BNL-NUREG-40867
CONF-880201--30

BIODEGRADATION OF ION-EXCHANGE MEDIA*

B. S. Bowerman, J. H. Clinton, S. R. Cowdery
Brookhaven National Laboratory
Upton, New York 11973

BNL-NUREG--40867

DE88 007381

INTRODUCTION

Nuclear power plants use organic ion-exchange materials for coolant chemistry control and removal of radioactive contamination from liquid wastes (1,2). These materials may be in the form of resin beads when ion exchange only is required, or powdered resins, if filtration is needed in addition to ion exchange properties. In the latter case, other filtration media, e.g., cellulosic materials, may be mixed with the powdered resins. While a wide variety of organic polymer based ion-exchange materials are commercially available, the most widely used are polystyrene polymers with divinyl benzene cross-linking.

Waste ion-exchange resins and waste filter media containing powdered resins are disposed of after solidification with binders such as cement or after dewatering in containers. Containers for dewatered resins may be either carbon steel liners or high density polyethylene high integrity containers (HIC) (3).

Several incidents involving dewatered ion-exchange resins and dewatered filter media have occurred in recent years, both during the dewatering process and during transport to the disposal site (4). In one incident, in January 1983, ion-exchange resins underwent an exothermic reaction during dewatering. Autoxidation appeared to be the likely cause of the exotherm, but biodegradation and/or metabolic by-products of biodegradation were believed to have contributed to the initiation of the exotherm.

Another incident involving powdered ion-exchange resins in a HIC occurred in September, 1984. The HIC became pressurized during transport to the disposal site. Subsequent measurements of CO_2 generation were made using a sample obtained from the same waste batch before it was shipped. It was concluded that pressurization of the HIC could have been caused by biodegradation (4).

While a significant body of information is available on the effects of irradiation on organic ion-exchange media (5-9), there is little known regarding the effects of biodegradation on these materials. Indeed, there are limited data available on the chemical characteristics of waste ion-exchange materials (8,9). In a preliminary study conducted at Brookhaven National Laboratory (BNL) (10), Francis and Quinby examined ion-exchange resin samples collected from the BNL High Flux Beam Reactor (HFBR). CO_2 generation from the resins was measured, and two bacterial strains were isolated.

*Work conducted under the auspices of the Nuclear Regulatory Commission.

The purpose of this study was to investigate further the potential for ion-exchange media (resin beads or powdered filter media) to support biological growth. A mixed microbial culture was grown from resin wastes obtained from the BNL HFBR by mixing the resins with a nutrient salt solution containing peptone and yeast extract. Bacterial and fungal growths appeared in the solution and on the resins after 7 to 10 days' incubation at 37°C. The mixed microbial cultures were used to inoculate several resin types, both irradiated and unirradiated.

EXPERIMENTAL

The two phases of this study consisted of (a) developing a mixed microbial culture from HFBR resin wastes, and (b) determining the ability of the culture to grow on different resin types.

Two types of resin were obtained from filter units servicing the fuel storage canal of the HFBR. Both samples were obtained by scooping material from the tops of resin beds just prior to a scheduled resin replacement in January, 1987. The samples were transferred to screw-topped plastic bottles in which they were stored until needed for this study. Aseptic techniques were not used for collection of the resins. Both samples contained radioactive contamination.

Cation bead resins were obtained from a pre-filter unit and consisted of Amberlite IR-200 resins (Rohm and Haas). The resins had been in service since February, 1984. They were an amber color and contained a noticeable quantity of white particulate matter. Mixed cation/anion bead resins were obtained from the main demineralizer unit. The HFBR mixed resins were originally obtained from Graver Company and consisted of Amberlite IR-200 cation resins and Amberlite IRA-400 anion resins, or equivalents. They had been in service since June, 1985.

The mixed microbial cultures were prepared by adding samples of the HFBR resins (3-5 grams) to flasks containing 100 ml of nutrient salts medium with a secondary carbon source. The composition of the nutrient salts medium is listed in Table I. The flasks were maintained under aerobic conditions using cotton plug closures, and incubated at 37°C. Six flasks were prepared: three using the HFBR mixed resins and three using the cation resins. One flask containing each resin type was sterilized in an autoclave to provide a comparison for visual observations.

Microbial growth developed in the HFBR resin cultures after 7 to 10 days (see below for details). These cultures were then used to provide inocula for culture tubes containing the nutrient salts solution in which the normal carbon source of yeast extract and peptone had been replaced by the addition of one of several types of ion-exchange bead resins. The purpose of this second stage of testing was to evaluate the ability of the mixed cultures to utilize ion-exchange media or organic ions sorbed on the media as a carbon source.

