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Modelling and Simulation of Hydrogen Cyanide Biodegradation in Cassava Wastewater using Natural Attenuation and Bioaugmentation

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Abstract

A dynamic model was developed to describe the degradation of hydrogen cyanide (HCN) in cassava wastewater using two strategies: natural attenuation and bioaugmentation. The model was validated experimentally by estimating biodegradation rate constants k_1=0.235day^{-1}; k_2=1.320 day^{-1} for natural attenuation and k_1=0.195day^{-1}; k_2=1.309 day^{-1} for bioaugmentation. Model validation results showed that the model was able to replicate the results of the experiment to a high level of confidence as seen in the excellent correlation between experimental and model predicted results. Simulation of the model revealed that the concentration of bound cyanide decreased with time in the course of bioremediation irrespective of the strategy adopted. Bioremediation by natural attenuation performed better than bioaugmentation as it resulted in the degradation of 90.5% of the bound cyanide content of cassava wastewater as against 85.6% obtained for bioaugmentation. A modelling exercise such as this is important as it will provide insight as to how the process responds under dynamic conditions and its amenability to control.

Keywords: Cassava wastewater, Hydrogen cyanide, Bioremediation,*Pseudomonas Aeruginosa*

Nomenclature

C_B	Concentration of bound cyanide (mg/L)
C_I	Concentration of intermediate cyanide (mg/L)
C_F	Concentration of free cyanide (mg/L)
r_1	Rate of linamarase catalysed reaction
r_2	Rate of lyase catalysed reaction
χ^2	Chi-squared value

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1.0 Introduction

Cassava (*Manihot spp*) is a shrubby and tuberous plant which exists in sweet and bitter varieties. It contains about 94% carbohydrate with low fractions of protein and vitamins making it one of the most important economic root crops grown in West Africa [1-3].Nigeria is the world's largest producer of cassava with a production capacity of 45 million tonnes per annum. This value represents about 57% of tropical root and tuber production [4,5]. Following closely after Nigeria are Thailand and Brazil in second and third respectively.

The cassava tuber contains linamarin (2-(b-D-glucopyranosyloxy)isobutyronitrile) and lotaustralin (methyl linamarin) as the primary cyanogenic glycosides. The concentration of these anti-nutritional and unsafe glycosides varies considerably between varieties and also with climatic conditions hence selection of cassava species to be grown, is of huge importance. The cyanide content of cassava root may range between 75 and 1000 mg (CN) per kg depending on the plant variety and soil conditions [6].

Unfortunately, large amounts of natural cyanoglycosides found in cassava are released into liquid effluents produced during the processing of cassava tubers into consumable food materials such as garri, fufu, cassava chips, cassava flour, starch, cassava bread etc [6,7]. The presence of cyanide in cassava wastewater is of concern to humans and animals as a result of its toxicitywith exposure typically resulting in serious harm to the central nervous, respiratory and digestive systems[8,9]. Environmental regulations typically stipulate the reduction of cyanide concentration in wastewater to levels below 0.2 mg/L before discharge into natural water bodies [10].

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Current wastewater treatments methods for cyanide removal employ chemical and physical methods which are often expensive and involve the use of hazardous reagents such as chlorine and sodium hypochlorite and in many instances, complete degradation of some cyanide complexes is not achieved [6,11,12]. Adopting biological treatment methods can be less expensive than chemical and physical methods, and much faster than natural degradation processes [13]. The success of biological treatment methods depends on the presence of microbes with the physiological and metabolic capabilities to degrade the pollutants in the contaminated environment [14]. Some microorganisms such as *Bacillus*,*Rhizopus*, *Fusarium*,*Pseudomonas*, and *Klebsiella oxytoca* have been reported to possess the metabolic capability to grow using cyanide as the sole nitrogen source under aerobic and/or anaerobic conditions. This makes these organisms suited for cyanide removal from cyanide containing wastewater [6,15].
This study is focused on the quantitative evaluation of HCN content in processed cassava wastewater and subsequent treatment using bioremediation techniques facilitated by *Pseudomonas aurugenosa*. The kinetics of the degradation process was evaluated using amathematical model derived by applying material conservation laws to the process. Simulation of the formulated model will provide an understanding of the dynamic behaviour of the process and how it responds to changes in operating conditions.

