Bioremediation of Irradiated Toxic Organic Compounds
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Biodegradation of Irradiated Toxic Organic Compounds

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Abstract

Bioremediation holds the promise as a cost effective treatment technology for a wide variety of hazardous pollutants. In this study, the biodegradation of organic compounds discharged together with radioactive wastes is investigated. Nuclear process wastewater was simulated by a mixture of phenol and strontium, which is a major radionuclide found in radioactive wastewater. Phenol was used in the study as a model compound due to its simplicity of molecular structure. Moreover, the biodegradation pathway of phenol is well known. Biodegradation studies were conducted using pure cultures of *Pseudomonas aeruginosa* and *Pseudomonas putida*. The rate of phenol degradation by both species was found to be higher in the test without strontium. This suggests some degree of inhibition in the degradation of phenol by strontium. There was no phenol degradation in the sterile controls. The results indicate the feasibility of the biodegradation of organic pollutants discharged in radioactive effluents by specialised microbial cultures.

Keywords: biodegradation, radioactive, wastewater, organic pollutants

1. Introduction

Radioactive wastes mostly originate from nuclear energy and radiochemical processing activities mainly from nuclear-power electricity generation (87%), radionuclide production for chemotherapy in the medical industry (1%), radioactive probe production and use in genetic studies (2%), and production of sterilisation agents in agricultural and several other industrial applications [1]. The volume of radioactive wastes is projected to increase significantly in the early part of the 21st century as most of the old generation reactors reach their design age and require decommissioning. Radioactive wastes are also produced during uranium mining and ore processing for the nuclear power generation industry. Uncontrolled release of radioactive wastes from the above mentioned activities still poses many problems to human life and the natural environment due to its toxicity and the long half-lives of the radioisotopes in the waste.

Presently, nuclear energy accounts for 17% of the world production of electricity. In South Africa, nuclear power generation amounts to 6% of the country's power supply. Estimates from the World Energy Council (WEC) indicate that nuclear energy will account for 60% of the world electricity by the year 2020. As countries take a fresh look at nuclear power generation as an intermediate solution towards replacing fossil-fuel steam-driven turbine power generation, the generation of toxic irradiated organic compounds from auxiliary processes such as treatment and reclamation of fuel is bound to increase [2].

Lately, more interest has been directed towards finding solutions that involve the use of cultures of microorganisms to treat contaminated sediments and waters impacted by nuclear waste. This is due to the perceived cost-effectiveness and efficiency of biological systems over the currently used chemical-based methods [3]. Additionally, biological processes offer a more environmental friendly solution to remediation of pollution as they normally occur under natural environmental conditions (pH = 7.0), and ambient temperature and pressure, without leaving hazardous chemical by-products [4].

There is little information on the bioremediation of the radioactive waste let alone biodegradation of irradiated organic compounds. The irradiated organic pollutants, even if not severely radioactive, are mostly recalcitrant in nature. Additionally, irradiated organics and radioactive actinides occur as mixed wastes which pose disposal challenges since different components of the waste must meet different regulatory requirements [5]. During treatment, other compounds in the mixture may inhibit the degradation of one component and different conditions may be required to treat different compounds. Hence there is a need to consider the impact of the interactions between several pollutants when deciding a suitable disposal option for radioactive waste.

This paper reports on a preliminary analysis of the organic compounds released in the fuel sphere processing for a Pebble Bed Modular Reactor (PBMR) process. Although the larger bulk of wastes are classified as low radiation level wastes (LLW), their proper treatment and disposal is of great importance in order to achieve feasibility of long-term operation of the new generation reactors. Preliminary studies conducted to treat the irradiated organic compounds in simulated waste streams have shown significant impacts of both the presence of nuclides and irradiated carbon species on the organic pollutant removal kinetics [6].

2 Materials And Methods

2.1 Reagents

$Sr(NO_3)_2$ arsenzo III reagent, Phenol and Folin-Ciocalteu,s phenol reagent were all purchased from Sigma. All other chemicals were of analytical grade either from Merck or Fluka.

2.2 Microorganisms, Growth Medium and Culture Conditions

P. putida and *P. aeruginosa* were used in this study due to their ability to degrade phenol [7-8]. Earlier studies showed the capability to biosorb and bioaccumulate radionuclides in these species [6, 9]. Additionally, the purified *Pseudomonas sp*. isolates were shown to be capable of degrading aromatic organic compounds [10-11]. *P. aeruginosa* and *P. putida* (of undetermined serotypes) were obtained from the Department of Microbiology and Plant Pathology, University of Pretoria, South Africa. The organisms were maintained on an agar medium and stored at 4 ± 0.5^{o}C for further use. The bacteria were grown in a nutrient broth.

2.3 Biodegradation Tests

The phenol degradation capability under radionuclide exposure conditions was investigated in batch systems comprised of 500 ml Erlenmeyer flasks containing 100 ml of culture in the growth medium. The preliminary experimental design is presented in Table I. The culture was incubated for 4 days at 28^{o}C in a lateral incubator shaker (Labcon SPL-M15) at an agitation rate of 120 rpm. Samples of 3 ml were taken aseptically at 24-hour intervals. All tests were carried out in triplicate.

