Characterization of Microbial Survival in the Presence of Radioactive ^{14}C Spiked Salts in Order to Minimize Waste from Graphite Moderated Nuclear Reactors

GA Pete

orcid.org/0000-0001-7325-6742

Mini-Dissertation submitted in *partial* fulfillment of the requirements for the *Master of Science in Applied Radiation Science and Technology* at the North-West University

Promoter: Prof M Mathuthu

Graduation May 2018
Student number: 17044324

NWU®
NORTH-WEST UNIVERSITY
NOORDWES-UNIVERSITEIT
UNIBESITI YA BOKONE-BOPHIRIMA

DECLARATION

I, the undersigned, hereby declare that the work contained in this Dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any University for a degree.

Signature: ....

Date: ……………..07 July 2017…………………

ABSTRACT

The Pebble Bed Modular Reactor (PBMR) is a high temperature, Gas (HTG) (helium) cooled reactor, which uses graphite as a moderator and a neutrons reflector. During the reactors operation, graphite is irradiated and contains radionuclides, such as actinides, fission and activation products.

This study investigated the removal of ^{14}C in irradiated graphite. Two experiments were performed. In the first experiment, non-irradiated graphite was exposed to a Mixed Culture (MC) of bacteria for 120 hours. The objective was to determine if bacteria thrives in the presence of inert graphite. Techniques used for graphite analysis were Scanning Electron Microscopy (SEM) and X-Ray Diffraction (XRD). SEM was used to verify the presence of bio-film on the graphite surface, which would indicate bacteria survival. XRD was used to determine whether there was a change in the graphite crystallographic structure, indicating possible metabolism of graphite carbon by the bacteria.

A second experiment was conducted with ^{14}C-containing salts chosen to represent the chemical form of ^{14}C on the surface of irradiated graphite. The MC bacteria were exposed to these salts for 120 hours as well. The bacteria samples were analysed using Denaturing Gradient Gel Electrophoresis (DGGE). The objective was to determine the diversity of the bacteria thriving in radioactive carbon species.

The results obtained from the analyses were as follows: SEM results indicated that bio-film is formed on the surface of the graphite, which indicated that bacteria can survive in the presence of graphite. The XRD results indicated that there was no change in the crystallographic structure of graphite whether or not there is bio-film formation.

The DGGE results indicated that there were bacterial species that survive in ^{14}C containing salts. When comparing the DGGE results of both salts, sodium bicarbonate was more dominant than sodium acetate in bacterial survival.

ACKNOWLEDGEMENTS

Firstly, I would like to thank God Almighty, my Creator, my strong of pillar, my source of inspiration for the wisdom, knowledge and understanding he bestowed on me during the writing of this dissertation. He has been the source of my strength throughout this programme.

Throughout my life, my late mother Constance Nonkuthalo Pete has always been there during difficult and trying times and I would like to dedicate this thesis to her. I must express my profound gratitude to her for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this dissertation. She was always keen to know what I was doing and how I was proceeding, although it is likely that she has never grasped what it was all about. I would not be who I am today without her love and support. I will miss her screams of joy whenever I reached a significant milestone and also just her general impudence. This accomplishment would not have been possible without her.

Writing this thesis has had a big impact on me. I would like to reflect on the people who have supported and helped me so much throughout this period. I would also like to acknowledge my supervisor Prof Manny Mathuthu of North-West University in the Centre of Applied Radiation Science and Technology. I am gratefully indebted to your very valuable guidance and comments on this thesis. You definitely provided me with the tools that I needed to successfully complete my dissertation. It was a period of intense learning for me, not only in the scientific arena but also on a personal level.

Finally, I am also grateful to my family members and friends who have supported me along the way.

Thank you very much, everyone.

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS AND ACRONYMS

Abbreviations or Acronyms	**Definitions**
CI	Consistency Indices
CS	Core Structure
CSC	Core Structure Ceramics
CBA	Core Barrel Assembly
DPP	Demonstration Power Plant
DGGE	Denaturing Gradient Gel Electrophoresis
DSSA	Denaturing Stock Solution A
DSSB	Denaturing Stock Solution B
FT-IR	Fourier Transform Infrared Spectroscopy
HTTG	High Temperature Test Reactor
HTGR	High Temperature Gas Reactor
HTR	High Temperature Reactor
IAEA	International Atomic Energy Agency
IR	Infrared Spectroscopy
LEU	Low Enriched Uranium
LILW	Low Intermediate Level Waste
MC	Mixed Culture
MWh	Mega Watt hour
NB	Nutrient Broth
NECSA	South African Cooperation of Nuclear Energy
PAUP	Phylogenetic Analysis Using Parsimony
PBMR	Pebble Bed Modular Reactor
PCR	Polymerase Chain Reaction
PWR	Pressurised Water Reactor
RI	Retention Indices
RS	Raman Spectroscopy
RCS	Reactivity Control System
RPV	Reactor Pressure Vessel
RSS	Reserve Shutdown System

RU	Reactor Unit
SEM	Scanning Electron Microscopy
SIMS	Secondary Ion Mass Spectroscopy
SG	Steam Generator
TBR	Tree Bisection Reconnection
XPS	X-Ray Photoelectron Spectroscopy
XRD	X-Ray Diffraction
ZR	Zymo Research

Table of Contents

CHAPTER 1: INTRODUCTION AND PROBLEM STATEMENT

1.1 Introduction: The Pebble Bed Modular Reactor

South Africa's electricity is mostly produced by coal fire stations and this amounts to about 93.5 percent, with an additional 4.5 percent coming from nuclear power from Koeberg Power Station, near Cape Town (www.wolrd-nuclear.org, accessed 18 August 2017). Hydro-electricity contributes 1.5 percent to the overall South Africa energy supply. However, there is a challenge pertaining to the hydro-electricity that South Africa is currently using. There are no more sites to build a hydro-electric power station which can mitigate against the country's electricity demand (www.gcis.gov.za, accessed 18 August 2017). With this challenge, the South African government, through the power utility called Eskom, started to explore the possibility of modelling the PBMR since 1993 (Kadak, 2005). The main intentions were to establish if the outcomes of the project could yield positive results. The ultimate goal was for the PBMR to form part of South Africa's safe and secure energy expansion programme which the country was planning to embark on. This was in light of having considered the advantages that PBMR presented (van Blerk, 2008).

Based on these challenges, it was decided that the PBMR project be explored further for possible inclusion in South Africa's future electricity generation mix. The outcomes of van Blerk's, (2008) study provided compelling evidence that, through the PBMR project the country could benefit both the energy supply and economically (Status report 70-PBMR, 2011).

The PBMR is a fourth generation reactor design, which incorporates passive safety features and requires no active cooling to prevent core overheating. It is a helium cooled reactor fuelled by uranium and moderated by graphite. Helium gas flows through the reactor core, a bed of fuel spheres or pebbles and carries the fission heat to a gas turbine-generator (Figure 1) (Fouries, 2005). The PBMR has a vertical steel pressure vessel, which is six (6) meters in diameter and approximately 20 meters high. It is lined with a 10 centimeter thick layer of graphite bricks, which acts as a reflector and transfers passive heat. The graphite brick lining has vertical holes for the control rods. There is a power convention unit that is joined to the reactor and it has a generator unit, and a closed circuit helium cycle (American Nuclear Society, 2011).

The reactor is designed to produce 400 MWh of electricity. The pebbles which contain the source of fuel are re-distributed within the reactor until the burn-up stage (Reitsma, 2004). Furthermore, the PBMR technology provides an alternative electricity generation source with an insignificant contribution to global warming through carbon emission. Such a technological advantage is considered beneficial to the economy and the environment (Koster, 2008). Figure 1 shows the layout of the PBMR.

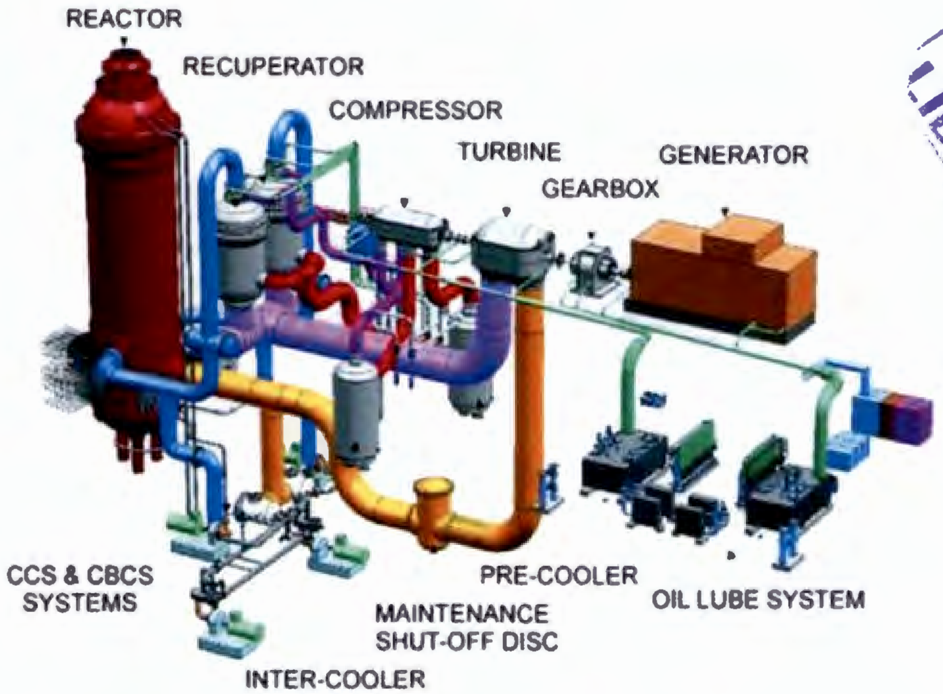

Figure 1: The schematic layout of the PBMR (Fouries, 2005; Mahlangu, 2009)

According to Koster (1998), the PBMR uses silicon carbide coated particles that are enriched using uranium oxide, encased in graphite to form a fuel sphere that is the size of a tennis ball. Helium is used as the coolant and energy transfer medium to a closed cycle gas turbine and generator system. When fully loaded, the core would contain 310 000 fuel spheres (Koster, 1998).

The pebbles contain graphite which provides additional moderation during reaction in the core. Inert helium is used to remove the heat that is generated in the reactor core, which in turn is used to turn turbines and generators. The helium coolant gets into the reactor vessel when it is approximately 500°C and moves down between the hot fuel spheres exiting at the bottom of the vessel after having been heated to a temperature of about 900°C. The hot gas gets into the first of three gas turbines, of which the first two drive the electrical generator. The coolant leaves the last turbine at about 530°C, after which it is cooled recompressed, reheated and returned to the reactor vessel (American Nuclear Society, 2004).

The PBMR is a modularised technology with one module consisting of the main building and generator that are housed in an area of about 4181 m^2 (113 x 37m). The height of the building is about 61.1 meters, and more than a third (22, 5 meters) is underground. Therefore, considering these dimensions, the two PBMR modules can fit on a soccer field (Nicholls, 2001).

During the PBMR operation, the fuel pebbles circulate through and out of the reactor at the bottom and are replenished on top. The circulating fuel design allows for on-line refuelling. Figure 2 provides a cross-sectional view of the core design, including the graphite reflector blocks, the annular pebble bed region and the fuel outlet chute (Fechter, 2008).

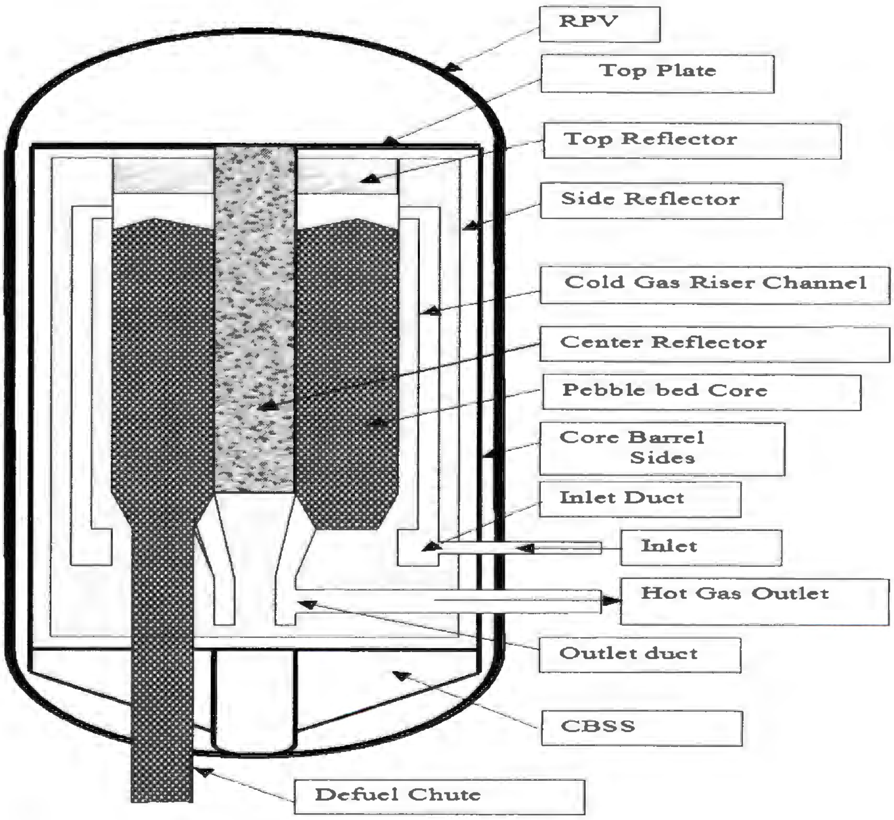

Figure 2: PBMR Vessel Cross-section (Koster, 2008)

The average core height is 11 meters and the annulus thickness is fixed at 0.85 meters. The centre reflector (2 meters in diameter) and side reflectors are manufactured from nuclear grade graphite blocks that are stacked in columns forming the geometry of the core (Koster, 2008). A PBMR fuel pebble is approximately the size of a tennis ball, as shown in Figure 3 below.

Figure 3: PBMR fuel spheres compared to the size of a tennis ball (George, 2001)

When it is full, the core would contain about 330 000 fuel spheres and 110 000 pure graphite spheres, of which the latter is added for neutron moderation and heat conduction (Nicholls, 2001).

The first coating layer deposited on the kernels is porous carbon, which allows fission products to collect without over-pressuring the coated fuel particles. This is followed by a thin coating of pyrolitic carbon (a very dense form of heat treated carbon), followed by a layer of silicon carbide (a strong refractory material), followed by another layer of pyrolitic carbon. The porous carbon accommodates any mechanical deformation that the uranium oxide particle may undergo during the lifetime of the fuel. The pyrolitic carbon and silicon carbide layers provide an impenetrable barrier designed to contain the fuel and the radioactive products of the nuclear reactions (Nicholls, 2001). The fuel particles are referred to as TRISO particles, reflecting their TRistructural (porous carbon, SiC and pyroltic carbon) ISOtropic nature (Slabber, 2006). In order for the PBMR project to be fully realized through execution a technical and governmental strategic decision must be taken. This is with the view that the country will note this consideration when looking at waste management and minimization thereof (Dunzik-Gougar, et al., 2008) .

The PBMR fuel is based on a German AVR technology with uranium oxide forming the kernel of each fuel particle as shown in Figure. 4 below (George, 2001).

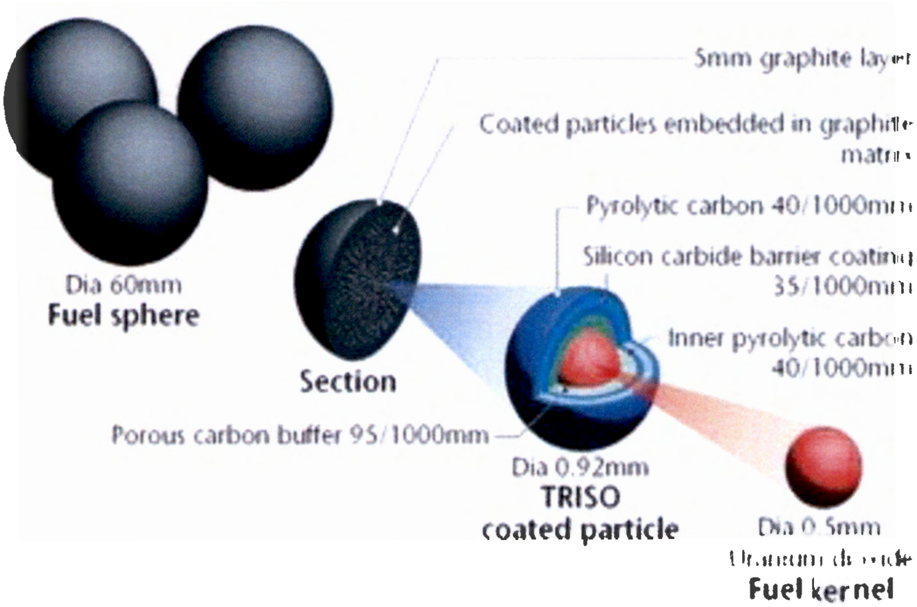

Figure 4: Anatomy of a PBMR fuel sphere containing TRISO coated particles (Mathibe, 2008)

Table 1 below gives details pertaining to the fuel sphere design.

Table 1: Pebble Design Consideration (Reitsma, 2004)

Fuel Spheres	Units	Values
Pebble radius	cm	3.0
Thickness of fuel free zone	cm	0.5
Density of graphite in matrix/fuel free zone	g/cm^3	1.74
U-235 enrichment of uranium	wt%	9.6%
Coated Particles		
Kernel diameter	µm	500
Kernel density	g/cm^3	10.4
Coating Material		C/C/SiC/C
Layer Thickness	µm	95/40/35/40
Layer Densities	g/cm^3	1.05/1.90/3.18/1.90

The reactor core is a very vital component in the reactor as it allows the on-going loading and off-loading process of fuel spheres. Every fuel sphere is allowed an approximate 10 times cycle in the reactor core. Once the fuel sphere has gone through the cycle, it is tested for mechanical strength and reliability by a scrap separator (Reitsma, 2004).

Once the fuel spheres are considered burned, fresh fuel spheres are inserted at the same position to restock the core. The rate of restocking the core is commutated through the detached burned fuel and the necessary additional reactivity. Every particle is numbered in positions externally to the core. The regulation of adding new fuel spheres, this ensures that the reactor core reactivity is retained at the design value for standard operation (Koster, 2008).

Due to the on-going refuelling processes in the reactor, the reactor is accessible all the time because there is a need to maintain and examine any movement of components. Therefore, this requires that caution be taken when designing any on-line maintenance activities that must be undertaken in order to preserve inevitable reactor shut downs (van Blerk and Kozak, 2008).

