

## Bioremediation of Organic Pollutants in a Radioactive Wastewater: Evaluation of Feasibility of Treatment

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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 (a major radionuclide found in radioactive waste water). 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.

### 1. Introduction

Radioactive wastes mostly originate 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 (UK Department for Environment, Food and Rural Affairs [UK-DFE], 2002). The volume of radioactive wastes is project 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 (UK-DFE, 2002) 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.

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 (Lutze and Ewing, 1988).

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 (Gadd, 1996). Additionally, biological processes offer a more environmental friendly solution to remediation of pollution as they normally occur under natural environmental conditions (pH  $\approx$  7.0, ambient temperature and pressure) without leaving hazardous chemical byproducts (Chen *et al.*, 2001).

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. Irradiated organics and radioactive actinides occur as mixed wastes which pose disposal challenges since different components of the waste must meet different regulatory requirements (Roberts, 1998). 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 is 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 (Francis, 1998).

## 2. Materials and Methods

### 2.1 Reagents

Sr(NO<sub>3</sub>)<sub>2</sub> 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 (Panke *et al.*, 1998; Wang and Qu, 1995). Earlier studies showed the capability to biosorb and bioaccumulate radionuclides in these species (Standberg *et al.* 1981; Francis, 1988). Additionally, the purified *Pseudomonas sp.* isolates were shown to be capable of degrading aromatic organic compounds (Verge *et al.* 2000; Taylor 2001). *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  $4 \pm 0.5^\circ\text{C}$  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 500ml Erlenmeyer flasks containing 100ml of culture in the growth medium. The preliminary experimental design is presented in Table 1. The culture was incubated for 4 days at  $28^\circ\text{C}$  in a lateral incubator shaker (Labcon SPL-M15) at an agitation rate of 120 rpm. Samples of 3ml were taken aseptically at 24 hour intervals. All tests were carried out in triplicate.

Table 1. 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.

### 2.4 Analytical Methods

#### *Spectrophotometric Analysis of $\text{Sr}^{2+}$*

The common aqueous species of Strontium is  $\text{Sr}^{2+}$ . A  $30\mu\text{l}$  of the sample solution was transferred to a test tube containing 1.97ml of 0.1M-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 (Box, 1983). 1ml 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 in a UV/VIS spectrophotometer (WPA, Light Wave II, Labotech, South Africa).

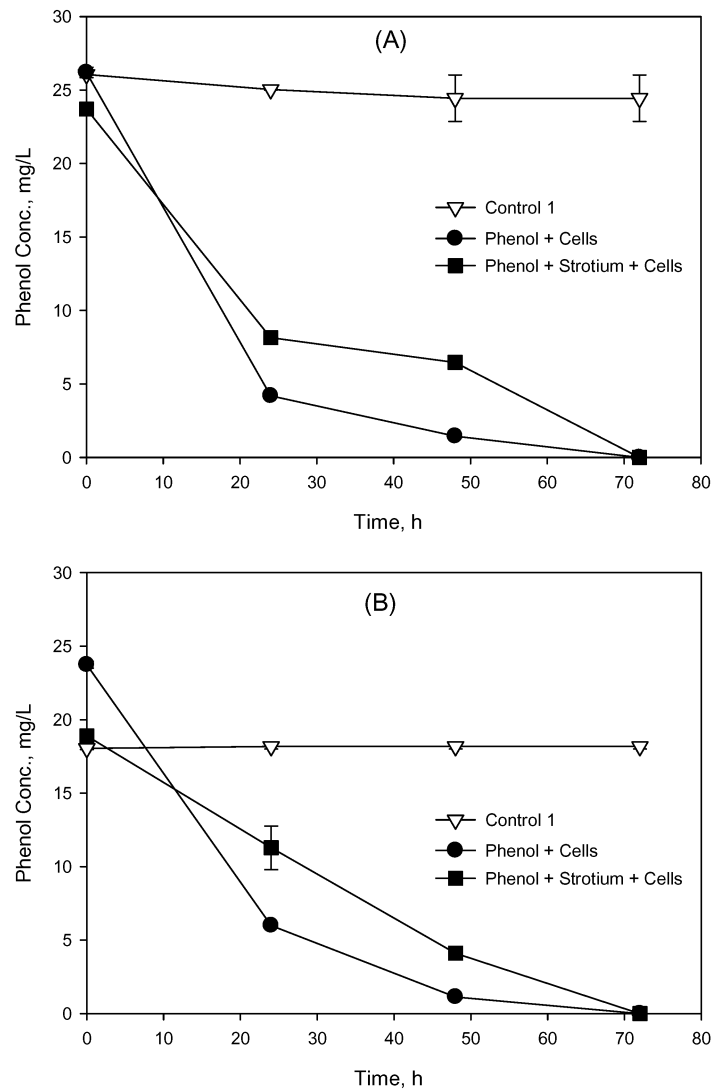
## 3. Results and Discussion

### 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 optimise experimentation and log-growth conditions as the treatment of the actual organic waste is expected to be carried out in a suspended culture systems. 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

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



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

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*.

