CHAPTER ONE

1.0 INTRODUCTION

Cassava is a very cheap source of carbohydrate and is the main carbohydrate source in the diet of the teeming population of the third world countries where it is largely grown. Banjoko et al. (2008) posited that cassava is a supplementary staple food of more than 200 million Africans aside from its uses as livestock feed particularly for monogastrics. Cassava is the most widely distributed major food crop with a high content of cyanogenic glycosides. It is also known as Manioc (manihot esculentz), yuca tapioca, or guacamate. Other foods such as sweet potatoes, yams, maize, millet, bamboo sugarcane, peas and beans, as well as kernel of almond, lemon, lime, apple, pear, cheery, apricot, prune and plum (Fiksel et al, 1981) contains cyanide.

Cassava as an important root crop in the tropics, widely grown throughout the tropical Africa, Asia, and South America contains 1mg/g of cyanide while cereals and grains contain cyanide of 0.001 to 0.45μg/g, 0.07 to 0.3μ/g for Soya proteins, 0.1to 3mg/g for lima bean (Honig et al, 1983).

Cyanide is both widely available and easily accessible throughout the world. The compound is not frequently encountered, as it is a potential terrorist agent, used as poison and contaminant in the past. It has the ability to cause significant social disruption and demands special attention to public health preparedness. Cyanide refers to the anion radical and the compounds capable of releasing cyanide may be inorganic or organic in nature. Inorganic compounds may be simple (eg AgCN, KCN) or complex (eg. A[CN]y, A[M]x[CN]y). Organic compounds may be glycosides or nitriles. It is toxic to humans and is a substance that is formed in combination with other
chemicals in the environment. In a drinking water, maximum acceptable concentration of 0.2mg/l (200μg/l) for free cyanide has been set. Free cyanide is defined as the sum of the cyanide present as either HCN or CN\(^-\). Hydrogen cyanide is a colorless liquid with an odor characteristic of bitter almonds and a vapor pressure of 107.6 KPa at 27.7\(^o\)C; it is completely miscible in water. Potassium cyanide, a white granular powder and sodium cyanide a white crystalline solid are both readily soluble in water. Cyanide is both man-made and naturally accruing substance found in food and water.

1.1 STATEMENT OF THE PROBLEM

The research in this study is to determine the inhibition of the cassava wastewater degradation by cyanide. Wastewater is an inevitable substance released during cassava starch processing. They are either a by-product of initial production process or they arise when the cassava tubes are indiscriminately discharged to a nearby water body. The most serious problem with cyanide itself is its potentialities of poisoning drinking water when it leaves in the ground and it is extremely effective reversible inhibition of cytochrome oxidase. A concentration of 1mM KCN is sufficient to inhibit oxygen consumption by mitochondria from a vertebrate source by >98%. Since human life generally involves the activities that result in accumulation of wastes. It is of importance in this research to know principal reduction effects of harmful chemicals on the wastewater before disposal to rivers to decrease totally its harmfulness to aquatic life.
1.2 SIGNIFICANCE OF THE STUDY

Observation has made it known that incomplete fermentation of cassava wastewater results in health problems, both in human and aquatic organisms; due to the inhibitory action of cyanide in cassava food, wastewater and the peels used as food for livestock’s. Therefore, this study will help to know the properties of cyanide as an inhibiter to the degradation of cassava wastewater and its effects and the possible means of removal of such toxic bioactive enzyme. Nevertheless to enhance safety to the environment, ensure that the discharge of wastewater from cassava to water bodies is reduced drastically.

1.3 OBJECTIVES OF STUDY

The objectives to this study are as follows:

To examine whether cassava wastewater degradation is actually inhibited by cyanide content during its fermentation

To determine the inhibition factor $K_i$ by using a statistical regression method and graphical method.

To investigate the type of inhibition involved in the waste degradation.

To determine its rate of degradation of cyanide in cassava wastewater.
1.4 SCOPE OF STUDY

This project was limited to analyzing inhibition of cassava (manioc) wastewater degradation by cyanide and to determine the kinetics of the inhibition constants, with the inhibition types involved.

1.5 LIMITATION OF STUDY

The basic limitations encountered in this research were inconsistent power supply and ineffectiveness/non-functional problem of some of the laboratory equipment/apparatus. Also there is human error/parallax error in taking the reading, though an acceptable result was achieved after the experiment.
CHAPTER TWO

2.1. GENERAL INFORMATION ON CASSAVA (MANIHOT ESCALENTA CRANTZ)

Cassava (manihot escalenta crantz) is an important tropical root crop providing energy to about 500 million people, as a very cheap source of carbohydrate and is the main carbohydrate source in the diet of the teeming population of the third world countries where it is largely grown. Apart from being the supplementary staple food of many Africans, it is also used as livestock feed particularly for monogastrics (Banjoko et al, 2008). Traditional African food products such as garri and fufu are obtained from cassava by series of operations such as grating, dewatering, fermenting and roasting. In these processes, it generates waste which includes wastewater and solid waste. But in this work, wastewater will be considered more due to its significant contribution to the environment. Cassava wastewater causes environmental pollution and aesthetic nuisance.

The major waste is from garri processing product and fufu production. The nutritional importance of cassava and its by products is however constrained by a number of factors. According to Iyayi et al (1997), the utilization of most of the agro-industrial by-products is plagued by their high level of structurally non-starch polysaccharide (NSP). These NSP include cellulose, hemicelluloses, pectin and lignin. Cassava by-products are also reputedly high in anti-nutrients like hydrogen cyanide (HCN), polyphenels (tannins) and Phyate and low in protein (Akpan and Ikeneboneh, 1995). The fallout of these constraints on the animals includes low digestibility, poor feed intake and reduced animal performance (Alawa, and Amadi, 1990; Adegbola, and Oduozo, 1992). In Nigeria today, 15 million tonnes of cassava are used for
processing of garri and fufu, in the processing of cassava fermented products, the roots are normally peeled to get rid of the out coverings, a then brown outer cover and a thicker leathery parenchymate inner cover.

The peels are also problematic to the environment, since its disposal to the land and ferments or decays to give out foul odor, polluted odor and are poisonous when inhaled by man or animals when dumped in a large amount near house holds. Therefore, they cause infection and other land problems that may take a long time to notice, rendering the soil unproductive, due to the biological and chemical reactions taking place between the continuously fermenting peels, soil and the surrounding vegetation. If these peels could make up 10% of the wet weight of roots, they constitute an important potential resource if properly harnessed biotechnologically.

**2.2. DESCRIPTION OF CASSAVA PRODUCTION**

**2.2.1 THE PRODUCTION OF CASSAVA GLOBALLY**

From research carried out by Dr Patrick Collard of the Department of Bacteriology, University of Ibadan, the world’s production of cassava as at 1959 was analyzed as shown below, through the analysis, Africa was the highest/greatest producer and consumer of cassava root and food respectively.
Table 2.1 World cassava production in 1959

<table>
<thead>
<tr>
<th>AREA</th>
<th>QUANTITY (TONS/YR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>North And Central America</td>
<td>612</td>
</tr>
<tr>
<td>South America</td>
<td>34.237</td>
</tr>
<tr>
<td>Asia</td>
<td>19.951</td>
</tr>
<tr>
<td>Africa</td>
<td>36.035</td>
</tr>
<tr>
<td>Oceanic (Pacific Country)</td>
<td>125</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>827.223</strong></td>
</tr>
</tbody>
</table>

Conducted By Dr Collard, P., Department of Bacteriology, University of Ibadan

Also statistically, according to FAO estimates, 172 million tonnes of cassava was produced worldwide in 2000. Africa accounted for 54%, Asia 28%, Latin America and Caribbean for 19% of total world production. In 1999 Nigeria produced 33 million tonnes making it the world’s largest producer. The average yield in Nigeria was 10.6 tonnes per hectare, while in 2002 was estimated to be 184 million tonnes. FAO of United Nations clarify that Thailand is the largest country in exporting of dried cassava with a total of 77% of world export in 2005, Victoria with 13.6% as second largest exporting country, Indonesia 5.8% and Costa Rica 2.1%. These were natural to Brazil and were imported into Nigeria by the early visiting Portuguese sailors and traders. Owing to its case of cultivation and viability, it has spread throughout Nigeria and has become the staple food of the country, used in so many ways of processing and needs.
Table 2.2 World’s Production of Cassava for 2000 and 2002

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>AFRICA</td>
<td>54%</td>
<td>56%</td>
<td>92.88</td>
<td>103.04</td>
</tr>
<tr>
<td>ASIA</td>
<td>25%</td>
<td>30%</td>
<td>48.1</td>
<td>55.2</td>
</tr>
<tr>
<td>AMERICA</td>
<td>19%</td>
<td>22%</td>
<td>32.68</td>
<td>40.04</td>
</tr>
</tbody>
</table>

Wikipedia, the free encyclopedia.

Oyenuga in his research in 1961 observed that cassava thrives successfully from sea level to an elevation of about 8300ft (1067m) but does not flourish at an altitude of higher than 4000ft (1219m). He observed also that a higher product of cassava was obtained from the southern states of the country than the Northern States and reported of having the capacity of yielding more than thirteen times as much as energy’s per hectare as maize or guinea corn. (Oyenuga, 1961 and Hahu et al, 1973).

Table 2.3 Nigeria States Cassava Production

<table>
<thead>
<tr>
<th>STATE</th>
<th>AREAS(Km²)</th>
<th>PRODUCTION</th>
<th>YIELD (ton/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern</td>
<td>572</td>
<td>1525</td>
<td>5878</td>
</tr>
<tr>
<td>Southern</td>
<td>298</td>
<td>1788</td>
<td>9869</td>
</tr>
<tr>
<td>Eastern</td>
<td>376</td>
<td>1474</td>
<td>8644</td>
</tr>
<tr>
<td>Western</td>
<td>260</td>
<td>1425</td>
<td>8730</td>
</tr>
</tbody>
</table>

Oyenuga, 1961 and Hahu et al, 1973
2.2.2. CASSAVA PRODUCTS AND VARIETIES

Cassava was grouped into two major varieties, via

The bitter variety known as manihot aliligsinanpoti and Sweet variety known as manihot palmate stapf.

They are used in processing different types of food from cassava root crop, using different methods. Examples are processing of garri, tapioca, chips, starch and fufu (known as Santana white or akpu igbo) etc. At the processes of cassava food, waste was generated as solid and liquid waste.

2.3. GENERATION OF CASSAVA WASTEWATER

Fermentation either naturally or with selected microbial inoculums has also been extensively used to enhance the nutrient potentials of cassava and its by-products both for human and livestock consumption. Borgstrom, 1968 considered the scope of bioconversion of cassava and its by-product through fermentation as the oldest form of food biotechnology, in which wastewater can be generated strongly.

Thus, two different wastes are obtained from cassava through different processes of the product. As mentioned earlier, it is of two groups, the solid waste and liquid waste.

