BIODEGRADATION OF CYANIDE FROM CASSAVA MILL EFFLUENT IN EBELLE COMMUNITY OF ESAN LAND

BY

CHUKWUMA, CHINYERE ANASTASIA
MATRICULATION NUMBER: 1401010020

DEPARTMENT OF BIOLOGICAL SCIENCES
COLLEGE OF BASIC AND APPLIED SCIENCES
SAMUEL ADEGBOYEGA UNIVERSITY.
OGWA, EDO STATE.

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES, SAMUEL ADEGBOYEGA UNIVERSITY, OGWA, EDO STATE.

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELOR OF SCIENCE (B. Sc Hons) DEGREE IN MICROBIOLOGY.

JUNE, 2018.

CERTIFICATION

This is to certify that this project work was carried out by Chukwuma, Chinyere Anastasia with Matriculation Number: 1401010020 in the Department of Biological Sciences, College of Basic and Applied Sciences, Samuel Adegboyega University, Ogwa, Edo state under my Supervision.

……………………....   ..…………………

DR K. E. ENERIJIOFI   **DATE**

(SUPERVISOR)

APPROVAL

This is to certify that this project work was accepted in partial fulfilment of the requirements for the award of Bachelor of Science degree (B. Sc. Hons) in Microbiology in the Department of Biological Sciences, Samuel Adegboyega University, Ogwa, Edo State.

……………………………… ……………………………

PROF. (MRS) F. M. OGBE **DATE**

(HEAD OF DEPARTMENT)

DEDICATION

This project work is dedicated to My Beautiful Redeemer for His wisdom and grace and also to my loving papa Late Mr. Benedict C. Udechukwu for inspiring me through his passion, courage and zeal for life.

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My highest praise and thanksgiving to the Glorious God for giving me the wisdom and grace to successfully complete this research work.

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ABSTRACT

The cyanide component of cassava mill effluent CME is highly toxic to man and it environment. This research was assessed using various concentrations of cyanide with variable concentrations of pH values, inoculum size and phenol, an inhibitory substance. The heterotrophic bacterial and fungal counts were 6.32 x 10^8cfu/ml and 2.87 x 10^8cfu/ml respectively. The microorganisms isolated and characterized were: *Staphylococcus aureus, Bacillus*, *Escherichia coli*, *Lactobacillus, Micrococcus*, *Klebsiella, Pseudomonas*, *Salmonella, Corynebacterium, Aspergillus niger*, *Penicillium*, *Fusarium* and *Saccharomyces* species. The physicochemical parameters; pH (4.81), electrical conductivity (4860uS/cm), cyanide (17.13mg/l), chemical oxygen demand (2041.20mg/l), biological oxygen demand (1490.08mg/l), total dissolved solids (2478.60mg/l), cations and heavy metals such as Chromium (19.44 mg/l), Manganese (136.08mg/l), Iron (340.20 mg/l) and Nickel (121.50mg/l) were above the Federal Environmental Protection Agency standard for effluent discharge. *Bacillus, Pseudomonas* and *Aspergillus* species which had the highest turbidity were used for the batch biodegradation studies. The result revealed that cyanide concentration of about 30ppm at pH 6 with inoculum size 6.5ml gave the highest cyanide degradation ability of 32.73% using *Pseudomonas* sp. at a residence time of 8 days. It was also found that the same organism gave the best degradative ability in the presence of phenol, an inhibitory substance. The findings revealed that *Pseudomonas* sp. and *Bacillus* sp. can be utilized for remediating cassava mill effluent contaminated environment containing cyanide.

CHAPTER ONE

1.0 INTRODUCTION

1.1. BACKGROUND OF STUDY

Biodegradation is the breakdown of materials through the aid of bacteria, fungi, or additional biological means, Vert *et al*. (2012). Eskander and Saleh, (2017) defines biodegradation as the fragmentation of all organic materials carried out by life forms comprising mainly of bacteria, fungi, protozoa and other organisms. Through this biologically natural process, toxic contaminants are converted into less lethal or harmless substances. It can be described as an action leading towards a change in the chemical composition and structure of contaminant caused by biological activity leading to naturally occurring metabolite end products (Bachmann *et al*., 2014; Jabir and Mustafa, 2016).

Cyanide is a group of compounds which contains a C≡N group: one atom of carbon linked with one atom of nitrogen by three molecular bounds, Moradkhani *et al.* (2018); Nwokoro and Uju Dibua (2014); Razanamahandry *et al*. (2017). In the environment, cyanides can be found in many different forms (Kuyucak and Akcil, 2013; Mirizadeh *et al*., 2014). It is also defined as a toxic nitrogen compound produced by living organisms comprising algae, bacteria, fungi, and plants as part of a defence mechanism against predation (Maniyam *et al*., 2013). Nevertheless, these natural sources of cyanide are inconsequential in pollution of the environment in comparison to cyanide production through anthropogenic activities (Zohre *et al*., 2017). Cyanide is lethal to humans and animals (Parker-Cote *et al*., 2018; Tiong *et al*., 2015; Uzoije *et al*., 2011) and thus wastewater containing cyanide poses a threat to aquatic organisms and terrestrial organisms that utilise water on the mainland (Mekuto *et al*., 2013).

Cyanide compounds are fast acting poison that interferes mainly with the cellular respiration process resulting in a number of illnesses and diseases and even death (Ewa *et al*., 2017). The unconscious consumption of large quantities of poorly processed foods such as cassava is more often a frequent cause of cyanide poisoning, Bradbury *et al*. (2011); Kamalu and Oghome, (2012). Microorganisms such as *Bacillus* sp., *Brevibacterium nitrophilous*, *Corynebacterium nitrophilous*, *Klebsiella oxytoca*, *Pseudomonas* sp. and *Rhodococcus* UKMP-5M have been reported to be proficient in cyanide degradation (Ibrahim *et al*., 2015).

Cassava, *Manihot esculenta* is classified among the family *Euphoribiaceae* and is grown almost entirely within the tropics. The tubers are fairly abundant in carbohydrates (85-90%) with a minimal amount of protein (1.3%), in addition to cyanogenic glucoside (Linamarin and Lotaustialin) present in cassava (Eze and Onyilide, 2015; Morgan and Choct, 2016; Sen and Ajit, 2015). It is a perennial shrub grown for edible tubers purported to have instigated in Tropical America was introduced to Africa around 16th century. It is widely cultivated, account for over half of the root tuber crops grown in African countries and one of the major food crops produced in Nigeria with Esan land inclusive (Enerijiofi *et al*., 2017b). Industrially cassava is important for the making of industrial alcohol, livestock feed, starch, textile and also for the making of cassava flour, macaroni and variety of beverages, Ani and Agbugba (2017); Igbinosa (2015); Omomowo *et al*. (2015). In Nigeria cassava processing is done mainly at subsistent level serving as a source of food and income. Commercially, it provides foreign exchange for the Government, Jackson *et al*. (2013).

Presently, the techniques intricate in cassava processing are denoted as rudimentary and unstandardized, requiring technical principles and awaiting possible upgrade (Omomowo *et*

al., 2015). Nigeria being the chief producer of cassava in the world accounts for 57,000 million tons annually according to Food and Agriculture Organisation of the United Nations FAO, (2015). The increased importance of cassava in agricultural and economic development and in food security particular in Nigeria has given its processing and wastes handling more attention, Okunade and Adekalu, (2013).

Cassava mill also known as Cassava processing plant was invented in 1919 and established in 1934 and is widely used in Nigeria, most exclusively in the southernmost part where cassava is the main agricultural produce (Enerijiofi *et al*., 2017b; Eze and Onyilide, 2015). It is used in grinding peeled cassava tubers which are drained for 2-4 days and then baked in pans overhead the fire to produce Garri, a major staple food. Cassava mill effluent (CME) are produced from various stages involved in processing like washing, grating and moisture extraction process (Enerijiofi *et al*., 2017b). The cassava mill effluent (CME) from traditional grating during processing is a major cause of environmental degradation, contaminating agricultural farmlands, streams and affecting bio diversities (Chinyere *et al*., 2013, 2016, 2018; Ezeigbo *et al*., 2014; Izah *et al*., 2018; Izonfuo *et al*., 2013). The discharged CME contains large amount of hydrocyanic acid, organic matter and water in the shape of peels and sieves from the pulp as waste products (Enerijiofi *et al*., 2017a). The rate of increase of heavy metals in the environment from several anthropogenic sources like cassava mill effluent should be a source of alarm for ecologists (Izah *et al*., 2016). These heavy metals that are discharged into the surroundings tend to persist indefinitely, accumulating in living tissues through food chain causing severe diseases to man (Akpoveta *et al*., 2010; Enerijiofi and Ajuzie, 2012).The fact that microorganisms are ever-present in nature is proven, so also is the age long fact that

microorganisms not only the source of diseases in humans or deterioration of materials but also beneficial in the manufacturing industries and degradation or transformation of both organic and inorganic substances (Ajuzie *et al*., 2015).

Hydrocyanic (HCN) is produced in cassava, when two major cyanogenic glucosides (linamarin and lotaustralin) are hydrolyzed and the acid released when the cassava roots is ruptured (Okolie *et al*., 2012). Therefore making it very toxic for consumers to eat the cassava root and leaves raw (Emmanuel *et al*., 2012). Several cases of cassava poisoning have been recorded in Nigeria, all resulting to improper fermentation and processing of cassava (Ifeabunike *et al*., 2017). It was reported that workers exposed to HCN for more than 5 years indicated an upsurge in symptoms such as abdominal colic, headache, changes in taste and smell, irritation of throat, lacrimation, nervous instability, pericardial pain, vomiting and weakness. As a consequence of poor processed cassava products, acute intoxication, with symptoms of dizziness, nausea, diarrhoea and sometimes death were observed in humans (Ifeabunike *et al*., 2017). Priya *et al*. (2011) also reported Atherogenic index of plasma (AIP) and triglyceride increase in the blood of cassava workers. It was reported that tropical ataxic neuropathy (TAN) or comparable deteriorating neuropathies that results in poor vision mainly amongst older people in Nigeria who have consumed cyanide chiefly from garri over a lengthy period of years (Izah *et al*., 2018).

Ebelle community of Esan Land is into subsistence farming with main focus on cassava which are processed into Garri. The cassava mills located in these areas seldom have proper discharge channels for the effluent which contains hydrogen cyanide and upon accumulation are harmful to the environment. Cassava waste water is discharged in the adjoining farm lands and bushes.

The deposition and accumulation of the cassava mill effluent over time poses serious problems to the surroundings and the public health of its teeming citizens and residents. The waste water from cassava processing could serve as a substitute source of mineral fertilizers, animals' feeds, biofuel and source of portable of water if treated with microorganisms thereby reducing contamination of the environment.

1.2. AIMS AND OBJECTIVES

The goal of this investigation was to employ the aid of microorganisms in cassava mill effluent to biodegrade and remove cyanide from cassava mill effluent, with specific objectives such as:

(a) isolation of microorganisms found in cassava mill effluent.

(b) characterisation of the isolated microbes.

(c) screening of the characterised microbes for cyanide biodegradation potentials.

(d) treatment of the effluent using screened microbes.

(e) evaluating the effects of cyanide on the environment (Ebelle).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. INTRODUCTION

Cassava production has significantly improved through the last decade and the view of cassava as the "food of the poor" is now changing, according to FAO, (2013). In Nigeria, over the years, cassava (*Manihot esculenta*) has become a key source of food thus providing nourishment for her teeming population. Nigeria is the leading producer of cassava in the world accounting for nearly 20% of global output (Izah *et al*., 2017).

However, in the international market, Nigeria is not a dynamic participant in cassava trade since majority of her cassava is directed at the local food market. The production techniques are principally subsistence in nature and therefore incapable to support industrial level demands (FAO, 2014). Any excess cassava is either processed on the farm or traded to indigenous processors. This subsistence cassava production method has generated large volume of cassava effluent, which when disposed unto the environment, mainly on soil deposits certain chemical compounds such as cyanide which is toxic to microorganisms, plant and animal cells. The disposal of these effluents have become a persisting problem as there is no precise disposal method. However, the cassava mill effluent contains certain microorganisms that has the ability to degrade the chemical compound contained in it (Mbanzibwa *et al.,* 2011) with biodegradation is potentially the cheapest means to dispose of cyanide (Ibrahim *et al*., 2015).

2.2. BIODEGRADATION

A very broad sense, in the environment, nothing is waste because virtually everything becomes recycled. In the microbiological sense, biodegradation simply means the breakdown of all

biological materials that is undertaking by life forms encompassing mainly bacteria, fungi, protozoa and other organisms. The harmful toxic pollutants are altered into less deadly or non-hazardous substances through this biologically natural process. The secondary metabolites, intermediate molecules or any waste products from one organism becomes the nourishment for others, substantiate carbon source and energy and they are further working-on breaking down the remain organic matter (Eskander and Saleh, 2017). Natural forces of biodegradation, abatement of wastes and eradication of most of ecological pollutants can be achieved by harnessing microbial communities. Dependent on the nature of the micro-organisms, they may eat a portion of the organic molecule only, destroying the intact parent material in a practice identified as primary biodegradation, or they may consume it completely in a procedure acknowledged as ultimate biodegradation (Eskander and Saleh, 2017). The energy producing part of the metabolic activity consumes oxygen, resulting in the immediate formation of carbon (iv) oxide, water and mineral salts in a process recognised as mineralisation (Jabir and Mustafa, 2016).

2.3. TYPES OF BIODEGRDATION

2.3.1. Aerobic Biodegradation

Eskander and Saleh, (2017) defined this as the degradation of compounds by microorganisms in the presence of oxygen. In aerobic biodegradation, microorganisms transform oxygen to water in the course of altering other components to simpler products. Aerobic biodegradation is also known as aerobic respiration (Moran *et al*., 2006).

Aerobic respiration is the means of disintegrating organic compounds with the aid of oxygen (O_2). In aerobic respiration, microbes use O_2 to oxidize an amount of the carbon in the

contaminant to carbon (iv) oxide (CO_2), with the rest of the carbon used to produce new cell mass. In this process reduction of O_2 occurs, producing water. Thus, the vital by-products of aerobic respiration includes carbon dioxide, water and an enlarged community of microorganisms (National Research Council, 1993).

2.3.2. Anaerobic Biodegradation

This is the degradation of compounds in the shortage of oxygen by microorganisms, National Research Council (1993). Anaerobic respiration is defined as a process in which microorganisms require the use of a chemical entity with the exception of oxygen as an electron acceptor. Common substitutes for oxygen are nitrate, sulphate, and iron (National Research Council, 1994). During anaerobic respiration, nitrate (NO_3^-), sulphate (SO_4^{2-}), metals such as iron (Fe^{3+}) and manganese (Mn^{4+}), or even carbon (iv) oxide (CO_2) may substitute the role of oxygen, receiving electrons from the degraded waste product. Thus inorganic chemicals are used as electron acceptors in anaerobic respiration. In addition to new cell matter, the by-products of anaerobic respiration may include nitrogen gas (N_2), hydrogen sulphide (H_2S), reduced forms of metals and methane (CH_4), depending on the electron acceptor (National Research Council, 1994). Anaerobic processes are employed in treating wastewater with high concentrations of recyclable biological materials such as concentrated animal manure slurry, biosolids, domestic wastewater and food processing wastes. The three biochemical reactions that characterize anaerobic processes are:

- Hydrolysis: An enzyme facilitated transformation of complex organic compounds into simple compounds.

- Acidogenesis: Is the use of bacteria in the alteration of basic compounds into substrates for methanogenesis (acetate, carbon dioxide, formate, hydrogen).
- Methanogenesis: Is the conversion of methanogenic substrates by bacteria into methane and carbon (iv) oxide (Leverenz *et al.*, 2002).

