

## Bioremediation of Soil Contaminated with 2,4-D Herbicide Using Bioslurry Reactor

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### ABSTRACT

**E**x-situ bioremediation of 2,4-D herbicide-contaminated soil was studied using a slurry bioreactor operate at aerobic conditions. The performance of the slurry bioreactor was tested for three types of soil (sand, sandy loam and clay) contaminated with different concentration of 2,4-D, 200, 300 and 500 mg/kg soil. Sewage sludge was used as an inexpensive source of microorganisms which is available in large quantities in wastewater treatment plants. The results show that all biodegradation experiments demonstrated a significant decreases in 2,4-D concentration in the tested soils. The degradation efficiency in the slurry bioreactor decreases as the initial concentration of 2,4-D in the soils increases. A 100 % removal was achieved at initial concentration of 200 mg 2,4-D/kg of sandy soil after 12 days and 92 % at 500 mg 2,4-D/kg sandy soil after 14 days. Clay soil represented minimum removal efficiency among the three soils, 82 % at initial concentration of 200 mg 2,4-D/kg clay soil after 12 days and 72 % for 500 mg 2,4-D/kg clay soil after 14 days.

Abiotic conditions were performed to investigate the desorption efficiency of the contaminant from soil to liquid phase through the three soils. In abiotic reactor the results showed that the rate of desorption for sand and sandy loam soils were nearly the same, it varied between 0.102-0.135 day<sup>-1</sup> at different initial concentration of 2,4-D. While for clay soil the desorption rate varied between 0.042- 0.031 day<sup>-1</sup> at different initial concentration of 2,4-D. The decrease in desorption rate in clay soil refers to the characteristic of clay soil, (fine texture, high organic matter and high cation exchange capacity compared with the other soils) that may retain the 2,4-D in the organic matter and the clay minerals.

**Keywords:** 2,4-D removal; soil contamination; biodegradation process; sewage sludge.

### المعالجة البيولوجية للتربة الملوثة بمبيد الاعشاب 2,4-D باستخدام المفاعل البيولوجي

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#### الخلاصة

تمت دراسة المعالجة البيولوجية للتربة الملوثة بمبيد الاعشاب 2,4-D في مفاعل بيولوجي يعمل تحت الظروف الهوائية. اجريت دراسة اداء المفاعل البيولوجي لثلاثة أنواع من التربة (رملية وزراعية وطينية) ملوثة بتراكيز مختلفة من الـ 2,4-D 200 و 300 و 500 ملغم/كغم من التربة. استخدمت حمأة الصرف الصحي كمصدر رخيص الثمن لتجهيز المفاعل بالكائنات الحية الدقيقة والذي تتوفر بكميات كبيرة في محطات معالجة مياه الصرف الصحي.

بينت النتائج العملية أن جميع التجارب للمفاعل البيولوجي أدت الى انخفاض ملحوظ في تراكيز الـ 2,4-D في التربة. ان كفاءة التحلل البيولوجي في المفاعل البيولوجي تناقصت مع ازدياد التركيز الابتدائي للـ 2,4-D في التربة واعطت إزالة 100% عند تركيز 200 ملغم / كغم من التربة الرملية بعد 12 يوما و 92% إزالة عند تركيز 500 ملغم / كغم من التربة الرملية بعد 14 يوما. اظهرت التربة الطينية اقل كفاءة إزالة بين انواع الترب الثلاثة حيث ان نسبة الازالة بلغت 82% عند تركيز 200 ملغم / كغم من التربة الطينية بعد 12 يوما و 72% عند تركيز 500 ملغم / كغم من التربة الطينية بعد 14 يوم. استخدم المفاعل عند الظروف اللاحيوية (اي بدون اضافة الاحياء المجهرية) وذلك للتحقق من كفاءة انتقال الملوثات من التربة بانواعها الثلاث إلى السائل الموجود في المفاعل. حيث بينت النتائج ان معدل انتقال المبيد من التربة الرملية والزراعية الى السائل كانت متقاربة وبلغ معدل الانتقال بين (0.102-0.135 day<sup>-1</sup>) لتراكيز مختلفة من الـ 2,4-D في التربة بينما بالنسبة للتربة الطينية كان معدل انتقال المبيد من التربة الى السائل بين (0.042-0.031 day<sup>-1</sup>) لتراكيز مختلفة من الـ 2,4-D في التربة الطينية. ان انخفاض معدل انتقال المبيد من التربة الطينية الى السائل في المفاعل البيولوجي سببه طبيعة التربة الطينية والتي تمثل نوعا من الدقائق والمحتوى العالي من المواد العضوية وزيادة السعة التبادلية الكاتيونية مقارنة مع الترب الأخرى.

**الكلمات الرئيسية:** إزالة 2,4-D ، تلوث التربة، عملية التحلل البيولوجي، الحمأة المنشطة.



## 1. INTRODUCTION

A pesticide is generally a chemical or biological agent (such as virus, bacterium, antimicrobial- or disinfectant) that through its effect deters, incapacitates, kills or otherwise discourages pests. Target pests can include insects, plant pathogens, weeds, mollusks, birds, mammals, fish, nematodes (round worms) and microbes, that destroy property, cause nuisance, spread disease or are vectors for disease. Pesticides can be classified by target organism, (e.g. herbicides, insecticides, fungicides, rodenticides, and pediculicides), chemical structure (e.g. organic, inorganic, synthetic, or biological (biopesticide)), and physical state (e.g. gaseous(fumigant)). Biopesticides include microbial pesticides and biochemical pesticides, **Tiryaki, and Temur, 2010**.

Herbicides are widely used to control unwanted plant species that are competing for light, water, and nutrients with the wanted plant species. Whenever herbicides are applied, they are transported to areas distant from the original site of application, ends up to rivers and streams. 2,4-Dichlorophenoxyacetic acid (2,4-D) is a common system herbicide used in the control of broadleaf weeds. It is the most widely used herbicide in the world, and the third most commonly used in North America, **USEPA, 2007**.

2,4-D is a synthetic auxin (plant hormone), and as such it is often used in laboratories for plant research and as a supplement in plant cell culture media. It is a major ingredient in Agent Orange (one of the herbicides and defoliants used by the U.S. military as part of its chemical warfare program, during the Vietnam War. 2,4-D is the active ingredient in several formulations of herbicides recommended for the control of broadleaf weeds, cereal crops such as wheat, corn, oats, and barley, and the cane crops. It is also widely used to control dandelions and other broadleaf weeds in lawns, rangeland, and pastures. Other uses include the control of aquatic weeds, some woody vegetation, and site preparation and conifer release in forests, **USEPA, 2007**.

2,4-D is classified by both ANVISA (Brazilian National Agency for Sanitarian Vigilance) and WHO (World Health Organization) as a hormonal herbicide of level II toxicity. After its application in field, the excess of the herbicide is easily transferred to the groundwater, due to its high solubility in water. Even after a long period of disuse, considerable amounts of either 2,4-D or its main product of degradation, 2,4-dichloro-phenol (2,4-DCF), might be found in surface waters, and groundwater as well, **Amarante, et al., 2003**.

Ingestion of high doses of 2,4-D have shown a moderate acute toxicity to lower mammals and humans (gastrointestinal irritation, spasms to the myocardio, depression of the central nervous system, and damage to liver and kidney, **Kwangjick, et al., 2001**. Studies on the environmental effects of 2,4-D indicate that this herbicide may be toxic to aquatic invertebrates, phytoplankton, and fish, although the extent and degree of toxicity and damage vary among the species, **Villalobos, et al., 1996; Cheney, et al., 1997**. 2,4-D present in soil water, as well as adsorbed on the colloidal hydroxides of Fe and Al and the organic matter of soils and sediments, may pose a potential risk to the aquifers and the trophic chain, **Dejonghe, et al., 2000**.

The remediation methods currently available for the treatment of pesticide contaminated soil are, low temperature thermal desorption, incineration, bioremediation and phytoremediation.

