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UPTAKE AND RETENTION OF Cs^{137} BY A BLUE-GREEN ALGA IN CONTINUOUS FLOW AND BATCH CULTURE SYSTEMS[1]

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Abstract

The uptake and retention patterns of the blue-green alga, Plectonema purpureum, were determined by exposing laboratory cultures to various concentrations of Cs^{137} in both continuous flow and batch tests. A simplified laboratory apparatus was devised to provide a continuous flow of radioactive culture media through multiple flasks containing the filamentous algae, which were set up in parallel. In each type of test, the maximum uptakes of Cs^{137} by the algae were proportional to the concentrations of Cs^{137} in the media. However, algal uptakes were higher by a factor of 7 in the continuous flow tests than in batch tests. Only 10% of the Cs^{137} was retained by the algae three days after the algae were transferred into cesium-free media.

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Introduction

The thermal waters of nuclear reactor effluents at the Savannah River Plant produce abundant growths of sessile blue-green algae which are chronically exposed to low-level concentrations of mixed fission products and radioactive corrosion products. Although it has been demonstrated by Williams and Swanson (1958), Rice and Willis (1959), and Williams (1960) that green algae and diatoms adsorb and/or absorb radioactivity from their environment, very little work has been done with the blue-green algae. Since routine monitoring data show that blue-green algae concentrate radioactivity from the water by factors as great as 10,000, cooperative studies with the Limnology Department of the Philadelphia Academy of Natural Sciences were initiated to investigate the uptake and retention patterns of specific radionuclides by the dominant genera of blue-green algae in the reactor effluents. Plectonema purpureum, one of the most common forms, was selected for the initial study.

Methods

The continuous flow culture systems described in the literature (Myers and Clark 1944, Rice 1961) were complex units designed for the continuous culture of unicellular organisms in a single growth chamber. Since our aim was to simulate conditions in a river, it was necessary to devise a method whereby a new quantum of water was continuously passing over a growing colony of algae. A diagram of this continuous flow culture system is shown in Figure 1.

Besides the advantages noted above, this type of system permits: 1) growth and/or radioactivity studies of long duration which are not possible in batch culture, 2) the maintaining of fairly constant radioactivity and nutrient concentrations, and 3) the maintaining of a colony of algae without undue loss due to unusual current effects often produced in continuous flow systems.

The basic unit of the system is a Sigmamotor Model T-8 pump[2] equipped with a variable-speed transmission. The design of this unit prevents the contact of culture media with the pump mechanism, thus facilitating radioactivity containment and bacterial control. The tube plates of the pump were modified to permit uniform pumping through six identical tubes simultaneously. A wide selection of flow rates can be obtained by varying the tubing size or the pump speed.

Algal inoculums were placed on tared Millipore® filter membranes[3] and a slight vacuum was applied to firmly position the inoculums on the membranes. This prevents the algae from being carried away by the current. The filter membranes were then placed in the bottoms of thirty 125-ml sidearm filter flasks. Influent medium was pumped from the reservoir through six Tygon tubes[4] (1/16 in. ID) to six connecting manifolds. Culture medium passing through each manifold was distributed to the bottoms of five algal flasks through individual hypodermic needles (0.013 in. ID). Effluent medium was discharged by gravity flow from the sidearm of each algal flask through rubber tubing (1/4 in. ID) to individual discharge containers.

Although it was not practical to maintain aseptic conditions in the continuous flow system, the effects of bacterial growth were minimized by sterilization of equipment and filtration of media. Type HA Millipore® filter membranes were used to filter the media prior to storage in the reservoir. The influent media to the algal flasks were refiltered by pumping through medium (10 to 15 μ and fine (4.0 to 5.5 μ) pore size fritted-glass-disk filters connected in series. All batch test media and equipment were sterilized prior to use. In order to measure the bacterial uptake of Cs^{137}, bacteria which had been growing in the algal nutrient media were cultured separately and radioanalyzed. Bacterial uptake of Cs^{137} was found to be insignificant compared to algal uptake.

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Plectonema were cultured in modified Chu 10 media (Gerloff, Fitzgerald, and Skoog 1950, Gerloff *et al.* 1950) enriched by the addition of trace amounts of zinc, manganese, aluminum, boron, lithium, and cobalt (see Table 1). Deionized distilled water was used as a base for the media. The initial pH of the media was adjusted to 7.3 to approximate the pH of effluent streams. Cultures were maintained at 35° ± 2°C under continuous illumination of about 350 ft-c with no shaking or aerating. Five cultures were sacrificed for statistical considerations at the end of each exposure period in both the continuous flow and batch tests. Algal cultures were separated from the media by filtration through type HA Millipore® filter membranes. Membrane and residue were washed with cesium-free media and dried prior to weighing and radioanalyzing.