2.4. MICROORGANISMS INVOLVED IN BIODEGRADATION

The term microorganism covers a vast variety of life form, Bacteria, Blue-green cyano bacteria, Algae, Lichens and Fungi together with Protozoa. An essential feature of microbial life is the probability to degrade any naturally occurring compounds. This is known as microbial omnipotence. Exception to this rule are a few man made compounds such as highly polymerized materials (resins, plastics), halogenated compounds and others. Those are called Xenobiotics, because they are strange to the living world and organisms had little time to adapt to these compounds and develop degradative enzymes (Philippe, 2017).

2.5. REQUIREMENTS FOR BIODEGRADTION

Biodegradation is an important attenuation mechanism of contaminants concentration in groundwater and soil. Biodegradation upon occurrence, limits migration of pollutants and diminishes contaminants mass in the subsurface. Biodegradable plumes are commonly less widespread than non-biodegrading counterparts and conveyed at slower relative rate. Biodegradation is a biochemical reaction which is facilitated by microorganisms. In a broad-spectrum, an organic compound is oxidized by an electron acceptor which in itself is reduced. Under aerobic conditions, or lethal environmental conditions, oxygen functions as an electron acceptor. The oxidation of the organic compounds coupled with the reduction of molecular

oxygen is called aerobic respiration. However in the lack of oxygen, microorganisms can utilise organic chemicals or inorganic anions as alternative electrons acceptors in a term referred to as anaerobic biodegradation. Anaerobic biodegradation sometimes occur underneath fermentative, denitrifying, iron reducing, sulphate-reducing or methanogenic conditions. There are six requirements for biodegradation which according to Eskander and Saleh, (2017) are

1. The presence of the appropriate microorganisms
2. An energy source such as organic moiety that is used by the organisms for cell maintenance and growth.
3. A carbon source.
4. An electron acceptor such as O_2, NO_3^-, SO^{2-}_4, CO_2.
5. Nutrients such as nitrogen, phosphorus, calcium, magnesium, iron, trace elements.
6. Acceptable environmental conditions *e.g.*, appropriate pH, salinity levels and temperature.

2.6. FACTORS AFFECTING CONTAMINANT BIODEGRADATION

2.6.1. Biological Factors

2.6.1.1. Rates of contaminant degradation

Dependent upon the degree of contaminant degradation are the concentration of the contaminant and the sum of catalyst present. In this context, the quantity of catalyst present denotes the number of organisms able to metabolize the contaminant and also the volume of enzyme(s) produced by each cell. Thus, any factor that affects concentration of contaminant, the amount of microorganisms present or the expression of specific enzymes by the cells can increase or decrease the level of contaminant degradation. It should be well-known that the

frequency of degradation is generally not constant and independent of contaminant concentration but rather decreases as the concentration of contaminant decreases. Any factor that affects concentration contaminant, the microbial population present or the expression of specific enzymes by the cells can increase or decrease the frequency of contaminant degradation (Philippe, 2017).

2.6.1.2. Extent of contaminant degradation

The extent to which contaminants are disintegrated is basically a function of the specific enzymes involved their affinity for the contaminant and the accessibility of the contaminant. The affinity of an enzyme is an essential characteristics of the enzyme that is determined by its structure. This affinity varies among different enzymes and even among enzymes with identical functions produced by different populations of microorganism, Eskander and Saleh (2017).

2.6.1.3. General indicators and microbial physiology

2.6.1.3.1. Carbon: nitrogen: phosphorus (C: N: P) ratios

Microbial cells are largely comprised of carbon (C), nitrogen (N) and phosphorus (P) at an average C: N: P ratio of 50:14:3. In order for unrestricted microbial growth to occur, adequate amounts of these nutrients must be available in a usable form and in suitable proportions. It is imperative to know that measures of total carbon, nitrogen and phosphorus do not certainly reflect the amounts that can actually be used by microorganisms for growth as they may be physically or chemically unavailable (Philippe, 2017).

2.6.1.3.2. Nutrient availability

Organic compounds serve as sources of carbon and can be estimated centred on concentrations of total organic carbon (TOC). All organic chemicals available in a sample, including compounds that are absence or not readily metabolized, are included in measures of TOC. Thus, TOC tends to overemphasize the carbon present to microorganisms. In general, the total count of organisms present is proportionate to the quantity of carbon available assuming other nutrients are not limiting. Thus, the microbial density of surface soils is greater than subsurface soils which in turn are generally greater than in aquifer sediments. Provided that carbon is present in sufficient amounts, the totality of nitrogen available to microorganisms in the form of organic nitrogen, ammonia (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) can significantly affect the rate of contaminant degradation. A C:N ratio of less than 40 suggests that adequate nitrogen is present. Microorganisms can utilize both soluble inorganic and organic forms of phosphorus. Phosphorus limitation can occur when the C:P ratio is more than 120:1 and a C:N:P proportion of 100:10:1 is considered optimal. Increase in the concentrations of calcium (Ca) and magnesium (Mg) may precipitate phosphates reducing the amount available for microbial metabolism. In most soils additional nutrients such as sulphur (S), Ca, Mg, potassium (K), as well as trace amounts of metals, are typically found in adequate supply for metabolic needs (Philippe, 2017).

2.6.1.3.3. Terminal electron acceptors

The metabolism or breakdown of organic contaminants by microorganisms often entails oxidation of the compound and this requires that the electrons resulting from these processes be used in reducing other compounds. A diverse range of compounds (organic and inorganic)

can be utilised by microorganisms as terminal electron acceptors by microorganisms (Table 2.1). The energy derived by the organism from these respiratory processes varies. Aerobic respiration produces the maximum energy and methanogenesis the least (the processes are itemised in Table 2.1 in the order of most to least energy conserved). At the first approximation, the contaminant degradation rate is roughly relative to the sum of energy obtained, Eskander and Saleh (2017).

Table 2.1: Compounds (Organic and Inorganic) that can be utilized as terminal electron acceptors by microorganisms

Electron acceptor	**Chemical symbol**	**Process**
Oxygen	O_2	Aerobic respiration
Nitrate and nitrite	NO_3 and NO_2	Aerobic respiration (denitrification)
Ferric iron	Fe^{3+}	Anaerobic respiration
Manganese	Mn^{4+} and Mn^{2+}	Anaerobic respiration
Sulphate	SO_4^{2-}, $S_2O_3^{2-}$ and SO_3^{2-}	Anaerobic respiration (sulphate reduction)
Organic compounds	Many	Fermentation
Carbon dioxide	CO_2	Methanogenesis

2.6.1.3.4. Soil respirometry

Soil respirometry provides a measure of oxygen (O_2) consumption or carbon dioxide (CO_2) production in soils and is an indication of net aerobic biological activity *in situ*. The determination is typically made by measuring the consumption of oxygen by contaminated soils over time and comparing the rate to that observed with soils from a nearby region that is not contaminated. Increased O_2 use, measured as lower O_2 levels, is taken as an indication of increased respiratory activity that is potentially due to metabolism of contaminant(s). Moreover, the method is useful only for monitoring aerobic respiration and does not include anaerobic respiration or other processes that may be embroiled in contaminant degradation (Philippe, 2017).

2.6.1.4. Temperature

Temperature is directly influenced by the rate of biodegradation by controlling the rates of enzyme catalysed reactions. The rate of biodegradation is decreased by roughly one-half for each 10ºC decrease in temperature. Rates of biodegradation are generally exceedingly low at 0ºC. Conversely, higher soil temperatures result in higher microbial metabolic activity and higher rates of biodegradation up to a maximum of about 65ºC. As a result, biodegradation rate may fluctuate seasonally and metabolic activity of microorganisms itself can increase soil temperature. It ought to be noted that most research studies stated in the scientific literature have employed temperatures that range from 20–25ºC and therefore, the reported rates of contaminant degradation are higher than would be expected in subsurface environments where average soil temperatures at depth are around 10ºC. A better estimate of rates of natural biodegradation based on literature values is possible when soil temperature is known (Philippe, 2017).

2.6.1.5. Moisture

Moisture (water) controls the rate of contaminant metabolism as it influences the kind and amount of soluble constituents which are obtainable as well as the pH and osmotic pressure of aquatic and terrestrial systems. As mentioned above, the volume of water available in the pore spaces of soil also affects the exchange of oxygen. Under saturated conditions, oxygen can be consumed faster than it is replenished in the soil, vapour space and the soil can become anaerobic. This can impede the degree of biodegradation and cause major changes in microbial metabolic activity to occur. Conversely, soil moisture content should be between 25–85% of the water holding capacity, and a range of 50–80% is optimal for biodegradation. Water holding capacity of soil is sometimes expressed as the field capacity which is the percentage of water residual in a soil once it becomes saturated and gravitational drainage has stopped. The volume of water present in the pore spaces of soil also affects the exchange of oxygen. Under these conditions, oxygen can be consumed faster than it is replenished (Eskander and Saleh, 2017).

2.6.1.6. pH

Soil pH is a degree of the acidity or alkalinity of water. Biodegradation occurs under a wide-range of pH; nevertheless, a pH of 6.5 to 8.5 is most optimal for biodegradation in most aquatic and terrestrial systems and values ranging from 5 to 9 are considered acceptable. Soil pH may affect nutrients availability. For example, the solubility of phosphorus, an important nutrient in biological systems, is maximal at a pH value of 6.5 and decreases at pHs that are either higher or lower than this value (Philippe, 2017).

2.6.2. Environmental Factors

2.6.2.1. Geologic and hydrogeologic factors

2.6.2.1.1. Adsorption and absorption

The binding of an organic compound to the surface of a solid is called adsorption. The extent of adsorption that occurs is determined by the relative affinity of the compound for a solid matrix, the surface area of the matrix and the volatility or solubility of the compound in water. In general, hydrophobic chemicals will tend to adsorb to insoluble organic matter and other hydrophobic materials. Similarly, hydrophilic chemicals may bind to minerals.

Absorption is an analogous process wherein a contaminant penetrates into the bulk mass of the soil matrix. Adsorption and absorption can both decrease the obtainability of the contaminant to most microbes and the level of chemical metabolism is proportionally reduced (Eskander and Saleh, 2017).

2.6.2.1.2. Contaminant migration in groundwater

Contaminant migration within an aquifer is controlled by many physical and chemical features of the contaminants, and of the hydrogeologic setting in which they are found. Hydraulic conductivity is a unique characteristics of the primary aquifer that must be understood to effectively predict contaminant migration and evaluate the possibilities for adding materials to enhance bioremediation. It may vary horizontally and vertically and is measured in units of length per time, such as feet per day or centimetres per second, reflecting the rate of water

movement through the rock or unconsolidated materials. Particle size and rock type affect hydraulic conductivity, with smaller particle size or increasing density of rock, resulting in lower rates of hydraulic conductivity. Typically, hydraulic conductivities for soils in the scope of $10^{-5} – 10^{-3}$ cm/sec will be convenient to biodegradation process, although values below 10^{-5} cm/sec may be acceptable if other site specific conditions are favourable. Other factors to consider are dispersion and diffusion of contaminants. Dispersion is the mechanical mixing and distribution of the contaminants that occurs within the aquifer and includes diffusion, the movement of contaminants along a concentration gradient owing to their kinetic energy. These are primarily physical processes that reduce contaminant concentrations within the plume and are of particular importance for sites where natural attenuation is the proposed remedy (Phillippe, 2017).

2.6.2.2. Bioavailability

The fraction of contaminant actually available to microorganisms is said to be bioavailable. The concentration of bioavailable contaminant is often less than the total concentration measured following solvent extraction or other methods. It is imperative to note that the rate and extent at which contaminant is degraded is proportionate to the concentration of bioavailable contaminant and not the total concentration, Eskander and Saleh (2017).

2.6.2.3. Soil Matric Potential

The soil matric potential indicates a measure of the essential energy needed to overcome capillary and adsorptive forces and thus mirrors the work that microbial cells must do to aid in extracting water from the soil. The soil matric potential is strongly dependent on soil type and is correlated with soil moisture content. Soil moisture contents of 50–80% roughly correspond

to matric potentials within the range of –0.01 to –0.015 Mpa (–0.1 to –0.15 bar) in sands and –0.03 to –0.05 Mpa (–0.3 to –0.5 bar) in moderate to fine textured soils. In general, microbial activity is low in soils with a matric potential of about –0.1 Mpa (–1 bar) then stops approximately at –8 Mpa (–80 bars). Matric potentials that range from –0.5 Mpa (–5 bars) to –1.5 Mpa (–15 bars) are generally considered adequate. If the matric potential is too low then contaminant degradation rates will also be low, Eskander and Saleh (2017).

2.6.2.4. Redox Potential

The soil oxidation-reduction potential offers a capacity of the electron density of the system. Biological energy is obtained through the oxidation of compounds in which electrons are transferred to several more oxidized compounds referred to as electron acceptors. A low electron density (Eh greater than 50 mV) an indicator of oxidizing, aerobic conditions, whereas high electron density (Eh less than 50 mV) designates reducing, anaerobic conditions. High positive Eh values (+100 mV to +400mV) indicate well aerated conditions that are optimal for biodegradation. It is crucial to note that Eh may vary considerably over a very small distance within soils and it is generally not practicable to directly measure this parameter for a specific site. As many microbial processes, improving the natural conditions in the biodegradation processes is a significant goal in order for the microbial physiological and biochemical activities to be directed towards biodegrading the target contaminants. Deviation of the environmental factors that is reported to influence microbial growth and bioactivity, away from the potential conditions will reduce the rates of microbial growth and transformation of target substrate and possibly cause the failure of the biodegradation process. The potential of the

process may also be limited by the toxicity of the pollutants to the degrading microorganism (Phillippe, 2017).

The initial plan of the biodegradation thus involves modification of the substrate to yield a product that is an intermediate or following further metabolism, is converted to an intermediate in there iniquitous metabolic sequences. This need to convert the synthetic molecule to intermediate is characteristics of both aerobes and anaerobes as they derive carbon and energy from the substrate.

Several conditions must be fulfilled for biodegradation to occur in an environment. According to Eskander and Saleh (2017), these include the following:

1. An organism that has the required enzymes to cause biodegradation must exist. The mere existence of an organism with the appropriate catabolic potential is essential but not adequate for the process to occur.

2. That organism must be bountiful in the environment comprising of the chemical. Although some microorganism are present in essentially every environment near the earth's surface, particular environments may not contain an organism with the necessary enzymes.

3. The chemical should be available to the organism having the necessary enzymes. Many chemicals persevere even in environments containing the biodegrading species but are not degraded simply because the organism is inaccessible to the compound that it would otherwise metabolize. Inaccessibility may result from the substrate being in a different environment from the organism, *e.g.*, in a solvent immiscible with water or adsorbed to solid surfaces.

4. If the initial enzyme bringing about the degradation is extracellular, the bond acted upon by the enzyme must be exposed for the catalyst to function. This case differs because of adsorption of many organic molecules.

5. Should the enzymes catalysing the initial degradation be intracellular, the molecule must penetrate the surface of the cell to the internal sites where the enzyme acts. Alternatively, the products of an extracellular reaction must penetrate the cell for the transformation to proceed further.

6. Because the population or biomass of bacteria or fungi acting on many synthetic compounds is initially small the condition in the atmosphere must be conducive to allow for proliferation of the potentially active microorganisms.

2.7. CYANIDE

Cyanide is a triple-bonded compound possessing a single negative charge consisting of a single atom of carbon in the oxidation state of +2 and one atom of nitrogen in the -3 oxidation state. (Dwivedi *et al*., 2011; Moradkhani *et al*., 2018; Nwokoro and Uju Dibua 2014). It is a very poisonous chemical which is usually found jointly with other chemicals to form cyanide compounds. Examples of cyanide compounds are: Hydrogen cyanide (HCN), Potassium cyanide (KCN), and some cyanide compound are produced by the action of some cyanide compound bacterial, fungi and algae and it is found in a number of foods and plants (Abdullahi and Saba, 2014; Kuyucak and Akcil, 2013; Mirizadeh *et al*., 2014).

