

Kinetics of Bioremediation of Shinko Drainage Wastewater in Jimeta-Yola Using *Bacillus Subtilis*

Yusufu Luka¹, Haruna Mavakumba Kefas², Jackson Robinson Genza³, Bukhari Auwal Abdul-hamid⁴.

^{1,2,3,4}Department of Chemical Engineering, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria.

Abstract— The use of *Bacillus subtilis* microorganism to study the kinetics of bioremediation of Shinko drainage wastewater used for agriculture and channeled into River Benue was carried out. The bioremediation was observed for a period of twenty six days at an interval of two days. A bioreactor was used under aerobic condition to achieve the degradation of contaminant. *Bacillus subtilis* was isolated from Shinko drainage wastewater and effective in reducing the substrate concentration from 65.35 mg/l to 15.59 mg/l for bioaugmented sample. While that of indigenous bacteria was reduced from 65.35 mg/l to 22.05 mg/l. The kinetic models obtained for waste reduction are: $S=65.35+2.5217t$ (Zero Order indigenous); $S=65.35+3.052t$ (Zero Order bioaugmented); $S=28.56e^{(-0.0265t)}$ (First Order indigenous); $S=22.65e^{(-0.0369t)}$ (First Order bioaugmented).

Keywords— Bioremediation, *Bacillus subtilis*, Kinetics, wastewater

I. INTRODUCTION

Pollutants are readily associated with surface water and Shinko drainage wastewater which has many tributaries from Jimeta-Yola metropolis is not an exception. Organic containing wastewater needs careful handling before they can be reused or discharged to the receiving water bodies [1; 2; 3].

Global attention has been drawn on ways to sustain the environment using microorganism to remediate environmental pollutants. Reasons, physical and chemical treatment are costly and can lead to production of toxic substance [4]. Therefore, bioremediation involves the use of microorganism to reduce or remove the pollutants from contaminated area which may lead to restoration of the original natural substance without further disruption to the local environment [5; 6].

Biodegradation requires the conversion of pollutants by microorganism into energy, cell mass and biological products. Organic pollutant under aerobic condition degrades to give stable product, water, a mixture of carbon dioxide and other gases [7; 8]. Similar procedure adopted by [4] is used to measure the extent of bioremediation by increase in biomass growth and reduction in substrate concentration.

Shinko drainage wastewater is used for irrigation and consumed by domestic animals as well as directly channeled into River Benue in Jimeta-Yola. The study carried out by many researcher including [1] shown that the river contained heavy metal at Shinko location. Many developing country in the world including Nigeria take advantage of wastewater from domestic and industries for irrigation and rearing of animals

due to lack of knowledge on its pollution status and its availability for use without treatment [9; 10].

The main focus of this study is to determine how effective *Bacillus subtilis* is in degradation of the contaminants in Shinko drainage wastewater under aerobic condition using bioreactor. Furthermore, kinetics parameters for the rate of degradation or reduction of substrate (chemical oxygen demand) with time as well as biomass growth were obtained.

II. MATERIALS AND METHODS

About 4 l of wastewater were collected from shinko drainage using sterilized plastic containers to be conveniently handled in the laboratory. The sample was preserved as stated by standard method [4]. Ten-fold serial dilution technique of analysis was used to enumerate and isolate *Bacillus subtilis* from the wastewater collected. Twenty eight grams (28 g) of nutrient agar powder was measured and added to 1 l of distilled water. The mixture was sterilized for 15 mins at 121 °C and allows cooling to 37°C. Later, 0.1 ml from the serially diluted samples from the reacting vessels plated with cultured media in petri dish. Its composition is such that it promoted the growth of *Bacillus subtilis* while inhibiting the growth of other bacteria. The petri dish is placed in the incubator at a temperature of 37±1 °C for 2 h. The colonies were formed by reproductive process of binary fission and the *Bacillus subtilis* members were measured as number of colonies per liter. This procedure is repeated for other samples. Then a broth culture was prepared for bioaugmentation. About 1 l of water samples were poured into each of the two suspended growth plastic bioreactor of volume 3.5 l. The first bioreactor was tagged control (indigenous) and the second bioreactor was labeled bioaugmented. For the bioaugmented, $10 \times [10]^{10}$ cfu/l of isolated broth pure cultures of *Bacillus subtilis* was introduced into the bioreactor making the total volume to be 1 l. The system was operated at a control temperature of 37°C and optimum pH of 7 [11]. The substrate removal was measured by recording the chemical oxygen demand concentration (COD) reduction at the interval of two days for 26 days [4; 12].