Culture tubes containing 0.25 g of the test resins and 5 mL of the nutrient salts medium were inoculated with the mixed microbial culture grown from the HFBR resins. The nutrient medium was identical to that shown in Table I, except that the peptone and yeast extract were excluded so that the

Table I

Composition of Nutrient Salt Solution

Ingredient	Amount
$(NH_4)_2SO_4$	1.32 g
Na_2HPO_4	1.42 g
KH_2PO_4	0.54 g
$MgSO_4 \cdot 7H_2O$	0.50 g
$Ca(NO_3)_2$	0.50 mg
$FeSO_4 \cdot 7H_2O$	0.50 mg
Peptone	0.10 g
Yeast extract	0.10 g
Deionized water	to 1000 mL

only carbon available was the ion-exchange medium. Transfers were made aseptically with a microbiological transfer loop. Mixed microbial cultures from both HFBR cation and mixed resins served as inocula. Additional inoculations from the sterilized control flasks served to confirm that all transfers were aseptic and provided a basis for visual observations of growth.

Two types of mixed resin beads (LOMI and IRN) and one filter medium (EX) were evaluated. All were equilibrated with the nutrient salts medium (minus peptone and yeast extract) to expend ion-exchange sites before being placed in the test tubes. Additional tests were conducted with LOMI and IRN resins equilibrated with organic acid anions and ferrous ions to simulate decontamination wastes. Descriptions of these materials are provided in Table II.

LOMI stands for Low Oxidation State Metal Ion and represents a dilute chemical process whereby a reagent containing picolinic acid and formic acid is added to coolant water to decontaminate cooling systems in nuclear power plants. The dilute chemicals are removed from the water with ion-exchange resins. Cations are removed with a "standard" strong acid cation resin such as IRN-77 (Rohm and Haas), while picolinic and formic acid anions are collected on weak base polyacrylic anion resins, i.e., IONAC A-365 (Sybron).

The two sets of LOMI resins had been prepared for use in another BNL study (11) and consisted of mixtures of two parts IONAC A-365 to one part IRN-77. One set of resins (LL) had been "loaded" with picolinic and formic acid. For this study, the resin mixture was equilibrated with ferrous sulfate solution. The other set (LC) in "as-received" condition was equilibrated with the nutrient salts mixture.

In addition to the LOMI process, other decontamination processes are available which utilize different organic acid reagents. The more commonly used chemicals are ethylenediamine-tetraacetic acid (EDTA), citric acid, and oxalic acid, usually in combination. After decontaminations are completed, the water streams are cleaned up by ion-exchange resins.

Table II

Resin Types Evaluated for Biodegradation Potential

Designation	Description
LC	LOMI resins equilibrated with nutrient salt solution (IONAC A-365: IRN-77 = 2:1)
LL	Simulated LOMI decontamination resin wastes (picolinic acid and formic acid anions on IONAC A-365, ferrous ion on IRN-77)
IRN	IRN-77 and IRN-78 resins, equilibrated with nutrient salts (IRN-78:IRN-77=1:1)
EOC	Simulated decontamination wastes (EDTA, oxalic and citric acid anions on IRN-78; ferrous ion on IRN-77)
EC	As in EOC, but without oxalic acid
EX	Ecodex filter medium equilibrated with nutrient salt solution
N	Blank: nutrient salt solution, no carbon source

Two simulated decontamination resin wastes were prepared: one containing all three organic acids (EOC), and one with EDTA and citric acids sorbed on the resins (EC). Both simulations consisted of a one-to-one mixture of cation to anion resins (IRN-77 and IRN-78), respectively. Before mixing, the cation resins had been equilibrated with ferrous sulfate, and the anion resins were loaded with the appropriate organic acids. The organic acids were sorbed onto the resins by equilibrating a solution of equimolar amounts of the acids with the resins.

A duplicate set of all the bead resin types was prepared and subjected to gamma radiation; the total dose was approximately 100 Mrad. These samples were tested to determine whether radiation-damaged resins would provide a better carbon source for the mixed microbial culture.

RESULTS AND DISCUSSION

Both cation and mixed HFBR resins provided a ready source of microorganisms for additional tests. Addition of the resin wastes to sterile solutions containing nutrient salts and a secondary carbon source resulted in visible evidence of growth in the solutions and on the resins as summarized in Table III. Bacterial growth, indicated by turbidity in the solutions, appeared within 4 to 6 days in the flasks.

With the exception of one of the flasks containing mixed resins (AM-1), all the HFBR resins exhibited fungal growth. The fungi grew in tufts or

clumps either attached to the resins or floating in the medium. Microscopic examination of the fungal growth showed that the mycelium was composed of hypha (filaments), which had no septa (cell wall divisions). Spores from the fungal growth were similar in all the flasks containing it, suggesting that only one type of fungi was present in the HFBR resin wastes.

