

## Evaluation of First and Second Order Degradation Rates and Biological Half-lives in Crude Oil Contaminated Soil

Mathew C. Menkit<sup>1</sup> and Ani Kingsley Amechi<sup>1\*</sup>

<sup>1</sup>Department of Chemical Engineering, Faculty of Engineering, Nnamdi Azikiwe University, P.M.B 5025, Awka, Anambra State, Nigeria.

### **Authors' contributions**

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aim:** The aim of the study was to investigate crude oil degradation using first and second order kinetic models, microbial activity using dehydrogenase assay.

**Place and duration of Study:** Department of soil science, University of Nigeria Nsukka, Enugu State from October 2015 to March 2016.

**Methodology:** Characterisation and microbial analysis of Goat manure and crude oil contaminated soil were investigated. Dehydrogenase assay was used as a measure of microbial activity microbial count using Heterotrophic plate count was also investigated. Experimental data were fitted into both first and second order kinetic models and biological half-lives in order to evaluate the kinetic parameters and half-lives.

**Results:** The physiochemical characterisation showed that Goat manure contained valuable sources of soil nutrient and organic matter, which enhanced the bioremediation process. The result obtained from the physiochemical characterisation of the control sample showed the inadequacies of soil nutrient in the crude oil contaminated soil. The microbial activity (DHA) indicated an increase in microbial activity in both the untreated crude oil contaminated soil and the Goat manure treated contaminated soil due to the presence of crude oil in the soil. Microbial count using heterotrophic

\*Corresponding author: E-mail: [meche\\_segofs@yahoo.com](mailto:meche_segofs@yahoo.com);

plate count indicated that higher colonies were recorded in Goat manure. 70% degradation of crude oil was achieved on the 14<sup>th</sup> day of treatment, whereas only 21% was achieved in the control sample. The kinetic parameters obtained indicated that the first order kinetic model and biological half-life gave a better result (higher degradation rate and lower biological half-life) than the second-order kinetic model.

**Conclusion:** In this study, we have shown that the bioremediation of COCS using GM as organic nutrient enhanced CO degradation. However, an increase in microbial count and dehydrogenase assay was observed in the GM treated COCS. The obtained kinetic parameter suggests that the first order kinetic model gave a better result (high degradation rate constant and lower biological half-life) for the studied CO degradation.

*Keywords: Crude oil; heterotrophic plate count; goat manure; dehydrogenase activity.*

## ABBREVIATIONS

CO : Crude Oil  
GM : Goat Manure  
HPC : Heterotrophic Plate Count  
DHA : Dehydrogenase Assay  
COCS : Crude Oil Contaminated Soil

## 1. INTRODUCTION

Globally, the predominant energy supply of the world over the years is to a great extent dependent on crude oil (CO) products. However, spillage resulting from exploitation processes, refining and transporting of the product is of significant environmental concern especially in developing countries like Nigeria.

Therefore, it is vital that these CO contaminants that adversely affect aquatic and terrestrial habitat be reduced to a tolerable level in the environment [1]. Thus, the need to develop and implement an effective remediation technology to reduce the threat caused by this contaminant becomes imperative [1,2].

However, CO contaminated soil is often poor in organic matter and generally low in the microbial population [3,4], thus, lack the essential nutrient to support plant growth. The technologies currently available (physical and chemical) for the remediation of contaminated soil are accompanied with its challenges like environmentally unacceptable, capital-intensive [5,6] and might not be an option for developing countries. There have been lots of researches and innovations in the area of remediating contaminated soil mainly due to increasing pressures from the public and government policies.

Previous works reported by many researchers on the use of organic manure in bioremediation of contaminated soil have been accepted globally mainly due to the ecological

compatibility of the process and reusability of the remediated soil [7]. The advantages of organic manure over the conventional methods in bioremediation include higher biodegradability, abundance microbial population and cheap.

