BIOREMEDIATION OF TEXTILE INDUSTRIES EFFLUENTS USING SELECTED BACTERIAL SPECIES IN KANO, NIGERIA

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DEDICATION

This work is dedicated to Allah Subhanahu Wata'ala who gave me the ability to complete this thesis, to my beloved parents Mallama Aishatu Ibrahim and Mallam Galadima A. Shuaibu Dagona, who taught me the best kind of knowledge to have is that which is learned for its own sake, however they also taught me that even the largest task can be accomplished if it is done one step at a time. Finally to my beloved siblings who were always beside me with encouragement and motivation.

DECLARATION

I declare that the work in the Thesis titled **''Bioremediation of textile Industries effluents using selected bacterial species in Kano, Nigeria''** has been performed by me in the Department of Microbiology under the supervision of Drs. S.E. Yakubu and S.A. Ado

The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

_____ _____

GALADIMA Adamu Dagona Date

CERTIFICATION

This thesis titled **''BIOREMEDIATION OF TEXTILE INDUSTRIES EFFLUENTS USING SELECTED BACTERIAL SPECIES IN KANO, NIGERIA''** by GALADIMA Adamu Dagona meets the regulations governing the award of the degree of master's in science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Release of untreated textile effluents, especially into water bodies makes the environment unhealthy, and thereby affecting entire life of humans, plants and other aquatic animals. This eventually affects the quality of water and limits its utilization. This study was carried out to determine the bioremediation potentials of bacteria species isolated from textile effluents of two industrial sites in Kano, Nigeria. Physicochemical characterization of textile effluents collected from Sharada and Chalawa industrial estates was carried out. The results showed high rates of contaminants and heavy metals. Temperature of discharge ranged from 35°C to 37°C and pH of the effluents was slightly above neutral level but within the permissible limits and ranged from 7.15 to 7.2. Using morphological and biochemical characteristics, fifteen (15) bacterial isolates were identified from the effluents sample and out of these, nine (9) of the isolates were selected for further studies based on their ability to degrade textile effluents and grow on minimum basal medium efficiently and rapidly. The biodegradation and decolourisations ability of these isolates were carried for ten days and the results were expressed in percentages with *Bacillus subtilis* having (99.60 %), *Pseudomonas aeruginosa* (99.60 %), *Pseudomonas flourescens* (96.00%), *Bacillus* brevis (95.60 %,), *Alcaligenes faecalis* (95.00%), *Pseudomonas putida* (92.00%) *Bacillus licheniformis* (91.60%), *Aeromonas hydrophila* (90.20%) and *Bacillus megaterium* (89.00%) Three microbial consortia were therefore developed and tested for their effectiveness in the bioremediation: Consortia 1 comprised of *Pseudomonas aeruginosa, P. putida,* and *Bacillus subtilis* and was able to degrade 99.70% of textile effluent within ten days, consortia 2 comprised of *Pseudomonas aeruginosa, P. putida, Bacillus subtilis* and *P. fluorescence* degraded by 96% and consortia 3 comprised of *Pseudomonas aeruginosa, P. putida, Bacillus subtilis, Pseudomonas fluorescence* and *Alcaligenes faecalis* degraded by 92%. Reduction efficiencies of different contaminants were evaluated and compared. Results indicated effectiveness of the investigated species for removal of the target contaminants ranged from 48% to 71% while, removal of heavy metal ranged from 50% to 99% was established. Analysis of variance of the results revealed that, there was statistically significant differences ($p \leq 0.05$) in pH and DO after bioremediation. while there was no statistical significant difference in the reduction efficacy of BOD , COD, TSS, TDS, EC, however, adsorption of heavy metals (Cr, Cd, Cu, Fe, Mn, Ni, Pb, and Zn) showed significant difference ($p \leq 0.05$), between individual organisms and the consortia after bioremediation . The selected bacterial species represent a promising tool for application in bioremediation of textile industrial effluents and the biodegradation potential observed would increase the applicability of these microorganisms for treatment of textile effluents before disposal to appropriate channel.

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LIST OF ABBREVIATION

pH	Negative logarithm to base 10 of hydrogen ion concentration
COD	Chemical Oxygen Demand
BOD	Biochemical Oxygen Demand
DO	Dissolved Oxygen
TEXtEL	Textile Effluent
NESREA	National Environmental standard Regulation Agency
ATSDR	Agency for Toxic Substances and Disease Registry
WHO	World Health Organisation
MBM	Minimum Basal Medium
FEPA	Federal Environmental Protection Agency
USEPA	United State Environmental Protection Agency
EPA	Environmental Protection Agency
ANOVA	Analysis of variance
TSS	Total dissolved solids
TDS	Total suspended solids
EC	Electrical Conductivity
CTIA	Consortia
NSA	National Academy of Science

CHAPTER ONE

INTRODUCTION

The continuing industrial development has led to a corresponding increase in the amount of wastewater generation leading to a consequential decline in levels and quality of the natural water in the ecosystem. Textile industries consume over 7 x 10^5 tons of dyes annually and use up to 1 litre of water per kg of dye processed and are one of the largest pollutants of the environment (Mutambanengwe *et al.,* 2007). However, there is increasing concern on the impact in effective treatment of textile effluents as they introduce secondary pollutants during the remediation process which is quite costly to run, maintain, and clean up. Research on biological treatment has offered simple and cost effective ways of bioremediation of textile effluent. Microbial decolourisation and degradation is an environmentally friendly and cost-competitive alternative to chemical decomposition processes (Verma and Madamwar, 2003).

Textile industries produce considerable amounts of effluent characterized by large amounts of suspended solids, high COD, fluctuating pH, high temperature, and a mixture of dyes (Robinson *et al*., 2001). Untreated textile wastewater can cause rapid exhaustion of dissolved oxygen if it is directly discharged into the surface water sources hence they are toxic to biological life. The high alkalinity and traces of chromium, where it was employed in dyes, adversely affect the aquatic life as well as interfere with the biological treatment process (Babu *et al*., 2000; Robinson *et al*., 2001; Zaharah *et al.*, 2004).

Heavy metals beyond permissible limits cause direct toxicity to all living beings. Metallic effluents can have ecological impacts on water bodies leading to increased nutrient load especially if they are essential metals. Heavy metals such as Zinc, lead, nickel, cadmium and chromium can bio-accumulate through the food chain to toxic level in man.

These metals in effluent may increase fertility of the sediment and water column and consequently lead to eutrophication, which in open water can progressively lead to oxygen deficiency, algal bloom and death of aquatic life (Ashoka *et al.,* 2000). There are physical and chemical methods, which in spite of costs, do not always ensure that the contaminants are completely removed (Hardman *et al*., 1993).

Bioremediation is the use of organisms to break down and thereby detoxify dangerous chemicals in the environment; it employs both plants and microorganisms. The presence of dyes in the effluent poses a biggest problem since they are recalcitrant and toxic. A very small amount of dye can be visible in water, thus decreasing the transparency of the water which leads to inhibition of sunlight penetration and consequently photosynthesis. Both aerobic and anaerobic processes have been successfully used for degrading the textile effluent, but the best appears to be a combination of both. Most studies on metabolism of organic contaminants have been performed with bacteria especially in context of bioremediation (Glazer, 1997). Bacteria generally are easier to culture and they grow more rapidly than fungi. They are more amenable to molecular genetic manipulation. Bacteria such as *Pseudomonas* and *Bacillus* have been shown to degrade the azo- or reactive dyes from textile industry effluent in a process often referred to as bioleaching (Ashoka *et al*., 2000). Of all the technologies that have been investigated, bioremediation has emerged as the most desirable approach for cleaning up many environmental pollutants (Lovely, 2003).

1.1 Statements of Research problem

Hazardous and harmful waste is constantly produced by textile industries all over the world. Environmental pollution arising from activities of chemicals in textile industries remains a burden in Nigeria due to inappropriate treatment of effluent and lack of environmental awareness. In many Nigerian cities like Kano, Kaduna, Lagos and Port Harcourt, the textile factories daily discharge million of litres of untreated effluents in the

form of wastewater into public drains that eventually empty into rivers (Olayinka and Alo, 2004).

Enormous volumes of effluents are generated at different stages of textile manufacturing, as a result of the use of copious amount of chemicals and dye. Technological advance has increase in diversity and complexity of synthesized textile dyes. A large proportion of these are azo dyes and can pass through normal water treatment system resulting in aesthetically unappealing water (Stolz, 2001; Pearce *et al.*, 2003; Pandey *et al.*, 2007). Industrialization in Nigeria has partially turned the environment into dumping site for wastewater. Water pollution by textile effluents affects man and aquatic ecosystem directly and this is due to large variability of the composition of textile wastewaters and chemicals, thus the need for such a research.

1.2 Justification

Environmental pollution has been recognized as one of the major concern of the modern world. The increasing demand of water and dwindling supply has made the treatment and reuse of industrial effluents an attractive option. Untreated industrial effluents diminish the water quality. The ability of microorganisms to degrade and metabolize a wide variety of compounds has been recognized and exploited in various bio treatment processes (Khehra *et al.,* 2005). Synthetic dyes are extensively used in wide range of industries amongst which textile processing industries are the major users. Almost all synthetic dyes used in textile industries are resistant to conventional wastewater treatment method (Robinson *et al.*, 2001). Microbiological treatment methods are attractive due to their cost effectiveness, and diverse metabolic pathways and versatility of microorganisms involved (Singh *et al*., 2004; Pandey *et al*., 2007).

1.3 Aim

The aim of this study is to determine the bioremediation potentials of bacteria isolated from Textile Industry effluents.

1.4 Specific Objectives

1. To determine the physico-chemical characteristics of the textile effluents.
2. To isolate and characterize selected bacteria from textile effluent samples.
3. To determine the bioremediation potentials of the selected bacteria isolated from textile industries.
4. To carry out biodegradation of textile effluent using selected bacteria spp and a combination of such bacteria.

CHAPTER TWO

LITERATURE REVIEW

2.1 Industrial Wastes and Pollution

Rapid population growth in Sub- Saharan Africa has brought about a tremendous increase in urbanization with attendant increase in the volume of domestic and industrial wastewater. Water pollution is primarily associated with domestic and industrial waste; both types of wastewater pose threats to water quality which may be classified into health hazards and sanitary nuisances. In most parts of Sub-Saharan Africa, people have no access to potable water. World Bank reported that about 19,000 tons of hazardous waste is produced annually in Nigeria and the waste comes mainly from steel, metal processing, pharmaceuticals, textiles, tanneries and oil refining industries (WHO, 2002). They are one of the largest water users and polluters (Babu et al., 2007). The public welfare concern over the effect of environmental pollution has increased substantially since the Industrial Revolution, mainly as a consequence of an enhanced understanding of the risk to human health. Much concern has concentrated on the visible effects of pollution, but the hidden effects are also of great importance. Wastewater treatment is not given the necessary priority it deserves and therefore, industrial waste and domestic sewage are discharged into receiving water bodies without proper treatment (Gasim et al., 2006). The situation is compounded by the fact that the common man in most of these countries does not have access to potable water, Water quality deterioration due to industrial effluent and municipal sewage discharge has been documented

in literatures (Babu *et al,*. 2000;Verman and Madamwar, 2003; Bhatt *et al.,* 2003 and, Prasad *et al.*, 2010).

Heavy Metals contained in the effluents (either in free form in the effluents or adsorbed in the suspended solids) from the industries have been found to be carcinogenic, (FEPA, 1991). A Study on water quality of River Ogun. Nigeria), in which industrial effluent from Lagos and Abeokuta is discharged, reported that the level of turbidity, TSS, COD and iron was very high in all the sampling sites (Akan *et al.,* 2009). Wynne *et al.* (2001); Jaji *et al.* (2007) and Akan *et al.*, (2009), in their findings noted that BOD and COD are above the discharge limit throughout in textile industries studied.

According to Bhatt *et al.* (2000), ‘’Dye in wastewater discharged from textile and dyestuff industries have to be treated due to their impact on water bodies and growing public concern over their toxicity and carcinogenicity. Thus, colour elimination in wastewater is today the principle problem concerning the textile industries, since it is the first contaminant recognized in textile wastewater and has to be removed before discharging into receiving water body (Robinson *et al.*, 2001).

All dyes used in the textile industry are designed to resist fading upon exposure to sweat, light, water, many chemicals including oxidizing agents, and microbial attack. During processing, up to 15% of the used dyestuff is released into the process water (Bhatt *et al.,* 2000). Many researches on the biodegradation capability of microorganisms especially bacteria and fungi have been reported and reviewed (Robinson *et al.*, 2001). To date, although research on biodegradation of reactive azo dyes by microbial consortia have been established internationally, limited studies on the decolourising capability of bacterial strains isolated locally have been reported and their potential in wastewater treatment

2.2 Textile industries

The textile industry is characterized by the large quantity of water consumption, and the varieties of chemicals used. Generally, there are a number of wet processes involved with high requirements for resource inputs, generating several types of wastewater (Babu *et al*., 2000). Other important features of this industry at unit level are high fluctuations in flow rates and waste concentration due to the factors of production size and process cycles. Liquid waste tends to dominate over air emissions and solid wastes in terms of severity of environmental impacts. Liquid waste arising from various steps of operations contains substantial pollution load in terms of organic matter and suspended matter. Chemicals may also adhere to these suspended particles. Wastewater is generally hot and alkaline, with a strong smell and colour due to the consumption of a variety of dyes and other chemicals in the dyeing processes (Robinson *et al*., 2001). Discharge of such effluents into aquatic bodies can cause lowering of dissolved oxygen, threatening aquatic life and downstream water users.

According to Robinson *et al.* (2001), Because of the high BOD, the untreated textile wastewater can cause rapid depletion of dissolved oxygen if it is directly discharged into the surface water sources. Therefore the effluents with high COD level are toxic to biological life.. The most studied step in textile processing regarding the treatment of the effluent is the dyeing step. Dyeing causes an easy recognition of pollution via colour. The most widely dyes use in most textile industries are: Procion Blue HERD (RB-160) azo dyes is a Copper complex, Remazol Violet 5R, (RV-5R) Vinylsul phonyl, monaazo and Remozol Brown GR (RBR-18) Diazo is a metal complex (Omar, 2009).

2.3 Textile effluent

Effluents from textile industries contain different types of dyes, which because of high molecular weight and complex chemical structures, show low level of biodegradability (Olayinka and Alo, 2004). Hence, direct deposition of these effluents into sewage networks, produce disturbances in biological treatment processes (Babu *et al.*, 2000). On the other hand, these types of effluents produce high concentrations of inorganic salts, acids and bases in biological reactors leading to the increase of treatment costs. Dyes are mostly stable in light and heat. Also, application of technologies which give them more stability in the environment The classic and conventional treatment methods for these types of effluents are based on chemical precipitation, activated sludge, chlorination and adsorption on activated carbon. In 1979, a study was done on the adsorption of dyes on waste textile fibers. Results were not satisfactory for all types of dyes; besides, separation of fibers from the effluent was not economically feasible (Babu *et al*., 2000).

2.4 Biodegradation of textile effluents

The understanding of microbial degradation and decolourisations of a dye is limited. However, results indicate that maximum dyes adopt reductive process of degradation. *Bacillus* spp, *Alcaligenes* spp, *Acinetobacter* spp are a few important bacteria useful in bioremediation of halogenated aromatic compounds and textile effluents (Olayinka and Alo, 2004).

Biodegradability of a compound is generally high if the compound occurs naturally in the environment, often compounds with a high molecular weight particularly those with complex ring structures and halogen substituent degrade more slowly than complex straight chain hydrocarbons or low molecular weight compounds (Olayinka and Alo, 2004). Whether synthetic compounds are metabolized by microorganisms is largely determined by the

structural features of the compound similar to naturally occurring compounds. The rate and extent to which the compound is metabolized in the environment is often determined by the availability of electron acceptors and other nutrients (Chen, 2002). Moreover decolourisation and degradation can also detoxify the effluent effectively without leaving any residues. In recent years, considerable interest has been generated in studying microbial azo dye degradation (Robinson *et al*., 2001). Environmental biotechnology relies upon the pollutant degrading capability of naturally occurring microbial consortium in which bacteria plays a central role. Wynne *et al.* (2001) and Stolz *et al.* (2001) noted that textile effluents are highly coloured and saline, contain non-biodegradable compounds, and are high in (BOD, COD). A combination of *P. aeruginosa, A. faecalis* and *P. putida* C15 was found capable of degrading all the dyes most efficiently compared to the other consortia (Omar *et al*., 2009). A temperature of about 29°C - 30°C and pH of 7.2 was reported for optimal degradation and decourisation of azo dyes Robinson *et al*., 2001) Previous studies indicates that pH of 7.00 was found to be most suitable for maximum decolourisation of dye effluent (Verma and Madamwar, 2002).

2.5.0 Physico-chemical parameters

The fate and transport of many anthropogenic pollutants are determined by not only hydrological cycles, but also physicochemical processes (Bhatt *et al*., 2000). Several works on water quality have focused on the physicochemical characteristics of waters. Growing populations may put stresses on natural waters by impairing both the quality of the water and the hydrological budget (Bhatt *et al*., 2000). The quality of given water body is governed by physical, chemical and biological processes, all of which inter play with one another and greatly influence productivity in water bodies. There is a great deal of investigations about fresh water quality (Gasim *et al*., 2006). Textile effluents are characterized by extreme fluctuations in many parameters such as Chemical oxygen demand, dissolve oxygen,

biological oxygen demand, pH and temperature (FEPA, 1991; Yusuf and Sonibare, 2004; Orisikwe 2009).

2.5.1 Temperature

Temperature is an important water quality parameter and is relatively easy to measure. Water bodies will naturally show changes in temperature seasonally and daily; however, all organisms have preferred temperature in which they can survive and reproduce optimally (WHO, 2006). Water temperature varies with season, elevation, geographic location, and climatic conditions and is influenced by streamside vegetation, groundwater inputs, and water effluent from industrial activities. Temperature of water has an extremely ecological consequence, which exerts a major influence on aquatic organisms with respect to selection/occurrence and level of activity of the organism. In polluted water, temperature can have profound effects on DO and BOD (FEPA, 1991). The effluent temperature was above the discharge limit of 45 ^{o}C in 23% of the samples collected (ATSDR, 2005**)**. Many aquatic organisms are sensitive to changes in water temperature. According to WHO (2006), Environmental policies require the monitoring of temperature in most urban and industrial locations, environmental permits are required to help minimize the temperature loading to water bodies.

2.5.2 pH

The pH of natural water can provide important information about many chemical and biological processes and provides indirect correlations to a number of different impairments. As the pH deviates from its normal range, it affects activities in biological processes, such as reproduction, cannot occur in acidic or alkaline waters. The pH and its changes may affect biological activities and bring about changes in the natural chemistry of water as well as pollution (Wagner *et al.*, 2002). The pH less than 6.5 or greater than 9.5 could markedly

impair the potability and Water pH is crucial for living organisms, biochemical processes and industrial water use (WHO, 2006). The pH is the indicator of the existence of biological life as most of them thrive in quite narrow and critical pH range (WHO, 2004). And also Low water pH accelerates heavy metals being washed away from sediments which are highly acidic and corrosive in water. The pH is typically monitored for assessments of aquatic ecosystem health, recreational waters, irrigation sources and discharges, livestock, drinking water sources, industrial discharges, intakes, and storm water runoffs.

2.5.3 Electrical Conductivity (EC)

Conductivity is the ability of a substance to conduct electricity. The conductivity of water is a more-or-less linear function of the concentration of dissolved ions. Conductivity itself is not a human or aquatic health concern, but because it is easily measured, it can serve as an indicator of other water quality problems. If the conductivity of a stream suddenly increases, it indicates that there is a source of dissolved ions in the vicinity. Therefore, conductivity measurements can be used as a quick way to locate potential water quality problems. Most freshwater sources will range from 0.001 to 0.1 µS/cm. The source of EC may be an abundance of dissolved salts due to poor irrigation management, minerals from rain water, runoff, or other discharges from industries. Conductivity ranges of four sample areas in Kano State, parameter varies but not very emphatic, so the values are from 169 to 264 µS/cm. The lower value 169µS/cm was recorded (Dan'azumi and Bichi, 2010) while a higher value of 1341 mg/l was reported from Kaduna refinery (Usman *et al.,* 2011).

