EVALUATION OF FIRST AND SECOND ORDER DEGRADATION RATES AND BIOLOGICAL HALF-LIVES IN CRUDE OIL CONTAMINATED SOIL.

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: characterization 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 characterization showed that Goat manure contained valuable sources of soil nutrient and organic matter, which enhanced the bioremediation process. The result obtained from the physiochemical characterization 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 plate count indicated that higher colonies were recorded in Goat manure. 70% degradation of crude oil was achieved on the 14th 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 great 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 [19]. Thus, the need to develop and implement an effective remediation technology to reduce the threat caused by this contaminant becomes imperative [19] [31].

However, CO contaminated soil is often poor in organic matter and generally low in the microbial population [9][3], 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 own challenges like environmentally unacceptable, capital-intensive [6] [12] 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 [30]. 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 [5] [14]. 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 fertilizing potency [29] [32].

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 characterize 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 volatilization 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 [33]. In this procedure, 10 g of soil from each sample (GM treated COCS and control sample) was put into a 50 ml beaker and 20ml 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

107 the extract into 50 ml of n-hexane. The absorbance of this solution was measured
108 spectrophotometrically at a wavelength of 400 nm using n-hexane as blank. The crude oil
109 concentrations in the soil were calculated with reference to a standard graph derived from fresh crude
110 oil diluted with n-hexane. The percentage removal of CO from the contaminated soil was calculated
111 using equation (1)

112 $$\% \text{ degradation } = \frac{\text{initial CO} - \text{finial CO}}{\text{initial CO}} \text{ x } 100 \quad (1)$$

113 Where; Initial CO is the Initial crude oil concentration in the soil at time t = 0.

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

115 **2.6 Microbial activity (Dehydrogenase assay)**

116 The soil dehydrogenase assay (DHA), was measured by reducing 2.3.5 triphenyl tetrazolium chloride
117 (TTC) according to [17]. Three replicates of 10g samples of GM treated COCS and the control sample
118 was mixed with 150mg $CaCO_3$, 1ml of 3%(w/v) TTC and distil water (10ml) and was incubated for
119 24hours at 30°C. After which, extraction with 25ml ethanol was performed. The extracts were filtered
120 and incubated for 1hour in the dark and the absorption was measured at 485nm (UV-1800
121 Shimadzu).

122 **2.7 Physiochemical characterization and microbial count**

123 Organic matter content (OMC) was determined using [2]. Total nitrogen was determined using the
124 Kjeldahl method [25]. Organic carbon (TOC) was determined using the Nelson and Sommers, (1996)
125 method [23]. The soil pH was determined using [11]. Available nutrients such as calcium, sodium,
126 magnesium, and potassium (Ca^{2+}, Na^{+}, Mg^{2+}, and K) were determined using the Mehlich 3 method
127 [24]. Soil organic phosphorus was determined using [26]. Estimation of live heterotrophic bacteria in
128 GM and COCS by heterotrophic plate count (HPC) was determined using method as described by
129 America public health association 1998 [4].

130 **2.8 CO degradation kinetics**

131 In this study, CO degradation kinetic parameters were evaluated using both the linearized forms of
132 first and second order kinetic models [9].

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

134 $$\ln[Ct] = -K_1 t + \ln[C_0] \quad (2)$$

135 Where [Ct] is the final concentration of CO in the at time t
136 [Co] is the initial concentration of CO in the at time t = 0
137 $-K_1$ is the CO degradation rate constant for the first-order kinetic model.
138 t is the time in days.

139 A graph of In[Ct] against time (t) in days will be a straight line graph with slope –K and In[Co] as the
140 intercept.

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

142 $$\frac{1}{[Ct]} = K_2 t + \frac{1}{[Co]} \quad (3)$$

143 Where $\frac{1}{[Ct]}$ is the final concentration of CO in the soil at time t
144 $\frac{1}{[Co]}$ is the initial concentration of CO in the soil at time t = 0
145 K_2 is the CO degradation rate constant for the second-order kinetic model.
146 t is the time in days.
147 A graph of $\frac{1}{[Ct]}$ against time in days will be a straight line graph with slope K_2 and $\frac{1}{[Co]}$ as the intercept.
148
149 **2.8.1 Biological half-life for CO degradation**

150 The biological half-life for CO degradation is the time taken by the microorganism to degrade half of
151 the initial CO concentration [9].