Bioremediation is the most attractive method for environmental protection due to its cost effectiveness and is unique by offering the potential for complete destruction. Bioremediation is a process that uses microorganisms or their enzymes to promote degradation and/or removal of contaminants from the environment, **Dobson, and Burgess, 2007**. The use of microbial metabolic



ability for degradation /removal of environmental pollutants provide an economic and safe alternative compared to other physicochemical methodologies. However, although highly diverse and specialized microbial communities present in the environment do efficiently remove many pollutants, this process is usually quite slow, which leads to a tendency for pollutants to accumulate in the environment and this accumulation can potentially be hazardous. Different strategies can be employed of improving the process of bioremediation, depending of the state of the contaminated environment. One of these strategies, biostimulation, involves promoting the growth of microorganisms at the contaminated site by introducing nutrients, surfactants and oxygen and as a consequence, the rate of biodegradation/ bioremediation can be increased. Another strategy, bioaugmentation or bioaddition, is the addition of microbial populations, indigenous, alien or genetically modified organisms (GMO), in places where there is an insufficiency of indigenous microorganisms, **Vidali, 2001; Silva, et al., 2004; Li, and Li, 2011.** Bioremediation is a versatile process that can be applied in-situ, at the contaminated site, or ex-situ, involving removal of contaminated material to be treated elsewhere. In-situ bioremediation technologies are more economical and release fewer pollutants into the environment; however, they may require longer treatment time frames than the ex-situ techniques, **Tabak, et al., 2005.** Treatment in a slurry bioreactor is considered to be one of the fastest ex-situ bioremediation methods. Soil slurry bioreactors consist of a mixture of soil in water in various ratios and greatly enhance rates over solid treatment systems by maximizing the contact between micro-organisms, contaminants, nutrients, and oxygen. In slurry bioreactors, the increase in soil moisture results in a larger amount of solubilized contaminant, therefore increasing bioavailability, **Weber, and Kim, 2005.**

Currently, there is wide variety of microorganisms (bacteria, fungi, yeasts and algae) that are being studied for use in bioremediation processes, **Bogacka, 2011; Ali, 2011.** Sewage sludge is a biomass waste generated from the regular biological activities of municipal wastewater treatment plants, it contains bacteria, fungi, yeast and protozoa. Rather than disposing the sludge, this waste material seems to be a promising way of turning it into a useful resource. Sewage sludge is also generally rich in nitrogen, which is essential for the growth of microorganisms. Furthermore, it has a high microbial diversity. The microorganisms overgrown in such wastewater systems can be utilized for treatment different types of contaminants including herbicides, **Bogacka, 2011.**

The aim of this research is to treat three types of contaminated soils, sand, sandy loam and clay with 2,4-D herbicide, using sewage sludge as an inexpensive, heterogeneous source of microorganisms, in ex-situ bioremediation process. The effect of using different concentrations of 2,4-D (200, 300 and 500mg/kg soil) on the bioremediation process was studied.

## 2. MATERIALS AND METHODS

### 2.1 Materials

2,4-D ( $C_8H_6Cl_2O_3$ ) was selected as a model herbicide in this study because of its unique behavior in soil. This chemical is both very degradable and vulnerable to leaching. 2,4-D used in this study was purchased from Fluka. Its purity is 95% and its solubility in water is 900mg/L at 20°C. Other chemicals are: methanol ( $CH_2CL_2$ ) of 99.9% purity was provided by Biosolve Chemicals; acetone ( $CH_3COH_3$ ) of purity > 99.8% was obtained from Fluka; acetonitrile ( $CH_3CN$ ) of 99.5% purity was obtained from BDH Chemicals Limited and Phosphoric acid of 85 % purity from SDF chemical limited .



## 2.2 Nutrients

Modified McKinney's medium was used for the bacterial growth. The composition of the minerals in one liter of growth media is shown in **Table 1** and the composition of trace elements is shown in **Table 2**. The medium was prepared by dissolving the inorganic chemicals in one liter of reverse osmosis water that resulted in a buffered solution of pH 6.5-6.7 which was then sterilized at 121°C.

## 2.3 Soil

Three types of soils were used in the present work: sand, clay and sandy loam. Sand and clay soil samples were obtained from the Ministry of Industry and Minerals (Iraq general establishment for geological survey and mineral investigations), while the third type of soil (sandy loam) was collected from agricultural soils in Baghdad City which was sieved to 2mm. The physical and chemical properties of the three soils are given in **Table 3**. The analysis was performed in the Ministry of Industry and Minerals (Iraq general establishment for geological survey and mineral investigations).

## 2.4 Preparation of 2,4-D Spiked Soil

To prepare the 2,4-D contaminated soil, a stock of 50, 30, 20 mg of 2,4-D was dissolved in 100 mL acetone. In a stainless steel container, a clean, air dried-soil was placed and sprayed with appropriate volume of this stock to give the desired contaminated soil. Mechanical mixing for 1 hour was applied to assure homogenization. The 2,4-D contaminated soil then dried for 24 hour at 35°C in order to evaporate the acetone, then stored in an air-tight stainless steel jar in a ventilated hood in the dark. Each batch of 2,4-D spiked soil was used within 2 weeks.

## 2.5 Microorganisms

Sewage sludge was used in the present study as 2,4-D degrading microorganisms. The sewage sludge was collected from the sludge drying bed in Al-Rustamiyah Sewage Treatment Plant, the old project in Baghdad city. A specified volume of sewage sludge providing at least  $10^7$  cells/mL was used in the experiments. The average mixed liquor volatile suspended solid of the sludge was measured according to, **Clesceri, et al., 1999** to be 5000 mg/L.

The number of bacteria in the sludge was estimated using the standard plate count which reflects the number of viable bacteria and assumes that the bacteria grow into single colonies.

## 2.6 Experimental Procedure

Sewage sludge of 20mL plus 60ml of nutrients were placed in three conical flasks (each of 250mL volume). Then 20mg of contaminated soil with concentration of 200, 300 and 500mg 2,4-D/kg of sandy soil were added in each flask. The flasks equipped on rotary shaker (from Heidolph, Germany), which operated at 200 rpm for 14 days. The required dissolved oxygen (2.5mg/L) was supplied by continuous mixing. The temperature was maintained at 25°C by inserting the rotary shaker inside the incubator. 2, 4-D concentration in both liquid and soil phases also the bacterial growth in liquid phase were measured in regular interval (mainly every 2 days), by removing 5mL sample from the aqueous phase by a clean syringe and 1gm of settled soil phase using a spatula. The same procedure was followed for sandy loam and clay soils. For control reactor the same procedure was followed but without using sewage sludge (i.e. 80mL of nutrients were placed in the conical flasks).

## 2.7 Extraction of 2,4-D from Soil Slurry

Air dried soil sample (0.25gm) at room temperature was extracted with 30mL of a mixture of acetonitrile-water-acetic acid 60:39:1, **Garibay, et al., 2005**. The suspension was vortexed, at maximum speed for 10 min. The solvent extract was recovered by centrifugation at 4000 rpm for 15 min to separate the liquid from the soil, the procedure was repeated twice before analysis by HPLC. The liquid was filtered through filter paper (grade No.1, Whatman, England) and then passed through 0.45  $\mu\text{m}$  PTFE syringe filter before analysis by HPLC.

## 2.8 Analysis

High performance liquid chromatography (HPLC), type Varian 9065, Netherlands, was used to identify the concentration of 2,4-D in the samples, at Environmental Engineering Department laboratories of Al-Mustansiriyah University. Analyses were carried out with the following conditions: wavelength of 282 nm, 1.5 mL/min flow rate and elution with an acetonitrile + aqueous phosphoric acid (1.0M, pH=3) solution (first using 60+40 solution by volume for 10 min and then 65+35 solution for 20 min). The HPLC was equipped with C18 column (250 mm long, 4 mm ID). The peak of 2,4-D was detected after 2.7 min.