III. RESULTS AND DISCUSSION

The results of biomass growth, substrate reduction, Zero order and first order kinetics for both indigenous (control) and bioaugmented have been illustrated in Figures 1-4, respectively.

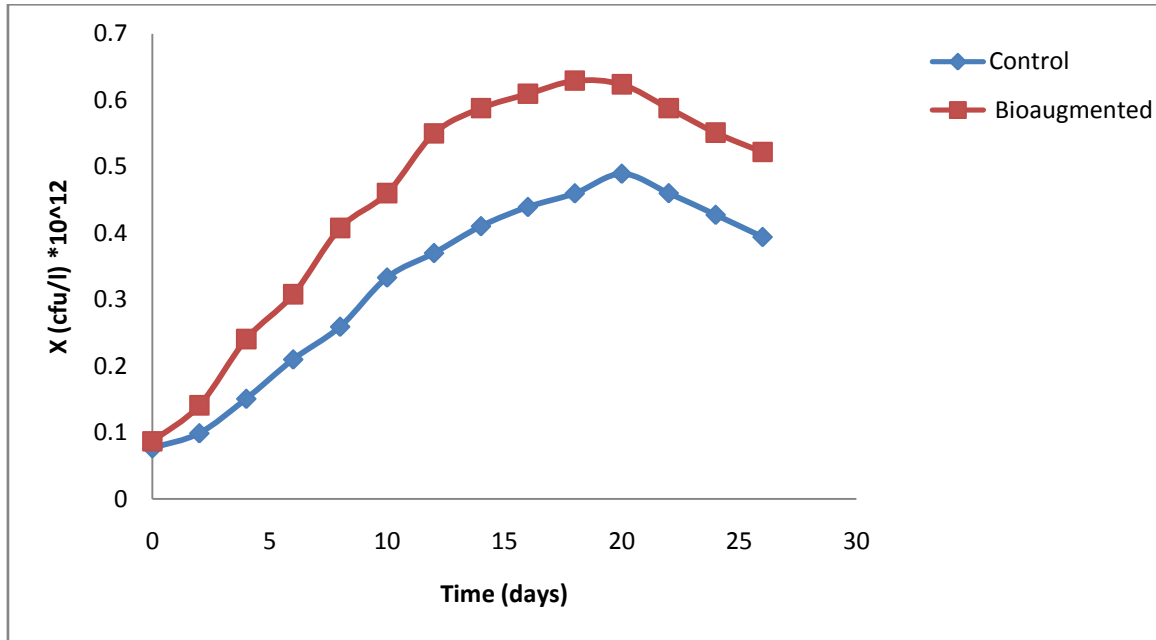
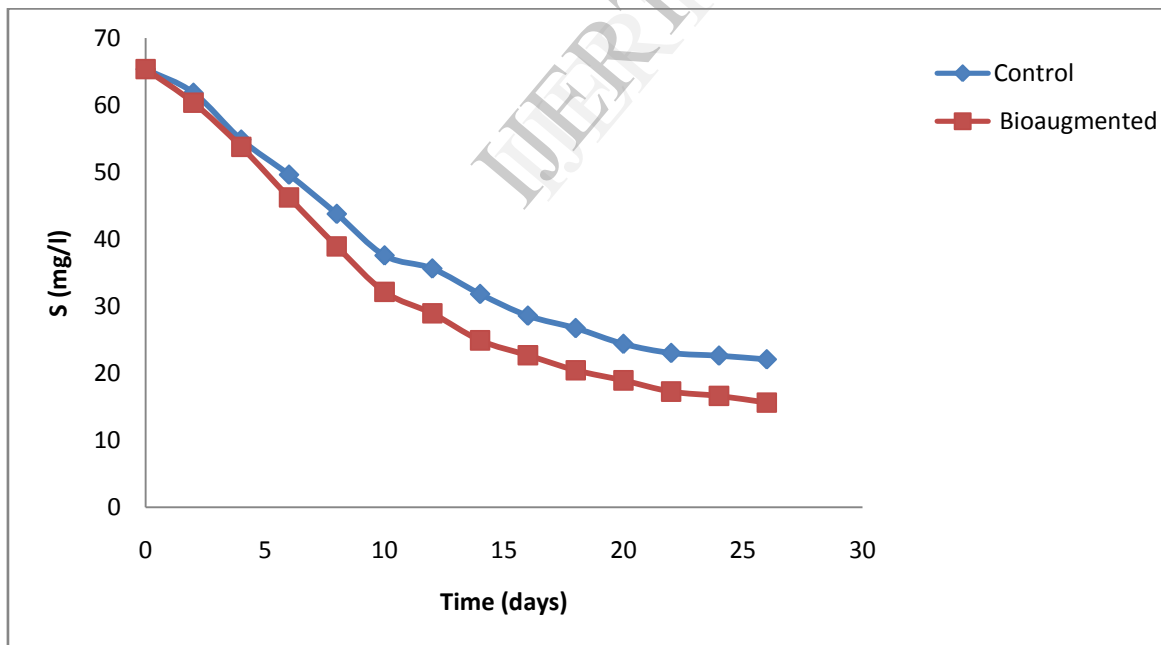


