Biological Removal of Radiocarbon-14 from Irradiated Graphite - 8357

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ABSTRACT

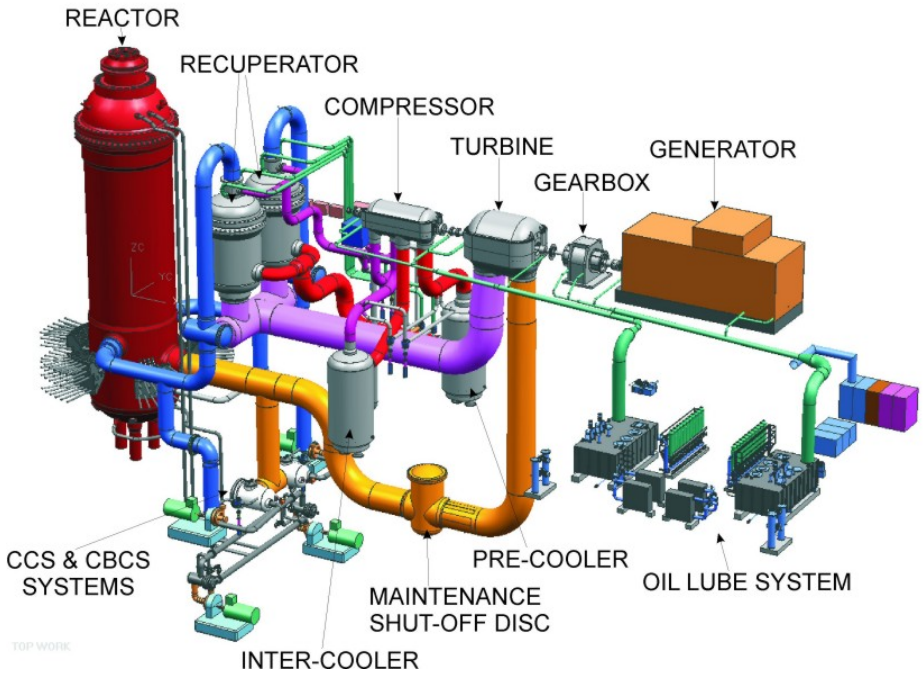
This paper reports on the preliminary study on the bioseparation of radiocarbon-14 (C-14) using a mixed-culture of microorganisms. The bioseparation principle is critically analyzed and is benchmarked against existing C-14 separation methods using physical chemical processes. The preliminary study indicated probable bioseparate of C-14 from solution prepared from a nuclear graphite mixture, even though the findings need to be verified. The current experiment consisted of a growth vessel and a biofilter operated in a closed loop. The biofilter was not installed for the purpose of treatment but rather as a method of isolation of microorganisms for further processing. Significant amounts of C-14 were detected in the trapped cells in the biofilter, significantly higher than in controls taken before adding carbon sources containing C-14. The microorganisms were grown under micro-aerobic conditions with graphite carbon and commercially purchased powdered carbon as the predominant supplied carbon sources. Small amounts of sucrose (500 mg/L) were added at 48 hour intervals to maintain culture balance. A proof of concept study is underway to determine the C-14 mass balance, characterize the microorganisms in the reactor, and establish the presence or absence of processes that might have affected the preliminary observations. This research represents an exploration into a new field using a new philosophy for treatment of C-14 in low-level waste

Key words: C-14 reclamation, waste minimization, ionization energy.

BACKGROUND

As the world becomes more sensitive to the problem caused by carbon emissions due to the impact on global warming, countries look at nuclear energy as one of the most viable intermediate solutions to replace the burning of fossil fuels. Apart from being relatively environmentally friendly, nuclear fuel (uranium) is more abundant and is required in smaller volumes than most carbon based fuels, e.g., coal. However, like fossil fuels, nuclear fuel is non-renewable, that is why it can only be used as a transitional solution as we seek more renewable energy sources. Worldwide, the use of nuclear energy is expected to increase from 17% of world energy to 60% in 2020 [1, 2]. In South Africa, coal generation accounts for 94% of the current supply – 6% is supplied by two Pressurised Water Reactors (PWRs) near Cape Town. Most of the coal generation plants in South Africa have reached their design age and thus will need to be replaced before the year 2025. The South African government has approved the construction of its demonstration unit for the fourth generation High Temperature Gas Cooled Reactor (HTGR) based on the pebble bed technology (Figure 1a). The long-term plan is to construct about 30 Pebble Bed Modular Reactor (PBMR) reactors at 165MW each to replace the aging coal generation plants and to meet future demands in the country (http://www.pbmr.co.za).