Solid waste: solid waste from cassava production is usually achieved in various processes depending on the type of cassava food prepared. But the basic solid waste are the peels, that is, the out and inner cover, others are gotten in a suspension or solution of the liquid water.
It was obtained by drying and weighing the residue removed by filtering of the sample when this residue was ignited, the volatile solid burned off. The liquid wastewater was normally generated from processing of garri or fufu. Garri was an end product of peeling, grating, dewatering, sieving and frying the resulting semi-solids mass, while that of fufu was the end product of cassava starch which consists of cleaning, peeling, chopping, pressing and straining of the cassava tubers. The cassava starch will be then either dehydrated or submitted to natural fermentation for the production of a sour cassava starch. These requires large amount of water and it estimated that the volume of combined wastewater from tapioca starch factory vary from 40 to 60m$^3$ per ton of starch in first-grade processing traditional labour intensive) and from 20-40m$^3$ per ton of starch in the second-grade processing highly mechanized, capital intensive, while the chips and pellets do not release wastewater because of it drying processes (Bengtsson and Triet, 1994). The water pollution problems are critical. The wastewater is highly acidic, sometimes as low as pH 2.6 and combined wastewater has been reported as ranging between pH 3.5 and 5.2 the low pH may harm aquatic organisms and prevent self-purification of the receiving water body. Suspended solids settle on the stream bed and spoil fish breeding areas, this is due to it toxicity.
Fig 2.1. Flow chart of sour cassava starch production (Demiate et al, 1999)
2.3.1. CASSAVA WASTEWATER TOXICITY

In developing countries and newly industrialized countries, there are frequent conflicts between traditional utilization of natural resources and industrial development. In addition to the loss of farmland to make room for industries, labour settlement, dumping site, etc in urbanized areas, waste from industries may degrade soil and water quality to such an extent that they no longer function normally. Cassava waste results in toxic effluents that when used for irrigation, may be considered a potential threat to crop (rice, vegetable, etc) and /or may kill aquatic food resources (fish, crustacean, etc) in the receiving water bodies. In most areas, cassava wastewaters are generated in large quantity because they are used for several purposes like food for man and utilized extensively for industrial purposes too as it used in production of paper, plywood, adhesives, sweeteners, monosodium glutamate, alcohol, amino acids, etc(Bengtsson and Triet, 1994). Prior to this, cassava was a major problem in disposal because of the high content of cyanide, which varies due to factors such as plant varieties, edaphic, climatic and other conditions. The content of cyanide peeled tubers may vary from 6 to 25 mg HCN/kg-1 fish weight, with a reported maximum of 434mg Kg-1. Because of the cyanide present in cassava, it is characterized as bitter or sweet according to the amount of cyanide present. In present of 50mg HCNkg-1 fresh weight is regarded as nontoxic, both higher than that is toxic or bitter and used mainly for industrial purposes.

The content of cyanide in cassava is in form of glycoside, that is, linamarin (93%) and lotaustralin(7%). There is general agreement that overestimate the actual cyanide toxicity to aquatic organisms and the analytically determination of HCN concentration in cyanide polluted
waters, is considered to be the most reliable index of toxicity (IRWIN, 1997). Therefore, the observed toxicity to aquatic life of simple and complex cyanide was attributed almost entirely to molecular (undissociated) hydrocyanide acid (HCN) derived from ionization, dissociation and photodecomposition of cyanide containing compounds. Hydrocyanide acid (HCN) is easily liberated from cassava cyanide glycosides by the enzyme linamarase in which case plant cells are physically disrupted. However, the ingestion of high concentration of cyanogenic glycosides from fresh cassava roots and leaves has been reported to be lethal in numerous species of animals. This was because the possibility of hydrolysis during digestion was not adequately understood, despite early reports that oral doses of pure linamarin produced physiological and biochemical changes in rats and chick embryos even in the absence of linamarase.

Toxicity in cassava may be acute and/or chronic. Acute toxicity results from ingestion of a lethal dose and death is caused by the inhibition of cytochrome oxidase of the respiratory chain by cyanide. Investigation in goat’s ingestion cassava leaves and also non-ruminants, like pigs when fed fresh uncooked tubers (Obioha, 1982). The chronic cyanide toxicity on animals affects both the growth and reproduction phase of development.

2.3.2 FERMENTATION OF CASSAVA AND ITS PRODUCTS

Fermentation of cassava and its by-products automatically qualifies such products as fermented foodstuffs, which according to Campbell-Platt (1994) are defined as animal or plant tissues subjected to the action of micro-organisms and/enzymes to give desirable biochemical changes
and significant modification of food quality. The need for fermentation was outlined by Steinkraus, (1995)

- Detoxification of food/feed anti-nutrients during the process of fermentation.
- Biological enrichment of food substrates with proteins, essential amino acids, essential fatty acids and vitamins.
- Enrichment of the fermented products through the impartation of an array of aroma flavours and textures.
- Preservation of the fermented products through the production of lactic acid, acetic aid, alcohol and alkali in the substrate as a result of fermentation.
- Decrease in cooking time and fuel requirements.

The outlined reasons have been achieved through fermentation of cassava and cassava by-products. Cassava and its by-products fermentation is popularly achieved in two-way techniques known as the liquid substrate fermentation and solid substrate fermentation techniques. The liquid substrate fermentation is described as the one in which water is always in a free state while food nutrients in the form of carbon, nitrogen, phosphorus and others are in a suspended or dissolved state. In regard to this technique, there is an order to ensure a successful fermentation in which the strict observance of aseptic inoculation of microorganisms which could only be economically feasible when done on an industrial scale, i.e., its application is yet to be suited to the traditional village setting where the bulk of the producers of cassava and its products are domiciled. Apart from the requirement of a sterile environment for the operation of submerged fermentation, enzymatic or acid treatment of starch is necessary when
yeasts are to be used as the microbial inoculums, also recovery of the cell mass could be tedious and might involve further processes like centrifugation/ultra-filtration before separation of cell biomass could be achieved (Balagopalan et al, 2002). While the solid substrate fermentation is a bio-system consisting of a solid, porous, water absorbing matrix, which can either be biodegradable or not of relatively high water activity on solid/gas interface in which air mixture of oxygen with other gases freely circulate under a relatively low pressure within the fermenting substrate (Raimbault, 1998).

2.4 CYANIDE

Cyanide refers to the $-\text{C}^0\text{N}$ anion radical. Compounds capable of releasing cyanide may be inorganic or organic in nature. Inorganic compounds may be simple (e.g., AgCN, KCN) or complex (e.g., $[\text{CN}]_y$, $A[M]_x[\text{CN}]_y$). Organic cyanide salts are often used in metallurgical industry activities such as metal surface treatment, but also in mining industries (example for gold extraction), which consume large amount of water and the effluents always contain cyanide that must be treated before disposal to the natural environment. It acts through the inhibition of cytochrome oxidasa in the respiration electron transport chain of the mitrochondria, impairing both oxidative metabolism and the associated process of oxidation phosphorylation (Holland, 1983 and Dreisenbach et al, 1987). Its outward acute effects resemble those of acute hypoxia. Interference in the oxidation process may also give rise to cardiac disturbance, seizures, unconsciousness and ultimately death to living organisms.
2.4.1 FORMS OF CYANIDE

Cyanide exists in three different forms, namely

FREE CYANIDE: This is a measure of the cyanide present as HCN or CN⁻. This is the one that mostly exists in cassava root crop.

TOTAL CYANIDE: Total cyanide measures all the cyanides including the iron cyanide complexes.

CYANIDE AMENABLE TO CHLORNATION: this measures simple metal cyanides and complex cyanide with the exception of iron cyanide (Atsdr, 1988).

2.4.2 CYANIDE IRREVERSIBLE TOXICITY AND INHIBITION

Generally, cyanide is classified as an irreversible enzyme inhibitor and therefore considered toxic (Speece, 1996; Mathews et al., 2000). Applications of anaerobic digestion for the treatment of cassava wastewaters show that, among the anaerobic processes, methanogenesis is the most sensitive to cyanide toxicity (Cuzin and Labat, 1992; Siller and Winter, 1998; Gijzen et al., 2000; Annachhatre and Amornkaew, 2001). In Gijzen et al. (2000), sludge activity measurements demonstrated that the effect of CN-inhibition on methanogenic activity was more pronounced for aceticlastic than for hydrogenotrophic methanogens. Therefore, modeling the toxicity and inhibition of cyanide will only consider the acetate degraders. Irreversible toxicity of cyanide is applied as a decay factor $I_{dec,Xac,cya}$. 
Cyanide inhibition occurs by blocking the active site of enzymes and therefore limits the substrate uptake (Mathews et al., 2000). The inhibition of cyanide is modeled by the Inhibition factor Icy to the acetoclastic methanogens.

![Flow chart of Angelidaki et al (1993) model.](image)

The conversion of organic matter to biogas is carried out by the simultaneous action of three groups of bacteria: acidogens (hydrolysis and acidogenesis), acetogens and methangens.
2.4.3 TOXICITY OF CYANIDE

There is a general agreement that cyanide is more toxic to freshwater fish under condition of low dissolved oxygen, that pH levels within the range 6.8 to 8.3 had little effects on cyanide toxicity but at enhanced toxicity acidic pH, that juveniles and adults were the most sensitive life stages tested and embryos sac fry the most resistant and substantial interspecies variability exists in sensitivity to free cyanide. Initial dose and water temperature both modify the biocidal properties of HCN to freshwater teleosts. At slowly lethal concentration (i.e., <10μg HCN/N), cyanide was more toxic at lower temperature, at high rapidly lethal HCN concentrations, cyanide was more toxic at elevated temperature. By contract, aquatic invertebrate were most sensitive to HCN at elevated water temperature regardless of dose (Bengtsson and Triet, 1994).

2.4.4. APPLICATION AND SOURCES OF CYANIDE

About 84% of domestic HCN production is used to produce organic cyanide, also known as nitriles including a crylonitriles, methylmethacrylate and adiponitrile(Irwin, 1997). In addition to their primary uses in the metals and electroplating industries and in the manufacture of synthetic fibers and plastics, various cyanide compounds have been used directly or as an intermediate to produce synthetic rubber, fumigants, rodenticides, insecticides, predator control agents, rocket fuels, paints and paint finishes, paper, nylon, pharmaceuticals, photographic, chemicals, mirrors, cement, perfume, bleaches, soaps, and detergents, riot control agent, dyes, pigment, fertilizer and weedicides(Irwin, 1997).
All these products are as a result of their sources, the anthropogenic source of cyanide in the environment include industrial processes, laboratories, fumigation operations, cyanogenic drugs, fires, cigarette smoking and chemical warfare operations.

It is present in many industries’ wastewaters like cassava wastewater, coal gasification, electroplaters, aluminum and plastics industries, etc. Thus cyanide compounds are useful to society in terms of their key role in synthetic and industrial processes, for certain fumigation and agricultural uses and for some therapeutic applications but serve no purpose in human body yet present in our food, air and water. From this fact, it is advised that raw cassava should not be taken unless when cooked or fermentation took place for some time to oxidize the effect of cyanide present.

2.4.5. TECHNIQUE ANALYSIS OF CYANIDE REDUCTION

A large number of procedures have been proposed for treatment of cyanides: Physical (dilution, membranes, hydrolysis (distillation), adsorption (minerals, activated carbon, resin), Complexation (acidification/volatilization, metal addition, flotation, solvent extraction) and/or Oxidation (bio-oxidation, catalytic, electrolytic chemical, photolytic) methods (Young and Jordan, 1995). In addition, alkaline chlorination of wastewater is one of the most widely used methods of treating cyanide waste. In this process, cyanogens chloride CNCI, is formed which at alkaline pH is hydrolyzed to the cyanate ion, CN\(^{-}\). If free and chlorine is present, CN\(^{-}\) can be further oxidized and it is converted to cyanate during chlorination of water supplied and complete oxidized to carbon dioxide and nitrogen. An alkaline pH favors the oxidation by
chlorine, where-as an acid pH favors volatilization of hydrogen cyanide into the atmosphere. (USEPA, 1980).

