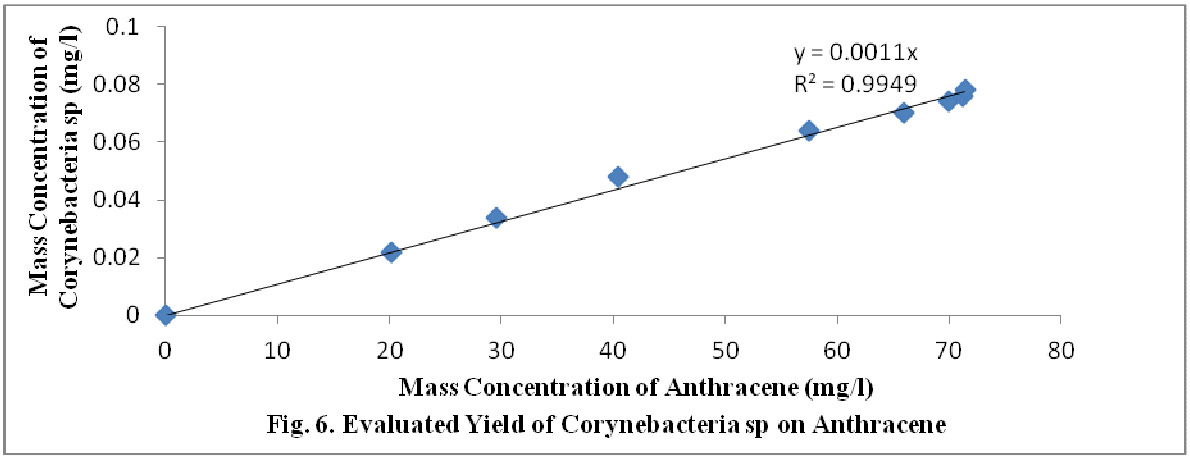

Fig. 6. Evaluated Yield of Corynebacteria sp on Anthracene

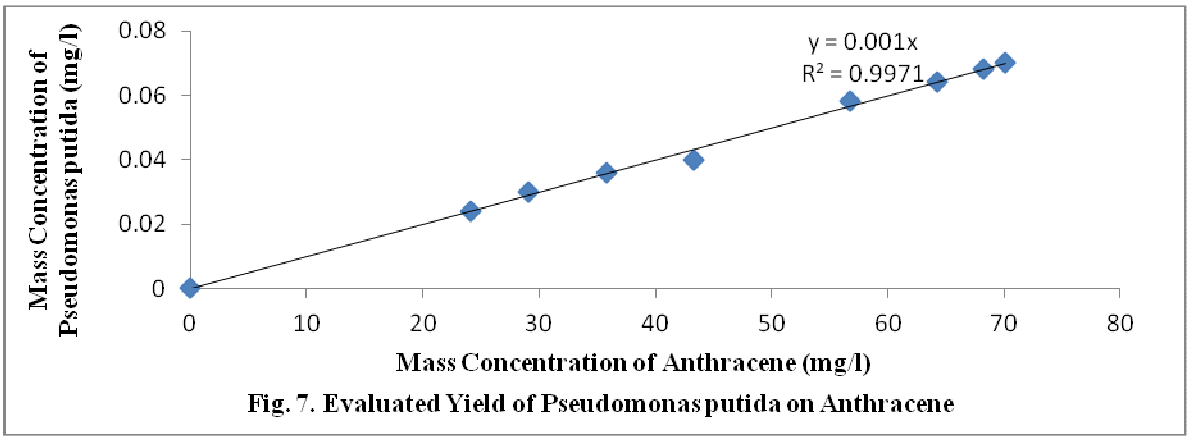

Fig. 7. Evaluated Yield of Pseudomonas putida on Anthracene

It was obtained based on equation (5) from Fig. 8 and 9 that the magnitude of maximum specific growth rate of *Corynebacterium sp and Pseudomonas putida* was 0.0747hr^{-1} and 0.0663hr^{-1} respectively. This indicates that conditions of anthracene as a substrate and degradation in the water was favourable to *Corynebacterium sp* compared with *Pseudomonas putida*. More so, Monod's kinetics coefficient of anthracene evaluated with magnitude of 10.0mg/l for the activity of *Corynebacterium sp* was lower compared with a magnitude of 13.03mg/l for the activity of *Pseudomonas putida*. This implies that *Corynebacterium sp* has higher affinity compared with *Pseudomonas putida* in anthracene contaminated environment which means, lower value of Monod's kinetics coefficient indicates higher value of affinity of the microbes and it can be attributed to the kinetics of the contaminated water medium and favourable conditions of the microbes. Furthermore, the R^2 value of the Fig. 8 and 9 depicts that the biodegradation kinetics data obtained for the anthracene by the activity of the *Corynebacterium sp* with a magnitude of 0.814 was more fitted compared *Pseudomonas putida* with magnitude of 0.677

