

## **Effect of Bio-Augmentation on Biokinetic Parameters in the Mesophilic Anaerobic Digestion of Poultry Droppings**

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**Abstract:-** The effect of bio-augmentation on biokinetics parameters in the mesophilic anaerobic digestion of poultry droppings was carried out. The parameters investigated include: the maximum specific growth rate, yield, COD removal efficiency, decay coefficient and the growth phases. It was observed that inoculation positively affect the lag phase, exponential growth phases, yield, specific growth rate and COD removal efficiency. This was achieved by integrated recycle of digested sludge into the reactor, whose value depended on the biomass yield, for the recycle stream to serve as inoculum.

**Keywords:-** Mesophilic Anaerobic digestion, biokinetic parameters, Inoculum, Poultry dropping

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### **I. INTRODUCTION**

Green plants capture solar energy by photosynthesis which is stored in biomass. Biomass, a high energy density system, such as trees, grasses, agricultural crops, residues, animal wastes and municipal solid wastes can be used as a solid fuel. Anaerobic digestion can be used to convert biomass by microorganisms in the absence of air to produce either alcohol or methane gas, which give energy on combustion. (Dara, 2006) Since biomass is obtained from photosynthesis, biomass energy could be considered to be another form of direct use of solar energy. Generally, generation of waste is a global problem that demands sustainable solution. In the past decades, the consumption of poultry in Nigeria and in many other developing countries has been on the increase. As a result of this growing poultry demand, there has been a corresponding increase in the poultry industry and consequently increasing amounts of organic solids by-products and wastes. Poultry droppings can be considered as a sustainable biomass; because a broiler produces approximately 11gDM/bird/day of poultry droppings while a layer generates 32.9gDM/bird/day (FAO, 2011). Biogas is produced from anaerobic digestion of poultry droppings which can be used in gas-engine electric generators and domestic cooking, while slurry from the digester could be converted into fertilizers (Hetal, 2000). Amidst these opportunities, poultry waste management in most countries, especially the developing countries can be best described as non-existence, or at best being ad hoc. Farmers compost poultry waste in heaped piles, emitting offensive odours, carbon dioxide, methane and leachate seepage and run-off to water sources, insects, aesthetic problems with its associated health and environmental concern (CDM, 2005; O'Mara, 1996). The development of better engineering systems for proper handling of poultry waste, rather than dumping them into the environment, is extremely important in protecting surface water, groundwater, soil, and maintaining air quality standards. Digesting poultry waste in anaerobic digester is a well-known option for poultry waste management; however, a successfully operating one could scarcely be found (CDM, 2005). Biological treatment of solid waste is a cost effective alternative to other waste treatment techniques and many experts regard biotreatment as the technology of the future (O'Mara 1996). A lot of work has been done on annexing waste for energy and soil conditioning. Yelebe and Puyates (2006) studied the biokinetics of aerobic digestion of municipal solid waste. In their work, Monod growth kinetics was used to model aerobic degradation of municipal solid waste in bioaugmented and non-bioaugmented batch reactors using a mixture of indigenous microorganisms isolated from the waste. Igogni *et al.*, (2006a; 2008b) estimated the kinetic parameters during anaerobic digestion of MSW and investigated the suitability of either batch or continuous (CSTR) digesters for anaerobic degradation of MSW in the production of biogas. Jiraphon *et al.*, (2010) developed dynamic model for anaerobic digestion of shrimp culture pond sediment to study the variables that affect biogas production process and optimization. Garcia-Ochoa *et al.*, (1999) developed kinetic model for anaerobic digestion of beef cattle manure. The purpose of this paper is to investigate the effect of bioaugmentation on biokinetic parameters in mesophilic anaerobic digestion of poultry droppings using recycle stream to serve as inoculum.

## II. MATERIALS AND METHOD

### 2.1 Sample Preparation

Fresh poultry droppings were collected from poultry farm of Agricultural Department in Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The non-biodegradable dirt such as feathers, etc. was manually sorted out, and the droppings were placed in an air-tight condition.