2.5.4 Total dissolved solids (TDS)

Waters with high total dissolved solids (TDS) are unpalatable and potentially unhealthy. Water treatment plants use flocculants to aggregate suspended and dissolved solids into particles large enough to settle out of the water column in settling tanks. TDS on

the other hand is equally important in water quality studies, though there was no serious health effect associated with TDS ingestion in water but some regulatory agencies (FEPA, 1991; NAFDAC, 2001) recommended a maximum TDS value of 500mg/l in drinking water supplies. Total Dissolve Solid concentration of 2200 mg/l in effluents has been reported by Jiunkins (1982) while Wesenberg (2003) reported a value of 2700 mg/l. High TDS is one of the major sources of sediment which reduce the light penetration into water and ultimately decrease the photosynthesis. According to WHO (2006), the palatability of water with TDS Level of less than 600.00 mg/l is generally considered good; drinking water becomes significantly and increasingly unpalatably at TDS Levels greater than about 1000.00mg/l. Therefore, a guideline value of 1000.00mg/l was established for TDS based on taste consideration (WHO, 2006).

2.5.5 Total suspended solids (TSS)

Total suspended solids (TSS), include all the particles suspended in water which will not pass through a filter. Suspended solids are present in natural water (WHO, 2004), sanitary wastewater, and many types of industrial wastewaters. It is observed that a suspended solid absorb heat from sunlight, causing increase in water temperature and subsequently decreases level of dissolved oxygen. Total suspended solids were extremely high up to 15,343.2 mg/l at two different sampled sites in one textile industry in Kaduna (Akan *et al.,* 2009). Total suspended solid levels were above the discharge limit of 2000 mg/l in 50% of the samples collected from Pakistan (Kumar, 2005). TSS levels in excess of 1000 mg/l were noted in 18% of the samples collected (Wesenberg, 2003). Some aquatic species are sensitive to prolonged exposure to TSS and thus, monitoring of TSS is an important criterion for assessing the quality of water (Akan *et al.,* 2009).

2.5.6 Dissolved Oxygen (DO)

The most important measure of water quality is the dissolved oxygen (Pearce *et al*., 2003). Dissolved oxygen (DO) is essential to all forms of aquatic life including the organisms that break down man-made pollutants. Oxygen is soluble in water and the oxygen that is dissolved in water will equilibrate with the oxygen in atmosphere (Akan *et al.,* 2009).

The DO of wastewater at sea level will range from 11 mg/l at 0^{o} C to 8mg/l at 25^{o} C. Concentrations of unpolluted fresh water will be close to 10 mg/l. In general, the concentration of dissolved oxygen will be the result of biological activity. The amount of free (not chemically combined) oxygen dissolved in water, wastewater, or other liquid is usually expressed in milligrams per litre, parts per million, or percent of saturation (Gasim *et al*., 2006).

2.5.7 Chemical Oxygen Demand (COD)

The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/l, required for degradation of the compound of wastewater to occur. The higher the COD value of wastewater, the more oxygen demand to discharge water bodies. Chemical oxygen demand Value of textile effluents was found to be in the ranges from 220–490 mg/l and 180–940 mg/l. The COD levels were 4500 mg/l and generally above the discharge limit of 2000 mg/l (Akan *et al*., 2009). Kumar (2005) reported that a high COD value show that the effluents have highly oxygen demanding wastes which cause the depletion of DO which is a fundamental requirement for aquatic life. The mean COD concentration of effluent in all the industries investigated range from 133.50 to 2399.00, in all the seasons sampled (Akan *et al.*, 2009). These values were higher than WHO and USEPA standard of 1000 mg/l for discharged of tanneries and textile effluent into surface water. Moreover, high COD produce

unaesthetic colour, endanger water supplies and decrease recreational value of water ways (Kumar, 2005)

2.5.8 Biochemical oxygen demand (BOD)

Biological oxygen demand (BOD) measures the amount of oxygen required by bacteria to break down to simpler substances, the decomposable organic matter present in any Wastewater or treated effluent (WHO, 2002).The high levels of BOD are indications of the pollution strength of the wastewaters. They also indicate that there could be low oxygen available for living organisms in the wastewater when utilizing the organic matter present. Biochemical Oxygen Demand was high by about 2-5 folds in two textile mill in Pakistan, (Nergis *et al.,* 2009). Industrial textile wastewater presents the additional complexity of dealing with unknown quantities and varieties of many kinds of dyes, as well as low BOD ratios, which may affect the efficiency of the biological decolourisation (Babu *et al*., 2000). Biochemical oxygen demand can also be used for evaluation of the efficiency of treatment processes, and it is an indirect measure of biodegradable organic compound in water, (FEPA, 2003). High BOD is often accompanied by low Dissolve oxygen (Gasim *et al*., 2006). Biochemical oxygen demand of the untreated textile wastewater can cause rapid depletion of dissolved oxygen if it is directly discharged into the surface water sources (Babu *et al.,* 2000).

2.6.0 Heavy metal and chemicals in the effluents

Although industrialization is inevitable, various devastating ecological and human disasters which have continuously occurred over the years implicate industries as major contributor to environmental degradation and pollution processes of various magnitudes (Dan'azumi and Bichi, 2010). Industrial wastes and emissions contain toxic and hazardous substance most of which can be detrimental to human health. These are: lead, chromium and mercury, (Dan'azumi and Bichi *et al*., 2010).

Several physicochemical methods have been widely used for removal of heavy metals from Industrial wastewater, from ion exchange, activated charcoal, chemical precipitation, chemical reduction and adsorption. The conventional methods used for the treatment of heavy metals from industrial wastewater present some limitations. There are still some common problems associated with these methods such as: they are cost- expensive and can themselves produce other waste problems, which has limited their industrial applications (Rengaraj *et al.,* 2001).

The application of microorganisms for the remediation of heavy metals in water is a recent field of research in environmental engineering. Some microorganisms have been identified to possess heavy metals removal capability from contaminated water. The dominant pollutants degraders in biofilters are bacteria and fungi. These simple organisms are capable of utilizing the substrate rapidly (Rengaraj *et al.,* 2001). Industrial effluents which are discharged from the textile industry contain a higher amount of metals especially chromium, cupper and cadmium (Rengaraj *et al.,* 2001). These effluents released on land as well as dumped in to the surface water which ultimately leaches to ground water can lead to contamination due to accumulation of toxic metallic components and result in a series of well documented problems in living beings because they cannot be completely degraded (Babu *et al.*, 2007). Industrial effluents offer a wide range of environmental problems, hence becoming more complex and critical. All metals in effluents cause serious health hazards due to unsafe disposal on soil and in water (Rengaraj *et al.,* 2001).

2.6.1 Cadmium

Cadmium content of textile industry effluent was reported to be 0.02 mg/l, in dye textile effluent (Dubey *et al.*, 2003). In another study, average value of cadmium was found to be 0.04 mg/l lower as compared to the value of (0.076 mg/l) observed in Bompai industrial

area, Nigeria while lower than 1.05 mg/l observed in Peshawar, Pakistan for textile effluents the maximum concentration in water is 0.5 mg/l (Lee *et al.*, 2002). Cadmium is a non-essential trace element that enters the environment via anthropogenic activities such as industrial effluent, sewage-sludge, fertilizers and pesticides. Cadmium adsorbs strongly to sediments and organic matter (Dos Santos, 2006). Cadmium has a range of negative physiological effects on organism, such as decreased growth rates and negative effects on embryonic development and children are likely to be exposed to cadmium when is highly toxic and absorbed in skin. And can cause long damage and irritation with shortness of breath dry throat, headache, vomiting, extreme restlessness or irritability etc (Lee *et al.*, 2002). Other potential long-term effects are lung damage and fragile bones (ATSDR, 2005).Cadmium can still be assimilated from anoxic sediments with high organic matter content (Chong and Wong, 2000). The maximum concentration of cadmium in water was 0.5 mg/l (WHO, 2006).

2.6.2 Copper

Copper is reddish metal that occurs naturally in rocks, soil water, industrial activities and sediment and has some practical uses in our society and are found in pipes, electrical wiring and coins. Copper was reported textile industry in Lagos in higher than the concentrations range from 4.0 mg/l to 5.14 mg/l, (Yusuf and Sonibare, 2004). The level of copper in the wastewater and sediment samples was above the WHO standard value of 0.5 ml/ to 1.00 mg/l for the survivors of aquatic organism (WHO, 2004). Copper is generally remobilized with acid-base ion exchange or oxidation mechanism (Orisikwe, 2009). Copper is an essential element for living organism, includes humans, and in small amounts necessary to our diet to ensure good health. However too much copper can cause adverse effect on health of living beings, effects include vomiting, diarrhoea, stomach cramps, and nausea. It

has also associated with liver damage and kidney disease. Maximum allowable concentration in drinking water was < 1.0 mg/l (FEPA, 1991).

2.6.3 Chromium

Chromium was found to be 1.202 mg/l higher than the value of 0.255 mg/l reported from textile industries effluent in Lagos metropolis, Nigeria (Ugoji and Aboaba, 2004). Effluents of textile industries were found to contain the average concentration of 1.70 and 0.75 mg/l (Yusuf and Sonibare, 2004). While in a similar study 0.45 to 2.14 mg/l of chromium was reported in textile industry effluent in Nigeria and in study chromium was reported rang of 0.5 to 1.57 mg/l (FEPA, 1999). Chromium can cause allergic reactions in the skin, damage the lungs, and asthma attacks maximum concentration of 0.1 mg/l was set up (ATSDR, 2005).

2.6.4 Iron

Textile effluent sample contain a concentration of iron released from India was said to 2.03 mg/l to 2.5 mg/l (Tariq *et al.,* 2006). Another study reveals that 0.351 mg/l irons was discharged from textile industry in Kano, and tend to increased significantly in rainy season to about 1.539 mg/l (Dan`Azumi 2010). Higher iron content may produce undesirable effects such as astringent taste, colouration, turbidity, deposits, and growth of iron bacteria in pipes affecting the acceptability of water for domestic use iron is essential element in human nutrition, and health effect of iron in drinking water may include warding off fatigue and anaemia (Kaushik, 2003). Iron cause conjunctivitis, choroidities and retinitis if it contacts with remain of tissue and concentration of iron oxide may enhance the risk of lung cancer. The maximum concentration of iron in drinking water was 0.5 mg/l to 10 mg/l (WHO, 2006).

2.6.5 Manganese

The concentration of manganese was reported as 0.988 mg/l for textile effluent. Depending upon the exposure route, manganese may be among the least toxic of the trace elements if ingested low IQ of children is attributed to high manganese intake and hence at high concentration lead to neurotoxins and has adverse effect on the brain (USEPA, 1999). Bremner, (1998) reported 1.75 mg/l concentration of manganese from textile industry effluent. In another study, higher value of 1.65 mg/l of manganese was reported by some workers in effluent from textile industry in Nigeria, while (1.02 mg/l) was reported by Yusuf and Sonibare (2004). However, it exceeded the USEPA maximum concentration of 0.5 mg/l for water samples.

2.6.6 Nickel

Background concentrations of nickel are usually quoted as being less than 50 mg/l Water levels range from 5 to 4 mg/l, with levels above 8 mg/l being indicative of contamination. The most obvious anthropogenic source of nickel is scrap metal waste, notably alloyed metals including stainless steel. Nickel is considered an essential trace element at very low concentrations. It does bio accumulate in aquatic systems, and as such elevations above normal concentrations can result in deleterious aquatic effects (ATSDR, 2005). The most common adverse health effect of nickel in humans is an allergic reaction. People can become sensitive to nickel when jewellery or other materials containing nickel are in direct contact with the skin. The International Agency for Research on Cancer (IARC) has determined that some nickel compounds are carcinogenic to humans (WHO, 2006).

2.6.7 Lead

Excess quantities of lead may impact human health, especially affecting small children (Orisikwe, 2009). Therefore a maximum allowable concentration of lead was been set at 0.05 mg/l of lead in drinking water. According to WHO (2004), average value of lead was 0.06 mg/l in textile effluent. However, 0.646 mg/l and 0.289 mg/l of lead were reported in wastewater samples of textile industry in Kaduna (Kaushik, 2003; Tariq *et al.,* 2006). Dubey, *et al.,* (2003) reported low value of lead range from 0.18 mg/l to 0.59 mg/l from effluent in one textile industry in India. In excess concentration, the negative effect of lead is considering the number one health threat to children, and the effects of lead poisoning can last a life time. Not only does lead poisoning stunt a child's growth, lead affect central nervous system, particularly in children and also damages kidneys and the immune system. The roles of lead pipes in solvency aluminium and dialysis

2.6.8 Zinc

Zinc occurs in natural in air, water and soil, but zinc concentration is rising unnaturally, due to addition of zinc through human activities. Most zinc is added during industrial activities, such as mining, coal and waste or sewage sludge from industrial areas. The levels of Zinc in the water and sediment samples contaminated by textile effluent were found to exceed the WHO guideline value of 3.00 mg/l and 6.00 mg/l (Babu *et al*., 2007). Effluents samples had zinc levels range between 0.07-5.14 mg/l as against lower set limit of 1.0 mg/l. High concentration of 1.57 mg/l and 1.07 mg/l was reported by (Akan *et al.,* 2009), similarly, Zn was the most abundant metal in the area which ranged from 0.264 to 0.947 mg/l for one textile industry studied in Pakistan **(**Kaushik, 2003; Tariq *et al.,* 2006). Zinc is a trace element that is essential for human health; the danger can be to unborn child when mothers absorbed large concentration of zinc and other health problem such as stomach cramps, skin

irritation, vomiting and anaemia (WHO, 2006).The maximum allowable concentration of zinc was < 1.0 mg/l (USEPA, 2005).

2.7.0 Bioremediation

Bioremediation is natural process of treatment of pollutants or waste (as in oil spill or industrial effluent, contaminated ground water, or an industrial process) by the use of microorganisms as bacteria and fungi that break down the undesirable substance (Dubey *et al* 2003). Another authority defined bioremediation as use the living systems or biological products to biodegrade anthropogenic and the objective being reduction of waste, and toxic chemical can be accumulated into natural cycles Bonaventura *et al.,* 1998). This eventually leads to actual reduction and degradation of wastes to CO2 and water, when there is organic compound (Bonaventura *et al.,* 1998). The aim of bioremediation is to biotransform toxic material into non toxic ones and makes accumulating anthropogenic waste enter natural bio-geo-chemical cycle more efficient and harmless. (Dubey *et al*., 2003). Thus, Bioremediation is a branch of biotechnology which deals with the method of solving and neutralizing environmental problems (Chen, 2002). It plays vital role in cleaning pollutants by using microorganisms. Bacteria are important microbes in this process because they breakdown the dead materials into organic matter and nutrients (Chen, 2003). Cycling and self-remediation are natural functions of life; which makes a great deal of sense for us to examine closely the natural biological processes in an effort to learn sound remediation techniques with which to ameliorate environmental damage (Dubey *et al.,* 2003)**.** Researchers are working towards understanding the biological background of bioremediation, its technical methods and Opportunity to improve the degradation by genetically engineered microorganisms (Babu *et al*., 2000).

All things in nature ultimately succumb to decay; much of this is natural consequence of the laws of thermodynamic **(**Dos Santos, 2006). Bioremediation/degradation of effluents is a natural process limited mainly by temperature, pH and scarcity of nutrient such as nitrogen, phosphate and oxygen. Microorganisms (primarily bacteria and fungi) are nature's original recyclers. Their capability to transform natural and synthetic chemicals into source of energy and raw materials for their own growth suggest that expensive chemical or physical remediation processes might be replaced with biological Processes (Sasikumar and Papinazath**,** 2003)

Bioremediation is reported to be most preferred and desirable, feasible way to accelerate or encourage the degradation of pollutant **(**Babu *et al.,* 2000; Gomez *et al.,* 200; Dubey *et al.,* 2003 and Dos Santos, 2006). Bioremediation does not involve only the degradation of pollutants; it can also be used to clean unwanted substances from water, air and soil (Gomez *et al*., 2000). Bioremediation is a process of environmental improvement in which organisms play a key role. Organisms adapt to their surrounding conditions over time, many microorganisms develop a way to use certain environmental pollutants as food sources and finally to obtain energy (Dubey *et al*., 2003). Effort to isolate bacterial culture capable of degrading azo dyes started in 1970s with report of a *Bacillus subtilis* (Gomez *et al.,* 2000). *Pseudomonas* species was isolated from aerobic dyeing house wastewater treatment facility as the most active degrader (Young and Juan, 2001).

2.7.1 Principle of bioremediation

The key players in bioremediation are bacteria—microscopic organisms that live virtually everywhere. Microorganisms are ideally suited to the task of contaminant destruction because they possess enzymes that allow them to use environmental contaminants as food and because they are so small that they are able to contact contaminants easily. In situ

bioremediation can be regarded as an extension of the purpose that microorganisms have served in nature for billions of years: the breakdown of complex human, animal, and plant wastes so that life can continue from one generation to the next. Without the activity of microorganisms, the earth would literally be buried in wastes, and the nutrients necessary for the continuation of life would be locked up in detritus (N.A.S, 2000).

The basic principle of bioremediation is the breakdown of organic contaminants into simple compounds like CO_2, water, salts and other harmless products. The capacity could be improved by applying the genetically modified microbes and plants. Bioremediation addresses most often the use of biological techniques to clean up pollution. A critical underpinning of this process is the ability to economically generate a sufficient biomass of the appropriate microbes to accomplish in weeks or months what would normally take nature years to do (Chen *et al.,* 2003). Typically, this is done either by applying a sufficient concentration of such microbes directly to the polluted area or by applying various concentrations of chemicals which in turn stimulate and foster the rapid growth of appropriate microorganisms.

Bioremediation is based on the idea that all organisms remove substances from the environment to carry out growth and metabolism (Zissi *et al.,* 2001). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (USEPA 2005). It is important to recognize that biological processes are dynamic given current knowledge, often lack the predictability of more conventional remediation technologies (Sasikumar and Papinazath**,** 2003). Thus, it is important to ensure that unacceptable risks do not develop in the future. These risks may include migration of contaminants to previously uncontaminated media and the failure of bioremediation to achieve acceptable contaminant concentrations.

2.7.2 Bacterial bioremediation

The wastewater management strategy for the future should meet the benefits of humanity is safety, respect principles of ecology, and compatibility with other habitability systems (Yang *et al*., 2003; Moosvi *et al*., 2005). For these purposes the wastewater management technologies relevant to application of the biodegradation properties of bacteria are of great interest. The selection of bacterial spp for the biological treatment depends upon the chemical composition of the dye effluent and the alkalis and salts used in the dyeing methods (Babu *et al*., 2000).

An NADH-dependant azoreductase of the strain *Bacillus* spp strain (SF) was found to be responsible for decolourisation of azo dyes (Maier *et al.,* 2004). The role of enzymes has been stressed in decolourisation of azo dyes. Enzymes involved in the degradation of azo dyes are mainly peroxidases (Zissi *et al.,* 2001). The microbes utilize carbon, nitrogen and sulphate found in effluent medium for their nutrition. Decolourisation percentage could be further increased and prolonged by supplementing the effluent medium with other cheaper effective carbon or energy source such as sucrose, starch and hydrolysed starch. Ability of the microbial isolates to utilize starch as a co-substrate could be encouraging from commercial point of view (Moosvi *et al*., 2005). Bacteria that are able to degrade effluent from textile industry in the presence of oxygen are *Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus* and *Mycobacterium.* They have been applied for bioremediation of pesticides (Vidali, 2001). Also the phosphate removal (phosphorus leads to eutrophication of lakes) is an important aerobic degradation carried out by certain heterotrophic bacteria (Rittman and McCarty, 2001). Some Bacteria such as *Bacillus* and *Pseudomonas* species are capable of storing energy in forms of intracellular polyphosphate so, this is a removal of phosphorus from the environment by biomass uptake (Rittman and McCartyn, 2001).

2.7.3 Development of bacterial consortia for biodegradation of effluents

Many researchers have mentioned that a higher degree of biodegradation and mineralization can be expected when co-metabolic activities within a microbial community complement each other. In such a 'consortium', the organisms can act synergistically on a variety of dyes and dye mixtures. One organism may be able to cause a biotransformation of the dye, which consequently renders it more accessible to another organism that otherwise is unable to attack the dye (Wilderer *et al.,* 2002) reported an example of this approach using a mixed culture containing at least four distinct microbial strains for the degradation of the diazo-linked chromophore in an industrial effluent. A consortium was reported to be effective in treating textile wastewater; it is probable that a mixed culture or consortium would be more effective in degrading toxic compounds in textile wastewater (Wilderer *et al.*, 2002). A mixed culture can adapt better to changing conditions during growth. As an example, the different conditions of textile wastewater after certain times may affect the growth of the consortia (Babu *et al*., 2007). Therefore, a consortium may be more effective in treating textile wastewater; bacterial consortium in textile effluent changed the colour from black to light brown. However pH was adjusted from 9.3 to 6.1, the biological oxygen demand was reduced from 1646 mg/l to 433 mg/l and the chemical oxygen demand was reduced from 3279 mg/l to 794 mg/l, (Saranraj *et al.,* 2010).