(a)



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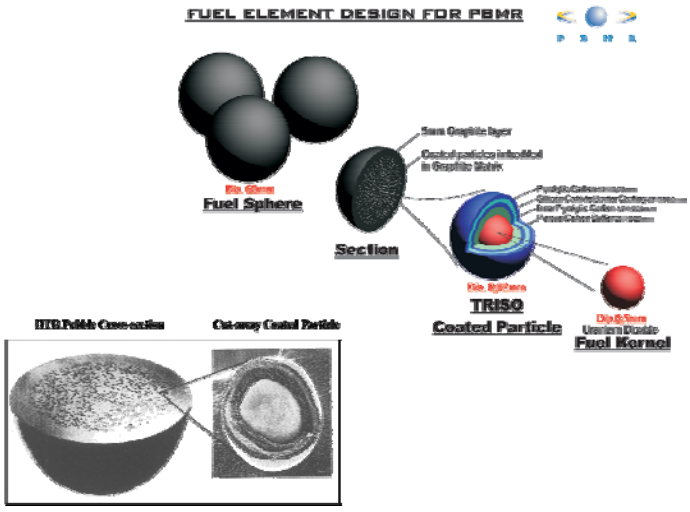

Figure 1.(a) Layout of the PBMR system and (b) Anatomy of the pebble [Courtesy, PBMR (PTY) Ltd, SA].

Advantages of the PBMR Technology

The main advantage of the PBMR technology is that it is inherently safe. The reaction elements (fuel kernels) are enclosed in carbon spheres protected by a graphite layer thereby eliminating the possibility of reactor melt down (Figure 1b). The operational fluid is inert gas helium instead of water typically used in traditional reactors. The strategic advantage of the pebble bed modular reactor is that they can be installed close to centres of industrial activity mostly along the cost.

Most of South Africa's coal fired electricity is generated by large scale plants built near the pit heads of two extensive coal producing areas, on the eastern side of the country. This requires the operation of long power lines from the coal rich areas to load centres away from the pit heads, which in turn results in high capital costs and transmission losses. Transporting coal to distant power stations is not an option as it is very expensive.

Shortcomings of the PBMR Technology

The Pebble Bed Reactor technology produces large volumes of low level radioactive waste (LLW). This is a result of the central design feature of the technology where the fuel kernels are nested with a relatively large amount of protective graphite (carbon) which must be replaced after exposure to radiation for a predetermined period. Expiry of the pebbles is determined by factors such as loss of structural integrity and increase of radioactive impurities mostly as actinides, irradiated gases, and C-14. The waste contains large amounts of carbon since the reactor pebbles and structural components are comprised of approximately 90% carbon. The C-14 is produced in the fuel, from core structural materials, and in reactor coolant, due to the presence of the (stable) parent isotopes N-14, O-17 and C-13 [3]. These stable isotopes are present as major components or impurities. These parent isotopes are involved in the three major types of reactions to produce C-14, as listed in Table 1.

Treatment Options

Microbial processes have been used in the treatment of conventional pollutants such as organic matter from municipalities and several waste compounds from industry. However, only a few biological processes have been investigated under severe radiation conditions [4-6]. Most of these studies concentrated on the microbial resistance to radiation and the ability to sustain metabolic activity while utilising ionizing radiation energy. Preferential metabolism of radioisotopes in aquatic systems has not been investigated at the laboratory or industrial process level. However, there are indications of preferential uptake of other carbon isotopes from ancient fossils [7].