2.4 Analytical Methods

Spectrophotometric Analysis of Sr^{2+}

The common aqueous species of Strontium is $Sr^{2+.}$ A 30 ml of the sample solution was transferred to a test tube containing 1.97 ml of 0.1 M-borate buffer at pH 9.0. The solution was visualised by the addition of 0.1 ml of 0.15% (w/v) Arsenzo-III reagent. The solution was then allowed to stand for 25 minutes. The absorbance was measured at 649 nm in a Hermle Z323 spectrophotometer against a reagent blank.

Analysis of Phenol

Phenol concentration was determined colorimetrically using the Folin-Ciocalteu Method [12]. 1 ml of sample solution or standard solution was added to 10 ml of distilled water and 1.0 ml of Folin-Ciocalteu reagent. The mixture was allowed to stand for 5 minutes and then 2.0 ml of sodium carbonate was added to the mixture. After 1 hour in a dark place the absorbance nm was measured at 750 nm in a UV/VIS spectrophotometer (WPA, Light Wave II, Labotech, South Africa).

Table I. Experimental design

Test	Properties
Control 1	Strontium + phenol, no cells
Control 2	Phenol + cells, no strontium
Object test	Phenol + strontium + cells

* Cells = *P. putida* or *P. aeruginosa* inoculated culture.

3 Results and Discussion

3.1 Growth Conditions

P putida and *P. aeruginosa* were grown in nutrient broth and phenol as a non-limiting substrate. Diluted cells were used to stimulate log-growth conditions, as the treatment of the actual organic waste will be carried out in a suspended culture system. The initial concentration of the cells, 1.5 mg/L and 1.2 mg/L (*P. putida* and *P. aeruginosa*, respectively), were used in the experiments.

3.2 Phenol Degradation in Nutrient Broth

The extent of phenol degradation for both *P. putida* and *P. aeruginosa* is shown in Figure 1a & b. Phenol at 25 mg/L initial concentration was completely degraded after 72 hours of incubation. There was no phenol degradation in the sterile controls. This showed that the radionuclide (strontium) did not react or form insoluble complexes with phenol, thus, all observed removal was due to biological processes. The rate of phenol degradation was higher in the test without strontium, for both cells, thus indicating some degree of inhibition of the phenol degradation process by strontium. The degradation rate at a point of 90% removal is presented in Table 2. The rate of degradation was higher in *P. putida* than in *P. aeruginosa.*

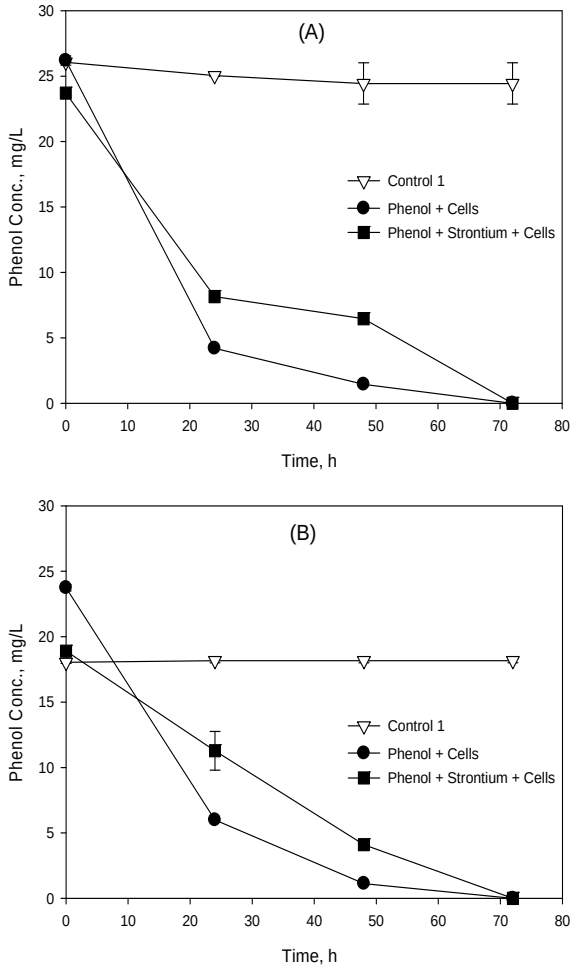

Fig. 1. Phenol degradation in (a) *Pseudomonas putida* and *(b) Pseudomonas aeruginosa* batch culture experiment.

Table II. Removal rates of Phenol

Cells	**Phenol with cells (mg/L/h)**	**Phenol +Strontium with cells (mg/L/h)**
P. putida	0.52	0.36
P. aeruginosa	0.47	0.31

During the biodegradation of phenol, the accumulation of a brown colour compound was observed in all cultures with phenol. However the brown colour was darker in the test with only phenol. The brown colour was not due to phenol photoxidation since the uninoculated phenol medium (Control 1) did not produce the brown colour. This observation was similar to that described in other reports in which the brown colour was attributed to the biologically mediated oxidation of phenol or derivatives of aromatic compounds of the same family [13-14]. Other researchers previously

suggested that this unidentified compound might be catechol, an intermediate of phenol degradation catalyzed by monooxygenase and dehydrogenase enzymes in phenol-degrading bacteria [14-15]. The accumulated aromatic compounds would require further treatment for complete mineralisation to occur. Further work in this study will involve the identification of these intermediates by high performance liquid chromatography (HPLC) analysis.