Preliminary characterizations of the bacterial growth in the HFBR resins has been reported elsewhere (12). At least nine distinct types of bacteria were isolated by streak plate subculture. Gram-staining and microscopic examination showed that all bacteria were rod-shaped; five were gram-negative.

Table III

Growth of Mixed Microbial Culture from HFBR Resins

Flask No.[a]	Observations and Types of Growth	Time to Develop
AC-1, AC-2	very turbid solutions; scum floating on solution surface	4-6 days
	slight fungal "halo" just above resin surfaces	8-10 days
AC-C	turbid solutions, no signs of growth	
AM-1, AM-2	turbid solution,	4-6 days
	small tufts of fungal-like growth on resins (AM-2 only)	20-25 days
AM-C	clear and colorless, no signs of growth	

[a]Key:
A = aerobic medium and cotton stopper,
C = cation resins from HFBR
M = mixed resins from HFBR.
Numbers after hyphen are replicates. C after hyphen indicates control sterilized after preparation for visual comparisons.

The development of a mixed microbial culture is significant in that it shows that a variety of microbes (both bacteria and fungi) will be present in resin wastes. Furthermore, these micro-organisms can remain viable for some time. Several sets of cultures were obtained from the HFBR resins over a period of five months. The first culture was started after the resins had been stored at room temperature in sealed containers for one month. After the removal of samples for the cultures, the containers were resealed and opened again as needed at three, four, and five months.

Microbial growths in the test tubes containing the different resin types varied considerably. All the test tubes containing ion-exchange media and nutrient salt solution exhibited some signs of microbial growth within a week or two after inoculation. In many cases, visible growth was limited to the surface of the liquid in the test tube. In those tubes with the heaviest

growths, the solutions were turbid, and fungal mycelial masses grew directly on and above the resin surfaces. Tables IV and V list the types of growth observed in unirradiated and irradiated samples, respectively.

Fungal growth (mycelia) occurred at the liquid surface in the test tubes, including the blank tests (N) which contained nutrient salt solution and no carbon source. The reason for growth in the latter case is not obvious. It may have been due to trace amounts of organic carbon in the water used to prepare the solution or adsorbed on the test tube glass surface, or there may have been enough carbon carried on the transfer loop to support a small amount of fungal growth initially. The fungal growths at the liquid surface in tubes containing resin beads and exhibiting growth in the solution or on the surface was generally heavier than that observed in the blank tests.

Table IV

Characteristics of Microbial Growth in Test Tubes with Unirradiated Resins

Resin Type	Inoculum Source	
	HFBR Cation Resins	HFBR Mixed Resins
LC	mycelia at liquid surface; slight turbidity in solution	mycelia at liquid surface and on resin beads; clear solution
LL	mycelia and nodules at liquid surface	mycelia at liquid surface
IRN	mycelia and nodules at liquid surface	mycelia at liquid surface
EOC	mycelia and nodules at liquid surface; turbid, green-tinted solution; mycelia and white granular material on resin surfaces	mycelia at liquid surface; turbid, green-tinted solution; mycelia and white granular material on resin surfaces
EC	same as EOC	same as EOC; definite mycelia growth on resin surfaces
EX	mycelia and nodules at liquid surface	mycelia at liquid surface
N	mycelia and nodules at liquid surface	mycelia at liquid surface

Table V

Characteristics of Microbial Growth in Test Tubes with Irradiated Resins

Resin Type	Inoculum Source	
	HFBR Cation Resins	HFBR Mixed Resins
LC	mycelia and nodules at liquid surface	no visible growth
LL	mycelia and nodules at liquid surface	no visible growth
IRN	mycelia and nodules at liquid surface; mycelia on resin surfaces	mycelia at liquid surface; mycelia on resin surfaces
EOC	mycelia and nodules at liquid surface; turbidity, floc and fungal growth in solution; mycelia and white granular material on resin surfaces	mycelia at liquid surface; turbidity, floc and fungal growth in solution; mycelia and white granular material on resin surfaces
EC	same as EOC	same as EOC

Except for the growths at the liquid surface, there were no marked growth differences between tubes with the same test resin and "different" inocula. In other words, inoculation with mixed microbial cultures from the HFBR cation resins did not result in growths that were drastically different compared to cultures from the HFBR mixed resins. However, at the liquid surface, inoculations from cation resins resulted in fungal mycelial growths and compact white nodules. Microscopic examination of the nodules showed dense aggregates of mycelia and bacteria. Tubes inoculated from the HFBR mixed resin did not have the nodules present.

Visible growth directly on or above the ion-exchange media would be more indicative of the biodegradation of the material. The heaviest fungal growth (mycelia) was observed in the solutions and on resin surfaces in test tubes containing mixed IRN resins loaded with EDTA, citric acid, and oxalic acid (EOC and EC). The solutions in these tubes were turbid, which may indicate extensive bacterial growth as well. A small amount of white granular material was present on the resin surfaces in the control samples in these tests. Clumps of mycelia in the test samples included a similar floc, or precipitate. IRN resins with no organic anions on them had similar but smaller amounts of growth develop in the same two week period.

