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BIOKINETICS OF MESOPHILIC ANAEROBIC DIGESTION OF POULTRY DROPPINGS

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ABSTRACT

Kinetic parameters for mesophilic anaerobic digestion of poultry droppings were determined experimentally in a batch reactor using mixed culture of indigeneous microorganisms isolated from the waste. The maximum rate of substrate utilization (K), yield coefficient (γ), saturation constant (K_s), endogeneous decay coefficient (K_d) ,COD removal efficiency(COD_{reff}) and maximum specific growth rate (μ_{max}) were found to be 1.09 day^{-1}, 0.09 , 73.015 mg/L , 0.065day^{-1} ,0.71 and 0.139 day^{-1} respectively. These biokinetics parameters if well annexed could prove invaluable in the efficient operation, design and control of bioreactors for mesophilic anaerobic digestion of poultry droppings for pollution control, energy and fertilizers for soil conditioning.

Keywords: *Poultry droppings, anaerobic digestion and kinetic parameters*

INTRODUCTION

Green plants capture solar energy by photosynthesis. The captured solar energy is stored in biomass. Biomass, a high energy density system, such as trees, grasses, agricultural crops, agricultural 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 themselves 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. Anaerobic digestion has been deemed one of the most useful decentralized sources of energy supply by the United Nations Development Programme. With the Clean Development Mechanism (CDM) arrangement under the Kyoto Protocol, industrialized countries with a commitment to reduce their own, invest financially in the implementation of anaerobic digestion systems in developing countries (UNFC, 2007). In the past decades, the consumption of poultry in Nigeria and in many other countries have 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 (FAO, 2011). Poultry droppings can be considered as a sustainable biomass; a broiler produces approximately 11 gDM/bird/day of poultry droppings while a layer generates 32.9 gDM/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, and the slurry from the digester could be converted into fertilizers (Hetal, 2006). 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 dump 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 concerns (CDM, 2005). 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). A clear understanding of the biokinetics of anaerobic digestion of poultry waste is the first step to optimization, control and operation of the digester.

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 Puyate (2009) studied the biokinetics of aerobic digestion of municipal solid waste has been studied extensively. In their work, Monod growth kinetics was used to model aerobic degradation of municipal solid waste in bioaugumented and non-bioaugumented batch reactors using a mixture of indigenous microorganisms isolated from the waste. Igoni *et al.,* (2006) and Igoni *et al*., (2008) have 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) has also developed kinetic model for anaerobic digestion of beef cattle manure. However, literature on biokinetics of mesophilic anaerobic digestion of poultry droppings is scare. The purpose of this paper is to present the biokinetics of mesophilic anaerobic digestion of poultry droppings.

MATERIALS AND METHOD

Sample Preparation

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

Determination of Moisture Content

The moisture content is required to determine 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 (2g) of the prepared sample was dried in an oven for four hours at 150^{o}C, until the weight of sample becomes constant and the moisture content was determined by

$$Moisture\ \ Content = \frac{W_1 - W_2}{W_1} x100 \quad (1)$$

Where W_1 = Initial weight of sample before drying, g

W_2 =Final weight of sample after drying, g

Batch Digester Experimental Set-Up

150g of prepared sample was transferred into 250 ml beaker, and distilled water was added to make up 80% moisture content and 20% Total Solid (TS) for optimum production of biomass. The diluted sample was divided equally into 35 universal bottles labeled A_1, A_2 , A_3, ...A_{34}, A_{35} . The initial pH, Chemical Oxygen Demand (COD), biomass concentration and microbial count analysis were determined and recorded as pH_0, COD_0 and X_0 respectively. Then, all the 35 universal bottles were incubated at 32°C for optimum digestion. Subsequently, samples were brought out daily, for pH, COD and Microbial Count analysis determination and recorded as pH_i, COD_i and X_i., Where i represent numbers of days count.

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. 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 9ml into the other test-tube using 10ml pipette. 1g of the prepared sample was put into the test-tube labeled 10^0. The mixture was homogenized, and 1ml of its content was transferred to the test tube labeled 10^{-1}. This mixture was also homogenized before 1ml was also 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-dish were labeled (10^{-3}, 10^{-3},10^{-4}, 10^{-4} and control). 5 ml of prepared sterile nutrient agar was also transferred into each of the 5 petri-dish and allowed to cool and solidified before 0.1 ml each from the corresponding named test-tube were inoculated into the surface of the petri-dish. 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, swarmy and rod-shape) were isolated from the poultry droppings. No colony was observed in the control.