Radiation as a Source of Metabolic Energy

Recent studies have shown that microorganisms from radiation exposed environments are capable of using ionising radiation as the source of energy for growth. Just as plants use the green pigment – chlorophyll – to trap energy in order to split water and generate electrons in the endothermal process of photosynthesis, certain species of fungi (*Wangiella dermatitidis* and *Cryptococcus neoformans*) have been shown to use the dark pigment (Melanin) to conserve energy from ionising radiation [4]. The biosynthetic pathway driven by ionising radiation is believed to have evolved during prolonged radiation exposure of microorganisms in ancient incidents such as the anomaly of the star Nemesis (undated) and the prolonged core reversals (superchrons) in the Kiaman and Cretaceous periods that may have resulted in a higher radiation dose at the surface of the earth [8,9]. The weakness of this process as a potential treatment processes is that it does not remove the source of radiation. However, radiation induced growth could result in the formation of biofilms which are known to be effective in providing shielding effect against toxicity and other damaging effects [10]. Microorganisms growing on radiation energy could have the benefit of growing well under organic carbon starvation conditions since organic carbon sources are non-essential for their growth in such environments. This could be beneficial for *in situ* bioremediation processes where

Table (i). Carbon-14 production mechanisms and cross-sections

Target isotope	Mechanism	Thermal cross-section (barns)	Isotopic abundance of the parent material (%)*
N-14	N-14(n, p)C-14	1.81	99.6349
C-13	C-13(n, γ)C-14	0.0009	1.103
O-17	O-17(n, α)C-14	0.235	0.0383

Source: International Union of Pure and Applied Chemistry (IUPAC), 1984.

the introduction of external organic carbon sources is not desirable (since external organics may act as potential pollutants).

Ionising Radiation Shielding by Biomass

Generation of biomass from the energy derived from radioactive decay could create a diverse microbial culture environment supporting a wider variety of microorganisms some of which may be capable of carrying out desirable hydroxylation processes of metallic pollutants and mineralization of recalcitrant organics. Uptake of the radioactive component of carbon in the systems is also desirable as it lowers the radioactive toxicity at the system level.

Bioseparation of C-14

Biomass has long been suspected of being capable of preferential sequestration of the carbon atom based on mass. Fossils from the earlier earth suggest higher concentrations of C-13 concentration in plants in an atmosphere with both C-13 and C-12 [7]. This phenomenon is not evident in the modern plants; the presence of this capability in microorganisms from extreme environments may not be likely due to preservation of archaic mechanisms in these organisms.

The objective of this study is to investigate the feasibility of the bioseperation of C-14 in consortium culture tested in the preliminary experiment. Further tests will be conducted using microorganisms from local sources to check the validity of the process and to determine the prevalence of the C-14 bioaccumulation microorganisms if feasible.

The results from this study have important ramifications for the development of radioactive waste from the nuclear power generation and medical industries.

PRELIMINARY STUDY

Microorganisms Growth Conditions

The microorganisms used in the preliminary experiment were available as a consortium supplied as humus from a radiation exposed environment such as a uranium mine. The cultures were cultivated in suspension in a completely mixed reactor aerated by vigorous mixing with sucrose as a supplementary carbon source. The initial conditions as described above were maintained for 24 hours before introduction of a C-14 rich carbon source which marked the beginning of the experiment.

Bioreactor Operation

A continuously mixed reactor (growth vessel) constructed from Pyrex panels (30 cm × 20 cm × 15 cm) was used to grow the above described consortium of bacteria with a mixture ground graphite (1500 – 2000 mg/L) and spikes of glucose (500 mg/L every 48 hours) an additional carbon source. The growth vessel was connected to a biofilter unit by submersible pump where particulate matter was isolated for further analysis (Figure 2). The pump was also used for recirculation and mixing.

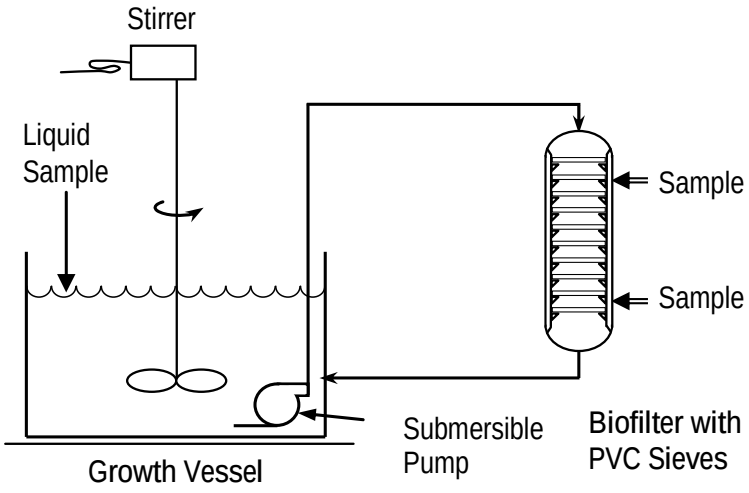

Figure 2. Preliminary experimental setup

EXPERIMENTATION

Waste graphite material was crushed into very small particles (micro-scale), in order to improve the bioavailability of the carbon in the graphite. It is recognized that graphite is a highly inert material thus the particle size necessary for effective bioavailability remains a subject for further