The potentials of microorganisms which are abundant in daily generated organic manure could be exploited for bioremediation processes. Bioremediation processes depend on enzymatic potentials of microorganisms to detoxify and transform the pollutants molecule into harmless products [8,9]. In this present work, consideration is given to the applicability of goat manure as an organic nutrient for the effective treatment of CO contaminated soil. Goat manure (GM) contains adequate amounts of nutrients needed by plants for optimal growth, the manure retains more nitrogen, thus increases its fertilising potency [10,11].

Accordingly, the objective of this work was to evaluate the kinetic parameters and biological half-lives using first and second order degradation kinetics, but also to characterise and investigate the microbial count and activity in the CO contaminated soil and GM. Thus, using GM as organic manure would the enhance contaminant degradation efficiency due to its abundance microbial population and also improve the soil properties of the contaminated soil.

## 2. MATERIALS AND METHODS

### 2.1 Soil Samples Collection

The soil used in this experiment was an agricultural soil with no history of crude oil contamination. The soil was collected from the surface horizon (0-30 cm). The soil sample was

sun-dried for one month, before passing it through a 2 mm particle size sieve for homogeneity and debris removal. The soil was transported to the Soil Science Laboratory of the University of Nigeria Nsukka for further analysis.

## 2.2 Preparation of Goat Manure (GM)

The GM was sourced from a farmhouse located at Umuchigbo Iji-nike in Enugu East Local Government area Enugu State Nigeria. The GM was sun-dried for two weeks, ground and passed through 2mm sieve for homogeneity before use and was transported to the Soil Science Laboratory of the University of Nigeria Nsukka for further analysis.

## 2.3 Preparation of CO Stock Solutions

CO stock solutions used in these experiments were prepared by weighing out (PCE analytical weighing balance PCE-6000) 100, 200, 300, and 400 g CO. Each of these CO was dissolved in 1.0 L of distilled water to give initial CO concentrations of 100 g/l, 200 g/l, 300 g/l, and 400 g/l. The soil was artificially contaminated by spiking the prepared CO concentrations on 100g of the soil sample. The contaminated soil samples were allowed to stay for twenty-one days before treatment with GM to allow for volatilisation and sorption of CO into the soil matrix.

## 2.4 Bioremediation Procedure

Four 250 ml Erlenmeyer glass flask was incubated with 100 g of soil. The prepared CO stock solutions were used to contaminate the soil artificially. The flasks were labeled A to D; each of the flasks labeled A to D was treated with 50 g of GM as an organic nutrient. Duplicate flasks with the same CO concentrations were labeled E to H and were used as the control sample (untreated COCS) to monitor CO degradation in the control sample. Composites Samples from each flask (treated and untreated COCS) were analyzed for heterotrophic plate count (HPC). Water contents of the samples were adjusted when necessary to aid microbial action. The samples were mixed twice on a weekly basis in order to maintain aerobic conditions for fifty-six days of remediation exercise.

## 2.5 Determination of CO Percentage Degradation

Final and initial CO concentrations in the COCS were determined by solvent extraction [12]. In

this procedure, 10 g of soil from each sample (GM treated COCS and control sample) was put into a 50 ml beaker and 20 ml n-hexane was added. The mixture was shaken vigorously on a magnetic stirrer for 15 min. This was to allow the n-hexane extract the crude oil from the soil sample. The solution was then filtered using Whatman filter paper, and the liquid phase extract (filtrate) diluted by taking 1 ml of the extract into 50 ml of n-hexane. The absorbance of this solution was measured spectrophotometrically at a wavelength of 400 nm using n-hexane as blank. The crude oil concentrations in the soil were calculated with reference to a standard graph derived from fresh crude oil diluted with n-hexane. The percentage removal of CO from the contaminated soil was calculated using equation (1):

$$\% \text{ degradation} = \frac{\text{initial CO} - \text{final CO}}{\text{initial CO}} \times 100 \quad (1)$$

Where

Initial CO is the Initial crude oil concentration in the soil at time  $t = 0$ .