2.4.6. REMOVAL OF CYANIDE CONSTRAINTS FROM CASSAVA.

Cyanide in cassava root crop can be eliminated or reduced through some concerted efforts, these effects include sun drying, oven drying, treatment with chemical preparation, steeping/retting or soaking in water, cooking and wetting/moistening (Badbury, 2004), but all these efforts are with varied levels of success, cost effectiveness and adaptability to local technologies. Fermentation either naturally or with selected microbial inoculums has also been extensively used to enhance the nutrient potentials of cassava and it’s by products both for human and livestock consumption. Fermentation, boiling and ensiling are efficient techniques for removing the constraint from cassava (Padmaja, 1995).

2.5. ENZYME INHIBITION.

The word inhibition is simply defined as reduction in enzyme activity through the binding of an inhibitor to a catalytic or regulatory site on the enzyme (Rogers and Gibon, 2009). Inhibition can be reversible or irreversible. Irreversible inhibition nearly always involves the covalent binding of a toxic substance that permanently disables the enzyme. This type of inhibition does not play a role in the fine control of enzyme activity and the reversible inhibitor to the enzyme which results in a temporary reduction in enzyme activity.

An inhibitor can take many different forms and can react with the cell in a variety of ways. Substrates or nutrients when present in the neighborhood of the cell can inhibit certain
pathways and accelerate others (Holly and Humphery, 1970). This is applicable in wastewaters certainly for the presence of substrates, for this reason cyanide inhibits the pathway in which degradation can take place. Nevertheless the permeability of cell wall is altered often or the activity of the enzyme is changed through complex formation with the inhibitor.

2.5.1. FORM OF INHIBITION

There are three basic of inhibition, namely

COMPETITIVE INHIBITION

Competitive inhibition is usually a close analogue of the substrate. It binds at the catalytic site but does not undergo catalysis. It wastes the enzymes time by occupying the catalytic site and preventing catalysis or put another way, the presence of an inhibitor decreases the ability of the enzyme to bind with its substrate.

An enzyme mechanism model of the action of a competitive inhibition (Ic) based on the standard model of a Michaelis-Menten enzyme where E+S leads to the E-S complex, which lead to product P.

\[
\begin{align*}
E + S & \quad \overset{k_2}{\underset{k_1}{\rightleftharpoons}} \quad ES \quad \overset{k_3}{\rightarrow} \quad E + P
\end{align*}
\]

\[
K_I = \frac{(E) (Ic)}{Eic}
\]

Fig 2.3. Model of a competitive inhibitor (Ic) interacting with enzyme (E) and all equations for the equilibrium found between Ic and E.
But the rate or product formation becomes

\[
dp \frac{dt}{dt} = \frac{V_m S}{K_m + S + \frac{V_m S}{K_1}}
\]

then the disisociation constant for the reaction between E and I is \( K_i \) where

\[
K_i = \frac{[E][S]}{EI}
\]

\( K_i \) = inhibition constant

\[
\text{And} = \frac{[E][S]}{ES} = \frac{(K_i + K_2)}{K_i} = \frac{K_m}{K_i}
\]

\( E_0 = E + ES + EI \)

\[
= E + ES + \frac{EI}{K_i} \hspace{1cm} \text{................................. 2.1}
\]

Simplifying equation 2.1, it becomes

\[
E_0 = E[1 + \frac{I}{K_i}] + ES
\]

\[
E = \frac{E_0 - ES}{1 + \frac{I}{K_i}} \hspace{1cm} \text{................................. 2.2}
\]

Substituting for \( E \)

\[
\frac{[E_0 - ES][S]}{(1 + \frac{I}{K_i})ES} = K_m
\]

\[
[E_0][S] - [ES][S] = ES[K_m (1 + \frac{I}{K_i})]
\]

\[
[E_0][S] = [ES][S] + [ES][K_m (1 + \frac{I}{K_i})]
\]
\[ E_0S = ES[S + K_m \left(1 + \frac{I}{K_i}\right)] \]

\[ ES = \frac{E_0S}{S + K_m(1+\frac{I}{K_i})} \]

\[ K = \frac{K_0S}{S + K_m(1+\frac{I}{K_i})} \] ..................................................... 2.3

- Non-Competitive Inhibition: This inhibitor does not bind to the catalytic site but binds to a second site on the enzyme and acts by reducing the turnover rate of the reaction. The binding of inhibitor and substrate is completely independent and the bind of the inhibitor results is total inhibition of the catalytic step.

\[
\begin{align*}
E + A & \overset{K}{\rightleftharpoons} EA \\
& \overset{k_1}{\longrightarrow} E + P \\
& + \\
& \overset{k_2}{\downarrow} I \\
& \overset{k_3}{\leftarrow} \overset{\downarrow}{k_4} \overset{k_5}{\rightleftharpoons} \overset{k_6}{\rightarrow} EIA
\end{align*}
\]

The association and disassociation rate \( k_1 \) and \( k_-1 \) are identical to \( k_3 \) and \( k_-3 \) (i.e., \( K_m \)) and similarly \( k_2 \) and \( k_-2 \) are equal to \( k_4 \) and \( k_-4 \) (i.e., \( k_1 \)).

But the complex enzymes reacting with the product, assumes equilibrium concentration of complex, the rate for product formation becomes

\[
\frac{dp}{dt} = \frac{Vm}{(1+\frac{K_m}{S})(1+\frac{I}{K_i})}
\]
- Uncompetitive Inhibition: This is another mechanism of inhibition. It does not bend to the enzyme but only the enzyme substrate complex. The enzyme kinetics equation example is

\[
E + A \xrightleftharpoons[k_{-1}]{k_1} EA \xrightarrow{+} E + P
\]

- Substrate Inhibition: This occurs when a second substrate molecule acts as an uncompetitive inhibitor binding to the enzyme-substrate complex to form an enzyme-substrate-substrate complex. One of the possible mechanism for substrate inhibition is the reduction the activity of the enzyme by complexing with the excess substrate.

\[
Es + S \xrightleftharpoons{k_2}{k_{-2}} ES_2 \xrightarrow{+} Es + P
\]

But the rate product formation becomes

\[
\frac{dp}{dt} = \frac{VmS(1+\beta S)}{S+Km+S^2/Ki}
\]

If the equilibrium concentration of the complex are assumed, where \( \beta, k_1, k_m \) are kinetic constants.

Note \( E + 1 = EI \), when a competitor for the enzyme is introduced, and the complex is biologically inactive.

- mixed inhibition
In mixed inhibition, the plots at different concentration (at fixed \([E_0]\)) will not intersect on either axis, nor the slope be the same, the pattern will be different from those characteristic of competitive, non-competitive and uncompetitive inhibition. This can be two forms; one is the competitive non-competitive inhibition that is where the pattern observed lies between those for competitive and non-competitive inhibition, and \(K_i\) (slope) > \(K_i\) (intercept), the plots cross to the left of the 1/K axis and above 1/S axis. The other is non-competitive-uncompetitive inhibition; in this case the pattern is intermediate between those for non-competitive and uncompetitive inhibition, and \(K_i\) (slope) < \(K_i\) (intercept), the plots cross to the left of the 1/K axis and below 1/S axis (Palmer T., 1981).

There are two processes by which inhibition may bind to the enzyme, from Michalis Meten equation

\[
E + I \leftrightarrow EI \quad \text{ (inhibitor constant } K_i) \]

And \(ES + I \leftrightarrow ESI\) \quad \text{ (inhibitor constant } K_1) \]

But \(K_i = \frac{[E][I]}{[EI]}\) and \(K_1 = \frac{[ES][I]}{[ESI]}\)

Recall \(\frac{[E][S]}{ES} = K_m\)

And \(E_0 = [E] + [ES] + [EI] + [ESI]\)

Developing the argument, without assuming \(K_i\) and \(K_1\) are identical

\[
E_0 = [E] + [ES] + \frac{[E][I]}{K_i} + \frac{[ES][I]}{K_1} \]
\[ E = E_0 - ES \left( 1 + \frac{I}{K'_I} \right) \]

\[ K = \frac{\frac{K_0 S}{S + \frac{K_0}{K'_I}}}{\frac{1 + \frac{I}{K'_I}}{S + \frac{K_0}{K'_I}}} \]

\[ K = \frac{K'_I S}{S + K_m} \]

\[ K_0' = \frac{K_0}{\left( 1 + \frac{I}{K'_I} \right)} \quad \text{and} \quad K_m' = \frac{\left( 1 + \frac{I}{K'_I} \right)}{\left( 1 + \frac{I}{K'_I} \right)} \]

It means that \( K_0' \) and \( K_m' \) intercept at x axis.
Taking the inverse of equation 2.7

\[ \frac{1}{K} = \frac{K_m'}{K_0'} (1/S) + \frac{1}{K_0'} \] 

2.8 (Mixed inhibition)

Where \( \frac{K_m'}{K_0'} = \frac{K_m}{K_0} \left(1 + \frac{I}{K_I}\right) \) 

2.9 (slope)

And \( \frac{1}{K_0'} = \frac{1}{K_0} \left[1 + \frac{I}{K_I}\right] \) 

2.10 (intercept at y axis)

2.6 CHARACTERISTICS OF CASSAVA WASTEWATER.

The influents from cassava floor industries are commonly analyzed to find the constituents of the waste, these results are mainly from the draining of the sedimentation tank in which the starch slurry was left to settle. The liquid residues generated from the washing and peeling of cassava roots generally contain a large amount of inert material with low COD and does not need biological treatment. These constituents are due to physical and chemical and biological constituents.

The physical constituents of this waste are odour, colour, Total solids, total suspended solid and volatile suspended solids.

While the chemical characteristics are the chemical oxygen demand, COD, Biochemical Oxygen demand. COD, pH, lactic acid, Acetic acid, Total nitrogen and phosphorus and the toxicity which is the cyanide found in cassava is the Biological characteristic of the wastewater.
2.7 ANAEROBIC AND AEROBIC PROCESSES OF WASTEWATER TREATMENT.

The experimental set up can be in two major ways Anaerobic and Aerobic conditions. Anaerobic digestion processes are biological degradation process in absence of Oxygen, but other diction acceptors are used. Oxygen may be poisonous for these types of micro-organisms (Kiestra and Eggers, 1986).

Thus, aerobic and anaerobic heterotrophic microorganisms use the fermentation process to reduce complex organic compounds to simple organic forms,

Heterotrophs are microorganisms that use organic carbon for the formation of new biomass, unlike the Autotrophic micro organisms that are important for the removal of nitrogen from wastewater.

Fermentation is the exothermic, enzymatic breakdown of soluble organic compound and does not depend on the presence of dissolved oxygen. Two stage are involved, the acid-fermentation and methane fermentation. End products of the acid fermentations process include Volatile fatty acids and alcohols and little reduction of BOD because most carbon is still in an organic form. During methane fermentation, portions of the acid fermentation are converted to methane and carbon dioxide gas. The result of this conversion provides a reduction in BOD.

Anaerobic micro organisms are rusted to the fermentation process in which methane can only be produced.
COHNS heterotrophic volatile + CO₂ + H₂O + CH₄ + energy + reddish
Orange compound Microbe fatty acids

But the aerobic micro organisms can further transform the VFA (and other bio-available organic compounds) into carbon dioxide and additional energy (Lehningr, 1973) through the process of respiration which requires oxygen. Oxygen acts as electron acceptor for catabolic degradation of the VFA. The aerobic system provides high rate wastewater treatment.

Volatile fatty acid + O₂ Aerobic energy + H₂O + residues.
Microbe

Prior to this, the industries practicing anaerobic treatment are the food and beverage manufacturing industries. Examples are sugar refineries, cassava, milk and dairy production, brewing and beverages fruit and vegetable canning etc.