Based on global best practices, the PBMR gives two self-regulating and different reactor shutdown systems. One of the systems comprises of control rods that travel within the side reflector. During normal operation of the reactor, these control rods maintain the reactor power output consistent with desired altitudes, which assist in power alterations outlet levels when preferred. In addition, the reactor permits refuelling of fuel spheres when its power is between 50% and 100% (Reitsma, 2004). The control rods are inserted into the side reflector when the reactor is at full power (Slabber, 2006).

The negative reactivity is caused by the Reserve Shutdown System (RSS), which consists of 18 vessels that are packed with borated graphite particles. These borated particles go into the outstanding borings, when there is an opening in the valve due to power being lost (Kadak, 2005).

The heat that is made throughout the nuclear reaction process inside the reactor core is transferred to the heat system and transformed into functional energy which is a form of steam produced when the water is heated, ultimately producing electricity. The steam that is produced is from the Steam Generator (SG) focal instrument. The produced steam originates from the helium gas which circulates on the inside of the SG pressure vessel. Whilst the heat flows inside the pressure vessel, water travels through the pipes to yield steam at a regulated pressure with the required temperature to be utilised in the production of electricity (American Nuclear Society, 2004; Reitsma, 2004). The circulator picks up cold gas which comes from the end of the SG, located where water that is circulating in the tubes is fed into and which is where the feed water enters the tubes (Reitsma, 2004).

1.2 PBMR Design

The PBMR is a generation IV technology and has advanced passive safety features. Amongst the advantages of the passive safety feature is the less reliance on human and engineered responses when an error happens during reactor operations. The inherent technology features are such that an automatic shutdown will occur resulting in a halt and only disperse decay heat which can be illustrated on a declining curve and will not cause any reactor core catastrophe or discharge of radionuclides into the atmosphere (Status Report 70-PBMR, 2011). The technological strength and advantages are attributed mainly to the design, the materials used, the fuel and the natural physics involved, rather than the engineered active safety systems in a Pressure Water Reactor (PWR) (Slabber, 2008).

The helium, used to transmit heat from the reactor centre gas turbines that generate the power, is chemically inert. It is non-combustible and cannot be associated with other chemicals. The inherently safe design, which makes the necessity of the safety grade backup structures and off campus emergency plans superseded, is important to the cost saving attained over other nuclear designs (Kessides & Kuznetsov, 2012). One of the essential design modifications between present generation reactors and High Temperature Gas - Cooled Reactors (HTGRs) with coated particle fuel, is the separate "containment" function of each fuel sphere. The characteristic scheme of these fuel spheres, combined with the cutting-edge design of the reactor, avoids a main or severe loss of containment (Slabber, 2006).

When compared with the vast atmospheric releases emanating from fossil-fuel energy, nuclear wastes occur in minute, exceedingly controllable quantities which can be dumped without harming people or the surroundings (Lalk, 2013). The main benefit of nuclear power reactor radioactive waste is that it is so small in volume compared to the corresponding waste from a coal-fired power station. One kilogramme of uranium in the PBMR fuel has a bigger energy output than 430 tonnes of the best coal with ash content of up to 40 percent. A large coal-fired power station uses approximately 2 200 trainloads of coal per year (six a day), while only two truckloads of fuel per week will be needed for 22 PBMR nuclear power reactors of the corresponding volume. The PBMR demo unit is located at Koeberg and ten truckloads are needed for the early load and only four truckloads per annum for the replacement of used fuel (Kadak, 2005).

A 165 MWh PBMR module will generate approximately 32 tonnes of used fuel pebbles per year, out of 32 tonnes uranium (Lalk, 2013). The storage of used fuel from PBMR will be simpler as compared to the storage of fuel rods that come from the nuclear reactors because of the cooling systems that is necessary to avoid failure of the fuel (Slabber, 2006). The pebble bed modular reactor has been designed to take care of radioactive waste in a safe manner. The reactor provides space for used fuel which can be stored in dry tanks for nuclear power stations whose operation life is expected to be 40-years. Whilst the used fuel is stored in the reactor, the fuel will not be removed from the site in which the reactor is constructed. Furthermore, once the reactor has been shut down, the used fuel can be stored safely in accordance with the IAEA safety standards on site for an additional 40 years before being taken for final disposal in which the following safe waste acceptance criteria for safe storage must be considered (Slabber, 2006):

- Firstly, the fission products are put in a layer of silicon carbide. This layer forms a protective layer around the fission products, thereby preventing pollution of the environment
- Secondly, the fuel has been put in a graphite sphere, which is a stable material. This means that the spheres will not disintegrate. The configuration of the spent fuel will therefore not change
- Finally, the density of spent fuel in each sphere is so small that the repository can accommodate millions of these spheres.

A PBMR module is made up of a graphite moderated helium cooled reactor where the gas is heated by the nuclear fission process and a direct cycle power conversion unit in which the heat is changed into electrical energy by means of a turbine-driven generator (Venter *et al*, 2005). This change happens in the main power system and uses a recuperative Brayton cycle that uses helium as the working fluid. A schematic layout of the cycle is shown in Figure 5 below.

The basic use of the reactor is to generate heat. This is done by sustaining a fission chain reaction in the centre. This fission chain reaction makes fuel fission products. The reactors' safety uses are all focused on keeping the liberation of the fission products from the fuel as low as possible. In the PBMR reactor, this is made possible through protecting the fuel by making sure that there is a safe shutdown of the reactor and the ability to take away the decay heat during all operating and accident conditions. The PBMR reactor is only able to do this in a natural and passive way by relying only on gravity, conduction, convection and thermal radiation (American Nuclear Society, 2004).

The PBMR reactor is based on the Germany developed High temperature Gas-Cooled Reactor technology. This includes but is not limited to the use of spherical fuel elements, referred to as pebbles, which are the same as the fuel which was developed for the German High Temperature Reactor (HTR) programmes (Venter *et al*, 2005).

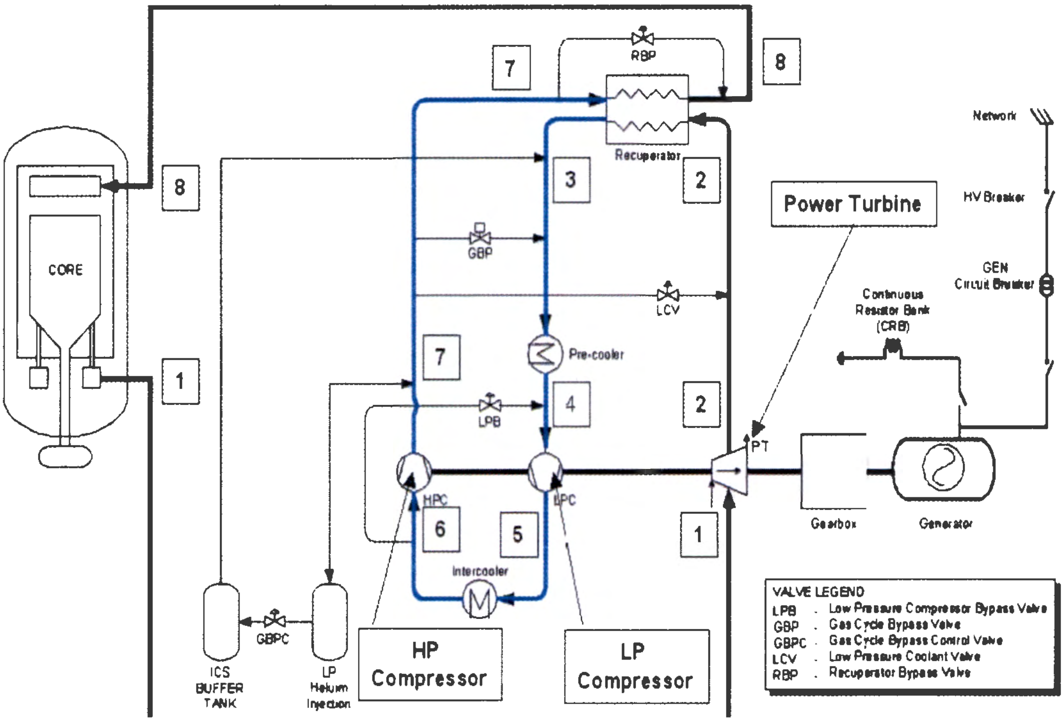

Figure 5: Layout of the PBMR Recuperative Brayton Cycle (Venter *et al*, 2005)

The Reactor Unit (RU) (Figure 6) is made up of the fuel, the Core Structures (CS), the Reactivity Control System (RCS) and the RSS, all contained inside the Reactor Pressure Vessel (RPV). The centre of the RU consists of an annulus filled with 60 mm fuel spheres. The volume of the annulus is large enough to ensure a low power density (~5 MW/m^3) and combined with the large heat capacitance of the RU results in the ability to survive a depressurised loss of forced cooling accident. The aspect ratio of the RU resembles a tall and slender cylinder to optimise the heat transfer area to pebble bed width, to enhance the passive heat transfer path whilst keeping the fuel and component temperatures within limits (Venter *et al*, 2005). The passive heat transfer path takes the fuels' heat to the environment after the RPV. The effectiveness of this heat transfer path enables the RU to withstand a depressurised loss of forced cooling without passing any of the component temperature limits, resulting in a design that does not need coolant to take away decay heat from the fuel (American Nuclear Society, 2004).

The fuel is based on proven TRistructural-ISOtropic nature (TRISO) coated fuel particles with Low Enriched Uranium (LEU) dioxide developed for the HTR-Modular reactor. TRISO fuel was extensively used in the AVR reactor as well as many other test facilities over a large range of temperatures, neutron fluences and burnups (Auwerda, 2014). The overall coated particle is approximately 1 mm in diameter. The fuel particles are moulded into 50 mm diameter graphite spheres, each containing approximately 14 500 coated particles. This 50 mm graphite sphere is then surrounded by a further 5 mm thick, fuel free, graphite layer resulting in an overall fuel sphere of 60 mm diameter. The fuel spheres are contained in an annular core volume shaped and supported by the graphite reflectors of the CS (Venter *et al*, 2005).

Venter et al, 2005 states that "The main parts of the CS are the metallic Core Barrel Assembly (CBA) and the Core Structure Ceramics (CSC)". The CSC is made up of the reflectors that are grouped into the top, centre-, side- and bottom reflectors. The basic structural material of the reflectors is purified graphite, made in wedge shaped blocks. The graphite blocks are put in vertical columns and supported by the CBA. The CBA is a steel cylindrical shell that is found and supported within the steel RPV. The inlet and outlet pipes to and from the RU connect to the CSC from where the gas flows through the pebble bed from the top to the bottom (Auwerda, 2014).

The flow scheme in the RU receives gas from the compressor via the two Core Inlet Pipes where it flows into an inlet plenum. The gas then gets distributed in riser channels in the side reflector that transports it up to the top of the pebble bed. The gas then flows down through the pebble bed where it is heated through the bottom reflector until it gets collected in the outlet plenum and transported back to the turbine through the Core Outlet Pipe. This flow scheme keeps the hot gas inside the graphite at all times, thereby protecting the metallic components of the CBA and RPV from the high temperature gas.

Reactivity control and shutdown of the core is accomplished by the RCS, with the RSS providing a diverse backup system for long-term shutdown of the core. The RCS is made up of 24 rods which have neutron absorbers that can be raised or lowered in channels in the Side Reflector. The RSS can put small graphite spheres with boron carbide B_4C into channels in the Centre Reflector, adding enough neutron absorption to keep the reactor subcritical at cold conditions (100°C and xenon free core). Both the RCS and RSS are designed as fail safe systems, by putting their respective neutron absorbers into the core under gravity alone, even if all the power falls away. A schematic vertical and horizontal cross-section through the RU is shown below in Figure 7 and Figure 8 respectively.

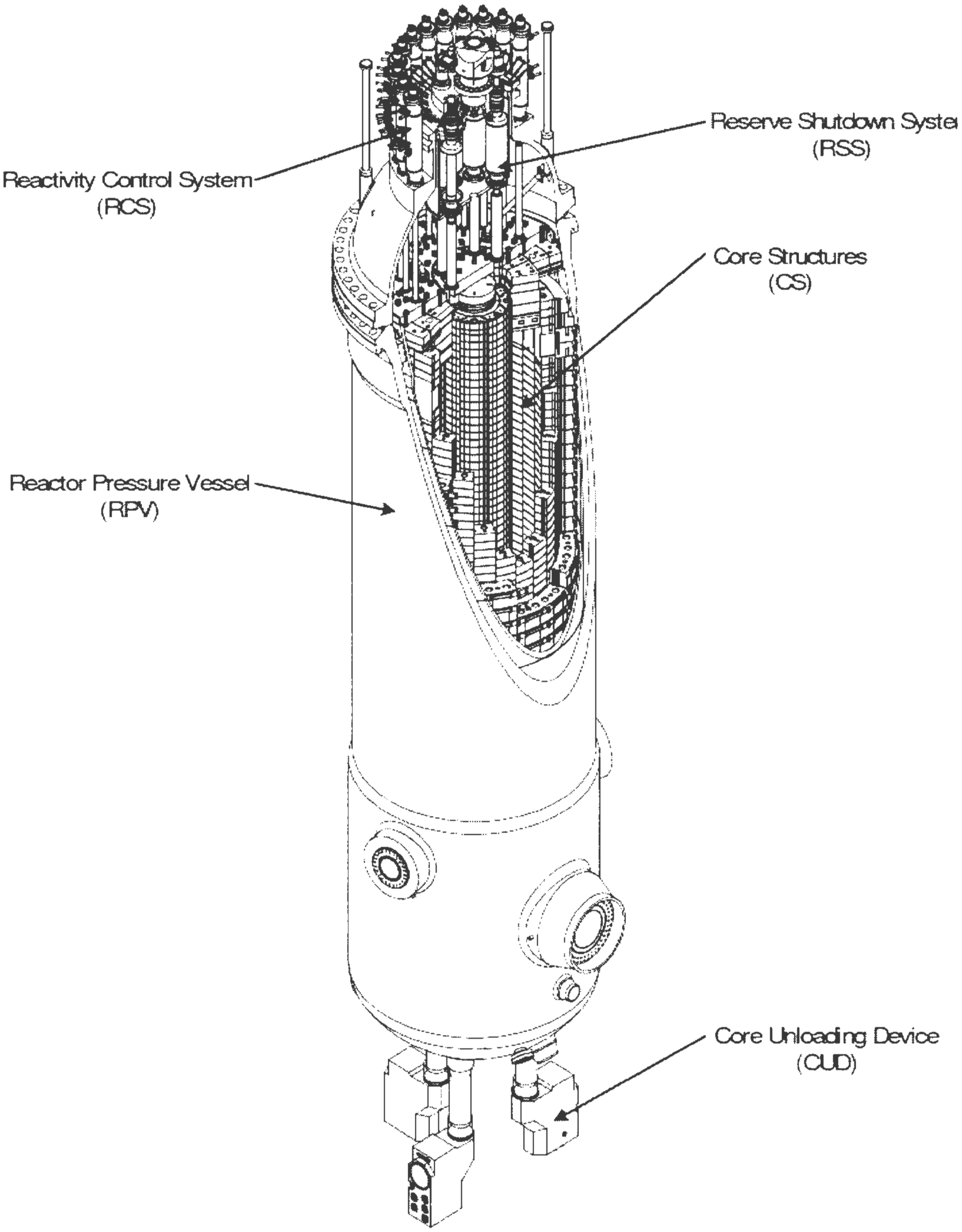

Figure 6: General Arrangement of the RU inside the RPV (Venter *et al.* 2005)

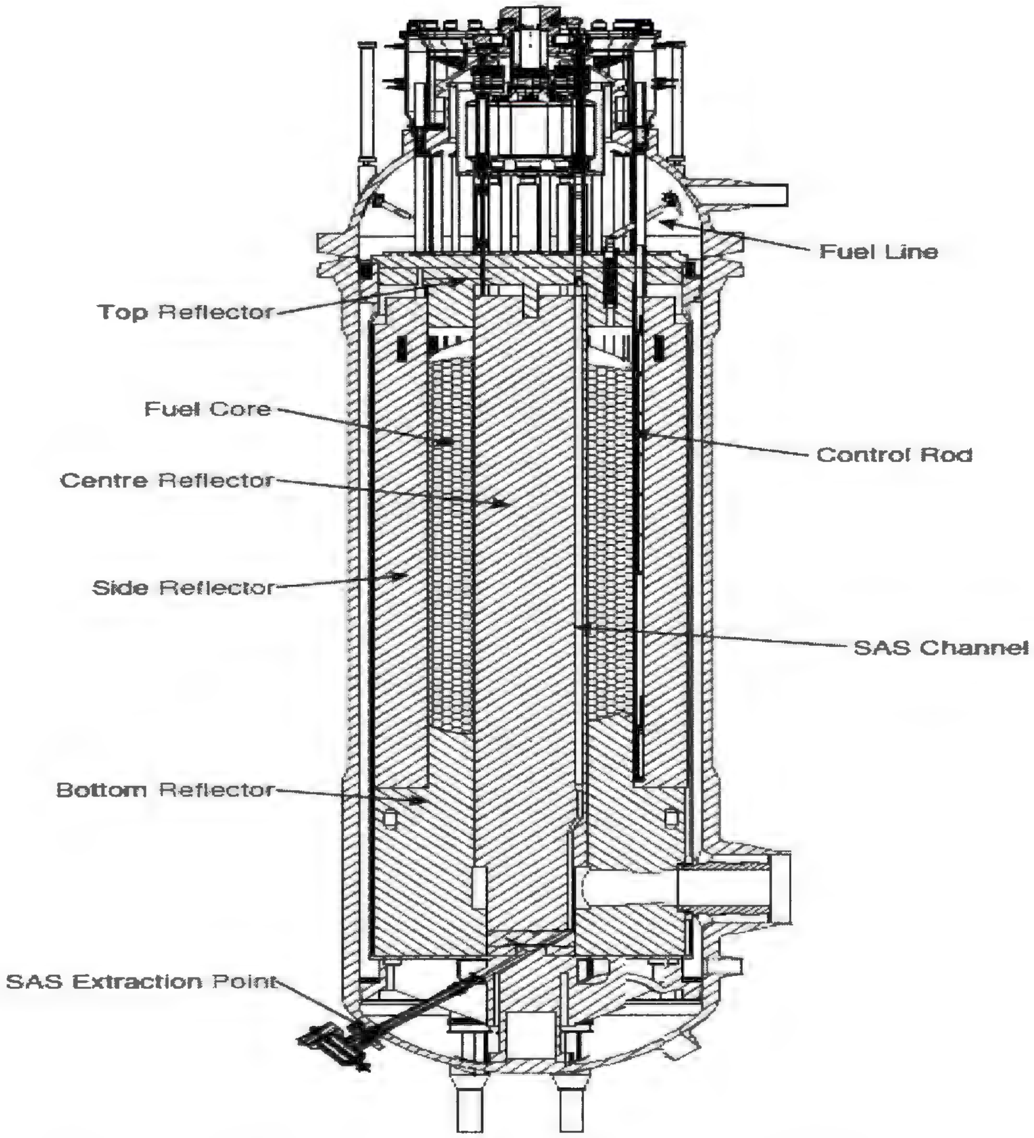

Figure 7: Vertical Schematic Section through the RU (Venter *et al*, 2005)