2.7.4 Types of bioremediation

Based on the basis of removal and transportation of wastes for treatment there are basically two methods.

i. *In situ* bioremediation.

ii. *Ex situ* bioremediation.

2.7.4.1 *''In situ''* Bioremediation

Both intrinsic and engineered bioremediation technology can be used in treatment of contaminants and toxins in the soil and water to remove harmful substance, the treatment of contaminant take place onsite without excavation. It is desirable option due to lower cost competitive and less disturbance of contaminated ground water (Chen, 2002). The site conditions are manipulated by inoculation with the degrading microorganisms and modification of pH and mixing aeration.

2.7.4.2 Intrinsic Bioremediation

Intrinsic bioremediation is an option when the naturally occurring rate of contaminant biodegradation is faster than the rate of contaminant migration (Chen, 2002). These relative rates depend on the type and concentration of contaminant, the microbial community, and the subsurface hydrogeo-chemical conditions (NAS, 2000). The ability of native microbes to metabolize the contaminant must be demonstrated either in field tests or in laboratory tests performed on site-specific samples. In addition, the effectiveness of intrinsic bioremediation must be continually monitored by analyzing the fate of the contaminants and other reactants and products indicative of biodegradation. In intrinsic bioremediation the rate-controlling step is frequently the influx of oxygen. When natural oxygen supplies become depleted, the microbes may not be able to act quickly enough to contain the contamination (NAS, 2000). Lack of a sufficiently large microbial population can also limit the cleanup rate. The microbial population may be small because of a lack of nutrients, limited availability of contaminants resulting from sorption to solid materials or other physical phenomena, or an inhibitory condition such as low pH or the presence of a toxic material (N.A.S, (2000).

Knowledge of the following key site characteristics are required to evaluate the likely success of intrinsic bioremediation. These are:

Bioavailability of contaminants: Microorganisms are ideally suited to the task of contaminant destruction because they possess enzymes that allow them to use environmental contaminants as food and because they are so small that they are able to contact contaminants easily; **The presence of mineral to buffer pH**: other environmental parameters, such as pH, specific conductivity and absence of inhibitory concentration of constituents indicate that conditions are favourable for microbial activity. Typically, microbial activity will be optimal at near neutral pH (approximate range 6 -8); **Level of the nutrients**: the extend and rate of bioremediation is probably limited by bioavailability of nutrient and organic compounds that saves as primary substrate; **adequate level of nutrient acceptor**: the compound that receives electrons (and there- fore is reduced) in the energy-producing oxidation-reduction reactions that are essential for the growth of microorganisms and for bioremediation. Common electron acceptors in bioremediation are oxygen, nitrate, sulphate, and iron; and finally the site should be **specific with high contaminants concentration** sufficient to support the microbial biodegradation and available substrates that may support the condition and growth of degrading microbes (Vidali. (2001); Chen, (2003).

2.7.4.3 Engineered Bioremediation

In some cases, it may be desirable to construct engineered systems to supply nutrients, electron acceptors or other materials that enhance the rate or extent of contaminant degradation. The location of environmental receptors or other liability issues dictate that steps be taken to optimize the rate of contaminant degradation in order to mitigate contaminant migration. The key site characteristics for engineered bioremediation are the same as for intrinsic remediation (Moosvi *et al.*, 2005).

2.7.4.4. ex *situ* Bioremediation

Bioremediation technologies that require removal of the contaminated matrix by excavation can be manipulated and treated elsewhere in some way through the use of slurry reactors, composting, biopiles or other technologies. Degradation can be facilitated by ex situ techniques which are easier to control, faster and able to treat wide range of contaminants and wastewater (Moosvi *et al.*, 2005).

2.7.4.5 Combination of Technologies

The bioremediation technology most suitable for specific site is determined by several factors, such as site conditions, indigenous microorganism population, quantity and toxicity of contaminant chemical present. Biological treatment technologies or source removal may be used to reduce the total amount of contaminant present at the site before, or concurrent with, bioremediation. For example, excessively contaminated soils may be excavated at the source of contamination, volatile contaminants may be vacuum extracted, or undissolved pools of contaminants may be pumped from aquifers. For simplification, the operations treatment can be classified into chemical, physical, and biological treatment (Babu *et al.,* 2007).

2.7.4.6 Bioaugmentation

Bioaugmentation is the addition of microorganisms to the environment that can metabolize and grow on specific compounds. The selection of the most appropriate strategy to treat a specific site can be guided by considering three basic principles: the amenability of the pollutant to biological transformation to less toxic products (biochemistry), the accessibility of the contaminant to microorganisms (bioavailability) and the opportunity for Optimisation of biological activity (Moosvi *et al.*, 2005).

2.7.4.7 Biostimulation

This involves the modification of the environment to stimulate existing bacteria capable of bioremediation. This can be done by addition of various forms of rate limiting nutrients and electron acceptors, such as phosphorus, nitrogen, oxygen, or carbon in the form of molasses (Chon and Wong, 2000). Additives are usually added to the subsurface through injection wells, although injection well technology for Biostimulation purposes is still emerging. Removal of the contaminated material is also an option, albeit an expensive one. Biostimulation can be enhanced by bioaugmentation. Chon and Wong (2000) stated that, the overall process is referred to as bioremediation and is an FEPA-approved method for reversing the presence of oil or gas spills. The primary advantage of Biostimulation is that bioremediation will be undertaken by already present native microorganisms that are well-suited to the subsurface environment, and are well distributed spatially within the subsurface (Carlucci *et al*., 2007). The primary disadvantage is that the delivery of additives in a manner that allows the additives to be readily available to subsurface microorganisms and the local geology of the subsurface (Carlucci *et al*., 2007).

2.7.4.8 Bioreactor

This method is a much faster means of waste bioremediation. It of course belongs to the *ex- situ* of bioremediation. You get a better control over the reaction conditions and the bacterial growth can be optimized. Bioreactors entail usually higher costs of equipment and require better knowledge of composition of waste and degradation pathways (Bonaventura *et al*., 1998).

2.7.4.9 Bioventing

This is the similar to Biostimulation. It involves the venting of oxygen through soil to stimulate the growth of introduce organism of to effect bioremediation (Chon and Wong (2000). This is used predominantly for soils contaminated with petroleum product. It is not suitable for removing halogenated gases that contribute to ozone layer damage.

2.7.4.1 Biofiltration

This approach is a promising method of cleaning gaseous and liquid streams. Filters concentrate pollutants and/or biodegrade them to a nontoxic forms. A biofilter can contain immobilized enzymes. An opportunity to immobilize enzymes is to embed them in a permeable membrane, in trickling filters and biofilters. The microorganisms are immobilized on a carrier or packing medium and bacteria are mainly dispersed in the circulating liquid. Slow biofiltration requires substantial land surface areas with microbiological activity in a small bed depth. The advantage of slow filtration is the fewer disturbances to build-up and trickling filters are packed-bed reactors in which the wastewater trickles over the rock or plastic media passage of air is possible, so that oxygen can be transferred to the microorganisms throughout the reactor (Rittman and McCarty, 2001).

The biofiltration process is based on transfer of contaminants from the air to the water phase and the bioconversion of pollutants to biomass, metabolic end-products is carbon dioxide and water. An application of biofiltration has been the removal of volatile organic compounds from air. Based on transfer of contaminants from the air to the water phase, and secondly conversion by microbes. Highest removals are achieved for Compounds that have a high water solubility and ability to be biodegraded. Some substances are also treated well, although they are insoluble in water, this shows that biofilm transport works even better than thought (Moosvi *et al.*, 2005).

2.7.4.10 Bio-assessment

The basic information to enhance biodegradative processes is the knowledge of the microorganisms present in a given site and food substrates they prefer to use, especially for *in situ* bioremediation. There is a need for an assessment of the environmental conditions however; the reaction of degradation in some cases enhanced by adding needed materials. Therefore, it is necessary to know what nutrients are already present. There is the possibility to accomplishing an initial assessment or a treatability study that investigates kinetics and degradation pathways (King *et al*., 1992). In an initial assessment, average hydrocarbon content and specific compounds are measured. The specific hydrocarbon degrading population is estimated by an analysis of the different present microorganisms. Wilderer *et al*. (2002) noted also important are BOD total organic carbon (TOC) and pH for wastewater. These parameters are necessary to provide degradation under ideal conditions (King *et al*., 1992).

2.7.5.11 Use of genetically engineered microbes

Another option is to use genetically engineered microbes. Despite the ability of many naturally occurring microbes to degrade a number of different chemicals (Olukanni *et al.,* 2006), a natural process of adaptation to the environment of microorganisms is the opportunity to exchange genetic material. This is possible via conjugation, transformation and transduction. It is a horizontal gene transfer without reproduction. Plasmids can be transferred, that encode for enzymes, which are able to degrade specific contaminants and thereby, open a new source of energy for the receiving bacteria. Researchers are interested in how the gene transfer is induced (Wilderer *et al.,* 2002). However, *Pseudomonas aeruginosa* secretes surfactants that solubilise oil and to enhance bioremediation, microorganisms are transfected with genes that improve their survival and competitiveness (Wilderer *et al.,*

2002). Microbes that normally thrive in a given environment could be transfected with degradative enzyme genes from bacterial species that are good biodegraders, but poorly adapted to the environment of interest (Bonaventura *et al.,* 1998). Combining plasmid from different strains within a single host, it is possible to an organism with multiple degradative capabilities **(**Olukanni *et al.,* 2006). According to Wilderer *et al.* (2002) operation with genetically engineered microbes brings about a certain amount of risk. The release of genetically engineered organisms and its effects is not completely understood. The first place for application of genetically engineered organisms is supposed to be bioreactors (Wilderer *et al*., 2002).

2.7.0 Monitoring Bioremediation

The process of bioremediation can be monitored by conducting routine analyses of the physicochemical parameters. This include: pH, BOD, COD, DO, EC, Temperature and total suspended solids (TSS) (Olukanni *et al.*, 2006).

2.8.0 Potential Advantages of Bioremediation Technologies

According to Moosvi *et al.*, (2005), the use of intrinsic or engineered bioremediation processes offers several potential advantages that are attractive to site owners, regulatory agencies and the public. These include: Lower cost than conventional technologies; Contaminants usually converted to innocuous products; Contaminants are destroyed, not simply transferred to different environmental media; Nonintrusive, potentially allowing for continued site use. Relative ease of implementation and lastly provide technique for cleaning up pollution by enhancing the same biodegradation processes that occur in nature (Olukanni *et al*., 2006).

2.8.1 Limitations for bioremediation

Bioremediation is not without its faults. It is limited to those compounds that are biodegradable. Not all compounds are subjected to rapid and complete degradation. There are some concerns that the product of bioremediation may be more persistent or toxic than the parent compound. Biological processes are highly specific, important site factors required for success include; the presence of metabolically capable microbial populations, suitable environmental growth conditions and appropriate levels of nutrients and contaminants. There are several limitations to bioremediation (Ghoreishi and Haghighi, 2003).

One major limitation has to do with the nature of the organisms the removal of pollutants by organisms is not a benevolent gesture. Rather, it is a strategy for survival. Most bioremediation organisms do their job under environmental conditions that suit their needs Vidali (2001), reported that consequently, some type of environmental modification is needed to encourage the organisms to degrade or take up the pollutant at an acceptable rate. In many instances the organism must be presented with low levels of the pollutant over a period of time. This induces the organism to produce the metabolic pathways needed to digest the pollutant. When using bacteria and fungi, it is usually necessary to add fertilizer or oxygen to the material containing the pollutant. This can be disruptive to other organisms when done in situ. Vidali (2001) noted that ‘’in situations where simple compounds and metals are being taken up it is likely that these pollutants are at toxic levels for the organisms.

These techniques are generally, the most desirable options due to lower cost and fewer disturbances since they provide the treatment in place avoiding excavation and transport of contaminants. In situ treatment is limited by the depth of the water that can be effectively treated (Carlucci *et al.*, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Design

This research involved Sampling of two sites in Kano Metropolis. One is located in Sharada (Site A) and the other one at Challawa (Site S). Some physicochemical analyses were carried out at the sites before and after bioremediation. However, Isolation, characterization of selected bacterial isolates and biodegradation/decolourisation potential of bacterial isolates was carried out for 10 days in the Laboratory of department of Microbiology, Ahmadu Bello University, Zaria, Nigeria.

3.2 Study Area

Kano lies on (Latitude 11°30^{I} N 8.30 ^{I}, Longitude11.5°N 8.5°^{I}E), in Northern Nigeria. The state has a total of area of 20,131 km^2 (7.14 sq mi) of land. Most industries in the city are textiles, tannery, chemical and allied. Kano City is located on the main watershed which separates the two main river basins in Challawa and Tamburawa (Dan' azumi and Bichi, 2010). The climate is characterized by well-defined wet and dry seasons. The wet season spreads from May to October, August usually being the wettest and dry season which lasts from November to April. Water pollution comes from domestic and industrial activities in which thousand tonnes of wastewaters flow into two water ways.

3.4 Sampling Points

The samples were collected from the discharge and drainage pipes of the two sites. Each sample was collected from the effluents paths of flow. The map of the sampling points of the two sites, that is Site A (Sharada) and Site S (Challawa), are presented in Figures 3.1 and 3.2.

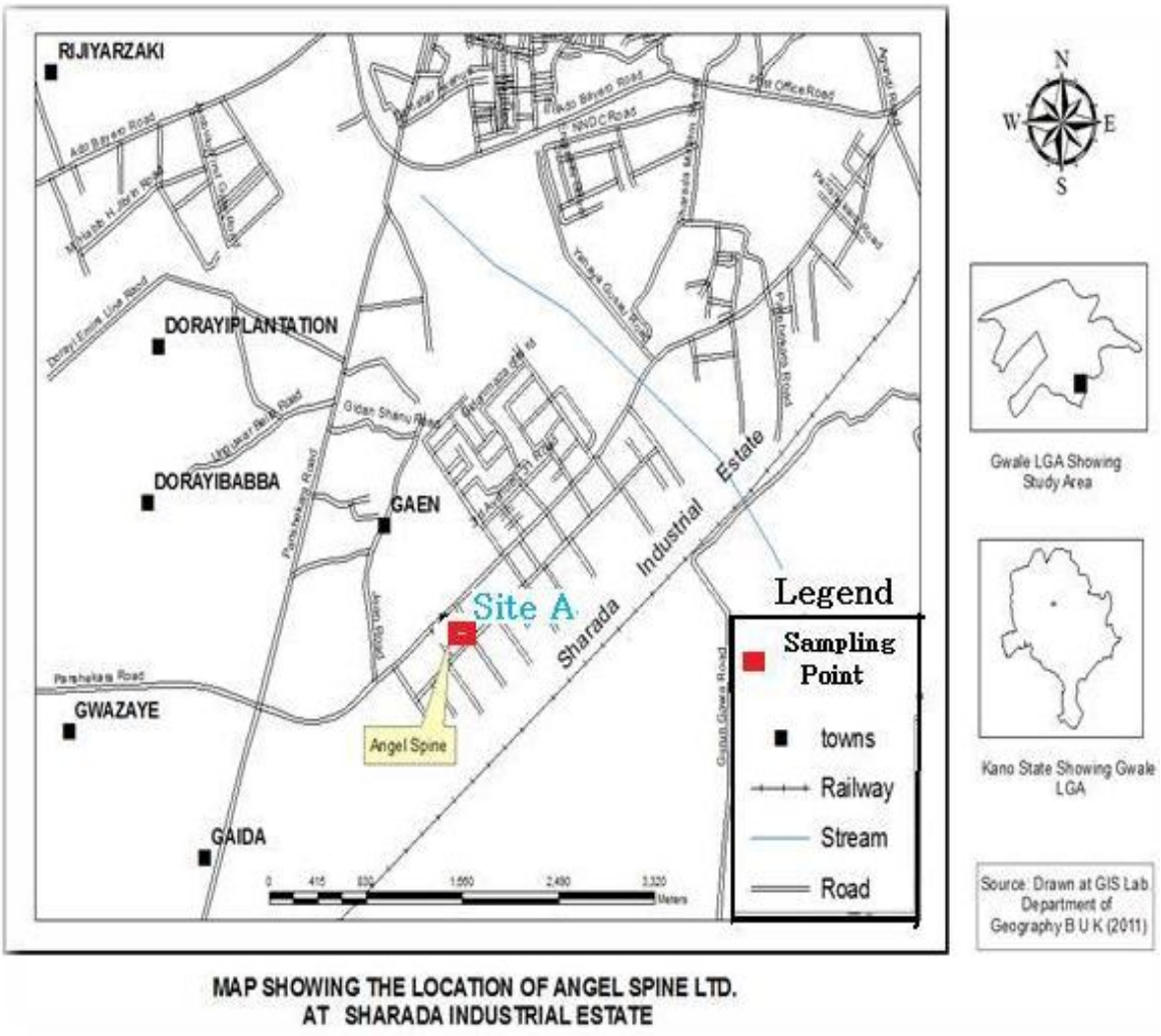


Figure 3.1. Sampling point at site A in Sharada Indusrial Estate in Gwale Local Government Area of Kano State

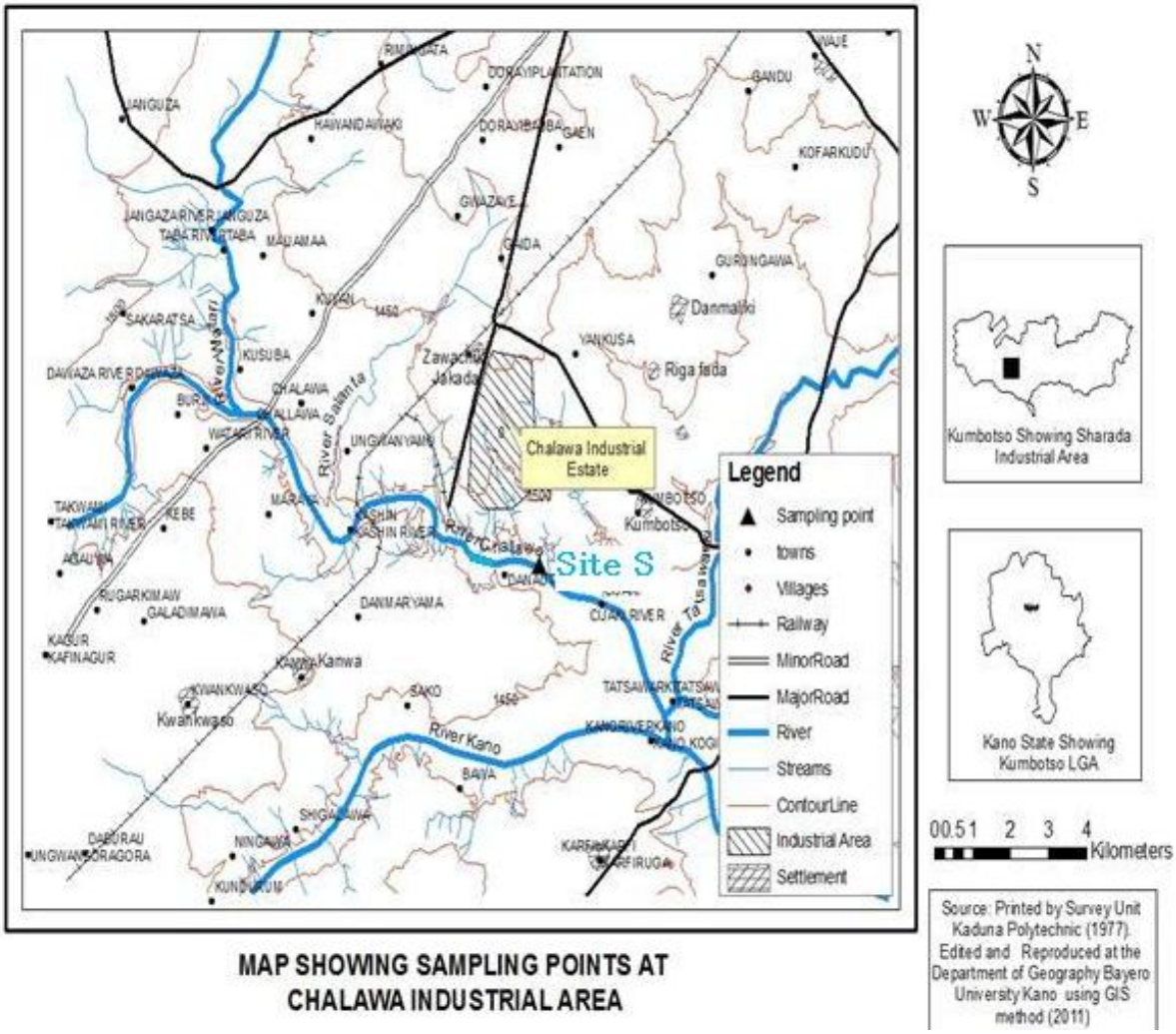


Figure 3.2 Sampling point at site S along Challwa Industrial Estate of Kumbotso Local Government Area of Kano State

3.3. Sample collection

.The samples were collected during the finishing step, chemical finishing, and mechanical finishing. Chemical finishing involves wet unit processes, while mechanical finishing involves dry unit operations, the former involves rinsing, washing, printing and dyeing processing which corresponding to the highest effluents volume discharge and hence the worsening environmental situation. Samples were collected in one Litre (1 L) plastic containers with screw caps from each point and transported in ice packs to Mcrobiology Laboratory, Ahmadu Bello University, Zaria, for analyses within 24 hours of collection.