Pilot experiments with an aerobic digester are also being undertaken in the synthetic detergent industry and in many other industries mentioned. These share the same characteristics a high concentration amount of toxic compounds of heavy metals and chlorinated hydrocarbons (Werstro and Egyers, 1986). One factor of big importance is the organic loading potential of an anaerobic reactor, which is certainly the complexity of the wastewater, particularly the fraction of insoluble organic matter.

With respect to the conversion rate of insoluble biodegradable pollutants, if should be clear that generally the hydrolysis (liquid fraction) is the limiting factor. This is particularly true for all lower ambient temperatures. In treating more complex types of wastewater, obviously the loading rates of the anaerobic treating-system will be dictated by:
The rate of hydrolysis which depend on the operation temperature, the size, shape and composition of the dispersed pollutants.

The efficiency of the system towards the removal of the dispersed pollutants, which in turn depends strongly on the settling, flocculation and absorption properties of these substance.

The efficiency of the system for retaining the exo-enzymes required for hydrolysis. Excreted exo-enzymes should not be washed out too rapidly.

2.8. EFFECTS OF ODOR IN FERMENTED CASSAVA WASTEWATER

Odor in fermented cassava wastewater are offensive, the importance of odor at low concentration in human terms is related primarily to the psychological stress they produce rather than to the harm they do to the body. These offensive odors cause poor appetite for consumption of cassava food, impaired respiration, nausea, vomiting and mental perturbation and can distort the interest of water consumption when highly discharged to the river without undergoing proper treatment, lower socioeconomic status, it also results in a decline in market and rental property values, tax revenues, payroll and sales, and very toxic at elevated concentration etc (Metcalf & Eddy, 2004).

2.9. CIRCUMSTANCES AFFECTING ODOR REMOVAL/DEVELOPMENT

Odor removal is influenced by so many circumstances as listed below

Type of aeration
2.9.1. DETERMINATION OF ODOR POLLUTION

Odor pollution can be determined using hydrogen sulphide (H\textsubscript{2}S) gas method, olfactometry analysis, and electronic noses.

The hydrogen sulphide (H\textsubscript{2}S) gas method used in determining odor, is a common gas component emitted from sewage work, it is used to measure odor strengths, because of its sensitive, easier, more reproducible and cheaper than the olfactometric methods.

Olfactometry analysis is another method of determining odor, is where panels of people are used to assess odor and to determine thresholds of human detection. Threshold odor numbers (TON) are defined as the number of dilutions at which 50% of the panelists can detect no odor or foul smell to distinguish a dilution odorous sample from odor-free air.

Another known as Electronic noses characterize odor without reference to its chemical composition. A sensor head, a sample vessel, inlet and outlets for pouring the sensor head and sample chamber and control system consisted in these and the strength of odor is measured
from a relationship between the electronic nose responses obtained from the sensor and the odor potentials. (Agunwamba, 2001)

2.9.2. PROCEDURES OF ODOR REMOVAL/CONTROL

Odor is one of the characteristic of wastewater and water. It always offensive stunk, unpleasant, pungent, putrid, suffocating, irritating, nauseating, because of the composition and substrate content of the waste. Odor in sewage is caused by volatile sulphur compounds, such as hydrogen sulphide and mercaptan (Hansen, 1998). Prior to nuisance to the environment, it is of important to control odor in wastewater to avoid its effects to heath and other environmental habitants.

Thereby odor removal can be achieved by chemical scrubbers, which in must cases not environmentally sound to the involvement of oxidizing agents, and it is an expensive method. Some of these agents are sodium hypochlorite, hydrogen peroxide, ozone, potassium permanganate, and sodium hydroxide (NaOH). NaOH is used in scrubbers, where H₂S concentration in the gas phase are high and in shock treatment to reduce microbial slime in sewers (Metcalf & Eddy, 2004). Also air, oxygen and chlorine are used in removal of odor in wastewater, but chlorine act as disinfectant for destruction of pathogen and control nuisance microorganisms and oxidation and destroy taste, color and odor compounds, as well as elimination of hydrogen sulphide, but not used commonly in scrubbing application of water facilities.

Activated carbon filtration and Biological methods are other methods in removal of odor. Nevertheless carbon adsorption is usually the most effective chemical for reducing the level of
odor and taste either in the treatment of water or wastewater. It is prepared from hard wood charcoal, lignite, nutshells, or other carbonaceous materials by controlled combustion to develop adsorptive characteristics. It is available in powdered and granular forms, can be applied through dry feed machine or as a slurry, introduced in any stage of processing before filtration or disperse the carbon. Because it adsorbs chlorine, therefore, these two chemicals should not be applied simultaneously, or in sequence without appropriate time interval. (Hammer and Hammer Jr, 2000).

Biological method, edofilter or bioscrubbers may be used. Bark, compost and peat are naturally occurring materials used as edofilts, as air flows through the biofilter, the pollutants are adsorbed onto the biofilm and subsequently degraded. The performances of biofilters are affected by moisture content, pH, accumulation of reaction products, and mechanical stability of the filter materials (Agunwamba, 2001).

2.10. FACTORS AFFECTING WASTEWATER RECLAMATION

Wastewaters are often times not easy to reclaim because of its composites, which influences it degradation. Degradation of wastewater is affected by different factors, via.

Temperature, pH, Sunlight, Photosynethic plant, Weather Condition, Aeration, Heavy Metals, Organic Chemicals, Viruses and Inorganic Salts. The outlined factors contribute to the degradation of wastewater for positive or negative results.

pH value is of high importance in water and wastewater treatment, because of its acidity and alkalinity impacts on the microbes in water. An alkaline pH of about 11 kills bacteria and
inactivates viruses, when it is more acidic, it causes the digester failure in anaerobic digester.

The pH-self regulation mechanism is as follows- whenever free ammonia (high for high pH) inhibits methanogenesis, acetic acids are accumulated, this causes an inhibition to acetogenesis and a consequent accumulation of propionic and butyric acid leading to inhibition of acidification. Since high values inhibit (thermodynamically) the generation of propionic and butyric acids, the volatile fatty acids (VFA) accumulation reduces the pH, causing a decrease in the free ammonia concentration and the inhibition of methanogenesis. Low pH values (<6) are expected to be inhibitory to all bacterial species and when pH drops to a low levels (<5.5) methane generation is stalled (Lyberatos and Skiadas, 1999). Therefore, bacteria are favored by pH level between 6.5 and 8.5.

Inorganic salt: As have mentioned in section 2.4 of this chapter, cyanide is an inorganic salt or organic salt form which act as an inhibitor in degradation of cassava wastewater, nor water content of it and more other inorganic salts like phenol, zinc, alcohol, mercuric chloride, and 2,4 dichlorophenoxyacetic acid, etc, affects the reclamation of wastewater as they decrease the effectiveness of degradation in metabolic activity. Aspects when chemical is added to activate the process involved in degrading of these constituents can reclamation be achieved.

Temperature: is the most important ambient condition for bacteria growth according to Ingraham, (1962). Its dependence on the biological reaction rate constant is very important in assessing the overall efficiency of a biological treatment process. Most enzymes and bacteria have optimum temperature of activity in the range 30-80°C above which they rapidly die or become inactive. But most biochemical reaction is small, usually from 5-90°C and constant fit
an Arrhenius-type of temperature relationship. Temperature does not only influence the metabolic activities of the microbial population but also has a profound effect on such factors as gas transfer rates and the settling characteristics of the biological solids.

\[ K^1 = A e^{-E/RT} \]

This is the Arrhenius-type of temperature relationship, where

- \( K^1 \) is the kinetic constant at temperature \( T^0 \)K,
- \( E \) is the activation energy
- \( R \) is the gas constant in cal/g –mol-K
- \( T \) is temperature
- \( A \) is the same as \( K_{\text{max}} \) that is maximum rate constant which have to be determined empirically.

Therefore, equation can be written as \( K = K_{\text{max}} \cdot \exp \left[- \frac{E}{RT} \right] \).
CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 SOURCES OF SAMPLES AND COLLECTION

The sample used in this study was collected from cassava processing plant where cassava is used in production of fufu and garri. This means that the processing plant was divided into two sections, via fermentation and the grinding department, the grinding department is also in two sub-sections which consist of the grinding and drying in which the wastewater can be collected (Ugwuanyi, 2008).

In detail, the fermentation sections for fufu production, soak or ferment cassava tubers with the peels. Thoroughly washed with clean water for four (4) days, fermentation in a clean container with sufficient water level, then the peels are removed and sifted in water for the removal of the fibres and compressed for the product of fufu and collect the wastewater for the research. Nevertheless the peels can be removed before fermentation also.

In the grinding and drying department, the cassava tubers were peeled and ground with the grinding machines, after which it is dried and waste is produced. This wastewater is discharged into the nearby drains;

This processing plant is not quite large, thereby this manual average production and average wastewater generated per day have not been estimated. Wastewater from cassava mainly contains starch and fibre as impurities. It may contain oil or grease from the lubricated parts of the machine also.
In the case of location of sample, the collection of samples to be used in determining the effect of cyanide is an important stage, but is often poorly conceived. Firstly the sample must be representative of the source that is to be evaluated. Secondly, the sampling equipment and techniques must be selected so that changes in the constituents to be analyzed will not occur between the sample collected and the time they are analyzed. However, sampling for ordinary chemical analysis requires no other precaution than collection in a dirt-free container. In
respect of this, the process of fermentation and shifting was done in the industry and the fermention cassava wastewater collected was stored in container and then transported to the laboratory for proper analysis. However, some tests were carried out immediately because of change with time, such as BOD test (Biochemical Oxygen Demand), coliform test, and cyanide test, and Do test (dissolved Oxygen test) in the laboratory.

3.2 LABORATORY SET UP.

Six plastic buckets were filled with cassava wastewater to a capacity of two litres (2.0l.).

The bucket was arranged in order of A₁, A₂, A₃, A₄, A₅, A₆, where the sample in A₁ was left as control and as some grams of chemicals were added, namely Sodium Hydroxide (NaOH). The chemical was added in the sample in 2%, 4%, 6%, 8%, and 10% to A₂ - A₆, respectively. Then the concentration of PH, cyanide, BOD, SS (suspended solid) was measured for 0 hours, 1 hour (0.42 day), 6 hours (0.025 days), 1 day, 3 days, 5 day, 8 day, 10 day, 15 day, 18 days and 20 days.

Therefore

Sample A₁: contained 0g of sodium hydroxide (i.e. control).

Sample A₂: contained 2g of sodium hydroxide

Sample A₃: contained 4g of sodium hydroxide

Sample A₄: contained 6g of sodium hydroxide

Sample A₅: contained 8g of sodium hydroxide

Sample A₆: contained 10g of sodium hydroxide
All samples were presented in such a way as to avoid any significant change in quality between the time of sampling and the actual testing.

3.3 SAMPLING TECHNIQUE

After the 4 days of fermentation process of cassava wastewater, a clean container was used to collect the samples. The interval between the test collection of the sample and the time of test was carried out and regulated. There are some inconsistencies due to factors such as electric supply and the entire samples for the whole experimental test on each time interval were collected at once.

Again, it was unsafe and unhygienic to store the cassava wastewater for weeks before the test would be conducted. The unused sample should be preserved in the refrigerator to avoid any further chemical activity like oxidation and degradation as the case may be. But it was not possible to preserve due to the large quantity of the samples involved.

3.4 pH TEST.

The pH of the sample was determined by simply dipping the pH meter (electrode) into the samples at readings, becomes steady the values was recorded.