Hydrogen cyanide (HCN) known also as Hydrocyanic acid and Prussic acid is an uncoloured or gas or pale blue liquid characterised by a faint bitter almond-like odour. It is a very frail acid, having an acid dissociation constant (p*K*a value) of 9.22 at 25°C (World Health

Organisation; WHO, 2009). It's completely miscible in water. Hydrogen cyanide is reported to have a characteristic odour of almonds or bitter almonds (National Poisons Information Service; NPIS, 2013). Owing to its small size and moderate lipid solubility, hydrogen cyanide is readily absorbed following inhalation, ingestion and dermal contact (Enerijiofi *et al*., 2017b; Scientific Committee on Occupational Exposure Limits; SCOEL, 2010).

Hydrogen cyanide is the chemical substance responsible for tissue hypoxia. Prolonged exposure to HCN may cause cardiovascular, neurological, respiratory and thyroid defects (Priya *et al*., 2011). Minimal concentrations of hydrogen cyanide (HCN) in the form of cyanogenic glycosides is contained in a number of edible plants. (United Kingdom Teratology Information Service; UKTIS, 2013; WHO, 2009). Notable examples are the kernels of wild almonds, apricots and black cherries, bamboo shoots, lima beans and cassava (WHO, 2009). Cyanogenic glycosides within plants may liberate hydrogen cyanide when the plant is damaged e.g. ground or chewed or enzymatically hydrolysed within the body (WHO, 2009). Cyanide level of these food stuffs can significantly increase if the soil on which they are planted is contaminated with it. (Ibrahim *et al*., 2015).

Cyanide is quite recalcitrant in the sense that it persists so long on any contaminated soil, Ewa *et al*. (2017). In the atmosphere, hydrogen cyanide half-life is 1–3 years (Republic, 2012).Various enzymes present in microorganism aids in the conversion of cyanide to a source of carbon and nitrogen (Ibrahim *et al*., 2015). The several researchers have been reviewed a bioremediation tests in different scale and conditions (Dash *et al*., 2009; Luque-Almagro *et al*., 2016).

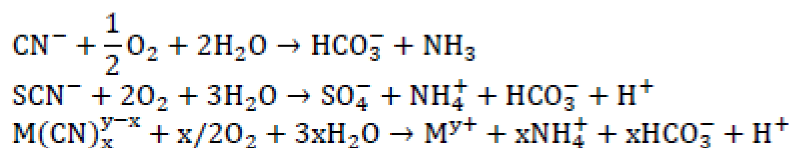
2.8. BIODEGRADATION MECHANISM OF CYANIDE

There are myriad groups of microorganism unearthed which can convert simple or complex cyanide compounds, including bacteria such as *Pseudomanas*, *Acinetobacter*, *Burkhoderia cepacia* and *Alcaligenes* sp., *Bacillus nealsonii* (Mohanraj *et al*., 2013), *Serretia marcescens* (Kumar *et al*., 2013), *Rhodococcus* UKMP-5M (Ibrahim *et al*., 2015), fungus such as *Fusarium oxysporum* (Akinpelu *et al*., 2015) then a few algae like *Chlorella* sp., *Arthrospira axima*, and *Scenedesmus obliquus* (Mirizadeh *et al*., 2014) and the usesful Actinomycetes *Streptomyces phaeoviridae* (Shete and Kapdnis, 2013). Motaung *et al*. (2012) stated recently that *Cryptococcus cyanovorans* sp. nov., a basidiomycetous yeast was sequestered from a soil contaminated by cyanide.

Cyanide serves as a nutrient for bacterial growth, acting as nitrogen source. Some bacteria make use of cyanide compounds both as a carbon and nitrogen source. Therefore, external supply of carbon source is no longer necessary for these bacteria. In the presence of cyanide other bacteria required glucose as carbon source for their survival (Bouari, 2012).

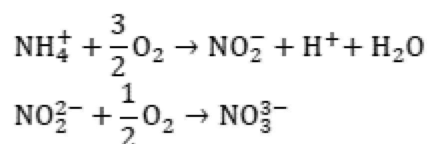
According to Razanamahandry *et al*. (2017), biodegradation occurs in two steps:

The principal step being the oxidative disintegration of cyanides and subsequent absorption and precipitation of free metals into the biofilm. Cyanide and thiocyanate are then converted to ammonia, carbonate and sulphate



$$CN^- + \frac{1}{2}O_2 + 2H_2O \rightarrow HCO_3^- + NH_3$$
$$SCN^- + 2O_2 + 3H_2O \rightarrow SO_4^- + NH_4^+ + HCO_3^- + H^+$$
$$M(CN)_x^{y-x} + x/2O_2 + 3xH_2O \rightarrow M^{y+} + xNH_4^+ + xHCO_3^- + H^+$$

In the second step, conversion of ammonia to nitrate through the conventional two step nitrification process shown as:



The ease of metal cyanides degradation depends on their chemical stability: Free cyanide is the most readily degradable, followed by metal cyanide complexes of Zn, Ni, and Cu; iron cyanide the least degradable.

2.8.1. Factors affecting Biodegradation of Cyanide in The Environment

The presence of microorganisms that have the physical and metabolic capacities to degrade the pollutants in the polluted environment ensures the success of biodegradation. Cyanide compounds are mostly found in natural surroundings and the microbial metabolic degradation of these compounds is thus possible. However, the following factors affect the process according to Dash *et al*. (2009); Ibrahim *et al*. (2015):

- Concentration of cyanide in the environment can have substantial effect in the treatment. For instance, acetonitrile of high concentration has demonstrated to be harmful to *Klebsiella oxytoca* due to the damage to nitrile hydratase, which is the nitrile degrading enzyme thereby inhibiting bioremoval of the compound by the microorganism.
- Biodegradation of cyanide compounds can be affected by nutrients availability. Carbon has been renowned as the restrictive factor in the biodegradation of cyanide compounds, which may make the biodegradation of industrially polluted soils not feasible.
- Aeration is very vital in the biodegradation of cyanide as oxygen is required during the degradation pathways.
- Cyanide toxicity can be paramount to anaerobic bacteria principally methanogens. Other contaminants existent at the contaminated areas may also affect bioremoval.

- Existence of high concentration of other pollutants can have negative effect on degradation of cyanide by swaying the native population and possibly hindering the proliferation of specific organism.

2.9. EFFECTS OF CYANIDE ON THE ECOSYSTEM

2.9.1. Cyanide Toxicity for a Living Environment

Cyanide is a carbon-nitrogen radical, which may be present in a wide diversity of inorganic and organic compounds. The toxicity of cyanide to existing cells is due to this three key mechanisms: resilient chelation to metals in metallo-enzymes; reaction with keto compounds to form cyanohydrin derivatives of enzyme substrates and reaction with Schiff-base intermediates during enzymic reactions to form stable nitrile derivatives, Ewa *et al*. (2017). Cyanides are available in several ecological elements such as air, soil, food, water, air exhaled and biological materials like blood, saliva and urine at the ratio of micrograms per litre to milligrams per litre (Donald, 2009).

Ewa *et* al. (2017); Moradkhani *et al*. (2018) reported that compounds comprising of cyanide ions are fast acting poison, as they interrupt the process of cellular respiration. The basic effect of cyanide activity includes combining with trivalent iron of cytochrome oxidase, which is an important enzyme of the respiratory chain (Fig. 2.1). This combination results in blocking of the intracellular respiratory and increasing synthesis of lactic acid. Although the obstruction of cytochrome oxidase has the most substantial impact, it ought to remember that the CN^{-} ions also impede other enzymes: glutamate decarboxylase, xanthine oxidase, superoxide dismutase, nitric oxide synthase and nitrite reductase. According to Ewa *et al*. (2017), cyanide ion can cause direct impairment to the nervous system by lipid peroxidation. Most sensitive to lethal

effects of cyanides are tissues with the swiftest oxygen metabolism, also affect brain and the heart muscle, however hypoxia results in the disorder of all body cells' functioning. Considering its high toxicity, effluents containing cyanide cannot be disposed without being exposed to treatment to diminish their cyanide contents to very minimal levels (<0.1 µg of CN- per litre) Abdullahi and Saba (2014); Ewa *et al*. (2017). At least, there are two forms of chronic cyanide poisoning in domestic animals which include hypothyroidism due to interference of iodide uptake by the follicular thyroid cell, sodium-iodide symporter by thiocyanate, a metabolite in the detoxification of cyanide, chronic cyanide and plant cyanide metabolite (e.g., various glutamyl β-cyanoalanines) -associated neuropathy toxidromes (e.g.cystitis ataxia syndromes in cattle, sheep, and goats, equine sorghum cystitis ataxia syndrome) (Okah *et al*., 2017; Rhian, 2014). The toxicity of cassava mill discharge is basically connected to its acidic pH and cyanide content. In acidic soils, plants are probable to take up toxic metals, preventing seed germination or eventual death (Enerijiofi *et al*., 2017a).

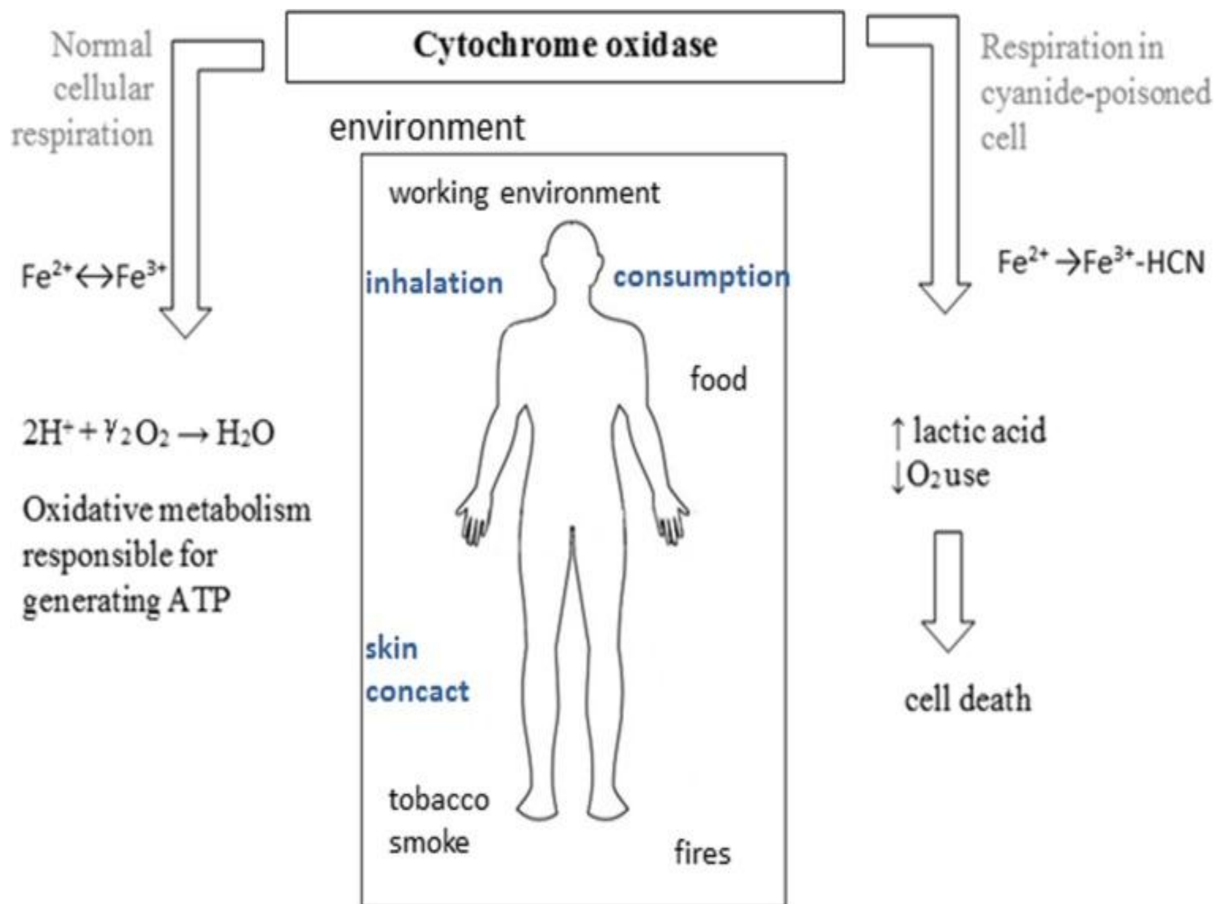


Fig. 2.1 Schematic illustration of the effect of cyanides on the human body (Ewa *et al*., 2017)

2.10. CASSAVA PRODUCTION

Cassava, *Manihot esculenta* Crantz is an imperative food security crop in Nigeria. It produces the food that feeds the populace also it provides the primary source of livelihood for the rural poor and raw materials for local Industries, Izah *et al*. (2018). It possesses a high poverty-reduction potential for Nigeria due to its low production cost (FAO, 2015; Organisation for Economic Co-operation and Development OECD, 2014). Its importance further relies on its capacity to grow in sub-optimal environments for example; conditions such as drought and low soil fertility are encountered in parts of Africa (Umunakwe *et al.,* 2015).

The mature plant usually takes one of two forms which includes either spreading stems or erect stems with several numbers of terminal branching (OECD, 2014). Cassava is grown also for industrialised purposes, such as starch production and for fermentation into ethanol (Adelekan, 2010; Ani and Agbugba, 2017). There are numerous significant prospects for improving cassava, especially in terms of compositional qualities, nutritional qualities, pest resistance and reduction of cyanogenic content (Halsey *et al.,* 2008; Rahman and Awerije, 2016).

Cassava processing using traditional methods is tasking, unproductive and also time consuming. Such problems arise in the grating and draining of the starchy fluid from the cassava dough since the orthodox methods available include processes that require a lot of labour and man hours. The problem is worsened when the quantities to be produced are very large (Ajala *et al.,* 2013). Effective cassava processing methods disintegrate the root tissue completely, thereby releasing an endogenous enzyme, linamarase; this endogenous β-glucosidase facilitates the hydrolysis of linamarin into glucose and acetone cyanohydrins. These chemical components will putrefy above pH 6 into volatile hydrogen cyanide (HCN) that is lost rapidly from the system (Lambri *et al*., 2013). Cassava varieties are categorized as bitter (glucoside content > 100 mg/kg fresh weight.) or sweet (glucoside content < 100 mg/kg fresh weight.) according to their HCN production level (Ariyomo *et al*., 2017; Kolawole, 2014).

Over hundreds of years numerous diverse methods have been established to increase processing of cassava roots, resulting in less residual cyanide. Some methods remove virtually all residual cyanogens but many methods leave behind appreciable amounts of cyanogens (Lambri *et al*., 2013). Generally the processing methods approved involve a combination of

procedures, such as flaking, slicing, fermentation, boiling, desiccating, hammering or milling and sieving. However, especially for the varieties high in HCN, the most popular and proficient processing method for their removal is fermentation (Nambisan, 2011). Actually, cassava is processed by fermentation in almost all parts of Africa and South America where fermentation plays a major socio-economic role by being a much desired practice in the rustic populations (Chelule *et al*., 2010; Nambisan, 2011). A combination of the microorganisms *Lactobacillus coryneiformis, Lactobacillus delbruckii* and *Saccharomyces* has proven effective in the fermentation of cassava to reduce cyanogens (Izah *et al*., 2017; Lambri *et al.,* 2013).