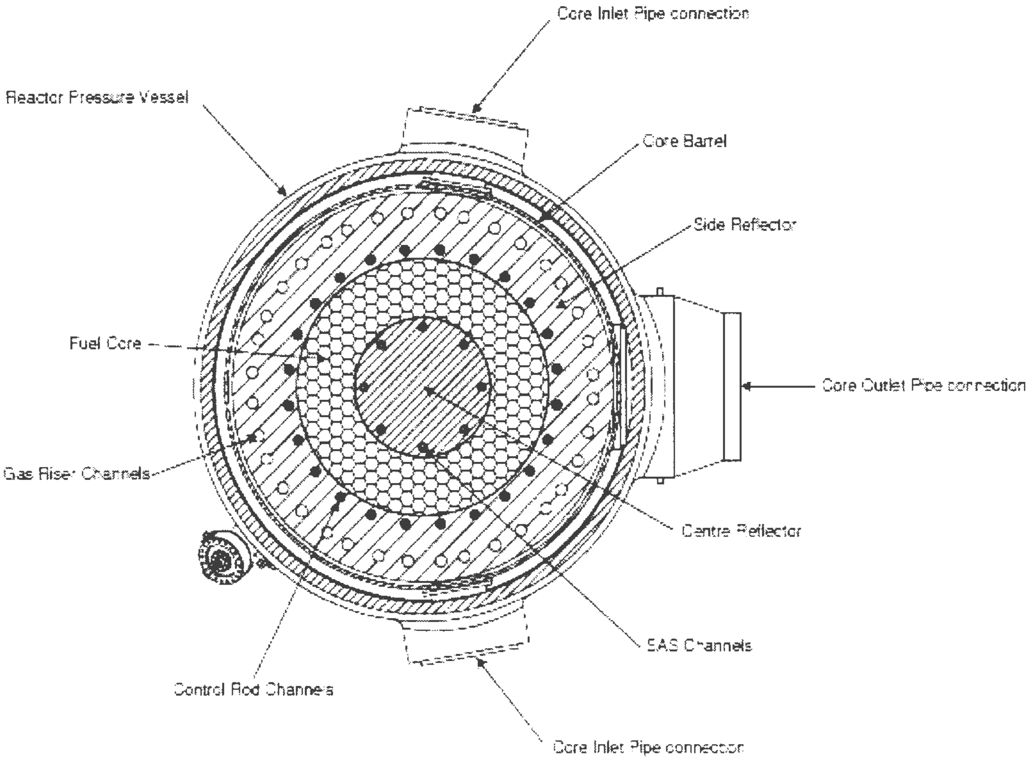

Figure 8: Horizontal Schematic Section through the RU (Venter *et al*, 2005)

1.3 The Features of a PBMR

The PBMR design enables a simplified process. The key structures of the effective PBMR layout according to (the Status report 70-PBMR, (2011) are:

- The on-line refuelling, through loading and off-loading of fuel spheres which enable high reactor availability. The PBMR on-line refuelling technology process permits the insertion of new fuel at the upper part of the reactor and the removal of used fuel to be at the lower part of the reactor (Cogliati, 2011). The ability for the reactor to be able to do such a refuelling process prevents the reactor from having scheduled shut downs. Furthermore, the modular model permits other reactor modules to be joined in supplying the steam to the turbine in the process plant. The independence of each module permits the creation of steam to continue uniformly, in the event where a reactor is detached resulting in condensed capacity
- The physical proficiency which is attributed to a high reactor vent temperature – In this section, there is highly automated control and protection of the

reactor core. At this stage of the reactor operation, the reactor is computerised for standard operational control and necessitates minimal reactor operator involvement. Furthermore, the comprehensive computerised protection system avoids major tool destruction.

The country's electricity demand is still lower than the capacity however, with moderate population growth, this will ultimately result in peak demand peak for electricity which will exceed demand if there is no intervention considered such as building new power stations. Furthermore, Eskom which is the current existing nuclear power station has an aging infrustructure which will reach the end of its operational life around the year 2025 (Kessides and Kuznetsov, 2012). Therefore, South Africa needs to make plans for producing the additional 20 000 MW of electricity required by 2025 (over and above the currently installed 39 000 MW) (Venter *et al*. 2005).

In addition, conventional power stations can increase the power by between 600 and 1000 MW. Usually, the time required for a power station that is powered by coal comprising of 6 x 600 MW units, is approximately eight years and that results in extra connection if economic growth is not as anticipated. If little duration is permitted, this will require plant operators to radically shorten the time it takes to make judgement on the prospect of additional capacity in the power plant. Eskom, like most plants globally, goes through short, sharp electricity demand in winter that is a challenge to accomplish with the gradual ramping features of the current large power stations (Venter *et al*, 2005).

Eskom considered all these and other challenges posed by conventional technologies and opted to pursue PBMR technology with the view to exploiting some of the inherent advantages the technology provides such as convenient deployment where it is needed and the time it takes to build and commission one (Lalk, 2013).

1.4 Problem Statement

As indicated previously, under standard operation lifecycle, approximately 3000 fuel particles are distributed every day and out of the 3000 fuel spheres, roughly 350 are cast-off as spent fuel and substituted with fresh spheres. The value or number of the fuel spheres depends on the number of cycles, which is measured primarily in relation to the fuel burn (Greyling, 2017). The balance of the fuel spheres disposed in the storage tank is verified on a daily basis and the fuel spheres that are considered to still be intact are returned to the reactor core (Cogliati, 2011). The balancing process must always be in compliance with the International Atomic Energy Agency (IAEA) regulatory standards or accounting and control when verified. Should there be any non-compliance, irregularities or illicit activities, this will be discovered rapidly by both the reactor operator and the IAEA. The IAEA monitors and controls the non-divergence of the spent fuel and this will be recorded before any large quantities can be removed (Status report 70-PBMR, 2011).

In addition to the physical protection systems that are in place to detect any unauthorised attempts to remove fuel, any deviations can be identified through the quick removal of fuel, making it difficult to reach the stability of the reactor. This requires modification in the primary upgrading to prevent the core from being uncotrollable if there is an addition of fuel spheres (Windes, Burchell and Bratton, 2007).

However, the disadvantage of the fuel spheres is the large amount of waste coming from the graphite which is attributed to the coated particles; thus has high transport cost implications. Moreover, with this disadvantage comes the advantage, which is the little unit heat manufacture which creates a possibility of 10 years of storage (van Blerk *et al*, 2008). The decision on the way forward largely dependents on the country's specific policy and economic status.

There are other types of radioactive waste in addition to the spent fuel that is produced during and after the reactor operation. The configuration of the waste generated from the operation of High Temperature Reactors (HTRs) is largely of a diverse nature with diverse properties. The waste property changes can be attributed to the helium because the gas escapes easily from any vessel. However, in order to continuously know the amount of gas in the reactor core, calculations must be done (van Blerk *et al*, 2008).

Radioactive waste is so small in volume when it is compared to the same waste from, a coal-fired power station. One kilogramme of natural uranium churns out the same energy output as 17 tonnes of coal with an ash content of up to 40 percent. About six trainloads of coal a day are used by a large power station that is fired by coal, while a nuclear power station with the same capacity uses only one large truckload of fuel per year (Windes *et al*, 2007).

According to Kadak (2008), the PBMR produces approximately 19 tonnes of used fuel pebbles in a year and from the 19 tonnes, just under one tonne results in depleted uranium. This is due to the silicon carbide coating on the fuel spheres and the radioactive decay particles are inaccessible for about a million years, which is longer than the activity of any of the radioactive products, including plutonium (Kadak, 2008).

Nevertheless, nuclear waste can be arranged proficiently and safely with the PBMR. Within the reactor, there is a dry storage tank which is allocated to store used fuel. The fuel is stored for a period of 40 years which is the operational life of the PBMR (Lalk, 2013). Once the time has elapsed, the used fuel will be taken away from the site. The process of storing fuel occurs after the reactor has been shut down, the used fuel is removed from the dry storage tanks where it was placed for a period of 40 years and deposited on site for an additional 40 years before it is deposited off for final storage and no decision has yet been made on the location of a site (Venter, Mitchell and Fortier, 2005).

The benefits that are brought about by the PBMR technology are that, it is capable of affording South Africa with inexpensive power generation coming from the coast. The competitive urge can place South Africa in the international global differentiation as it will be exceedingly economical compared to other methods of energy generation. Currently, most of South Africa's coal-fired electricity is produced by large-scale reactors which are located on the coal producing areas in the eastern side (Slabber, 2006). The location of the reactors, make the transportation of coal to other locations which are far from the coal producing areas expensive because it will result in high capital costs and possibly result in losing electricity during transmission (Venter *et al*, 2005).

The pebble bed reactors which were made in Germany almost 20 years ago, came about through the AVR pebble bed research reactor which produced 40 MWh, operating for about 22 years. This, therefore, gave an indication that the pebble bed technology works. The AVR reactor created heat by permitting the helium gas through the reactor core containing fuel sphere having uranium content. The viability of the technology was found to be safe, simple to operate and refuel, modular and economic which provided the advancement of the reactor technology leading to the pebble bed reactor concepts (Kadak, 2005). By utilising the direct and indirect cycle helium gas to produce power, the new reactor designs are aimed at achieving 45% efficiency compared with the 32% of the old designs. Furthermore, this type of design does not need complicated and expensive emergency core cooling systems as the centre cannot melt because the design does not use hot water.

Therefore, the challenges linked with sustaining high temperature water systems in the reactor are eradicated (Kadak, 2005). Based on the economic interest of the country, the direct cycle has the advantage of promising higher efficiencies upon total optimisation. Furthermore, the possibility of water leaks during normal operations thereby causing component corrosion and other intrinsic dynamics associated with water damage are avoided in the direct cycle. Therefore, this allows a high outlet temperature of the coolant that can be utilised in industrial applications and hydrogen production (Koster, 2008).

In the PBMR reactor, the pebbles are found in the reactor centre. The inside of the centre is made up of carbon blocks of graphite, these carbon blocks serve as reflectors and physical support of the reactor. Another additional feature in the PBMR reactor is the inclusion of the central graphite reflector, with the aim of allowing higher power level to be produced whilst retaining the efficiency of the external control rods in the outer reflector (Kadak, 2005).

The PBMR radioisotope inventory irradiated graphite has products from neutron activation of the graphite impurities and radionuclides that come from the fuel particles and move into the graphite in a number of ways (Wickham and Marsden, 2006). The authors argue that the most significant of the long-lived nuclides is ^{14}C, the bulk of which is generally found on the graphite component surface.

Worth noting is that the core structural part of the PBMR adds to the waste. Products such as neutron activation emanating as impurities of the irradiated graphite form part of the radioisotope inventory of the PBMR. One of the radioactive isotope that is of importance is ^{14}C due to the long half-life it possess. In addition to the long half-life of the ^{14}C is that, the element is forms part of the graphite crystallographic structure (Dunzik-Gougar, et al, 2008).

Carbon-14 is a radioactive isotope that decays by beta emission with a mid-life of 5730 years, and it interacts chemically in essentially the same way as the stable isotope carbon-12. Therefore, in our biosphere, carbon-14 would easily be incorporated into the carbon cycle and into carbon-based life. The combination of its lengthy life and the easy transport into the biosphere make ^{14}C a concern for long-term disposal (Wickham and Marsden, 2006). Removal of ^{14}C from the large volume of irradiated PBMR graphite may result in several benefits. First, the volume of radioactive waste requiring long-term disposal would be reduced. Secondly, ^{14}C could be incorporated into a stable waste form, from which release into the biosphere would be severely restricted. Finally, with the removal of much of the activity, the highly purified nuclear grade graphite may be more easily recycled for a variety of applications. This study investigated ways of minimising the radioactive waste produced as a result of ionising radiation which is a serious concern (Kadak, 2005).

1.5 Aim and Objectives

The work presented in this dissertation was designed and performed to support a larger project focusing on irradiated graphite remediation. The overall project plan was to perform proof-of-concept experiments to demonstrate that most ^{14}C can be removed from irradiated graphite surface via bioremediation

1.5.1 Aim

The aim of this research was to characterise Microbial Survival within Radioactive ^{14}C Spiked Salts in order to minimize graphite waste produced in Graphite Moderated Nuclear Reactors.

1.5.2 Objectives

The objectives of the study were to:

- Investigate if microbes can live in the presence of graphite, a chemically nonreactive and presumably inhospitable material
- Study the effect of microbes, living in the presence of graphite, on the phase structure of the material
- Characterize the nature of microbes living in the presence of a source of ^{14}C chemically representative of ^{14}C on the surface of irradiated graphite.
- Perform a proof-of-concept experiments to demonstrate that most ^{14}C can be removed from irradiated graphite surface via bioremediation.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Electricity demand in South Africa is increasing and has resulted in most parts of the country experiencing blackouts as the supply of the electricity can no longer meet the demand. Most of the South African businesses have had to close down because of blackout occurrences, which affected the country's economy (Van Wyk, 2013; Brown, 2017). A few years ago, South Africa was producing more electricity than it needed, which made the price of electricity supply cheap. The electricity generation was from coal. Now, that the demand is growing and the supply is low, this has resulted in an increase in the price of electricity (Rennkamp and Bhuyan, 2016). Furthermore, due to the growth in demand and the country's development in both business and infrastructure, the country has had to consider other options available for electricity generation and security. Options considered were hydropower, wind, solar or nuclear energy, as this would result in having an energy mix (Brown, 2017). The generation of power using other resources, such as hydropower, has been a problem because when using hydro-electricity, the area of demand needs to be close to the water source (Brown, 2017 and Lalk, 2013). Most areas in South Africa do not have dams or rivers close by. Wind and solar generated electricity are useful for small power needs in remote locations, but they cannot serve as base load power generators like coal and nuclear plants (Brown, 2017) (http://www.eskom.co.za; last opened on 04 March 2017).

Coal is the current choice for most power production in South Africa. However, its reserves are dwindling (www.universalcoal.com, accessed 18 August 2017) and the use of coal leads to large-scale pollution such as that currently affecting other developing countries including China (IRSN last access July 2017). Furthermore, the concentration of the current coal fleet is near the pit-heads of two extensive coal producing areas, inland on the eastern side where abundant coal reserve are located. Therefore the location of these plants requires long power lines from the coal-rich areas to load centrers away from the pit-heads, which in turn implies high capital costs and transmission losses and therefore poses a threat to electricity supply in the country. Transporting coal to power stations that are far away is expensive (Kessides et al. 2012). The chances for producing hydro-electric power or natural gas power in South Africa are severely limited as indicated previously (Kadak. 2005), due to the uncertain weather conditions resulting from the lack of strong winds or low water supply.

At the moment. the demand in South Africa is currently lower than the capacity. although it is expected that new capacity will have to be commissioned by 2030. Even moderate growth of 2.5 percent will mean that the peak electricity demand will still exceed capacity by then. In addition, Eskom's older power stations reach the end of their design life after 2025 (Kessides et al, 2012). South Africa will therefore need to acquire and make use of all natural resources to produce the extra 20 000 MW of electricity that will be required by 2025 (van Blerk *et al*. 2008). Eskom also experiences high demand in winter and this is difficult to accommodate with the slow ramping characteristics of the existing large power stations. These factors prompted the investigation into small electricity generation plants that can be placed near the demand points (Lalk, 2013).

South Africa has one nuclear power plant and is considering building three new nuclear plants to help meet the growing demand. One type of nuclear technology that has been considered by the South African government is the PBMR (Slabber, 2006).

The PBMR concept. which has a short construction lead-time. low operating cost and fast load-following characteristics, seems a good option (American Nuclear Society, 2004). It is a reactor that is changeable and flexible. It is being made in a modular fashion. which allows for extra modules to be added according to the demand. It is much less location-dependent than hydro-electric or fossil-fuelled power stations. The term modular emanates from the design focus that duplicate modules can be positioned in a block of 4 to 8 reactors to make up a large power station. The small size and modularity permits shortened construction duration and gives the utility an opportunity to grow to meet the demand that one large plant permits (Koster, 2008).

The goal was to develop this technology to be used locally and marketed internationally. which would improve the economy of the country. PBMR is a graphite moderated helium cooled reactor. with graphite comprising the bulk of the core structure and fuel pebbles. The nature and nuclear applications of graphite will be discussed in the following sections (Van Wyk, 2013).

Furthermore, another advantage that nuclear energy poses is that of environmental importance (Kadak, 2005). The environmental advantage that nuclear energy has is that there are no discharged greenhouse gases, insignificant quantities of fuel are necessary and the waste needed to be disposed of is small.

The cost implications with regards to the building of a nuclear power plant are higher when compared to other energy sources (Lalk, 2013). Nevertheless, the operation and maintenance cost of a nuclear power plant are quite low as opposed to the other sources of energy (Kadak, 2005). Globally, the public is concerned about the increasing discharge of greenhouse gases into the environment that emanates from the burning of fossil fuels such as coal.

Nuclear energy thus receives the highest scrutiny when the following issues are considered, even if it can be debated that nuclear energy is not a carbon dioxide emitter (Kadak, 2005):

- The safety of nuclear energy and the detrimental consequences associated with the incident or accident
- The unbalanced controlled climate
- The vague waste disposal solution that the global nuclear energy community is trying to disentangle.
- The PBMR is small, requiring a shorter construction time to meet the demand
- The on-line refuelling capabilities of the PBMR shortens the duration of the shutdown. Refuelling can be done during normal reactor operations (Cogliati, 2011)

The PBMR can be changed and is highly flexible. It is being made in a modular fashion to enable extra modules so that it may keep up with the demand. It is much less location-dependent than, hydroelectric or fossil-fuelled power stations. Furthermore, the advantage presented by a pebble bed modular reactor is that it provides a base load for power production which can be manipulated to the electricity needs of the society (American Nuclear Society, 2004).

The PBMR is based on the theory that the new brand of nuclear reactors should not be big. The commercial PBMRs would be sized to produce 165 MW nominally, which is approximately 18 percent of the output of a normal reactor such as the ones at Koeberg (Lalk, 2013). To maximise the sharing of support systems, the PBMR has been configured into a variety of options, of which the four-pack layout is the most affordable. The multi-module concept gives the option of having the plants being brought on line as soon as they are completed (Slabber, 2008).