Kinetics of Microbial Growth

The material balance for microbial growth for the anaerobic digestion of poultry droppings is given by

$$\begin{pmatrix} Rate\ of\ Accum \\ of\ materials\ in \\ \text{Re}actor \end{pmatrix} = \begin{pmatrix} Rate\ of\ materials\ flow \\ \text{int}o\ reactor \end{pmatrix} + \begin{pmatrix} Rate\ of\ Appearance\ or\ Disapp. \\ of\ materials\ due\ to \\ reaction \end{pmatrix} - \begin{pmatrix} Rate\ of\ Materials \\ flow\ out\ of\ reactor \end{pmatrix} \quad (1)$$

Equation (1) can be expressed mathematically as

$$\frac{d[X]}{dt}V_r = Q[X_i] + V_r r_{g_{net}} - Q[X] \quad (2)$$

where it is assumed that the death rate of microorganisms during the period of growth is zero or negligible compared to the growth rate, V_r is the volume of reactor, r_{gnet} is the net growth rate of microorganisms (mass/(volume x time)), [X] is the concentration of microorganisms, t is time.

For a batch digester where there is no flow (i.e.Q.=0), equation (2) becomes

$$\frac{d[X]}{dt}V_r = r_g V_r = \mu[X]V_r \qquad (3)$$

where μ is the specific growth rate(time^{-1}) of microorganisms which is related to substrate concentration,[S] in the form (Kiely 1997 ; Sincero and Sincero, 2004)

$$\mu = \mu_{\max}\frac{[S]}{K_S + [S]} \qquad (4)$$

Where [S] is the concentration of substrate, μ_{max} is the maximum specific growth of microorganism, K_s is the substrate concentration when μ is equal to $\mu_{max}/2$. Combining equations (3) and (4) gives the batch culture rate equation for cell growth as

$$\frac{d[X]}{dt} = \mu_{\max}\frac{[S][X]}{K_S + [S]} \qquad (5)$$

Taking into account die-off or kinetics and dynamics of death; an endogenous decay (rate constant, ,K_d) is incorporated into equation (5), the rate of increase of biomass becomes

$$\frac{d[X]}{dt} = \mu_{\max}\frac{[S][X]}{K_S + [S]} - K_d[X]$$

In the growth of microbial culture, not all the substrate is converted to cells. Hence, yield coefficient (γ <1) is introduced such that the rate of substrate utilization is related to the rate of cells formation. Yield is defined as (Kiely 1997).

$$-\gamma\frac{d[S]}{dt} = \frac{d[X]}{dt} \qquad (7)$$

Also, the substrate utilization rate can be modeled as (Bailey and Ollis, 1996)

$$\frac{d[S]}{dt} = \frac{-[K][S][X]}{K_S + [S]} \qquad (8)$$

which is the Michaelis-Menton equation and

$$K = \frac{\mu_{\max}}{\gamma} \qquad (9)$$

where K is the maximum rate of substrate utilization per unit mass of cells produced.

The death rate or decay of biomass can be expressed as(Kiely 1997 and Ogoni 2004).

$$-\frac{d[X]}{dt} = K_d[X]$$

(10)

where K_d is the endogeneous decay constant, day^{-1}

Determination of Kinetic Parameters

For a normal batch fermentation process,[S] is much larger than K_S for most of the growth period (i.e.[S]>>K_S) (Yelebe and Puyate 2009); Richard and Peacock 2006). Applying this condition in equation (5) yields

$$\frac{d[X]}{dt} = \mu_{\max}[X] \quad (11)$$

Separating variables in equation (11) and integrating gives

$$\ln[X] = \mu_{\max} t + \ln[X_o] \quad (12)$$

A plot of $\ln[X]$ against time for the period of cell growth in the bioreactor gives a straight line with a correlation coefficient of 0.983 of slope $\mu_{\max}$ equal to 0.139 day^{-1} as shown in Figure 2