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

153 $$T_{\frac{1}{2}} = \frac{\ln 2}{k_1} \quad (4)$$

154 Where K_1 is the first order rate constant and $T_1^{\frac{1}{2}}$ is the first order biological half-life (day^{-1}).

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

157 $$T_{\frac{1}{2}} = \frac{1}{k_2 [Co]} \quad (5)$$

158 Where K_2 (day^{-1}) is the second order rate constant and $T^{\frac{1}{2}}$ (gL^{-1}. day^{-1}) is the second order biological
159 half-life.

160

161 ## 3.0 RESULTS AND DISCUSSION

162 ### 3.1 Characteristics of crude oil contaminated soil and GM

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

167 The neutral pH of GM (7.2) from Table 1 was within the optimum range for microbial growth and
168 multiplication [21]. Also at neutral pH, the nutrient availability in GM was greater due to an equal
169 number of H^+ and OH^-. Similarly, in Table 1, GM was higher in organic matter (66.62%) compared to
170 the control sample (12.52%). This could be due to the presence of high degradable organic matter in
171 GM, while the low organic matter content of the control sample might be due to the effect of CO on
172 soil microbial population and nutrients. [3] reported that hydrocarbon contaminated soils are always
173 poor in organic matter with low microbial activity. However, the high organic carbon of the control
174 (51.46%) might be due to the presence of carbon in the CO which could have been converted to soil
175 organic carbon [7] [20]. The acidic pH (4.7) and low nutrients observed in the control sample as
176 shown in Table 1 could be attributed to the presence of the CO in the soil, which caused deficiencies
177 in soil essential nutrients [20]. The GM clearly showed the presence of some valuable soil nutrients,
178 which could support the indigenous microbial population Table 1.

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

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

Parameters	control sample (0-30cm)	GM	methods
pH	4.7	7.2	ASTMD4972-13 [11]
Organic matter %	12.52	66.62	ASTMD2974-14 [2]
Organic carbon %	51.46	27.04	Nelson, and Sommers, [23]
Kjeldahl Nitrogen %	0.81	3.82	Kjeldahl digestion [25]
Available Nutrients			
sodium (mg/l)	0.03	6.24	Mehlich 3 [24]
magnesium (mg/l)	0.16	3.07	Mehlich 3 [24]
calcium (mg/l)	0.19	1.88	Mehlich 3 [24]
phosphorous (mg/kg)	0.58	3.56	Bray no 1 Extract [26]
HPC (cfu/g)	0.4x10	3.9x10	APHA, 1998, [4]

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190 **3.2 Microbial count**

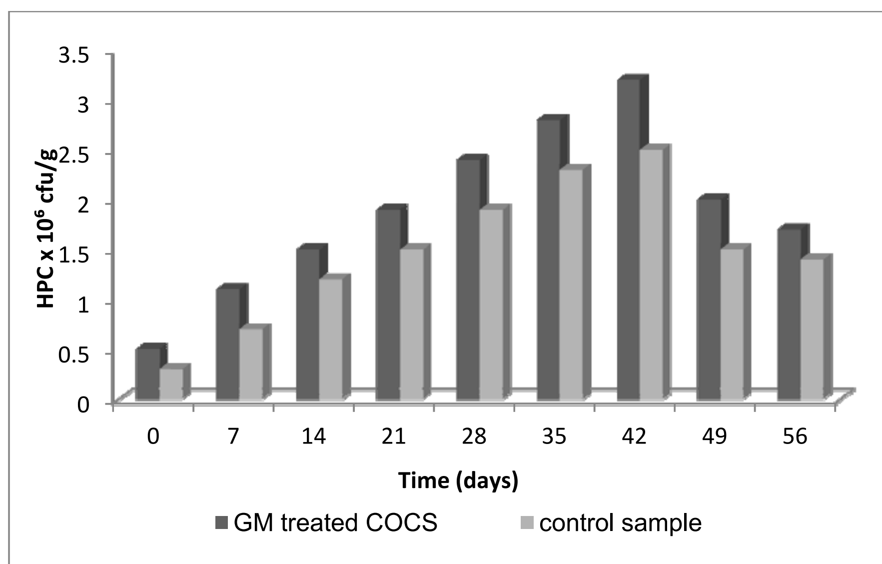
191 In the case of heterotrophic plate count (HPC) shown in (Fig 1), generally, an increase in HPC was
192 observed from 7 to 42nd day in both the control sample and GM treated COCS (Fig 2). Average counts
193 of microorganisms (Fig. 1) are expressed as log of CFU/g of sample and they correspond with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