2.10.1. Classification and Description of Cassava

The scientific term of cassava is *Manihot esculenta* Crantz, Integrated Taxonomic Information System; ITIS (2012). Cassava (*Manihot esculenta*) belongs to the family *Euphorbiaceae* (Jackson *et al*., 2014; Kolawole *et al.,* 2014). Three subspecies of cassava have been documented: *Manihot esculenta* sp. *esculenta* is the cultivated strain, and *M. esculenta* sp. *flabellifolia* and *M. esculenta* sp. *peruviana* are wild forms (Allem, 2002; OECD, 2014).

Cassava is a perennial shrub of one to five meters in height. Its leaf shape is palmate with three to nine lobes and covered with a shiny and waxy epidermis. Mature cassava plant usually takes the form of an erect stem with several amounts of terminal branching or a spreading stem. Species in the genus *Manihot* well adapted to the tropics, taking subshrubs forms to small trees and forms large, woody roots (Alves, 2002; OECD, 2014).

Cassava is cultivated basically for its large storage roots used primarily for human consumption (Onyenwoke and Simonyan, 2014). Although cassava has the lowest protein-to-carbohydrate

ratio among major crops, it plays an important dietary role in the diets of individuals worldwide (OECD, 2014; Prochnik *et al.,* 2012).

Analysis carried on the susceptibility of crops to the effects of climate alteration indicated that cassava may be better suited to survive the changes in climate than most staple crops in the tropics, making it a candidate for key food security crop of the future (OECD, 2014). Calculation indicates that cassava has the potentials to produce and store carbohydrate than any other grain or root crop. However, it fails to attain that potential because of the poor quality materials, sub-optimum agronomic practices including diseases and pests (Fermont *et al*., 2009; Jarvis *et al*., 2012).

Hydrogen Cyanide is released through hydrolysis of dual cyanogenic glucosides, primarily Linamarin and lower levels of lotaustralin, and hydrolysis initiated by physical tissue disruption. Linamarin is located in the vacuoles of plant cells while linamarase hydrolyses linamarin to release hydrogen cyanide, which is found in the plant cell wall (Permi *et al.,* 2007; OECD, 2014).

2.10.2. Origin of Cassava

Cassava is among the world's most significant food crops with a yearly output of about 34 million tonnes of tuberous roots. Pinpointing cassava's origin has been complicated. Cassava samples have been historically grown in humid lowland regions in Central and South America. Tissue samples are more easily obtained in arid regions, but it is thought that these areas are not the origins of cassava. Three questions are of concern pertaining to the origin of cassava: botanical origin i.e. the wild ancestors from which cassava descended; the geographical origin i.e. the area where the progenitor evolved in the geological past and agricultural origin i.e. the

area of initial cultivation (Asogwa *et al.,* 2013; ITIS, 2012; Stella *et al*., 2013). For this Write, We would pay attention to only the Agricultural Origin.

2.10.2.1. Agricultural Origin.

It is currently assumed that there is only one domestication site for cassava, possibly along the southern boundary of the Amazon basin, where *Manihot esculenta* sp. *flabellifolia* plants were initially amassed from the wild, domesticated and grown through vegetative propagation (Allem, 2002; OECD, 2014). A comprehensive molecular analysis established on the single - copy nuclear gene encoding glyceraldehyde 3-phosphate dehydrogenase designated that cassava was domesticated precisely from populations of *Manihot esculenta* sp. *flabellifolia* occurring along the southern perimeter of the Amazon basin in the Brazilian states of Acre, Mato Grosso and Rondônia and possiblely extending south into Bolivia. Later studies have confirmed a southern Amazonian domestication site (OECD, 2014).

2.10.3. Geographical Description of Cassava

Nine (9) African countries, three Latin American countries and seven Asian countries (nineteen altogether) are considered the major cassava growers producing one million tonnes or more of cassava annually (OECD, 2014). Nigeria, Thailand, Brazil, Indonesia and Democratic Republic of Congo are the topmost five cassava manufacturing countries in the world (FAO, 2015; OECD, 2014). The genus *Manihot* is distinct to Central America and South America, where Brazil and Mexico has the highest *Manihot* species with centre of diversity: Central Brazil, North eastern Brazil, Brazil, South Western Mexico, Western Mato Grosso and Bolivia (Nassar, 2000; OECD, 2014).

Cassava is limited to the tropical regions where the annual mean temperature is greater than 18°C, but few species can survive in frosty areas e.g. *Manihot neusana* and *Manihot grahamii* (Nassar and Ortiz, 2006). It is drought tolerant, has an optimum performance at areas with annual rainfall of 600 – 1500mm and temperature of 25 – 29°C (Nassar, 2000; OECD, 2014). Tropical areas with altitude up to 2000m, between latitudes of 30°N and 30°S with day length of 10 – 12 hours supports cassava growth (Vieira *et al*., 2012). Certain varieties of *Manihot* are developed for specific geographical regions. *Manihot glaziovii* (*Manihot carthagenensis* sp. *glaziovii*) has naturalized in Africa. It was introduced to Africa from Brazil as a source of rubber (Andersson and Vicente, 2010). Some species *Manihot pohlii*, *Manihot zehntneri* and *Manihot grahamii,* can be invasive in new areas while others are known as survival of drought and fire (Nassar and Ortiz, 2000; OECD, 2014).

2.11. ECOLOGICAL REQUIREMENT FOR CASAVA PRODUCTION

2.11.1. Soil

Cassava is grown on a variety of soils. It tolerates marginal, low-fertility acid soils better than many other staple crops. However, cassava is well-known to be sensitive to soils with high pH greater than 7.8 and elevated conductivity and/or sodium. The soil also should be rich in organic materials. Shallow soils should be avoided as it inhibits tuber expansion (OECD, 2014).

2.11.2. Rainfall

Cassava is extremely drought resistant and grown in several parts where rainfall is low and unpredictable. The annual rainfall should range between 2000mm to 3000mm (Ebukiba, 2010).

2.11.3. Altitude

Cassava strives at almost all altitudes. It grows on low to medium altitudes. It yields best at altitude range of one metre to about one thousand five hundred metres (1 – 1500m) Ebukiba (2010).

2.12. CASSAVA HARVESTING AND PROCESSING

2.12.1. Cassava Harvesting.

Depending on the cassava genotype, environment, soil type and intended use, cassava may be harvested within six to thirty six months after planting. This large harvest opening permits it to serve as a scarcity reserve crop. Within 24 – 48 hours of harvest, cassava roots undergo 'post-harvesting physiological deterioration or PPD' (Lebot, 2009; Onyenwoke and Simonyan, 2014).

2.12.2. Cassava Processing

Because of the short storage roots shelf life, cassava is processed for proper and longer storage. The figure 2.2 below summarizes the traditional cassava processing processes;

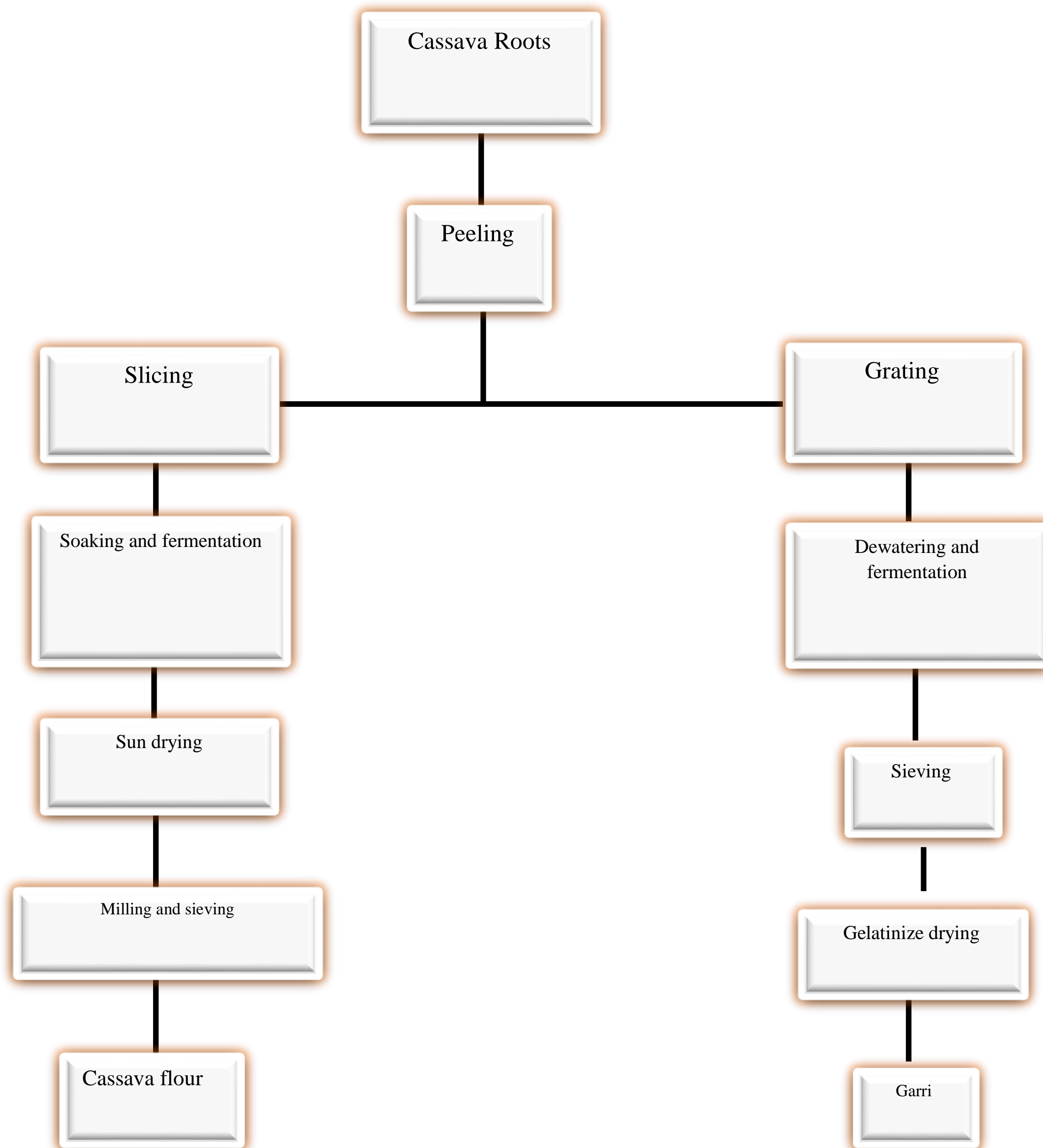


Fig 2.2. The Traditional Cassava Processing Processes (Eze and Onyilide, 2015)

2.13. CASSAVA PRCESSING INDUSTRY (CASSAVA MILL)

Cassava mill likewise known as Cassava processing Industry was created in 1919 and planted in 1934 and is used extensively in Nigeria, particularly in the southern region where cassava is

a major agronomic produce (Eze and Onyilide, 2015). This is where cassava production is carried on a large scale. Over time, cassava has evolved from being a peasant's crop to cash and industrial crop. Cassava in Nigeria serves two main purposes: 90% as human nourishment and only 5 to 10% as ancillary industrial material used typically as animal feed. About 10% of Nigeria's industrial demand comprises of high quality cassava flour (HQCF) used in producing biscuits and confectioneries, dextrin pre-gelled starch for adhesives, hydrolysates and starch for pharmaceuticals merchandises and seasonings while 70% of cassava undergoes processing as human food well-known as *garri* (Onyenwoke and Simonyan, 2014). Despite the increase in cassava production each year, the discharge of cassava effluent (waste water) has not been properly managed. Traditional production of garri is associated with emancipation of large amounts of water (effluent), hydrocyanic acid and organic matter in the form of peelings and sieves from the pulp as waste products (Eziegbo *et al.,* 2014). Around the cassava mills, this liquid waste is discharged indiscriminately and allowed to accumulate. Studies have shown that at toxic concentrations of the effluent, germination of cereal seeds are prevented (Eziegbo *et al.,* 2014; Olorunfemi *et al*., 2008). Long-term discharge of this effluent into the soil could result in a serious imbalance in the microbial population, which in turn could result in alteration of soil fertility toward a negative direction (Akpan *et al*., 2017). The high content of cyanide from the waste equally poses significant menace to humans and the environment, calling for regulations in the discharge of the effluent generated (Enerijiofi *et al.,* 2017b).

2.13.1. Microorganisms and Chemical Compounds Present in Cassava Mill Effluent

The effluent released into the ecological niche contains diverse microbes. Notably are bacterial and fungal species. The bacterial species include*: Aerococcus viridens, Bacillus subtilis,*

Bacillus sp*., Lactibacillus acidophilus and Corynebacterium manihot*. The fungal species commonly found are mold: *Aspergillus fumigatus, Aspergillus niger, Aspergillus repens, Articulospora inflata* and *Geotrichum candidum*. Yeast: *Candida utilis* and *Saccharomyces exguus* (Enerijiofi *et al*., 2017b; FAO, 2013*).*

Chemical compounds found in cassava effluent include Cyanide, Iron, Zinc, Manganese, Nickel, Cadmium, Vanadium, Chromium, and Lead. Cassava containing these compounds affects the soil, causing imbalance in biotic and abiotic entities of the ecosystem and reduction in soil fertility and crop yield (Eneriojiofi *et al*., 2017a; Etinosa and Igiehion, 2015).

2.14. IMPORTANCE OF CASSAVA

Cassava as a food and industrial crop whose significance relies on its roots since they accumulate starch (approximately 30–60% dry matter), and so, globally it is considered the alternative source of starch, after maize (FAO, 2013; Izah *et al*., 2018). Cassava is identified among world's most vital food crops with an annual output of over 34 million tonnes of tuberous roots. Africa is a continent of the world where around 600 million people are reliant on cassava for food (Ani and Agbugba, 2017; International Fund for Agricultural Development; IFAD, 2013; Kolawole *et al*., 2014). Cassava is manufactured largely by small-scale farmers using elementary farm implements and most of the cassava produced is used mainly for human consumption with less than 5% in industries. Cassava is important for the provision of calories for consumption and for income for both commercial and small scale farmers (Asogwa *et al.,* 2013; IFAD, 2013).

Ani and Agbugba (2017) states that 80% of energy needed for work comes from cassava. Hence, cassava is responsible for 80% percent of energy production when consumed. Cassava

is used for industrial purposes such as making of adhesives, bakery products, dextrin, dextrose, lactose, surcrose and glucose as well as industrial raw materials (Adul-Azeez, 2013; Stella *et al*., 2013). Food and beverage industries employs cassava in the making of chewing gum and jelly caramel. Pharmaceutical and chemical industries use the ethanol from cassava to produce cosmetics and drugs. In compounding of livestock feed, cassava is also used as a compounding agent (Adul-Azeez, 2013; Okeowo, 2015; Stella *et al*., 2013).

2.14.1. Nutritional Improvements on Cassava.

There is research ongoing into the enhancement of micronutrient and vitamin content like zinc, iron and vitamins of cassava through genetic engineering (Chavarriaga-Aguirre, 2016; Ukwuru and Egbonu, 2013). Modifying starch quality and enhancing the production of sugars in the storage roots is also under investigation. To increase protein content of cassava storage roots, tissue-specific production of an artificial storage protein is being attempted. Efforts are ongoing to enhance starch synthesis and accumulation for both food and industrial purposes and to reduce starch grain size, largely for industrial uses. In addition to directly improving the storage root quality, there are efforts underway to improve foliage quality, specifically the longevity of the leaves. Leaves that remain photosynthetic longer contribute to higher root yields and in regions where the leaves are also consumed, long-lived leaves add to the overall value of the crop (Stella *et al*., 2013; OECD, 2014).