According to Kadak, 2005 "The selection and the qualification of material grades are important in ensuring that the requirement of the PBMRs' normal and off-normal operating conditions are met and these include but are not limited to graphite for the reactor centre and internals".

According to Greyling, 2017 "The reference material for the permanent side reflector support blocks at the hot duct entrance and selected core support post blocks is SGL NBG-18 graphite grades. There are about 4000 graphite blocks in the reactor and 12 000 in total including dowels and fixation parts. The graphite blocks weigh up to 1000 kg".

2.2 Graphite use in a nuclear reactor

Graphite has been found to be a good moderator and reflector of neutrons in nuclear power plants and research reactors. (Wood, 2006). During construction, the moderator of the reactor is installed as an interlocking structure of graphite bricks. It has also been used as a matrix material for particle fuel to form spheres or cylindrical compacts (Mason, 1999).

Most of the graphite moderated reactors have aged and they are no longer in use. Decommissioning of these reactors includes management of the irradiated graphite as waste. The radioactive graphite coming from the reactor has different physical, chemical and radioactivity properties compared to other radioactive waste; hence the waste must be evaluated carefully to develop the best waste management plan (IAEA, 2006).

2.3 Manufacturing of graphite

Graphite is a composite material made from filler coke and pitch binder. Nuclear graphites are usually manufactured from isotropic cokes that are from petroleum or coal-tar and they are made so that they are almost isotropic or isotropic material (von Lersa, Vulpius, Steinmetz, Girke et al, and last accessed July 2017). Figure 9 highlights the important processing steps in the manufacturing of nuclear graphite. After baking, the artefact is saturated with a petroleum pitch and rebaked to harden the part. Impregnation and rebaking may be done a number of times in order to get the required density. Graphitisation usually happens at temperatures that are greater than 2500 °C (Lee, Kang and Roh, 2015). Extra halogen purification may be needed. The usual manufacturing times for a batch of graphite are 6–9 months (IAEA, 2000; Windes *et al*, 2007).

The formation and densification processes put property variations inside the billet. The properties will not be the same in the forming direction in comparison to the perpendicular direction. Moreover, a density gradient will form from the end of the billet to the core (Lee, Kang and Roh, 2015). These variations must be quantified for the selected graphite grades. In addition, property variations will be found from billet to billet in a batch and between production lots. In extruded graphite, in-billet variations will be significant and can exceed the lot-to-lot variations (Venter *et al*, 2005 and Windes *et al*, 2007). In isostatically moulded graphite, the variations will be smaller than for extruded graphite and will be in the order of the lot-to-lot variations. Finished graphite billets are machined to the complex geometries that are needed for the reactor components (fuel elements, reflector blocks, core support post, etc.). Figure 9 below shows a graphite reflector element from the High Temperature Test Reactor (HTTR) undergoing machining.

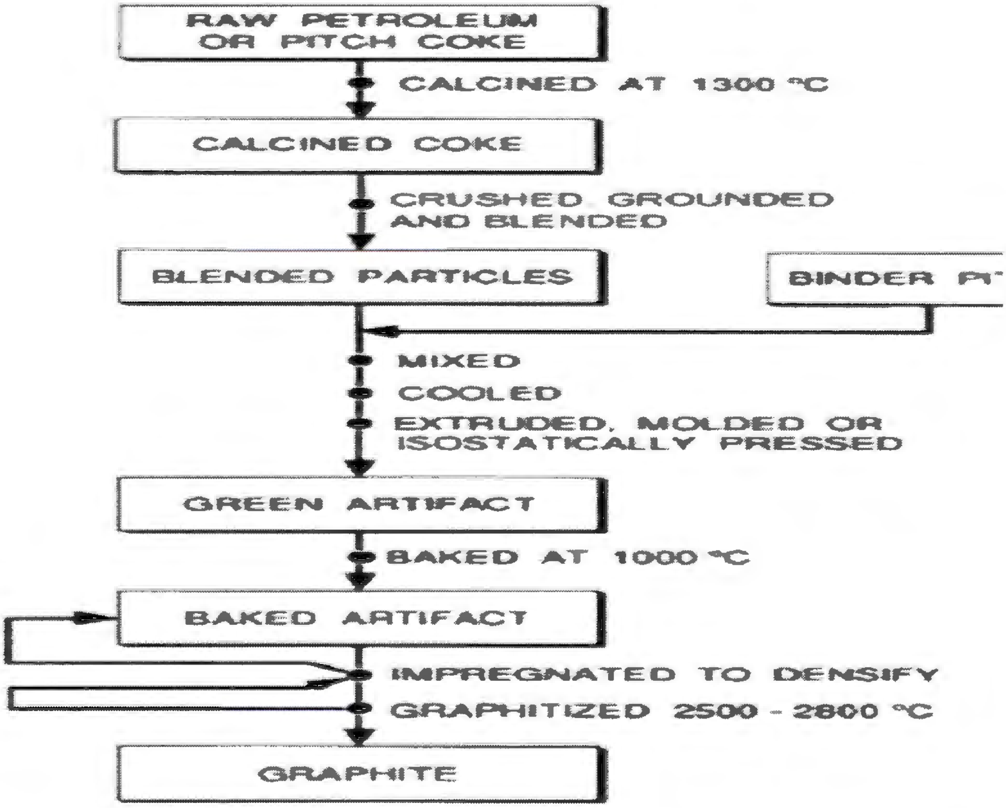

Figure 9: The process steps in the manufacture of nuclear graphite (Windes *et al*, 2007)

The properties of graphite are a direct consequence of the raw materials used in the manufacture and forming method. A heavy emphasis is placed on the type and source of the coke used in manufacture because the coke's properties largely dictate the properties and behaviour of conventionally manufactured graphite (Lee et al, 2015). In conventional nuclear graphite, the selection of the coke is paramount. Reactor designers desire isotropic irradiation behaviour (to minimise differential irradiation-induced dimensional changes and subsequent stress build-up) and modern nuclear graphite achieves this through a combination of an "isotropic" coke and the forming method (IAEA, 2000).

Secondary coke graphite uses a nonconventional manufacturing process to achieve the desired isotropic irradiation response and does not depend on starting with an "isotropic" coke. Essentially, anisotropic cokes are fabricated into graphite and then ground to become the starting "filler" in a conventional process (IRSN, last accessed July 2017). As a result of the long graphite manufacturing process, the time taken to develop new graphite grades, or to introduce a new source of coke, is significant (several years). The importance of the type and source of coke is discussed next.

The graphite that is being used in the nuclear industry is manufactured from either petroleum or pitch coke. These cokes differ significantly in their configuration, size and purity. The cokes that are used during the fabrication process are such that on graphitisation, they readily form the required graphite crystallographic structure (Lee et al, 2015). Some cokes may contain impurities that hinder the graphitisation process. For this reason, most impurities are removed by high temperature calcinations prior to graphite production (Marsden, 2002). The cokes are calcinated at a temperature between 900-1300 °C.

After the calcination process, the coke is graded and mixed with a suitable pitch binder. The resultant combination is formed into blocks, referred to as the green article (Marsden *et al*, 2002). The green article is practically soft and is cooled by submerging it in water. To drive off the volatility of the material and to bind the coke, the green article is placed in water at a temperature of 800 °C (IRSN, last accessed July 2017).

During the fabrication process, impurities such as nitrogen may be introduced to the graphite. Nitrogen is one of the main contributors to the formation of ^{14}C. The nitrogen impurity is present in air trapped in graphite pores during manufacture and also may be introduced later as an impurity in the coolant gas during the reactor operation (IAEA, 1999).

2.4 The irradiation of graphite

In a High Temperature Gas-Cooled Reactor such as the PBMR, the core and reflectors are constructed from graphite components (Mason, 1999), (IAEA, 2006). In this reactor, the graphite does not only serve as a moderator but also as a major structural component that gives way for transferring the helium gas. These graphite components change in their physical properties due to the irradiation process whilst the reactor is operating. The manner in which the graphite components change in their physical composition after irradiation is mostly dependent upon the manufacturing process (IAEA, 1999; Marsden, 2002). The irradiation of graphite in a reactor core leads to three types of changes in the material (Wickham, 2006):

- activation of impurities;
- changes in the physical and chemical properties and
- Radiation chemistry.

The activation process is largely inevitable where suitable parent isotopes are available. The interaction of fast neutrons with the graphite crystal structure leads to the displacement of carbon atoms and the creation of vacancies within the crystallites. Changes also occur as a result of fast neutron damage in other physical, mechanical and thermal properties of the graphite. In general, for graphite-moderated reactors, ^{14}C is the major isotope likely to be present from impurity activation which need to be considered in terms of possible entry to the food chain (Wood, 2006). Once the physical and chemical properties of the graphite have been modified, the radiation chemistry (e.g. substitution of ^{12}C by ^{14}C in chemical reactions; alteration of bond structure by radiation), of the irradiated graphite is also changed.

2.5 Graphite waste management

Graphite has been used as a moderator and reflector of neutrons in nuclear power plants that are well over 100 as well as in a number of experimental reactors. It is also used as matrix material for particle fuel, which is disposed throughout the operation. In some cases, the volume of the graphite involved is of the same order as the core itself. Bearing in mind that the core of a typical graphite moderated reactor may contain 2000 tonnes of graphite, the involved volume is large (IAEA, 1999).

Most of the older graphite moderated reactors are no longer in use and therefore decommissioning, planning and preparation is a now priority. Even after a number of years of irradiation, graphite still has most of its good mechanical components and is insoluble and not chemically reactive (IAEA, 1999). It appears to fulfil most of the necessities for a solid radioactive waste form suitable for disposal. However, the radionuclide inventory of irradiated graphite implies it cannot be taken by the disposal sites without particular conditioning (IAEA, 1999).

There are numerous radionuclides that are found in irradiated graphite and these include tritium and carbon-14, but also corrosion or activation products (^{57}Co, ^{60}Co, ^{54}Mn,^{63}Ni, ^{22}Na), fission products (^{134}Cs, ^{137}Cs, ^{90}Sr, ^{152}Eu, ^{154}Eu, ^{155}Eu, ^{144}Ce) and small amounts of trans-uranium elements (^{238}Pu, ^{239}Pu, ^{241}Am,^{243}Am) (IAEA, 1999). For this investigation, concentration was on the ^{14}C. The focus of this project will be to investigate the formation and location of ^{14}C in graphite.

2.6 Location and formation of ^{14}C in the graphite

According to the International Atomic Energy Agency (2006), ^{14}C is produced by three reactions in the gas-cooled, graphite moderated reactor core:

$^{14}N + n \rightarrow {}^{14}C + p$ in the coolant gas and graphite pores

$^{17}O + n \rightarrow {}^{14}C + \alpha$ in the UO_2 kernels of the fuel spheres

$^{13}C + n \rightarrow {}^{14}C + \gamma$ in the fuel spheres and structural graphite.

Due to the low isotopic abundance of ^{17}O (0.04%) and high retention capacity of TRISO-coated particles, the $^{17}O\ (n,\ \alpha)\ ^{14}C$ reaction is usually disregarded as an important contributor to the ^{14}C content of the graphite. The $^{13}C\ (n,\ \gamma)\ ^{14}C$ reaction may impact significantly to the ^{14}C content (Dunzik-Gougar et al, 2008). Marsden *et al.* 2002, "identified ^{13}C as the antecedent element for more than 30% of the ^{14}C inventory in irradiated graphite from carbon dioxide cooled Advanced Gas Reactors operated in the United Kingdom".

Despite the low probability of this reaction (i.e. ^{13}C has a small neutron cross-section and comprises 1.1% of natural carbon), the high quantity of carbon atoms in graphite makes the reaction important. Current research shows that most ^{14}C does not seem to be mixed into the graphite crystal lattice but is only found on the top of graphite crystallites and pores after neutron activation of ^{14}N impurity (Euratom, 2007).

As summarised by the International Atomic Energy Agency (2006) most of the ^{14}C is formed in the surficial pores of the graphite surface; this is because no significant amount of ^{14}C atoms can diffuse into the graphite matrix until the temperature of 1800 degrees is reached (IAEA, 2006). The distribution of this carbon isotope is uniform on the graphite structure, which leads to this isotope being the contributing factor to the graphite waste. Most of the ^{14}C that is obtainable in the irradiated graphite is formed by the ^{14}N reaction with the ^{13}C reaction being the second element or atom that contributes to the generation of ^{14}C. Since ^{14}N is located on the surface of the graphite, it can therefore be concluded that most of the ^{14}C formed will be on the surface.

There is a significant difference between the ^{14}C formed from ^{13}C and ^{14}C formed from the ^{14}N within the graphite. The former will be in graphitic form, with ^{14}C incorporated into the lattice or remaining trapped within the porosity. ^{14}C from the coolant or cover gas will be in the form of amorphous carbon or carbonaceous solids that have some elements of hydrogen and oxygen. It will be available mostly as deposits on the geometrical tops of the graphite and inside the pores (Tsang, 2005). The extent of the latter is reliant on the availability of permeable gas flows through the transport porosity for the times of reactor operation. Hence, more of the ^{14}C is expected to be inside the pores of the surficial graphite (Wood, 2006).

Table 2 below shows the carbon isotopes and their respective half-lives. For this project, the focus was on the ^{14}C isotope. Having an understanding of the half-life of the isotope in question will assist in comprehending the importance of the isotope. This also gives an understanding of how long will it take for the isotope to reduce its activity by half should it be bio-available in the environment.

Table 2: Half-lives of all the carbon isotopes

ISOTOPE	HALF-LIFE
^{9}C	127 ms
^{10}C	19.26 s
^{11}C	20.39 m
^{12}C	Stable (98.93)
^{13}C	Stable (1.07 %)
^{14}C	5730 y
^{15}C	22.45 ms
^{16}C	747ms

Table 2 above illustrates the different types of carbon isotopes and the isotope of major importance is ^{14}C. It decays by emitting a beta particle and has a long half-life, hence it must be prevented from entering the food cycle. For this reason, management of the ^{14}C isotope and the development of the management, method have been recognised to be critical issues in waste minimisation. It, therefore, becomes important to look into methods that have been used to separate isotopes that are available in the literature. Knowledge acquired from previously used methods for isotope separation paved the way forward on which method was suitable for the current investigation undertaken in this study. The insight provided the know-how on which method was better or if the project should explore another method. The following literature section describes the methods that have been explored and used before and are currently being used.

2.7 Methods that have been used before to separate isotopes

This section will be focusing on three isotope separation methods as listed below. The methods indicated describe the separation of uranium isotopes. These techniques are being used to enrich ^{235}U. The ^{235}U is to be separated from the ^{238}U via:

- Gaseous Diffusion
- Gas Centrifuge
- Electromagnetic Separation (Lamarsh and Baratta 2001)

2.7.1 Gaseous Diffusion

Gaseous diffusion is one of the methods that have been used globally in the developments of military programmes for the enrichment of uranium. This method is based on the separate molecular diffusion of a gaseous isotopic mixture through porous membranes (Philippe and Alexander, 2013).

In this method, the UF_6 is first converted from a solid to a gas using a combination of high temperature and pressure. Once the UF_6 has been converted into the gaseous phase, it is then piped through a series of porous membranes. The pores of this membrane are very small in such a way that the UF_6 gas can hardly pass through the membranes, but the system is designed in such a way that only the lighter isotope which is ^{235}U can pass through the membranes and the heavier isotope ^{238}U remains behind. On the other hand, since there is a small mass difference between the two isotopes, only a small enrichment capacity can be achieved, hence it requires that many stages of the gaseous diffusion be interconnected into hundreds more of these stages, in such a way that one diffusion output stage becomes the input of the other diffusion stage. However, this method requires enormous amounts of electricity to pump the gas and much space is needed for the plant (Lamarsh and Baratta, 2001).

2.7.2 Gas centrifuge

The gas centrifuge method is similar to the gas diffusion method because it also uses gas molecules and it operates on the principle of a rotating cylinder, in which the centrifugal force compresses the gas molecules in the cylinder to the outer wall. The velocity that is produced by the thermal agitation tends to redistribute the gas molecules throughout the cylinder. The lighter molecules such as ^{235}U, are then favoured by this result because of their greater velocity and so a higher concentration of the ^{235}U gathers near the centrer of the rotating drum (Lamarsh and Baratta, 2001).

2.7.3 Electromagnetic Separation

According to Lamarsh and Baratta (2001) "This method is an addition on a large-scale of the theory of the mass spectrometer. Ionised uranium gas is sped up by an electrical prospective into a magnetic field, where the more heavy particles move in arcs of large radii than the lighter particles". The split beams are then put together in distinctly spaced graphite receivers (Philippe and Alexander 2013). The uranium ion reacts with the graphite and makes uranium carbide, which is then chemically treated to get the separated isotopes. One of the advantages of the electromagnetic process is that separation of the uranium isotopes can be obtained in one pass through the machine (Lamarsh *et al*, 2001).

The above mentioned methods are not viable to be used for the separation of the carbon isotopes on the current pilot project because they require space, there are also some technical limitations associated with these methods and they are also expensive. The use of microbes as treatment is more advantageous when compared to the other methods, hence it is worth investigating due to the available historical information presented by various researchers which will be presented in the following sections and the cost-effectiveness associated therewith. Furthermore, it is indicated that from the release of radionuclides emanating from nuclear installations during the generation of nuclear power, the consequences of historical nuclear weapons testing during the 1950s include nuclear accidents such as the Chernobyl accident, Fukushima accident of 2011 and the storage methods of nuclear waste. This has caused the radionuclide to be bio-available in the environment and therefore initiated the interest in investigating the environmental outcome of these radionuclides (Lloyd and Renshaw, 2005).

2.8 **Use of Microbes in Waste Minimisation**

The distribution of micro-organisms in the atmosphere and their growth in different environmental conditions is an on-going process and of particular interest for this study, is the survival of micro-organisms in the presence of radionuclides, as this gives in-depth knowledge on their characterisation (Lloyd and Renshaw, 2005).

Microbes play a significant role in the minimisation of radioactive waste and have survived the harsh radioactive environment (Wang *et al*, 2014). As an example, microbes that have been found on or in radioactive disposal site are the sulphur-reducing bacteria and they fall under the endospore bacterial group (Lloyd and Renshaw, 2005). They are resistant to radiation, heat and aggressive chemicals. Another type is the aerobic bacteria, the *deinoccus radiophilus*, which can tolerate high doses of radiation (Pedersen, 2000). This characteristic of the bacteria has motivated this researcher to explore this aspect, through the use of microbes as a means to investigate the separation on ^{14}C and ^{12}C.