## 3. RESULTS AND DISCUSSION

### 3.1 2,4-D Partitioning between Soil and Liquid Phase in Abiotic Control Reactor

Abiotic control reactor represents a reactor with no microorganisms. The behavior of 2,4-D partitioning between soil and liquid in abiotic control reactor for three types of soil (sand, sandy loam and clay) were investigated in the present experiments. For each type of soil three different initial concentrations of 2,4-D (200, 300 and 500 mg/kg soil) were tested, **Figs.1-3**.

After startup of the reactor 2,4-D was desorbed from the soil to the liquid phase. It approached a steady state condition after nearly 12 days for sand and sandy loam soil. For clay soil the steady state appeared after 12-14 days. **Fig. 1** shows that at initial 2,4-D concentration of 200 mg/kg soil, the release of 2,4-D from the soil to the liquid phase reaches 97%, 95% and 72% for sand, sandy loam and clay soil respectively. **Fig. 2** shows that at initial concentration of 300mg 2,4-D/kg soil, the release of 2,4-D from the soil to the liquid phase reaches 86%, 89% and 72% for sand, sandy loam and clay soil respectively. While from **Fig. 3** at initial concentration of 500mg 2,4-D/kg soil, the release of 2,4-D from the soil to liquid phase reaches 89%, 88% and 58% for sand, sandy loam and clay soil respectively. These results were tabulated in **Table 4**. The results show that the release efficiency decreased with the increase of 2,4-D initial concentration. A high initial concentration of 2,4-D enhance the sorption into the soil. This observation was coinciding, **Boivin, et al., 2005**. Also a distinct decrease in the release efficiency was observed for clay soil, reaching 58% compared with 89% for sandy soil at initial concentration of 500mg 2,4-D/kg soil. This attribute to the fine texture of clay soil, high content of organic matter (1.967 %) and high value of cation exchange capacity (68.34meq/100mg), which enhance the sorption of 2,4-D in to the soil. **Bolan and Baskaran, 1996** in their study for adsorption/ desorption behavior and degradation of 2,4-D, examine 10 soils from New Zealand that have different organic matter and clay content. They observed that the extent of adsorption increased with the increase in soil organic compounds.

At early period of time desorption of pesticides follows first-order reaction kinetics, Eq.(1):

$$S_t = S_0 \exp[-k_0 t] \quad (1)$$

where  $S_0$  and  $S_t$  are the amount of 2,4-D (mg/kg) in the soil phase at time zero and  $t$ ,  $t$  the desorption period (day), and  $k_0$  is the rate of desorption ( $\text{day}^{-1}$ ). A linear relationship with  $R^2$  of more than 0.90% was obtained as illustrated in **Fig.4**.

It can be observed that the rate of desorption  $k_0$  for sand and sandy loam soils were nearly the same, it varies between 0.102-0.135  $\text{day}^{-1}$  at different initial concentration of 2,4-D. While for clay soil the desorption rate  $k_0$  varies between 0.031- 0.042  $\text{day}^{-1}$  at different initial concentration of 2,4-D. The decrease in desorption rate refers to the characteristic of clay soil, (fine texture, high organic matter and high cation exchange capacity) that may retain the 2,4-D in the organic matter and the clay minerals, as confirmed by **Bolan, and Baskaran, 1996; Smith, et al., 1992**.

### 3.2 2,4-D Partitioning Between Soil and Liquid Phase in Biological Active Reactor

The effect of microbial activity, bioaugmentation (adding of sewage sludge) and biostimulation (adding of nutrient) together on 2,4-D degradation using slurry bioreactor were investigated in these experiments.

2,4-D partitioning between soil and liquid phase for sand ,sandy loam and clay at different initial concentrations 200, 300 and 500 mg/kg soil are plotted in **Figs. 5-7**.

In all experiments the 2,4-D concentration in liquid phase increased initially reaching maximum concentration and then rapid degradation of 2,4-D was observed. The reason for this is that the desorption rate of 2,4-D is higher than the biodegradation rate during the first period of experiments. In the soil phase a continuous decrease in 2,4-D concentration continued until the end of the experiments. This result indicates that the desorbed 2,4-D in the liquid phase was subjected to continuous biological degradation therefore desorption was enhanced due to the partitioning effect.

**Fig.5** represents the partitioning between solid and liquid phases for 200mg 2,4-D/kg soil in the biological active reactor, for the three different soils. From this figure, the maximum concentration of 2,4-D in liquid phase can be observed to be 2.2, 6.8 and 1.4mg/L after two days of the reactor operation for sand , sandy loam and clay respectively.

For 2,4-D initial concentration of 300mg/kg soil as represented by**Fig.6**, the maximum concentration was achieved in liquid phase equal to 13.9 and 37mg/L after two days for sand and sandy loam soil and 30.2 mg/L after six days for clay soil.

For 500mg 2,4-D/kg soil, **Fig.7**, the maximum concentration in liquid phase was obtained to be 19, 12.4 and 18.9 mg/L after two days for sand ,sandy loam and clay soil respectively.

From the above results it can be concluded that 2,4-D in liquid phase increased until almost two days of the reactor operation reaching a maximum concentration and then decreased at different rates indicating that 2,4-D was subjected to continues biological degradation.

At early period of time the degradation of pesticides also follows first-order reaction kinetics, Eq.(2):

$$C_t = C_0 \exp[-k_1 t] \quad (2)$$

where  $C_0$  and  $C_t$  are the amount of 2,4-D (mg/kg) in the soil phase at time zero and  $t$ ,  $t$  the degradation period (days), and  $k_1$  is the rate constant of degradation ( $\text{day}^{-1}$ ).

A linear relationship with  $R^2$  of more than 0.93 was obtained as illustrated in **Fig.8**, revealing that the rate of degradation is directly proportional to the concentration of 2,4-D in the soil phase. The rate of degradation of organic pesticide has often been observed to follow first order reaction kinetics, **Cycon, et al., 2011; Plangklang, and Reungsang, 2010**.



$k_1$  for each type of soil with different concentrations of initial 2,4-D were tabulated in **Table 5**. From **Table 5** and **Fig.8**, it can be observed that the rate of degradation  $k_1$  decreased with the increase of initial concentration of 2,4-D.

**Macur, and Wheeler, 2007** studied the impact of 2,4-D application on soil microbial community structure. They showed that at high 2,4-D concentration (100 and 500mg/kg soil) a significant reduce in the diversity of 2,4-D degradation of bacterial strains was observed. They also illustrated that clay soil has minimum degradation rate among the three soils (sand, sandy loam, clay).

Considering the 2,4-D removal efficiency for the three soils in the present experiments as it is plotted in **Fig. 9** and listed in **Table 5**, the removal efficiency decreases as the initial concentration of 2,4-D in the soils increases. A 100 % removal at initial concentration of 200mg 2,4-D/kg soil after 12 days for sandy soil was achieved and this percent decreases to 92% at 500mg 2,4-D/kg soil after 14 days .

**Fournier, et al., 1981** found that the degradation rate of 2,4-D is highly dependent on the initial concentration (the higher the degradation, the lower the initial concentration).

Clay soil represent the less removal efficiency among the three soils , 82% for 200mg 2,4-D/kg soil after 12 days and 72% for 500mg 2,4-D/kg soil after 14 days . Clay soil characteristics enhance the sorption capacity and limits the release of 2,4-D to the liquid phase in which biological degradation is performed .

**Nikakhtari, et al., 2009** mentioned that the biodegradation rate in the bioslurry reactor with clay is slower than with sandy loam and sandy soil.

### 3.3 CFU Variation

CFU (Colony Forming Unit) was investigated in the present experiments as an important parameter indicating the microbial activity. **Figs. 10-12** show the biomass growth with 2,4-D degradation in the liquid phase during the bioreactor operation. Different initial concentration of 2,4-D (200, 300, 500 mg/kg) in sand ,sandy loam and clay soils were studied.

Initial microbial cell numbers of the sewage sludge which was utilized in the biodegradation operation were about  $200 \times 10^7$  CFU/mL.