### 2.2 Determination of Moisture Content

The moisture content is the amount of water to be added to get the required total solid (TS) to water ratio (20:80)% by weight (Reynold and Richard 1996). Two grams (2 g) of the prepared sample was dried in an oven for four hours at 150°C, until the weight of sample becomes constant and the moisture content was determined by

$$\text{Moisture Content} = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

where  $W_1$ = Initial weight of sample before drying, g

$W_2$ =Final weight of sample after drying, g

### 2.3 Enumeration and Isolation of Bacteria

Ten-fold serial dilution method of analysis was used to enumerate and isolated the bacteria responsible for digestion used in the study (Ofunne, 1999). Five test-tubes were sterilized and labeled  $10^0$ ,  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$ . 10ml of saline solution was transferred into the  $10^0$  test-tube and 9 ml into the other test-tubes using 10 ml pipette. 1g of the prepared sample was added into the test-tube labeled  $10^0$  and the mixture was homogenized. 1ml of its content was transferred to the test tube labeled  $10^{-1}$  test tube and the mixture was also homogenized, 1ml was transferred into  $10^{-2}$  test tube. In the same manner subsequent transfers were made to test tube labeled  $10^{-3}$  and from  $10^{-3}$  test tube into  $10^{-4}$  test tube. Five (5) sterilized petri-dishes were labeled ( $10^{-3}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-4}$  and control). 5 ml of prepared sterile nutrient agar were transferred into each of the 5 petri-dishes, allowed to cool and solidify before 0.1ml each from the corresponding test-tubes were inoculated into the surface of the petri-dishes. The inoculated medium was spread on the agar plates using a sterile bent glass rod. The inoculated plates were transferred into anaerobic jar and were incubated at 32°C for 24 hours. After incubation, the plates were examined and colonies that developed were counted and recorded, and taken as the total number of bacteria enumerated from the sample. Also, the cultural characteristics of the colonies were observed and three types of bacteria (spherical, swarthy and rod-shape) were isolated from the poultry droppings. No colony was observed in the control.

### 2.4 Preparation of Broth Culture

The preparation of the broth culture was carried out by inoculating the three types of bacteria isolated from the poultry dropping sample into 50 ml peptone water in the round bottom conical flask and then incubated at 32°C for 5 days.

#### Batch Digester Experimental Set-Up

150 g of prepared sample was transferred into 250 ml beaker, distilled water was added to make up 80% moisture content and 20% Total Solid (TS) for optimum production of biomass as discussed in literatures (Ofunne , 1997). The diluted sample was divided equally into two (2) beakers, labeled A and B. Beaker A was then inoculated with 20 ml of the mixed culture inoculums isolated from the poultry droppings and shaken properly. Then the content of beaker A was divided equally into 35 universal bottles labeled  $A_1$ ,  $A_2$ ,  $A_3$ , ...,  $A_{34}$ ,  $A_{35}$ . The same was repeated for beaker B without inoculation. The substrate and biomass concentrations were determined in terms of the chemical oxygen demand (COD) and the mixed liquor volatile suspended solids (MLVSS) respectively. The initial pH, Chemical Oxygen Demand (COD) and MLVS analysis were recorded. Then all the 70 universal bottles were incubated at 32°C for optimum digestion. These measurements were repeated daily for 35 days.

## III. RESULTS AND DISCUSSION

### 3.1 Effect of Bioaugmentation on Growth Phases

The microbial growth during the period of the experiment for both bioaugmented and non-bioaugmented reactors is presented in Figure 1. The first 4 days is the lag phase for the non-bioaugmented reactor while that of the bioaugmented was a period of only 2 days. The inoculation was responsible for the quick acclimatization of the microbes in the bioaugmented reactors, hence the reduced lag phase. Biomass concentration increased steadily from 7 mg/l to 10 mg/l in the non-bioaugmented (control) bioreactor within

the first 4 days; while in the bioaugmented bioreactor the increases was, from 16 mg/l to 20 mg/l within the first 2 days .