Equation (7) can be rewritten as

$$\gamma = \frac{-d[X]}{d[S]} \quad (13)$$

Equation (8) can be written as

$$\frac{dt}{d[S]} = -\frac{K_S + [S]}{K[S][X]} \quad (14)$$

Equation (14) can be expressed as

$$\frac{dt}{d[S]} = \frac{-K_S}{K[S][X]} - \frac{1}{K[X]} \quad (15)$$

Separating variables and integrating yields

$$\int_{t=0}^{t=t} dt = -\int_{S_o}^{S_e} \frac{K_S dS}{K[S][X]} - \int_{S_o}^{S_e} \frac{dS}{K[X]} \quad (16)$$

Therefore,

$$t = \frac{K_S}{K[X]} \ln\left[\frac{S_o}{S_e}\right] + \frac{[S_o]-[S_e]}{K[X]} \quad (17)$$

Equation (17) expresses the time, t required to degrade the substrate from concentration S_o to S_e, which is also called the time for batch digestion (Igoni *et al*., 2008). And [X] is the average cell mass concentration equals $(X_o+X_e)/2$ (Igoni *et al*., 2008; Reynold and Richard 1996). Equation (17) can be written as

$$\ln\left[\frac{S_o}{S_e}\right] = \frac{K[X]}{K_S} t - \frac{[S_o]-[S_e]}{K_s} \quad (18)$$

Equation (18) shows clearly that a plot of $\ln\left[\frac{S_o}{S_e}\right]$ against t will give a straight line, with a slope $\frac{K[X]}{K_s}$ and intercept of $-\frac{[S_o]-[S_e]}{K_s}$; from Figure 4, K and K_s are determined as 1.09day^{-1} and 73.015mg/l respectively.

Separating variables and integrating equation (10) yields

$$\ln[X] = -K_d t + \ln[X_o] \quad (19)$$

Equation (19) implies that a plot of ln[X] versus time in the death phase will give a straight line of slope $-K_d$. From Figure 5, K_d is 0.065 day^{-1}.

RESULTS AND DISCUSSION

Growth Phases

Figure 1 shows the result of the microbial growth during the period of the experiment. The first 4 days of the experiment is the lag phase where the microbial population increases steadily from $7.0x10^4$ cfu/l to $10x10^4$ cfu/l in the bioreactor. In this phase, the microorganisms are adjusting to the shock of a rapid switch to a new environment. The microbial population increases exponentially from $10x10^4$ cfu/l to $35x10^4$ cfu/l in the next 10 days; which is in agreement reported in literature (Yelebe and Puyate 2008; Nwabanne *et al*., 2009). This period is known as the exponential growth phase, which could be adduced to full adaptation of the microorganisms to their new environment, and their metabolic activities are at the maximum rate with the presence of abundant nutrients (i.e. substrate) to sustain microbial growth. It can also be seen from Figure 1 the rapid decline in microbial population from $35x10^4$ cfu/l to 8.8×10^4 cfu/l on day 15th to 35th respectively. This period of sharp decline of microbial population is regarded as the exponential death phase of the

microorganisms. The decrease in microbial population after day 15th may be attributed to non-favourable environmental conditions for cell growth resulting from any one or combination of the following factors: (i) exhaustion of nutrients (ii) production of toxic products, and (iii) existence of growth limiting nutrient (Reynold and Richard, 1996; Kiely 1997; and Bailey and Ollis, 1986).. In each of the stages of the growth, the population density of the microorganisms lies between the specified range of 10^5 to 10^9 per ml for anaerobic digestion(Kiely 1997).