A lag phase of almost 2 days was observed through all the experiments. The lag phase is related to the time required to develop a population of microorganisms that could effectively degrade the 2,4-D. The lag phase is a period of adaption of cells to a new environment. Microorganisms reorganize their molecular constituents when they transfer to a new medium. Depending on the composition of nutrients new enzymes are synthesized, the synthesis of some other enzymes is repressed, and the internal machinery of cells is adapted to the new environmental conditions. During this phase, cell mass may increase a little, without an increase in cell number density, **Yu, and Ruihong, 2006**.

After the lag phase a period of an active growth was observed. The microorganisms multiply rapidly and enter the exponential growth phase. The maximum CFU/mL observed in the present experiments was in the range of  $700 \times 10^7$  -  $1200 \times 10^7$  CFU/mL which achieved after almost six days of operation as shown in the **Figs. 10-12**. After the exponential growth, a stationery phase then a decline in microbial growth was observed, due to the nutrient (2,4-D) depletion.

It can be noticed that the maximum release of 2,4-D from the soil to the liquid phase happened during the lag phase period. The results show that when the 2,4-D concentration increased the biodegradation retarded due to inhibition created by the substrate and the toxicity of high 2,4-D concentration.



### 3.4 pH Variation

The pH variation in the liquid phase during the abiotic and biotic bioreactor operation was measured to track the microbial activity. **Fig. 13** shows the variation in pH for sand, sandy loam and clay for 200, 300 and 500 mg 2,4-D/kg soil. It can be noticed that the pH variation in the reactor with biological activity (biotic) was more obvious compared to the control reactor (abiotic). The variation could result from the products obtained from the biodegradation activity. The decrease in pH in the biological active reactors may be due to the formation of CO<sub>2</sub> from mineralization of the metabolic intermediates formed, **Mohan, et al., 2008**.

### 4. CONCLUSIONS

The main conclusions that can be drawn from the experimental work of this research are as follows:

- The rate of desorption in the abiotic reactor for sand and sandy loam soils were nearly the same, it varies between (0.102-0.135 day<sup>-1</sup>) at different initial concentration of 2,4-D. While for clay soil the desorption rate varies between (0.031-0.042 day<sup>-1</sup>) at different initial concentration of 2,4-D.
- In all biological active reactor experiments the 2,4-D concentration in the liquid phase increased initially reaching a maximum level and then rapid degradation of 2,4-D was observed. The maximum release of 2,4-D from the soil to the liquid phase happened during the lag phase period of microbial growth.
- The degradation of 2,4-D in the bioreactor follows first-order reaction kinetics. A linear relationship between  $\ln(C_t/C_0)$  vs.  $t$  with correlation coefficient  $R^2$  of more than 0.93 was obtained revealing that the rate of degradation was directly proportional to concentration of 2,4-D in the soil phase.
- The removal efficiency of 2,4-D in the bioslurry reactor decreases as the initial concentration of 2,4-D in the soils increases, it reached 100% at initial concentration of 200mg 2,4-D/kg sandy soil after 12 days and decreased to 92% at 500 mg 2,4-D/kg sandy soil after 14 days .
- Clay soil represent the less removal efficiency in the bioslurry reactor among the three soils , 82% for 200mg 2,4-D/kg clay soil after 12 days and 72% for 500 mg 2,4-D/kg clay soil after 14 days .
- The pH variation in biological active reactor was more obvious compared to the control reactor (abiotic) in all experiments.