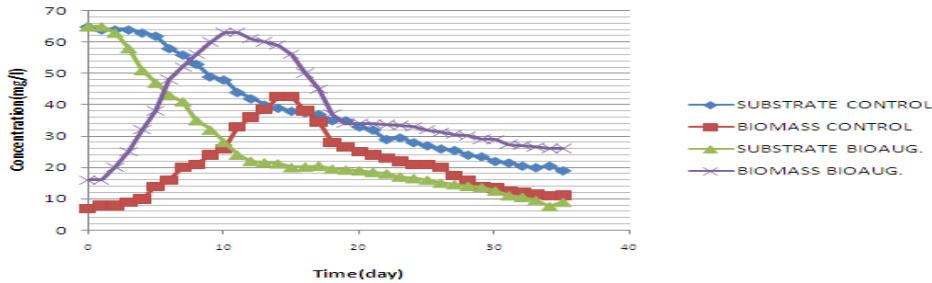


Figure 1: Plot of Microbial Population versus Days

This is the period the microorganisms are adjusting to the shock of a rapid switch to a new environment. The microbial concentration increases exponentially from 10 mg/l to 42.5 mg/l in the next 10 days for the reactors under control; which is the same number of days reported elsewhere ( Igoni *et al.*, 2006). In the bioaugmented reactor, the exponential increase was observed from 20 mg/l to 63 mg/l in the next 8 days. This period is known as the exponential growth phase, which could be deduced to full adaptation of the microorganisms to their new environment, and the metabolic activities at the maximum rate with the presence of abundant nutrients (i.e. substrate) to sustain microbial growth. Bioaugmentation decreased the exponential growth phase by two days but there was no significant effect on the stationary phase. It can also be seen from Figure 1 that a rapid decline in microbial concentration from 42.5 mg/l on day 15 to 16 mg/l on the 28<sup>th</sup> day for the control reactor, while 63 mg/l and 34 mg/l were observed on day 11 and 19 for the bioaugmented reactor. This period of sharp decline of microbial concentration is regarded as the exponential death phase of the microorganisms. The microbial concentration decreased steadily from 16 mg/l to 11.14 mg/l for 28 and 35 days respectively for the control reactors; whereas in the bioaugmented reactors, a steady decrease from 34 mg/l to 26 mg/l on 19 and 35 days. This decrease in microbial population may be attributed to non-favourable environmental conditions for cell growth may be due to exhaustion of nutrients, production of toxic products, and existence of growth limiting nutrient (Reynold and Richard, 1996; Kiely, 1997 and Ogoni, 2002)

### 3.2 Effect of Bioaugmentation on Kinetic Parameters

A plot of  $\ln[X]$  against time for the period of cell growth in the bioreactor gives a straight line of slope,  $\mu_{\max}$  (Yelebe and Puyate 2009) as shown in Figure 2.

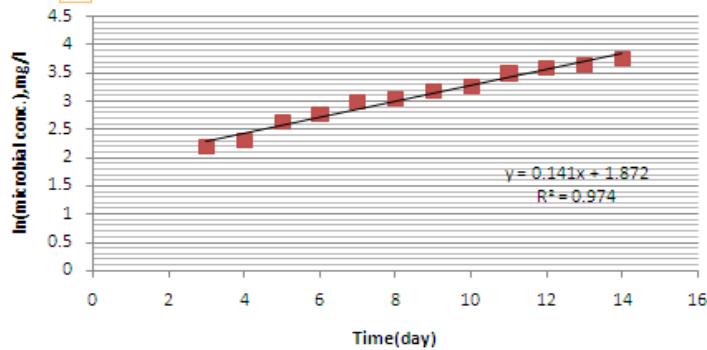


Figure 2: Plot of  $\ln[\text{Microbial Conc.}]$  against Time for control Bioreactor

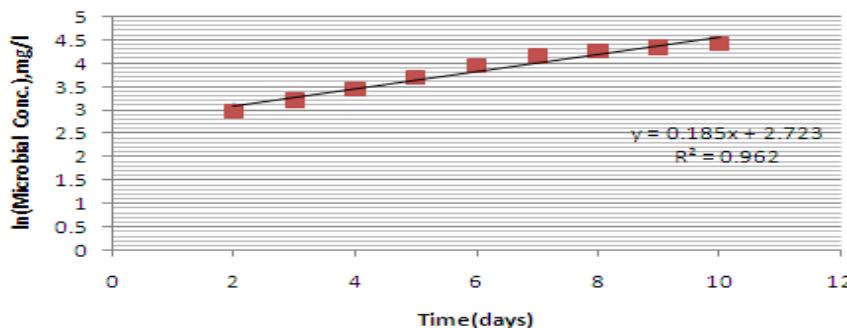


Figure 3: Plot of  $\ln[\text{Microbial Conc.}]$  against Time for Bioaugmented Bioreactor

From Figures 2 and 3, the  $\mu_{\max}$  for the control or non-bioaugmented reactor is  $0.141 \text{ day}^{-1}$  while that of the bioaugmented reactor is  $0.185 \text{ day}^{-1}$ ; 31 percent increase in the  $\mu_{\max}$ . The yield,  $\gamma$  the fraction of substrate converted to biomass, (mg/l of biomass/mg/l of substrate).The yield could be defined as (Kiely , 1997)

$$\gamma = \frac{-d[X]}{d[S]} \quad (2)$$

The yield calculated from the experimental data for non-bioaugmented and bioaugumented was 0.09 and 0.17 which lie between the specified range of 0.08 to 0.2 for anaerobic digestion.(Kiely, 1997). Bioaugmentation increases the yield by 97 percent. The main purpose of anaerobic digestion or treatment is the minimization of waste pollution potential and the provision of renewable energy. The COD removal efficiency for both the non-bioaugumented and bioaugmented reactors for the anaerobic digestion of poultry droppings is shown in Figure 3.