3.5 BIOCHEMICAL OXYGEN DEMAND (BOD) TEST

Biochemical oxygen demand is an important indication of the amount of organic matter in the sample, showing the richness of waste in organic matter. The biochemical oxygen demand (BOD) measures described therein constitute an empirical test in which standardized laboratory
procedures are used to determine the reactive oxygen requirement of wastewater, as well as efficient and polluted water.

BOD test procedure, measures the dissolved oxygen consumed by microbial life while assimilating and oxidizing the organic matter present in the water. High BOD indicates the presence of a large amount of organic pollution. Its data have wide application in sanitary engineering practices. It is the principal test applied to sewage and industrial wastewater to determine the strength in terms of oxygen required for stabilization. Also it is a major criteria used in stream pollution control where organic leading must be restricted to maintain desired dissolved oxygen level.

Nevertheless the 5-day’s incubation of sample at 20°C is generally adopted as a standard method, in which the reduction in dissolved oxygen concentration during the incubation period yields a measure of BOD as factor used in determining the size of certain units and used to evaluate the efficiency of treatment plant. It is also used in sewer rental charges calculations.

The rate of oxidation of carbonaceous organic matter in the BOD test is mathematically written as

\[ X = L_0 \left(1 - 10^{-kt}\right) \]

where \( L_0 = \) ultimate BOD

\[ X = \text{Amount of } D_0 \text{ at any time} \]

\[ t = \text{Time in days} \]

\[ k = \text{first order rate of reaction rate constant.} \]
Apparatus

a. Incubation bottle (300ml capacity)

b. Air incubator or water bath

Reagents

Distilled water

Phosphate Buffer solution pH 7.2

Magnesium sulfate solution. Dissolve 22.5cm$^3$ of MgSO$_4$.7H$_2$O in distilled water and dilute to 1 litre.

Calcium chloride solution. Dissolved 27.5g anhydrous CaCl$_2$ in distilled water and dilute to 1 litre.

Ferric chloride solution. Dissolved 0.25g of FeCl$_3$.6H$_2$O in distilled water and dilute to 1 litre

Procedure

Prepare dilute water by adding 1ml of phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride solution for each litre of distilled water.

Seeding was not necessary since microorganisms were already present in domestic waste. The dilution water was saturated with air for about 20 minutes.

Several solutions of the samples were prepared with dilution (i.e., dilution covered a wide range of BOD).
3.6 COLIFORM MPN TEST

Traditionally coliform organisms are used as indicators or water pollution originating from human and animal intestines, comprise of all the aerobic and facultative anaerobic, a gram-negative, non-spore forming rod-shaped bacteria which ferment lactose with gas formation within 48 hours at 35°C.

For the fact that coliforms originate from other sources than the animal and human intestines, a confirmatory test for the presence of E-coli shows that the water under examination is unsafe for human consumption. Coilform standard test may be carried out either by multiple tube fermentation technique or by the membrane filter technique, and each technique has been applicable with due consideration of the purpose of the examination. These tests are used over a broad range in water quality and were carried out on raw water as it passes through the treatment processes.

Reagents

Presumptive test: Mac Conkey Broth was used.

Procedures

A series of fermentation tubes were inoculated with appropriate graduated quantities, multiples and sub-multiples of 1ml of water to be tested. Bottles to contain 100ml of portion were prewarmed water both at 35°C. The concentration of nutritive ingredients in the mixture of medium and added portion of sample conformed to the requirement.
The amount of the water sample used for inoculating the lactose broth fermentation tube varied in size and number with chamber of the water under examination but in general were decimal multiples and submultiples of 1ml. The inoculated formation tubes were incubated at 35°C ± 0.5°C. After shaking gently, each tube was examined for gas formation at the end 24 ± 2hrs and if none had formed, and being trapped in the inverted Durham tubes, examine again at the end of 48 ± 3 hrs. The presence and absence of gas formation of each examination of the tube was recorded regardless of the amount.

3.7 CYANIDE TEST

Methods of testing cyanide

One of the methods for testing cyanide is reflux distillation. It is a procedure used to extract soluble cyanide salts and many insoluble cyanide complexes from waste and leachates. It is based on the decomposition of nearly all cyanide by a strong acid and a magnesium catalyst. (i.e., distillation of the sample in the presence of sulfuric acid readily converts the simple cyanides into HCN). Cyanide in the form of hydrochloride acid (HCN) is poured from the sample and captured into alkaline solution. The concentration of cyanide in the alkaline solution is then determined.

Apparatus

Modified caisson flask, Allihn-type condenser, Gas washer, suction flask, Air inlet consisting of a thistle tube, water aspirator, heating element and connection tube

Reagents
Sodium Hydroxide Solution and Mercuric Chloride Solution.

Application

This method is applicable to the determination of cyanide in drinking surface and saline water, domestic and industrial wastes. The titration procedure using silver nitrate with P-diethyl-aminobenza-rhodamine indicator is used for measuring concentration of cyanide exceeding 1mg/l (0.25mg/250ml of absorbing liquid), the sample contains more than 1mg/l of CN, transfers the distilled or a suitable aliquot dilute to 250ml to a 50ml Erlenmeyer flask. Add 10-12 drops of the paradimethylaminobenzarhodamine indicator; titrate with standard silver nitrate to the first colour change from yellow to brownish pink. Titrated distilled water using the same amount of sodium hydroxide and indicator as the sample. The analyst should familiarize herself with the end point of the titration and the amount of indicator to be used before the samples are actually titrated. Using the titrimetric method, calculate concentration of CN as follows.

5 ml burette may be used to obtain a precise titration, the titration is based on the following reaction, Ag + 2 CN₆ + [Ag (CN)]₂⁻; if all the cyanide has complexes and more silver nitrate is added, the excess silver combines with the paradimethylaminobenzarhodamine indicator to turn the solution yellow and then salmon color (brownish pink). When titrimetric method is used

\[
\text{mg/l CN} = \frac{(A-B)\times 1000}{ml \text{ of original sample}} \times \frac{N}{ml \text{ of sample}}
\]
where \( A \) = value of AgNO\(_3\) for filtration of sample

\[
B = \text{value of AgNO}_3 \text{ for titration of blank}
\]

\[
N = \text{normality of the solution.}
\]

Cyanide is decomposed by oxidizing agent like chlorine, sulphide and nitrates.

3.8 SUSPENDED SOLID TEST (SS)

Apparatus

The apparatus used for suspended solid test includes beakers, filter papers, desiccators, oven, weighing balance, etc.

Procedure

A beaker with a filter paper inserted into it was dried at a temperature of 103 – 105°C for 1 hr.

Allow the beaker to cool in desiccators. Weigh the empty beaker and filter paper, then record.

10ml of the sample was taken and filtered, using the weighed filter paper ad a vacuum device.

The filter paper was replaced in the beaker again in oven for 1 hour at a temperature 105°C. The filter paper and beaker were reweighed and the difference in weight was recorded as suspended solid.

The mg/l SS was calculated thus;

\[
\text{Mg/l SS} = \frac{\text{weight of SS (mg) x 1000}}{1m \text{ of sample}}
\]
3.9 DATA ANALYSIS PROCEDURE

Under steady state conditions, mass balance are written differently for substrate and biomass using Monod kinetic model the rate of formation of biomass or utilization substrate can be assumed. Therefore, from steady state completely mixed Monod reactor

\[
\text{ACCUMULATION = INFLOW-OUTFLOW + DECAY}
\]

Mathematically written as

\[
\frac{Vdc}{dc} = Qc_0 - Qc + V(-Kc) \quad 3.1
\]

\[
0 = Qc_0 - Qc - VKc \quad 3.2
\]

From Monod model \( K = \frac{X}{Y(K_m+S)} \)

where \( C = S = X \)

Substituting for \( K \) in equation 3.2

\[
Q_{so} - Qs - \frac{K_s SXV}{Y(K_m+S)} = 0 \quad 3.3
\]

\[
Q_{xo} - Qx - \frac{K_s SXV}{K_m+S} + K_d XV = 0 \quad 3.4
\]

But \( X_0 = 0 \)

Then \( - Qx - \frac{K_s SXV}{K_m+S} + K_d XV = 0 \quad 3.5 \)

Divide right through by \( XYQ \), obtains
\[ \frac{1}{Y} - \frac{K_d S}{Y Q (K_m + S)} = \frac{K_d V}{Y Q} = 0 \]  \[ 3.6 \]

but \( V/Q = \Theta \)

where \( \Theta \) is the detention time

\[ \frac{K_d S \Theta}{Y (K_m + S)} = \frac{K_d \Theta}{Y} + \frac{1}{Y} \]  \[ 3.7 \]

But from equation 3.3

\[ \frac{S_0 - S}{X} = \frac{K_d S \Theta}{Y (K_m + S)} \]  \[ 3.8 \]

\[ \frac{S_0 - S}{X} = \frac{K_d \Theta}{Y} + \frac{1}{Y} \]  \[ 3.9 \]

By inversion of equation 3.9

\[ \frac{X}{S_0 - S} = \frac{Y}{K_d \Theta} + Y \]

Since \[ \frac{S_0 - S}{X} = \frac{K_d S \Theta}{Y (K_m + S)} \]

Then \[ \frac{X}{S_0 - S} = \frac{Y (K_m + S)}{K_d S \Theta} \]  \[ 3.10 \]

Simplifying equation 3.10 further, it becomes

\[ \frac{X \Theta}{S_0 - S} = \frac{Y K_m}{K_o} \frac{1}{S} + \frac{Y}{K_o} \]  \[ 3.11 \]

Where \( K_d, Y, K_m, \) and \( K_o \) are constants, and can be determined using graphically or regression analysis. From the equations \( 1/Y, Y/K_o \) and \( K_d/Y, Y K_m/K_o \) is the intercept and slope of the both equation.
Furthermore, dividing equation 3.11 by Y, it then becomes

\[ \frac{X}{(S_0-S)Y} = \frac{K_m}{K_0} 1/S + \frac{1}{K_0} \]  

3.12

But from equation 3.10

\[ \frac{X}{(S_0-S)Y} = \frac{1}{K} \]  

3.13

since \( K = \frac{(S_0-S)Y}{\theta X} \)

Therefore \( \frac{1}{K} = \frac{K_m}{K_0} 1/S + \frac{1}{K_0} \)  

3.14 (for uninhibited inhibition)

Presenting equation 3.14 in a graphical method shows the Typical Lineweaver-Burk plot for the Michaelis-Menten equation which was called uninhibited inhibition, where \( K_m/K_0 \) and \( 1/K_0 \) were the slope and intercept respectively.