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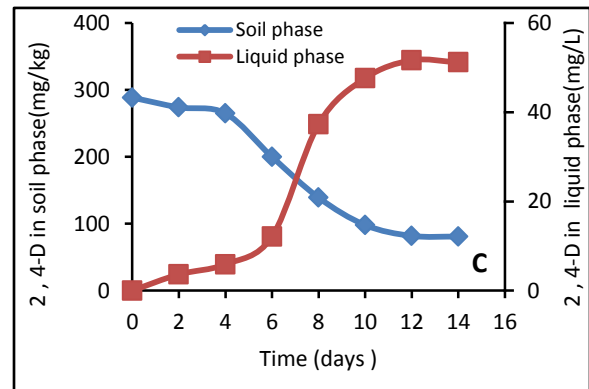
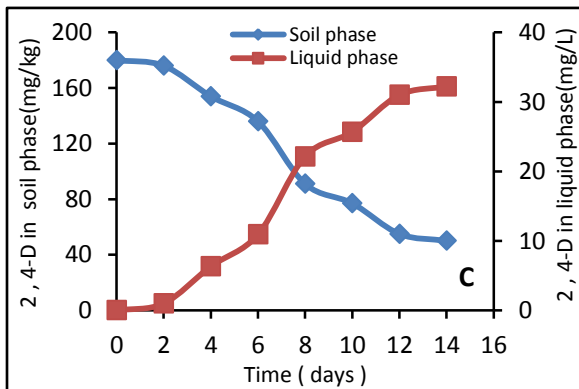
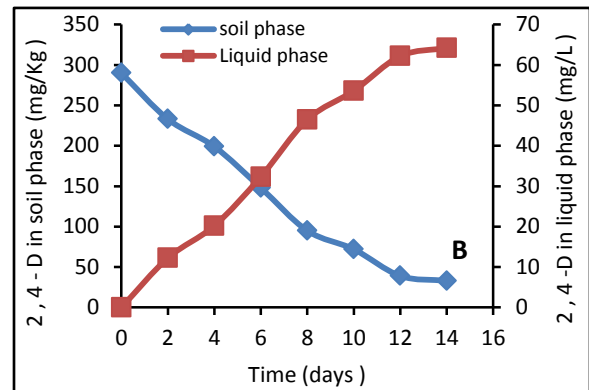
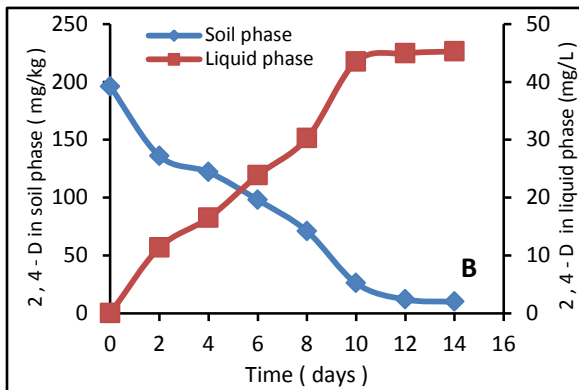
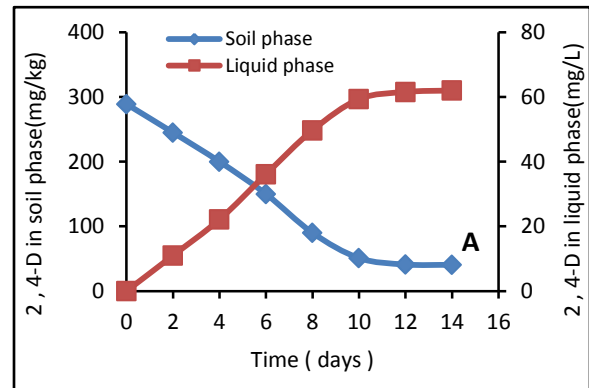
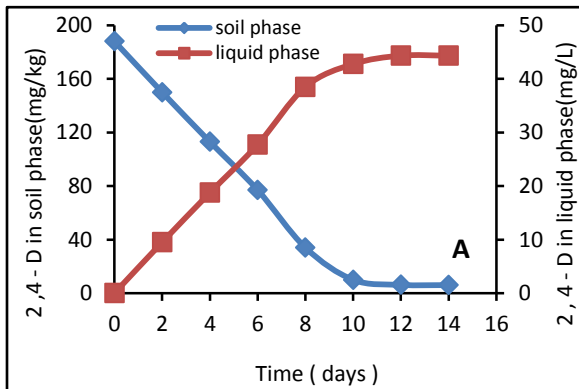
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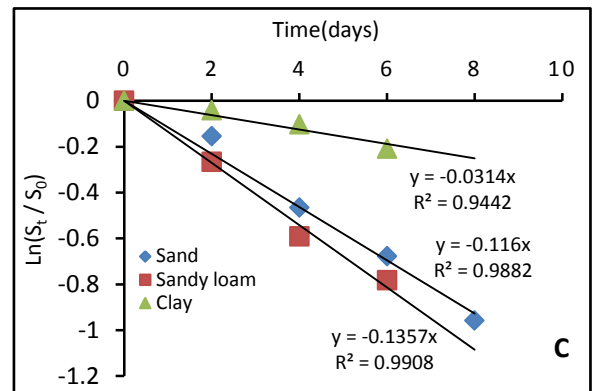
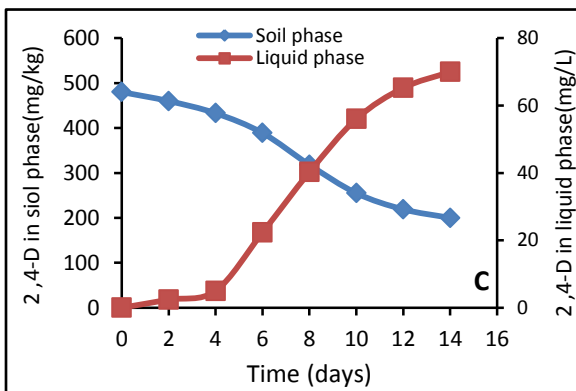
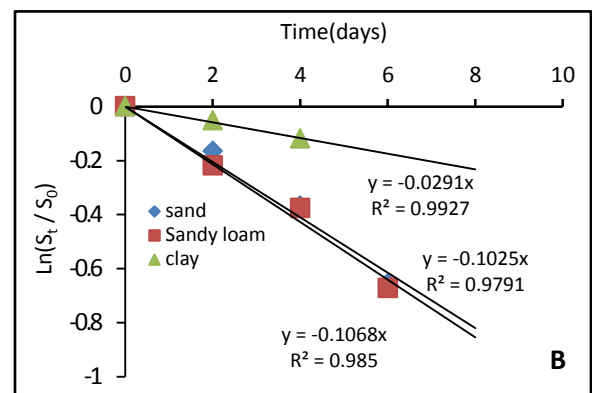
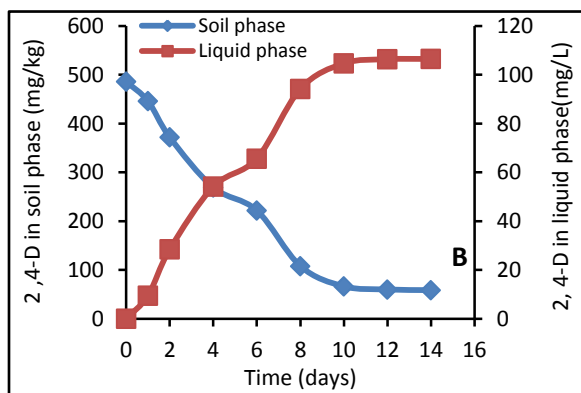
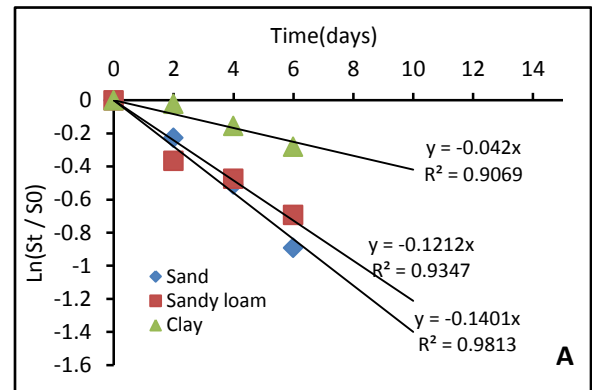
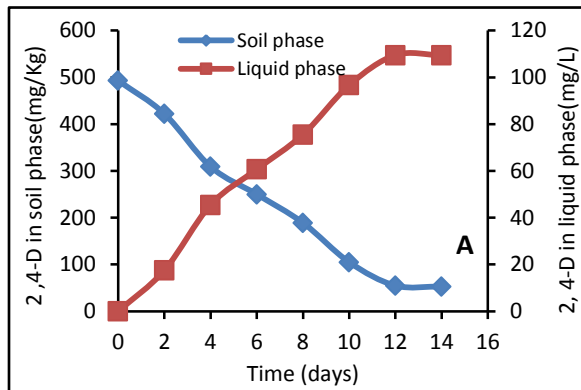
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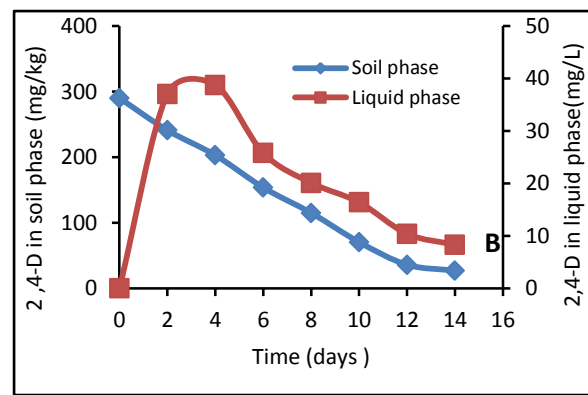
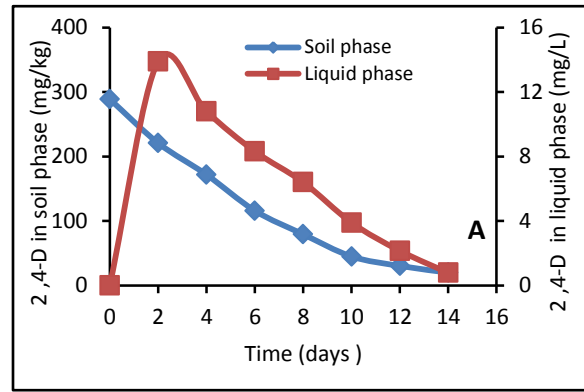
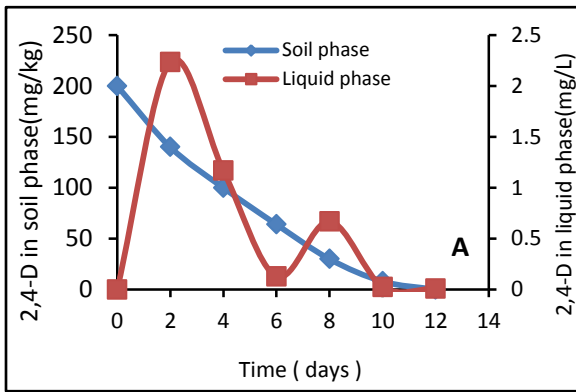
**Figure 1.** Partitioning between soil and liquid phase in abiotic control reactor using three types of soil (A) sand, (B) sandy loam, (C) clay. 2,4-D concentration = 200 mg/kg soil.

**Figure 2.** Partitioning between soil and liquid phase in abiotic control reactor using three types of soil (A) sand, (B) sandy loam, (C) clay. 2,4-D concentration = 300 mg/kg soil.