2.0 Materials and Methods

2.1 Cassava Variety and Microorganism

Cassava tubers of the bitter variety were obtained from a Cassava mill in Benin City, Edo State, Nigeria. Analytical grade sodium carbonate, potassium cyanide and hydrochloric acid were supplied by Griffin and George Ltd, Loughborough, England. They were used without further purification.Pure cultures of *Pseudomonas aeruginosa*were obtained from the Department of microbiology, Faculty of life sciences, University of Benin, Benin City, Edo State, Nigeria. *Pseudomonas aeruginosa* was activated in a nutrient broth on a shaker at 30 ^oC and 200rpm for 24 h. Subsequent cultivation and maintenance were carried out in a nitrogen-free sucrose (NFS) medium containing 2% sucrose, 0.2 g/L MgSO4·7H2O, 0.073 g/L $CaCl_2 \cdot 2H_2O$, 10µM $Na_2MoO_4 \cdot 2H_2O$, 20µM $FeCl_3 \cdot 6H_2O$ and 2µM phosphate buffer pH 7.4. The cell growth was counted every 6 hours for 80 hours [6].

2.2 Extraction of Cassava Wastewater

Twenty kilograms of the cassava tubers were manually peeled and washed. The cleaned tubers were mechanically grated to a slurry state. The mash was collected from the grater and put into sacs. A screw press was used to extract the cassava wastewater which was stored in a sterilised plastic container. The total volume of cassava wastewater collected at the end of the process was about 10 litres.

2.3 Bioremediation Studies

2.3.1 *Natural attenuation*

Natural attenuation studies were carried out to determine the efficiency of the indigenous microorganisms in the degradation of cyanide contained in cassava wastewater. Cassava wastewater of volume 2 litres was measured into a 2.5 litres glass beaker and maintained at an ambient temperature of 30^oC. Sampling was done on day one to determine the initial pH and concentration of cyanide in the wastewater. For subsequent analysis, the wastewater was agitated and 20 mL of it was sampled and filtered through a 0.45-µm membrane and the residual concentration of hydrogen cyanide was determined spectrophotometrically. The sampling process was continued for a period of 10 days[4].

2.3.2 *Bioaugmentation*

Cassava wastewater of about2 litres was measured into a 2.5 litres glass beaker and maintained at an ambient temperature of 30^oC. The indigenous microbial population was augmented with pure *Pseudomonas aeruginosa* culture. Sampling was done on day one to determine the initial pH and concentration of cyanide in the wastewater after which the cassava wastewater was inoculated with pure *Pseudomonas aeruginosa*. For subsequent analysis, the wastewater was agitated and 20 mL of it was sampled and filtered through a 0.45-µm membrane and the residual concentration of hydrogen cyanide was determined spectrophotometrically. The sampling process was continued for a period of 10 days[4].

2.4 Analytical Methods

The concentration of residual hydrogen cyanide in the cassava wastewater was determined using the alkaline picrate method as described by Bradbury et al. [16]. A UV-Vis spectrophotometer (PG Instruments model T70) was used to detect the absorbance peak of the samples at a wavelength of 490 nm. Theabsorbance values were then converted to concentration values using a standard cyanide curve. The pH of the tested samples was measured using a calibrated pH electronic meter (Unican model). The measurements were made in triplicates and the mean value was recorded.

2.5 Modelling Cyanide Degradation

For the current study, the hydrogen cyanide degradation mechanism was represented as follows:

$$Bound\ HCN \xrightarrow[Linamarase]{k_1} Intermediate\ HCN \xrightarrow[Hydroxynitrile\ \text{lyase}]{k_2} Free\ HCN \quad (1)$$

The reaction mechanism represented in Equation (1) is a series reaction type which may conform with a first order rate kinetics in which the rate of HCN reduction is directly related to its concentration. In formulating the model, the following assumptions were made:

- The degradation process proceeds isothermally
- There is negligible change in the volume of the reaction vessel
- No nuclear reaction occurs; hence rate of material generation is zero.
- The contents of the reactor are assumed to be perfectly mixed.