195 The HPC increased from1.1x10 CFU/g to 3.5x10 CFU/g in GM treated COCS, whereas it
196 increased from 0.4x10 CFU/g to 2.3x10 CFU/g in the control sample (Fig 1). This result showed
197 that the GM enhanced microbial growth which resulted in higher HPC compared to the control sample
198 [28] evaluated the presence of microorganisms (Bacteria and fungi) in soil samples amended with
199 different organic materials quantitatively using agar plate counts [27]. They observed that amendment
200 with different organic materials significantly affected microbial quantity.

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

203 The presence of CO was the main driver for the increase of CFU in the control sample. The increase
204 in numbers of HPC in both GM treated and control sample demonstrates how rapidly indigenous soil
205 microorganisms are able to adapt to new substrates [15].

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



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210 Fig.1, HPC plot of indigenous microbes versus time (values are ± standard error of three
211 measurements)

212

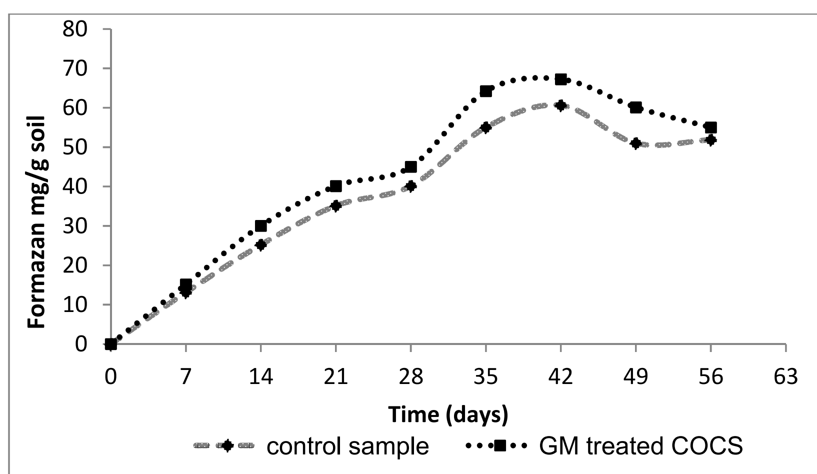
213

214 ### 3.3 Dehydrogenase assay (DHA)

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

218 The result in (Fig. 2) showed a high DHA activity in the control sample and GM treated COCS. The
219 increase in DHA of the control sample could be attributed to the low concentrations of CO used in this
220 study. This also suggests that at low concentrations, the inhibitory effect of CO contaminant on
221 microorganisms was negligible. However, previous studies reported that contaminated soil DHA was
222 dependent on the level of contamination [22].

223 [22] reported an increase in DHA of soil contaminated with petrol, diesel and engine oil at low
224 concentrations. Moreover, another cause of the increased DHA in the COCS could be attributed to
225 the ongoing biodegradation process at low CO concentration [1]. Consequently, the GM treated
226 COCS used in this study also recorded a high DHA. The result could be attributed to both the neutral
227 pH (7.2), which enhanced microbial growth proliferation and the ongoing CO degradation process (Fig
228 2). However, [27] reported that measuring soil enzymatic activities can provide information about the
229 function and structure of soil microbial communities in hydrocarbon-contaminated soils,



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231 Fig.2. DHA for GM treated COCS and control sample (values are ± standard error of three
232 measurements)