Figure1: Growth Profile of Biomass for Control and Bioaugmented Samples

Figure 2: Substrate Reduction by *Bacillus subtilis* for Control and Bioaugmented Samples

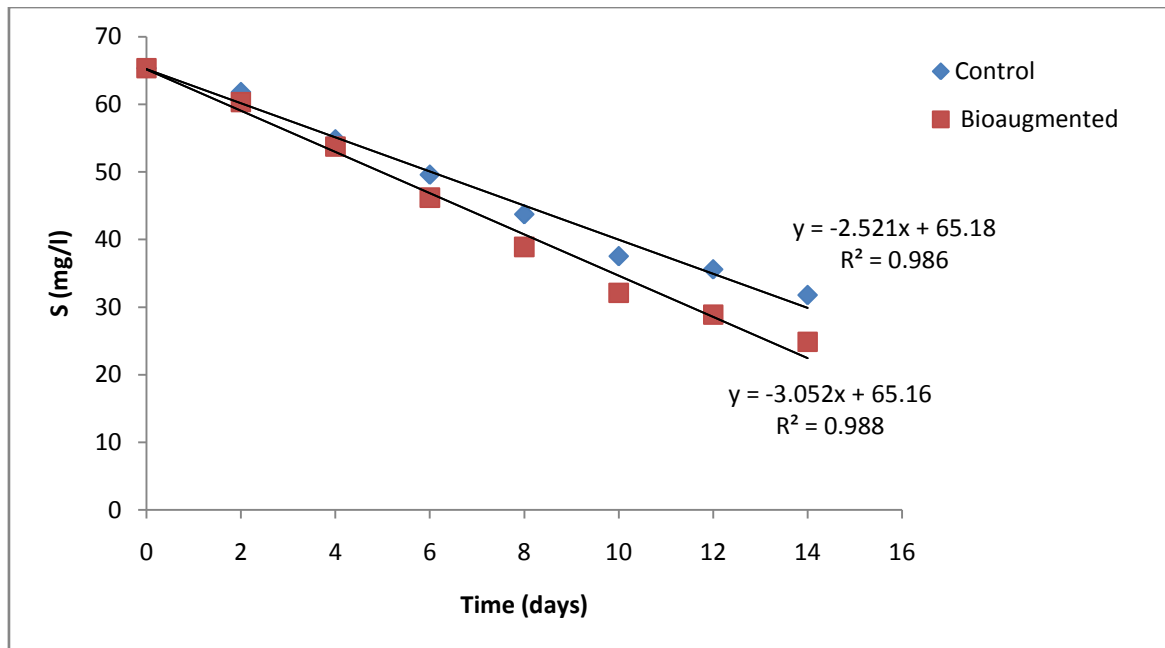


Figure 3: Testing Zero Order Kinetics for Substrate Reduction by *Bacillus subtilis* for Control and Bioaugmented Samples