Lloyd and Renshaw (2005), state that, "Other environments that have been impacted by the radioactive substances have not been investigated in-depth and this could be due to the radiation dose exposure allowed for an individual originating from the environment."
This, therefore, becomes a limiting factor in the knowledge fraternity, available to this area of interest. However, radioactive uranium isotopes have been investigated focusing on the uranium deposited on sediments as a result of nuclear waste, nuclear accidents or nuclear sites. Lloyd and Renshaw (2005) investigated sediments contaminated by leakage of waste containing high concentrations of alkali, nitrate, aluminate and chromate. From the collected sediment, there were about 11 aerobic heterotrophic bacteria discovered which included *Rhodococus and Nocardis*. One bacterial species that was discovered to have survived high radioactive dose levels was closely related to the *Deinococcus radiodurans* species. The dose level was at 20 kGy (Lloyd and Renshaw; 2005).

Another environmental investigation report indicated the case of water samples collected from the repository for Russia's low-level liquid radioactive waste. In this case, anaerobic denitrifiers, fermenters, sulfate-reducers and methanogens were characterised. Of interest were the results obtained from the sampled water, which showed that the microbial cell count was higher than background as compared to other areas of the repository sample areas (Pedersen, 2000). The results obtained also indicated that the ionising radiation environment presented by the radionuclides does not hinder the growth of the micro-organisms. Furthermore, some of these radionuclides come from the waste generated from the stages of a nuclear fuel cycle, which have different radiation levels and volume. The ultimate possible routine to be taken for handling the waste is either disposal or reuse (Figueroa *et al*. 2017).

The reduction of soluble U(VI) to insoluble U(IV), has been achieved through the biosorption of U(VI) by the micro-organisms. This also gave more insight into the interaction process occurring between the micro-organisms and the ionising radiation environment. Another micro-organism that reduced the U(VI) is the sulfate-reducing *Desulfosporosinus spp.* The mechanisms of reduction used by the sulfate-reducing bacteria such as *Desulfovibrio* micro-organisms were reported (Lloyd and Renshaw, 2005).

In the investigation, the micro-organism make up in the anaerobic environment was controlled by the subsequent microbes formed by hydrolysis and the use of growth media as an energy source. Selected bacteria from dissimilar substrate niches (e.g. glucose vs. lactate vs. acetate) are capable of uranium reduction (Figueroa *et al*, 2017). Most micro-organisms do not grow as a result of the energy that is got from the uranium/organic carbon redox couple. The reduction of uranium shows a problem and an unusual environment to use the organic carbon as an energy source.

The micro-organism needs to also use extra organic carbon in conjunction with its energy producing electron acceptor to grow and remain active. At the same substrate level, there are micro-organisms that do not lessen uranium and also are in competition for the same organic compounds. Lloyd and Renshaw, (2005) highlight that carbon flow through non-uranium reducing micro-organisms at all substrate levels could result in no uranium reduction in an apparently appropriate anaerobic environment. The authors argue that the structure of the active microbial ecology is also sensitive to the solution phase chemical composition of the water.

The use of this separation method is preferable when compared to the other separation methods, because there is not much space required and it is not as costly. In addition, microbes use carbon as an energy source which is an advantage for this study because that is the investigated element as compared to the methods which are more generic in their respective applications. Therefore, the method to be used and investigated for applicability and success was through the use of micro-organisms. Findings acquired from this research will provide a better understanding of micro-organisms isotopic separation methods that impact on the mobility of ^{14}C. This information is useful in waste minimisation, the design of disposal nuclear waste in deep geological sites as well as nuclear bio-remedial measures. In terms of the deep geological disposal sites, outcomes of this study provided insight into factors to consider when conducting a safety assessment of the geological disposal site for nuclear waste.

The Low-Intermediate Level Waste (LILW) national inventory of waste that is thought to be generated by the PBMR Demonstration Power Plant (DPP) is highlighted in Table 8 below. Berk *et al*, (2008), describe the assumptions made to get the national inventory, including the screening of radionuclides considered to be of no importance in terms of safety in the long run. The radionuclides and activities presented in Table 8 show an improved approximation of the nuclides and associated activities that can be anticipated at the end of the PBMR DPP operational life (van Blerk *et al*, 2008).

Table 3: Nuclide inventory of the potential future PBMR waste in 2036, and the national inventory of Low-Intermediate Level Waste (LILW) used in the 2007 Vaalputs Post-Closure Radiological Safety Assessment (Blerk *et al*, 2008).

Radionuclide	PBMR LLW (Bq)	PBMR ILW (Bq)	Total LILW	Radionuclide	PBMR LLW (Bq)	PBMR ILW (Bq)	Total LILW
Am-241			5.55E+09	Pu-239			4.94E+08
Am-242m			4.18E+06	Pu-240			3.71E+08
Am-243			2.37E+07	Pu-241			1.10E+10
C-14	3.75E+12	1.45E+12	2.60E+14	Pu-242			1.79E+06
Cd-113m			5.55E+06	Se-79			3.13E+05
Cl-36			5.17E+04	Sm-151			1.75E+08
Cm-243			1.86E+09	Sn-121m			3.36E+05
Cm-244			6.85E+08	Sn-126			6.38E+05
Cm-245			3.49E+05	Sr-90	1.48E+11	5.71E+10	6.20E+11
Cm-246			1.10E+05	Tc-99			2.95E+10
Co-60			2.22E+14	Th-230			8.61E+00
Cs-135			2.75E+05	U-232			2.10E+04
Cs-137	4.58E+13	1.77E+13	1.37E+14	U-233			1.98E+01
Eu-154			2.84E+08	U-234			1.68E+12
H-3	3.94E+16	1.52E+16	5.46E+16	U-235			1.12E+11
Ho-166m			3.81E+03	U-236			1.68E+05
I-129			1.23E+08	U-238			4.99E+11
Ni-63			1.45E+07	Zr-93			1.36E+07
Np-237			3.58E+07	Tru			1.77E+10
Pu-238			2.28E+09	**Total**	**3.94E+16**	**1.52E+16**	**5.52E+16**

CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction

The ^{14}C, is in a reactive form rather than in the crystal structure of the graphite, and the graphite used in this investigation was the exact same type as used in PBMR fuel spheres before irradiation. However, the source of ^{14}C was from the introduction of the radioactive salts. The initial stage of the experiments was using graphite chucks, observing if the microbes could grow in the presence of graphite in the media. This was through visually observing the growth medium turning milky or turbid from a clear solution overtime (Wickham *et al*, 2006).

The second stage of analysis focused on investigating if there was a bio-film formation on the surface of the graphite chucks. These samples were analysed using the SEM described below in **Section 3.2.** This type of analysis method will be discussed later in the report and the results obtained therefrom.

Furthermore, another set of experiments were explored in parallel with the flasks that contained the graphite chucks. In this set of experiments, the graphite was milled into a powder form in order to understand its crystallography. The analysis of these samples was done using the XRD - hence the samples had to be in a powdered form. Details on how the samples were prepared will be discussed in **Sections 3.3**.

Non-radioactive graphite and samples were used in the experiments conducted using the SEM and XRD. The ***positive results*** obtained from both analyses, then enabled the project investigations to proceed and radioactive ^{14}C spiked salts were used.

3.2 Scanning Electron Microscope (SEM)

SEM is a piece of equipment that has the ability to magnify scanning images up to at a high resolution of 3D level, which cannot be achieved by a naked eye. Therefore, the instrument assists in investigating samples at a surface plane. The resultant images obtained are helpful in evaluating and in acquiring surface information from the graphite specimen. The principal advantage of this instrument is that it has a highly focused electron beam that scans across the surface of the specimen (Sujata *et al*, 1991).

The beam creates backscattered, secondary electrons and x-rays in a highly restricted area. These signals can be seen and the results of the analysis are shown as a specific intensity on a screen at a place that represents the location of the electron area. After some time, information about the whole area is shown on the screen, which ends up in a complete 3D image as with a television image. The SEM is highly flexible and the quality of the image is related to its resolution and contrast (Sujata *et al*, 1991). SEM was chosen to do an analysis on the graphite to see if the bacteria can stick to the graphite top to create a bio-film. Biofilms are created by the colonisation of bacteria onto a solid surfaces. SEM is used for the direct study of the surface of solid items by scanning with high energy beams of electrons. Bio-film creation would show a mutual relationship between the attachments of the bacteria onto the graphite. Attachment will not happen if the graphite is toxic to the microbes.

3.2.1 Sample Preparation

These experiments were performed at the same temperature of 22°C where the bio-reactors were closed with foil to avoid chemical reactions, and then batch experiments were done. The advantage of having batch experiments was that they were easy to operate and observe; duplicate reactors were also prepared to ensure consistency of the experimental results and to monitor changes occurring throughout the experiments.

For the SEM analysis, samples are prepared by removing the graphite chunks from the flasks and placing them in a sterile bottle containing a fixing solution (Sujata *et al.*, 1991). The fixing solution comprised of 2ml of gluteraldehyde, 10 ml of 0.0075 M phosphate buffer and 8 ml of H_2O was added to the mixture.

Twenty-two test tubes were used for the SEM sample preparation. The graphite chunks were then inserted into the test tube and the fixing solution added. The samples were then left for 30 minutes after which the fixing solution was removed, leaving the graphite chunks in the test tubes. The chunks were then washed three times with 0.075 M $NaPO_4$ buffer diluted 1:1 in double distilled water for 15 minutes to remove glutaraldehyde. A buffer of 700 ml, 0.15 M KH_2PO_4- Na_2HPO_4 at pH 7.4, KH_2PO_4 (molecular mass = 136.09), 2.04 g, $Na_2HPO_4.2H_2O$ (molecular mass = 177.99) 16.02 g was prepared and filled up to 700 ml with distilled water.

Furthermore, the chunks were dehydrated by four different concentrations of ethanol for 15 minutes each and the particles washed with 70%, 80% and 90% of ethanol. A metal tray was used which was covered with a carbon tape. Graphite samples were then placed in a sequence on the tray. The samples of graphite particles that are used for sampling were left for three days in 100% ethanol before they could be analysed using the SEM. The samples were dried overnight with a crystal drying point coated with gold.

3.3 X-Ray Diffraction (XRD)

This technique has enhanced the knowledge of the function and components of a material in biological and organic polymers by giving structural data at a molecular level. An XRD instrument is made up of an x-ray generator, goniometer, sample holder and x-ray detectors such as photographic film or a movable proportional counter (Jenkins, 2000).

An XRD instrument is used to measure crystal structure, grain size, texture and the residual stress of material and compounds which is done through the interaction of x-ray beams and the sample (Burchell *et al.* 2007). For this investigation, attention was given to the crystal structure measurement. The wavelength of x-rays is of the same order of magnitude as the distance between atoms in a molecule. The diffraction is obtained when the crystal diffracts x-rays passing through it at a certain angle (IAEA, 2000).

There exist many parameters that must be covered in the explanation of the XRD analysis which are:

- Diffraction
- Powder xray Diffraction(XRD)
- Crystallography
- x-ray Diffraction Crystallography (Cullity, 1977)

3.3.1 Diffraction

According to Venter (2005) diffraction happens as waves meet with a normal structure whose repeat distance is almost the same as the wavelength. Jenkins (2000) argues that "diffraction is a wave property of electromagnetic radiation that causes the radiation to be bent as it passes by an edge or through an opening and it results in disturbance that gives out dark and bright rings, lines or spots depending on the geometry of the object causing the diffraction".

3.3.2 Powder X-ray Diffraction

Powder XRD is the most widely used XRD method for characterising materials. As the name suggests, the sample is normally in powder form, and has small grains of single crystalline material that have to be analyzed. Powder means that the crystalline domains are randomly oriented (Thermo ARL Applied Research Laboratories S.A. 1999). Therefore, when the 2-D diffraction pattern is noted, it shows concentric rings of scattering peaks corresponding to the various d spacing in the crystal lattice. "The position and the intensities of the peaks are used for identifying the underlying structure of the material" (Venter, 2005). The phase identification is important because the material properties are highly dependent on the structure. Powder XRD is the technique that was used for investigation in this dissertation as the prepared specimen was a powder. The protocol we used was according to Jenkins, (2000).

3.3.3 Crystallography

Crystallography is the science of getting the arrangement of atoms in solids by measuring the angles of crystal faces relative to theoretical reference axes (Jenkins, 2000). Crystallographic methods depend on the analysis of the pattern that comes from a sample that is targeted by a beam, in this case, an x-ray beam. The understanding of the crystal structure is a vital prerequisite for getting to know about crystallographic defect. Materials do not form in a single crystalline, but poly-crystalline form such that the powder diffraction method plays the most important role in the structural determination (Venter, 2005). Crystallography is then a useful tool applied to identify the phases of a crystal. When performing material processing, crystallography then helps to determine the compounds and phases present in the material" (Burchell, 2017).

The use of this analysis technique helps in acquiring the crystallographic and property information from the graphite samples, thereby achieving one of the project objectives that were set, which was to understand if the microbes had an impact on the graphite phase structure.

3.3.4 Sample preparation

The XRD instrument that was used to determine if microbes living in the presence of graphite affect the graphite structure was the PANalytical Empyrean Diffractometer of CuK$_{\alpha}$ radiation (λ = 0.15418 nm) and graphite samples with and without biofilms were used in this experiment.

For the XRD analysis method, powdered graphite samples (Cullity, 1997), were exposed to two types of media namely, Styriakova and Nutrient Broth (NB) media along with the MC bacteria. The range of the experimental graphite masses varied from 1g to 4g. The mixture of both the graphite and the media was poured into a flask and allowed to run for a duration of three weeks before analysis was done using the XRD. The Styriakova Media was composed of NaH_2PO_4, $(NH_4)_2SO_4$, $NaHCO_3$ and Glucose (at different masses ranging from 0 g to 4 g). For XRD, a particle size of less than 50 µm was required, therefore the chunk samples had to be milled with a hand grinding method using mortar and pestle. When an x-ray strikes a sample (graphite powder), the particles of the sample become excited to higher energy states and on returning to ground state they emit characteristic X-rays of the absorbed energy (Thermo ARL Applied Research Laboratories S.A, 1999; Venter, 2007). This XRD spectrum is a signature of the mineralogy of the sample (i.e. the structure of the graphite sample before and after irradiation in the reactor is determined).

3.4 Denaturing Gradient Gel Electrophoresis (DGGE)

3.4.1 Sample Preparation

Based on the results obtained from both the SEM and XRD analysis methods, the findings motivated the project to proceed to investigate the third project objective being; to determine the nature of microbes living in the presence of a source of ^{14}C chemically representative of ^{14}C on the surface of irradiated graphite. Therefore, to meet this objective the DGGE method of analysis was used (University of Toledo, 2001).

A mixed culture (MC) of bacteria were inoculated into the Styriakova Medium and left for 120 hours (Assih and Ouattara, 2002). Sodium acetate and sodium bicarbonate salts were added to the medium, mixed in an Erlenmeyer flask which acted as a bio-reactor. During operation of the bio-reactor, carbon free air was allowed to flow in, to assist in circulating the gas produced by the bacteria, within the reactor (Ercolini, 2003). This gas is carried from the bio-reactor by the carbon free gas through the tube furnace for oxidation and therefore trapped in the NaOH solution where samples are retrieved for analysis via the Liquid Scintillation Counter, (the Packard Tricard model 4430).

In the case of the molecular work, interest is only in the bacteria that are growing in the bio-reactor. After 120 hours of reactor operation, samples were collected for analysis. The mixture was centrifuged then the supernatant pipetted out leaving the bacterial pellet at the bottom of the centrifuge tube. The pellet was then used to do the DNA extraction, PCR and DGGE. The reason behind the 120 hours is that the investigation was for the population dynamics and the survival of the bacteria after it is exposed to radiation for a longer duration. In the PBMR there might be a need to expose the bacteria to the irradiated graphite for longer periods since the graphite is inert. The research investigated which salt is preferred by the bacteria and which bio-reactor had more bacterial growth between the two salts and which type of species indicated dominance over the others; this was obtained by doing DGGE investigation (Ercolini, 2003).

Total genomic DNA was then taken from the samples using a ZR Soil Microbe DNA kit (Zymo Research, Inqaba Biotec, Pretoria, South Africa), based on the manufacturer's specifications. The ZR Soil Microbe DNA Kit is made for the easy, fast isolation of humic-free, PCR-quality DNA from microbes. The kit can be used to isolate DNA from tough-to-lyse bacteria, fungi, protozoa and algae that inhabit a range of samples. Samples were added to ZR Bashing Bead Lysis Tube where microbes were rapidly and efficiently lysed by bead beating in a unique designed lysis buffer. Fast-

Spin column technology was used to isolate the DNA which was subsequently filtered to remove humic acids/polyphenols that inhibit PCR (Figueroa *et al*, 2017).

Polymerase Chain Reaction (PCR) was then used to amplify a portion of the 16S rRNA of the DNA extracted from PBMR samples. PCR was used as an efficient method to create a precise gene replication through targeting. The aim of targeting was to obtain additional information about the DNA compilation of the PBMR samples. Target sequence within the gene was amplified using the oligonucleotides, "PRUN518r 5'-ATT-ACC-GCG-GCT-GCT-GG-3' (Siciliano *et al.*, 2003) and pA8f-GC 5'-CGC-CCG-CCG-CGC-GCG-GCG-GGC-GGG-GCG-GGG-GCACGG-GGG-GAG-AGT-TTG-ATC-CTG-GCT-CAG-3" (Fjellbirkeland *et al.*, 2001). Each PCR reaction mixture (20 µl) had ~27 ng/µl of genomic DNA as template, 10.3 µl sterile distilled MilliQ water, 2.5 µl PCR buffer with $MgCl_2$ (10×), 2 µl dNTPs (2.5 µM), 1 µl primer PRUN518r (50µM), 1 µl primer pA8f-GC (50 µM) and 0.2 µl Supertherm *Taq* (5U/µl) (Southern Cross Biotechnology). A negative control (all reagents and no template DNA) was added to rule out contamination. The tubes were then placed in a Perkin-Elmer GeneAMP® 2700 thermal cycler. Following incubation at 95 °C for ten minutes, the reaction mixture was subjected to 35 cycles of denaturing at 94 °C for 30 seconds, annealing at 58°C for 30 seconds and elongation at 72 °C for one minute. Once the cycles were completed, a final elongation step was performed at 72°C for ten minutes to complete DNA synthesis. An aliquot of the amplified DNA was analysed by agarose gel electrophoresis on a 1.5% (w/v) TAE gel in the presence of appropriate molecular weight marker (1 kb). The PCR products were then stored at 4°C, until further analysis.