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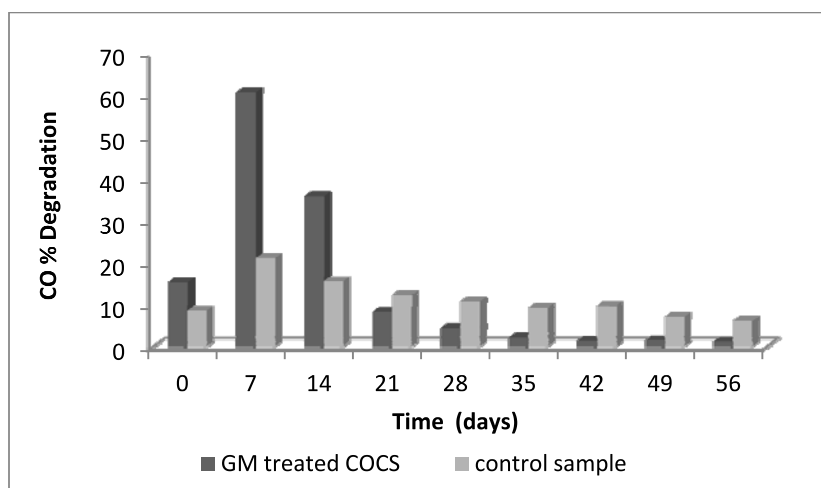
234 ### 3.4 CO degradation process

235 (Fig.3) shows the degradation profile of COCS as a function of time in GM treated COCS and control
236 sample. It could be observed that CO degradation commenced from 7 to 21days and continued up to
237 the fifty-six day. Percentage CO degradation of 60% was achieved within the first 14 days in GM
238 treated COCS, whereas only 21% of the CO contaminant was degraded in the control sample. The
239 inability of the control sample to support the bioremediation process has been previously reported.

240 [19] reported that only 29.5% of the polyaromatic hydrocarbons (PAHs) were degraded in
241 contaminated soil without organic co-substrate (control).

242 There was a noticeable positive correlation between the increase in HPC of the microorganisms and
243 the decrease in the CO contaminant of GM treated COCS during the bioremediation process. This
244 showed that the indigenous microorganisms in GM were able to utilize the CO contaminant. [19]
245 found that native microorganisms present in the soil and organic amendments were more effective as
246 they were more adapted to the soil environmental conditions.

247 It was observed in (Fig. 3), that the CO degradation was observed to be fast during the first fourteen
248 days of treatment after, which a gradual degradation was observed in GM treated COCS [15] [8].
249 During the investigation periods, there was no significant reduction of CO in the control sample, which
250 could be attributed to lack of the organic co-substrate to support the indigenous microorganisms.



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252 Fig.3 plot of CO degradation versus time (values are ± standard error of three measurements)
253

254 **3.5 Evaluation of First and Second order CO biodegradation rates and half-lives**

255 Data obtained from the CO degradation process were fitted to the linearized forms of first and second
256 order kinetic models of equations Eqns. 2 and 3, respectively. The models were used to evaluate the
257 kinetic parameters for CO degradation for both GM treated COCS and control sample. The kinetics
258 parameter obtained from the first and second-order kinetic model are shown in Tables 2 and 3,
259 respectively.

260 The first order degradation rate constant (K_1), was obtained from the slopes of the linear plots of the
261 natural log of the final CO concentration (InCt) versus time (Figs 4 and 5). Similarly, the slopes for the
262 linear plots for the inverse of the final CO concentration (1/Ct) versus time were used to obtain the
263 second order degradation rate constant (K_2) (Figs 6 and 7). The correlation coefficient (R^2) shown in
264 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 [32].

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 [10] [13][12].

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 14days 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 [18]. 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

GM treated COCS				Control			
Conc. (g/L)	K_1 (day^{-1})	T½(days)	R^2	Conc. (g/L)	K_1 (day^{-1})	T½(days)	R^2
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

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

GM treated COCS				Control sample			
Conc. (g/L)	K_2 (day^{-1})	$T_2½$($g/L.day^{-1}$)	R^2	Conc. (g/L)	K_2 (day^{-1})	$T_2½$($g/L.day^{-1}$)	R^2
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

295

296 $T_2^{\frac{1}{2}}$ ($gl^{-1}.day^{-1}$) is the second order biological half-life. K_2 (day^{-1}) is second order CO degradation rate
297 constant, K_1 (day^{-1}) is the first order CO degradation rate constant. $T_1½$ (days) is the first order
298 biological half-life.