Final CO is the final crude oil concentration in the soil at time  $t = t$

## 2.6 Microbial activity (Dehydrogenase Assay)

The soil dehydrogenase assay (DHA), was measured by reducing 2.3.5 triphenyl tetrazolium chloride (TTC) according to [13]. Three replicates of 10 g samples of GM treated COCS and the control sample was mixed with 150 mg  $\text{CaCO}_3$ , 1ml of 3% (w/v) TTC and distil water (10 ml) and was incubated for 24 hours at 30°C. After which, extraction with 25ml ethanol was performed. The extracts were filtered and incubated for 1hour in the dark and the absorption was measured at 485 nm (UV-1800 Shimadzu).

## 2.7 Physiochemical Characterisation and Microbial Count

Organic matter content (OMC) was determined using [14]. Total nitrogen was determined using the Kjeldahl method [15]. Organic carbon (TOC) was determined using the Nelson and Sommers, (1996) method [16]. The soil pH was determined using [17]. Available nutrients such as calcium, sodium, magnesium, and potassium ( $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ , and K) were determined using the Mehlich 3 method [18]. Soil organic phosphorus was determined using [19]. Estimation of live

heterotrophic bacteria in GM and COCS by heterotrophic plate count (HPC) was determined using a method as described by America public health association 1998 [20].

## 2.8 CO Degradation Kinetics

In this study, CO degradation kinetic parameters were evaluated using both the linearized forms of first and second order kinetic models [3].

The linear first order CO degradation kinetic model is presented in equation (2):

$$\ln[C_t] = -K_1t + \ln[C_0] \quad (2)$$

Where

$[C_t]$  is the final concentration of CO in the at time t

$[C_0]$  is the initial concentration of CO in the at time t = 0

$-K_1$  is the CO degradation rate constant for the first-order kinetic model.

t is the time in days.

A graph of  $\ln[C_t]$  against time (t) in days will be a straight line graph with slope  $-K$  and  $\ln[C_0]$  as the intercept.

The linear second order CO degradation kinetic model is presented in equation (3):

$$\frac{1}{[C_t]} = K_2t + \frac{1}{[C_0]} \quad (3)$$

Where

$\frac{1}{[C_t]}$  is the final concentration of CO in the soil at time t

$\frac{1}{[C_0]}$  is the initial concentration of CO in the soil at time t = 0

$K_2$  is the CO degradation rate constant for the second-order kinetic model.

t is the time in days.

A graph of  $\frac{1}{[C_t]}$  against time in days will be a straight line graph with slope  $K_2$  and  $\frac{1}{[C_0]}$  as the intercept.

### 2.8.1 Biological half-life for CO degradation

The biological half-life for CO degradation is the time taken by the microorganism to degrade half of the initial CO concentration [3].

The first order biological half-life is presented in equation (4):

$$T_{\frac{1}{2}}^1 = \frac{\ln 2}{k_1} \quad (4)$$

Where  $K_1$  is the first order rate constant and

$T_{\frac{1}{2}}^1$  is the first order biological half-life ( $\text{day}^{-1}$ ).

The biological half-life for second order degradation is dependent on the initial CO concentration as shown in equation (5):

$$T_{\frac{1}{2}}^2 = \frac{1}{k_2[C_0]} \quad (5)$$

Where  $K_2$  ( $\text{day}^{-1}$ ) is the second order rate constant and  $T_{\frac{1}{2}}^2$  ( $\text{g}L^{-1} \cdot \text{day}^{-1}$ ) is the second order biological half-life.

## 3. RESULTS AND DISCUSSION

### 3.1 Characteristics of Crude Oil Contaminated Soil and GM

Some of the physiochemical properties of the GM and control sample are shown in Table 1. GM was selected to determine the effect of animal residue used as an organic nutrient in COCS during the bioremediation process. The obtained results indicated that GM contained a valuable nutrient source that could support the indigenous microbial activities during the bioremediation process.