PCR products were then subjected to DGGE according to the method described by Muyzer *et al.* (1993). Some 10 µl of the each PCR product was loaded onto 40 - 60% and 44 - 55% denaturing gradient gels (Table 3). Gels were run at 70 V for 17 hours at a constant temperature of 60 °C. Selected bands were picked under blue light from the DGGE gels using a sterile scalpel and forceps. Each band was assigned a number for sequence analysis. The gel fragments were then placed into 25 µl filter-sterilized deionised water and allowed to stand overnight to dissolve. The dissolved fragments were then subjected to PCR as described in the previous section.

Table 4: Denaturing gradient table showing volumes in milliliters of DSSA (denaturing stock solution A): 8% acrylamide in 0.5x TAE (40 mM Tris, 20 mM acetic acid, 1nM EDTA (pH 8.3) buffer) and DSSB (denaturing stock solution B: 8% acrylamide, 7M urea, 40% formamide in 0.5x TAE buffer) mixed to form a gradient within the gel.

Denaturing percentage	**DSSA (ml)**	**DSSB (ml)**
30	10.2	4.4
35	9.4	5.1
40	8.7	5.8
45	8.0	6.5
50	7.3	7.3
55	6.5	8.0
60	5.8	8.7
65	5.1	9.4

3.4.2 Sequencing

Amplified products from DGGE bands were cleaned using 15µl sterile water, taking the volume to a 0.5 ml Eppendorf sequencing tube, adding 2 µl of sodium acetate (3 M) and 50 µl ethanol (95%), and allowing it to stand on ice for ten minutes. The tubes were then centrifuged at 10 000 rpm for 30 minutes. The ethanol solution was taken out, the pellet washed with 150 µl ethanol (70%), and the tubes centrifuged again for five minutes at 10 000 rpm. The ethanol was aspirated and the pellet vacuum dried for approximately ten minutes. These samples were then taken to Inqaba for sequencing using the oligonucleotides PRUN518r (Siciliano *et al.*, 2003). Nucleotide sequences were analysed with BioEdit v.5.0.9.1 (Hall, 2001) and their identity was verified by sequence match searches against the RDP database (Available from http://rdp.cme.msu.edu, accessed 21 April 2017).

3.4.3 Phylogenetic analysis

Sequencing the PCR product from the DGGE bands was done using primer K. DNA sequences were outsourced to Inqaba Biotec (Pretoria, South Africa). Each sequence was put to a BLAST analysis on the GenBank and RDP databases and matching hits, with e-values closest to 0.0 indicating a statistically plausible match, were chosen for alignment. Raw electropherogram data was analysed using the Vector NTI suite (Contig Express, AlignX) of bioinformatics software, to generate initial sequence alignments. Sequences were aligned with Clustal X (Thompson *et al.* 1994) and inserted gaps were treated as missing data. Ambiguously aligned regions were not included in the data set before analysis. Phylogenetic analysis was based on parsimony using PAUP 4.0b10 (Phylogenetic Analysis Using Parsimony) (Swofford, 2000). Heuristic searches were done with the random addition of sequences (1000 replicates), tree bisection reconnection (TBR), branch swapping, MULPAR-effective and MaxTrees set to auto-increase. Phylogenetic signal in the data sets was assessed by evaluating tree length distributions over 100 randomly generated trees. The consistency Index (CI) and retention indiex (RI) were determined for all data sets. Phylogenetic trees generated from sequences were rooted with *Thermotoga maritima* as monophyletic out-group to the remaining taxa. Bootstrap analyses were done, taking groups with 70% consistency, to show confidence in branching points (1000 replicates) for the most parsimonious trees generated.

CHAPTER 4: RESULTS AND DISCUSSIONS

4.1 Scanning Electron Microscope (SEM)

Below, are figures illustrating graphite microscopic images. All the scanning electron microscope measurements were done at the University of Pretoria SEM Laboratory using the SEM (JSM 6390LV Scanning Electron Mircoscope (SEM), Jeol, supplier: Advanced Laboratory Solutions). The objective of the SEM analysis was to investigate whether there is bio-film formation on the graphite samples which would indicate bacterial growth in the presence of graphite.

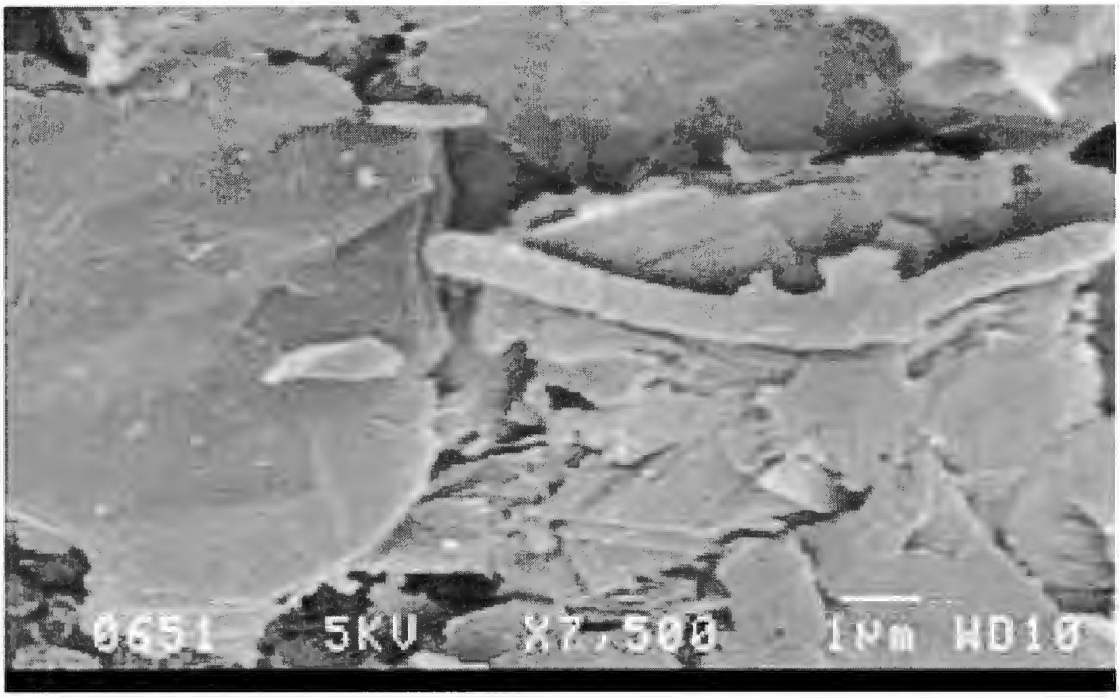

Figure 10: Illustration of a non-exposed graphite sample to the bacteria

Figure 10 is an image of a control graphite sample. This is a graphite sample that was not exposed to the micro-organisms and the growth media, it will be used for comparison in the figures to follow of the SEM images taken when exposed to different environments.

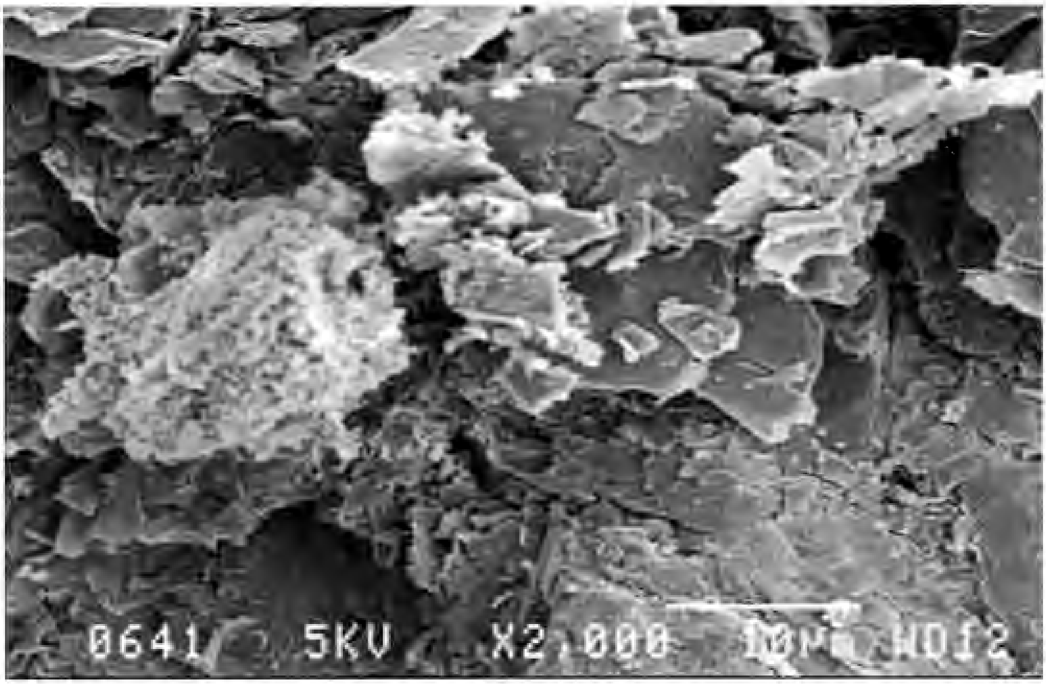

Figure 11: Graphite chunk that was exposed to the Nutrient Broth sample

Figure 11 is a graphite chunk that was exposed to the NB growth media and the MC. It illustrates that there are some rod-shaped species, which seem to have multiplied over time, and these are bacteria. It is also evident that there are different species present on the graphite surface. This is due to the different shapes and sizes observed from the image. When compared to Figure 10 which serves as a reference sample image, it was observed that there is a major difference which could be attributed to the introduction of the micro-organisms and NB medium with which the graphite chunks were exposed. These micro-organisms are the bacteria living in the NB.

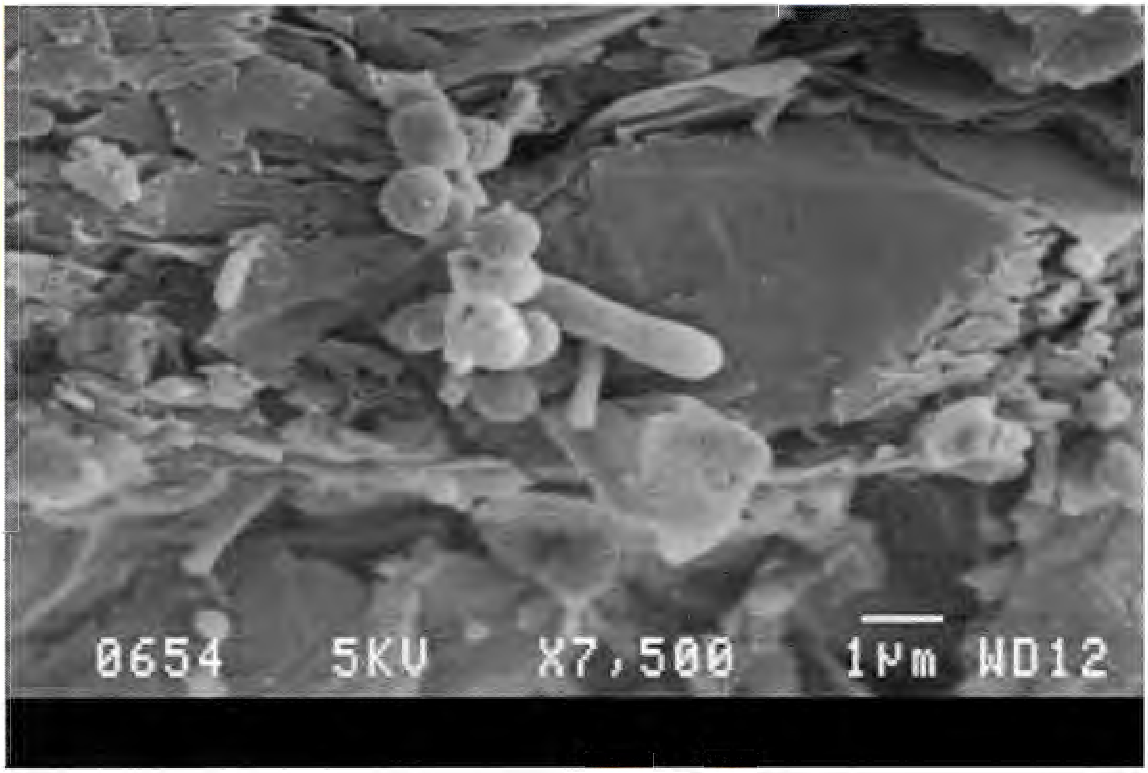

Figure 12: Illustration of Nutrient Broth sample over time

Results obtained in Figure 12 above illustrates that the growth rate of the micro-organisms has multiplied overtime. When comparing Figure 11 and 12 it is evident that the cell growth rate has increased overtime. Based on the observations made from both figures, it is evident that the longer the micro-organisms are afforded the time to grow, the greater the bio-film formation on the surface of the graphite chunks.

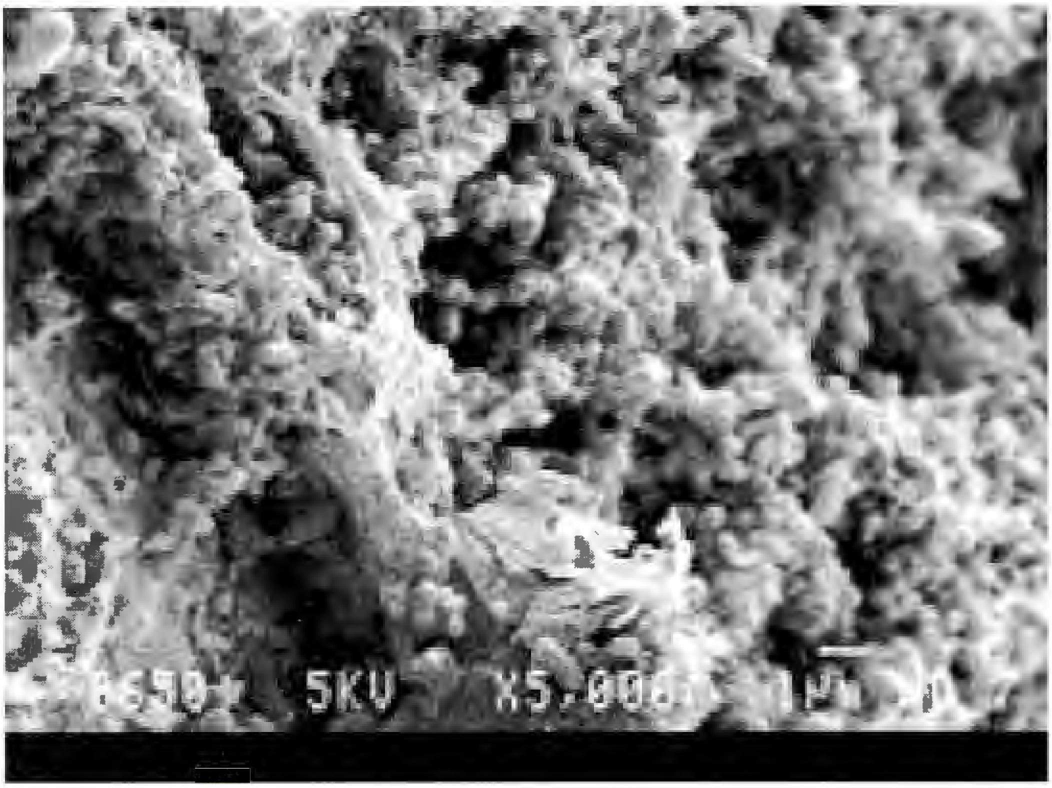

Figure 13: Styriakova Medium sample

Figure 13 is a graphite sample that was not exposed to a different bacterial growth medium. From the image, it is evident that there was more bacterial growth as compared to images illustrated in Figures 11 and 12. Therefore, it was evident that the growth environment presented by the Stryriakova medium was more favourable as compared to the media used in Figure 11 and 12. Another interesting factor that should be taken into consideration is that Figures 11, 12 and 13 show different bio-film formations. That is, the shapes of the bacterial species on the surface are different. This gives rise to the possibility of having multiple bacteria species, hence the DGGE analysis technique is necessary. As mentioned before, all figures indicate that there was bio-film formed on the surface of the graphite, hence it then became worth investigating whether there was any change in the crystallographic structure of the graphite.

The use of SEM gave a clear indication that micro-organisms can or do grow and survive in the presence of the inert graphite, which was a good indication since it was expected that no micro-organism can survive and that the graphite might be toxic to the micro-organisms.

4.2 X-Ray Diffraction (XRD)

The graphite samples were sent to the XRD laboratory at NECSA for evaluation. The main focus of this evaluation was to investigate the crystallographic structural arrangement of the graphite samples. This technique is important because of its non-destructive application. The evaluation was performed on all the samples that were treated as explained previously. The instrument operation was performed by the XRD Lab Technician (Mr. T. Ntsoane) in the researchers' presence and observation. These samples were of non-radioactive graphite. The instrument used was the XRD (model: D8 Discover with GADDS-Modular X-Ray Diffraction System, Maker: Bruker, Supplier: Bruker South Africa). Results are shown in Figure 14 below.

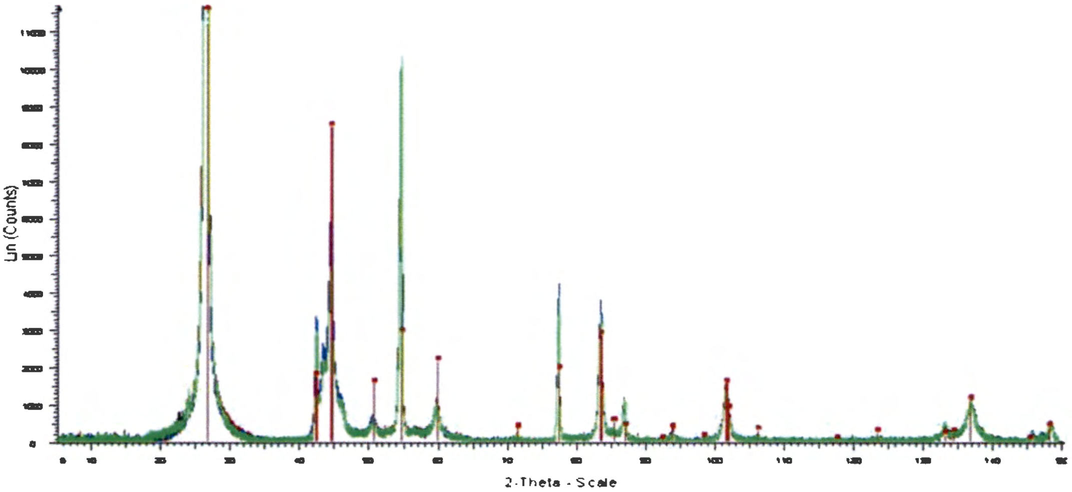

Figure 14: Superimposed diffraction pattern of graphite samples doped with different fractions of glucose. The pattern shows no glucose present.