3.5.0 Analysis of physicochemical Parameters-

3.5.1 pH

The pH was determined by placing a pH probe (Hanna instrument C-99- USA) into the sample in a 250 ml conical flask and allowed to equilibrate for 3 minutes and pH meter was read and recorded accordingly.

3.5.2 Temperature

The temperature of water and effluent was also determined on the field by lowering a mercury thermometer (Hanna instrument C-99- USA) into the sample and allowed to equilibrate for 4 minutes and reading was taken to the nearest degree Celsius (^{o}C).

3.5.3 Electrical conductivity (EC)

The electrical conductivity was determined by placing a conductivity probe (Hanna instrument C-99- USA) into the sample in a 250 ml conical flask and allowed to equilibrate for about 3 minutes and the electrical conductance in micro second per centimetre (µs/cm) was recorded.

3.5.4 Dissolved Oxygen (DO)

Dissolved oxygen of the effluent samples was determined using Jenway Model 9070 (Hanna instrument C-99-USA) waterproof DO-meter. The protective cap of the DO meter was removed from the probe. Membrane module was taken and held in the vertical position. The probe was calibrated prior to measurement with the appropriate traceable calibration solution of 5% sodium sulphate in accordance with the manufacturer's instruction. The probe was immersed into the effluent samples to be analysed and the readings were recorded at the point of sample collection.

3.5.5 Determination of Chemical Oxygen Demand (COD)

Fifty (50 ml) of sample was taken into a refluxing flask and several boiling stones were added. Then 0.1 g HgSO4 was added to the solution and 5 ml of concentrated H_2 SO_4 was also added to the solution. To ensure that $HgSO_4$ dissolved completely, the solution was swirled slowly while adding Sulphuric acid, then 0.1 g of Ag_2SO_4 was added to this solution and finally Potassium dichromate was added. Thorough mixing of the solution was ensured by swirling the flask in a water bath to prevent any volatile substances that may have escaped from the liquid state. The flask was then attached to a condenser and further cooling was carried out and 20 ml of sulphuric acid was added to the solution in the flask continuing cooling and swirling to mix the solution. The solution was refluxed for 1 hour. A blank run (using 50 ml distilled water instead of sample) was simultaneously conducted with the same procedure after cooling; the solution was then transferred to an Erlenmeyer flask. The reflux flask was rinsed thrice, pouring the rinsing water to the Erlenmeyer flask. The solution was diluted to about 300 ml and about 8 drops of phenanthroline ferrous sulphate was added to the solution as an indicator. The solution was titrated against the Mohr's salt and the titre volume required for the colour change from blue-green to reddish blue was noted. The

procedure was repeated for the blank run. Below is formular used to calculate COD: (COD = 8000 x (Vbl – Vs) ^{x} $\left[\frac{m}{original\ volume\ of\ sample\ taken\ in\ mg/l}\right]$ (APHA, 2001)

Where,

Vbl = Titre volume for the blank

Vs = Titre volume for the sample M = Morality of Mohr's solution.

3.5.6 Determination of Biochemical Oxygen Demand (BOD)

Biochemical Oxygen Demand ($BOD_{-5)}$ was determined using DO HI9146 (Winkler) method of DO determination, Microprocessor Dissolved Oxygen Meter. The amount of sample to be analysed was measured, clean calibrated thermometer was placed into the sample; temperature was stabilized at 20°C ±1°C in the refrigerator. DO instrument was turned on for 30-60 minutes. After aeration, 1 ml each of the potassium phosphate, magnesium sulphate, calcium chloride, was diluted according to manufacturer's instruction. Dilution was placed at constant temperature to maintain the initial temperature until sample dilutions and analyses began. The initial and final (after 5 days ± 4 hours) DO concentration of was measured as (D_1) of each sample and each dilution blank. Temperature was checked using air incubator with laboratory thermometer to ensure that the temperature has been maintained. At the end of 5 days ± 4 hours, BOD bottle was removed from incubator, and was poured off the water seal and ground-glass stopper, and final DO concentration (D_2) was measured . The DO_1 uptake (DO_2 days – DO_5 days) in the dilution water should not be greater than 0.2 mg/l and preferably not more than 0.1 mg/l.

For each test bottle meeting the 2.0-mg/L minimum DO depletion and the 1.0-mg/L residual DO, calculate BOD5 as follows:

The formula for calculating BOD is stated below.

$$BOD_5\ (mg/l) = \frac{D_1 - D_2}{P}$$ (APHA, 2005)

Where,

D_1= DO diluted sample immediately after preparation (in mg/l)

D_2= DO diluted sample after 5 day of incubation at 20°C ±1°C (in mg/l)

P= decimal volumetric fraction of sample used.

3.5.7 Determination of Total Suspended Solids (TSS)

Before sampling, glass fibre filters were prepared first by soaking them in distilled water, drying them at 103°C and weighing and recording their weight. Sample bottles were dried, and weighed glass fibre filters were poured onto a filtering flask – wrinkled side up. Sample bottle was shaken first, and then water was poured on the pump. The amount of water needed to filter may change according to water conditions. One hundred ml of sample was filtered with paper with porosity 0.8 mm. Filtered, was recorded with volume of water filtered. Filter paper was dried 103°C to 105°C, and was allowed dry at room temperature, and weighed. It was dried, and re-weighed. This was repeated until the filter reached a constant weight. Final end weight was recorded. This increase in weight representing TSS was calculated by using the equation,

$$TSS = \frac{A - B \times 100}{C}$$

Where,

A = End weight of the filter

B = Initial weight of the filter

C = Volume of water filtered (APHA, 2001)

3.5.8 Total dissolved solids (TDS)

Total dissolved solid (TDS) was determined by evaporating the waste samples to dryness (AOAC, 1990). In this method, 50 ml of sample was transferred to a weighed

evaporating dish, and evaporated to dryness by heating for 1-2 hours at 180°C to a constant weight. A total dissolved solid was calculated as follows:

$$mg/l\ of\ TDS = \frac{mg\ residue}{ml\ of\ Sample} \times 1000$$

3.6. Determination of heavy metals in effluent Samples

Determination of Cu, Zn, Mn, Fe, Cr, Cd, Ni and Pb was made directly on each final solution using Standard method of heavy metal content as described by (APHA, 2001).. Each of the metals was analysed by using Atomic Adsorption Spectrophotometer (AAS -model-GBC-932 plus Chem Tech – USA). The wastewater samples were digested as follows: hundres millilitre of the sample was transferred into a beaker and 5ml concentrated HNO_3 was added. The beaker with the content was placed on a hot plate and evaporated down to about 20 ml. The beaker was then cooled and another 5ml concentrated HNO_3 was also added. The beaker was covered with watch glass and returned to the hot plate. The heating was continued, and then small portion of HNO_3 was added until the solution appeared light coloured and clear. The beaker wall and watch glass was washed with distilled water and the sample was filtered to remove any insoluble materials that could clog the atomizer. The volume was adjusted to $100cm^3$ with distilled water, the result was read and in mg/l (APHA, 1995).

3.6.1 Sample Preservation and Laboratory Analysis

The samples were preserved by adding 1.5m1 concentration of HNO_3 to each 1 litre of sample and the pH adjusted to 2.0 by HCl and recorded using pH meter. The sample was stored in a refrigerator at about 4^oC, for subsequent analysis. As samples may contain particulate or organic materials, pre-treatment in the form of digestion was required before analysis. Nitric acid digestion was employed in accordance with (APHA, 1995). The digested

sample was taken for Atomic Adsorption Spectrophotometer (AAS) analysis. The analysis began with selection and adjustments of various units of the machine (lamp selection, wavelength selection, slit adjustment and flame adjustment) and the machine was standardized by aspirating distilled water to get zero absorbance. A standard solution of 1000 mg/l for all the metals was prepared, and from them working solutions (with concentrations within the range of 0-5 mg/l) was prepared by serial dilution (APHA, 1995). The standard solution was taken through the same digestion technique as mentioned. After digestion, the solutions was taken to AAS and the absorbance value read and recorded. A graph of absorbance vs. concentration (the calibration curves) was plotted. The sample was then aspirated into the machine and the absorbance value read and recorded. The concentration (in mg/l) was obtained by interpolating/extrapolating the values of absorbance from the calibration curve. The procedure was repeated for all the samples. This was carried out in the Department of Agriculture, Bayero University, and Kano.

3.7 Isolation of bacterial isolates from textile effluents

Serial dilution of the effluent sample was made and 0.1 ml each of the respective dilutions was plated onto Nutrient agar (NA), MacConkey agar, and Blood agar and Centrimide agar using the spread plate technique. The plates were incubated at 30 ^{o}C for 24 to 48 hours before enumeration of the colonies. The isolates obtained were purified and subcultured on same media, stored on slants and were maintained at temperature of 4^{o}C for further usage.

3.8.0 Microscopic examination

A sterile wire loop was used to transfer a small portion of the prepared bacterial colony into a drop of distilled water; it was then emulsified to a make a thin film and allowed to air dry before it was heat fixed. The slide was gram stained and observation was made

under x100 objective (oil immersion), Gross morphology and detailed features were observed.

3.8.1 Identification and characterization of bacterial species

Pure colony of each bacterial isolate was identified presumptively on the basis of the following features: colonial morphology, pigmentation, cell morphology, and Gram-staining reaction. Isolates were further characterized biochemically using the Microgen TMGnA+B-ID System, identification system for all *Enterobacteriaceae* and extensive range of oxidase-positive Gram Negative rods bacteria and Bacillus-ID, an Identification system for the Mesophilic *Bacillus* Specie was also carried out using, Microgen Bioproducts (U.K.),

3.8.2 Principle of the test for *Bacillus*-ID

The Microgen Bacillus-ID identification system consists of 2 microwell strips (labelled BAC 1 and BAC 2), each containing 12 dehydrated substrates for the performance of either carbohydrate fermentation tests or other biochemical based tests. The last well in the second strip is a carbohydrate fermentation control well for use as a reference well in the interpretation of these tests. The selection of the substrates included in the test panel was determined using computer based analysis of all available substrates for the identification or differentiation of this group of organisms according to the manufacturers instruction. Identification of isolates was achieved by recording the results visualised by a colour change after 24 and 48 hours incubation at 30°C and the addition of appropriate reagents (Indole, Nitrate and VP tests) after 48 hours. These results were then analysed using the Microgen Identification System Software (MID-60). This test strip and report form is shown in Appendices I and II.

3.8.3 Principle of the test for *Enterobacteriacea* -ID

The microgen GN-ID system comprises two separate microwell test strips GN A and GN B). Each microwell test strip contains 12 standardised biochemical substrates which have been selected on the basis of extensive computer analysis of published database for identification of the family *Enterobacteriacea* and commonly encountered non-fastidious oxidase positive and Gram negative rods bacteria. Dehydrated substrates in each well were reconstituted with a saline suspension of the organism to be identified. If the individual substrates are metabolised by the organism, a colour change occurs during incubation, or after addition of specific reagents. The various sugar tests are: Lysine, Ornithine, H_2S, Glucose, Mannitol, Xylose, ONPG, Nitrate, Indole, Urease, VP, Citrate, TDA, Gelatine, Malonate, Inositol, Sorbitol, Rhamnose, Sucrose, Lactose, Arabinose, Adonitol, Raffinose, Salicin and Argine. The permutations of the metabolised substrates were interpreted using the Microgen Identification System Software (MID-60) to identify the test organism, according to manufacturers' instructions. Microgen ID well and report form are shown in Appendies III and IV.

3.9 Screening of bacterial isolates for biodegradation potential

Three millitre suspension for each bacterial species isolated was inoculated into a basal medium containing 0.5% 1%, 1.5 %, 2 %, and 2.5 % inoculate with 100 ml of the textile effluent were incubated at 30°C for 24 – 48 hours. At the end of the incubation period the growths of each bacterial species was determined spectrophotometrically and the species with the highest level of growth and rapid degradation/decolourisation were further selected for biodegradation experiment.

3.9.1 Biodegradation of textile effluents using selected bacterial species

Three separate flasks of 250 ml set up were mounted for each identified isolate. One was to examine the action of individual bacterial isolates, the second set was to determine the action of combined culture (consortia) and third flask set up contained no bacterial inoculums and therefore saved as control. The isolates for the consortium were selected based on three criteria; ability to grow on minimum basal medium and also ability to degrade textile effluents. Three consortia were developed using combinations of three to five isolates. Inoculation was done in proportion of 1:1:1. Trace of yeast extract (0.6%), sucrose (3.5%), $MgSO_4$.7H2 (0.02%), and Na_2CO_3 (1.0%) were added to the effluents as co-substrates to help maintain the culture as stated by Senan *et al.,* (2004). The pH was adjusted to 7 ± 0.2 using sodium hydroxide and hydrochloric acid solution. Then, the flasks were sterilized at 121°C for 15 minutes. The sterilised flasks were inoculated under aseptic condition with 3 ml suspension of selected bacteria species into 250 ml Erlenmeyer flasks containing 200 ml of sterile effluents. The flasks were incubated on an orbital shaker at 200rmp for 10 days at room temperature. Samples were drawn at 48 hour intervals for observation. Three millilitre of the each sample solution was filtered and centrifuged at 5000 rpm for 20 minutes. Biodegradation/Decourisation of effluents was determined by monitoring the decrease in absorbance at the maximum wavelength of effluents (λmax. 523nm) by using a UV-Visible spectrophotometer (UV-1700 Pharmaspec, Shimadzu Made in China). The decolourisation activity (%) was calculated the by following formula and all the assay were done in triplicate. Thus, the absorbance was calculated using this equation

$$\% \, Decolourisation = \left[\frac{Initial \, Absorbance - Final \, Absorbance \,]}{Initial \, Absorbance} \right] \times \quad 100$$

3.10 Statistical analysis

The means comparison between physicochemical parameters of the two sampled Sites, analysis of heavy metal Adsorption among the various isolates before and after bioremediation was determined using one-way analysis of variance. Where there were differences between the means, post hoc test was carried out using Duncan multiple range tests to rank the mean values. Level of significance was set at $p \leq 0.05$, differences between mean values were considered to be significant when $p \leq 0.05$ otherwise they were considered not significant ($p > 0.05$). Percentages were used to express biodegradation process of the effluent by all isolates in both sites and the consortia.

CHAPTER FOUR

RESULTS

Analysis of physico-chemical parameters of effluents before bioremediation experiments revealed the characteristics of the effluents sampled from two textile industries located in Sharada (site A) and Challawa (site S) Industrial Estates of Kano State, Nigeria (Table 4.1). The initial pH values of effluent for both sites were near alkaline range from 7.15 to 7.2. Similarly, temperature values of the effluents for sites A and S was between 35.60°C to 37°C. Total suspended solid (TSS) for Site A was 2245 mg/l, Site S recorded the highest TSS of 2505 mg/l. Total dissolved solid (TDS) value recorded for site A was 1940 mg/l while site S had 2105mg/l. Concentration of COD in effluent from site A was 2743 mg/l while Site S had 2831 mg/l. Biochemical oxygen demand (BOD) values for site A and S were 1622 mg/l and 1902 mg/l respectively. The result for Electrical conductivity (EC) showed concentrations of 2577 µS/cm for site A and 3050 µS/cm for site S. Dissolved oxygen (DO) values of effluents were 5.55 mg/l and 6.66 mg/l for sites A and S respectively.

Table 4.2: shows the biochemical characterisation of the isolates from the effluents of the two sites. Gram positive and Gram negative rods were isolated. A total of 15 bacterial isolates were recovered; five belong to *Pseudomonas* genera while six belong to *Bacillus* genera. Others were *Alcaligenes faecalis, A. hydrophila, Actinobacillus* species and *Burkholderia cepacia* from Sharada (site A) and Challawa (site S). The reaction of various biochemical tests is presented in Appendix I. Fifteen (15) bacterial isolates were selected and screened. Nine bacterial isolates exhibited high growth potential when inoculated on minimum basal medium at different concentrations with rapid degradation of textile effluents in 48 hour, and they were presumptively selected for bioremediation/decolourisation studies. These are shown in Table 4.3.

Table 4.1: Physico-chemical parameters of industrial effluents before bioremediation for Sites A and Site S

Sites	pH	Temp	TSS	TDS	COD	BOD	EC	DO
A	7.20 ± 0.00	37.00 ± 0.00	1940.0 ± 0.00	2245.00 ± .00	2743 ± 0.00	1622 ± 0.00	2577 ± 0.00	6.33 ± 0.00
S	7.15 ± 0.05	35.50 ±0.05	2105.50±5.50	2504.50± 0.50	2831.50 ± .50	1902.50± 0.50	3050.5± 0.05	5.55 ± 0.05

Key:
pH= Negative logarithm to base 10 of hydrogen ion concentration.
Temp = Temperature of effluent.
TSS = Total suspended solids.
TDS = Total dissolved solids. = Chemical oxygen demand.
COD = Chemical oxygen demand
BOD = Biological oxygen demand.
EC = Electrical conductivity.
DO= Dissolved oxygen.

Site A- Sharada Industrial Estate

Site S-Challawa Industrial Estate

Table 4.2: Identification and biochemical characterizations of bacterial genera isolated from textile industry effluent samples from Site A and Site S

Isolate NO	Octal Code	Test System	Site	Identified Genera
PA47	447530001	MID27T	A	*Pseudomonas aeruginosa*
PA23	444052000	MID27T	A	*Alcaligenes faecalis*
PA12A	667640041	MID27T	A	*Aeromonas hydrophila*
PA4	447530001	MID27T	A	*Pseudomonas aeruginosa*
BS1	554710001	MID27T	A	*Pseudomonas shigelloides*
BS10	777760731	MIDBAC	A	*Bacillus licheniformis*
PA12B	55453000	MID27T	A	*Actinobacillus spp*
BS12	752441444	MID27T	A	*Burkholderia cepacia*
PA27	601200211	MID27T	A	*Pseudomonas putida*
PA22	01000101	MIDBAC	S	*Bacillus brevis*
BS12	77776073	MIDBAC	S	*Bacillus licheniformis*
BS33	42233210	MIDBAC	S	*Bacillus lentus*
BS6	213600011	MIDBAC	S	*Bacillus megaterium*
BS4	30000111	MIDBAC	S	*Bacillus subtilis*
BS5	440402013	MID27T	S	*Pseudomonas fluorescence*

PA = Isolates Code Number for oxidase positive- Gram positive Rods

BS = Isolates Code number for *Bacillus* Spp

Octal Code = Digits generated at the end of observation for each isolate 8 digits for *bacillus* 9 for oxidase positive Gram negative rods bacteria

MIDBAC = Microgen identification system for *Bacillus* spp

MID27T = Microgen identification system for *Enterobacteriacea* non fastidious oxidase positive and negative bacteria gram negative rods.