The neutral pH of GM (7.2) from Table 1 was within the optimum range for microbial growth and multiplication [21]. Also at neutral pH, the nutrient availability in GM was greater due to an equal number of  $H^+$  and  $OH^-$ . Similarly, in Table 1, GM was higher in organic matter (66.62%) compared to the control sample (12.52%). This could be due to the presence of high degradable organic matter in GM, while the low organic matter content of the control sample might be due to the effect of CO on soil microbial population and nutrients. Aion et al. [4] reported that hydrocarbon contaminated soils are always poor in organic matter with low microbial activity. However, the high organic carbon of the control (51.46%) might be due to the presence of carbon in the CO which could have been converted to soil organic carbon [22,23]. The acidic pH (4.7) and low nutrients observed in the control sample as shown in Table 1 could be attributed to the presence of the CO in the soil, which caused deficiencies in soil essential nutrients [23]. The GM clearly showed the presence of some

valuable soil nutrients, which could support the indigenous microbial population Table 1.

On the other hand, the heterotrophic plate count (HPC), was used to estimate the number of live heterotrophic bacteria in GM and control sample. The result showed an increase in the heterotrophic bacteria for GM indicating the presence of abundance microbial population that could support the bioremediation process. However, the control sample, recorded low population of heterotrophic bacteria which could be attributed to microbial competition for the scarce nutrient in the CO contaminated soil.

### 3.2 Microbial Count

In the case of heterotrophic plate count (HPC) shown in (Fig 1), generally, an increase in HPC was observed from 7 to 42<sup>nd</sup> day in both the control sample and GM treated COCS (Fig. 2). Average counts of microorganisms (Fig. 1) are expressed as log of CFU/g of sample and they correspond with the counts of microbes in the control sample and GM treated COCS.

The HPC increased from  $1.1 \times 10^5$  CFU/g to  $3.5 \times 10^6$  CFU/g in GM treated COCS, whereas it increased from  $0.4 \times 10^5$  CFU/g to  $2.3 \times 10^6$  CFU/g in the control sample (Fig. 1). This result showed that the GM enhanced microbial growth which resulted in higher HPC compared to the control sample [24] evaluated the presence of microorganisms (Bacteria and fungi) in soil samples amended with different organic materials quantitatively using agar plate counts [25]. They observed that amendment with different organic materials significantly affected microbial quantity.

The changes in HPC during the bioremediation process show that the level of active bacteria particularly heterotrophic bacteria increased in both GM treated and control sample.

The presence of CO was the main driver for the increase of CFU in the control sample. The increase in numbers of HPC in both GM treated and control sample demonstrates how rapidly indigenous soil microorganisms are able to adapt to new substrates [26].

The findings of the present study suggest the presence of CO degrading bacteria in the COCS as an increase in the CFU was observed in both controls and GM treated COCS. However, GM had a stronger stimulatory effect in the COCS (Fig. 1).

### 3.3 Dehydrogenase Assay (DHA)

The dehydrogenase assay (DHA) was used as a major pointer of microbial enzymatic activities in the investigated COCS. DHA was determined for both the control sample and GM treated COCS incubated with TTC (Fig. 2).

The result in (Fig. 2) showed a high DHA activity in the control sample and GM treated COCS. The increase in DHA of the control sample could be attributed to the low concentrations of CO used in this study. This also suggests that at low concentrations, the inhibitory effect of CO contaminant on microorganisms was negligible. However, previous studies reported that contaminated soil DHA was dependent on the level of contamination [27].

**Table 1. Physiochemical properties and HPC of GM and control sample**

Parameters	control sample (0-30 cm)	GM	methods
pH	4.7	7.2	ASTMD4972-13 [17]
Organic matter %	12.52	66.62	ASTMD2974-14 [14]
Organic carbon %	51.46	27.04	Nelson, and Sommers, [16]
Kjeldahl nitrogen %	0.81	3.82	Kjeldahl digestion [15]
Available nutrients			
sodium (mg/l)	0.03	6.24	Mehlich 3 [18]
magnesium (mg/l)	0.16	3.07	Mehlich 3 [18]
calcium (mg/l)	0.19	1.88	Mehlich 3 [18]
phosphorous (mg/kg)	0.58	3.56	Bray no 1 Extract [19]
HPC (cfu/g)	$0.4 \times 10^5$	$3.9 \times 10^6$	APHA, 1998, [20]