Furthermore, when the rate for product formation is given as

\[ \frac{dp}{dt} = \frac{K_0S}{K_m+S+I_{km}/Ki} \]  

3.15

If \( \frac{dp}{dt} = K \)

Then, \( K = \frac{K_0S}{K_m+S+I_{km}/Ki} \)  

3.16

Taking the inversion of equation 3.16 and simplifying it

\[ \frac{1}{K} = \frac{K_m(1+I/K_i)+S}{K_0S} \]  

3.17

Simplifying the RHS of the equation,
\[ \frac{K_m(1 + \frac{I}{K_i})}{K_0S} + \frac{S}{K_0S} \]

\[ \frac{1}{K} = \frac{K_m(1 + \frac{I}{K_i})}{K_0S} + \frac{1}{K_0} \]  \hspace{1cm} (3.18) \hspace{1cm} \text{(for competitive inhibition)}

But if the rate for product formation is given as

\[ \frac{dp}{dt} = \frac{K_0}{(1 + \frac{K_mS}{K_i})(1 + \frac{I}{K_i})} \]  \hspace{1cm} (3.19)

\[ K = \frac{K_0}{(1 + \frac{K_mS}{K_i})(1 + \frac{I}{K_i})} \]  \hspace{1cm} (3.20)

Taking the inversion of equation 3.20

\[ \frac{1}{K} = \frac{(1 + \frac{K_mS}{K_i})(1 + \frac{I}{K_i})}{K_0} \]  \hspace{1cm} (3.21)

By expansion of the RHS, it becomes

\[ \frac{1 + \frac{I}{K_i} + \frac{K_m}{K_i}}{K_0S} \]  \hspace{1cm} (3.22)

Dividing the nominator and denominator by \( k_0 \), the equation gives

\[ (1 + \frac{I}{K_i}) \frac{1}{K_0} + \frac{K_m}{K_0S} \left(1 + \frac{I}{K_i}\right) \]  \hspace{1cm} (3.23)

Rearranging equation 3.23, then

\[ \frac{1}{K} = \frac{K_m}{K_0} \left(1 + \frac{I}{K_i} \right) \frac{1}{S} + \left(1 + \frac{I}{K_i}\right) \frac{1}{K_0} \]  \hspace{1cm} (3.24) \hspace{1cm} \text{(for noncompetitive inhibition)}
CHAPTER FOUR

DATA ANALYSIS, RESULTS AND DISCUSSION

4.1 DATA ANALYSIS AND PRESENTATION OF RESULTS

Six samples of the cassava wastewater were put in the measure of 2000ml each in six plastic buckets. The buckets were left open to air since the condition used is aerobic.

Sample A₁ = 0.0g of NaOH

Sample A₂ = 2.0g of NaOH

Sample A₃ = 4.0g of NaOH

Sample A₄ = 6.0g of NaOH

Sample A₅ = 8.0g of NaOH

Sample A₆ = 10.0g of NaOH.

The samples were collected in the times allotted in the order of 0hour, 1hour(0.042 days), 3hours(0.125 days), 1day, 3days, 5days, 8days, 10days, 15days, 18day, 20days. After which the parameters measured were plotted against time. Graphical method and statistical regression analysis were used to determine the constants, $K_m$, $K_c$, $K_d$, and $Y$ using equation 3.9 and 3.11. Graphically, using equation 3.9, plotting $(S_0 - S)/X$ versus $\Theta$ is linear.
**Fig 4.1 Variation of pH with Time**

**Fig 4.2 Variation of BOD\textsubscript{5} with Time**
Fig 4.3 Variation of Suspended Solid (SS) with Time

Fig 4.4 Variation of coliform with Time
Fig 4.5 Variation of Cyanide with time

DETERMINATION OF K_{d}, Y, K_{m} AND K_{o} USING THE GRAPHS BELOW

Using the mathematical models in sub-title 3.8 of chapter three for the Michaelis-Menten of kinetic inhibition, regression analysis was carried out and was graphically presented below showing the variation of the rate of concentration reaction against time \( \Theta \) for different grams of sodium hydroxide added to the experiments to determine the Monod constants \( K_{d}, Y, K_{m} \) and \( K_{o} \).
Fig 4.6 Rate of concentration reaction versus time $\Theta$ for the determination of $Y$ and $K_d$ for the addition of 0g NaOH

Fig 4.7 Determination of $K_d$ and $K_o$ for the addition of 0g NaOH
Fig 4.8 Rate of concentration reaction versus time $\Theta$ for the determination of $Y$ and $K_d$ for the addition of 2g NaOH

Fig 4.9 Determination of $K_m$ and $K_o$ for the addition of 2g NaOH
Fig 4.10 Rate of concentration reaction versus time $\Theta$ for the determination of $Y$ and $K_d$ for the addition of 4g NaOH

Fig 4.11 Determination of $K_m$ and $K_o$ for the addition of 4g NaOH
Fig 4.12 Rate of concentration reaction versus time $\Theta$ for the determination of $Y$ and $K_d$ for the addition of 6g NaOH

Fig 4.13 Determination of $K_m$ and $K_o$ for the addition of 6g NaOH
**Fig 4.14** Rate of concentration reaction versus time θ for the determination of Y and K_d for the addition of 8g NaOH

**Fig 4.15** Determination of K_m and K_o for the addition of 8g NaOH
Fig 4.16 Rate of concentration reaction versus time $\Theta$ for the determination of $Y$ and $K_d$ for the addition of 10g NaOH

Fig 4.17 Determination of $K_m$ and $K_o$ for addition of 10g NaOH
To determine the value of specific growth rate time, $K$,

Equation 3.14 was used for the presentation of graph to determine the slopes and intercepts which enables the determination of $k$ values for different grams of NaOH added.

![Graph 4.18](image1.png)

**Fig 4.18 Variation of $1/K$ with $1/S$ for the calculated specific growth rate at 0g NaOH**

![Graph 4.19](image2.png)

**Fig 4.19 Variation of $1/K$ with $1/S$ for the calculated specific growth rate at 2g NaOH**
Fig 4.20 Graph of $1/K$ with $1/S$ for the calculated specific growth rate at 4g NaOH

Fig 4.21 Variation of $1/K$ with $1/S$ for the calculated specific growth rate at 6g NaOH
Fig 4.22 Variation of $1/K$ with $1/S$ for the calculated specific growth rate at 8g NaOH

![Graph showing variation of $1/K$ with $1/S$ for the calculated specific growth rate at 8g NaOH with an observed correlation coefficient $R=0.6392$.]

Fig 4.23 Variation of $1/K$ with $1/S$ for the calculated specific growth rate at 10g NaOH

![Graph showing variation of $1/K$ with $1/S$ for the calculated specific growth rate at 10g NaOH with an observed correlation coefficient $R=0.9054$.]
GRAPHS OF SODIUM HYDROXIDE AS AN OXIDIZING AGENT (NaOH) SHOWING THE TYPES OF INHIBITIONS

Fig 4.24 Variation of $1/K$ with $1/S$ for the cases of competitive inhibition and no inhibition

Fig 4.25 Variation of $1/K$ with $1/S$ for the cases of competitive non-competitive inhibition and no inhibition
Fig 4.26 Variation of $1/K$ with $1/S$ for the cases of non-competitive inhibition and no inhibition

\[ y_n = 68.21x + 0.372 \]
\[ R^2 = 0.473 \]

\[ y_{nc} = 241.0x + 1.751 \]
\[ R^2 = 0.548 \]

Fig 4.27 Variation of $1/K$ and $1/S$ for the cases of competitive inhibition and no inhibition

\[ y_c = 241.5x + 0.805 \]
\[ R^2 = 0.796 \]

\[ y_n = 400.4x + 0.801 \]
\[ R^2 = 0.396 \]
4.2 DETERMINATION OF THE INHIBITION CONSTANT $K_i$

FROM THE GRAPHS ABOVE, USING THE EQUATION OF EACH OF THE INHIBITION TYPES OBTAINED, $K_i$ WILL BE EVALUATED.

Evaluation of $K_i$ For the competitive inhibition using equation 4.18

\[
\frac{1}{K} = \frac{K_m}{K_0}(1 + \frac{I}{K_I}) \frac{1}{S} + \frac{1}{K_0}
\]

where \( \frac{K_m}{K_0}(1 + \frac{I}{K_I}) = \text{slope} = 333.0003 \)

But \( K_m = 5645.2622 \text{ mg/l} \)

\( K_0 = 22.2222 \text{ day}^{-1} \)

Given that $I = 184 \text{ mg/l}$

Therefore \( \frac{5645.2622}{22.2222}(1 + \frac{184}{K_I}) = 333.0003 \)

\[
254.0368 + \frac{46745.7712}{K_I} = 333.0003
\]

\( K_1 = 591.954 \text{ mg/l} \)

Substituting in the main equation

\[
\frac{I}{K} = 254.0368(1 + 0.3108)(0.0271) + 0.094
\]

\[
\frac{I}{K} = 9.1181
\]
Evaluation of $K_i$ for the competitive non-competitive inhibition (mixed inhibition) using equation 2.8

$$\frac{l}{K} = \frac{K_m'}{K_0'} \left( \frac{1}{s} \right) + \frac{1}{K_0'}$$

$$\frac{1}{K_0'} = \frac{1}{K_0} \left[ 1 + \frac{l}{K_i} \right] = 3.681 \text{ (intercept)}$$

$$\frac{1}{K_0} = 1.9429$$

$$l = 184$$

Therefore

$$1.9429 \left( 1 + \frac{184}{K_i} \right) = 3.681$$

$$K_i = 205.6807 \text{ mg/l}$$

But $\frac{K_m'}{K_0'} = \frac{K_m(1 + \frac{l}{K_i})}{K_0} = 1222 \text{ (Slope)}$

$$\frac{K_m}{K_0} = 807.5753$$

$$807.5753 \left[ 1 + \frac{184}{K_i} \right] = 1222$$

$$K_i = 358.554 \text{ mg/l}$$

Substituting the values $K_i$ in the mixed inhibition equation

$$\frac{l}{K} = \frac{K_m}{K_0} \left( 1 + \frac{l}{K_i} \right) \frac{1}{s} + \left( 1 + \frac{l}{K_i} \right) \frac{1}{K_0}$$
\[ \frac{I}{K} = 18.1008 \]

Evaluation of \( K_i \) for the non-competitive inhibition using equation 4.24

\[ \frac{I}{K} = \frac{K_m}{K_0} \left( 1 + \frac{I}{K_i} \right) \frac{1}{S} + \left( 1 + \frac{I}{K_i} \right) \frac{1}{K_0} \]

\[ \frac{K_m}{K_0} \left( 1 + \frac{I}{K_i} \right) = 241.0 \]

\[ \left( 1 + \frac{I}{K_i} \right) \frac{1}{K_0} = 1.751 \]

\[ [1 + \frac{184}{K_i}][-0.0197] = 1.751 \]

\[ K_i = 2.0471 \text{ mg/l} \]

For \( \frac{K_m}{K_0} \left( 1 + \frac{I}{K_i} \right) = 241.0 \)

\[ 164.9357[1 + \frac{184}{K_i}] = 241.0 \]

\[ K_i = 398.980 \text{ mg/l} \]

Substituting the value for non-competitive inhibition

\[ \frac{I}{K} = 164.9357(1 + 0.4612)(0.0086) + (1+89.8876)(-0.0197) \]

\[ \frac{I}{K} = 0.2821 \]

Evaluation of \( K_i \) for the competitive inhibition using equation 4.18

\[ \frac{I}{K} = \frac{K_m}{K_0} \left( 1 + \frac{I}{K_i} \right) \frac{1}{S} + \frac{1}{K_0} \]
\[
\frac{K_m}{K_0} \left(1 + \frac{I}{K_i}\right) = 400.4
\]

\[K_m = -611.381 \text{ mg/l}\]

\[K_0 = -0.4432 \text{ day}\]

\[
\frac{-611.381}{-0.4432} \left(1 + \frac{184}{K_i}\right) = 400.4
\]

\[K_i = 259.254 \text{ mg/l}\]

Substituting the value of \(K_i\) in the above equation

\[
\frac{I}{K} = 10.7063
\]

Table 4.1  Summary of the results for the values of \(K_i\) was listed below for different grams of the oxidizer added to the wastewater and the identification of the types of inhibition involved.