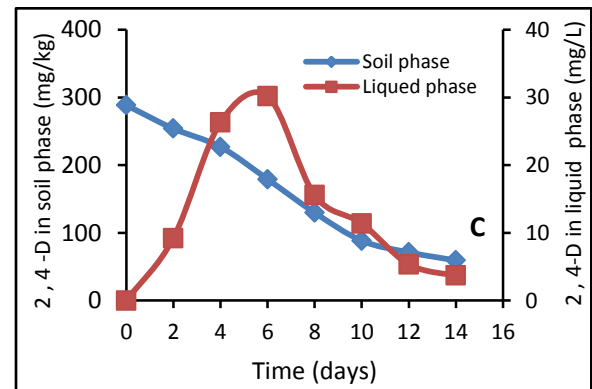
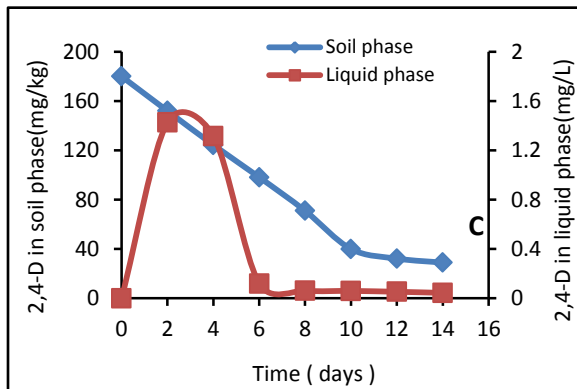
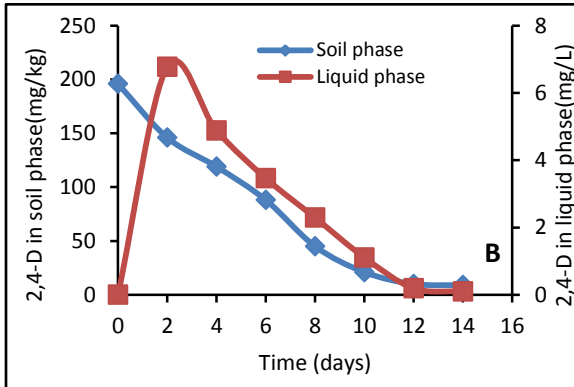


**Figure 3.** Partitioning between soil and liquid phase in abiotic control reactor using three types of soil (A) sand, (B) sandy loam, (C) clay. 2,4-D concentration = 500 mg/kg soil.

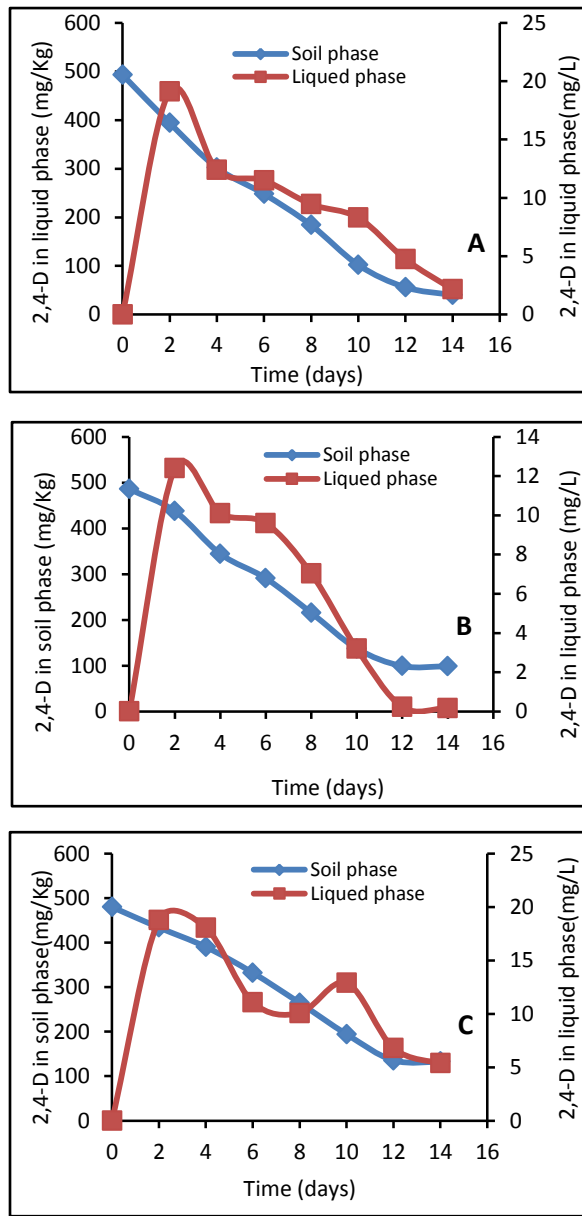
**Figure 4.** First order rate constant for 2,4-D in abiotic control reactor at different initial concentrations, (A) 200 mg/kg soil, (B) 300 mg/kg soil, (C) 500 mg/kg soil.



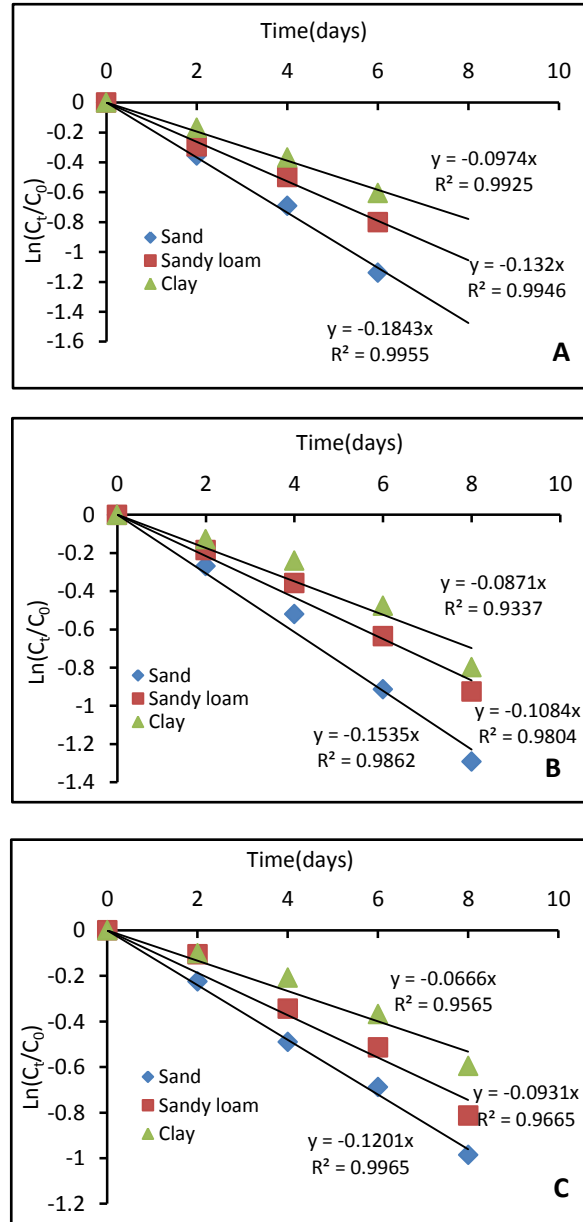
**Figure 5.** Partitioning between soil and liquid phase in bioslurry reactor using three types of soil (A) sand,(B) sandyloam, (C) clay. 2,4-D concentration = 200mg/kg soil.