Figure 14 and 15 indicated that there is no change done by the micro-organisms to the crystallographic structure of the graphite hence the peak heights and widths are not different. This is apparent that there is no change in the samples with or without glucose and the MC in Figure 15.When observing the peak at 2Θ (2 theta) = 27°, 45°, 52° and 60° for both Figure 14 and 15, the same counts are noted. According to Wang, *et al.* (2014), this could be attributed to the diagnostic peak of the graphite, glucose and that of the MC. When analysing Figure 14 it was noted that there was no difference observed in the patterns, that is, with graphite exposed to different glucose concentrations. Therefore, this implies that the glucose did not have any significant impact to the graphite since the peaks patterns are the same when compared to the graphite that was not exposed to the glucose in Figure 15. The observation made in Figure 15 becomes important when making a comparison of figures to follow in the discussion section. One important factor derived from Figure

15 is that graphite still maintains its inert crystallographic structure even in the presence of a glucose dissolved medium.

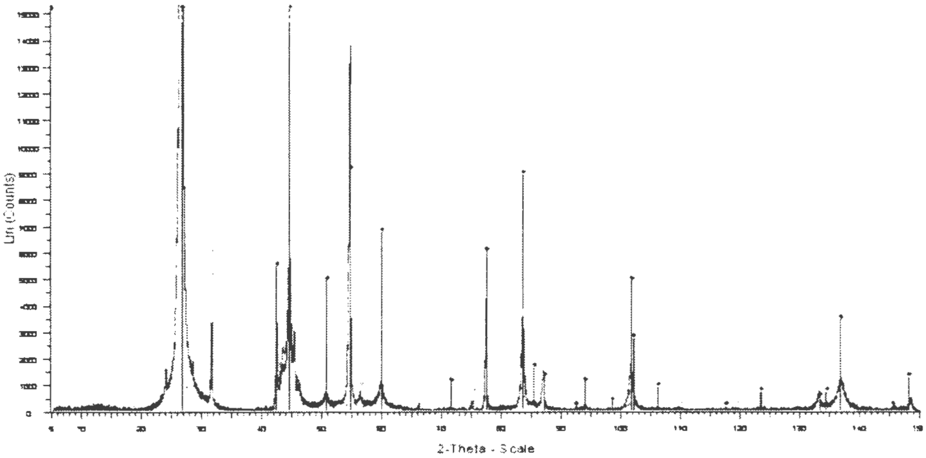

Figure 15: Superposed diffraction patterns of Nutrient Broth MC (NBMC) graphite samples and NB graphite only

Figure 15 above illustrates two diffraction patterns of the graphite chunks when exposed to the NB medium alone and when exposed to the NB medium with the MC inoculum. The diffraction pattern indicated in red is that of the NB medium with graphite only and the diffraction pattern illustrated in blue is that of the graphite exposed to the NB and the MC. The justification of having the two experiments conducted was to gain insight as to whether there is any change brought about by the MC inoculum. This difference was expected to be observed and noted in the diffraction pattern where there was a mixed culture of micro-organisms. However, according to Figure 15, it was evident that there is no difference in the graphite crystallographic structure. With or without the inoculum of the MC micro-organisms, there was no change in the crystallographic structure observed.

The lack of change in the results of Figure 14 and 15 implies that graphite maintained its crystallographic structure even in the present of the MC. The MC is used as the nutrient contained in the NB medium, acted as an energy source for micro-organisms to survive in the presence of the inert graphite without having any impact on its crystallographic structure.

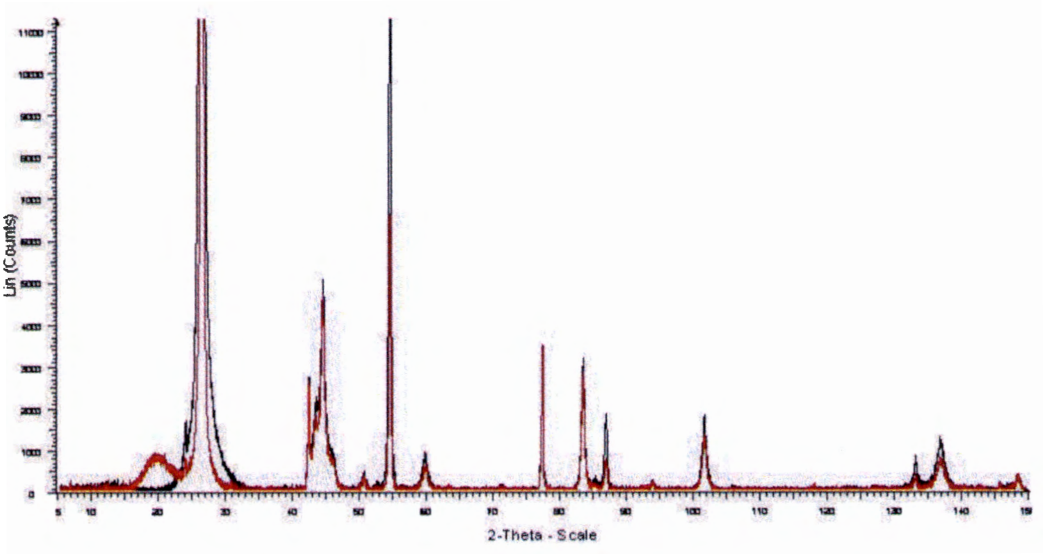

Figure 16: Superposed diffraction patterns of pure graphite sample and emulsified graphite. The hump on the emulsified pattern is a contribution from the amorphous component.

The above Figure 16, illustrates the results obtained when experimenting with an emulsifier which is the diffraction pattern in red and the **black** diffraction pattern was that of a pure graphite sample which was not exposed to either glucose, NB or MC inoculum. The pure graphite pattern was serving as a control in this regard, however, Figure 16 indicates that something different occurred when the graphite was combined with the emulsifier. This is shown by the hump (at 2-theta = 20°) that is observed and this could be due to the amorphous component caused by the contribution of the emulsified pattern, in the sample and there is also a difference when compared to other figures. A small fraction is amorphous and the other fraction could still be crystalline, because of the hump revealed by the spectrum at the beginning and some smooth peaks which signify crystallinity, therefore there is a possibility that the emulsifier worked.

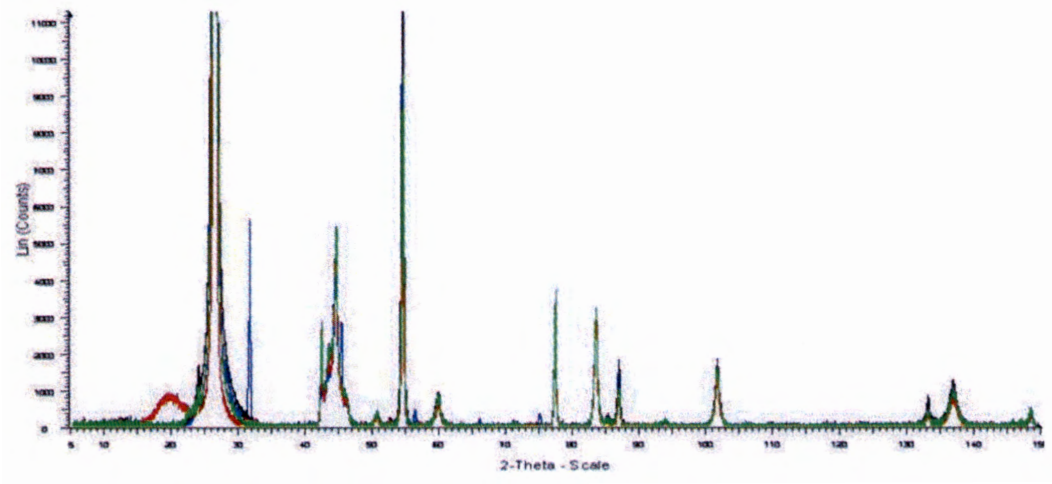

Figure 17: Superposed diffraction patterns of graphite, emulsifier graphite, cultured and glucose-doped graphite samples

The pattern colours are; red- the graphite with the emulsifier, **black-** the pure graphite, blue-graphite in the NB, green- graphite in the NB and MC inoculum. Figure 17 is a combination of all the figures embedded together, showing that the only impact was from the emulsifier as shown at 2-theta = 20°.

4.3 Denaturing gradient gel electrophoresis

We then investigated the nature of microbes living in the presence of a source of ^{14}C chemically representative of ^{14}C on the surface of irradiated graphite. A tentative correlation can be made between band intensities and relative species richness within a sample. For example sequence four has a much darker band on the DGGE gel (Figure 20) than sequence 14, indicating that there may be a higher number of these bacteria in the sample than those found for sequence 14. If the bacterial species in question has a desirable/expected metabolic process from a particular sample and this appears to be the dominant organism, then by extrapolation this process is occurring (Figueroa *et al.*, 2017).

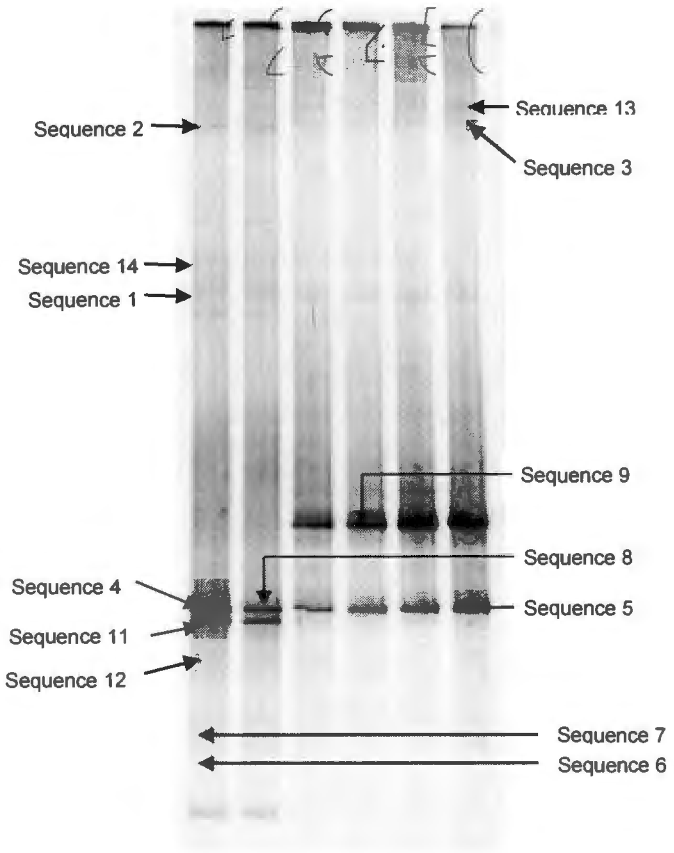

Figure 18: 45-55% denaturing gradient gel of graphite samples (Lane 1 and 2- control sample; Lane 3 and 4- Acetate sample; Lane 5 and 6- Bicarbonate sample)

4.4 Sequencing

DGGE band sequences grouping with known BLAST results provide a tentative identification of organisms within each sample (Figure 18). Bootstrap values can be construed as a percentage support for the branches forming each clade and thus for tentative identification of the bacterial species (Mohapatra, *et al.*, 2010)

Figure 18 depicts a phylogenetic relationship between micro-organisms that mediate the enzymatics reduction and/or oxidation of the sodium acetate and sodium bicarbonate. The major physiological characteristics and types of the acetate and bicarbonate redox transformations are enzymatically mediated by the micro-organisms (Suzuki *et al*, 2006).

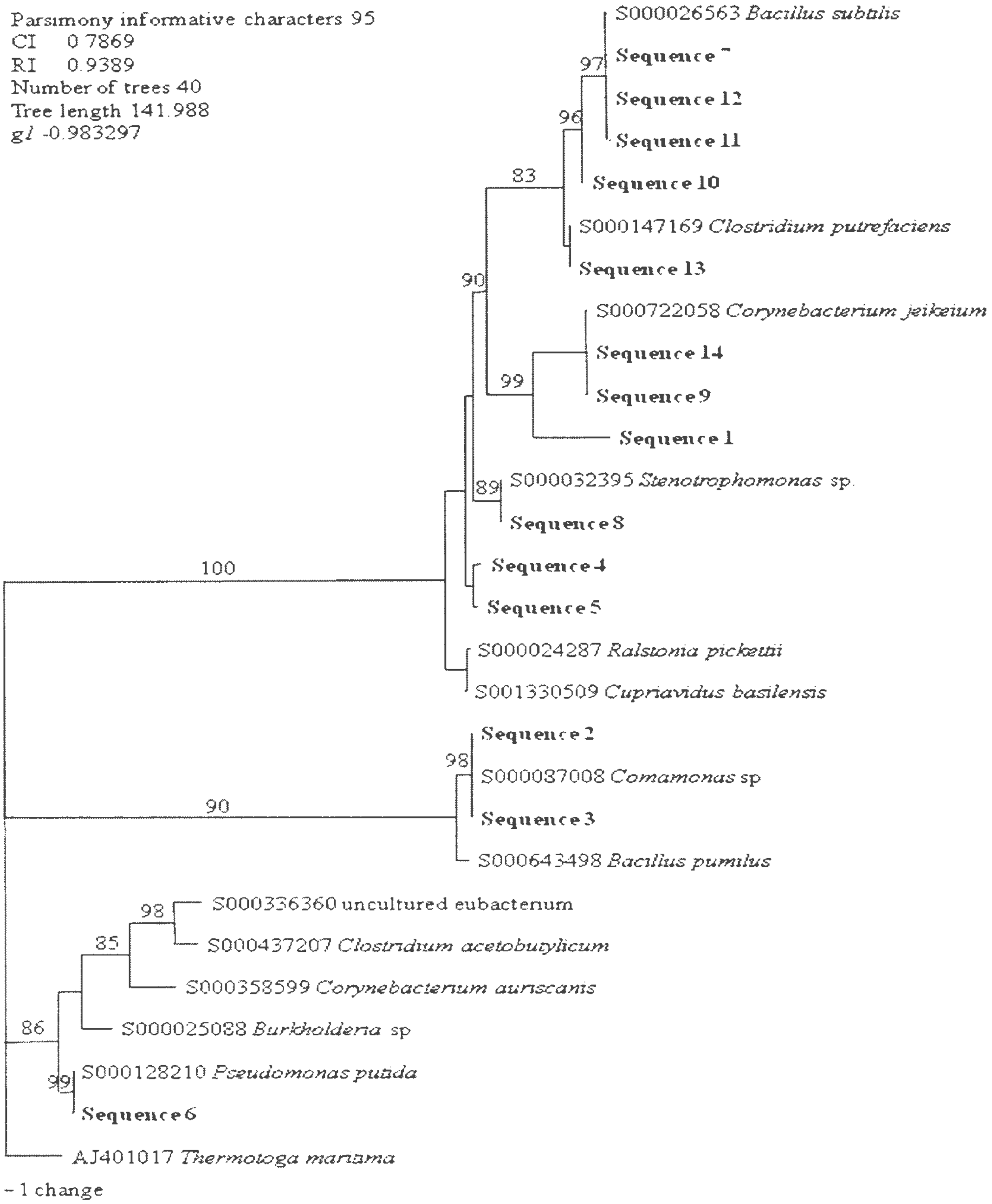
Parsimony informative characters 95
CI 0.7869
RI 0.9389
Number of trees 40
Tree length 141.988
g1 -0.983297

Figure 19: Phylogeny of partial16S rRNA gene amplicons recovered from the DGGE gels in Figure 20 (bootstrap values are indicated in above branches)

Table 5 below depicts the various organisms tentatively identified from our PBMR samples. From the literature, it is clear that these micro-organisms possess specific characteristics which enable them to survive in the presence of the radioisotopes (acetate and bicarbonate) (Lloyd *et al.*, 2005).

Table 5: Organisms identified in this study from MCs using DGGE

Organism identified (This work)	**Isolated**	**Unique ability (This work)**
Corynebacterium jeikeium	*Corynebacterium* species have been isolated from amniotic membranes and spent nuclear pools (Sing *et al.*, 2006)	Radiation resistant micro-organisms. Able to oxidise C2-C4 alkanes (Aviam *et al.*, 2004).
Corynebacterium auriscanis		
Clostridium acetobutylicum	*Clostridium* species have been isolated from different environments such as soil, decomposing biological material, lower gut of mammals and waste water (Suresh *et al.*, 2007)	*Clostridium* specie has been used in bioremediation approaches to treat nitroaromatic contaminants (Sembries and Crawford 1997)
Clostridium putrefaciens		
Bacillus subtilis	*Bacillus* species have been isolated from a nuclear power plant (Chicote *et al.*, 2004)	n-alkane utilising strain (Wentzel *et al.*, 2007)
Bacillus pumilus		
Pseudomonas putida	*Pseudomonas* species have been isolated from nuclear power plant reactor contaminated with metals (Chicote *et al.*, 2004). Gleeson *et al.* (2006) has isolated *Pseudomonas* on weathered K-feldspar	A patent has been registered in which this organism is used to treat precious metals which contains refractory carbon (United States Patent 5244493)
Stenotrophomonas	Constructed wetland (Nicomrata *et al.*, 2008), crude-oil samples (Yoshida *et al.*, 2005), anaerobic reactor (Assih *et al.*, 2002), and soil (Emerson and Moyer 1997)	Able to degrade high molecular weight polycyclic aromatic hydrocarbons (Boonchan *et al.*, 1998). The organism is able to degrade ethylene glycol, which is a toxic byproduct from polyester hydrolysis (Kim *et al.*, 2003)

Comamonas spp	Species isolated from soil and a spent nuclear pool (Chicote *et al.*, 2004)	Possibly radiation resistant (Chicote *et al.*, 2004)
Cupriavidus basilensis	Isolated from environments with high heavy metal contaminants (Nies 1999)	Able to degrade xenobiotics in the presence of high heavy metal concentrations (Springael *et al.*, 1993)
Burkholderia spp	*Burkholderia* species have been isolated from various environments: soil (LiPuma *et al.*, 2002), crude-oil samples from Japanese oil stockpiles (Yoshida *et al.*, 2005); human patients suffering from cystic fibrosis (LiPuma *et al.*,2002) and iron ore (Valverde *et al.*, 2006)	Showed to be able to fractionate carbon and hydrogen isotopes

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The uses of SEM for analysis improved the understanding of the process that occurred in the nuclear reactor experiments where graphite was exposed to microbes. This was indicated by the bio-film formation on the sample surface. As this was the initial method of analysis it gave positive results, which motivated to use other analysis techniques namely XRD and DGGE. The final graphite chunks samples from the SEM experiment indicated that the bio-film precipitated on the sample surface suggesting that there was no inhibition caused by the graphite to the micro-organisms (Wang *et al*, 2014).