The model was obtained from material conservation law applied to the reaction system. The general form of the conservation law is written as:

$$\begin{pmatrix}\text{Rate of accumulation}\\ \text{of component i within}\\ \text{the reactor}\end{pmatrix} = \begin{pmatrix}\text{Rate of flow of}\\ \text{component i into}\\ \text{the reactor}\end{pmatrix} - \begin{pmatrix}\text{Rate of flow of}\\ \text{component i out of}\\ \text{the reactor}\end{pmatrix} + \begin{pmatrix}\text{Rate of generation}\\ \text{of component i within}\\ \text{the reactor}\end{pmatrix} \quad (2)$$

For a batch process with no inlet and outlet streams, Equation (2) reduces to:

$$\begin{pmatrix}\text{Rate of accumulation}\\ \text{of component i within}\\ \text{the reactor}\end{pmatrix} = \begin{pmatrix}\text{Rate of generation}\\ \text{of component i within}\\ \text{the reactor}\end{pmatrix} \quad (3)$$

Equation (3) was applied to all components within the reaction vessel to obtain the equations describing the material balance of all components as follows:

$$\frac{dC_B}{dt} = -r_1 \quad (4)$$

$$\frac{dC_I}{dt} = r_1 - r_2 \quad (5)$$

$$\frac{dC_F}{dt} = r_2 \quad (6)$$

The rates of both reactions are described by the following equations:

$$r_1 = k_1 C_B \quad (7)$$

$$r_2 = k_2 C_I \quad (8)$$

Where : C_B Represents concentration of bound cyanide(mg/L)
C_I Concentration of intermediate cyanide(mg/L)
C_F Concentration of free cyanide(mg/L)
k_1 and k_2 biodegradation rate constants (min^{-1})

The model for hydrogen cyanide degradation is described by the system of Equations (4) to (8) which is a set of linear differential and algebraic equations (DAEs) and describes the dynamic behaviour of the concentration of all chemical species present in the reaction vessel.

3. Results and Discussion

3.1 Model Validation and Estimation of Kinetic Parameters

Model validation is typically the next step after developing a model for any process. This is done by estimating some of the model parameters which are not known to a high level of accuracy [17]. For the current study, an experimental validation of the formulated reactor model was carried out by estimating the biodegradation rate constant k_1 and k_2 for both the natural attenuation and bioaugmentation processes. The biodegradation model was simulated and the result of the simulation was compared with experimental results to determine validity.The numerical solver used for the parameter estimation exercise is 'MAXLKHD' which is based on a sequential quadratic programming code [18]. The parameter estimation was based on the maximum likelihood formulation, which attempts to determine values for the uncertain physical and variance model parameters that maximize the probability that the model will predict the measurement values obtained from the experiments [18].

Tables 1 and 2 show the estimated parameters k_1 and k_2, the optimal estimated value, 95% confidence interval, 95% t-value and standard deviation for the case of natural attenuation and bioaugmentation respectively. The t-value shows the percentage accuracy of the estimated parameters with respect to the 95% confidence interval. The 95% confidence interval shows that an estimated parameter will have a 95% probability of being within the stated interval. The small magnitude of the confidence intervals and the standard deviation obtained in both cases indicates that the estimated parameters were accurate to a high level of confidence. The weighted residual and χ^2-value are statistical tools used to test how well a model fits experimental data. A good fit results if the weighted residual is less than the χ^2-value. As shown in Tables 1 and 2, the χ^2-value and weighted residual were 11.055 and 30.144 respectively for the case of natural attenuation while the values were 15.677 and 30.144 respectively for the case of bioaugmentation. Since the weighted residual is less than the χ^2-value, then it can be inferred that the model results show a good fit and proper correlation with the experimental results.