Results

Algal data for the continuous flow and batch cultures were obtained under similar test conditions except for the volume of media used. Fresh medium was pumped through the continuous flow cultures at a rate of 15 ml per hour. The batch test inoculums were cultured in single 50 ml aliquots of medium which were not changed during the exposure period. Although the growth for the first three days was comparable in both tests, the seven-day growth of the continuous flow cultures was 1.7 times that of the batch cultures. Since preliminary data on the batch uptake tests indicated a gradually reduced growth rate, these tests were terminated after seven days.

In both the continuous flow and batch tests, algae were exposed to media containing 100, 200, and 400 disintegrations per minute (d/m) of Cs^{137} per of media. All of the Cs^{137} used in these tests was found to be in ionic form; the procedure outlined by Rice and Willis (1959) was used. In the continuous flow tests, at a flow rate of 15 ml per hour, the Cs^{137} concentrations available to the algal cultures remained constant. Concentrations in the batch test media, with no flow, decreased due to algal uptake. Maximum algal concentrations of Cs^{137} increased linearly with the concentration of Cs^{137} in the media. The maximum concentration factor (radioactivity per gram of algae/radioactivity per ml of medium) of the continuous flow cultures (1,700) was higher by a factor of 7 than that of the batch cultures (250). The algal uptake patterns (Figure 2 and Table 2) show that the continuous flow cultures had a much higher initial uptake of Cs^{137} than the batch cultures. After the high initial uptake, the Cs^{137} per gram of continuous flow algae decreased because the growth rate exceeded the uptake rate. The concentration of Cs^{137} in the dividing parent cells was reduced approximately 50% at each cell division. In the continuous flow cultures, subsequent cell divisions occurred before maximum concentrations of Cs^{137} were attained in the daughter cells. The converse occurred in the batch cultures.

The Cs^{137} retention patterns of the Plectonema were determined by subculturing radioactive algae in cesium-free media. The algae which were exposed for 7 days in media containing 100, 200, and 400 d/m of Cs^{137} per ml were then washed with, and subcultured in, cesium-free media and the retention measured for 21 days.

Data in Figure 3 and Table 3 show that the algae retained only 10% of the initial radioactivity after 3 days in the cesium-free environment. The rapid loss of Cs^{137} was apparently due to leaching, since only 5% of the loss could be attributed to dilution by cell division. Throughout the remainder of the tests, the concentrations of Cs^{137} in the continuous flow cultures were further reduced both by leaching and by cell division. The Cs^{137} released from the algal cells was removed by a media flow rate of 15 ml per hour. In the batch tests, there was no evidence of further loss by leaching after the third day. The Cs^{137} released during the first three days remained in the environment and may have been absorbed by the algae during the latter part of the experiment.

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Footnotes

[1] The information contained in this article was developed during the course of work under Contract AT(07-2)-1 with the U. S. Atomic Energy Commission.

[2] Sigmamotor, Inc., Middleport, N. Y.

[3] Registered trademark, Millipore Filter Corp., Bedford, Mass.

[4] U. S. Stoneware Co., Akron 9, Ohio.

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Table 1. Compounds added to modified Chu 10 media.

Compound	Grams/Liter (multiply by 10^{-3})
$ZnSO_4 \cdot 7H_2O$	2.0
$MnSO_4 \cdot H_2O$	1.4
$AlCl_3 \cdot 6H_2O$	3.6
H_3BO_3	2.0
LiCl	1.0
$CoCl_2 \cdot 6H_2O$	1.0

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Table 2. Cs^{137} uptake in continuous flow and batch culture systems. (See also Figure 2.)