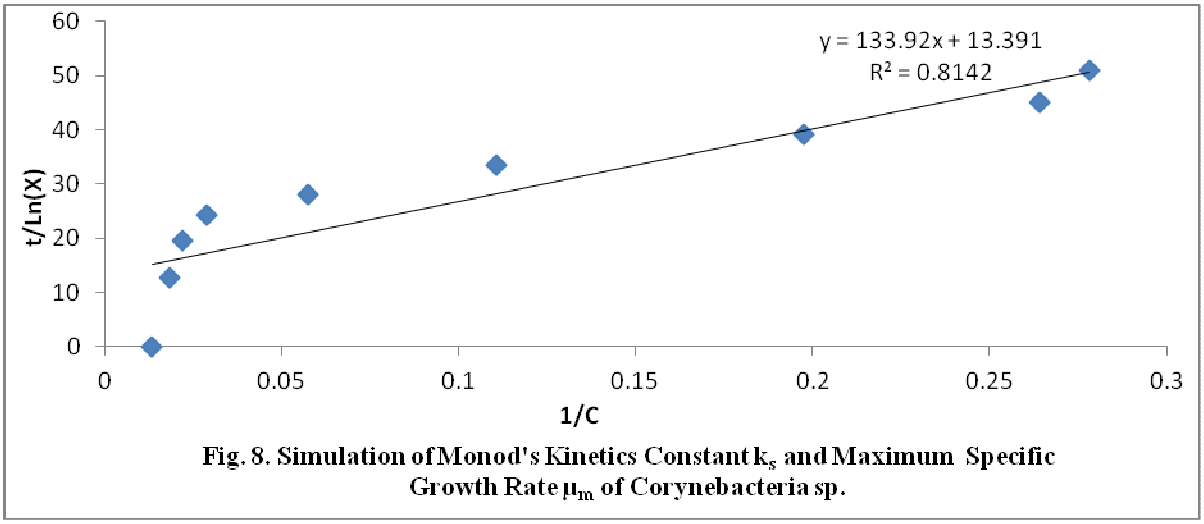

Fig. 8. Simulation of Monod's Kinetics Constant k_s and Maximum Specific Growth Rate μ_m of Corynebacteria sp.

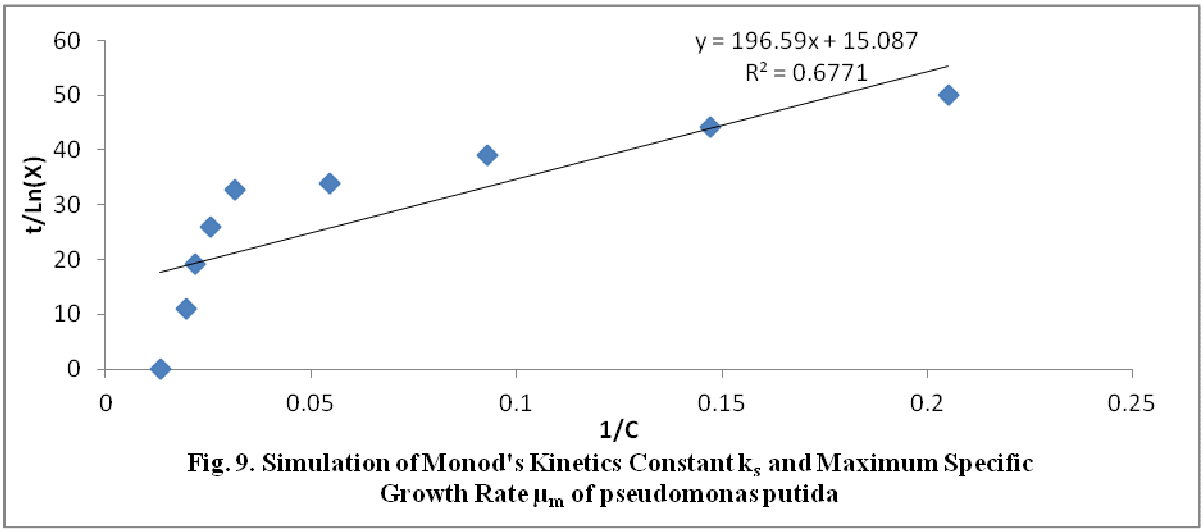

Fig. 9. Simulation of Monod's Kinetics Constant k_s and Maximum Specific Growth Rate μ_m of pseudomonas putida

Apart from the maximum specific growth rate and Monod's kinetics coefficient parameters employed for the evaluation of the biodegradation as a tool and suitability of microbes for removal of the threat that might be posed by anthracene in contaminated wastewater, it was also obtained that about 95.2 percent of the anthracene was degraded by the activity of the *Corynebacterium sp* while 93.5 percent of the anthracene was degraded by the activity of the *Pseudomonas putida* which indicates that degree of effectiveness was more than that of *Pseudomonas aeruginosa* (312A) with degradation rate (3.90 mg L^{-1} day^{-1}) of seventy one percent of anthracene utilized with the addition of the 250 mg L^{-1} of medium after 48 days as stated in Jacques et al [11] work, Mathew et al [16] which isolated *Micrococcus luteus* from crude oil polluted environment for degradation of about 91.7% anthracene and *Pseudomonas citronellolis* (222A) showed an intermediate level of degradation of about fifty one (51%) percent of anthracene by Jacques et al [11]. More so, Kumar et al [13] research resulted that *Pseudomonas sp* degraded 74.8% anthracene supplemented in BSM medium at 0.1% with Acridine orange induced plasmid curing but antharcene utilized was less than that of *Corynebacterium sp and Pseudomonas putida.*

[IV] CONCLUSIONS

Based on the results of the evaluated biodegradation kinetics parameters of the anthracene with activity of the *Corynebacterium sp and Pseudomonas putida* in a contaminated water, it can be deduced that both *Corynebacterium sp and Pseudomonas putida* were effective for removal of the threat posed by anthracene to minimum level using biodegradation methods as an alternative to other physical and chemical methods. Though, the product formation and mechanism were not determined but about 95.2 percent and 93.5 percent of anthracene was utilized and degraded by *Corynebacterium sp and Pseudomonas putida* respectively in anthracene contaminated water which indicates that both microbes were effective for the biodegradation of anthracene in a contaminated environment. More so, *Corynebacterium sp* may be preferred for the bioremediation of anthracene in a contaminated environment due to little variation in the utilization of the anthracene compared with *Pseudomonas putida*.

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