Table 1: Statistical information for parameter estimation (natural attenuation)

Parameters	Optimal estimate	95% confidence interval	95% t-value	Standard deviation
k_1	0.235	0.0003	708.71	0.0002
k_2	1.320	0.0011	1200.11	0.0005
Weighted residual		χ^2-value	Comment	
11.055		30.144	Good fit: weighted residual less than χ^2 value	

Table 2: Statistical information for parameter estimation (bioaugmentation)

Parameters	Optimal estimate	95% confidence interval	95% t-value	Standard deviation
k_1	0.195	0.0248	7.8769	0.0118
k_2	1.309	0.0850	15.4043	0.0406
Weighted residual		χ^2-value	Comment	
15.677		30.144	Good fit: weighted residual less than χ^2 value	

Table 3 shows the values of the biodegradation rate constants k_1 and k_2 estimated as well as values reported in literature. It can be observed that the values obtained in this work are comparable with those obtained by other researchers. The differences might have resulted from the operating procedures and conditions of the study as well as differences in the species of cassava used. It should also be noted that the rate constants for the case of natural attenuation were higher than those obtained for bioaugmentation. This is an indication that bioremediation via natural attenuation is a better option for decontaminating cyanide containing cassava wastewater.

Table 3: Comparison of estimated parameters with literature values

Method	Value of rate constant (day^{-1})		Reference
	k_1	k_2	
Natural attenuation	0.235	1.320	This work
Bioaugmentation	0.195	1.309	This work
Natural attenuation	0.754	1.550	Uyigue and Omotioma [1]
Natural attenuation	0.380	0.596	Ofuya et al. [19]

Figures 1 and 2 respectively show the overlay plots of bound and free cyanide concentrations obtained for natural attenuation studies. The error bars indicate the standard deviation of the measurements from the mean. These plots display a comparison between the experimental and model predicted results in terms of trend and correlation. Figure 2 shows the variation of bound cyanide concentration with time for both experimental and model results. It is evident from the plot that the model was able to replicate the experimental concentration of bound cyanide to a high level of confidence. This is an indication that the model exhibits a good fit with the experimental data as seen in the excellent correlation between the experimental and model predicted results. In spite of the difference in trend showed in Figure 2 compared with Figure 1, a similar correlation was however observed i.e. the model was also able to replicate the experimental concentration of free cyanide to a high level of confidence. Figures 3 and 4 respectively show the overlay plots of bound and free cyanide concentrations obtained for bioaugmentation studies. The correlation between experimental and model predicted results indicates that the model was able to replicate the experimental results to a high level of confidence. This shows validity of the model developed.

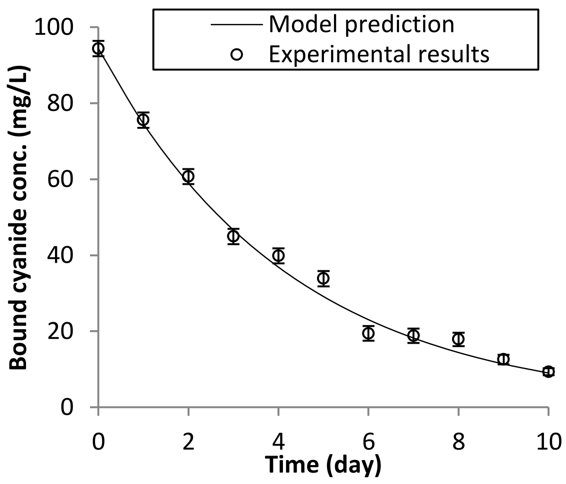

Figure 1: comparison between experimental and model predicted bound cyanide concentration for natural attenuation

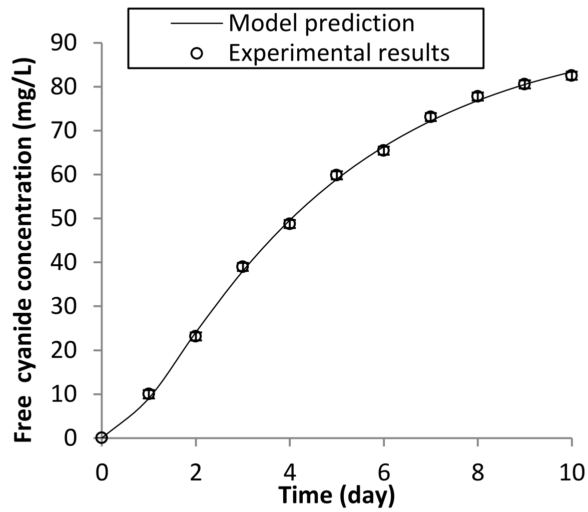

Figure 2: comparison between experimental and model predicted free cyanide concentration for natural attenuation