Site A = Sharada Indusrrial Estate Site S = Challawa Industrial Estate

Table 4.3: Biodegradation potentials of bacterial isolates in degrading textile effluents growth on minimum basal medium efficiently within 24 to 48 hours

Identified Isolates	0.5v/v Texeft MBM	1% Texeft MBM	1.5% Texeft MBM	2% Texeft MBM	2.5% Texeft MBM
Bacillus subtilis	+	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+	+
Pseudomonas flourescens	+	+	+	+	+
Bacillus brevis	+	+	+	+	+
Alcaligenes faecalis	+	+	+	+	+
Pseudomonas putida	+	+	+	+	+
Bacillus licheniformis	+	+	+	+	+
Aeromonas hydrophila	+	+	+	+	+
Bacillus megaterium	+	+	+	+	+
Bacillus licheniformis	–	+	+	–	+
Pseudomonas aeruginosa	+	–	–	+	+
Pseudomonas shigelloides	–	+	–	+	–
Burkholderia cepacia	+	–	–	–	–
Actinobacillus spp	–	–	–	–	+
Bacillus lentus	–	–	–	–	–

Key: TEXEFT = Textile effluent, MBM = minimum basal medium, + = Growth.— = No Growth

Table 4.4 shows the results of physico-chemical parameters after bioremediation with various isolates from Site A and indicated that the effluents samples were slightly acidic. The values ranged from 6.53 to 6.66. The highest temperature of the effluent samples was 30.60°C while others had 30.10°C. Total suspended solid (TDS) reduction efficiency exhibited by all isolates ranged from 797.30 mg/l to 898 mg/l with 53% to 60 % reduction. Similar reduction was recorded for TDS after bioremediation with various isolates; the range was from 1017 mg/l to 1085 mg/l with 55% and 52 % respectively. The highest reduction was observed with *Pseudomonas putida* which showed 50% reduction. Biochemical oxygen demand (BOD) after bioremediation by *Pseudomonas aeruginosa* was 665.30 mg/l (59% reduction), the least reduction was observed with *A. hydrophila* (725.90 mg/l) representing 54% reduction. Similar result was shown for EC which was reduced from 779 µS/cm to 1051 µS/ (59% to 70% reduction). Dissolved oxygen ranged from 7.10 – 9.24mg/l after bioremediation.

The results of the physico-chemical parameters after bioremediation of the effluent sample for site S as presented in Table 4.4 were near acidic pH and ranged between 6.12 and 6.97. However, sample effluents treated with *Alcaligenes faecalis* showed near neutral pH (6.97) while when treated with *B. brevis* the effluents had a pH of 6.12. Similarly, temperature range demonstrated by effluents sample was 30.30 to 31.40°C. Total suspended solid (TSS) reduction capability was 1097 mg/l with *B. brevis* reduced by 60 % while *P. flourescens* had 48%. The efficiency in reduction of TDS as shown by *B. Subtilis* was 965 mg/l by (61%) while *B. licheniformis* exhibited the least reduction by 1238 mg/l with 50% reduction. However, chemical oxygen demand was highly reduced by *B. brevis* by 997 mg/ (62%) and 1037 mg/l was reduced by *B. subtilis* with (53%). *Bacillus subtilis* effectively reduced BOD to 705 mg/l (63%) while less reduction was recorded by *B. licheniformis* by 50%. Electrical conductivity result revealed that *P. fluorescens* had reduced EC to 1328 µS/cm (56%), while *B. brevis* had the highest reduction by 944 µS/cm (69%). Increase in DO of the effluents was observed after bioremediation with the isolates ranged between 7.92 mg/l to 9.66 mg/l.

Table 4.4: means of physico-chemical parameters of effluents sample for Site A and S before and after bioremediation by all isolates

	Isolates	pH		Temp (°C)		TSS		TDS	
		Before	After	Before	After	Before	After	Before	After
A	*P. aeruginosa*	7.20 ± 0.00	6.65 ± 0.13	37.00 ± 0.00	30.10 ± 0.40	1940.00 ± 0.00	797.30 ± 137	2245.00 ± 0.00	1045.50 ± 178
	P. putida	7.20 ± 0.00	6.56 ± 0.23	37.00 ± 0.00	30.10 ± 0.27	1940.00 ± 0.00	898.20 ± 157	2245.00 ± 0.00	1017.50 ± 168
	B. megaterium	7.20 ± 0.00	6.53 ± 0.20	37.00 ± 0.00	30.60 ± 0.37	1940.00 ± 0.00	822.90 ± 130	2245.00 ± 0.00	1033.30 ± 216
	A. hydrophila	7.20 ± 0.00	6.66 ± 0.19	37.00 ± 0.00	30.10 ± 0.31	1940.00 ± 0.00	856.70 ± 142	2245.00 ± 0.00	1085.50 ± 314
S	*B. licheniformis*	7.15 ± 0.05	6.96 ± 0.15	35.50 ± 0.05	30.30 ± 0.30	2105.50 ± 5.50	898.50 ± 161	2504.50 ± 0.50	1238.10 ± 223
	B. subtilis	7.15 ± 0.05	6.41 ± 0.11	35.50 ± 0.05	30.60 ± 0.30	2105.50 ± 5.50	89 9.90 ± 215	2504.50 ± 0.50	1119.30 ± 210
	A. faecalis	7.15 ± 0.05	6.97 ± 0.26	35.50 ± 0.05	31.40 ± 0.83	2105.50 ± 5.50	1006.30±227	2504.50 ± 0.50	1145.70 ± 243
	P. fluorescens	7.15 ± 0.05	6.75 ± 0.90	35.50 ± 0.05	30.70 ± 0.33	2105.50 ± 5.50	1079.90±238	2504.50 ± 0.50	1226.70 ± 250
	B. brevis	7.15 ± 0.05	6.12 ± 0.20	35.50 ± 0.05	30.40 ± 0.40	2105.50 ± 5.50	846.30 ±180	2504.50 ± 0.50	965.30 ± 201

Key:
pH= Negative logarithm to base 10 of hydrogen ion concentration.
Temp = Temperature of effluent.
TSS = Total suspended solids.
TDS = Total dissolved solids. = Chemical oxygen demand.

Table 4.4: Means of physico-chemical parameters of effluents sample for Sites A and S before and after bioremediation by all isolates (continue)

Site	Isolates	COD (mg/l)		BOD (mg/l)		EC (µS/cm)		DO (mg/l)	
		Before	After	Before	After	Before	After	Before	After
A	*P. aeruginosa*	2743 ± 0.00	804.40 ± 88	1622 ± 0.00	665.30 ± 86	2577 ± 0.00	1051.7 ± 209	6.33 ± 0.00	9.02 ± 0.54
	P. putida	2743 ± 0.00	852.90 ± 100	1622 ± 0.00	689.70 ± 97	2577 ± 0.00	852.70 ± 118	6.33 ± 0.00	8.88 ± 0.60
	B. megaterium	2743 ± 0.00	847.10 ± 96	1622 ± 0.00	744.30 ± 110	2577 ± 0.00	838.50 ± 12	6.33 ± 0.00	8.03 ± 0.45
	A. hydrophila	2743 ± 0.00	824.30 ± 92	1622 ± 0.00	725.90 ± 106	2577 ± 0.00	779.60 ± 98	6.33 ± 0.00	7.83 ± 0.44
S	*B. licheniformis*	2831.50 ± 0.50	1251.10 ± 269	1902 ± 0.50	937.90 ± 193	3050.5 ± 0.05	1251.9± 279	5.55 ± 0.05	8.38 ± 0.41
	B. subtilis	2831.50 ± 0.50	1341.30 ± 290	1902 ± 0.50	705.30 ± 206	3050.5 ± 0.05	1138.3± 279	5.55 ± 0.05	9.66 ± 0.58
	A. faecalis	2831.50 ± 0.50	1195.90 ± 257	1902 ± 0.50	920.90 ± 191	3050.5 ± 0.05	1064.4± 202	5.55 ± 0.05	8.16 ± 0.40
	P. fluorescens	2831.50 ± 0.50	1268.50 ± 269	1902 ± 0.50	805.60 ± 189	3050.5 ± 0.05	1328.1± 325	5.55 ± 0.05	7.92 ± 0.33
	B. brevis	2831.50 ± 0.50	1074.70 ± 225	1902 ± 0.50	907.90 ± 193	3050.5 ± 0.05	944.50± 177	5.55 ± 0.05	8.10 ± 0.10

Key

COD = Chemical oxygen demand
BOD = Biological oxygen demand.
EC = Electrical conductivity.
DO = Dissolved oxygen

Results for physico-chemical parameters of the effluents after bioremediation with the consortia revealed acidic pH range of 6.50 – 6.76 (Table 4.5). The temperature showed by all sample effluents ranged from 29.70ºC to 30.20ºC. Effluent sample treated consortium 1 had the highest temperature (30.20ºC). The reduction capability of TSS was shown by CTIA 3 to be 1115 mg/l with (47%) and 60% reduction was recorded by CTIA 1. However, CTIA 3 reduced TDS by 1187 mg/l with (52%), while much removal was demonstrated by CTIA 1 with 1102 mg/l by 56%. Consortium 2 was reduced by 1234 mg/l with (56%), less reduction of 1281mg/l by (54%) was shown in CTIA 3. Biochemical oxygen demand was removed by CTIA 1 with 59% and the least was observed in CTIA 3 with 57% reduction. Furthermore, EC was efficiently reduced with CTIA 1 by 899 mg/l and CTIA 2 by 940 mg/l with 71% and 69% respectively. Dissolved oxygen increased after bioremediation by 7.10 mg/l with CTIA 3 while CTIA 1 had higher efficacy (increased by 9.84 mg/l).

Adsorption by various isolates from Site A reveals that *Aeromonas hydrophila* demonstrated highest efficacy in adsorption of cadmium of 0.04 mg/l (96%) while *P. aeruginosa* had the least adsorption of 0.5 mg/l (58%) (Figure 4.1). A similar result revealed by *Pseudomonas aeruginosa* adsorbed Cu from 1.02 to 0.03 mg/l (97% reduction) while *A. hydrophila* had the ability to adsorbed 0.5 mg/l (50%). However, the adsorption rates of Cr by all isolates ranged from 0.05 mg/l to 0.93 mg/l (that is, 69% to 98%) after bioremediation. Iron and nickel had the highest concentration of 4.21 mg/l and 3.11 mg/l respectively. After bioremediation with *P. Putida* and *A. hyddrophila,* they showed high efficiency of adsorption by 0.08 mg/l (98%) and 0.23 mg/l (93%) in that order, while the least was recorded by *P. aeruginosa* (0.9 mg/l) 78% and *B. megaterium* (0.73) with 76% respectively. Lead was greatly adsorbed by *B. megaterium* from 2.4 mg/l to 0.2 mg/l with 92% and the least of 0.4 mg/l by (83%) was uptake by *P. putida*. Manganese adsorption ranged from 0.7 mg/l to 0.9 mg/l with 56% to 65% by all isolate.

Table 4.5: Means of physico-chemical parameters after bioremediation by consortia

Consortia	pH	Temp (°C)	TSS (mg/l)	TDS (mg/l)	COD (mg/l)	BOD (mg/l)	EC (µS/cm)	DO (mg/l)
CTIA 1	6.50 ± 0.15	30.10 ± 0.23	848.10 ± 246.96	1102.70±224.58	1246.80±268.94	780.90±268.94	899.30±258.90	9.84 ±0.50
CTIA 2	6.76 ± 0.18	29.70 ± 0.26	1162.50±268.75	1158.50±228.28	1234.70±284.63	783.30±187.20	915.30± 63.97	7.53 ±0.68
CTIA 3	6.66 ± 0.99	30.20 ± 0.30	1115.50±235.75	1187.70±234.83	1281.00±290.90	807.00±187.20	940.90±275.25	7.10 ±0.48

Key:
pH = Negative logarithm to base 10 of hydrogen ion concentration.
Temp = Temperature of effluent
TSS = Total suspended solids
TDS = Total dissolved solids
COD = Chemical oxygen demand
BOD = Biological oxygen demand
EC = Electrical conductivity
DO = Dissolved oxygen
CTIA 1- *Pseudomonas aeruginosa*, *P. putida, Bacillus subtilis*

CTIA 2 – *Pseudomonas aeruginosa*, *P. putida, Bacillus subtilis* and *P. fluorescens*

CTIA 3 – *Pseudomonas aeruginosa, Bacillus. Subtilis, Pseudomons putida, P. fluorescens* and *A. faecalis*

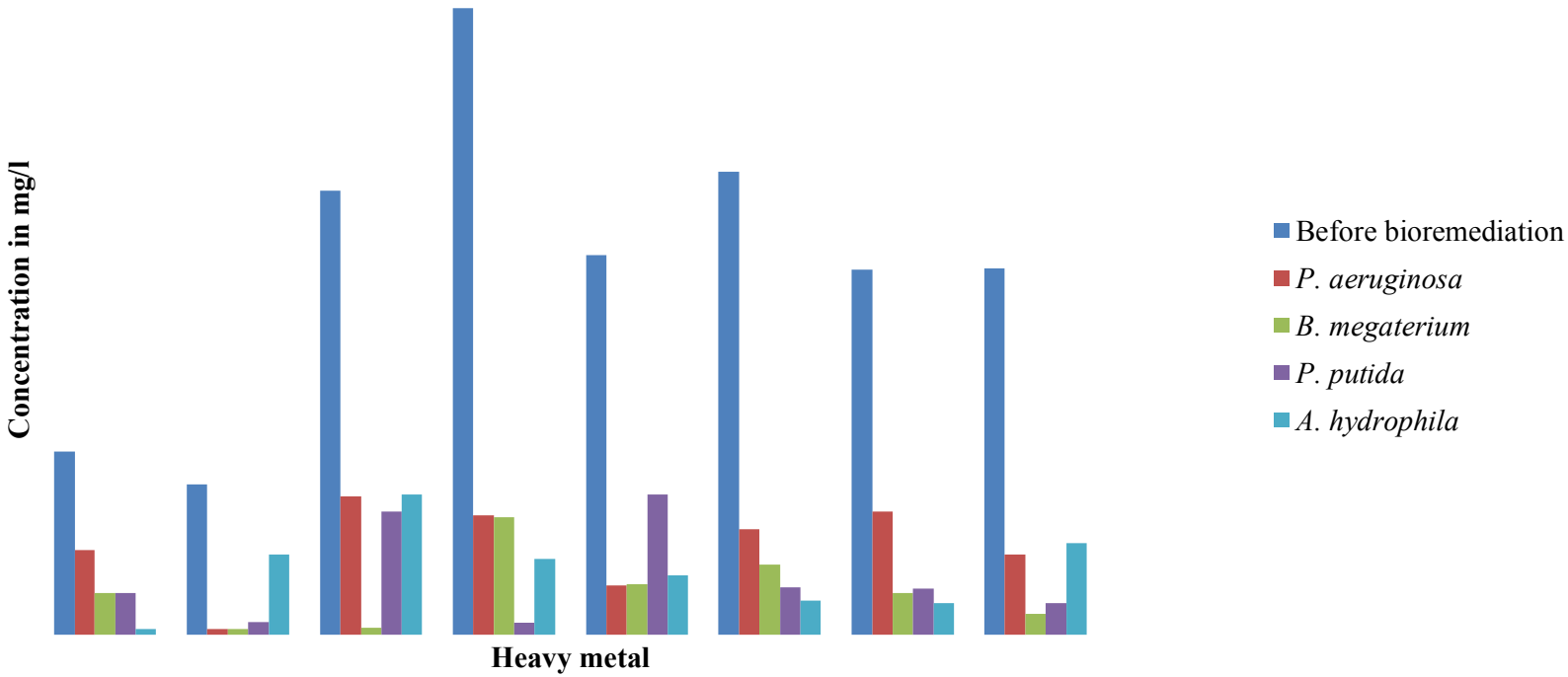


Figure 4.1 Heavy metals concentration before and after bioremediation by isolates from site A
Key

Cd = cadmium, Cu = copper, Cr = chromium, Fe = iron, Mn = manganese, Ni = nikel, Zn = zinc, and Pb = Lead

Figure 4.2 shows detailed trends of adsorption of heavy metals by bacterial isolates before and after bioremediation for site S. Adsorption rate of cadmium ranged from 0.03 mg/l (98%) to 0.8 mg/l (66%) by all isolates. Similarly the concentration of copper was 1.06 mg/l, after bioremediation *B. subtilis* demonstrated high efficacy in adsorption by 0.5 mg/l with (53%) while *P. fluorescens* had the least adsorption ability by 0.8mg/l (52%). However, chromium was adsorbed most by *Pseudomonas fluorescens* from 2.98 mg/l to 0.05 mg/l with (98%) while *B. subtilis* exhibited low adsorption by 1.2 mg/l with (59%).

The concentrations of iron were high in the effluents at site S was (4.21 mg/l). *Pseudomonas fluorescens* adsorbed much ion content of 0.2 mg/l (95%) while the *A. faecalis* had the least adsorption (1.3 mg/l) by 70%. Manganese recorded a concentration value of 2.55 mg/l after bioremediation the efficacy recorded by all isolates ranged was from 56% to 65% of removal. Nickel is next to iron in terms of concentration, after bioremediation *Bacillus. subtilis* had adsorbed Nickel from 3.11 mg/l to 0.01 mg/l (99%), while 0.6 mg/l (81%) was adsorbed by *Pseudomonas fluorescens*. A remarkable adsorption of Zn was recorded with *B. brevis* by 0.02 mg/l with (99%) while the least adsorption of Zn was recorded by *P. fluorescens* (0.5 mg/l) with 78%. The adsorption of heavy metal by consortia before after bioremediation is depicted in Figure 4.3. Consortia 3 recorded high adsorption of cadmium from 2.06 mg/l to 0.04 mg/l (98%) while consortium 1 had the least of 0.06 mg/l (97%). However consortium 3 adsorbed copper to 0.03 mg/l (97%) from the initial concentration of 1.06 mg/l while, consortium 1 had least uptake of copper by 0.5 mg/l with (53%). Chromium was adsorbed from 2.3 mg/l to 0.04 mg/l (98% reduction) by all the 3 consortia. Consortium 2 had the highest efficacy for adsorption of nickel and lead; each recorded 0.01 mg/l (98%) while the least was adsorption was showed by consortia 1 and 3 with 97% each. Zinc and iron was absorbed by all the consortia ranged from 0.04 to 0.12 mg/l with 94% to 98% and 0 0.01 to 0.12 mg/l by 99% to 97% respectively.