Efforts to reduce the release of cyanide from cassava tissues focus on either reducing the production of the cyanogenic glycosides or increasing the rate of breakdown of the glycosides. In the first instance, the approach is to use anti-sense constructs to reduce the synthesis of a cytochrome P450 that catalyses the first step in the synthesis of linamarin and lotaustralin. In

the second case, the approach is to increase the synthesis of hydroxynitrile lyase which catalyses the disintegration of acetone cyanohydrin into acetone and hydrogen cyanide (Chavarriaga-Aguirre, 2016; Taylor *et al*., 2012).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. DESCRIPTION OF THE STUDY AREA

In Igueben Local Government Area of Edo State is located Ebelle my study area with geographical coordinates 6° 30' 0" North, 6° 12' 0" East. It is naturally humid and characterized by a bimodal rainfall pattern particularly in July and September. The annual mean rainfall is about 1,650nm, with a mean annual temperature of 37.3^{o}C. The average mean relative humidity is 73.2% (Nigerian Meteorological Agency; NIMET, 2012). Ebelle town is mainly an agrarian economy and the residents are mainly farmers with cassava tubers being the greatest farm output. The sample was amassed from Cassava mill established in the year 2012, by the FADAMA (III) project which is being operated by Mr Philip Osajie.

3.2. COLLECTION OF SAMPLE

Raw cassava mill effluents was collected from cassava processing mill site at Ebelle in Igueben Local Government Area of Edo state. The cassava mill effluent sample was collected using sterile plastic four (4) litre containers and conveyed to the laboratory in ice pack containers. The sample was used immediately it arrived the laboratory.

3.3. STERILIZATION OF EQUIPMENT AND MEDIA

All glass wares used were thoroughly washed, air dried, wrapped in aluminum foil and sterilized in an oven at 160^{0}C for 2hrs. Inoculating loops and needles were sterilized by dipping in 70% ethanol and flamed using a Bunsen burner. The culture media utilized were weighed according to the manufacturer's instruction, dissolved in 1000mls of distilled water and sterilized by autoclaving at 121^{0}c for 15mins in a well stopped conical flasks.

3.4. DETERMINATION OF PHYSICOCHEMICAL PARAMETERS

The physicochemical parameters were determined using the method of American Public health Association APHA (2011). Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD),Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), pH, temperature, phosphate (PO_4^{3-}), nitrate (NO_3^-), alkalinity, chloride (Cl^-), Sulphide (SO_4^{2-}), electrical conductivity (EC), Turbidity and cyanide content (CN). The DO and BOD was determined using the DO and BOD meter while pH, temperature and conductivity were determined using pH meter, thermometer and conductivity meter respectively. In the appendix section the methods for the above physicochemical parameters are well spelt out in appendix A.

3.5. EFFLUENT HEAVY METALS AND CATIONS ANALYSIS

The heavy metals were determined according to the method described by Izah *et al*. (2017). Aqua/Regia Digestion (ASTM D 3974 - 99) method was adopted for heavy metal determination. The flame atomic absorption spectrometry (FAAS) (GBC Avanta PM A6600) was calibrated with prepared working solutions from stock solutions (Accu Standards, 1,000 mg/l) for each of the respective metals to be analysed. The heavy metals were analysed at varying wavelength of 213.9nm, 324.70nm, 232.0nm, 248.3nm, 279.5nm, 357.90nm, 217.00nm and 240.70nm for zinc, copper, nickel, iron, manganese, chromium, lead and cobalt respectively. The concentrations of cations like Na^+, Ca^{2+}, Mg^{2+} and K^+ were determined with a flame photometer, model PFP-7 by aspiration while the concentrations of the heavy metals were analysed using atomic absorption spectrophotometer, model PG 550.

3.6. MICROBIOLOGICAL ANALYSIS

3.6.1. Media Used and Their Preparations.

Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were the media used for growing heterotrophic bacterial and fungal respectively. They were prepared in accordance with the manufacturer's instructions as detailed in appendix B.

3.6.2 Determination of Total Heterotrophic Bacterial Count

3.6.2.1 Serial dilution of Cassava Mill Effluent (CME) Samples

Ten - fold serial dilution of the cassava mill effluent sample was prepared as defined by Cheesbrough (2006). One (1) millilitre of the sample was standardised in 9mls of distilled water. Thereafter, One (1) millilitre of homogenized cassava mill effluent sample was aseptically transferred into a second tube containing 9mls of distilled water. The procedure continued until the tenth tube which gave rise to ten - fold dilution (10^{-10}).

3.6.2.2. Inoculation and Enumeration

For bacteria growth Nutrient agar was used. aliquot one 1ml of appropriate ten - fold serial dilution (10^{-3}, 10^{-6}, 10^{-9}) of the cassava mill effluent sample was inoculated into the Nutrient agar plates containing fuscin applying pour plate method (Cheesbrough, 2006). The inoculated petri dishes were incubated at 37ºC for 24hrs in an incubator for the enumeration of the total heterotrophic bacterial counts. The colony counter (Model- Labtech) was used to count visible discrete colonies on inoculated plates and expressed in colony forming units per millilitre (cfu/ml).

3.6.2.3 Characterisation and Identification of Bacterial isolates

The distinct colonies were sterilized by sub - culturing into freshly prepared Nutrient agar plates. Characterization and identification of pure cultures of bacterial isolates were based on their biochemical, cultural and morphological characteristics (Cheesbrough, 2006).

3.6.3 Determination of Total Heterotrophic Fungal Counts

3.6.3.1. Inoculation and Enumeration.

Potato Dextrose Agar (PDA) was used in growing of fungal species. Aliquot 1ml of appropriate ten - fold serial dilution (10^{-3}, 10^{-6}, 10^{-9}) of the cassava mill effluent sample was inoculated into Potato Dextrose agar plates containing streptomycin in triumvirates using pour plate technique (Cheesbrough, 2006). The inoculated plates were kept at room temperature of 28^{0}C for 72hrs and thereafter the visible discrete colonies were counted using colony counter (Model-Labtech) and expressed in colony forming units per millilitre (cfu/ml).

3.6.3.2. Characterization and Identification of fungal isolates

The discrete fungal isolates were further purified by sub culturing into Potato Dextrose agar plate. Pure cultures of fungal isolates were observed based on cultural characteristics and microscopically using Lactophenol blue. They were recognised centred on the provisions of Barnett and Hunter (1972).

3.7. UTILIZATION OF CASSAVA MILL EFFLUENT (CME) BY MICROBIAL ISOLATES

3.7.1. Isolation of Cyanide Degrading Microbes

Cyanide-degrading microorganisms were segregated from a cassava mill effluent samples and purified by repeatedly transferring the cells to enrichment medium. For enrichment of

microorganisms Nutrient broth was used and the sample cultivated in a 500ml Erlenmeyer flask containing 100ml nutrient broth, with 1% Cyanide Concentration .To screen cyanide degrading bacterium, 10ml of culture from 1.5×10^8 was transferred into 500ml Erlenmeyer flask containing 100ml of buffer medium BM (K_2HPO_4 4.35g, NaOH 4g and 10 ml of trace salts solution ($FeSO_4 \cdot 7H_2O$ 300 mg, $MgSO_4 \cdot 7H_2O$ 180 mg, $CoCl_2$ 130 mg, $CaCl_2$ 40 mg, $MnCl_2 \cdot 4H_2O$ 40 mg and MoO_3 20 mg in 1 litre deionized water) and 0.1% yeast extract containing 1% of cyanide was incubated at 30°C, 150rpm. This process was repeated three times by inoculating in fresh medium with 10% (v/v) of the previously grown culture. Cyanide-degrading bacteria were isolated after 8 days on a 2-day basis by smearing on nutrient agar medium. Colonies that differed mainly in the morphology were selected and obtained pure cultures by continuous sub-culturing. The bacteria isolated were verified for their Gram reactions, other biochemical and physiological tests (Mirizadeh *et al*., 2014).

3.8. CYANIDE DEGRADATION EXPERIMENT

The method of Arutchelvan *et al.* (2005) and Mirizadeh *et al*. (2014) was used. Inoculated bacterial and fungal strains with distinct morphological colonies in nutrient broth for 24 hours. For the purpose of strains comparison study, removal of cyanide was determined every 48 hours for 8 days. At regular intervals of 48 hours samples were taken and tested for cyanide reduction. Non-inoculated medium served as control. The effect of substrate concentration, pH, inoculum size and phenol were determined.

3.8.1. Effect of Substrate concentration (Cyanide)

The modified method of Arutchelvan *et al*. (2005) was used to analyse the impact of substrate concentration, five substrate concentrations of 30ppm, 60ppm, 90ppm, 120ppm and 150ppm

were used in the study while all other given parameters remained constant. Each flask containing 100ml medium was added and sterilized after regulating the pH to desired value. The initial cyanide concentration was sustained at 15ppm adding 1ml (1mg) from the stock solution whose concentration was 1000 mg/l. Similarly, prepared the other flasks to a last volume of each 100ml with initial cyanide concentration of 0, 50,100,150,200 and 250 ppm in the Nitrogen free Glucose (NFG) medium ($Na_2HPO_4.2H_2O$[50mM]7.098g/L, KH_2PO_4[100mM]-13.609g/L,$MgSO_4.7H_2O$[1mM]-0.246g/L,$CaCl_2$[0.1mM]-0.11g/L, Dextose[0.8%]-8 g/L) also with 4%(v/v) of Inoculum.

3.8.2. Effect of pH

The effect of pH on degradation of cyanide was determined by maintaining pH ranges from 4 to 8 100ml of NFG medium were prepared at a cyanide concentration of 15ppm in different flasks labelled with pH ranging 4, 5, 6, 7 and 8. All flasks were subjected to sterilization before adding 6ml of inoculum according to the method of Arutchelvan *et al*. (2005).

3.8.3. Effect of Inoculum Size

In other to assess the effect of inoculum variation in degradation the cyanide, prepared another set of labelled flasks with different inoculum loadings; 2.5ml, 3.5ml, 4.5ml, 5.5ml and 6.5ml containing the same species and regulated to a final volume of 100ml of the same medium. The solution was maintained at pH 6 and the initial concentration of cyanide as 15ppm according to the method of Arutchelvan *et al*. (2005).

3.8.4. Effect of Phenol

The method of Arutchelvan *et al*. (2005) stated NFG medium with pH 6 was dispensed in each of the 5 flasks. Subsequently, after sterilization and cooling, inoculated the content with 6ml of culture and added with 0.30%, 0.50%, 0.70%, 0.90% and 1.10% of phenol concentration. Cyanide concentration as 15ppm was maintained and experimentations were carried out.

3.9 ANALYTICAL METHODS

3.9.1 Growth determination

Cell growth was examined by determining the optical density (O.D) of 1 ml culture at 660 nm through Spectrophotometry (GENESYS 10 UV-Vis Scanning, Thermo Scientific) and expressed as OD660 nm according to the method of Kandasamy *et al.* (2015).

3.9.2. Degradation Efficiency

The degradation efficiency (DE) of cyanide degrading bacterium was calculated as shown in following formula below

$$DE\ (\%) = \frac{Ic - Rc}{Ic} \times 100$$

Where, Ic = Initial concentration of cyanide (mg/l) and Rc = Residual concentration of cyanide (mg/l).

CHAPTER FOUR

4.0 RESULTS

The concentration of the different physicochemical parameters and heavy metals of the raw cassava mill effluent analysed were revealed in Table 1. The pH of 4.81 reported was highly acidic while the electrical conductivity was high with 4860 uS/cm. Other major pollutants reported were chemical oxygen demand (2041.20mg/l), nitrate (140.94mg/l), sulphate (257.58mg/l) and phosphate (102.06mg/l). The heavy metals recorded were Nickel (121.20mmg/l), Iron (340mg/l) and Chromium (19.44mg/l). The cyanide content was 17.13mg/l.

The results in Table 2 indicates that the total heterotrophic bacterial count (6.32 x 10^8cfu/ml) was higher than the total heterotrophic fungal count of (2.87 x 10^8cfu/ml).

Table 3 detailed the presence of bacterial isolates such as *Lactobacillus, Micrococcus, Staphylococcus aureus, Bacillus, Escherichia coli, Klebsiella, Pseudomonas, Salmonella and Corynebacterium* species.

Table 4 recorded the fungal isolated which were *Aspergillus and Fusarium, Penicillium and Saccharomyces* species.

Figure 4.1 (Appendix C) unveiled the ability of microbial isolate to utilise 1% cyanide. It showed that all isolates had the ability to utilize cyanide. *Bacillus* species had the highest turbidity value of 0.543nm among the bacterial isolates while the least was *Salmonella* with

0.236nm. However, of all fungal isolated *Aspergillus* species had the highest of 0.407nm while *Saccharomyces* had the least of 0.239nm.

It was establish that *Pseudomonas* sp. performed better because it was able to reduce the substrate concentration of 30ppm and 60ppm to 20.18ppm (32.73%) and 49.43ppm (17.62%) respectively at a residence time of 8 days while *Bacillus* sp. was most effective in the reduction of 120ppm and 150ppm to 99.68ppm (16.93%) and 127.06ppm (15.29%) respectively at residence time of 8 days. In summary, Figure 4.2(Appendix D) details the smaller the concentration the better the degradation efficiency.

Figure 4.3 (Appendix E) revealed the ability of microbial isolates to degrade cyanide at varying pH while other parameters remained constant. It was noted that pH 6 gave the highest reduction in cyanide concentration. *Pseudomonas* sp. at pH 6 had 11.05ppm, 7.89ppm, 7.81ppm and 7.65ppm at day 2, 4, 6 and 8 respectively.

The findings in Figure 4.4 (Appendix F) showed that at the inoculum size of 6.5ml, *Bacillus* species gave the most efficient degradation rate of 8.69ppm at day 8 as compared with other volumes of inoculum used in this study.

Figure 4.5 (Appendix G) details the cyanide degradation efficiency of the microbial isolates at varying concentration of Phenol with other parameters at constant. *Pseudomonas* sp. gave the best 12.04ppm at 0.30% phenol concentration, *Bacillus* sp. at 0.50% and 0.70% was 13.68ppm and 14.46ppm respectively while at 0.90% and 1.10% *Pseudomonas* sp. gave a reduction value of 14.18ppm and 14.43ppm respectively.

Table 4.1: Physiochemical Properties of Cassava Mill Effluent

Physicochemical parameters	Units	Concentration	FEPA Effluent Limitation Guideline (1991) mg/l
Ph		4.81	6-9
Electrical Conductivity	uS/cm	4860	1000
Chlorine	mg/l	34.02	600
Alkalinity as Bicarbonate	mg/l	27.65	NA
Total Suspended Solid	mg/l	29.65	30
Total Dissolved Solid	mg/l	2478.60	2000
Turbidity	NTU	166.74	300
Chemical oxygen demand	mg/l	2041.20	40
Dissolved oxygen	mg/l	0.63	40
Biochemical oxygen demand	mg/l	1490.08	10
Cyanide	mg/l	17.13	0.2
Sulphate	mg/l	257.58	50
Nitrate	mg/l	140.94	1.0
Phosphate	mg/l	102.06	5.0
Ammonium nitrogen	mg/l	0.97	NA
Calcium	mg/l	156.98	100
Magnesium	mg/l	58.32	100
Sodium	mg/l	680.40	200
Potassium	mg/l	1506.60	NA
Zinc	mg/l	58.32	1.0
Copper	mg/l	72.90	1.5
Chromium	mg/l	19.44	0.5
Lead	mg/l	0.29	0.5
Manganese	mg/l	136.08	0.5
Iron	mg/l	340.20	20
Nickel	mg/l	121.50	1.0

Legend:
NA: Not Available.

Table 4.2: Enumeration of Bacterial and Fungal Counts (x 10^8 cfu/ml)

	THBC (cfu/ml)	THFC (cfu/ml)
Cassava effluent	6.32	2.87

Legend: THBC – Total Heterotrophic Bacterial Count; THFC – Total Heterotrophic Fungal Count.