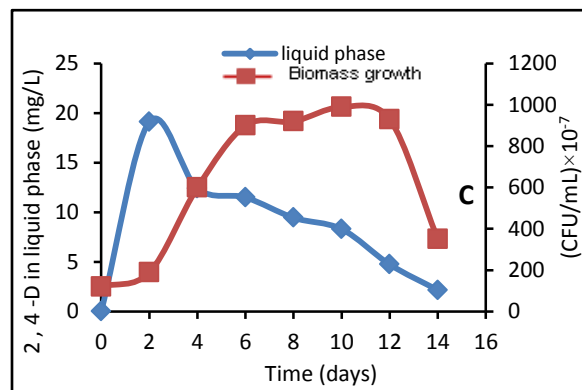
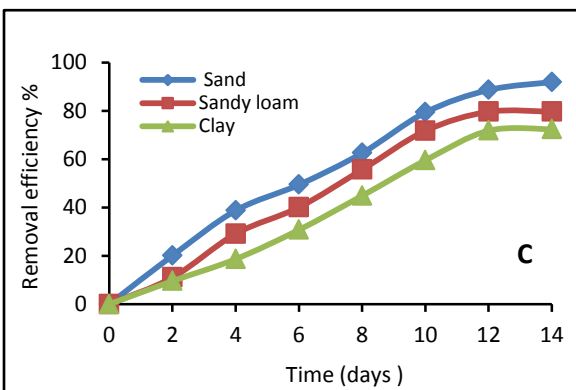
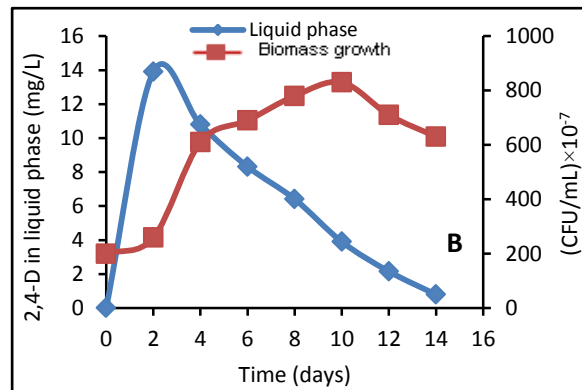
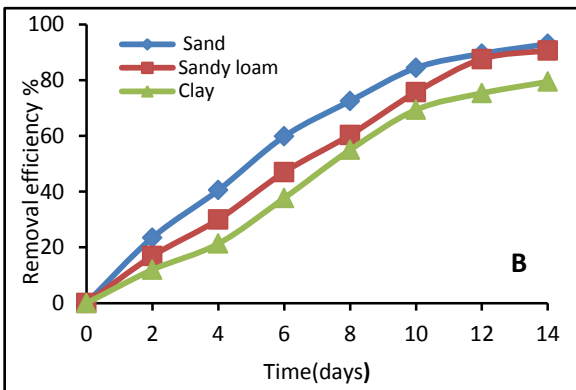
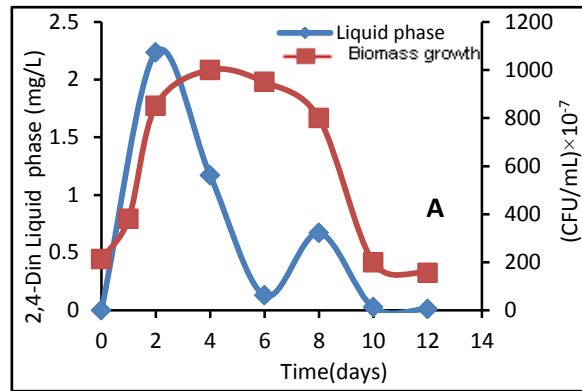
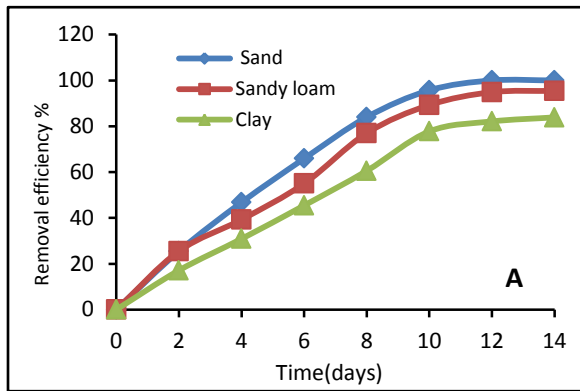
**Figure 6.** Partitioning between soil and liquid phase in bioslurry reactor using three types of soil (A) sand, (B) sandy loam, (C) clay. 2,4-D concentration = 300 mg/kg soil.



**Figure 7.** Partitioning between soil and liquid phase in bioslurry reactor using three types of soil, (A) sand, (B) sandy loam, (C) clay. 2,4-D concentration = 500mg/kg soil.



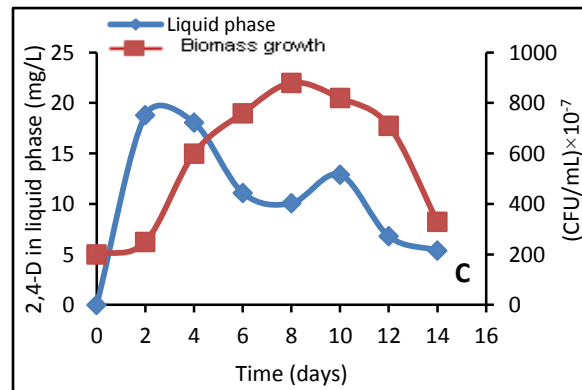
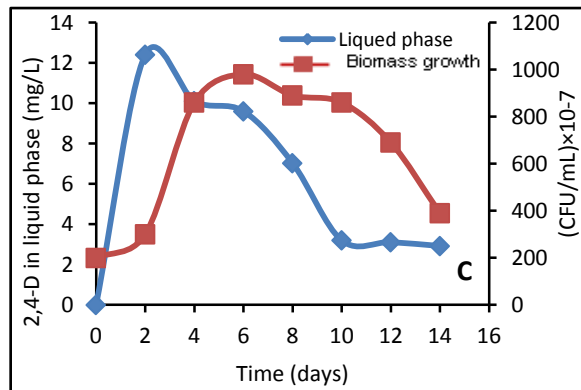
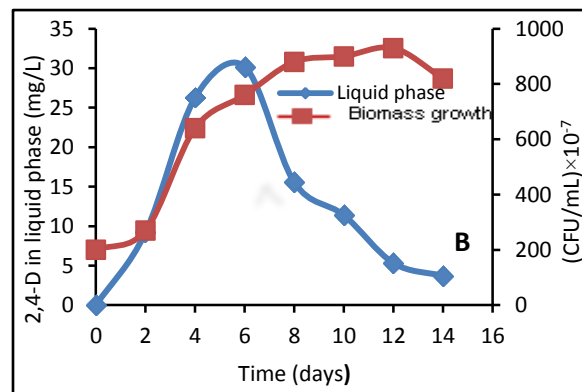
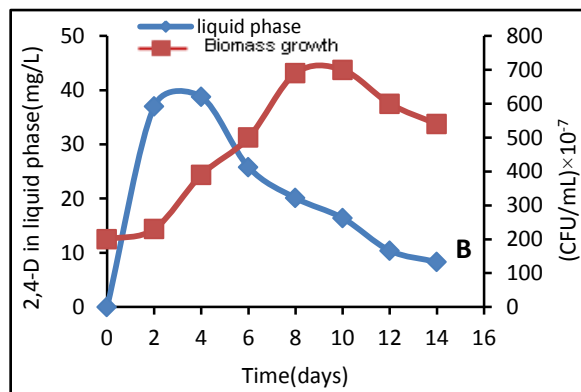
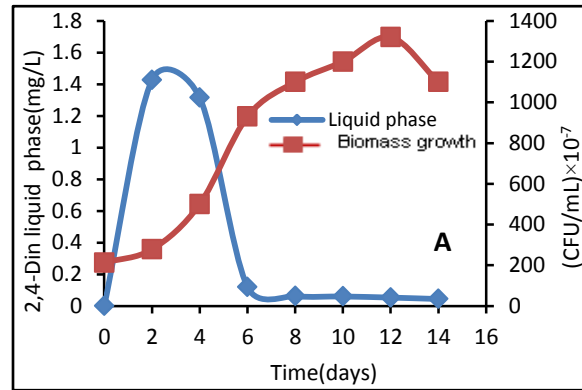
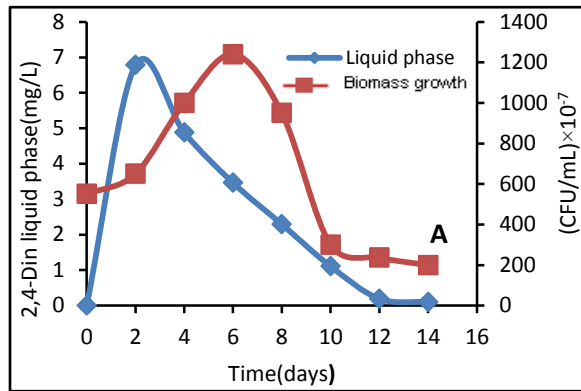
**Figure 8.** First order rate constant for 2,4-D in bioslurry reactor at different initial concentrations, (A) 200mg/kg soil, (B) 300mg/kg soil, (C) 500mg/kg soil.