LOMI resins exhibited small amounts of fungal growth on the resins equilibrated with nutrient salts (LC). However, the resins containing picolinate, formate, and ferrous ion (LL) showed no visible signs of growth.

Fungal growth and turbidity was more pronounced in the irradiated EOC, EC, and IRN resins, compared to the unirradiated samples. The opposite effect was seen in the LOMI resins (LC); irradiated LC samples had no fungal growth on resin surfaces.

After 2 to 3 weeks, streak plates from cultures of each resin-type were prepared on nutrient broth agar. Either bacterial or bacterial and fungal growth was obtained from all cultures, including those with no added carbon source, that had received inocula from flasks containing live microbes. Thus, although culture conditions might not be suitable for growth, bacterial and fungal cells or spores remained viable within the culture medium.

The heavy growths observed may not represent the "real world" waste situation, since excess water and the full spectrum of inorganic nutrient salts were provided. However, the fact that a mixed microbial culture was derived from real HFBR resin wastes suggests that some microbial activity is present in all dewatered resin wastes, and remains viable for at least several months.

CONCLUSIONS

The main conclusions of this study are: a mixed microbial culture can be grown from actual ion-exchange resin wastes provided nutrient salts, a secondary source of carbon, and excess water are added to the wastes, and the use of a mixed microbial culture is appropriate for evaluating the potential for ion-exchange media to support biological activity.

The effects of environmental factors such as resin type, chemicals sorbed on the resins and radiation damage were examined in this study. Heavier growths were seen in IRN resins subjected to 100 Mrad of gamma irradiation. Some organic chemicals used in dilute chemical decontamination processes encourage heavier microbial growths.

The growth evaluations in this study were qualitative. Further research quantifying the ability of micro-organisms to metabolize ion-exchange media would be useful, since biodegradation could have potentially adverse effects on the long-term stability of the wastes or waste containers. Specific topics which should be investigated include: 1) establishing the long-term viability of mixed microbial cultures in dewatered resin wastes, and 2) identifying the characteristics of respiration and metabolism of microbes in dewatered resins. The latter topic is important because the effects of biodegradation cannot be predicted accurately without basic information on how rapidly microbes utilize the nutrients available in the wastes. Specific data on microbial metabolism under expected disposal conditions, e.g., sealed containers at low (15° to 20°C) temperatures, should be obtained.

REFERENCES

1. W. E. Clark, "The Use of Ion-Exchange to Treat Radioactive Liquids in Light-Water-Cooled Nuclear Reactor Power Plants," NUREG/CR-0143, ORNL/NUREG/TM-204, 1978.

2. K. H. Lin, "Use of Ion-Exchange for the Treatment of Liquids in Nuclear Power Plants," ORNL-4792, 1973.

3. R. L. Jolley, et al., "Low-Level Waste from Commerical Nuclear Reactors, Vol. 2, Treatment, Storage, Disposal, and Transportation Technologies and Constraints," ORNL/TM-9846/V2, 1986.

4. B. S. Bowerman and P. L. Piciulo, "Technical Consideration Affecting Preparation of Ion-Exchange Resins for Disposal," NUREG/CR-4601, 1986.

5. T. E. Gangwer, M. Goldstein, and K. K. S. Pillay, "Radiation Effects on Ion-Exchange Materials," BNL-50781, 1977.

6. K. J. Swyler, C. J. Dodge and R. Dayal, "Irradiation Effects on the Storage and Disposal of Radwaste Containing Organic Ion-Exchange Media," NUREG/CR-3383, 1983.

7. K. J. Swyler, C. J. Dodge, and R. Dayal, "Assessment of Irradiation Effects in Radwaste Containing Organic Ion-Exchange Media," NUREG/CR-3812, 1984.

8. D. R. MacKenzie, M. Lin, and R. E. Barletta, "Permissible Radionuclide Loadings for Organic Ion-Exchange Resins from Nuclear Power Plants," NUREG/CR-2830, 1983.

9. P. L. Piciulo, "Technical Considerations for High Integrity Containers for the Disposal of Radioactive Ion-Exchange Resin Waste," NUREG/CR-3168, 1983.

10. A. J. Francis and H. Quinby, "Microbial Degradation of Spent Reactor Resins - A Preliminary Study," BNL-30897, 1981.

11. J. W. Adams and P. Soo, "Decontamination Impacts on Solidification and Waste Disposal," Quarterly Progress Report, October-December 1986, WM-3246-1, May 1987.

12. P. Soo, et. al., "Low-Level Waste Package and Engineered Barrier Study," Quarterly Progress Report, July-September 1987, WM-3291-5, December 1987.