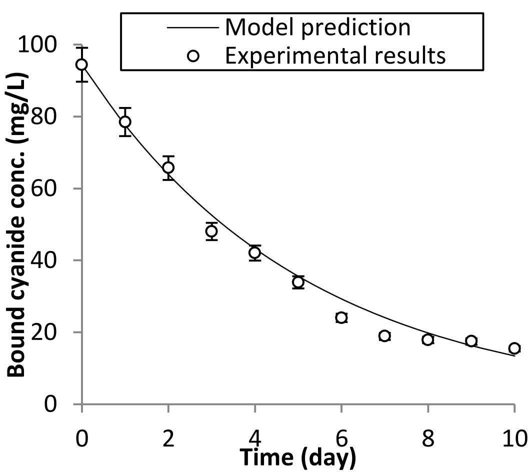

Figure 3: comparison between experimental and model predicted bound cyanide concentration for bioaugmentation

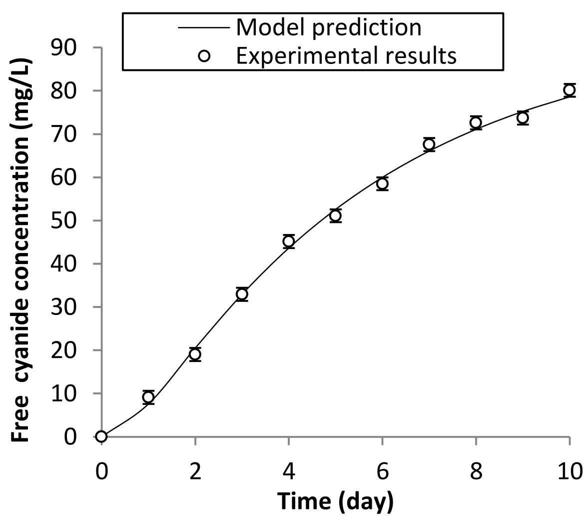

Figure 4: comparison between experimental and model predicted free cyanide concentration for bioaugmentation

3.2 Model Implementation

In order to investigate the dynamic behavior of the batch biodegradation of hydrogen cyanide, the formulated model was implemented in an advanced equation oriented process modelling software. Figure 5 show the concentration of bound, intermediate and free cyanide obtained for natural attenuation studies. The concentration of bound cyanide showed a gradual and progressive decrease in the wastewater in the course of bioremediation. This is an indication of the degradation of cyanide in cassava wastewater by the indigenous microorganisms. Despite the potential toxicity of cyanide to organisms, many microorganisms still possess the capability to metabolically utilise cyanide as a carbon source. By making use of the enzymes hydratase and amidase, these organisms are able to convert cyanide to considerably less toxic susbtances such as formamide through hydration and ammonia and formic acid through hydrolysis [4,20].Over the course of 10 days of bioremediation, the concentration of cyanide reduced by as much as 90.5%.Bioremediation via natural attenuation is facilitated by the indigenous microorganisms present in the wastewater. Uyigue and Omotioma [1], applied natural attenuation to the biodegradation of cyanide in processed cassava wastewater in order to study the kinetics of the degradation process. They reported that hydrogen cyanide concentration was reduced from 12.5 to about 0.1 mg/L during a 10 days treatment period. Figure 5 shows that the concentration of intermediate cyanide increased initially until it reached a maximum value after which it decreased progressively till the end of the process. The trend observed can be explained by the fact that the bound cyanide is initially converted to intermediate cyanide. This accounts for the initial increased observed for the intermediate cyanide concentration. The intermediate cyanide is subsequently converted to free cyanide which accounts for the decrease in intermediate cyanide concentration observed in later stages of the process. The concentration of free cyanide increased with bioremediation time. The increase corresponds to the decrease in the bound cyanide concentration as the bound cyanide is the precursor to the free cyanide.