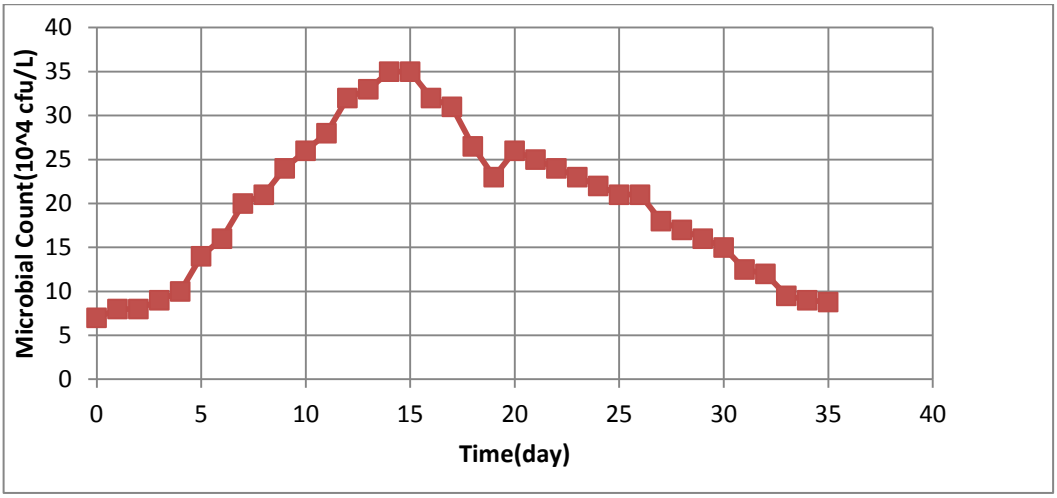


Figure 1: Plot of Microbial Population versus Days

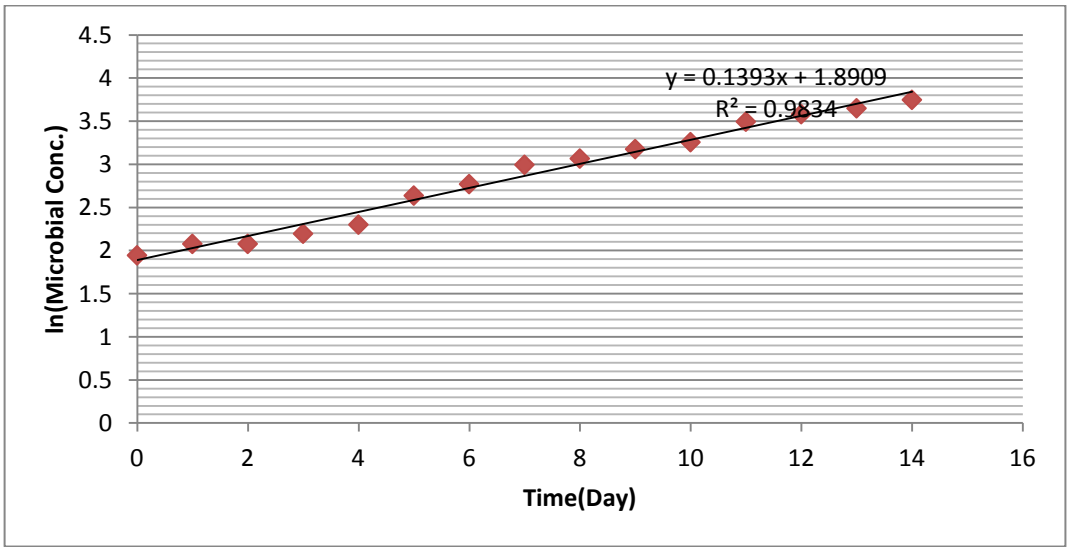


Figure 2: Plot of ln[Microbial Conc.] against Time in the Growth Phase

The yield, γ is the fraction of substrate converted to biomass, (mg/l of biomass/mg/l of substrate). The yield calculated from the experimental data is 0.09, which lies between the specified range of 0.08 to 0.2 for anaerobic digestion (Kiely 1997). (See Figure 2). The low yield explains why anaerobic digestion is not considered as a complete processor of wastewater on its own, but an addendum to existing conventional aerobic processes; anaerobic digestion reduce high organic loads to the magnitudes of COD that can be accommodated in conventional aerobic processes, most typically activated sludge (Kiely 1997).