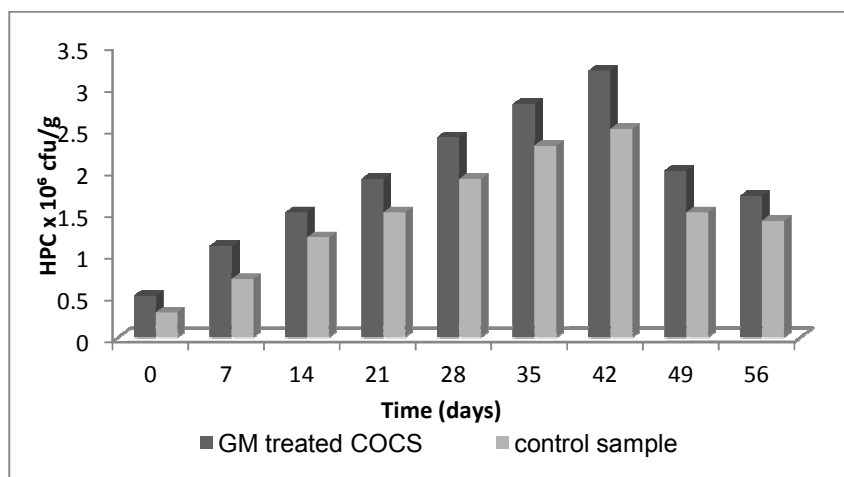


Fig. 1. HPC plot of indigenous microbes versus time (values are  $\pm$  standard error of three measurements)

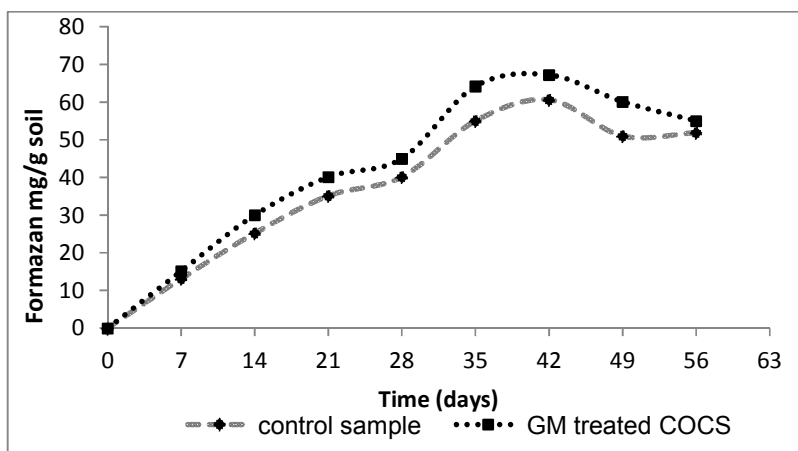


Fig. 2. DHA for GM treated COCS and control sample (values are  $\pm$  standard error of three measurements)

Wolińska, et al. [27] reported an increase in DHA of soil contaminated with petrol, diesel and engine oil at low concentrations. Moreover, another cause of the increased DHA in the COCS could be attributed to the ongoing biodegradation process at low CO concentration [28]. Consequently, the GM treated COCS used in this study also recorded a high DHA. The result could be attributed to both the neutral pH (7.2), which enhanced microbial growth proliferation and the ongoing CO degradation process (Fig 2). However, [25] reported that measuring soil enzymatic activities can provide information about the function and structure of soil microbial communities in hydrocarbon-contaminated soils.