investigation. The crushed graphite was supplemented with powdered carbon obtained commercially from medical sources to supplement the fine particle range of the crushed sample. The target here was to simulate as closely as possible the composition of the pulverized graphite pebbles in a carbon reclamation system.

Standard C-14 was supplemented proportionally to the anticipated C-14 composition of radioactive nuclear graphite. The reactor was operated without C-14 in the reactor for 24 hours, then with C-14/C-12 added for 48 hours.

Liquid samples from the growth vessel were examined periodically under an optical microscope for changes of morphology and microbial species composition. So far, approximately 16 different cell types were identified. The actual role of each cell type was not determined in the initial study. However, the observations were useful to monitor the culture conditions in the vessel.

The reactor foamed extensively after operation for 48 hours with the following possible outcomes: (1) the accumulation of a relatively hydrophobic substance or surfactant, (2) the predominance of hydrophobic bacteria as the reactor become more anaerobic, or (3) production of anaerobic gases. As the reactor was operated under batch conditions, the first possible outcome is most likely.

C-14 uptake by the bacteria was analysed from the captured cells on the biofilter trays. C-14 was determined using a combination of scintillation count and mass spectrometry to determine content and degree of decay.

Model of C-14 Removal

So far, the only feasible model for the removal of C-14 from the suspended culture growth vessel is bioaccumulation. The biological removal of C-14 was simply measured by the disappearance of C-14 from the growth vessel and reappearance of C-14 in the cells. Unfortunately, the comparative analysis of the C-14 disappeared versus C-14 recovered from trapped cells was incomplete. A more detailed analysis was, however, conducted for the C-14 accumulated in the cells.

C-14 in the Biomass

The samples were provided to the external laboratory (CSIR, Pretoria) without detailed background information on the C-14 content to avoid bias. Samples A and B were provided whereby Sample B was a set of biofilter trays collected from the biofilter reactor before adding C-14 (24 hours operation) and Sample A was a set of biofilter trays removed after 48 hours operation under a radioactive carbon source. Note that during the first 24 hours circulation was done in the absence of C-14 isotopes and the last 48 hours circulation was done with C-14 isotopes present in the mixture.

The samples were dried and combusted under an oxygen stream to produce carbon dioxide. The produced CO_2 was subjected to standard cryogenic and chemical purification processes to yield a clean sample for admission into the detector system. An additional clean filter was treated

separately in order to determine its carbon yield. The samples were counted in proportional gas counters, using the standard method for radiocarbon analysis which requires a replicate measurements analysis on different counters. The replication procedure was not performed due to time constraints.

The additional sample (without biomass) was used to determine additional the background C-14 in CO_2 in the dead-gas used for volume correction when the amount of gas produced during the burning of a sample was insufficient for analysis. The analysis showed that C-14 in the filter material could be approximately 6000 years old where as the C-14 in the cells was much recent indicating the high probability that the C-14 in the cells was bioaccumulated. The analysis also showed quantitatively that the Sample A corrected after operation under C-14 for 48 hours contained twice as much C-14 than the Sample B (collected before operation under C-14).

The measured C-14 activity of the samples comprised of a component contribution from the filter and from the bacteria. The activity of the filter was not known therefore only the scenario relating to the potential contribution of the filter to the overall activity of the samples was considered. Even with the lack of precision in the analysis, the preliminary outcome indicated that the filters did not have any C-14 activity and that the observed difference was related to the radioactivity of the bacteria.

PROOF OF CONCEPT RESEARCH

Rationale

A proof of concept experiment is required to state with certainty whether the current consortium culture sufficiently bioaccumulate C-14 to warrant a more detailed study towards developing a process. This is necessitated by the fact that certain critical elements were not studied during the preliminary experiment.