Concentration	Cs^{137} Uptake* (disintegrations per minute per gram of algae) ± 1σ				
Days Exposure →	1	3	7	14	
	CONTINUOUS FLOW MEDIA				
100 d/m/ml	18.1 ± 2.1	14.8 ± 3.3	6.08 ± 1.62	4.48 ± 1.88	
200 d/m/ml	24.0 ± 4.9	17.3 ± 0.9	10.8 ± 3.3	9.14 ± 2.27	
400 d/m/ml	78.7 ± 26.0	62.0 ± 6.6	24.4 ± 7.8	12.7 ± 3.9	
Days Exposure →	1	2	3	4	7
	BATCH MEDIA				
100 d/m/ml	2.56 ± 1.36	1.69 ± 0.88	1.43 ± 0.24	1.55 ± 0.18	2.03 ± 0.47
200 d/m/ml	2.98 ± 0.33	3.44 ± 1.19	4.13 ± 1.34	5.02 ± 3.63	5.81 ± 2.03
400 d/m/ml	7.73 ± 1.19	7.85 ± 1.60	8.98 ± 2.34	8.33 ± 0.62	9.91 ± 2.07

* Multiply all uptake values by 10^4.

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Table 3. Cs^{137} retained in continuous flow and batch culture systems. (See also Figure 3.)

Concentration	Cs^{137} Retained (% per gram of algae) ± 1σ					
Days in Cs-Free Media →	0	1	3	7	14	21
			CONTINUOUS FLOW MEDIA			
100 d/m/ml	100.0 ± 3.5	63.7 ± 52.6	8.31 ± 3.25	1.06 ± 0.60	0.403 ± 0.066	0.482 ± 0.218
200 d/m/ml	100.0 ± 21.1	19.1 ± 7.6	5.14 ± 1.58	2.65 ± 0.81	1.09 ± 0.63	0.992 ± 0.352
400 d/m/ml	100.0 ± 7.3	-	26.4 ± 28.3	2.79 ± 0.85	2.22 ± 1.03	0.942 ± 0.256
			BATCH MEDIA			
100 d/m/ml	100.0 ± 31.1	24.7 ± 10.4	13.0 ± 6.4	9.27 ± 4.02	7.01 ± 2.32	8.11 ± 4.05
200 d/m/ml	100.0 ± 25.7	10.4 ± 3.0	9.98 ± 2.81	8.53 ± 3.59	6.06 ± 2.74	5.58 ± 3.12
400 d/m/ml	100.0 ± 14.5	9.36 ± 6.74	10.3 ± 3.2	7.78 ± 1.81	7.28 ± 3.02	7.00 ± 2.71

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Figures

FIG. 1. Diagram of a continuous flow culture system.

FIG. 2. Cs^{137} uptake in continuous flow and batch culture systems. (The curves show uptake of radioactivity by algae after exposure to media of different activities.)

FIG. 3. Cs^{137} retained in continuous flow and batch culture systems. (The curves show % of initial radioactivity retained by algae in cesium-free media after 7 days exposure to media of different activities.)