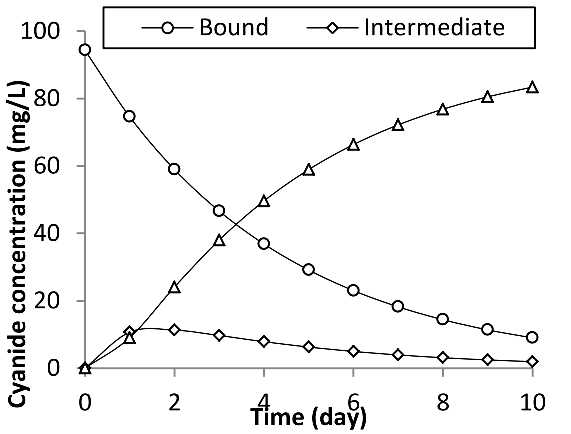

Figure 5: comparison between experimental and model predicted bound cyanide concentration for bioaugmentation

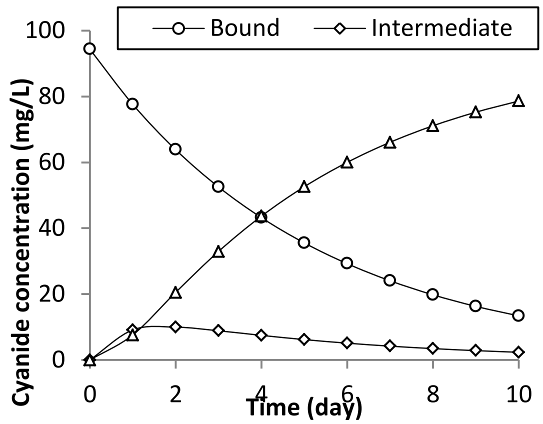

Figure 6: comparison between experimental and model predicted free cyanide concentration for bioaugmentation

Figure 6 show the concentration of bound, intermediate and free cyanide obtained for bioaugmentation studies. Trends similar to those presented in Figure 5 were obtained for the bound, intermediate and free cyanide concentration. The concentration of bound cyanide decreased in the course of bioremediation indicating the degradation of cyanide in cassava wastewater by the exogenously added microbial population. A reduction level of 85.8% was recorded in the concentration of cyanide in the wastewater.

Figures 7 to 9 compare the concentration of bound, free and intermediate cyanide concentrations respectively obtained for the cases of natural attenuation and bioaugmentation. It can be observed from the Figures that natural attenuation performed better than bioaugmentation as a strategy for the bioremediation of cyanide containing cassava wastewater. This is evident in the reduction level of 90.5 and 85.8% recorded for natural attenuation and bioaugmention respectively.

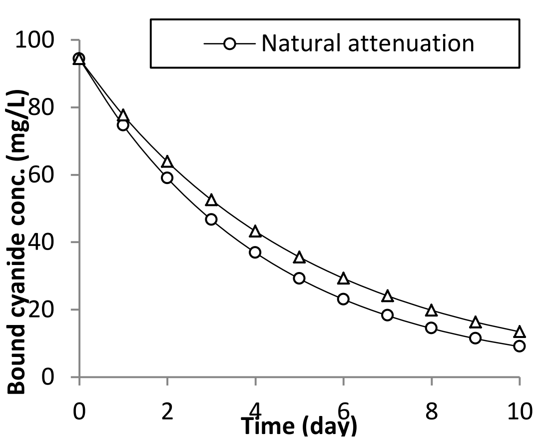

Figure 7: comparison between bound cyanide concentration obtained for natural attenuation and bioaugmentation