Materials and Methods

The bacteria used in the preliminary experiment will be used in the proof of concept research with improvements in the culturing method to determine with certainty the required growth conditions for the consortium. A closed system will use for the reactor vessel to allow control of aeration conditions (that the reactor may be operated aerobically – with air bubbled through the reactor – or anaerobically with no air supply) (Figure 3). An external pump will be used to feed the biofilter system to allow better control of flow and the effect of mixing at different rates will be studied. The reactor system will be equipped with a gas analyser, pH and ORP meter, DO meter, and TOC analyser. Trays removed at predetermined intervals will be analysed for cell composition and C-14 content.

Carbon Mass Balance Analysis

The sampling plan shown in Figure 3 will make it possible to conduct a comprehensive mass balance of the C-14 removed from the suspension and the C-14 accumulated in cells. Gas analysis

will allow us to determine the amount of C-14 lost through gases. A high value of C-14 in the gas phase could indicate losses through catabolism and low to no bioaccumulation. C-14 lost through mineralization may be more difficult to treat than the C-14 accumulated in cells since the C-14 in the gas phase could require rather expensive scrubbers to remove.

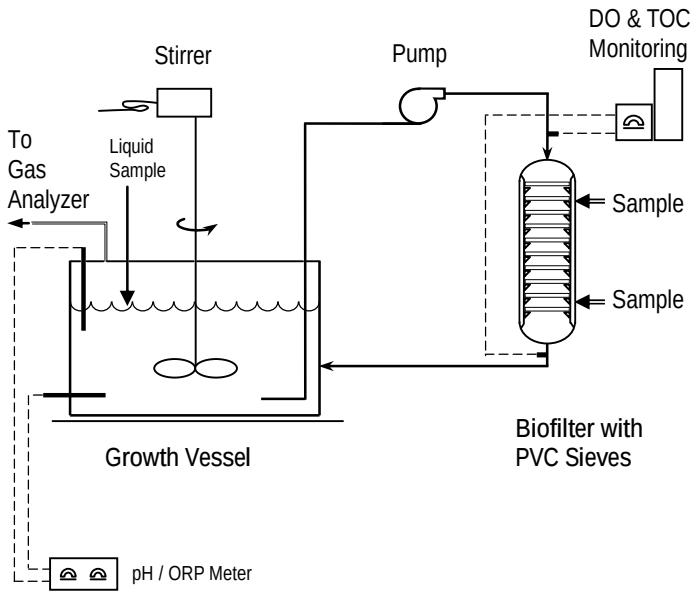

Figure 3. Proof of concept experimental setup.

The system as presented in Figure 3 will also be used to evaluate the ratios of C-14:C-12 at every point of the metabolic chain. Variations in the ratio of C-14:C-12 may indicate the flux of C-14 in the system and thus will be used to validate the process of bioseperation.

A different experiment will be conducted in vitro to determine the preferred energy source for the microorganisms. This will be done specifically to determine if the cultures derive energy out of the radioactive decay of C-14. This test is valid as it seeks to explain why the culture thrived under organic carbon starvation.

Characterization of Microorganisms

The function of the different species of microorganism in the current microbial consortium is not known. It is suspected that there are interdependencies between organisms in the culture that might be lost if conventional culturing methods are used. Culturing will therefore be conducted under conditions very similar to the current growth conditions.

Two molecular methods will be used for microbial culture characterisation, i.e., (1) reverse transcription-polymerase chain reaction (RT-PCR) and (2) denaturing gradient gel electrophoresis

(DGGE), both methods are available at the University of Pretoria. More detailed analysis will involve the 16S rRNA fingerprinting by determining terminal restriction fragment length polymorphisms [16].

CONCLUSION

Primary results show with a high degree of certainty that cells accumulated as demonstrated by the increase in C-14 activity in cells trapped on biofilter (sieve) trays. Observed metabolic activity indicated that the process was possible under very dissolved oxygen suggesting growth under micro-aerobic to anaerobic conditions. There is need to verify the results with more detailed measurement of C-14 in solution and C-14 evolved as $^{14}CO_2$ through the process of respiration. There is also need to verify the status of radioactive decay in the solution as to where C-14 contributes to available energy sources in the systems. The results from this research are extremely important as they might form a basis for future treatment of expired graphite for reuse.

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