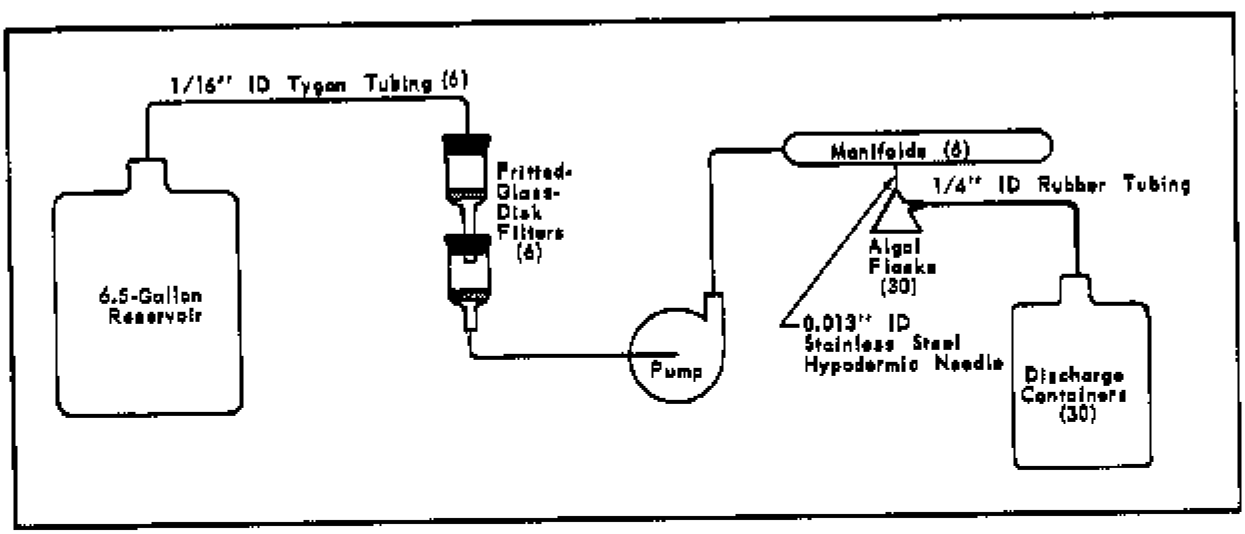

FIG. 1 DIAGRAM OF A CONTINUOUS FLOW CULTURE SYSTEM

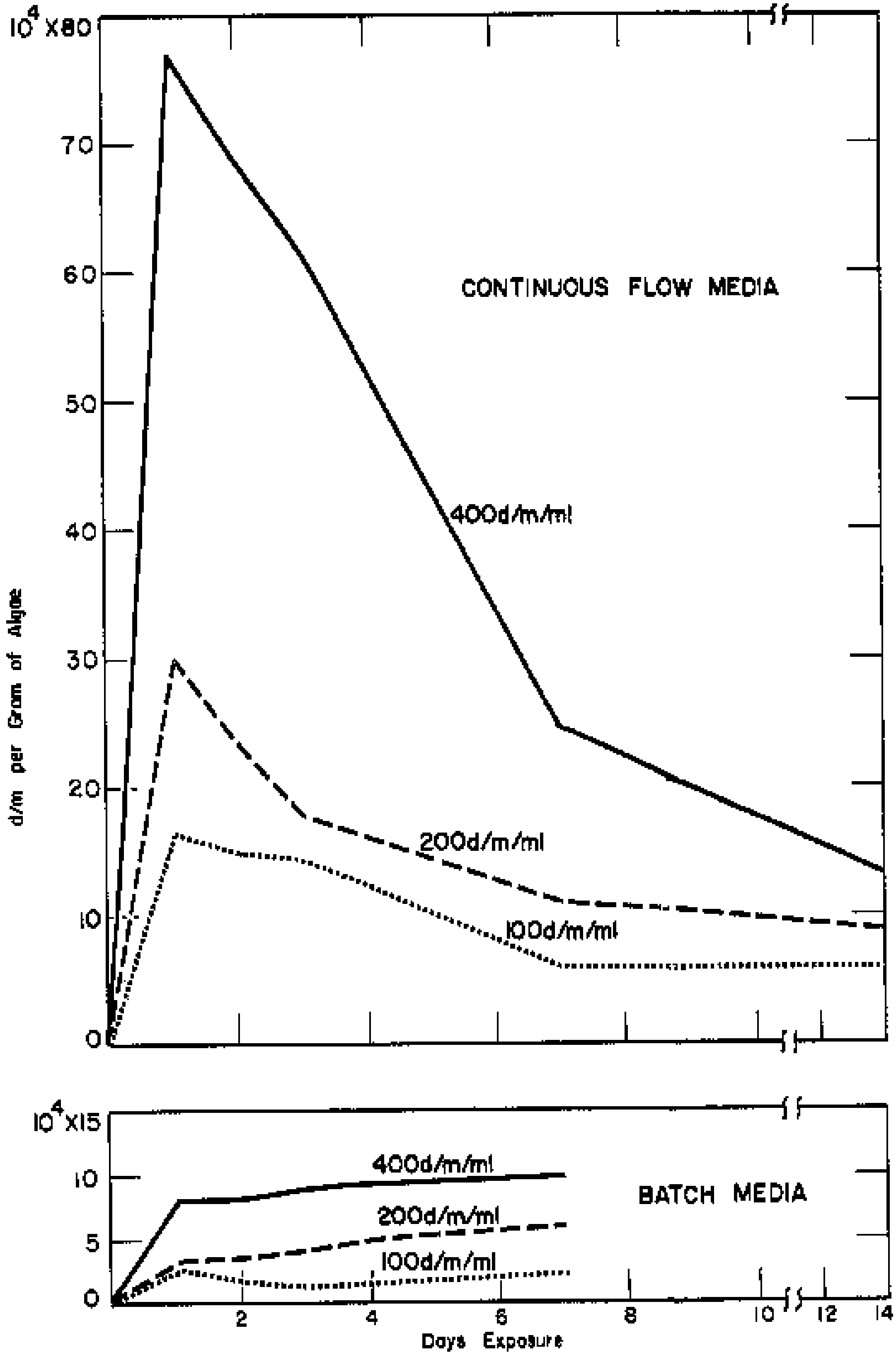

FIG. 2. Cs^{137} UPTAKE IN CONTINUOUS FLOW AND BATCH CULTURE SYSTEMS. (The curves show uptake of radioactivity by algae after exposure to media of different activities.)