### 3.4 CO Degradation Process

(Fig. 3) shows the degradation profile of COCS as a function of time in GM treated COCS and control sample. It could be observed that CO degradation commenced from 7 to 21 days and continued up to the fifty-six day. Percentage CO degradation of 60% was achieved within the first 14 days in GM treated COCS, whereas only 21% of the CO contaminant was degraded in the control sample. The inability of the control sample to support the bioremediation process has been previously reported. Sayara, et al. [1] reported that only 29.5% of the polyaromatic hydrocarbons (PAHs) were degraded in contaminated soil without organic co-substrate (control).

There was a noticeable positive correlation between the increase in HPC of the microorganisms and the decrease in the CO contaminant of GM treated COCS during the bioremediation process. This showed that the indigenous microorganisms in GM were able to utilize the CO contaminant. Sayara, et al. [1] found that native microorganisms present in the soil and organic amendments were more effective as they were more adapted to the soil environmental conditions.

It was observed in (Fig. 3), that the CO degradation was observed to be fast during the first fourteen days of treatment after, which a gradual degradation was observed in GM treated COCS [26,29]. During the investigation periods, there was no significant reduction of CO in the control sample, which could be attributed to lack of the organic co-substrate to support the indigenous microorganisms.

### 3.5 Evaluation of First and Second order CO Biodegradation Rates and Half-lives

Data obtained from the CO degradation process were fitted to the linearized forms of first and second order kinetic models of equations Eqns. 2 and 3, respectively. The models were used to evaluate the kinetic parameters for CO degradation for both GM treated COCS and control sample. The kinetics parameter obtained

from the first and second-order kinetic model are shown in Tables 2 and 3, respectively.

The first order degradation rate constant ( $K_1$ ), was obtained from the slopes of the linear plots of the natural log of the final CO concentration ( $\ln Ct$ ) versus time (Figs 4 and 5). Similarly, the slopes for the linear plots for the inverse of the final CO concentration ( $1/Ct$ ) versus time were used to obtain the second order degradation rate constant ( $K_2$ ) (Figs 6 and 7). The correlation coefficient ( $R^2$ ) shown in Tables 2 and 3 indicated that the biodegradation data fitted well to both first and second order kinetic models. The first and second order biological half-life for both GM treated COCS and control was evaluated using Eqns. 4 and 5, respectively and the values were shown in Tables 2 and 3.

The results obtained for GM treated COCS from both first and second order kinetic model, indicated a higher degradation rate constants ( $k_1$  and  $k_2$ ) and consequently a lower half-life compared to the control. This phenomenon indicated that the rate of the CO degradation in GM treated COCS was faster [11].

On the other hand, the first order rates for CO contaminant degradation were higher than the second order indicating that the first order kinetic model performed better at all CO concentrations [30,31,6].

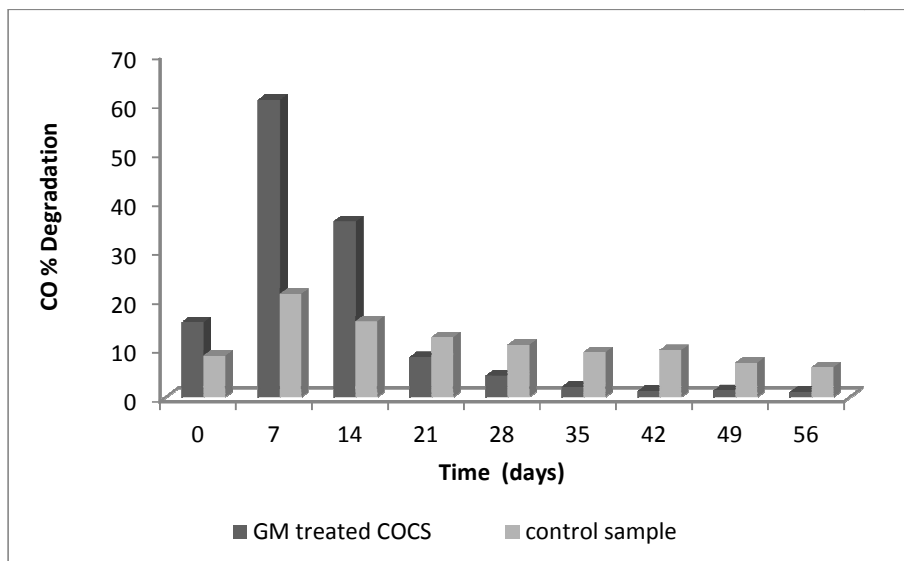


Fig. 3. Plot of CO degradation versus time (values are  $\pm$  standard error of three measurements)