The use of all the three analysis methods was complimentary because the limitations of one technique were the covered by another method. The use of microbes to minimise radioactive waste is cost-effective and does not need much machinery to operate; it was evident in this project. Another aspect that should be noted is that the results obtained in this investigation are preliminary results and as such, they are subject to confirmation. This is because, once this is implemented to a larger scale and irradiated graphite is used, it gives rise to a different set of conditions than the ones used in this study.

The XRD analysis showed that there was not a significant difference brought about by the microbes to the crystallographic structure. This could be due to the experimental duration and the unbroken graphite carbon bonds which made it difficult for the microbes to process the carbon as an energy source (Boyanov *et al*, 2011).

The DGGE results assisted in bacterial species identification. The analysis indicated that sodium bicarbonate had more bacterial growth when compared to the sodium acetate (Wang *et al*. 2014).

It, therefore, can be concluded that the objectives of this project have been achieved. Microbes can survive and grow in the presence of inert graphite and radioactive environment.

The characteristics of the micro-organisms identified are:

- Radiation resistant
- Historically the species have been used in bioremediation approaches to treat nitroaromatic contaminants
- Used in the treatment of metals which contain refractory carbon
- Able to degrade high molecular weight polycyclic aromatic hydrocarbons and ethyl glycol which is a toxic byproduct from polyester hydrolysis
- Able to fractionate carbon and hydrogen isotopes.

SEM results indicate that bio-film is formed on the surface of the graphite, therefore indicating that bacteria can survive in the presence of graphite. Whereas, results of XRD analysis indicated that there was no change in the crystallographic structure of graphite whether or not there is bio-film formation.

XRD provides information on bulk material properties, not sample surfaces. Since microbial action occurred on the graphite surface, any effect on the surface structure would not likely be evident via XRD analysis. To identify surface structural changes, graphite surfaces could be analysed via techniques designed to identify surface molecular features. Possible methods include Fourier transform infrared spectroscopy (FT-IR), infrared spectroscopy (IR), Raman spectroscopy (RS), secondary ion mass spectroscopy (SIMS), and x-ray photoelectron spectroscopy (XPS).

The DGGE results indicated that there were bacterial species that survive in the presence of the ^{14}C containing salts. When comparing the DGGE results of both salts, it was indicated that the sodium bicarbonate was more preferred than the sodium acetate salt. Various bacterial species identified were, *Burkholderia spp, Cupriavidus basilensis, Comamonas spp, Stenotrophomonas, Pseudomonas putida, Bacillus pumilus, Bacillus subtilis, Clostridium putrefaciens, Clostridium cariscanis and Corynebacterium jeikeium.* The significe of the identified bacteria was that, different bacteria from the mixed culture had survived in the presence of the radioactive salts.This is of pramount importance when conducting further studies to focus solely on each identified bacteria. Based on the results obtain from utilising pure graphite that was not irradiated, it can be conlcuded that the micro-organisms do not have an impact on the structure of the graphite due to the inert crystallographic structure. Therefore, the results obtained can be transposed into the actual irradiated gaphite since carbon-14 forms part of the graphite crystallophic structure. It can therefore, be concluded that the microbes used in this investagation did not consume the carbon in the graphite, but consumed the carbon-14 in the salts.

5.2 Recommendations

Even though the initial objectives of this study have been achieved, much work still needs to be done to form part of the complete waste minimisation project. For the completion of this project the following needs to be done:

- XRD analysis needs to be conducted on irradiated graphite after microbes have been introduced and compared to the XRD results of the non-radioactive graphite
- A detailed study that entails an investigation with each of the bacterial species identified in this dissertation. That is, there is need to repeat the experiment with each bacterial species at higher activities for each salt.
- Investigations should be made to manage the radioactive bacteria, thereby removing the radioactivity from the bacteria then dispose. The reason for separation is:
  - Bacteria are not allowed to be present at the disposal site in Vaalputs, due to the corrosion problems they cause
  - ^{14}C would then be used for medical purposes
- Investigate at a larger scale with irradiated graphite. The bacterial species that would be found to be dominant in the presence of irradiated graphite would then be compared to the bacterial species identified in the dissertation.

REFERENCES

1. American Nuclear Society (2004), The Pebble Bed Modular Reactor Layout and Neutronics Design of the Equilibrium Cycle, *PHYSOR- The Physics of Fuel Cycles and Advanced Systems:* Global Development, Chicago, Illinois.
2. Assih, E. A., Ouattara, A. S., Theirry, S., Cayol, J-L., Labat, M. & Macarie, H. (2002). Stenotrophomonas acidaminiphila sp. Nov., strictly aerobic bacterium isolated from an upflow anaerobic sludge blanket (UASB) reactor. *International Journal of Systematic and Evolutionary Microbiology,* **52**: 559-568.
3. Auwerda G.J, (2014). Department of Radiation Science and Technology, Core Physics of Pebble Bed High Temperature Nuclear Reactors, ISBN 978-94-6295-047-4.
4. Aviam, O., Bar-Nes, G., Zeiri, Y.& Sivani, A. (2004). Accelerated Biodegradation of Cement by Sulfur-Oxidizing Bacteria as a Bioassay for Evaluating Immobilization of Low-Level Radioactive Waste. *Applied and Environmental Microbiology,* **70** (10): 6031-6036.
5. Boonchan, S., Britz, M. L. & Stanley, G. A. (1998). Surfactant-enhanced biodegradation of high molecular weight polycyclic aromatic hydrocarbon by *Stenotrophomonas maltophilia. Biotechnology and Bioengineering,* **59** (4): 482-494.
6. Boyanov, M. I., Fletcher, K.E., Kwon, M.J., Rui, X., Loughlin, E.J.O., Loffler, E.F.& Kemner, K.M., (2011). *Solution and Microbial Control on the Formation of Reduced U(IV) species.*
7. Brown Lynne, (2017). Quarterly System Status Speech by the Minister of Public Enterprises, Available from. http://www.eskom.co.za/AboutElectricity/Documents/24012017MinBrownSpeechStatusGrid.pdf (Accessed on 17 July 2017
8. Burchell, T., Bratton, R.& Windes, W. (2007). NGNP Graphite Selection and Acquisition Strategy, *ORNL/TM-2007/153*, Oak Ridge National Laboratory.
9. Chicote, E., Moreno, D., Garcia, A., Sarro, I., Lorenzo., P.& Moretero, F. (2004). Biofilming on the walls of a spent nuclear fuel pool with radioactive ultrapure water. *Biofiulming*, 20: 35-42.
10. Chirwa, E. (2007). Minimization of Radioactive Waste, Literature Review Report. *PBMR Document*, WM-PC-222-20595.

11. Chirwa, E. (2008). Minimization of Waste Project: Phase I work with 14C Research Plan. *PBMR Document 089813*

12. Cogliati, J.J; (2011). Pebble Bed Pebble Motion: Simulation and Applications

13. Cullity, B. D. (1977). Elements of X-Ray Diffraction. *Second Edition*

Emerson, D. and Moyer, C. (1997). Isolation and characterization of novel iron-oxidizing bacteria that grow at circumneutral pH. *Applied and Environmental Microbiology*, 63 (12): 4784-4792.

14. Dunzik-Gougar. M, van Ravenswaay. F, Kuczynski. L and Slabber. J.M. F.Microbial Treatment of Irradiated Graphite for Separation of Radioisotope 14C From Bulk Graphite 12C. Fourth International Topical Meeting on High Temperature Reactor Technology, Volume 2, Washington, DC, USA, September 28–October 1, 2008

15. Euratom, "CARBOWASTE," (2007). Seventh Framework Programme, Nuclear Research and Training Activities, Annex-1, Description of Work, CARBOWASTE, Treatment and Disposal of Irradiated Graphite and Other Carbonaceous Waste Grant agreement for: Large-Scale Integrating Collaborative Project, Grant agreement no.:211333.

16. Ercolini, D. (2003). PCR-DGGE Fingerprinting: novel strategies for detection of microbes in food.

17. Fechter, M. (2007). Fuel Sphere Matrix Graphite Material Datasheet. PBMR Document, 025013.

18. Figueroa, L. A., Honeyman, B.D. & Ranville, J.F. (2017). Coupled microbial and chemical reactions in Uranium bioremediation, last viewed in 2017.

19. Fourie, P. (2005). PBMR 400 MWT: *Production and Emission of carbon-14 Report*. Document No.: 017808.

20. Fjellbirkeland, A., Torsvik V. & Øvreås, L. (2001). Methanotrophic diversity in an agricultural soil as evaluated by denaturing gradient gel electrophoresis profiles of *pmoA*, *mxaF* and 16S rDNA sequences. *Antonie van Leewenhoek*, **79:** 209-217.

21. George, A. P. (2001). *Introduction to the Pebble Bed Modular Reactor*. PBMR Document No.: 009949-185.

22. Gleeson, D. B., Kennedy, N.M., Clipson, N., Melville, K., Gadd, G.M.& McDermott, P. (2006). Characterization of bacterial community structure on a weathered pragmatic granite. *Microbial Ecology*,51: 526-534

23. Greyling T, Pebble Bed Modular Reactor (PBMR)- A Power Generation Leap into the future. Pebble Bed Modular Reactor (Pty) Ltd. Accesses 2017

24. Hall, T. (2001). BioEdit v 5.0.9.1 North Carolina State University Raleigh, NC.

25. http://www.eskom.co.za; last opened on 04 March 2017

26. http://www.world-nuclear.org/information-library/country-profiles/countries-o-s/south-africa.aspx, accessed 18 August 2017
27. http://www.gcis.gov.za/sites/www.gcis.gov.za/files/docs/resourcecentre/yearbook/2013-4Energy.pdf, (accessed 18 August 2017)
28. http://www.universalcoal.com/our-projects/coal-mining-in-south-africa/ (accessed 18 August 2017)
29. IAEA. (1999). Nuclear Graphite Waste Management Technical Committee Meeting Held In Manchester United Kingdom.
30. IAEA. (2006). Characterization, Treatment and Conditioning of Radioactive graphite from Decommissioning of Nuclear Reactors.
31. Intitut De Radioprotection et de Surete Nucleaire (IRSN), accessed 2017.
32. Jenkins R. (2000) X-ray Techniques: Overview, Encyclopaedia of Analytical Chemistry, R. A. Meyers (Ed.) pp. 13269–13288, Ó John Wiley & Sons Ltd, Chichester.
33. Kadak, A.C. (2005). A future for nuclear energy: pebble bed reactors, *Int. J. Critical Infrastructures,* Vol. 1, No.4, Department of Nuclear Engineering 24-202, Massachusettes Institution of Technology.
34. Kessides. I.N and Kuznetsov. V, (2012). Small Modular Reactors for enhancing energy security in developing countries.
35. Kim, C. H., Han, S. H., Kim, K. Y., Cho, B. H., Kim, Y. H., Koo, B. S. & Kim, Y. C. (2003). Cloning and expression of pyrroloquinoline Quinone (PQQ) genes from a phosphate solubilizing bacterium *Enterobacter intermedium. Current Microbiology,* 47: 457-461.
36. Koster, A. (2008). Technical description of the PBMR Demonstration Power Plant.PBMR Document No.: 016956.
37. LalK. J. (2013). Talking lessons learned from the PBMR project forward: A holistic view.
38. Lamarsh, J. R & Baratta, J.B. (2001). Introduction to Nuclear Engineering. Third Edition.
39. Lee Sang-Min, Kang Dong-Su and Roh Jea-Seung, (2015). Bulk graphite: material and manufacturing process, carbon letters vol.16, No. 3, 135-146.
40. LiPuma, J. J., Spilkeer, T., Coenye, T., LiPuma, C. F. G .J. J., Spilker, T., Coenye, T. & Gonzalez, C. F. (2002). An epidemic Burkholderia cepacia complex strain identified in soil. *The Lancet,* 359 (9322): 2002-20030.
41. Lloyd, J. R. & Renshaw, J. C. (2005). Bioremediation of radioactive waste: radionuclide-microbe interactions in laboratory and field-scale studies.
42. Management of waste containing tritium and carbon-14, International Atomic Energy Agency Vienna. (2004). *Technical Report Series No. 421.*

43. Marden, B. J., Hopkinson, K. L. & Wickham. (2002).The chemical form of carbon-14 within graphite. A report produced for Nirex.
44. Mahlangu. J. (2009) Influence of temperature on the microstructure of silicon carbide layer of the PBMR fuel particles.
45. Mathibe, C., Evaluation of the efficiency of magnetic isotope scrubber in the Pebble Bed Modular Reactor. 2008
46. Mohapatra, B. R., Dinardo, O., Gould, W. D. & Koren, D.W. (2010).Biochemical and genomic facets on the dissimilatory reduction of radionuclides by micro-organisms- A review.
47. Morash, B., Richnow, H. H., Schink, B., Vieth, A. & Mechkenstock, R. U. (2002). Carbon and hydrogen isotope fractionation during aerobic bacterial degradation of aromatic hydrocarbons. *Applied and Environmental Microbiology*, **68** (10): 5191-5194.
48. Muyzer, G., De Waal, E. C. & Uitterlinden, A. G. (1993). Profiling of complex microbial communities by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, 59: 695-700.
49. Nicholis. D.R. (2001). The Pebble Bed Modular Reactor.
50. Nicomrata, D., Dicka, W. A., Dopsonc, M. & Tuovinen, O. H. (2008). Bacterial phylogenetic diversity in a constructed wetland system treating acid coal mine drainage. *Soil Biology & Biochemistry*, 40: 312-321.
51. Nies, D. H. (1999). Microbial heavy metal resistance. *Applied Microbiology and Biotechnology*, 51: 730-750.
52. Pedersen, K. (2000). Microbial processes in radioactive waste disposal. *Department of Cell and Molecular Biology*. Microbiology Göteborg University.
53. Pebble Bed Modular Reactor (Pty) Ltd Limited (PBMR). Status report 70-Pebble Bed Modular Reactor. (10 August 2011).
54. Reitsma, F. (2004). The Pebble Bed Modular Reactor Layout and Neutronics Design of the Equilibrium Cycle. Reactor and Radiation Analysis.
55. Sembries, S. and Crawford, R.L. (1997). Production of *Clostridium bifermentas* spores as inoculum for bioremediation of nitroaromatic contaminants. *Applied and Environmental Microbiology*, 63 (5): 210-214.

56. von Lensa W, Vulpius D, Steinmetz H.J, Girke N, Bosbach D, Thomauske, Julich, Banford A.W, Bradbury D, Grambow B, Grave M.J, Jones A.N, Petit L, Pina G (2017). Treatment and disposal of irradiated graphite and other carbonaceous waste, (2007). *Nuclear Research and Training Activities grant agreement for: Large- Scale Integrating collaborative project.* Grant agreement No.:211333.

57. Siciliano, S. D., Germida, J.J., Banks, K. & Greer, C. W. (2003). Changes in microbial community composition and function during a polyaromatic hydrocarbon phytoremediation field trial. *Applied and Environmental Microbiology,* 69: 483-489.

58. Sing, R., Gupta, P., Purohit, S., Kumar, P., Vaijapurkar, S. G. & Chackarkar. (2006). Radiation resistance of the microflora associated with amniotic membranes. *World Journal of Microbiology and Biotechnology,* 22: 23-27.

59. Slabber, J. (February 2006). Technical Description of the PBMR Demonstration Power Plant. PBMR Document No.: 016956.

60. Springael, D., Diels, L., Hooyberghs, L., Kreps, S. & Mergeay, M, (1993). Construction and characterization of heavy metal resistant haloaromatic-degrading *Alcaligenes eutrophus strains*. *Applied and Environmental Microbiology,*59: 334-339

61. Sujata, K. and Hamlin M. Jennings. (1991). Advances in Scanning Electron Microscope.

62. Suresh, K., Prakash, D., Rastogi, N. & Jain, R. K. (2007). *Clostridium nitrophenolicum* sp. Nov., a novel anaerobic p-nitrophenol-degrading bacterium, isolated from a subsurface soil sample. *International Journal of Systematic and Evolutionary Microbiology,* 57: 1886-1890.

63. Suzuki, Y. & Suko, T. (2006) Geomicrobiological factors that control uranium mobility in the environment: Update on recent advantages in the bioremediation of uranium-contaminated sites.

64. Thermo (1999). ARL Applied Research Laboratories S.A., Chapter 7: Basics of X-ray Diffraction.

65. University of Toledo (2001). Laboratory for Microbial Ecology, Department of Earth, Ecological and Environmental Science.

66. Valverde, A., Delvasto, P., Peix, A., Velázquez, Santa-Regina, I., Ballester, A., Rodrĭgiez-Barrueco, C., Garcĭa-Balboa, C. & Igual, J.M. (2006).*Burkholderia ferrariae* sp. Nov. isolated from an iron ore in Brazil. *International Journal of Systematic and Evolutionary Microbiology,* 56: 2421-2425.

67. van Wyk, Jo-Ansie (2013). South Africa's Nuclear Energy Future. *South African Institute of International Affairs (SAIIA) Occassional Paper No. 150.* Governance of Africa's Resources Programme. Pp 1 – 36

68. van Blerk, J. J, Kozak, M. W. (2008). Environmental Impact Assessment for a 400 MW(t) Pebble Bed Modular Reactor Demonstration Power Plant, Aquisim Consulting (Pty) Ltd.
69. Venter, A. M. (2005). Elements of neutron diffraction. NECSA.
70. Wang, Z., Tebo, B. M., Giammar. (2014). Effect of Mn(II) on UO_2 Dissolution under Anoxic and Oxic Conditions.
71. Wentzel, A., Elligensen, T. E., Kotlar, H-K., Zotchem, S. B., Throne-Holst, M. (2007). Bacterial metabolism of long-chain n-alkanes*Applied Microbiology and Biotechnology.*76: 1209-1221.
72. Wenzel, U., Herz, D., Schmidt, P. (1979). Determination of ^{14}C in spent HTGR fuel elements*Journal of Radioanalytical Chemistry, Vol 53, and No. 1- 2.*
73. Wickham, A. J. and B. J. Marsden (principal authors). (2006). "IAEA Characterization, Treatment and Conditioning of Radioactive Graphite from Decommissioning of Nuclear Reactors." IAEA- TECDOC-1521.
74. Windes, W., Burchell, T., Bratton, R. (2007). Graphite technology development plan. INL/EXT-07-13165. The Idaho National Laboratory (INL).
75. Wood, C. (2006). *Graphite Decommissioning.*Options for graphite treatment, recycling or disposal including a discussion of Safety-Related Issues.
76. Yoshida, N., Yagi, K., Sato, D., Watanabe, N., Kuroishi, T., Nishimoto, K., Yanagida, A., Katsuragi, T., Kanagawa, T., Kurane, R., Tani, Y. (2005). Bacterial communities in petroleum oil in stockpiles. *Journal of Bioscience and Bioengineering* 99 (2): 143-149.