Table 4.3: Cultural, Morphological and Biochemical Characteristics of Bacterial Isolates

CULTURAL CHARACTERISTICS	A	B	C	D	E	F	G	H	I
Shape	Round	Round	Round	Round	Round	Round	Round	Round	Irregular
Colour	Creamy	Creamy	light green	Milky	Milky	Milky	Pale green	Milky	Milky
Size	Large	Large	Small	Large	Large	Small	Large	Large	Large
Elevation	Raised	Flat	Flat	Raised	Flat	Flat	Flat	Flat	Flat
Transparency	Opaque	Opaque	Opaque	Transparent	Opaque	Opaque	Opaque	Transparent	Opaque
MORPHOLOGY									
Gram stain	Positive	Positive	Positive	Positive	Negative	Negative	Negative	Negative	Positive
Cell type	Rod	Rod	Cocci	Rod	Rod	Rod	Rod	Rod	Rod
Cell arrangement	Chains	Pair	Clusters	Pair	Pair	Single	Single	Single	Single
BIOCHEMICAL TEST									
Citrate utilization	-	+	+	+	+	+	+	+	-
Spore forming	-	-	-	+	-	-	-	-	-
Catalase production	+	+	+	+	+	-	-	-	+
Indole	+	-	-	+	+	+	+	+	+
Motility	+	+	-	+	+	+	-	+	-
Methyl red	+	-	-	-	+	+	-	+	+
Vogues prauskeur	-	+	+	+	+	-	+	-	+
Coagulase test	-	-	+	-	-	-	-	-	-
Oxidase test	+	+	+	-	-	+	+	-	+
FERMENTATION TEST									
Lactose	+	-	+	+	+	+	-	+	+
Glucose	+	+	+	+	+	+	-	+	-
Galactose	+	+	-	+	+	-	+	+	+
Maltose	+	+	+	+	+	+	+	+	-
Raffinose	-	-	-	-	+	-	+	-	+
Mannitol	-	-	+	-	-	-	-	-	-
PROBABLE IDENTITY	*Lactobacillus* sp.	*Micrococcus* sp.	*Staphylococcus* aureus	*Bacillus* sp.	*Escherichia coli*	*Klebsiella* sp.	*Pseudomonas* sp.	*Salmonella* sp.	*Corynebacterium* sp.

Table 4.4: Microscopic and Macroscopic Characteristics of Fungal Isolates

Isolate	Cultural	Microscopic examination	Fungal isolates
CN1	Black fluffy colonies with reverse side yellow	Simple septate and branched conidia in chains.	*Aspergillus niger*
CN2	White and cottony hyphae with reverse side pinkish	Non septate hyphae with sporangiosphore and rhizoid	*Fusarium* sp.
CN3	Milky whitish colonies, and white on reverse	Spherical to ova shape fungi Stained positive on gram reaction	*Saccharomyces* sp.
CN4	Green flat colony with reverse side dirty white	Brush-like condidia, septate branching conidiphore was smooth/rough walled.	*Penicillium* sp.

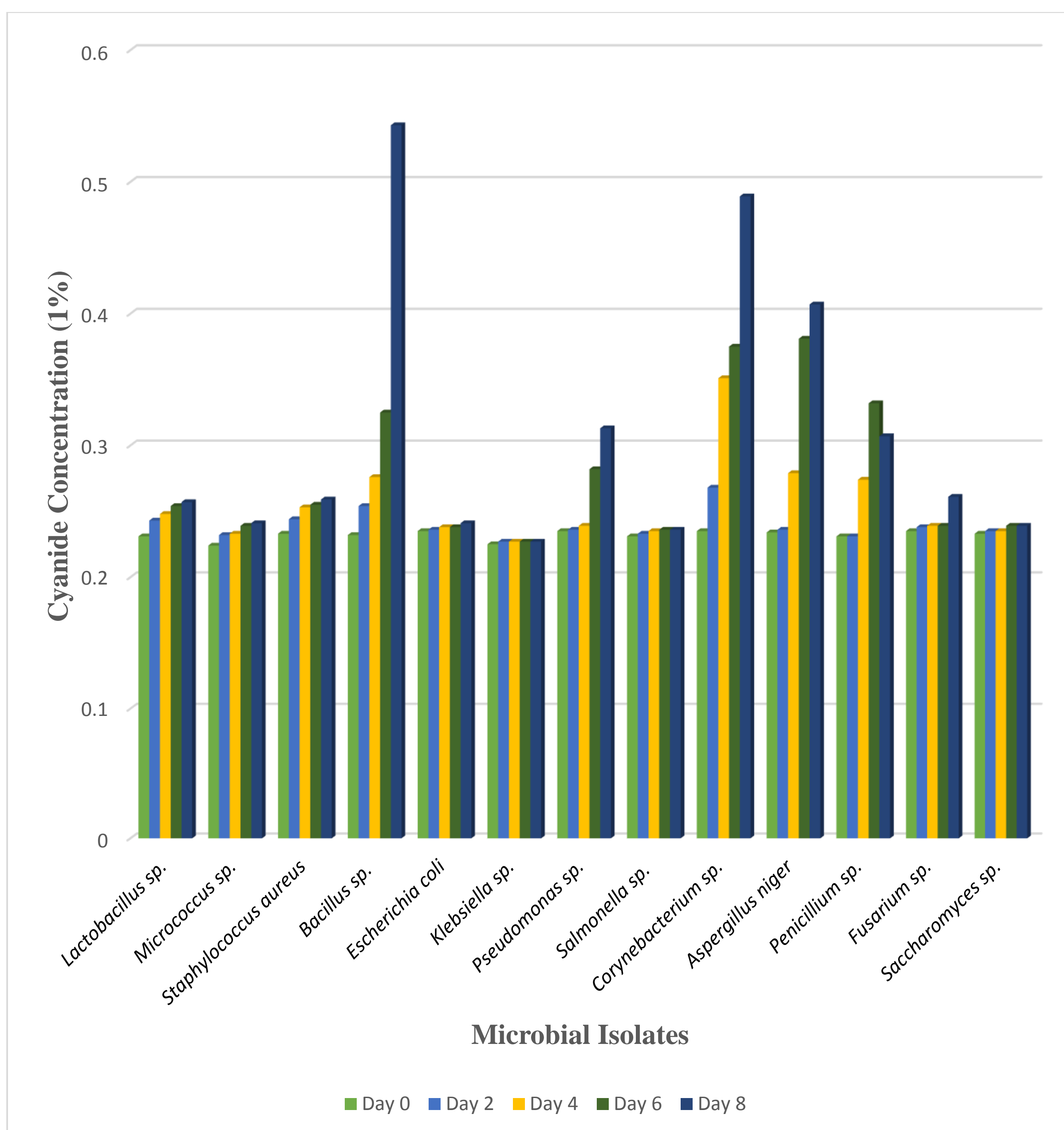


Figure 4.1: Isolation of cyanide degrading microbes (bacteria and fungi) with mineral salt medium containing 1% cyanide

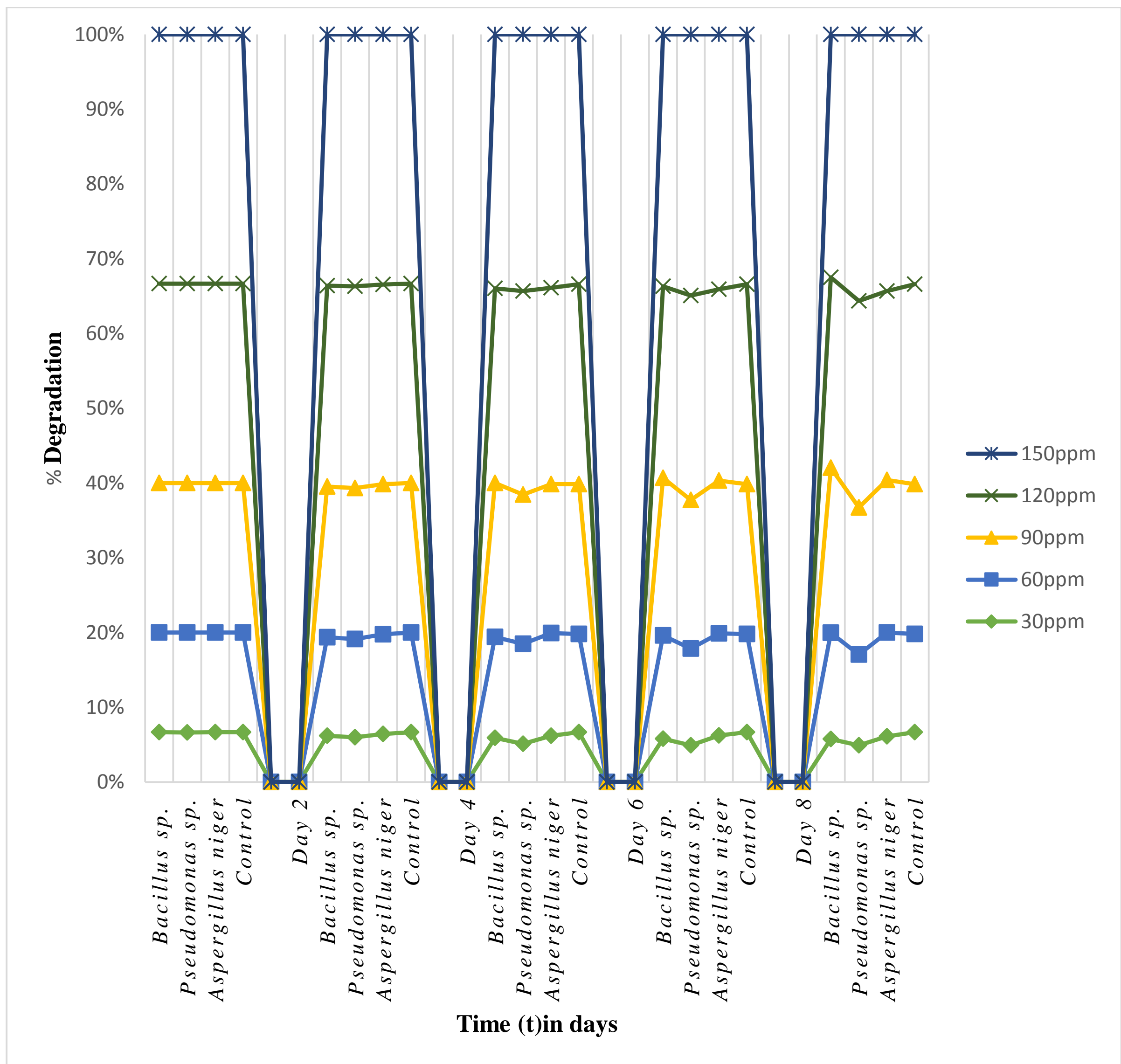


Figure 4.2: Effect of Substrate Concentration (Cyanide)

Inoculum size = 6ml of 1.5 x 10^8cfu/ml, pH =6.

Table 4.5: Effect of pH

At interval of 2 days, Cyanide Concentration = 15ppm, Cell suspension=6 ml of 1.5 x 10^8

Day 0	pH 4	pH 5	pH 6	pH 7	pH 8
Bacillus sp.	15.34	15.42	15.33	15.37	15.44
Pseudomonas sp.	15.28	15.36	15.41	15.38	15.42
Aspergillus niger	15.43	15.31	15.35	15.42	15.38
Control	15.33	15.41	15.42	15.37	15.41

Day 2	pH 4	pH 5	pH 6	pH 7	pH 8
Bacillus sp.	12.54	11.89	11.55	13.33	15.11
Pseudomonas sp.	14.11	11.43	11.05	13.97	15.25
Aspergillus niger	14.68	13.75	12.76	14.78	15.31
Control	15.14	15.38	15.19	15.36	15.4

Day 4	pH 4	pH 5	pH 6	pH 7	pH8
Bacillus sp.	10.89	10.35	8.63	12.78	14.98
Pseudomonas sp.	13.77	10.85	7.89	12.43	15.06
Aspergillus niger	14.11	12.98	12.04	14.31	15.34
Control	15.05	15.33	15.14	15.31	15.39

Day 6	pH 4	pH 5	pH 6	pH 7	pH 8
Bacillus sp.	10.67	9.83	8.46	12.27	14.68
Pseudomonas sp.	13.63	10.63	7.81	11.81	14.76
Aspergillus niger	13.83	12.59	11.68	14.17	15.03
Control	14.90	15.02	14.84	15.00	15.08

Day 8	pH 4	pH 5	pH 6	pH 7	pH 8
Bacillus sp.	10.46	9.64	8.29	12.02	14.39
Pseudomonas sp.	13.36	10.42	7.65	11.57	14.46
Aspergillus niger	13.55	12.34	11.45	13.88	14.73
Control	14.89	15.01	14.83	14.89	15.07

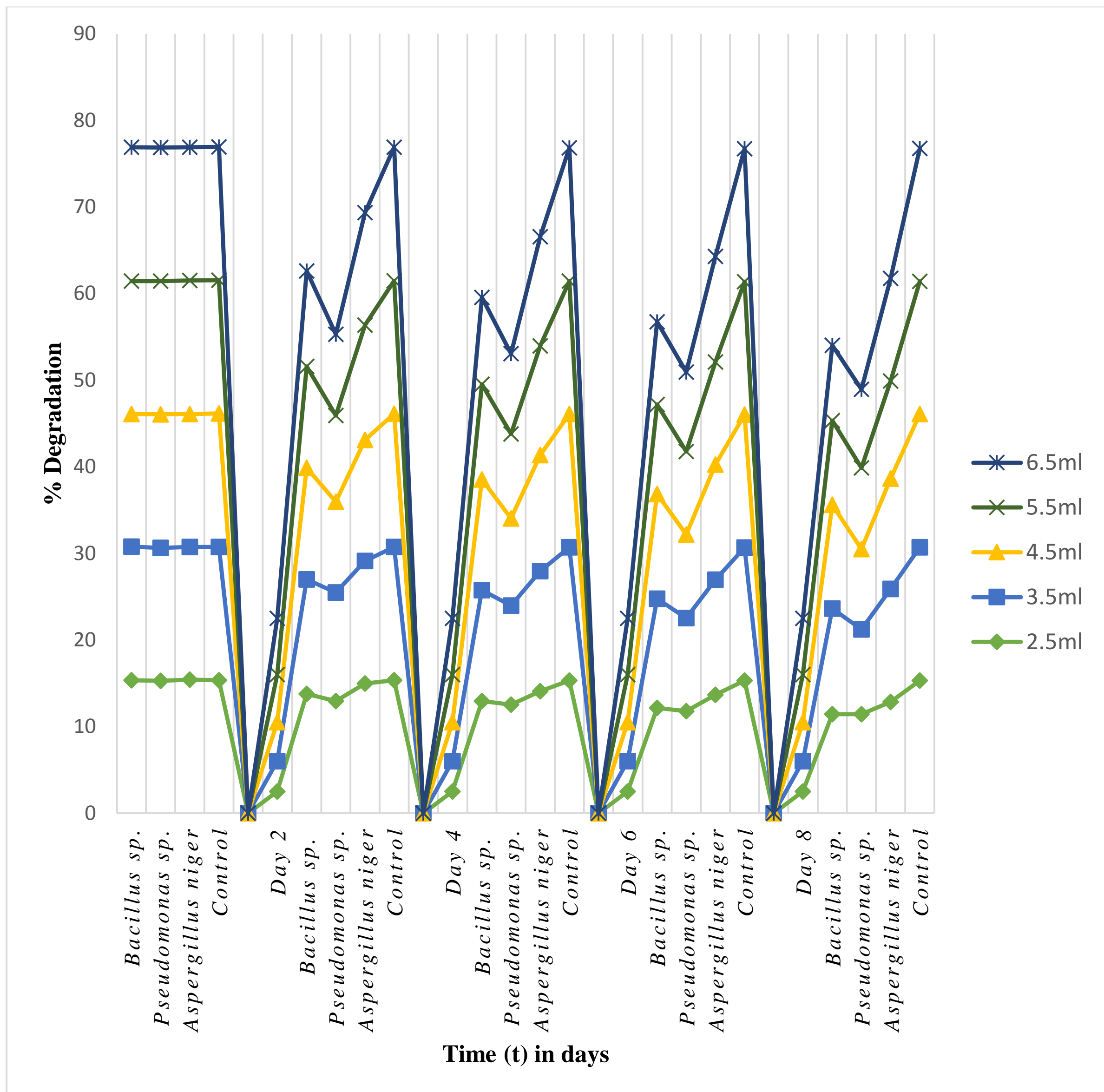


Figure 4.4: Effect of Inoculum size

pH = 6, Cyanide Concentration = 15ppm.