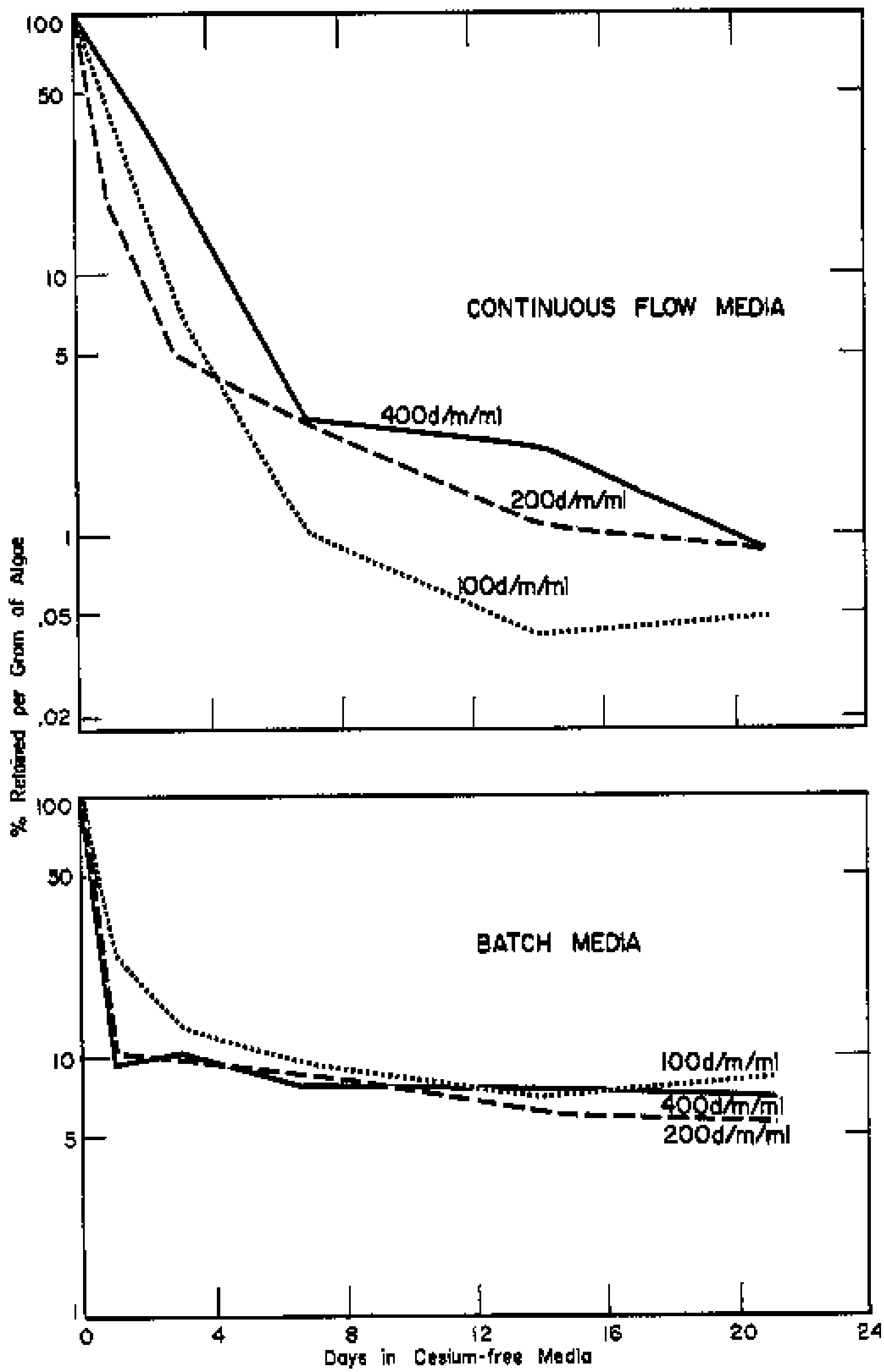

FIG. 3. Cs^{137} RETAINED IN CONTINUOUS FLOW AND BATCH CULTURE SYSTEMS. (The curves show % of initial radioactivity retained by algae in cesium-free media after 7 days exposure to media of different activities.)