<table>
<thead>
<tr>
<th>Inhibition types</th>
<th>Competitive</th>
<th>Mixed</th>
<th>Non-competitive</th>
<th>competitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOH (g)</td>
<td>2g</td>
<td>4g</td>
<td>6g</td>
<td>10g</td>
</tr>
<tr>
<td>(K_i)</td>
<td>591.954</td>
<td>358.554</td>
<td>398.980</td>
<td>259.254</td>
</tr>
</tbody>
</table>
4.3 DISCUSSION OF RESULT

Bacteria are favored by pH level between 6.5 and 8.5. With respect to this the pH of the sample varies with time, as it increases and decreases showing its effect to converge to its neutral state in Fig 4.1. The BOD values in Fig 4.2, decrease with time showing the reduction of the pollutants as they are exposed to air in effect reducing the cyanide contents with time enabling degradation. In Fig 4.3, the suspended solid in the wastewater was observed to be reducing; resulting in the decrease in turbidity of the water and in Fig 4.4, the coliform results indicate that in each day, there is reduction of pollution of the wastewater. Again, the introduction of sodium hydroxide as an oxidizing agent reduces the amount of cyanide as it decreases with time in Fig 4.5 allowing the degradation of biological activities. Figures 4.6-4.17 give the results for the Monod kinetic constant, the growth yield “Y”, death rate “K_d”, maximum Specific growth rate constant “K_0” and Michealis Menten constant “K_m” which enable the achievement of the Specific growth rate result K of biological activities in the reactors.

Progression of these results using Line Weaver Burk Model leads to the finally summarized result in Table 4.1 above showing different inhibition types obtained at different concentration reactions of the oxidizing agent added using regression analysis. The inhibition constant also varies with the increase in variation rate of the oxidizing agents added to reduce cyanide concentration for biodegradation. Two competitive inhibitions were observed from the graph of the addition of 2g and 10g of sodium hydroxide to the waste for the reduction of the concentration of the inhibitor, indicating that the substrate concentration is low at 2g NaOH which makes the inhibitor to compete favorably with the substrate, resulting to high degree of
inhibition, but at 10g NaOH the substrate concentration is high making the inhibitor to be much less successful in competing with the substrate, then the inhibition degree becomes lower.

For 4g and 6g of the oxidizing reagent, mixed inhibition and non-competitive inhibition were observed respectively from the plotted graphs that show the variation of $1/k$ with $1/S$ above from Figures 4.18 to 4.22. The non-competitive inhibition produces an ideal end complex since the inhibitor combines with an enzyme molecule not minding whether a substrate molecule bound or not, (i.e., the presence of the inhibitor on the enzyme does not prevent that complex from reacting with the substrate). This is because the substrate does not affect the inhibitor binding, thereby the total enzyme concentration was effectively reduced by the inhibitor, decreasing the value of $K_0$ and not altering $K_m$. The observed mixed inhibition form is known as competitive non-competitive inhibition showing that from the graph $K_i$(slope) is greater than $K_i$(intercept), i.e., $K_i > K_1$ and the plots cross to left of the $1/k$ axis, and is above the $1/S$ axis.

Competitive inhibition was observed mathematically since the calculated $K_m$ (Michealis Menten constant) was changed and $K_0$ (maximum specific growth rate constant) was unchanged, while for the non-competitive $K_m$ was unchanged and $K_0$ was changed.
CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

This research made it possible to analyze the chemical component of cassava wastewater and the effects of cyanide on its degradation. The wastewater was subjected to laboratory tests and an oxidizing agent was added to ensure effective degradation. The additions of sodium hydroxide oxidizes cyanide content to cyanate. Positively using the statistical regression and the Lineweaver-Burk plot equation, presentations of the plots classifies the different types of inhibition involved, at different grams of oxidization agent added in buckets $A_2 - A_6$. Also results show that cyanide reduces with time. Finally, the results show that the inhibition factors vary at different percentage increases of the oxidizer.

5.2 RECOMMENDATION

This research is very important to sanitary engineers, environmentalists, enzymologists and others whose interests are on investigation of the level of pollution in water bodies and on the environments by toxic chemicals like cyanide. Since cassava is being utilized extensively for industrial purposes, like production of paper, plywood, adhesives, sweeteners, alcohol, and amino acid, the waste obtained from all these measures are more acidic. It is highly proposed that the waste undergo proper treatment before discharge to rivers. Research has made known that low concentration of less than 0.3mg/L of cyanide may result in massive fish kills. Hence
the analytical result of this research is useful and noteworthy for the design of treatment plants. Untreated sewage from cassava industries should not be used for irrigation or in fish ponds. Since it produces chromosomal aberration in some plants and prevents self purification of the receiving water bodies.
REFERENCES


influenced by protein supplementation and processing” Proc. 33rd Annual Conf. of NSAP, Ayetoro, Ogun State Nigeria 373-376.


Irwin, J., (1997). “Environmental Contaminants Encyclopedia Entry on Cyanide(s) in General” Pg 7-64.


Email: arogers@bral.gov.


Ugwuanyi, H.K. (2008). “Inhibition of Cassava Wastewater Degradation by Cyanide” Undergraduate Project, Civil Engineering Department UNN.


# APPENDIX

## pH TEST OF VALUES

<table>
<thead>
<tr>
<th>Time(days)</th>
<th>0</th>
<th>0.042</th>
<th>0.125</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>8</th>
<th>10</th>
<th>15</th>
<th>18</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>0g-NaOH</td>
<td>4.71</td>
<td>4.47</td>
<td>4.64</td>
<td>4.62</td>
<td>7.2</td>
<td>8.27</td>
<td>8.35</td>
<td>9.04</td>
<td>9.13</td>
<td>9.29</td>
<td>9.46</td>
</tr>
<tr>
<td>2g-NaOH</td>
<td>4.67</td>
<td>4.53</td>
<td>4.44</td>
<td>4.69</td>
<td>6.1</td>
<td>7.58</td>
<td>8.09</td>
<td>8.93</td>
<td>9.09</td>
<td>9.3</td>
<td>9.36</td>
</tr>
<tr>
<td>4g-NaOH</td>
<td>4.8</td>
<td>4.59</td>
<td>4.69</td>
<td>4.7</td>
<td>6.13</td>
<td>7.28</td>
<td>7.82</td>
<td>8.56</td>
<td>8.95</td>
<td>9.16</td>
<td>9.22</td>
</tr>
<tr>
<td>6g-NaOH</td>
<td>4.87</td>
<td>4.83</td>
<td>4.79</td>
<td>4.9</td>
<td>6.26</td>
<td>7.05</td>
<td>7.45</td>
<td>8.32</td>
<td>8.49</td>
<td>8.6</td>
<td>8.74</td>
</tr>
<tr>
<td>8g-NaOH</td>
<td>5.01</td>
<td>4.97</td>
<td>4.95</td>
<td>5.16</td>
<td>6.4</td>
<td>6.92</td>
<td>7.11</td>
<td>7.76</td>
<td>8.2</td>
<td>8.52</td>
<td>8.64</td>
</tr>
<tr>
<td>10g-NaOH</td>
<td>5.06</td>
<td>5.08</td>
<td>4.99</td>
<td>5.02</td>
<td>6.65</td>
<td>6.98</td>
<td>7.1</td>
<td>7.69</td>
<td>7.61</td>
<td>7.49</td>
<td>7.67</td>
</tr>
</tbody>
</table>

## BOD₅ TEST OF VALUES (mg/l)

<table>
<thead>
<tr>
<th>Time(days)</th>
<th>0</th>
<th>0.042</th>
<th>0.125</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>8</th>
<th>10</th>
<th>15</th>
<th>18</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>0g-NaOH</td>
<td>341</td>
<td>262</td>
<td>190</td>
<td>162</td>
<td>101</td>
<td>62</td>
<td>42</td>
<td>29</td>
<td>22</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>2g-NaOH</td>
<td>388</td>
<td>297</td>
<td>230</td>
<td>193</td>
<td>133</td>
<td>92</td>
<td>64</td>
<td>49</td>
<td>39</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>4g-NaOH</td>
<td>473</td>
<td>351</td>
<td>290</td>
<td>235</td>
<td>171</td>
<td>120</td>
<td>103</td>
<td>89</td>
<td>53</td>
<td>40</td>
<td>35</td>
</tr>
<tr>
<td>6g-NaOH</td>
<td>498</td>
<td>392</td>
<td>322</td>
<td>265</td>
<td>201</td>
<td>147</td>
<td>125</td>
<td>111</td>
<td>104</td>
<td>82</td>
<td>62</td>
</tr>
<tr>
<td>8g-NaOH</td>
<td>514</td>
<td>420</td>
<td>357</td>
<td>291</td>
<td>251</td>
<td>200</td>
<td>187</td>
<td>162</td>
<td>159</td>
<td>126</td>
<td>102</td>
</tr>
<tr>
<td>10g-NaOH</td>
<td>562</td>
<td>479</td>
<td>381</td>
<td>304</td>
<td>281</td>
<td>236</td>
<td>214</td>
<td>199</td>
<td>189</td>
<td>162</td>
<td>147</td>
</tr>
</tbody>
</table>
### SUSPENDED SOLID OF VALUES (SS) (mg/l)

<table>
<thead>
<tr>
<th>Time(days)</th>
<th>0</th>
<th>0.042</th>
<th>0.125</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>8</th>
<th>10</th>
<th>15</th>
<th>18</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>0g-NaOH</td>
<td>1.6</td>
<td>1.4</td>
<td>1.4</td>
<td>3.2</td>
<td>3.0</td>
<td>0.8</td>
<td>4.0</td>
<td>2.2</td>
<td>3.8</td>
<td>4.8</td>
<td>2.6</td>
</tr>
<tr>
<td>2g-NaOH</td>
<td>1.0</td>
<td>2.4</td>
<td>1.6</td>
<td>0.8</td>
<td>0.2</td>
<td>0.6</td>
<td>3.6</td>
<td>3.6</td>
<td>13</td>
<td>6.4</td>
<td>2.6</td>
</tr>
<tr>
<td>4g-NaOH</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>2.0</td>
<td>0.4</td>
<td>0.6</td>
<td>9.0</td>
<td>6.0</td>
<td>8.2</td>
<td>8.2</td>
<td>0.6</td>
</tr>
<tr>
<td>6g-NaOH</td>
<td>0.6</td>
<td>0.6</td>
<td>0.8</td>
<td>1.2</td>
<td>0.2</td>
<td>1.4</td>
<td>6.4</td>
<td>4.6</td>
<td>7.8</td>
<td>11.6</td>
<td>4.0</td>
</tr>
<tr>
<td>8g-NaOH</td>
<td>2.4</td>
<td>1.6</td>
<td>0.4</td>
<td>1.2</td>
<td>2.6</td>
<td>4.0</td>
<td>2.8</td>
<td>3.8</td>
<td>8.0</td>
<td>7.8</td>
<td>2.6</td>
</tr>
<tr>
<td>10g-NaOH</td>
<td>1.2</td>
<td>1.2</td>
<td>1.0</td>
<td>0.6</td>
<td>1.4</td>
<td>1.8</td>
<td>2.8</td>
<td>6.0</td>
<td>5.8</td>
<td>8.0</td>
<td>3.6</td>
</tr>
</tbody>
</table>