The main objectives of anaerobic digestion or treatment are minimization of waste pollution potential and the provision of renewable energy. The COD removal efficiency of anaerobic digestion of poultry droppings is shown in Figure 3. In Figure 3, the COD removal efficiency for the bioreactors for the anaerobic digestion of poultry droppings is 0.71; which falls between the range 65% to 85% for anaerobic digestion (Gunjan 2010). The higher the COD removal efficiency, the higher the biogas production and lower the residual unreacted organics (Gunjan 2010; Bailey and Ollis, 1986). The value of the kinetic parameters, K and K_s are determined as 1.09 day^{-1} and 73.015 mg/l respectively, which indicates that the anaerobic digestion of poultry droppings will require inoculation to speed up the process instead of allowing it to depend on self-generation and subsequent regeneration. The pH of the bioreactor during the period of experimentation is shown in Figure 6; which ranges from 6.2 to 7.5. The volatile fatty acids produced from the hydrolysis of complex particulate organic matter is supposed to depress the pH of the bioreactors, but the bicarbonate formed when the CO_2 is produced, which is soluble in water reacts with hydroxide ions, is responsible for the observed pH, which lies within the range of 6 to 8 for anaerobic digestion reactions (Kiely 1997;)

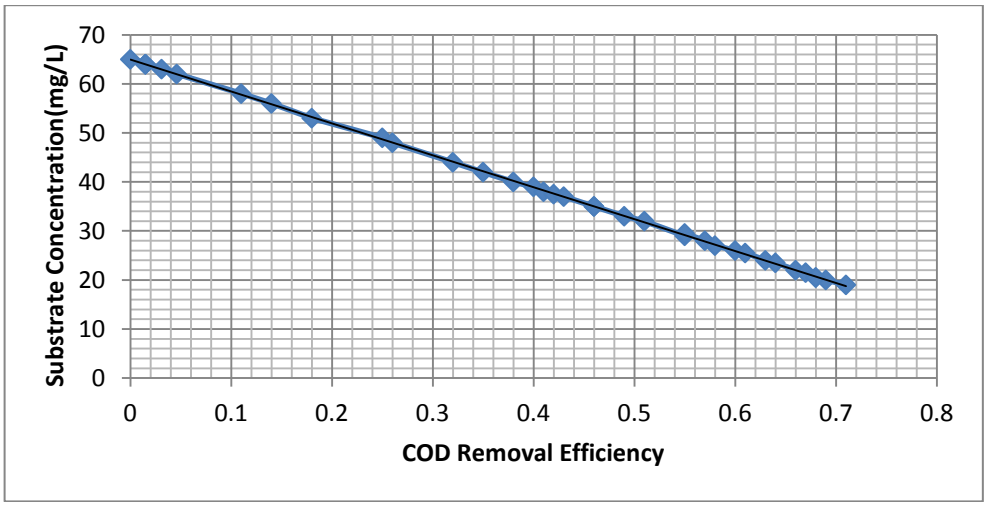


Figure 3: A Plot of Substrate Conc. against COD Removal Efficiency

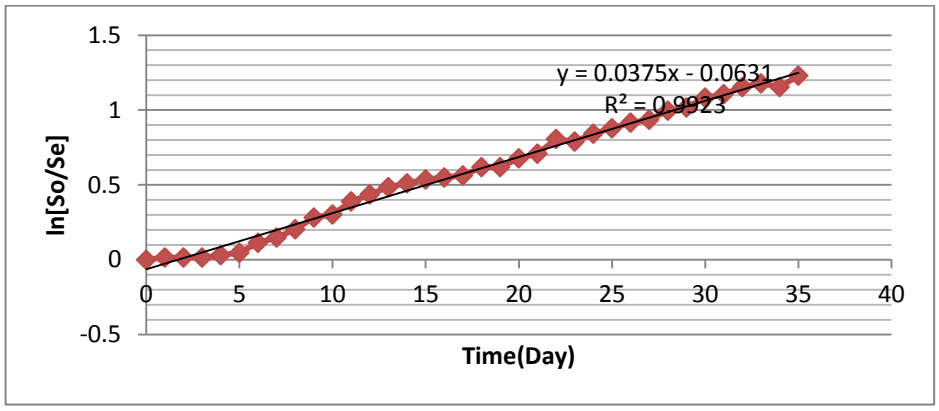


Figure 4: A Plot of $\ln\left[\frac{S_o}{S_e}\right]$ against time