The biological half-life for the CO degradation process was evaluated for both first and second order kinetic model using Eqns. 4 and 5, respectively as shown in Tables 2 and 3. From the first order biological half-life in Table 2, the microorganisms in GM treated COCS took 14 days to degrade half of the initial concentration of 100 mg/l, whereas the microorganisms inherent in the control samples took 18 days to degrade half of the same initial CO concentration [32]. A Similar result was also observed in the second order biological half-life where the GM treated COCS gave a better result (lower half-life and higher degradation rate constant).

However, it could be observed from Tables 2 and 3 that as the CO concentrations increased, the CO degradation rate constant increased. This observation indicated that the lowest degradation rate constants were recorded at the lowest CO concentration in both GM treated COCS and the control sample suggesting that higher CO concentration might be satisfying the microbial carbon need. Also, the time taken by the microorganisms to degrade half of the initial CO contaminant was dependent on the initial CO concentration as more time was taken to degrade lower CO concentrations. However, the inhibitory effects of CO were not observed within the CO concentration range.

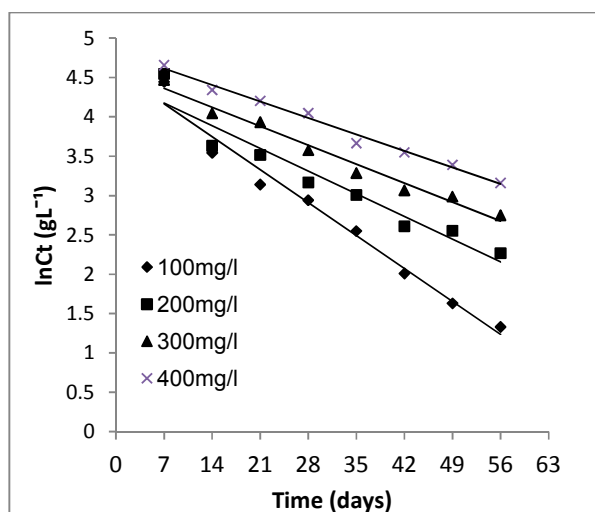
**Table 2. First-order CO degradation rate constants and biological half-lives**

Conc. (g/L)	GM treated COCS		R <sup>2</sup>	Conc. (g/L)	Control		R <sup>2</sup>
	K <sub>1</sub> (day <sup>-1</sup> )	T <sub>1/2</sub> (days)			K <sub>1</sub> (day <sup>-1</sup> )	T <sub>1/2</sub> (days)	
100	0.0299	24	0.928	100	0.0249	35	0.901
200	0.0344	20	0.957	200	0.0296	28	0.891
300	0.0402	17	0.961	300	0.0353	21	0.906
400	0.0518	14	0.977	400	0.0428	18	0.911

**Table 3. Second-order CO degradation rate constants and biological half-lives**

Conc. (g/L)	GM treated COCS		R <sup>2</sup>	Conc. (g/L)	Control sample		R <sup>2</sup>
	K <sub>2</sub> (day <sup>-1</sup> )	T <sub>2/2</sub> (g/L.day <sup>-1</sup> )			K <sub>2</sub> (day <sup>-1</sup> )	T <sub>2/2</sub> (g/L.day <sup>-1</sup> )	
100	0.00018	31	0.902	100	0.00012	45	0.901
200	0.0021	28	0.915	200	0.0011	35	0.891
300	0.0035	20	0.952	300	0.0022	28	0.906
400	0.0059	19	0.967	400	0.0033	21	0.901

$T_{2/2}^{\frac{1}{2}}$  (gl<sup>-1</sup>.day<sup>-1</sup>) is the second order biological half-life.  $K_2$  (day<sup>-1</sup>) is second order CO degradation rate constant,  $K_1$  (day<sup>-1</sup>) is the first order CO degradation rate constant.  $T_{1/2}$  (days) is the first order biological half-life.