Table 2. Removal rates of Phenol

Cells	Phenol with cells (mg/L/h)	Phenol +Strontium with cells (mg/L/h)
<i>P. putida</i>	0.52	0.36
<i>P. aeruginosa</i>	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 photooxidation 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 (Gibson *et al.* 1968b; Reardon *et al.* 2000). Other researchers previously suggested that this unidentified compound may be catechol, an intermediate of phenol degradation catalyzed by monooxygenase and dehydrogenase enzymes in phenol-degrading bacteria (Reardon *et al.* 2000; Loh and Chua, 2002). 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 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 solo supplied carbon source.

### 3.3 Inhibition characteristics

Metabolic intermediates may inhibit the biodegradation of organic aromatic compounds such as phenol (Reardon *et al.* 2000). 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 that the rate of phenol degradation was higher in the test without strontium. The significance of the inhibition effect of strontium was confirmed using a One Way Repeated Measures Analysis of Variance (ANOVA).

## 4. Conclusion

Both the *P. aeruginosa* and *P. Putida* cultures were capable of degrading phenol under mild exposure concentrations of 25 mg/L. The rate of phenol biodegradation was significantly inhibited by the presence of strontium as determined by (ANOVA). 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.

## 5. Acknowledgement

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## 6. References

- Box J. D. (1983). Investigation of the Folin-Ciocalteu phenol reagent for the determination of polyphenolic substances in natural waters. *Water Research*, 17, 511-525.
- Chen K.-C., Lin Y.-H., Chen W.-H., Liu Y.-C. (2001). Degradation of phenol by PAA-immobilized *Candida tropicalis*. *Enzyme and Microbial Technology*, 31 (4), 490-497.
- Francis AJ (1998). Biotransformation of uranium and other actinides in radioactive wastes. *Journal of Alloys and Compounds*, 271, 78-84.
- Gadd GM (1996). Influence of microorganisms on the environmental fate of radionuclides. *Endeavour*, 20, 150-156.
- Gibson DT, Koch JR, Schuld CL and Kallio RE (1968b). Oxidative degradation of aromatic hydrocarbons by microorganisms. II. *Biochemistry*, 7, 3795-3802.
- Loh KC and Chua SS (2002). Ortho pathway of benzoate degradation in *Pseudomonas putida*: induction of meta pathway at high substrate concentrations. *Enzyme and Microbial Technology*, 30, 620-626.
- Lutze W. and Ewing R.C. (1988) *Radioactive Waste Forms for the Future*, North-Holland, Amsterdam, p. 778.
- Panke, S., Sanchez-Romero, J. M., and De Lorenzo, V. (1998). "Engineering of quasi-natural *Pseudomonas putida* strains for toluene metabolism through an ortho-cleavage degradation pathway." *Appl. Environ. Microbiol.*, 64 (2), 748-751.
- Reardon KF, Mosteller DC and Rogers JD (2000). Biodegradation kinetics of benzene, toluene and phenol as single and mixed substrates for *Pseudomonas putida* FI. *Biotechnology and Bioengineering*, 69, 385-400.
- Roberts CJ (1998). Management and disposal of waste from sites contaminated by radioactivity. *Radiation Physical Chemistry*, 31, 579-587
- Strandberg GW, Shumate II, SE and Parrott JR (1981). Microbial cells as biosorbents for heavy metals; accumulation of uranium by *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*. *Applied and Environmental Microbiology* 41, 237-245.
- Taylor GT (2001). Evaluation of the potential of biodegradation of the disposal of cutting fluid from the machining of uranium. *International Biodeterioration and Biodegradation*, 47, 215-204.
- Department for Environment, Food and Rural Affairs and UK Nirex Ltd (2002) "The 2001 UK Radioactive Waste Inventory" , Defra/RAS Report 02.004; Nirex Report N/042, October 2002.
- Verce MF, Ulrich RL and Freedman DL (2001). Transition from cometabolic to growth-linked biodegradation of vinyl chloride by a *Pseudomonas* sp. isolated on ethane. *Environmental Science and Technology*, 35, 4242-4251.
- Wang, Y. T., and Qu, M. (1992). Substrate interactions during biodegradation of phenols by a *Pseudomonas* sp., *Proc. of the 65th Annual Water Environment Federation Conference and Technical Exposition*, Alexandria, VA, 63.