Table 4.6: Effect of Phenol

Inoculum size = 6ml of 1.5 x 10^8cfu/ml, pH =6, Cyanide Concentration = 15ppm

Day 0	0.30%	0.50%	0.70%	0.90%	1.10%
Bacillus sp.	15.34	15.42	15.33	15.37	15.44
Pseudomonas sp.	15.28	15.36	15.41	15.38	15.42
Aspergillus niger	15.43	15.31	15.35	15.42	15.38
Control	15.33	15.41	15.42	15.37	15.41

Day 2	0.30%	0.50%	0.70%	0.90%	1.10%
Bacillus sp.	13.88	15.01	15.27	15.31	15.43
Pseudomonas sp.	13.25	14.95	15.25	15.29	15.4
Aspergillus niger	14.96	15.13	15.33	15.4	15.38
Control	15.33	15.41	15.42	15.37	15.41

Day 4	0.30%	0.50%	0.70%	0.90%	1.10%
Bacillus sp.	12.78	14.69	15.06	15.28	15.41
Pseudomonas sp.	12.41	14.25	15.19	15.23	15.03
Aspergillus niger	13.99	15.21	15.28	15.39	15.36
Control	15.32	15.39	15.4	15.36	15.41

Day 6	0.30%	0.50%	0.70%	0.90%	1.10%
Bacillus sp.	12.52	13.96	14.76	14.67	15.10
Pseudomonas sp.	12.29	13.97	15.04	14.47	14.73
Aspergillus niger	13.71	14.75	14.82	15.24	15.05
Control	15.32	15.37	15.4	15.35	15.41

Day 8	0.30%	0.50%	0.70%	0.90%	1.10%
Bacillus sp.	12.27	13.68	14.46	14.38	14.80
Pseudomonas sp.	12.04	13.69	14.74	14.18	14.43
Aspergillus niger	13.44	14.46	14.53	14.93	14.75
Control	15.32	15.37	15.4	15.35	15.41

CHAPTER FIVE

5.0. DISCUSSION

The results of physicochemical quality (nutrient, heavy metals, oxygen-related parameters and general physicochemical parameters) of this study revealed that the major pollutants of cassava mill effluent were pH (4.81), electrical conductivity (4860 uS/cm), Sulphate (257.58 mg/l), Nitrate NO_3^- (140.94 mg/l), Phosphate PO_4^{3-} (102.06 mg/l), Cyanide CN^- (17.13 mg/l), Chemical Oxygen Demand (2041.20 mg/l), Biochemical Oxygen Demand (1490.08mg/l), Total Dissolved Solid (2478.60 mg/l), Calcium (156.98 mg/l), Sodium (680.40 mg/l), Zinc (58.32 mg/l), Copper (72.90 mg/l), Chromium (19.44 mg/l), Manganese (136.08mg/l), Iron (340.20 mg/l) and Nickel (121.50 mg/l). This is because the above-mentioned physicochemical parameters exceeded the Federal Environmental Protection Agency; FEPA, (1991) recommended standard for effluent discharge. The pH detailed was acidic and this could be traced to the high cyanide content recorded from the cassava mill effluent. The high electrical conductivity observed could be due to an increase in the concentration of dissolved salts as reported by (Enerijiofi *et al*., 2017a; Etinosa and Igiehion, 2015). The low chloride reported could be charted to low use of chlorinated water in the cassava processing procedures. This is not surprising as chlorinated water is mostly not utilized in the study area because of its rural status. The high concentration of TDS could be attributed to the high organic components of the cassava effluent which required high utilization of dissolved oxygen leading to the low dissolved oxygen and high biological oxygen demand. This results agreed with earlier studies which was corroborated by earlier studies (Ebukiba, 2010; Enerijiofi *et al*., 2017b). Enerijiofi *et al*. (2017b) reported most of the concentration of the physicochemical parameters were higher than FEPA (1991) regulatory recommended standards for the discharge of industrial

effluent. All heavy metals analysed exceeded the limits specified by FEPA (1991) with exception of Magnesium (58.32 mg/l) and Lead (0.29 mg/l). The high heavy metals noted in this study could be traced to the anthropogenic input into the environment. This could have resulted from corrosion of the metal parts of farming implement used in harvesting, flaking, washing, and treatment as well as milling machines concerned with cassava processes in collaboration with the work of Etinosa and Ozede (2015).

The colony enumeration has shown that the cassava mill effluent contained more heterotrophic bacterial and fungal load. This revealed that the cassava mill effluent contained more nutrient for microbial growth and proliferation. This study acknowledged a diversified range of bacterial and fungal isolates from cassava mill effluent. They comprised *Lactobacillus* sp., *Micrococcus* sp., *Staphylococcus aureus*, *Bacillus* sp., *Escherichia coli, Klebsiella* sp., *Pseudomonas* sp., *Salmonella* sp. *Corynebacterium* sp., *Aspergillus niger, Penicillium* sp., *Fusarium* sp. and *Saccharomyces* sp. are natural flora of soil and linked with degradation of organic matter. This diversity reported could be based on the fact that cassava mill effluent is prone to microbial attack. The association of the microbial isolates observed with cassava mill effluent had earlier been documented by several researchers. (Chinyere *et al*., 2016; Enerijiofi *et al*., 2017b; Izah *et al*., 2018; Orji and Ayogu, 2018). Enerijiofi *et al*. (2017b) reported bacteria such as *Lactobacillus* sp*., Acetobacter* sp*., Pseudomonas* sp*., Micrococcus* sp*., Staphylococcus aureus, Corynebacterium* sp. and *Bacillus* sp. while the fungal isolates included *Aspergillus* sp*., Rhizopus* sp*., Saccharomyces* sp. and *Penicillium* sp.

The microbial (bacterial and fungal) isolates specified different turbidity values. This designated their ability to degrade cassava mill effluent differently. This entails that the cassava mill effluent contained ample source of degradable nutrients which makes it predisposed to microbial degradation as they did harbour microorganisms that exhibited great potentials in utilizing cassava mill effluent as their primary carbon and energy source (Enerijiofi *et al*., 2017a). However, the prevalence of indigenous microbes is not a direct indication that the indigenous microbes principally utilize cassava mill effluent as their carbon and energy sources, rather may utilize cassava mill effluent as their secondary carbon and energy sources. *Bacillus, Pseudomonas* and *Aspergillus* species had the maximum cyanide utilizing ability (turbidity) of all the microbial isolates screened, hence they were exploited for the biodegradation study. This further attested that these microorganisms have the capabilities to grow favourably in cassava mill effluent and alter them to non – toxic products. A similar observation was made by Enerijiofi *et al*. (2017b) in the findings of biodegradation potentials of cassava mill effluents using indigenous microorganisms.

The result revealed that as the substrate concentration was increased, the degradation efficiency reduced. *Pseudomonas* sp. degraded cyanide best to the tune of 32.73% at a residence period of 8 days. From this, it can be concluded that increased substrate will inhibit the degradation process. Kandasamy *et al*. (2015); Mekuto *et al*. (2013); Mirizadeh *et al*. (2014) also suggested similar findings.

The pH concentration also plays a major role in the biological activity. As per the observations made in the batch reaction process it was found that the maximum of 49% degradation was

achieved at pH 6. From this, it can be concluded that the microbial isolates works well in acidic, slightly acidic and neutral condition in concordance with Kwon *et al*. (2002); Ibrahim *et al*. (2015).

It was evident that the degradation percentage amplified when the biomass concentration was increased. At a concentration of 6.5 ml *Bacillus* species gave the optimum degradation efficiency. This result also revealed that with an increase in biomass concentration the residence time have also been reduced considerably. The observation corroborates with the findings of Arutchelvan (2005).

It was recorded that with increase of phenol concentration in the cyanide wastewater, the degradation of cyanide reduces due to inhibitory action of phenol and the residence time increases with interference of phenol. As per the observation made during the experiment, it was establish that the organisms(*Bacillus* sp., *Pseudomonas* sp. and *Aspergillus niger*) were more active in degrading the phenol rather degrading the cyanide in correlation to the findings of Neetu and Chandrajit (2016); Singh *et al*. (2018); Syed and Jabeen (2015).

5.8. CONCLUSION AND RECOMMENDATION

Pseudomonas, Bacillus and Aspergillus species were the most dominant microbial (bacterial and fungal) isolates which showed the highest ability of improving cassava mill effluent by reducing the cyanide content under precise cultural conditions. Since, this study has unveiled the potentials of biodegradation of cyanide from cassava mill effluent. It is recommended that further leaps be taken from this study in a bid to exploring newer, more effective, less costly and better satisfactory methods of cassava mill effluent management before eventual

emancipation into the environment. This study therefore recommends that contamination caused by CME in the environment is mostly from acidity due to cyanide and heavy metals recorded.

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APPENDICES

APPENDIX A

PROCEDURES FOR DETERMINING PHYSICOCHEMICAL PARAMETERS

1. pH

The hydrogen ion concentration (pH) of the sample was measured using a HMDPHM 80 digital pH metre. The pH meter was calibrated before and after reading using freshly prepared pH buffers (7.00), (4.00) and (9.00). The electrode probe was inserted into a glass beaker containing about 20 ml of the cassava mill effluent (CME) sample and the result was read from the screen and recorded.

2. Electrical conductivity

The electrical conductivity of sample was measured using a HMDPHM 80 digital EC metre. The EC meter was calibrated before and after reading. The electrode probe was inserted into a glass beaker containing about 20 ml of the CME sample and the result was read from the screen and recorded. The ability of a solution to conduct an electrical current is governed by the migration of solutions and is dependent on the nature and numbers of the ionic species in that solution. This property is called electrical conductivity. The permissible limit for electrical conductivity (EC) is 1000 µScm-1. EC of the collected sample was 4860 µS cm^{-1}.

3. Chloride

An aliquot of 50ml of sample was measured into a conical flask to which about 0.5ml of potassium chromate indicator is added and titrated against standard silver nitrate till silver dichromate ($AgCrO_4$) starts precipitating. Blank titration with only the reagents and no CME sample was also performed.

4. Alkalinity Determination

A 50ml CME sample was measured in a conical flask. Two drops of methyl orange indicator was added and the resulting mixture titrated against the standard 0.1M HCL solution to the first permanent pink colour at pH 4.5. The following equation was used in the calculation.

Alkalinity mg ($CaCO_3$)/L = AxNx50, 000 1ml sample

Where A = ml of acid used. N= Normality of standard acid used

5. Total Suspended Solid

The known volume of vigorously shaken sample (50ml) is filtered into a pre-weighed glass fibre filter disk fitted to suction pump, and washed successively with distilled water. The filter is carefully removed from the filtration apparatus and dried for an hour at 103-105 ^{o}C in an oven, cooled in desiccator and weighed for constant weight

6. Total Dissolved Solid

A 50ml well-mixed sample was measured into a beaker. The WTW TDS/ Conductivity meter probe was immersed in the sample and its conductivity recorded.

7. Turbidity

The turbidity of the respective CME sample was determined using a spectrophotometer. Five millilitres of the sample was dispensed into a cuvette and placed in the light chamber and the absorbance was measured at a specific wavelength using distilled water as blank. The turbidity values were recorded in nepholometer turbidity unit (NTU).

8. Chemical oxygen demand (COD)

The COD value for the CME sample were determined using the colorimetric procedure. HACH COD reagents (high range), COD reactor (HACH) and HACH DR 2010 Spectrophotometer

were utilized. A measured 10mg/l of the sample was added to 5 ml of high range COD reagent (HACH). This mixture was placed in a COD reactor for about 1h and upon cooling, the absorbance of the mixture was read at a specified wavelength of 346nm using a HACH DR 2010 Spectrophotometer.

9. Dissolved Oxygen (DO)

250 ml DO bottle were filled to the brim with CME sample, taking care to minimize contact with air. 10 ml of the CME sample solution was measured to which 2 drops of starch indicator was added. The resulting dark blue solution was titrated against a colourless 0.0125M Thiosulphate solution.

10. Biological oxygen demand (BOD)

Determination of the Dissolved Oxygen (DO) using Wrinkler's method on a suitable portion of the seeded water was carried out. An incubation bottle was filled to the brim with the remainder of the diluted CME sample. The bottle was screw capped and incubated in the dark for 5 days at 20°C. On the 5th day, the DO value was determined. The BOD value was the result of the DO value divided by the percentage dilution.

11. Nitrate

Aliquots of 0.1, 0.2, 0.3 and 0.4ml of nitrate stock solution were measured into different 100ml volumetric flasks. Two millilitre (2ml) of 0.1M sodium hydroxide (NaOH) was added followed by the addition of 1ml of brucine to each. The mixtures were diluted to 100ml mark. A straight line graph was plotted at the absorbance of 543nm with UV-Visible spectrophotometer against concentration which passed through the origin. An aliquot of 2ml of 0.1M sodium hydroxide (NaOH) solution and 1ml of brucine was added to a 50ml sample. The mixture was allowed to

stand for 15 to 20 minutes and the nitrite concentration was determined at wavelength of 543nm with UV-Visible spectrophotometer. A blank analysis was run with all the reagents without the sample for all the analysis.

12. Sodium

The filter of the flame photometer is set to 589nm (marked for Sodium, Na). By feeding distilled water the scale is set to zero and maximum using the standard of highest value. A standard curve between concentration and emission is prepared by feeding the standard solutions. The CME sample is filtered through filter paper and fed into the flame photometer and the concentration is found from graph or by direct readings.

13. Potassium

The filter of the flame photometer is set at 766.5nm (marked for Potassium, K) the flame is adjusted for blue colour. The scale is set to zero and maximum using the highest standard value. A standard curve of different concentration is prepared by feeding the standard solutions. The CME sample is filtered through the filter paper and fed into the flame photometer. The concentration is found from the standard curve or as direct reading

14. Sulphate

100ml of the sample is filtered into a Nessler's tube containing 5ml of conditioning reagent. About 0.2g of barium chloride crystals is added with continued stirring. A working standard is prepared by taking 1ml of the standard, 5ml of conditioning reagent and made up to 100ml, to give 100 NTU. The turbidity developed by the sample and the standards are measured using a Nephelometer and the results are tabulated

15. Phosphate

To 50ml of the filtered CME sample, 4ml of ammonium molybdate reagent and about 4-5 drops of stannous chloride reagent is added. After about 10 min but before 12 min, the colour developed is measured photometrically at 690nm and calibration curve is prepared. A reagent blank is always run with same treatment with distilled water as sample. The value of phosphate is obtained by comparing absorbance of sample with the standard curve and expressed as mg/L.