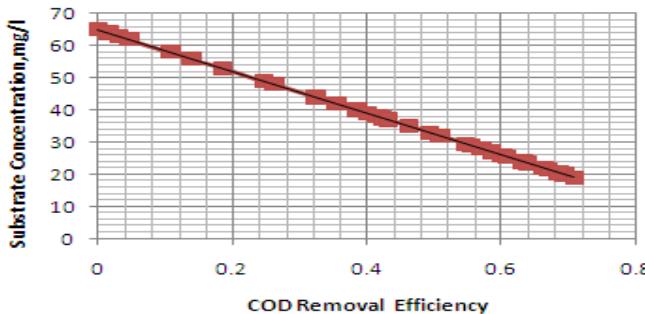


Figure 4: Plot of Substrate Conc. against COD Removal Efficiency for Control Bioreactors

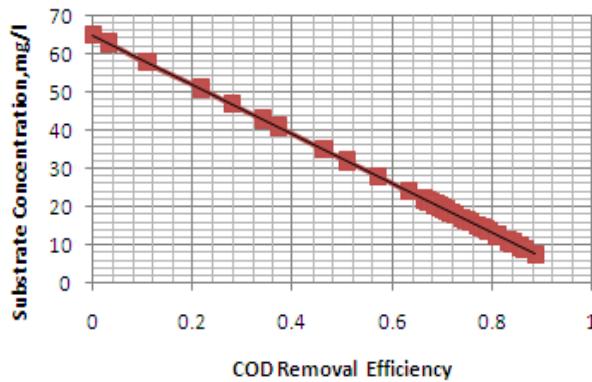


Figure 5: Plot of Substrate Concentration against COD Removal Efficiency for Bioaugmented Reactors

In Figure 3, the COD removal efficiency for the non-augmented bioreactor for the anaerobic digestion of poultry droppings is 0.71; while 0.88 was observed for the bioaugmented reactor. The higher COD removal efficiency implies higher biogas production and lower residual unreacted organics (Gunjan , 2010 ; Bailey and

Ollis 1986). Nwabanne et al., (2009) also demonstrated that a plot of  $\ln\left[\frac{S_o}{S_e}\right]$  against time with a straight line

is an indication of a first order biokinetics. From Figures 6 and 7, both the control and bioaugmented reactors gave a straight line graph; showing that bioaugmentation does not affect the order of the reaction.

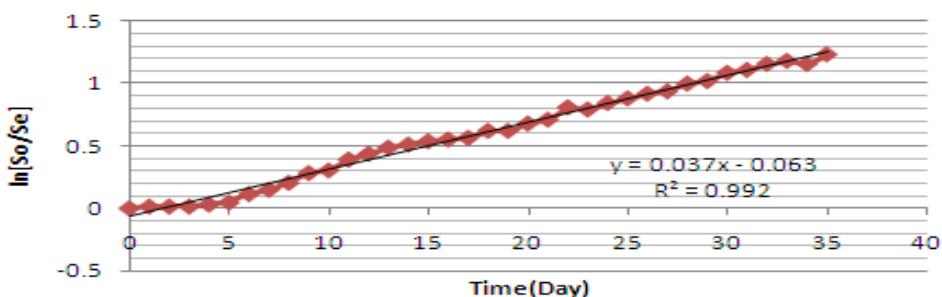


Figure 6: A Plot of  $\ln\left[\frac{S_o}{S_e}\right]$  against time for the Control Reactors