**Fig. 4. First order plot of lnCt versus time for GM treated COCS**



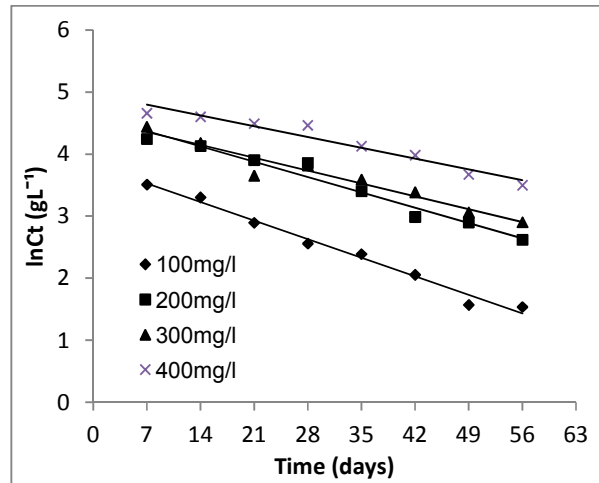


Fig. 5. First order plot for lnCt versus time (days) for the control sample

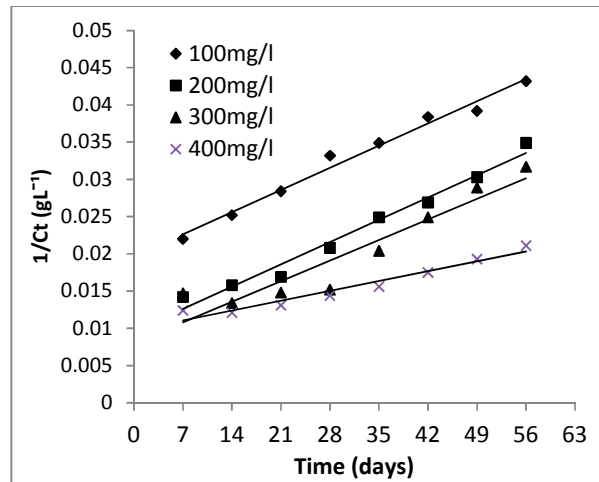


Fig. 6. Second order plot for 1/Ct versus time for GM treated COCS

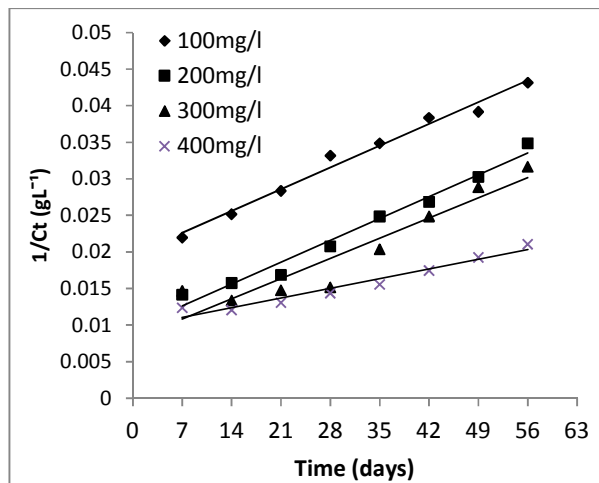


Fig. 7. Second order plot for 1/Ct versus time (days) for the control sample

#### 4. CONCLUSION

In this study, we have shown that the bioremediation of COCS using GM as organic nutrient enhanced CO degradation. However, an increase in microbial count and dehydrogenase assay was observed in the GM treated COCS. The obtained kinetic parameter suggests that the first order kinetic model gave a better result (high degradation rate constant and lower biological half-life) for the studied CO degradation.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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