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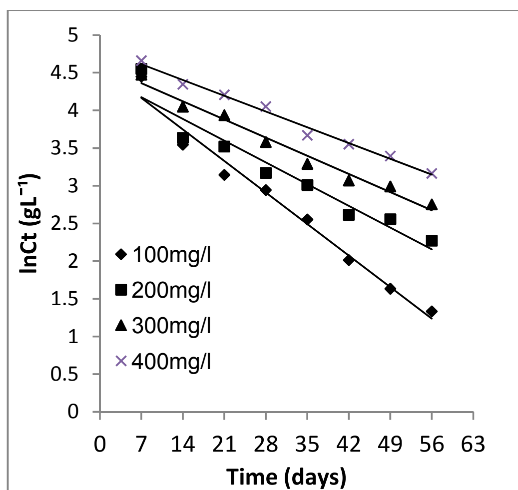

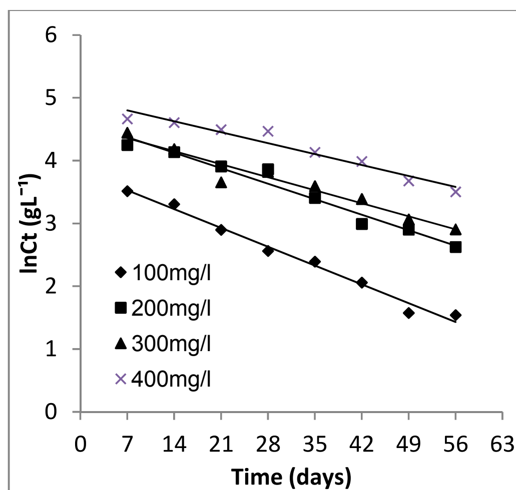

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302 Figs.4 first order plot of InCt versus time
303 for GM treated COCS

Figs.5 first order plot for InCt versus time (days) for the control sample

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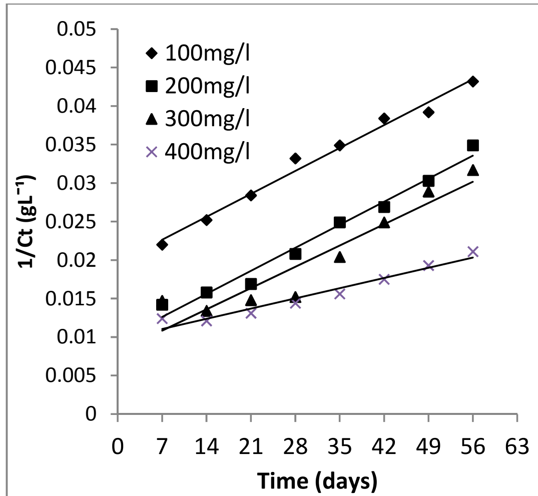

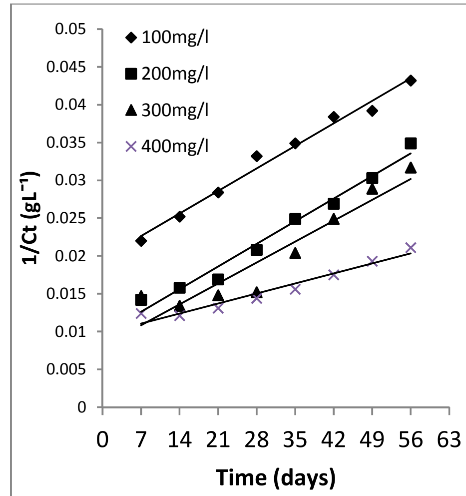

308 Figs.6 second order plot for 1/Ct versus time
309 for GM treated COCS

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

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311

312 **CONCLUSION**

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