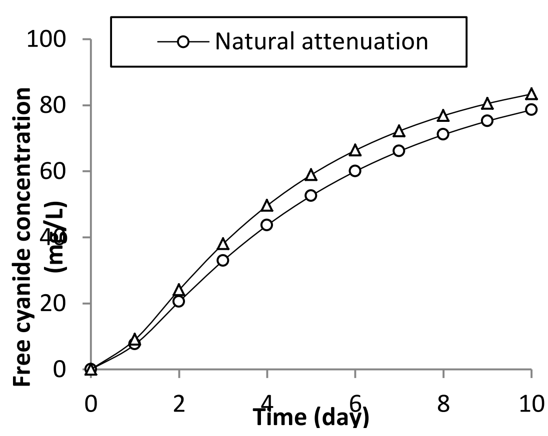

Figure 8: comparison between free cyanide concentration obtained for natural attenuation and bioaugmentation

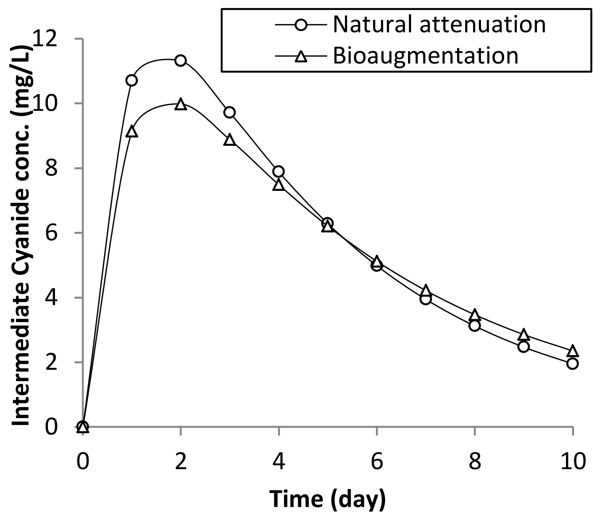

Figure 9: comparison between intermediate cyanide concentration obtained for natural attenuation and bioaugmentation

Oliveira et al. [3]used aerobic and combined anaerobic/aerobic reactors for the biotreatment of cassava meal industry wastewater. They reported over 95% reduction in the concentration of cyanide in the wastewater after 50 days of treatment. In another study, Akcil et al. [15] investigated the biological treatment of cyanide by natural isolated bacteria (*Pseudomonas* sp.). They observed that cyanide treatment was generally effective over a wide pH range, with the optimum being approximately 10.5. They further noted that *Pseudomonas* sp. reduced the cyanide concentration from 200mg/L down to 1mg/L within 70 hours of treatment. Campos et al. [21]reported cyanide reduction levels of 96% for the bioremediation of cyanide contaminated wastewater using *Fusarium oxysporum* CCMI 876 and *Methylobacterium sp*. RXM CCMI 908. These results show the importance of bioaugmentation as a strategy for bioremediation. However some reports like the case of this study, have shown that natural attenuationcould actually perform better than bioaugmentation as the introduction of enriched external cultures of microorganisms may not significantly enhance the efficiency of the bioremediation process[22]. The better performance observed for the case of natural attenuation might be because the indigenous microorganisms are already acclimatised to the wastewater environment as against the exogenously added microorganisms which are foreign to the wastewater environment [23]. It is also possible that the exogenously added microbial population might not possess the metabolic pathways required to metabolise hydrogen cyanide into harmless forms; hence they cannot significantly improve the degradation efficiency of the indigenous microbial population[24].

3.3 Conclusion

Kinetic modelling is gaining increasing interest in different fields of research due to its wide range of application. The present study investigated the use of modelling tools to describe the trends in the biodegradation of hydrogen cyanide in cassava wastewater. The following conclusions can be drawn from this study.

- The batch biodegradation of hydrogen cyanide in cassava wastewater was accurately represented by a validated dynamic model
- The model upon validation was able to replicate the experimental results to a high level of confidence as seen in the excellent correlation between experimental and model predictedresults
- Kinetic parameters can be estimated accurately using experimentally validated models.
- The concentration of cyanide decreases with time in the course of bioremediation irrespective of the strategy adopted
- Natural attenuation performed better as a bioremediation strategy for decontaminating cassava wastewater with a cyanide reduction level of 90.5% as against 85.8% recorded for the case of bioaugmentation

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