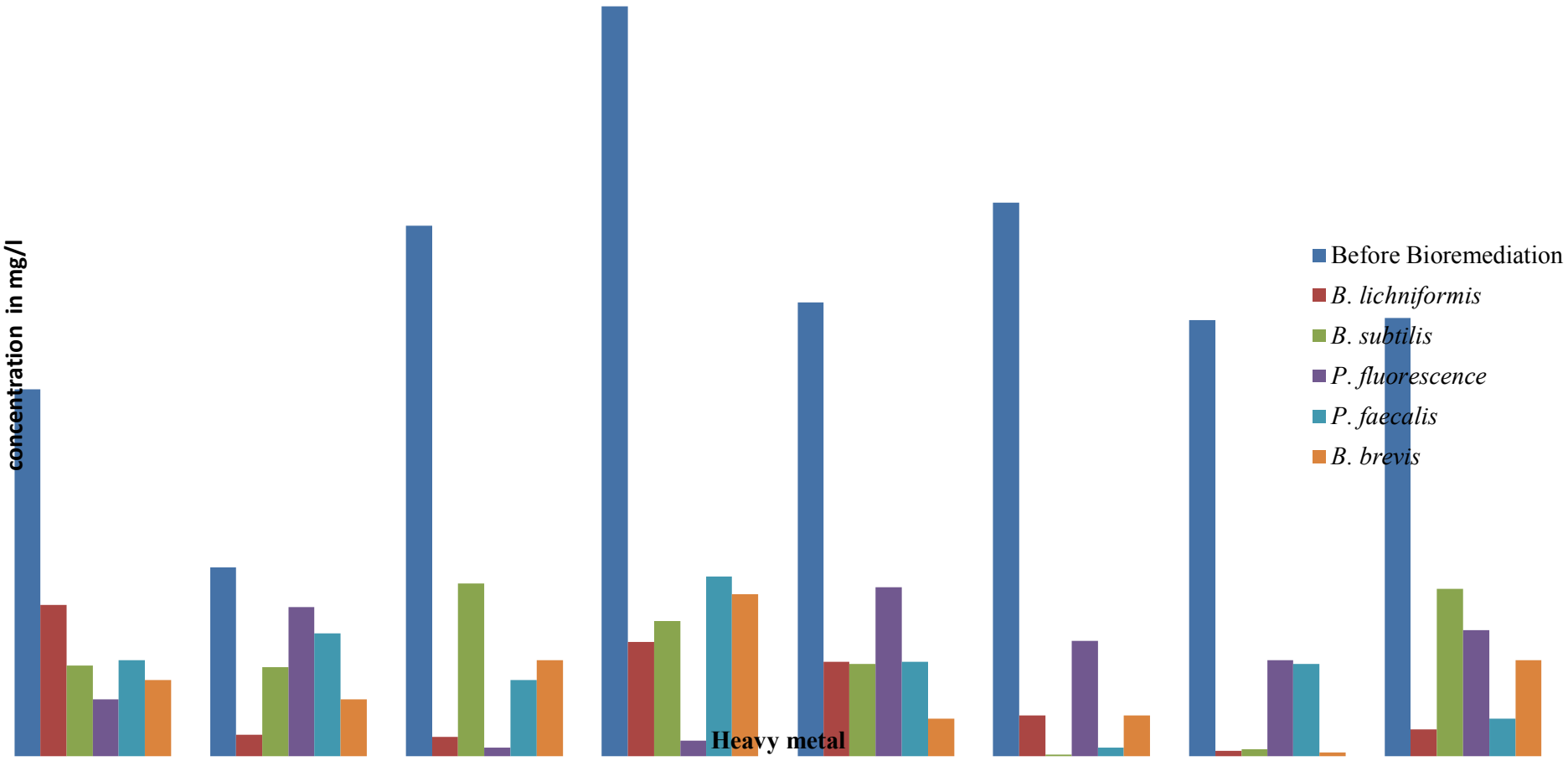


Figure 4.2: Heavy metals concentration before and after bioremediation for site S
Cd= cadmium, Cu= copper, Cr- chromium, Fe- iron, Mn- manganese, Ni- nikel, Zn- zinc, and Pb- lead

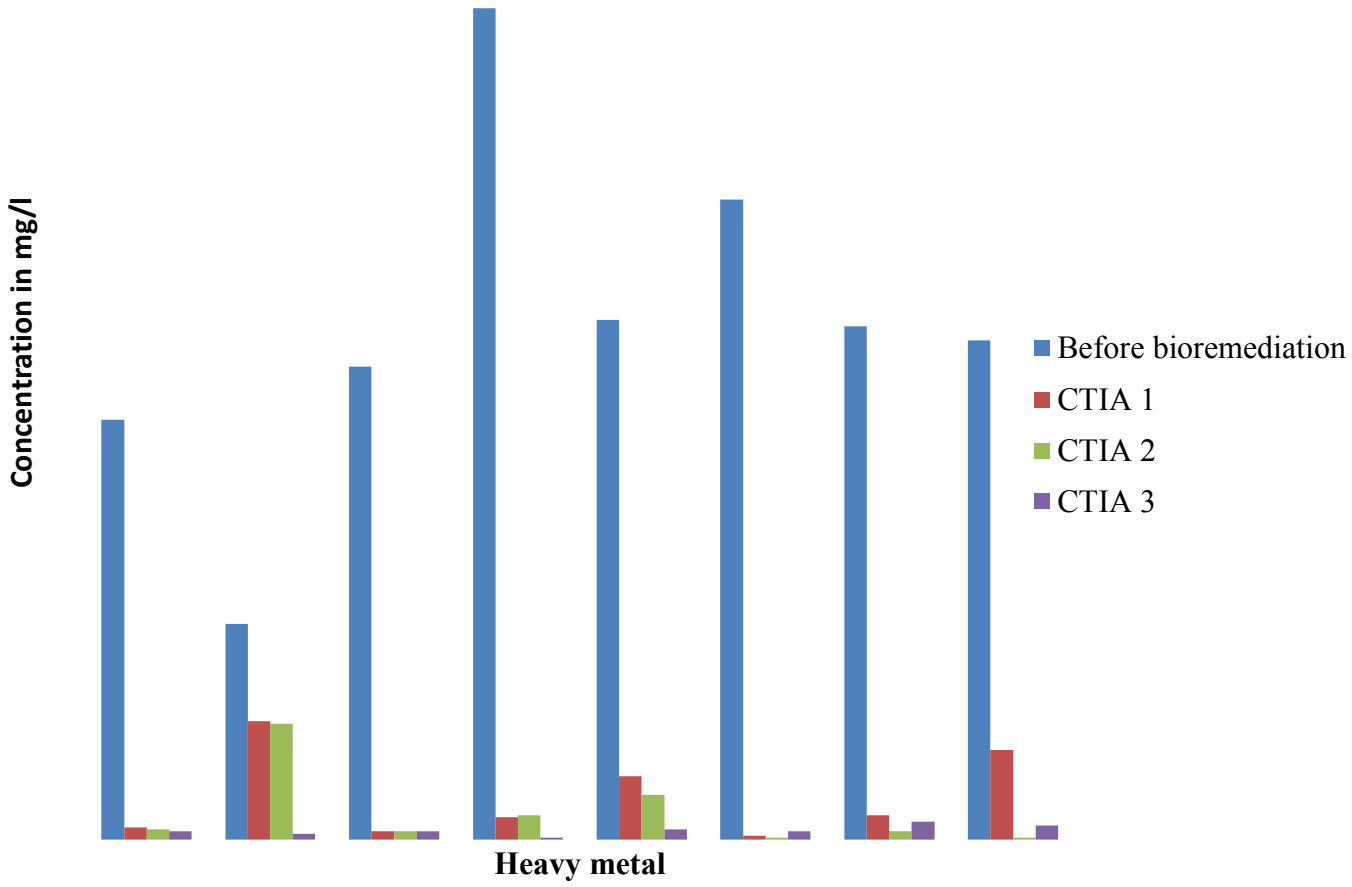


Figure 4.3: Heavy metals concentration before and after bioremediation by consortia

Cd cadmium, Cu copper, Cr chromium, Fe iron, Mn manganese, Ni nikel, Zn zinc, and Pb lead

CTIA 1- *Pseudomonas aeruginosa*, *P. putida* and *Bacillus subtilis*

CTIA 2 – *Pseudomonas aeruginosa*, *P. putida, Bacillus subtilis* and *P.fluorescens*

CTIA 3 – *Pseudomonas aeruginosa*, *P.putida, Bacillus subtilis, P.fluorescens* and *A. faecalis.*

Table 4.6: Showed overall adsorption of heavy metals obtained after bioremediation for both sites and the consortia after bioremediation. The higher adsorption of cadmium were demonstrated by *Aeromonas hydrophila, B. licheniformis,* consortia 2 and 3 with 0.04 mg/l, 0.8 mg/l, 0.05 mg/l and 0.04 mg/l respectively from the sample effluents where as *P. fluorescens* showed the least adsorption of 0.54 mg/l. The highest adsorption of Cu was demonstrated by consortium 3 (0.03 mg/l) and *A. faecalis* had the least efficacy of adsorption by (0.84 mg/l). However, *B. megaterium, B. subtilis, P. fluorescens,* consortia 1, 2 and three showed the highest adsorption with 0.83 mg/l, 0.97 mg/l, 0.43 mg/l, and 0.04 mg/l respectively. *Pseudomonas aeruginosa, Aeromonas hydrophila and B. licheniformis* exhibited low adsorption (0.93 mg/l, 0.94 mg/l and 0.11 mg/l). Iron was remarkably adsorbed by consortia 3 (0.01 mg/l), while less was exhibited by *P. aeruginosa, B. megaterium, and B. licheniformis* (0.80 mg/l, 0.08 mg/l and 0.64 mg/l). Manganese was adsorbed by consortium 3 while *P. putida,* and *B. subtilis* had the least adsorption ranging from 0.34 mg/l to 0.52 mg/l. Similarly, consortia 1 and 2 exhibited the highest rate of adsorption of nickel (0.02 mg/l and 0.01 mg/l respectively).

The results of adaptability and biodegradation of textile effluent by bacteria isolates for site A showed in the first day experience a gradual increase in biodegradation or decolourisation (Figure 4.4). However, on the third day *Pseudomonas aeruginosa* decolourised the effluent by 12.00% while *A. hydrophila* decolourised by 4.90%. There was no decolourisation/degradation in the control flask. Degradation/decolourisation continued on the 5th day, *Pseudomonas aeruginosa* degraded by 84% of sample effluents while 35.6 % was achieved by *B. megaterium.* The same pattern was observed as days increased; so the biodegradation increased. The highest Decolourisation/ degradation was by *P. aeruginosa* was (99.2%), while the least was observed in *B. megaterium* (89.00%).

Table 4.6: means comparison of heavy metals after bioremediation of the two sites and the consortia

Isolates	Cd	Cu	Cr	Fe	Mn	Ni	Zn	Pb
P. aeruginosa	0.57 ± 0.00^{b}	0.04 ± 0.00^{hi}	0.93 ± 0.85^{a}	0.80 ± 0.05^{c}	0.33 ± 0.26^{bc}	0.71 ± 0.00^{a}	0.83 ± 0.05^{a}	0.54 ± 0.00^{hi}
P. putida	0.28 ± 0.00^{ed}	0.44 ± 0.01^{g}	0.05 ± 0.00^{b}	0.79 ± 0.00^{f}	0.34 ± 0.00^{a}	0.47 ± 0.00^{d}	0.28 ± 0.24^{bc}	0.14 ± 0.00^{e}
B. megaterium	0.28 ± 0.23^{ed}	0.09 ± 0.00^{d}	0.83 ± 0.01^{e}	0.08 ± 0.00^{c}	0.94 ± 0.01^{bc}	0.32 ± 0.00^{c}	0.31 ± 0.00^{cd}	0.21 ± 0.00^{f}
A. hydrophila	0.04 ± 0.00^{f}	0.54 ± 0.00^{c}	0.94 ± 0.00^{a}	0.51 ± 0.00^{e}	0.40 ± 0.00^{bc}	0.23 ± 0.00^{e}	0.21 ± 0.00^{e}	0.62 ± 0.00^{b}
B. licheniformis	0.85 ± 0.50^{f}	0.12 ± 0.00^{h}	0.11 ± 0.00^{a}	0.64 ± 0.00^{c}	0.53 ± 0.00^{b}	0.23 ± 0.00^{g}	0.03 ± 0.00^{de}	0.15 ± 0.00^{g}
B. subtilis	0.51 ± 0.00^{dc}	0.50 ± 0.00^{g}	0.97 ± 0.00^{e}	0.76 ± 0.00^{f}	0.52 ± 0.00^{a}	0.01 ± 0.00^{b}	0.04 ± 0.00^{b}	0.94 ± 0.00^{a}
A. faecalis	0.32 ± 0.00^{bc}	0.84 ± 0.00^{a}	0.05 ± 0.00^{d}	0.09 ± 0.00^{a}	0.95 ± 0.00^{b}	0.65 ± 0.00^{f}	0.54 ± 0.00^{b}	0.71 ± 0.00^{e}
P. fluorescens	0.54 ± 0.00^{a}	0.69 ± 0.00^{f}	0.43 ± 0.00^{e}	1.01 ± 0.00^{d}	0.53 ± 0.00^{b}	0.05 ± 0.00^{e}	0.52 ± 0.00^{e}	0.21 ± 0.00^{f}
B. brevis	0.43 ± 0.00^{d}	0.32 ± 0.00^{e}	0.54 ± 0.00^{c}	0.91 ± 0.00^{b}	0.21 ± 0.00^{cd}	0.23 ± 0.00^{e}	0.02 ± 0.00^{e}	0.54 ± 0.00^{c}
CTIA 1	0.06 ± 0.00^{ef}	0.58 ± 0.01^{b}	0.04 ± 0.00^{e}	0.11 ± 0.00^{f}	0.31 ± 0.00^{bc}	0.02 ± 0.00^{g}	0.12 ± 0.00^{de}	0.44 ± 0.00^{d}
CTIA 2	0.05 ± 0.00^{f}	0.57 ± 0.01^{b}	0.04 ± 0.00^{e}	0.12 ± 0.00^{f}	0.22 ± 0.00^{cd}	0.01 ± 0.00^{g}	0.04 ± 0.00^{ed}	0.01 ± 0.00^{i}
CTIA 3	0.04 ± 0.00^{f}	0.03 ± 0.00^{i}	0.04 ± 0.00^{e}	0.01 ± 0.00^{g}	0.05 ± 0.00^{d}	0.04 ± 0.00^{f}	0.09 ± 0.00^{ed}	0.07 ± 0.00^{gh}

Means with the same superscript are not significantly different; others differ significantly ($p < 0.05$)

Cd = cadmium, Cu = copper, Cr = chromium, Fe = iron Mn = manganese, Ni = nikel, Zn = zinc and Pb = Lead

CTIA-1- *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Bacillus subtilis*

CTIA-2- *Pseudomonas aeruginosa*, *Pseudomonas putida, Bacillus subtilis* and *P.fluorescens*

CTIA-3- *Pseudomonas aeruginosa*, *Pseudomonas putida, Bacillus subtilis, P.fluorescens* and *A. faecal*

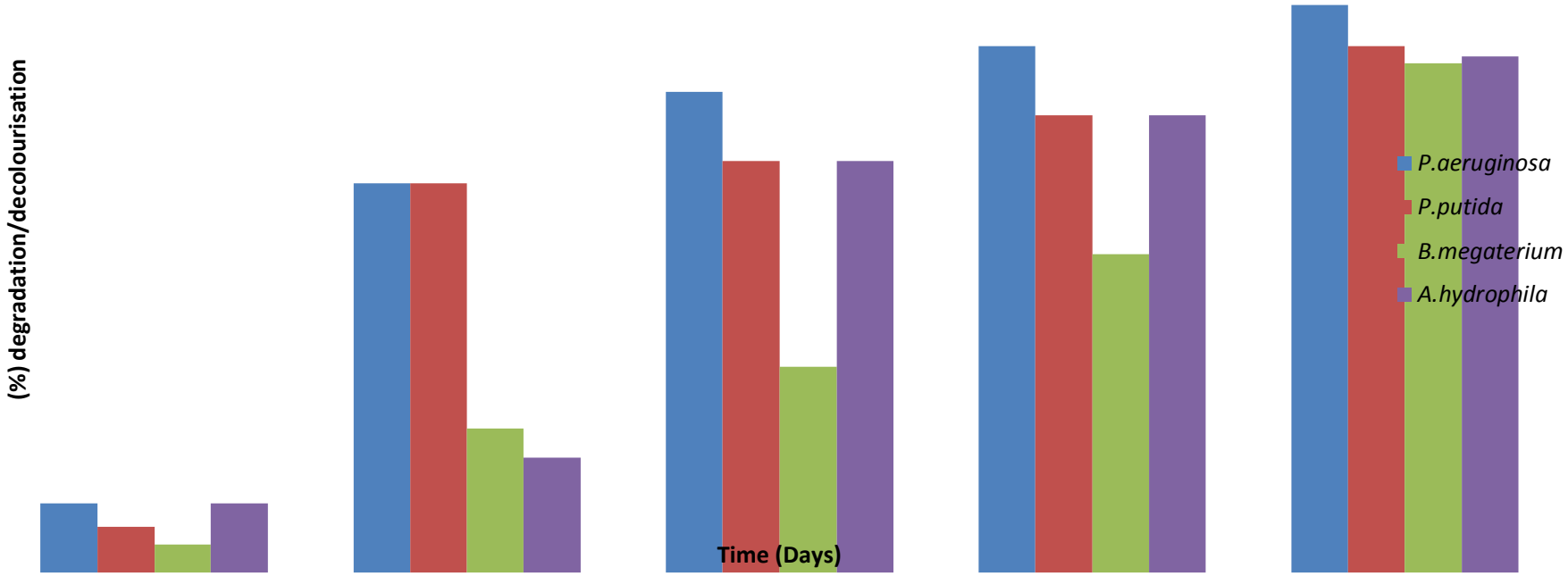


Figure 4.4: Biodegradation/ Decolourisation by isolates from Site A for ten days.

The results result of biodegradation of the effluents for site S is shown in Figure 4.5. The range of activity on decolourisation of effluent on the first day was, 15.00%, by *B. licheniformis* and *P. fluorescens* while the highest percentage of decolourisation was observed with *B. subtilis, A. faecalis* and *B. brevis* each by 20% and on the third day by, *B. subtilis* was (72.00%).

However, biodegradation continued until the 10^{th} day, and a remarkable degradation was observed in *Bacillus subtilis* indicating to be the most effective biodegrader of textile effluent with 99.6 % among the isolates from Site S, while *A. faecalis* had the least biodegradation of 91.60%.

The Degradation/decolourisation efficiency of different combinations of selected isolates obtained revealed that, in the first day, consortium 1 and 2 had degraded 8% each while the least degradation was demonstrated by consortium 3 (Figure 4.6). A rapid degradation were observed in the 5th day, consortium 1 recorded 68.0% and 69.0% by CTIA 3. Biodegradation proceeded up to day ten. Consortium 1 exhibited highest degradation of 99.7%, while, consortium 3 had decolourised by 92.0% on the 10^{th} day of the biodegradation assay.

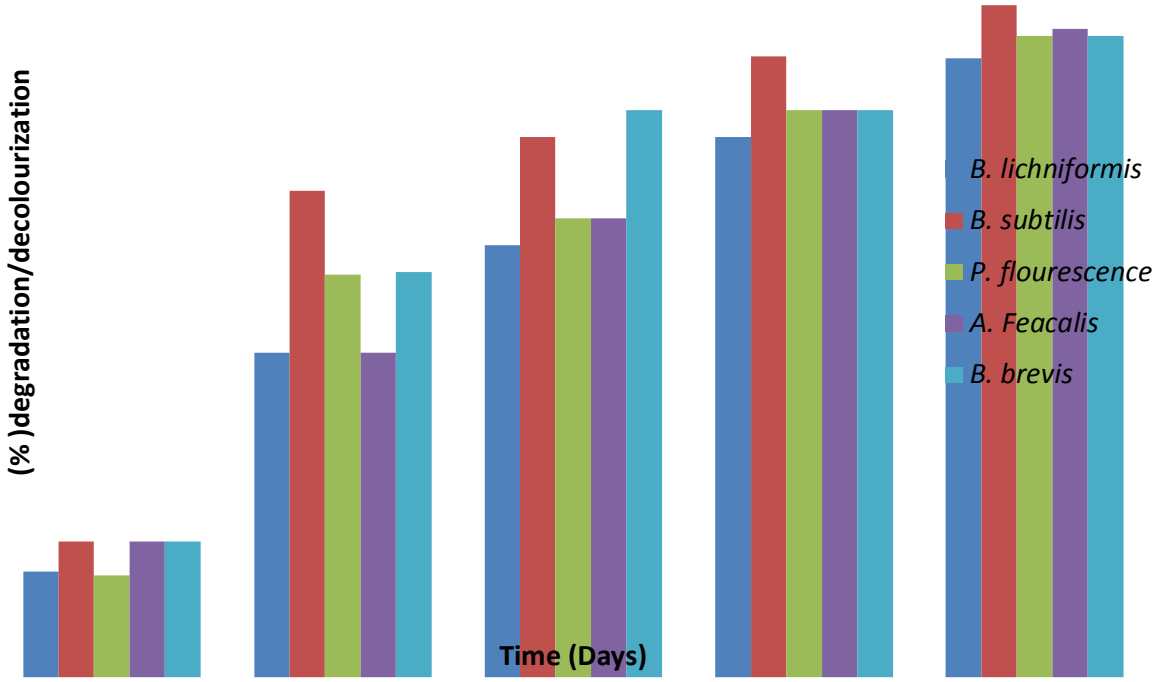


Figure 4.5: Biodegradation/decolourisation by isolate from Site S for ten days.