The partial degradation of phenol in these cultures may be due to the existence of other more easily degradable carbon sources coming from the nutrient broth, such that phenol is not a preferred carbon source in the system. The removal of the observed intermediates will be investigated using mineral medium with phenol as the sole supplied carbon source.

3.3 Phenol Degradation in Mineral Medium

As stated earlier, partial degradation of phenol observed when growing on nutrient broth was attributed to cometabolism. In order to achieve complete biodegradation, the biodegradation of phenol was tested in a basal mineral medium. The basal mineral medium did not contain any carbon source.

Phenol was added to the basal mineral medium after autoclaving as the only carbon source. Biodegradation was carried out under the same conditions as earlier stated except that the initial concentration of phenol and strontium were increased to 100 mg/L respectively. The extent of phenol degradation for both *P. putida* and *P. aeruginosa* is shown in Figure 2a & b. There was no phenol degradation for the first 90 hours for *P. putida* and 110 hours *P. aeruginosa* due to acclimation. Phenol at 100 mg/L initial concentration was completely degraded after 132 hours of incubation. There was negligible phenol degradation in the sterile controls. There was no accumulation of a brown colour compound during biodegradation however the incubation time was increased from 72 hours to 132 hours.

The use of simple mineral medium will be preferred over complex medium such as nutrient broth in industrial or actual treatment process because there will be no formation of intermediates. Also the cost of using complex media and subsequent treatment of the additional generated waste makes it more expensive than using simple mineral medium.

3.4 Inhibition Characteristics

Metabolic intermediates may inhibit the biodegradation of organic aromatic compounds such as phenol [14]. For organic radioactive waste, inhibition can also occur due to the radionuclides hence the degree of inhibition is increased. This is evident from our results since the rate of phenol degradation was higher in the test without strontium than in the experiments with strontium.

The significance of the inhibition effect of strontium was confirmed using a One Way Repeated Measures Analysis of Variance (ANOVA). In the statistical test the power

of the test was significant between cultures with cells and the control (without cells). The significance of strontium inhibition on phenol degradation was marginal in both cases (Figures 1a &b and 2a &b).

4 Conclusion

Both the *P. aeruginosa* and *P. Putida* cultures were capable of degrading phenol under mild exposure concentrations of 25 mg/L in a complex nutrient medium. Phenol degradation was observed after 90 hours of incubation for *P. Putida* and 110 hours for *P. aeruginosa* in a simple mineral medium. There was accumulation of intermediates during the biodegradation. The rate of phenol biodegradation was significantly inhibited by the presence of strontium as determined by (ANOVA). Biodegradation of phenol using simple mineral medium resulted in complete degradation of phenol i.e. there was no accumulation of intermediates. The study demonstrates the feasibility of utilising a biological process in the treatment of irradiated organic compounds in low-level waste emanating from nuclear and medical processing effluents.

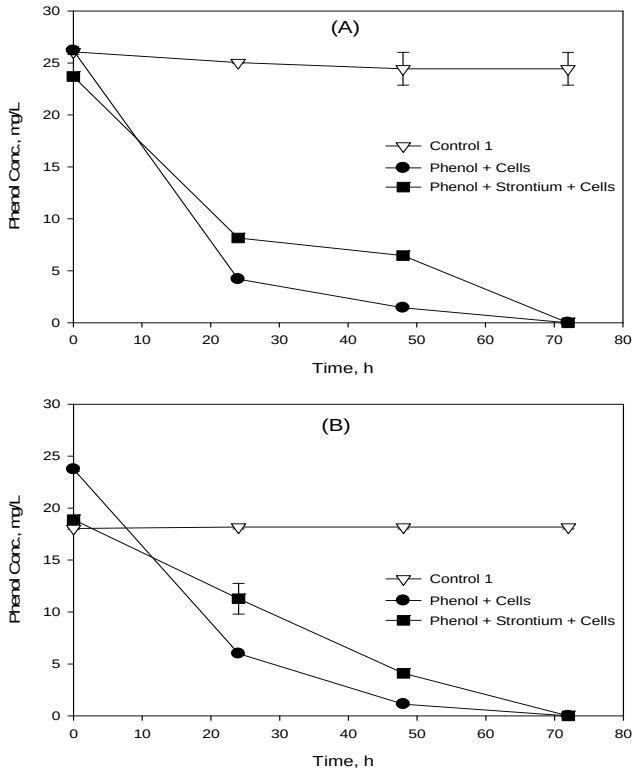

Fig. 2. Phenol degradation in *(a) Pseudomonas putida* and (b) *Pseudomonas aeruginosa* batch culture experiment

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