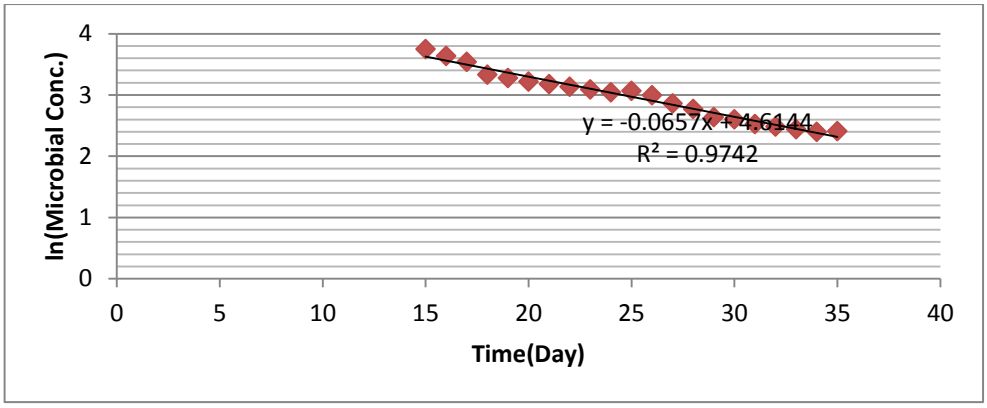
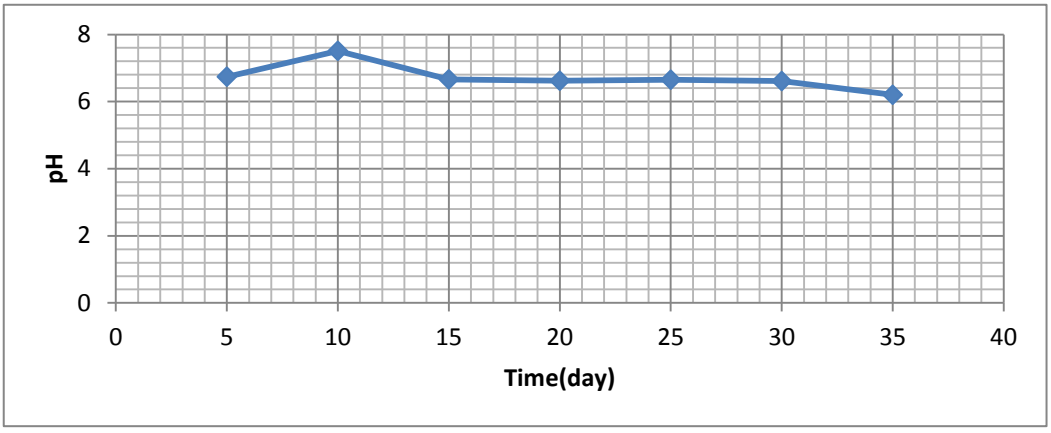


Figure 5:A Plot of ln[X] against time in the Death Phase



CONCLUSION

Anaerobic digestion is the use of microbial organisms in the absence of oxygen for the stabilization of organic materials by conversion to methane and inorganic products including CO_2.It reduces the pollution potential of waste, eliminates pathogens; improve the fertilizer/fuel value of waste product and produces biogas as energy source. The kinetic parameters for anaerobic digestion of poultry droppings, K, $COD_{\mathrm{Re}ff}$, K_s, γ, K_d, and $\mu_{\max}$ were determined ;which is the first step in the utilization of anaerobic digestion for the control of poultry induced pollution and energy production. The value of the kinetic parameters indicates that the anaerobic digestion of poultry droppings will require inoculation to speed up the process instead of allowing it to depend on self-generation and subsequent regeneration. The effect of bioaugumentation on kinetic parameters is the focus of future investigations.

NOMENCLATURE

[X] Biomass concentration, mg/L
[X_e] Concentration of biomass in effluent, mg/L
[X_o] Initial biomass concentration, mg/L
$\mu_{\max}$ Maximum specific growth rate, day^{-1}
μ Specific growth rate, day^{-1}
K Maximum rate of substrate utilization, day^{-1}
K_d Endogeneous decay coefficient, day^{-1}
K_s Half velocity constant, mg/L
Q Flow rate, volume/time
S_o Influent substrate concentration, mg/L
S_e Effluent substrate concentration, mg/L
γ Biomass

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