16. Calcium

A known volume (50ml) of the sample is pipetted into a clean conical flask, to which 1ml of sodium hydroxide and 1ml of iso-propyl alcohol is added. A pinch of murexide indicator is added to this mixture and titrated against EDTA until the pink colour turns purple

APPENDIX B

PROCEDURES USED IN MEDIA PREPARATION

1. NUTRIENT AGAR (NA)

1.1. Composition

Nutrient Agar

Beef Extract…………………..…………..3.0 g

Peptone……..…………………..…………5.0 g

Agar……………………………………….15.0 g

Distilled Water…………………………….……1000 ml

1.2. Preparation

1. Dissolve the dehydrated medium in the appropriate volume of distilled water i.e. 28 g dehydrated nutrient agar in 1000 ml distilled water.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder
3. Sterilized the medium by **autoclaving (121°C for 15 min)**
4. Dispense the medium in to tubes or plates. Left the agar medium to solidify and store.
5. Determine the pH of the medium (pH 6.8 +/- 0.2) with a pH meter and adjust if necessary.

2. POTATO DEXTROSE AGAR (PDA)

2.1. Composition

Commercial PDA Powder (20 gm dextrose, 15 gm agar, and 4 gm potato starch)

2.2. Procedure

1. Dissolve 39 gm of Commercial PDA Powder in 1 Litre of distilled water
2. Boil while mixing to dissolve and autoclave for 15 min at 121°C.

3. Dispense the medium in to tubes or plates. Left the agar medium to solidify and store.
4. Determine the pH of the medium (pH 6.8 +/- 0.2) with a pH meter and adjust if necessary.

APPENDIX C

PROCEDURE FOR MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF BACTERIAL ISOLATES

1. MORPHOLOGICAL CHARACTERISTICS

1.1. Gram Staining

A smear of the bacteria was made on a sterile slide, it was heat fixed using a spirit lamp and stained with crystal violet and left to stand for 60 seconds and rinsed with clean water. Few drops of iodine was added to cover the smear and left for 60 second and washed away with clean water. Few drops of the decolourizer (ethanol) was added and left to stand for 3 minutes and then washed off with clean water. The counter stain safranin was added and left to stand for 60 seconds and then rinsed. It was left to dry and viewed on the microscope by oil immersion for clearly view.

2. BIOCHEMICAL CHARACTERISTICS

2.1. Citrate Utilization Test

This test showed the ability of some bacterial to utilize citrate as their sole carbon source. Deoxycholate citrate agar with bromothymol blue as pH indicator was used. Slants of Simmon's citrate agar were prepared in test tubes as instructed by the manufacturer. Using a sterile straight wire loop, a normal saline suspension of the test organism was first streaked on

the slants and then stabbed. The test tubes wee incubated at 37^0C for 72 hours. Alkaline pH shown by intense bright colour indicated citrate utilization.

2.2. Spore forming

A smear of the bacteria was made on a sterile slide, it was heat fixed using a spirit lamp. Covered the smear with a piece of absorbent paper and saturate the paper with malachite green stain solution and steam for about five (5) minutes, keeping the paper moist by adding more dye as required. Washed the slide with distilled water. Counter stain with 0.5% safranin for 30 seconds, washed and blot dry. Examined the slide under microscope at 100^x for the presence of endospores. The endospores are bright green and vegetative cells are brownish red to pink.

2.3. Catalase

This is a test done to identify microorganisms that produce the enzyme catalase with mediates the breakdown of hydrogen peroxide. A 24 hour old culture is used to carry out the test. The sterile loop was used to make a homogenous suspension on the slide. A drop of hydrogen peroxide (H_2O_2) was added to the suspension and the occurrence of the effervescence indicated a positive reaction while its absence indicated a negative reaction.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

2.4. Indole Test

Indole test is used to determine the ability of an organism to split amino acid tryptophan to form the compound indole. It is used to differentiate enteric (members of family Enterobacteriaceae). The bacterium to be tested is inoculated in tryptone broth and incubated

at 37°C. Few drops of kovac's reagent, Ehrlich's reagent is more sensitive in detecting indole production in anaerobes and non-fermenters.

2.5. Methyl Red (MR) Test

The methyl red (MR) test detects the production of sufficient acid during the fermentation of glucose. An organism from an 18-24 hours pure culture is used and lightly inoculated into the medium. It is incubated aerobically at 37° C for 24 hours. After, 24 hours of incubation, 1ml aliquot of the broth in a clean test tube is re-incubated for an additional 24 hours. 2 to 3 drops of methyl red indicator is added to the aliquot. A **Positive Reaction** shows a distinct red colour while **Negative Reaction shows** a yellow colour.

2.6. Vogues Proskauer (VP) Test

This is a test used to detect acetoin in a bacterial broth culture. A tube of VP broth is inoculated with a pure culture of the test organism and incubated for 24 hours at 35ºC. 0.6ml of 5% alpha naphthol is added, followed by 0.2 ml of 40% KOH. Shake the tube gently to expose the medium to atmospheric oxygen and allow the tube to remain undisturbed for 10 to 15 minutes. A positive test is represented by the development of a red colour 15 minutes or more after the addition of the reagents indicating the presence of diacetyl, the oxidation product of acetoin. The test should not be read after standing for over 1 hour because negative Voges-Proskauer cultures may produce a copper like colour, potentially resulting in a false positive interpretation.

2.7. Coagulase Test

Coagulase test is used to differentiate *Staphylococcus aureus* (positive) from Coagulase Negative *Staphylococcus*. Pick few colonies of Staphylococci from culture in a drop of normal saline on two ends of clean glass slide. Label one as "test" and the other as "control". The control suspension serves to rule out false positives due to auto agglutination. Mix a drop of rabbit or human plasma with the test suspension. Observe agglutination or clumping of cocci. Agglutination within 5-10 seconds is considered as positive. Some strains of *S. aureus* may not produce bound coagulase, and such strains must be identified by tube coagulase test.

2.8. Oxidase

This test was carried out using freshly prepared oxidase reagent. After placing filter paper in a clean petri dish, 2 to 3 drops of oxidase reagent (1% of aqueous solution of tetra methyl phenylene diamine dihydrochloride) was placed on filter paper. With the aid of the sterile glass rod, a colony of each the isolates was picked a smeared on the filter paper in the petri dish. The development of the blue-black colour within few seconds on the filter paper indicated a positive result

3. FERMENTATION TEST

This test is based on identifying if a microorganism can ferment lactose, glucose, galactose, maltose, raffinose and mannitol as a source of carbon. An inoculum of pure culture is transferred aseptically to a sterile tube of 0.1g of Peptone, 0.1g (lactose, glucose, galactose, maltose, raffinose and mannitol) and 0.1g phenol red. The inoculated tube is incubated at 35-37^0c for 24 hour and the results are determined. A positive test is indicated with a colour change

from red to yellow, indicating a pH change to acidic while a negative test is indicated with a colour change from red to magneta or hot pink indicating pH change to base/alkaline.

APPENDIX D

Table 4.5: Isolation of cyanide degrading microbes with mineral salt medium containing 1% cyanide

Microbial Isolates	Day 0	Day 2	Day 4	Day 6	Day 8
			Bacterial Isolates		
Lactobacillus sp.	0.231	0.243	0.248	0.254	0.257
Micrococcus sp.	0.224	0.232	0.233	0.239	0.241
Staphylococcus aureus	0.233	0.244	0.253	0.255	0.259
Bacillus sp.	0.232	0.254	0.276	0.325	0.543
Escherichia coli	0.235	0.236	0.238	0.238	0.241
Klebsiella sp.	0.225	0.227	0.227	0.227	0.227
Pseudomonas sp.	0.235	0.236	0.239	0.282	0.313
Salmonella sp.	0.231	0.233	0.235	0.236	0.236
Corynebacterium sp.	0.235	0.268	0.351	0.375	0.489
			Fungal Isolates		
Aspergillus niger	0.234	0.236	0.279	0.381	0.407
Penicillium sp.	0.231	0.231	0.274	0.332	0.307
Fusarium sp.	0.235	0.238	0.239	0.239	0.261
Saccharomyces sp.	0.233	0.235	0.235	0.239	0.239

APPENDIX E

Table 4.6: Effect of Substrate Concentration

Inoculum size = 6ml of 1.5 x 10^8cfu/ml, pH =6.

Day 0	30ppm	60ppm	90ppm	120ppm	150ppm
Bacillus sp.	29.98	60.14	90.07	120.03	149.98
Pseudomonas sp.	29.96	60.07	89.96	120.05	149.95
Aspergillus niger	29.99	60.09	89.99	120.02	150.04
Control	29.97	60.02	90.03	120.04	149.99

Day 2	30ppm	60ppm	90ppm	120ppm	150ppm
Bacillus sp.	27.65	58.76	90.01	120.01	149.98
Pseudomonas sp.	26.78	58.32	89.92	120	149.95
Aspergillus niger	28.94	59.89	89.98	120.02	150.04
Control	30.01	60.01	90.03	120.04	149.99

Day 4	30ppm	60ppm	90ppm	120ppm	150ppm
Bacillus sp.	25.58	58.46	89.11	112.81	146.98
Pseudomonas sp.	22.13	57.79	86.32	117.60	148.45
Aspergillus niger	26.98	59.56	86.38	114.02	147.04
Control	29.97	59.03	90.01	120.02	150.01

Day 6	30ppm	60ppm	90ppm	120ppm	150ppm
Bacillus sp.	24.05	57.29	87.33	106.04	139.63
Pseudomonas sp.	20.80	54.32	83.73	115.25	146.97
Aspergillus niger	26.17	57.18	85.52	107.18	142.63
Control	29.97	59.03	90.01	120.02	150.01

Day 8	30ppm	60ppm	90ppm	120ppm	150ppm
Bacillus sp.	22.60	55.57	86.45	99.68	127.06
Pseudomonas sp.	20.18	49.43	80.38	112.94	145.50
Aspergillus niger	24.60	56.03	82.10	101.82	138.35
Control	29.98	59.01	89.99	120.01	150.02

Legend: PPM-Parts per million

APPENDIX F

APPENDIX G

Table 4.9: Effect of Inoculum size

pH = 6, Cyanide Concentration = 15ppm

Day 0	2.5ml	3.5ml	4.5ml	5.5ml	6.5ml
Bacillus sp.	15.34	15.42	15.33	15.37	15.44
Pseudomonas sp.	15.28	15.36	15.41	15.38	15.42
Aspergillus niger	15.43	15.31	15.35	15.42	15.38
Control	15.33	15.41	15.42	15.37	15.41

Day 2	2.5ml	3.5ml	4.5ml	5.5ml	6.5ml
Bacillus sp.	13.76	13.24	12.88	11.69	11.05
Pseudomonas sp.	12.93	12.55	10.48	9.97	9.36
Aspergillus niger	14.97	14.16	13.97	13.26	12.98
Control	15.33	15.39	15.4	15.37	15.41

Day 4	2.5ml	3.5ml	4.5ml	5.5ml	6.5ml
Bacillus sp.	12.93	12.84	12.75	10.99	10.06
Pseudomonas sp.	12.54	11.42	10.06	9.77	9.27
Aspergillus niger	14.07	13.88	13.41	12.60	12.59
Control	15.31	15.39	15.4	15.35	15.39

Day 6	2.5ml	3.5ml	4.5ml	5.5ml	6.5ml
Bacillus sp.	12.16	12.59	12.11	10.33	9.55
Pseudomonas sp.	11.79	10.74	9.66	9.58	9.17
Aspergillus niger	13.65	13.32	13.28	11.84	12.21
Control	15.31	15.34	15.38	15.34	15.37

Day 8	2.5ml	3.5ml	4.5ml	5.5ml	6.5ml
Bacillus sp.	11.43	12.21	11.99	9.71	8.69
Pseudomonas sp.	11.44	9.77	9.27	9.38	9.08
Aspergillus niger	12.83	13.06	12.75	11.25	11.85
Control	15.31	15.39	15.38	15.33	15.35

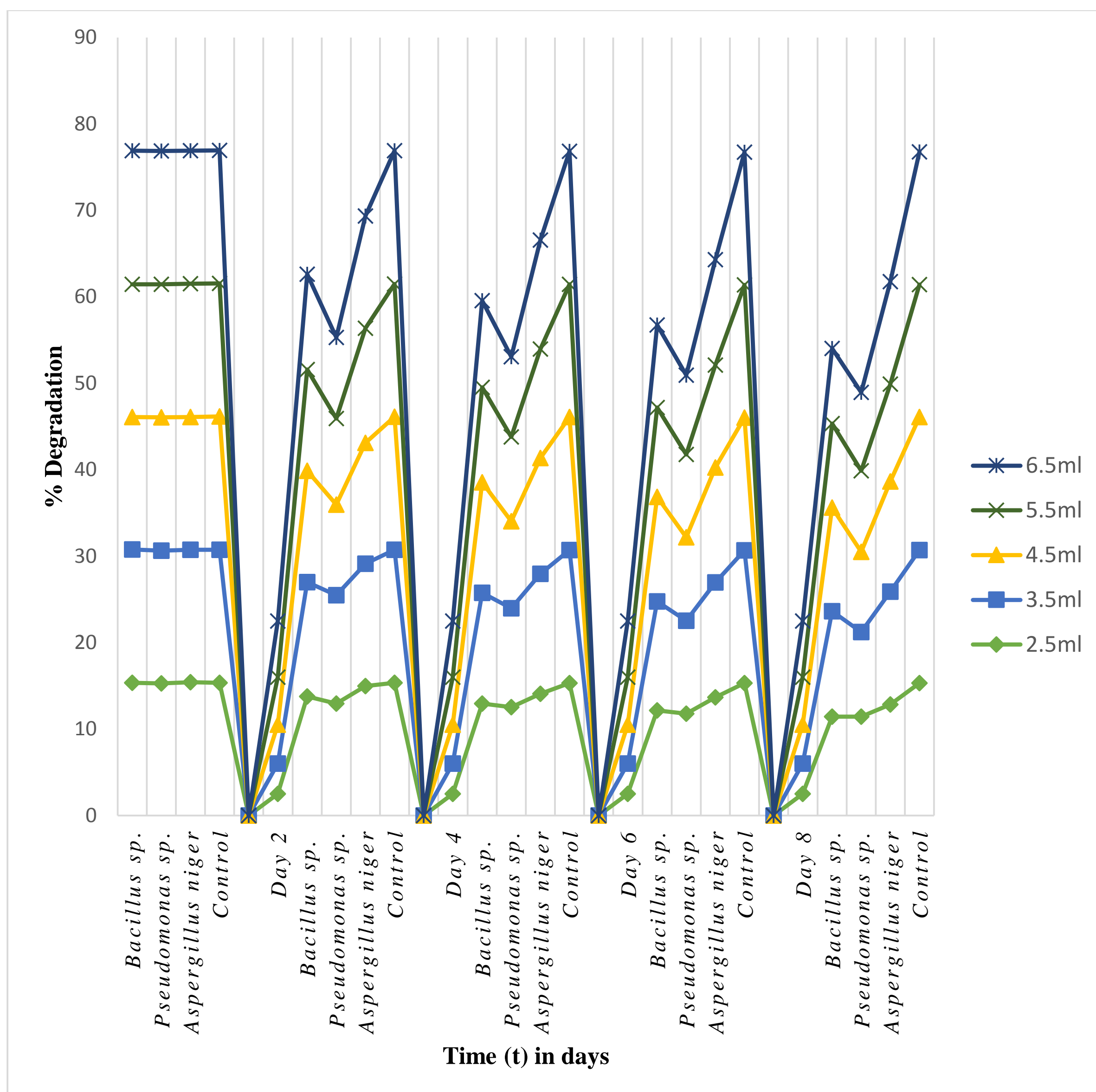

Figure 4.3: Effect of Inoculum size

pH = 6, Cyanide Concentration = 15ppm.