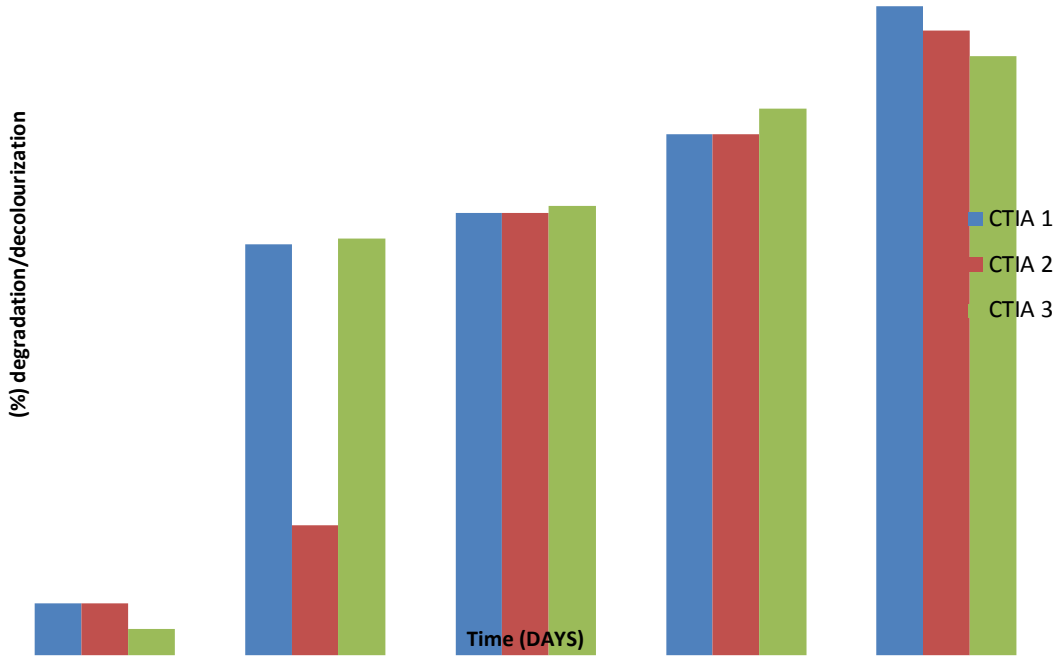


Figure 4.6: Biodegradation/ decolourisation by Consortia for Ten days

CTIA 1- *Pseudomonas aeruginosa*, *P. putida, Bacillus subtilis* (1:1)

CTIA 2 – *Pseudomonas aeruginosa*, *P. putida, Bacillus subtilis* and *P. fluorescens* (1:1)

CTIA 3 – *Pseudomonas aeruginosa*, *P.putida, Bacillus subtilis, P.fluorescens* and *A. faecalis.*(1:1)

CHAPTER FIVE

DISCUSSION

5.1 Physico-chemical parameters

The upsurge in the search for cost effective and environmentally sound alternatives to the conventional methods for dealing with wastes has been reported by Ugoji and Aboaba (2004). In this present study, the results of the physico-chemical characteristics of textile effluents indicated that, the effluents were highly polluted before bioremediation for both industries studied. This is in agreement with Olayinka *et al*. (2004); Awomeso *et al.* (2010), who reported high levels of pollutants from twelve sampled areas in Lagos contaminated by textile effluents. The effluent discharged by these industries leads to serious pollution of groundwater and soil, which ultimately affects the livelihood of inhabitant of the area. In this present study pH of the effluent Table 4.1 samples were slightly alkaline when compared to acidic pH of the dyeing effluent in previous study (Al-ghouti, 2003). In this present study, the electrical conductivity, was found to be 3050 mg/l, 2577; however, total dissolved solids was for site A 2504 mg/l and site S was 2245 mg/l and TSS 2105 mg/l, 1940 values for Site A and Site S respectively, they were above the limit of discharge , but when compared with textile effluent in India, Pakistan and Lagos was below discharge limit of 670 mg/l, 787 mg/l and 574 mg/l for EC, TDS and TSS respectively (Gark and Kaushik, (2007). High values of all physicochemical parameters in Table 4.1 suggest the presence of excess of dissolved matter in textile effluents. High values of TDS are one of the common sources of sediments which reduce the light penetration into water and ultimately decreased the photosynthetic activities. The decrease in photosynthetic rate reduces the DO level of wastewater by microorganism in the current sample. Electrical conductivity (EC) at different sampling

points generally higher; this might be connected with the release of effluents containing chemical salts during processing of dye in the textile industry. However, this may probably be due to high organic and inorganic compounds from various chemicals used during processing stages in textile industry. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. High values 1622 mg/l; 1902 mg/l and 2743; 2831 mg/l of BOD and COD respectively were noted prior to bioremediation in the present study in comparison to low values of BOD, (1501 mg/l) COD (1234 mg/l) in one effluent study by (Vandevivre *et al*. (1998) High BOD and COD levels are another indicator of an increased load of organic pollutants in the effluent.

5.2. Identification and biochemical characterisation and potential of bacterial isolates

Fifteen (15) bacterial isolates were identified using morphology and biochemical characterization from Site A (Sharada) and S (Challawa) industrial estates (Table 4.2.). Nine (9) bacterial isolates were screened and selected for biodegradation/decolourisation studies, based on their efficacy to degrade effluent and grow on MBM efficiently Table 4.3. These isolates probably have acquired natural adaptation to survive in the presence of the textile effluents, and had the degradative enzymes for degradation of effluents. This support the findings of Prasad *et al*. (2010), who isolated and characterised thirty bacterial isolates and found only three potential degraders of textile effluent belonging to *Bacillus* and *Pseudomonas* spp. while Kayode-isola *et al.* (2008) Usman *et al.* (2012) isolated *Bacillus cereus, Bacillis subtilis* and *Pseudomonas* spp isolated from refinery effluent, diesel oil and textile effluent. However, Saranraj *et al*. (2009) isolated five bacteria species in textile effluent *Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia* and *Escherichia coli.* Subsequently after biodegradation with various isolates the pH drops to slightly nearly neutral (Table 4.4, 4.5 4.6). The pH of the effluents affects the physico-chemical attributes of wastewater which in turn adversely affects aquatic life, plants and

animals. This changes the permeability which results in polluting underground resources (Buckey, 1992). In the present study, the temperature of the effluent after bioremediation ranges from 29°C to 37°C, while in comparison with report of effluents temperature by others range from 40°C to 47°C, Vandevivre *et al.* (1998) in the current studies after bioremediation (4.6) the result shows that there is statistical significant difference in pH which bring to near neutral by all isolates and consortia. However, dissolved oxygen also showed significant difference, the increased after bioremediation with *B. subtilis* 9.84, CTIA 1. 9.66 and *P. aeruginosa* with 9.02 mg/l. However, all the remaining isolates demonstrated relatively high efficacy in reduction of physico-chemical parameters but there is no statistical difference among all the isolates of the study. This is in line with findings by, Abdulrahim *et al.* (2009), who reported biodegradation of wastewater with optimum pH 6.50 at 30.0 °C, and Jaji *et al.* (2007) reported pH of 7.0 and temperature ranges from 30- 33 °C in all the sampling sites studied in biodegradation of wastewater. The increase in DO level in the effluents after bioremediation with *P. aeruginosa, B. Subtilis* CTIA 1 might be due to oxygenic nature of bacterial. The overall analyses of increase in DO and the removal of TSS, TDS, COD, BOD, and EC observed in this study agree with the observation by (Vijayakumar *et al.,* 2005). This agrees with the findings of Prasad and Bhaskara (2010); Ajao *et al.* (2011) and Samuel *et al.* (2011). In this current study, the decrease in Biochemical oxygen demand range was 50% to 63%. Total suspended solid reduction efficacy ranged by all isolates was from 48% to 60%, while total dissolve solid reduction ranged was from 50% - 60%. However the reduction efficacy demonstrated by isolates for COD was 53% to 71% levels suggests the fact that the process of bioremediation is in progress. However, this might be due to acid catalysis during nucleophilic addition reactions and probably isolates acquire positive charges that can interact with the chromophores found in effluent, as those high levels of these contaminants are often indicated in waste water containing substances that can

be biologically degraded. Remarkable reduction of all the contaminants was observed after bioremediation with various isolates from the two sites (Table 4.4, 4.5). This agreed with the findings of Ranjamohan and Karthikayam, (2004); Ajao *et al*. (2011); Salisu and Mustapha, 2010) who reported high degree of reduction in BOD having 97%, TSS (85%), TDS and (96%) by Bacillus and *Pseudomonas aeruginosa* and *Alcaligenes faecalis*. Studies show that after biodegradation/decolourisation with *Pseudomonas* species, *Bacillus megaterium*, *A. faecalis* and *B*. *Subtilis* and *B*. *cerus* showed great potential in reduction of COD, BOD, TSS, TDS, to acceptable limit Sigh *et al*. (2003). The ability of *Bacillus* and *Pseudomonas* spp in removal of COD, to 67% BOD, (78%) TSS (64%), TDS 60% and EC 40% reduction have been demonstrated by several workers (Vijayakumar *et al*., 2005; Togo *et al.*, 2008 and Usman *et al*., 2012).

5.3 Heavy metals in textile effluents sample

The presence of heavy metals in the current study, were found to be higher for both sites studied, cadmium, ranges (1.23 to 2.06 mg/l chromium,(2.98 to 2.32) copper(1.06 to 1.01 mg/l), iron 4.21 to 4.05), nickel 3.11 to 3.4 mg/l), zinc (2.52 to 2.45 mg/l)and lead (2.45 to 2.46 mg/l) which is the same other of magnitude reported in the study by (Naeem *et al*. (2009). Heavy metals presents as impurities in dye effluents or chelated as part of dye molecules. In metal complex dyes, the metal is coordinated or forms a chemical bond with the organic dye molecules. Thus, it is an indispensable constituent of the dye and governs the fastness absorb the colours. The highest value of heavy metal ions in the effluents severely affects the soil fertility and depletes the soil and its nutrients. In this present study the levels of heavy metals concentrations have contributed during production into the textile wastewater. Besides, the variations of the heavy metals concentration in wastewater sample were due to the different types of dyestuff used in different production of the threads when the samples were taken. Concentration of heavy metals in the present study could be a

serious environmental nuisance if a large Volume of such effluents is released into the environment on a regular basis without proper treatment. In line with the findings by Yusuff and Sonibare (2004), it is reported that heavy metals have been associated with the textile effluents because of copious use of chemical in dye processes. However, analysis of heavy metal using One-way analysis of variance (4.6) showed significant difference at ($p \leq 0.05$) in the adsorption of all heavy metals by isolates ranged from 54% to 98% removal. Consortium 1 performed best compared to individual organisms adsorbed all metals ranges 0.01 mg/l and 0.03 mg/l with 95% to 99%. Literatures have documented that bacterial isolates had high affinity for metals uptake and accumulated toxic metals by a variety of mechanisms (Wang *et al.,* 2010; Mathiyazhagan *et al.,* 2011). This is in agreement with this current work in which *Bacillus megaterium*, *P. fluorescens, P. aeruginosa, P. putida*, *P. fluorescens* and *A. faecalis* were reported to have the potential of adsorption of heavy metals Cd from 2.00 to 0.05 mg/l (97%), Fe from 4.30 to 1.00 mg/l (76%), Cr was adsorbed 2.0 to 0.03(85%) mg/l and Ni was adsorbed to 0.54 mg/l (54%) in wastewater from textile industries.

Other studies demonstrated the capability of several bacterial species and consortia involved in adsorption of Cr, Cu, Fe, Cd and other toxic metal from textile effluent which ranged from 57% to 70% of adsorption (Wang *et al.,* 2010; Ajao *et al.,* 2011; Mathiyazhagan *et al.,* 2011). A study showed that the temperature and the pH of water were essential parameters that affect the microbial growth, metal utilisation and activity on water of textile industry (Omalay *et al*., 2008; Mathiyazhagan *et al.,* 2011). In this present study, a room temperature of 29°C to 31°C and near neutral pH of 6.66 to 6.97, this has effectively aided the adsorption of metal and might be due to the enzymatic activities of bacterial cell in uptake of metals.

This agrees with the findings of Muller *et al.* (2001) who reported that nearly neutral environment and average temperature were effectively enhanced by the capacity of metal

adsorption. Dan`azumi and Bitchi (2010) analysed heavy metal in Kano from Challawa industrial estate and found out high concentrations of metal ions. Most wastewaters, especially industrial effluents contain different types of chemical and heavy metal as demonstrated by the study of bioflocculant which produced many bacterial species capable of uptake of heavy metals as well as decolourising effluent samples from textile industries Muller *et al.* (1992); Mathiyazhagan and Natarajan. (2011).

5.4 Biodegradation/decolourisation of textile effluents samples

The biodegradation/decolourisation obtained in this study were expressed in percentages with *Bacillus subtilis* having 99.60%, *Pseudomonas aeruginosa* (99.20%), *Pseudomonas fluorescens* (96.00%), *Bacillus brevis* (95.60%), however , *Alcaligenes faecalis* had 95.00%, *Pseudomonas putida* (92.00%), *Bacillus licheniformis* (91.60%), *Aeromonas hydrophila* (90.20%), and *Bacillus megaterium* (89.00%). The role of some bacterial spp for the decolourisation and degradation of textile dyes have also been reported by (Jumarkar *et al*., 2006; Olukanni *et al*., 2006; Togo *et al*., 2008).r, Chen *et al.* (2003); Senan *et al.,* (2004) reported the isolation and screening of bacteria capable of decolourising various azo dyes from industrial effluent samples collected from wastewater treatment sites contaminated by dyes. Iyang (2006); Prasad *et al.* (2010) and Samuel *et al.* (2011) isolated bacterial spp that are potential degraders of hydrocarbon and textile effluent belonging to *Bacillus* spp and *Pseudomonas* spp.

Our findings is line with the findings of Saranraj *et al.* (2010) who reported *Pseudomonas aeruginosa* (97.33%) as a potential degrader of dye effluent. Others include *A. faecalis* (98.44%), *Bacillus subtilis* (99.05%), *Aeromonas hydrohpila* (87.27%) and *Klebsiella pneumonia (*92.03%). In contrast to this study, Ajibola *et al*. (2005); Chimezie and Thomas, (2011) checked the ability of *Staphylococcus aureus, Bacterioides fragilis, Bacillus subtilis, Bacillus cereus, Alcaligenes faecalis, Aeromonas hydrophila, Escherichia*

coli and *Peptostreptococcus* spp in degradation of dye effluent with percentage ranges 75% of 90.00% with temperature of 31°C to 35°C and pH range of 7-8. The effectiveness of microbial decolourisation depends on the adaptability and activity of selected microorganisms (Abdulrahman, 2009). A number of microorganisms have been studied to unfold their degradative abilities in bioremediation of pollutants (Melvin, 2006). Biodegradation of textile effluent using *B. licheniformis* and *B. megaterium* has been documented by (Omalay *et al*., 2008; Praveen *et al.*, 2009). *Bacillus brevis* have the ability to decolourize textile effluent sample in twelve days Muller *et al.* (1992) All the samples exhibited effluents degrading capabilities. This is indicated by their growth in the medium which is clearly shown in decrease in absorbance density of the sample cultures Figures 4.4, 4.5, and 4.6. The variation in degrading/decolourisation demonstrated by bacterial cultures and consortia might be due to different microorganisms which have complementary and different Degrading capabilities.

The results of this present work with bacteria disagree with reports that degradation/decolourisation could be achieved in more than fifteen days (Abdulrahim *et al*., 2009; Ajao *et al*., 2011). However, our findings established with that of Mutambanengwe (2007), who reported a similar study were carried out with *E. coli*, *bacillus* spp, *A. feacalis* and *Aeromonas hydrophila* pure culture with degradation rates of 96%, 89%, 90% and 93% respectively, within five to ten days, this is attributable to the fact that bacterial spp require shorter time-span for maximum efficiency which reiterates the potential of bacterial bioremediation.

This current study showed that degradation/decolourisation continued gradually up to the 10th day with individual bacterial isolates whereas in consortia treatment, there was slow decolourisation during initial days of exposure. This sped up on the 5th to 7th days of treatment (Figures 4.4, 4.5, 4.6). Control shows no decolourisation which probably confirms

that biodegradation is as a result of metabolic activities of the introduced microbes. This can be attributed to the physiological difference and the decolourisation enzymes ability of bacterial spp used. This supports the findings of Omar *et al.* (2009), who reported that, when each pure culture was tested individually, they showed less decolourisation. In the current work, sucrose, yeast extracts $NaCO_3$, were use as co-substrate to maintain the isolates in the culture. This also tallies with a number of studies which reporedt that yeast extract, sucrose, maltose and sodium carbonate are commonly added as co-substrate for efficient textile effluents decolourisation by bacterial spp. This might be due to the metabolism of yeast extract which is considered essential for regeneration of NADH (Prasad and Bhaskara, 2011). Many pure cultures like *Pseudomonas* spp*, Klebsiella*, *Aeromonas hydrophila* and *Pseudomonas fluorescens* have exhibited effectiveness in decolourisation of different composition of effluent from textile industry with supplement of yeast extract and sucrose (Elisangela *et al.,* 2009). This is also in agreement with the present work. It was noted that although the percentage degradation was not up to 100 %, some of the liquid appeared colourless, indicating efficient decolourisation. The highest percentage (99.70%) degradation was observed in CTIA 1 (Figure 4.6). These indicated the synergistic effects of the consortia used for the biodegradation; however the most potential degraders, when subjected to bioremediation individually were *Pseudomonas aeruginosa* (99.20%) and *B. subtilis* (99.6 %). However, similar studies reported high biodegradation/decolourisation with two, three and seven consortia with almost 100% than those tested individually (Omar *et al.,* 2009; Saranraj *et al.,* 2009). This was supported by Wesenberg *et al*. (2003), Agarry *et al*. (2008) who reported that mixed culture had a higher percentage decolourisation of textile effluents than the individual isolates. This is also in accordance with study conducted by Wynne *et al.* (2001) and Stolz, (2001). Probably the synergy of microbial systems was effective in the degradation of textile effluent as well as other pollutants.

Conclusion

Although Bioremediation/degradation is a challenging process to both the textile industry and the wastewater treatment analysts, the result of this study and literature suggest a great potential for bacteria to be used to remove pollutants from textile effluents. Interestingly, the evidence for bacterial bioremediation of effluent from textile wastewaters was established. The reduction in BOD, COD, TSS, TDS, EC and adsorption of metal ions are appreciable. The removal efficiency in the level of pollutants and heavy metals adsorption paved way for the adoption of the bacteria spp which were used in this study. These findings established that the bacteria were adaptive in nature and can degrade contaminants. The ability of the bacteria to adapt and degrade effluents from textile at high concentration gives it an advantage for treatment of effluents from textile industry. It was evidently clear that Consortium 1, *B. subtilis, P. aeruginosa, P. putida, A. hydrophila Pseudomonas fluorescens, B. licheniformis, A. faecalis, B. brevis*, *B. megaterium,* consortium 2 and 3 were capable of bioremediation of textile effluents and represent a promising tool for application in biodegradation of textile industries effluents at large scale.

Recommendations

i. The bacterial spp should be screened in the laboratory for pathogenicity and toxicity before use on the field in order to avoid cross infection to plants, humans and other animals.

ii. Simple and rapid microbiological tools are required to monitor bioremediation efficacy. This will provide important information on the effective ways of harnessing environmental pollution and will give microbial ecologists further insight in response of microbial communities to pollutants.

iii. More avenues of research have arisen from bioremediation study. It is recommended to characterise the predominant bacteria using both molecular method and conventional techniques to enable control, consistency and predictability of the degradation processes. In consequence, this will lead to standardisation of the effluent treatment process.

iv. Application of the study to more dyes and identification of end products of the dyes using mass spectrometry is required to confirm the fate of aromatic amines.

v. A more complete study should be conducted on the operative parameters for the reduction of all pollutant indicators by the use of microbial organism to support efficient wastewater treatment.

vi. From the findings it is recommended that, all the tested pure culture and the consortia of bacteria used in the study should be further used in large scale as an alternative treatment system for industrial textile effluent before discharging to appropriate channels.