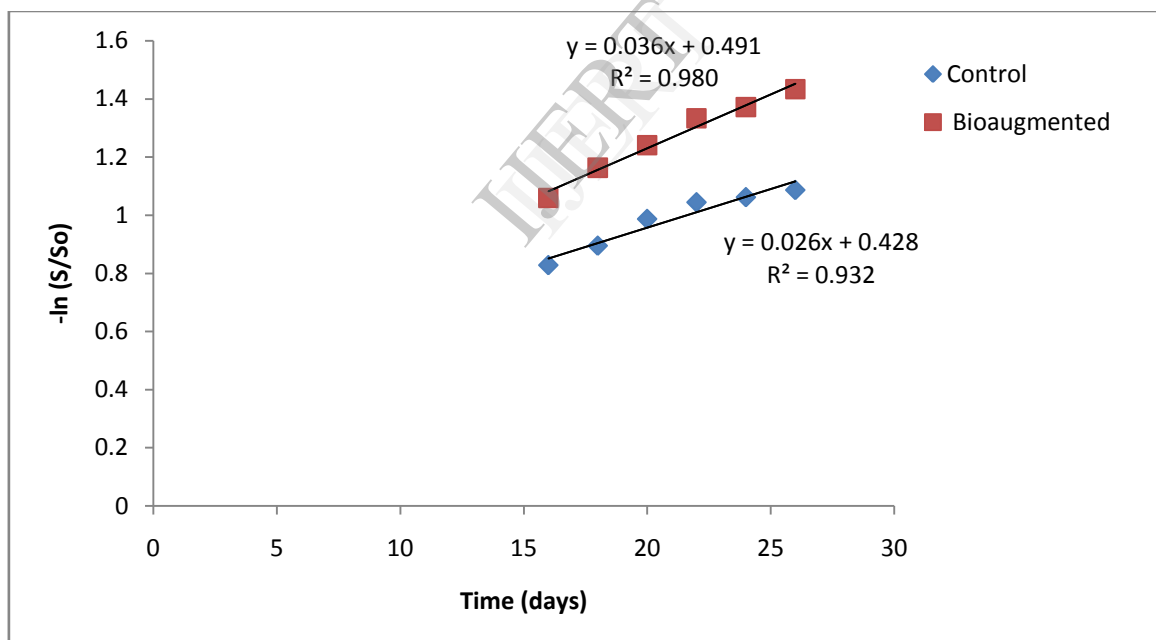


Figure 4: Testing First Order Kinetics for Substrate Reduction by *Bacillus subtilis* for Control and Bioaugmented Samples

Growth profile of biomass is shown in Figure 1.

any particular time compared to the control sample using *Bacillus subtilis*. There was no lag time observed in the microbial growth. This might be due to the fact that *Bacillus subtilis* in the bioaugmented and indigenous (control) sample of wastewater were not exposed to entirely different environment before being introduced to the bioreactor [7; 13]. After 20 days, bacteria stopped multiplying with the

Bioaugmented sample gives a better growth for biomass at maximum value of 48.93×10^{10} cfu/l for indigenous sample while at 18 days the bacterial stopped multiplying with a maximum value of 62.92×10^{10} cfu/l for bioaugmented sample which indicate the beginning of death phase for the two different samples.

Substrate reduction by bacteria is presented in Figure 2. The result shows the ability of *Bacillus subtilis* to utilize the pollutants as a source of carbon for its growth as well as

degrading the pollutants in Shinko drainage wastewater. Also better results were obtained with bioaugmented sample compare to indigenous bacteria from Shinko drainage wastewater[14].

From literature, zero order is used to observe rate of reduction at high substrate concentration and First order at moderate or low substrate concentration and the overall reaction can be regarded as shifting order reaction [4; 12; 13; 15]. The results obtained in Figure 3 depicted that substrate reduction fit Zero order from 65.35 mg/l to 31.8 mg/l with rate of -2.5217 for indigenous bacteria and from 65.35 mg/l to 24.87 mg/l with rate of -3.052 for bioaugmented sample, respectively. Using the results obtained for rate constant (k) and initial substrate concentration (So) into the integrated rate expression for Zero order which is represented by Equation 1 [4; 13]

$$S = S_0 - kt(1)$$

The kinetic models for substrate reduction at high concentration (testing Zero order Kinetic) using *Bacillus subtilis* are represented by Equation 2 and 3

For indigenous

$$S = 65.35 + 2.5217t(2)$$

For Zero Order bioaugmented

$$S = 65.35 + 3.052t$$

(3)

Figure 4 illustrated that First order testing for substrate reduction fit from 28.56 mg/l to 22.05 mg/l with rate 0.0265 for indigenous bacteria and from 22.65 mg/l to 15.59 mg/l with rate 0.0369 for bioaugmented sample, respectively. Similarly, using the results obtained for rate constant (k) and initial substrate concentration (So) into the integrated rate expression for First order which is represented by Equation 4 [4; 12; 13]

$$S = S_0 e^{-kt}(4)$$

Therefore, the kinetic models for substrate reduction at moderately or low concentration (testing First order kinetic) using *Bacillus subtilis* are represented by Equation 5 and 6

For First Order indigenous

$$S = 28.56e^{-0.0265t}(5)$$

For First Order bioaugmented

$$S = 22.65e^{-0.0369t}(6)$$

IV. CONCLUSION

Based on the result of this study, the following conclusion can be made:

- Bioaugmentation increase the efficiency of remediation because better results were recorded compare to that of indigenous bacteria.
- Zero order kinetic model fit high substrate concentration whereas First order kinetic model fit low substrate concentration. The kinetic models agree with Monod equation.
- The kinetic models and bioreactor if implemented at site will help in removing contaminant in Shinko drainage wastewater before it is being used for other purposes.

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