**Figure9.** Removal efficiency for bioslurry reactor using three types of soil at (A)200 mg 2,4-D/kg soil, (B) 300mg2,4-D/kg soil , (C) 500mg2,4-D/kg soil .

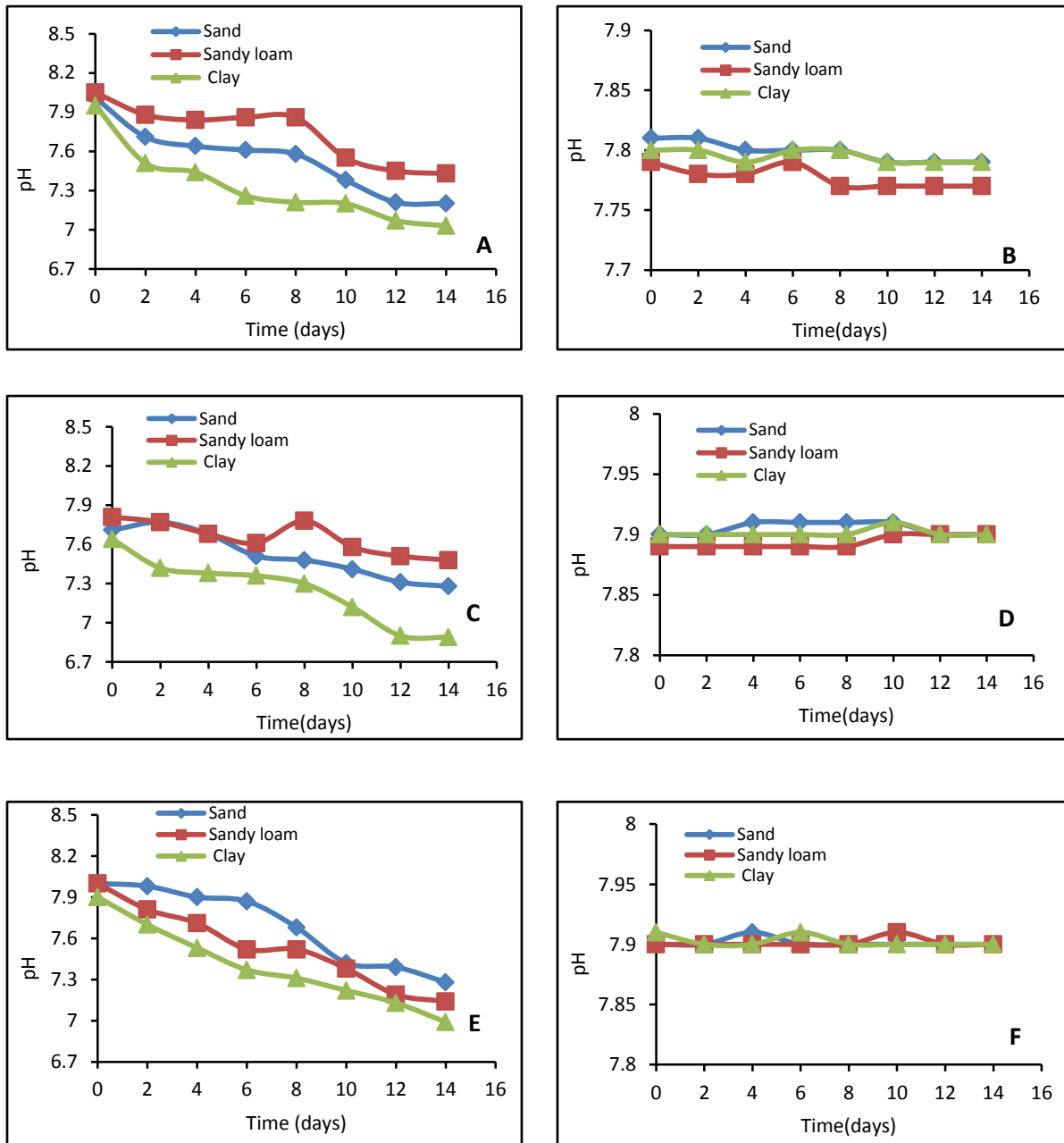
**Figure10.** Biomass growth and 2,4-D degradation in liquid phase, during bioslurry reactor operation for sandy soil. 2,4-D concentration (A) 200mg, (B) 300mg, (C) 500 mg/kg soil.





**Figure11.** Biomass growth and 2,4-D degradation in liquid phase, during bioslurry reactor operation for sandy loam soil. 2,4-D concentration (A) 200mg, (B) 300mg, (C) 500 mg/kg soil.

**Figure12.** Biomass growth and 2,4-D degradation in liquid phase, during bioslurry reactor operation for clay soil. 2,4-D concentration (A)200mg,(B) 300mg, (C) 500 mg/kg soil.



**Figure 13.** Variation of pH during biotic and abiotic reactor, (A)200, (C)300, (E) 500 mg 2,4-D/kg soil, for biotic reactor,(B)200, (D)300, (F)500 mg 2,4-D/kg soil for abiotic reactor.

**Table 1.** Modified McKinney's medium in 1 liter of reverse osmosis water.

Substance	Mass or Volume
$\text{KH}_2\text{PO}_4$	420 mg
$\text{K}_2\text{HPO}_4$	375 mg
$(\text{NH}_4)_2\text{SO}_4$	237 mg
NaCl	30 mg
$\text{CaCl}_2$	30 mg
$\text{MgSO}_4$	30 mg
$\text{Fe}(\text{NH}_4)_2\text{SO}_4$	10 mg
Trace element	1mL

**Table 2.** Trace elements composition in 1 liter of reverse osmosis water.

Substance	Mass(mg)
$\text{H}_3\text{BO}_3$	300
$\text{CoCl}_3$	200
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	100
$\text{MnCl}_2$	30
$\text{Na}_2\text{MoO}_4$	30
$\text{NiCl}_2$	20
$\text{CuCl}_2$	10

**Table 3.** Characteristics of the three soils.

Soil type	Soil texture		Soil pH	OMC %	CEC (meq/100mg)
Sand	Sand	98.93%	8.75	0.027	0.071
	Silt	1.07%			
	Clay	-----			
Sandy loam	Sand	88.03%	8.61	0.28	1.42
	Silt	11.97%			
	Clay	-----			
Clay	Sand	2.3%	8.05	1.967	68.34
	Silt	-----			
	Clay	97.7%			

OMC: Organic matter content

CEC: Cation exchange capacity



**Table 4.** Release efficiency and rate of release of 2,4-D from the three soils with different concentration.

Initial 2, 4-D conc. (mg/kg)	Sand				Sandy loam				Clay			
	$k_0$ (day <sup>-1</sup> )	R <sup>2</sup>	Residue (mg/kg)	Release (%)	$k_0$ (day <sup>-1</sup> )	R <sup>2</sup>	Residue (mg/kg)	Release (%)	$k_0$ (day <sup>-1</sup> )	R <sup>2</sup>	Residue (mg/kg)	Release (%)
200	0.140	0.98	6	97	0.121	0.93	10	95	0.042	0.91	50	72
300	0.102	0.98	40.5	86	0.106	0.99	33	89	0.029	0.99	80.8	72
500	0.116	0.99	53	89	0.135	0.99	58.7	88	0.031	0.94	200	58

**Table 5.** Removal efficiency of 2,4-D and rate of degradation in the shake flask bioreactor.

Experiment Time (days)	Initial 2, 4-D conc. (mg/kg)	Sand			Sandy loam			Clay		
		$k_1$ (day <sup>-1</sup> )	R <sup>2</sup>	Removal efficiency %	$k_1$ (day <sup>-1</sup> )	R <sup>2</sup>	Removal efficiency %	$k_1$ (day <sup>-1</sup> )	R <sup>2</sup>	Removal efficiency %
12	200	0.184	0.996	100	0.132	0.995	95	0.097	0.993	82
14	300	0.153	0.986	93	0.108	0.9804	91	0.087	0.934	80
14	500	0.120	0.997	92	0.0931	0.967	80	0.067	0.957	72