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Appendix I: Isolation identification and biochemical characterisation of bacteria isolates

Lab. #	GRXC	XI	MOT	NIT	LYS	H_2S	0RN	GLU	MAN	XYL	ONP	IND	UR	VP	CIT	TDA	GEL	MAL	SUC	LAC	ARA	RHA	SOR	ADO	RAF	SAL	ARG	Identified (Genus)
PA4	+	+	-	-	+	-	-	+	+	+	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	+	+	*Ps.aeruginosa*
PA22	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	+	+	+	*Bacillus brevis*
PA23	+	+	+	+	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	*Alcaligene faecalis*
PA12A	+	+	+	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	+	-	-	+	-	-	*A. hydrophila*
PA12B	+	+	+	+	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	+	-	-	+	-	-	*Actinobacillus spp*
PA27	+	+	+	+	+	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	+	-	-	+	-	-		*Pseudomonas putida*
PA47	+	+	-	-	+	-	-	+	+	+	+	-	+	-	+	+	-	-	-	-	-	-	+	-	-	+	+	*Ps.aeruginosa*
BS1	+	+	+	-	=	-	-	-	+	+	+	-	+	-	+	+	-	-	-	-	-			-	-	+	+	*Ps.shigelloides*
BS4	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+	-	-		-	+	*Bacillus subtilis*
BS5	+	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-		-	-	+	+	-	-	+	-	-	*Ps.flourescens 25*
BS6	+	+	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	+	-	-	+	-		-	-	-	.	*Bacillus megaterium*
BS10	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	+	_	+	+	*Bacillus licheniformis*
BS12A	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	+	_	+	+	*Bacillus licheniformis*
BS12B	+	+	+	-	-	+	+	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	+	-	-	*Burkholderia cepacia*
BS33	+	+	+	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	*Bacillus lentus*

Key

GRXC = GRAM REACTIO, XI= OXIDASE, MOT= MOTILITY, NI= NITRATE, LYS= LYSINE, GLU= GLUCOSE, XYS= XYLOSE, ORN= ORNITHINE, H_2S= , MAN= MANOSE , XL= XYLASE, IND= INDOLE, UR= UREASE, VP= , CIT= CITRATE, MAL= MALTOSE, SUC= SUCROSE, LAC= LACTOSE, ADO= ADONITOL , RAF= RAFINOSE, SAL= SALICIN, ARG= ARGINE and GEL= GELATINE

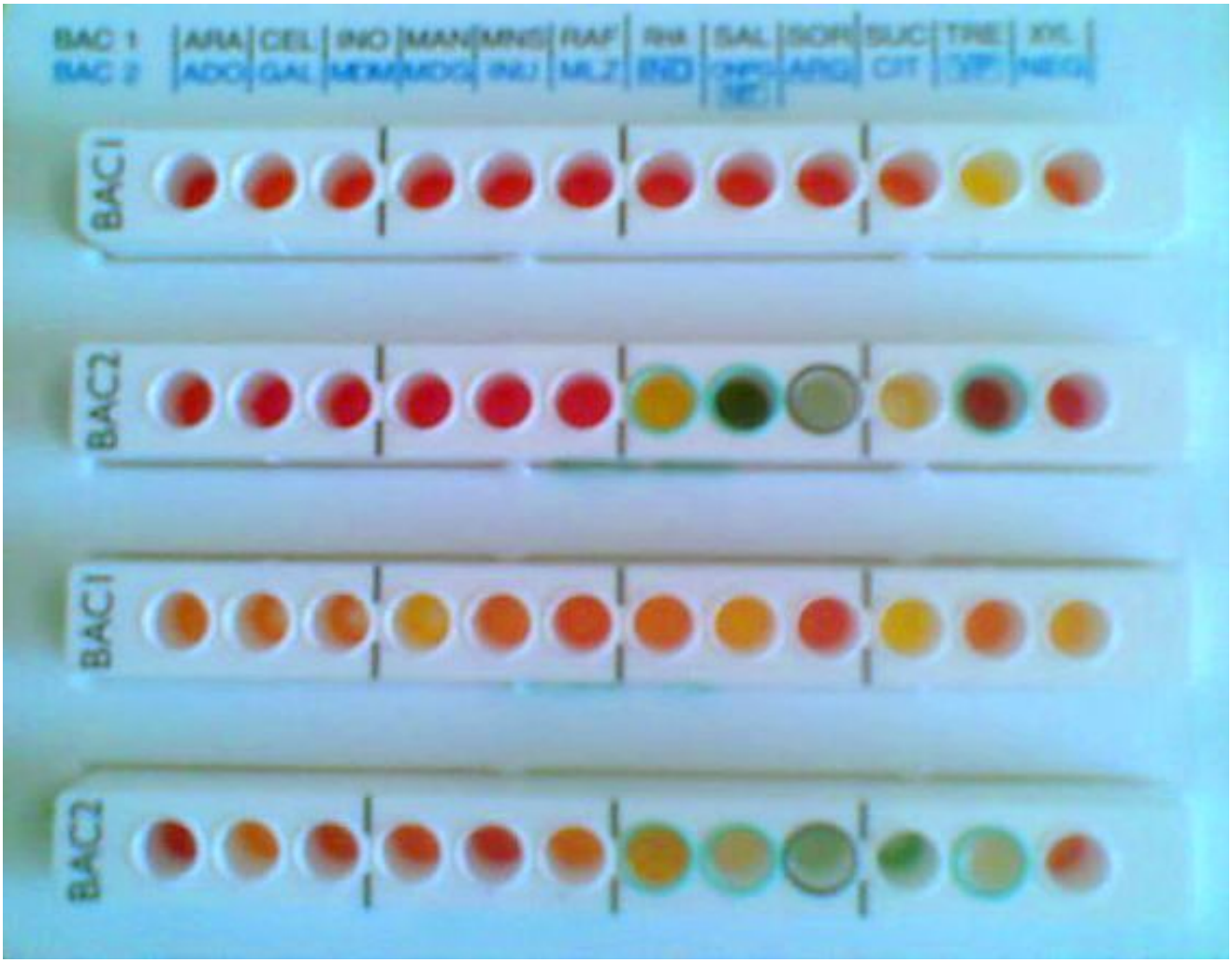
PA4-PA47= ISOLATES NUMBER

BS1-BS33 = ISOLATES NUMBER

Ps= Pseudomonas

A. =Aaeromonas

Appendix II: Microgen Test strips for Identification of *Bacillus* Spp



Key;

BAC 1 Contains various sugar tests

BAC 2 Control well of the test

Appendix III: Microgen *Bacillus*-ID 24 test report form

MICROGEN BACILLUS-ID 24 TEST
REPORT FORM

Lab. No.

Specimen Type:

Date:

MICROGEN BIOPRODUCTS

	Bacillus Strip 1 (BAC 1)												Bacillus Strip 2 (BAC 2)											
Well Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	20	24
Reaction	ARA	CEL	INO	MAN	MNS	RAF	RHA	SAL	SOR	SUC	TRE	XYL	ADO	GAL	MDM	MDG	INU	MLZ	IND	ONPG	ARG	CIT	VP	NIT	NEG
24 hours																									
48 hours																									
Reaction Index	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	
Sum of Positive Reactions																									

Profile No:

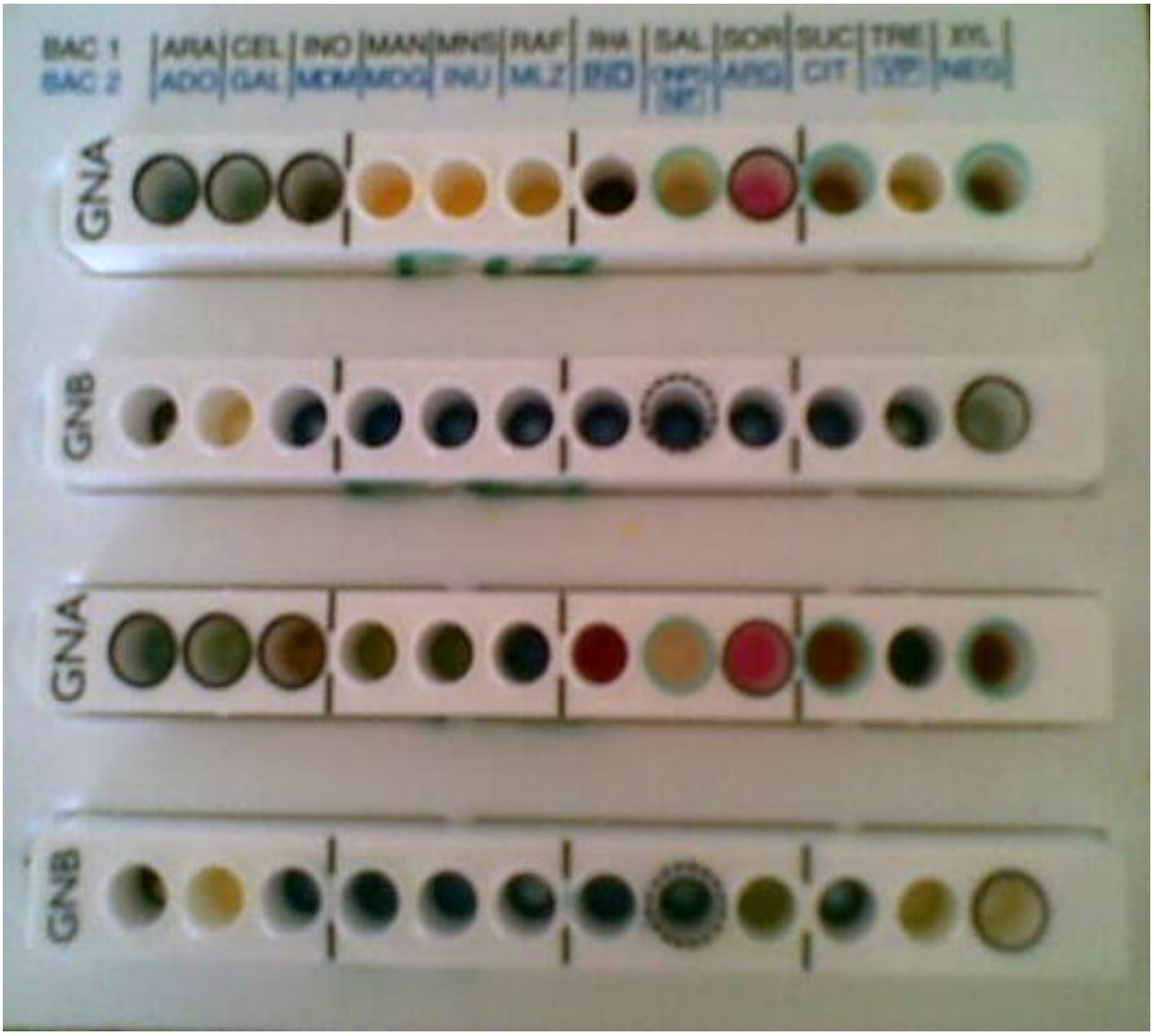
Final Identification:

WN6675/2005/02

Key:

Sum of positive gives 8 digit octal code for final identification of the organism.

Appendix IV: Microgen Test strip for Identification of *Enterobacteriacea*

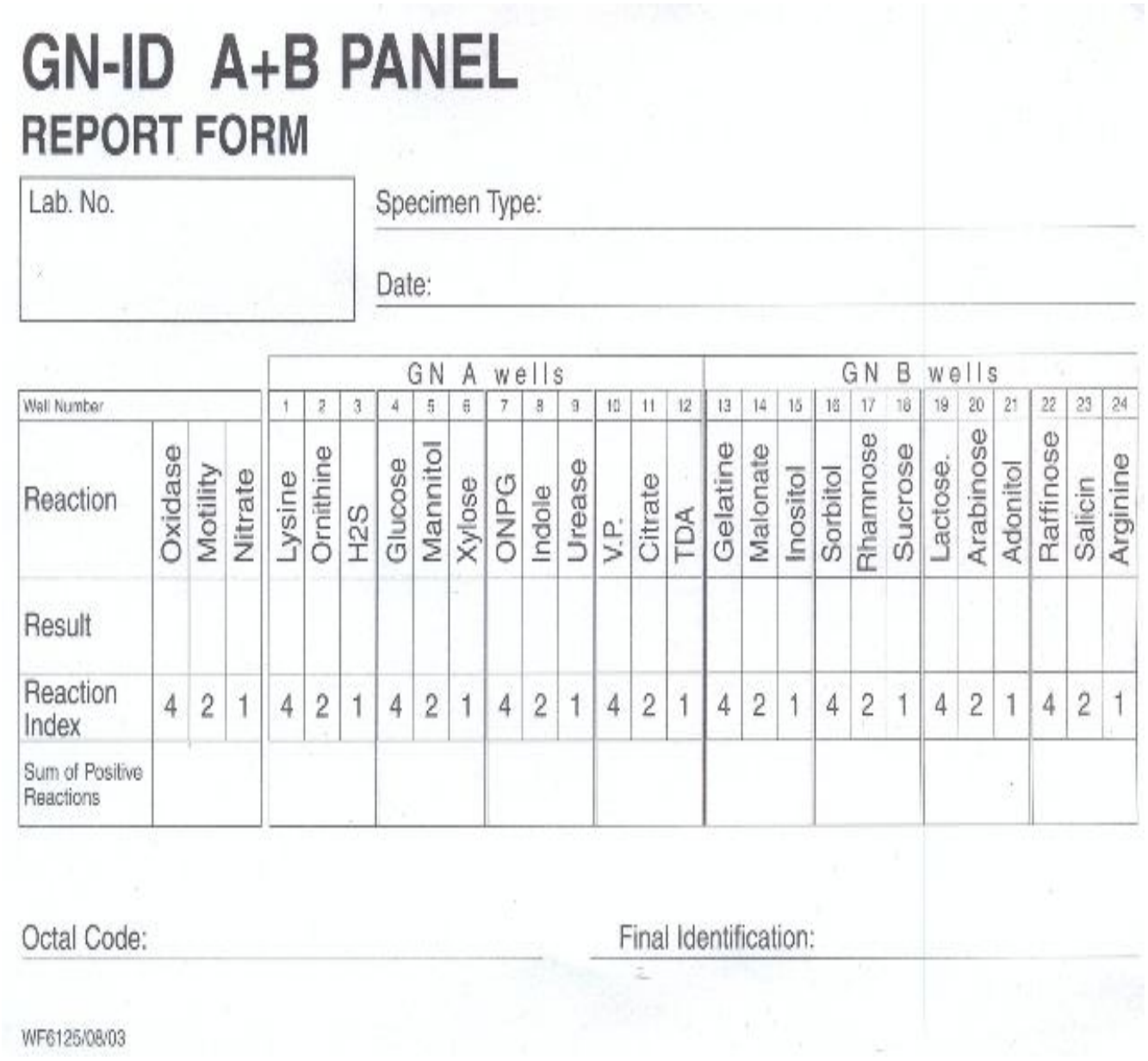


Key:

GNA Oxidase positive organism plus GNA oxidase negative organism

Which give oxidase posive organism Octal Code of nine (9) digit

Appendix V. Microgen GN-A+B Panel report form for *Enterobacteriacea*



GN-ID A+B PANEL
REPORT FORM

Lab. No.

Specimen Type:

Date:

				GN A wells												GN B wells											
Well Number				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Reaction	Oxidase	Motility	Nitrate	Lysine	Ornithine	H2S	Glucose	Mannitol	Xylose	ONPG	Indole	Urease	V.P.	Citrate	TDA	Gelatine	Malonate	Inositol	Sorbitol	Rhamnose	Sucrose	Lactose.	Arabinose	Adonitol	Raffinose	Salicin	Arginine
Result																											
Reaction Index	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1
Sum of Positive Reactions																											

Octal Code:

Final Identification:

WF6125/08/03

key

Octal Code: sum of positive (9) digits for final identification of organism

Appendix VI: Mean comparison of physico-chemical parameters for both sites and the consortia after bioremediation

Isolates	pH	Temp (°C)	TSS (mg/l)	TDS (mg/l)	COD (mg/l)	BOD (mg/l)	EC (mg/l)	DO (mg/l)
P. aeruginosa	6.65 ± 0.13^{ab}	30.10 ± 0.40	797.30 ± 137	1045.50 ± 178	804.40 ±1 88	665.30 ± 860	1051.7 ± 209	9.02 ± 0.54^{cd}
P. putida	6.56 ± 0.23^{c}	30.10 ± 0.27	898.20 ± 157	1017.50 ± 168	852.90 ± 100	689.70 ± 970	852.70 ± 118	8.88 ± 0.60^{bc}
B. megaterium	6.53 ± 0.20^{c}	30.60 ± 0.37	822.90 ± 130	1033.30 ± 216	847.10 ± 960	744.30 ± 110	838.50 ± 120	8.03 ± 0.45^{bc}
A. hydrophila	6.66 ± 0.19^{ab}	30.10 ± 0.31	856.70 ± 142	1085.50 ± 314	824.30 ± 920	725.90 ± 106	779.60 ± 980	7.83 ± 0.44^{ab}
B. licheniformis	6.96 ± 0.15^{bc}	30.30 ± 0.30	898.50 ± 161	1238.10 ± 223	1251.10 ± 269	1037.90 ± 193	1251.9 ± 279	8.38 ± 0.41^{bc}
B. subtilis	6.41 ± 0.11^{ab}	30.60 ± 0.30	899.90 ± 215	1119.30 ± 210	1341.30 ± 290	1020.30 ± 206	1138.3 ± 279	9.66 ± 0.58^{d}
A. faecalis	6.97 ± 0.26^{a}	31.40 ± 0.83	1006.30 ± 227	1145.70 ± 243	1195.90 ± 257	1008.90 ± 191	1064.4 ± 202	8.16 ± 0.40^{bc}
P. fluorescens	6.75 ± 0.90^{a}	30.70 ± 0.33	1079.90 ± 238	1226.70 ± 250	1268.50 ± 269	1010.60 ± 189	1328.1 ± 325	7.92 ± 0.33^{ab}
B. brevis	6.12 ± 0.20^{b}	30.40 ± 0.40	846.30 ± 180	965.30 ± 201	1074.70 ± 225	997.90 ± 193	944.50 ± 177	8.10 ± 0.10^{bc}
CTIA 1	6.50 ± 0.15^{c}	30.20 ± 0.30	1115.50 ± 235.75	1187.70 ± 234.83	1281.00 ± 290.90	807.00 ± 187.20	940.90 ± 275.25	9.84 ±0.50^{de}
CTIA 2	6.76 ± 0.18^{ab}	29.70 ± 0.26	1162.50 ± 268.75	1158.50 ± 228.28	1234.70 ± 284.63	783.30 ± 187.20	915.30 ± 263.97	7.53 ±0.68^{ab}
CTIA 3	6.66 ± 0.99^{ab}	30.10 ± 0.23	848.10 ± 246.96	1102.70 ± 224.58	1246.80 ± 268.94	780.90 ± 268.94	899.30 ± 258.90	7.10 ±0.48^{a}

Means with the same superscript are not significantly different; others differ significantly (P < 0.05)

CTIA-1- *Pseudomonas aeruginosa*, *P. putida* and *Bacillus subtilis*

CTIA- 2- *Pseudomonas aeruginosa*, *Pseudomonas putida, Bacillus subtilis* and *P. fluorescens*

CTIA-3- *Pseudomonas aeruginosa*, *Pseudomonas putida, Bacillus subtilis, P.fluorescens* and *A. faecalis*

Appendix VII physicochemical analysis of both Sites and consortia ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
pH	Between Groups	5.915	11	0.538	1.917	0.045*
	Within Groups	30.294	108	0.280		
	Total	36.208	119			
Temp	Between Groups	20.501	11	1.864	1.219	0.283
	Within Groups	165.076	108	1.528		
	Total	185.578	119			
TSS	Between Groups	1675363.667	11	152305.788	0.378	0.962
	Within Groups	4.347E7	108	402523.780		
	Total	4.515E7	119			
TDS	Between Groups	799672.367	11	72697.488	0.156	0.999
	Within Groups	5.045E7	108	467122.811		
	Total	5.125E7	119			
COD	Between Groups	4803432.492	11	436675.681	0.845	0.596
	Within Groups	5.584E7	108	517014.297		
	Total	6.064E7	119			
BOD	Between Groups	2342486.600	11	212953.327	0.754	0.684
	Within Groups	3.050E7	108	282407.772		
	Total	3.284E7	119			
EC	Between Groups	3171732.967	11	288339.361	0.565	0.853
	Within Groups	5.511E7	108	510233.620		
	Total	5.828E7	119			
DO	Between Groups	18.447	11	1.677	0.729	0.030*
	Within Groups	248.402	108	2.300		
	Total	266.849	119			

*significant ($p \leq 0.05$)

Appendix VIII: Analysis of heavy metals for both sites and consortia after bioremediation

		Sum of Squares	df	Mean Square	F	Sig.
Cd	Between Groups	1.526	11	0.139	14.514	<0.001*
	Within Groups	.115	12	0.010		
	Total	1.641	23			
Cu	Between Groups	1.404	11	0.128	1717.766	<0.001*
	Within Groups	.001	12	0.000		
	Total	1.405	23			
Cr	Between Groups	3.646	11	0.331	263.388	<0.001*
	Within Groups	.015	12	0.001		
	Total	3.662	23			
Fe	Between Groups	3.126	11	0.284	637.214	<0.001*
	Within Groups	.005	12	0.000		
	Total	3.132	23			
Mn	Between Groups	1.644	11	0.149	13.132	<0.001*
	Within Groups	.137	12	0.011		
	Total	1.781	23			
Ni	Between Groups	1.364	11	0.124	5702.176	<0.001*
	Within Groups	.000	12	0.000		
	Total	1.364	23			
Zn	Between Groups	1.498	11	0.136	13.209	<0.001*
	Within Groups	.124	12	0.010		
	Total	1.622	23			
Pb	Between Groups	1.302	11	0.118	270.404	<0.001*
	Within Groups	.005	12	0.000		
	Total	1.307	23			

* Significant ($p \leq 0.05$)