Figure 7: Plot of  $\ln[So/Se]$  against Time for Bioaugmented Reactors

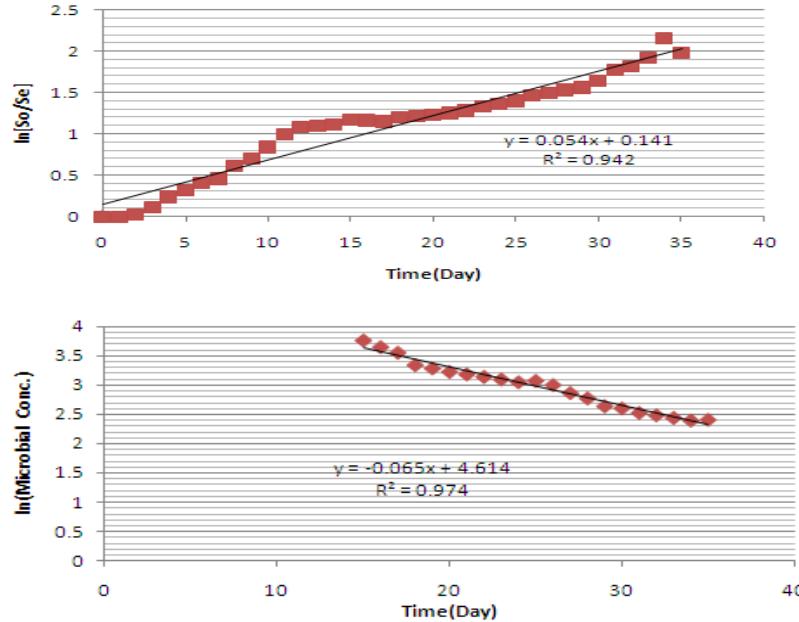


Figure 5: A Plot of  $\ln[X]$  against time in the Death Phase for Control Reactors

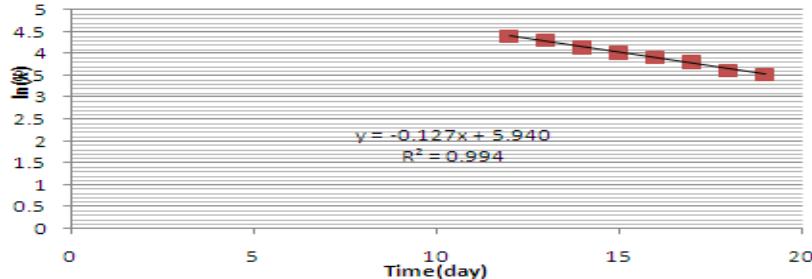


Figure 9: Plot of  $\ln[X]$  against Time for the Death Phase of Bioaugmented Reactor

A plot of  $\ln[X]$  versus time in the death phase will give a straight line of slope  $-K_d$  (Kiely 1997). From Figures 8 and 9,  $K_d$  for the control and the bioaugmented reactors is  $0.065 \text{ day}^{-1}$  and  $0.127 \text{ day}^{-1}$  respectively. As expected, the microbes in the bioaugmented bioreactor decay faster due to faster exhaustion of nutrient and production of metabolic products and space. But in the design of anaerobic digester, the reactor should be designed for the period of cell growth.

#### IV. CONCLUSION

The inoculation of the reaction broth with indigenous anaerobes in the mesophilic anaerobic digestion of poultry droppings tremendously affected the biokinetic parameters. In the growth phases, there is shorter lag phase period and shorter exponential growth phase with a higher specific growth. The decay coefficient in the inoculated reactor is high as compared to the non-inoculated reactor, signifying a higher death rate. However, in design of biological systems, the target is the exponential growth phase for optimum reactor performance. Shorter period of exponential growth phase implies small hydraulic retention time; signifying small reactor size, and hence a lower reactor cost. Also, a higher COD conversion efficiency is obtained in the inoculated reactors, which means a higher biogas production potentials and lower residual unreacted organics. In practice, inoculation or bioaugmentation could be achieved by the recycle of digested sludge as inoculums and raw sludge as feed. The value of recycle ratio used should be a function of the biomass yield for the anaerobic digestion of the poultry droppings for the recycle stream to serve as inoculum, and it lies between 0.08 and 0.2 for anaerobic digestion (Kiely 1997). Inoculation is recommended in digester design in the mesophilic anaerobic digestion of poultry droppings. However, the performance evaluation of reactor types in the mesophilic anaerobic digestion of poultry droppings is the focus for further investigations.

## NOMENCLATURE

- [X] Biomass concentration, mg/L  
[X<sub>e</sub>] Concentration of biomass in effluent, mg/L  
[X<sub>o</sub>] Initial biomass concentration, mg/L  
 $\mu_{\max}$  Maximum specific growth rate, day<sup>-1</sup>  
[S<sub>0</sub>] Influent Concentration, mg/L  
[S<sub>e</sub>] Effluent Concentration, mg/L  
K Maximum rate of substrate utilization, day<sup>-1</sup>  
K<sub>d</sub> Endogeneous decay coefficient, day<sup>-1</sup>

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