### COLIFORM TEST OF VALUES

<table>
<thead>
<tr>
<th>Time(days)</th>
<th>0</th>
<th>0.042</th>
<th>0.125</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>8</th>
<th>10</th>
<th>15</th>
<th>18</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>0g-NaOH</td>
<td>240</td>
<td>39</td>
<td>64</td>
<td>150</td>
<td>240</td>
<td>460</td>
<td>460</td>
<td>240</td>
<td>1100</td>
<td>2400</td>
<td>2400</td>
</tr>
<tr>
<td>2g-NaOH</td>
<td>15</td>
<td>15</td>
<td>20</td>
<td>93</td>
<td>150</td>
<td>210</td>
<td>120</td>
<td>28</td>
<td>150</td>
<td>150</td>
<td>1100</td>
</tr>
<tr>
<td>4g-NaOH</td>
<td>4</td>
<td>7</td>
<td>11</td>
<td>64</td>
<td>23</td>
<td>150</td>
<td>28</td>
<td>11</td>
<td>75</td>
<td>39</td>
<td>93</td>
</tr>
<tr>
<td>6g-NaOH</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>11</td>
<td>9</td>
<td>15</td>
<td>20</td>
<td>4</td>
<td>43</td>
<td>28</td>
<td>15</td>
</tr>
<tr>
<td>8g-NaOH</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>9</td>
<td>3</td>
<td>23</td>
<td>3</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>10g-NaOH</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>3</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
CYANIDE TEST OF VALUES (mg/l)

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>0</th>
<th>0.042</th>
<th>0.125</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>8</th>
<th>10</th>
<th>15</th>
<th>18</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>0g-NaOH</td>
<td>780</td>
<td>750</td>
<td>605</td>
<td>530</td>
<td>510</td>
<td>405</td>
<td>300</td>
<td>203</td>
<td>150</td>
<td>93</td>
<td>88</td>
</tr>
<tr>
<td>2g-NaOH</td>
<td>560</td>
<td>520</td>
<td>410</td>
<td>390</td>
<td>380</td>
<td>215</td>
<td>175</td>
<td>133</td>
<td>65</td>
<td>34</td>
<td>30</td>
</tr>
<tr>
<td>4g-NaOH</td>
<td>480</td>
<td>420</td>
<td>360</td>
<td>235</td>
<td>155</td>
<td>130</td>
<td>109</td>
<td>93</td>
<td>67</td>
<td>50</td>
<td>21</td>
</tr>
<tr>
<td>6g-NaOH</td>
<td>300</td>
<td>210</td>
<td>151</td>
<td>170</td>
<td>144</td>
<td>120</td>
<td>79</td>
<td>68</td>
<td>53</td>
<td>30</td>
<td>17</td>
</tr>
<tr>
<td>8g-NaOH</td>
<td>230</td>
<td>205</td>
<td>150</td>
<td>141</td>
<td>120</td>
<td>91</td>
<td>64</td>
<td>50</td>
<td>44</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>10g-NaOH</td>
<td>194</td>
<td>176</td>
<td>143</td>
<td>120</td>
<td>94</td>
<td>74</td>
<td>40</td>
<td>18</td>
<td>9</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

DETERMINATION OF Y, K_d, K_m, AND k_0 FOR THE DIFFERENT SAMPLES.

Using \( \frac{S_0 - S}{X} = \frac{K_d \theta}{Y} + \frac{I}{Y} \)

the inverse of above equation then becomes

\[
\frac{6X}{S_0 - S} = Y \frac{K_m}{K_0} \left( \frac{I}{S} \right) + \frac{Y}{K_0}
\]

The two equations were gotten from the steady state equation in which the Monod constant was obtained.

The Monod constants are K_d, k_m, K_0, y
For the plot of $\frac{S_0 - S}{x}$ with $\Theta$, $\frac{K_d}{Y}$ becomes the slope, while $\frac{l}{Y}$ is the intercept and $\frac{eX}{S_0 - S}$ against $\frac{l}{s}$, $\frac{YK_m}{K_0}$ becomes the slope, while $\frac{Y}{K_0}$ the intercept in graphs presented.

Using the two equations to obtain the constants for the 0g of NaOH as plotted in fig 4.1 and 4.2

$$\frac{K_d}{Y} = 1.1787$$

$$\frac{l}{Y} = 71.8482$$

$$Y = 0.0139 \text{ mg/mg BOD}$$

$$K_d = 0.0164 \text{ day}^{-1}$$

Then, $\frac{YK_m}{K_0} = 3.5311$

$$\frac{Y}{K_0} = 0.00063$$

Then $K_0 = 22.0635 \text{ day}^{-1}$

$$K_m = 5604.9206 \text{ mg/l}$$

Using the two equations to obtain the constants for the 2g of NaOH as plotted in fig 4.3 and 4.4

$$\frac{K_d}{Y} = 2.5214$$

$$\frac{l}{Y} = 70.1762$$

$$Y = 0.0142 \text{ mg/mg BOD}$$

$$K_d = 0.0359 \text{ day}^{-1}$$
Then,

\[ \frac{YK_m}{K_0} = 4.7286 \]

\[ \frac{Y}{K_0} = 0.0013 \]

Then \( K_0 = 0.0915 \text{ day}^{-1} \)

\( K_m = 30.4859 \text{ mg/l} \)

Using the two equations to obtain the constants for the 4g of NaOH as plotted in fig 4.5 and 4.6

\[ \frac{K_d}{Y} = 9.1390 \]

\[ \frac{I}{Y} = 100.7702 \]

\( Y = 0.0099 \text{ mg/mg BOD} \)

\( K_d = 0.0907 \text{ day}^{-1} \)

Then,

\[ \frac{YK_m}{K_0} = 7.9949 \]

\[ \frac{Y}{K_0} = 0.0192 \]

Then \( K_0 = 0.5156 \text{ day}^{-1} \)

\( K_m = 416.40 \text{ mg/l} \)
Using the two equations to obtain the constants for the 6g of NaOH as plotted in fig 4.7 and 4.8

\[ \frac{K_d}{Y} = 4.8731 \]

\[ \frac{l}{Y} = 7.5402 \]

\[ Y = 0.1326 \text{ mg/mg BOD} \]

\[ K_d = 0.6463 \text{ day}^{-1} \]

Then, \[ \frac{YK_m}{K_0} = 14.1295 \]

\[ \frac{Y}{K_0} = 0.0195 \]

Then \[ K_0 = 6.8 \text{ day}^{-1} \]

\[ K_m = 724.5897 \text{ mg/l} \]

Using the two equations to obtain the constants for the 8g of NaOH as plotted in fig 4.9 and 4.10

\[ \frac{K_d}{Y} = 2.6124 \]

\[ \frac{l}{Y} = 53.5588 \]

\[ Y = 0.0187 \text{ mg/mg BOD} \]

\[ K_d = 0.0489 \text{ day}^{-1} \]
Then,

\[
\frac{YK_m}{K_0} = 33.3441
\]

\[
\frac{Y}{K_0} = -0.0592
\]

Then \( K_0 = -0.3159 \) day\(^{-1}\)

\[
K_m = -0.5723333 \text{ mg/l}
\]

Using the two equations to obtain the constants for the 10g of NaOH as plotted in fig 4.11 and 4.12

\[
\frac{K_d}{Y} = 3.4277
\]

\[
\frac{I}{Y} = 31.0415
\]

\[
Y = 0.0322 \text{ mg/mg BOD}
\]

\[
K_d = 0.1104 \text{ day}^{-1}
\]

Then,

\[
\frac{YK_m}{K_0} = 44.4166
\]

\[
\frac{Y}{K_0} = -0.0726
\]

\[
K_0 = -0.4435 \text{ day}^{-1}
\]

\[
K_m = -611.7989 \text{ mg/l}
\]
Additionally, Monod constant $K$ is given as

$$K = \frac{(S_0 - S)Y}{\theta}$$

Taking the reciprocal of $K$, Lineweavers-plot equation was obtained

Therefore

$$\frac{1}{K} = \frac{K_m}{K_0} \frac{1}{S} + \frac{1}{K_0}$$ (Lineweavers-plot equation called uninhibited inhibition)

Calculating for $k$ using the equation above for the different grams of sodium hydroxide added to the cassava wastewater, the intercept $\frac{1}{K_0}$, obtained is used, where $\frac{K_m}{K_0}$ is the slope.

Therefore the calculation of $K$ for 0 gram of sodium hydroxide equals to

$$\frac{1}{K} = \frac{K_m}{K_0} \frac{1}{S} + \frac{1}{K_0}$$

$$\frac{K_m}{K_0} = 254.0364$$

$$\frac{1}{K_0} = 0.0450$$

$K_0 = 22.2222$ day$^{-1}$

$$K_m = 5645.2622$ mg/l

Substituting the values in the equation, then

$$254.0368(0.02708) + 0.0450$$

$$\frac{1}{K} = 6.9243$$
\[ K = 0.1444 \text{ time}^{-1} \]

Calculation of \( K \) for 2 gram of sodium hydroxide equals to

\[ \frac{1}{K} = \frac{K_m}{K_0} \left( \frac{1}{S} \right) + \frac{1}{K_0} \]

\[ \frac{K_m}{K_0} = 333.0003 \]

\[ \frac{1}{K_0} = 0.0949 \]

\[ K_0 = 10.5374 \text{ day}^{-1} \]

\[ K_m = 3508.9574 \text{ mg/l} \]

Substituting the values in the equation, then

\[ 333.0003 \times (0.0179) + 0.0949 \]

\[ \frac{1}{K} = 6.0556 \]

\[ K = 0.1651 \text{ day}^{-1} \]

Calculation of \( K \) for 4 gram of sodium hydroxide equals to

\[ \frac{1}{K} = \frac{K_m}{K_0} \left( \frac{1}{S} \right) + \frac{1}{K_0} \]

\[ \frac{K_m}{K_0} = 807.5753 \]

\[ \frac{1}{K_0} = 1.9429 \]


\[ K_0 = 0.5147 \text{ day}^{-1} \]

\[ K_m = 415.6546 \text{ mg/l} \]

Substituting the values in the equation, then

\[ 807.5753(0.0118) + 1.9429 = 11.4723 \]

\[ \frac{1}{K} = 11.4723 \]

\[ K = 0.0872 \text{ day}^{-1} \]

Calculation of K for 6 gram of sodium hydroxide equals to

\[ \frac{1}{K} = \frac{K_m}{K_0} \left( \frac{1}{S} \right) + \frac{1}{K_0} \]

\[ \frac{K_m}{K_0} = 164.9357 \]

\[ \frac{1}{K_0} = -0.0197 \]

\[ K_0 = -50.7614 \]

\[ K_m = -8372.3670 \]

Substituting the values in the equation, then

\[ 164.9357(0.0086) - 0.0197 \]

\[ \frac{1}{K} = 1.3987 \]

\[ K = 0.7149 \]
Calculation of $K$ for 8 gram of sodium hydroxide equals to

\[ \frac{1}{K} = \frac{K_m}{K_0} + \frac{1}{K_0} \]

\[ \frac{K_m}{K_0} = 1836.58 \]

\[ \frac{1}{K_0} = -3.1646 \]

\[ K_0 = -0.3159 \]

\[ K_m = -580.351 \]

Substituting the values in the equation, then

\[ 1836.58(0.0055) - 0.3159 \]

\[ \frac{1}{K} = 9.3853 \]

\[ K = 0.1022 \]

Calculation of $K$ for 10 gram of sodium hydroxide equals to

\[ \frac{1}{K} = \frac{K_m}{K_0} + \frac{1}{K_0} \]

\[ \frac{K_m}{K_0} = 1379.397 \]

\[ \frac{1}{K_0} = -2.2562 \]

\[ K_0 = -0.4432 \]
\[ K_m = -611.381 \]

Substituting the values in the equation, then

\[ 1379.397(0.0042) - 0.4432 \]

\[ \frac{1}{K} = 5.3503 \]

\[ K = 0.1869 \]