

The Use of Glass Microbeads in Ecological Experiments with Bacteriophagic Nematodes¹

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Abstract: A system that uses microbeads for the culture of bacteriophagic nematodes is described. Glass-bead culture was found to simulate a soil microcosm more closely than did agar culture in terms of CO₂ production, number of nematodes produced, and nematode size. *Key Words:* bacteriophagic, natural environment, glass microbeads.

Bacteriophagic soil nematodes have generally been cultured on agar with defined nutrients and bacteria. The results may vary markedly from results in soil, however, in part because of the physical difference between the two systems. Clapham (2) observed that length between field samples and nematodes grown in nutrient agar differed, and the effects of soil particle size on phytophagous nematode growth and activity have been reported by Baines (1), Ponchillia (7), and Robbins and Barker (8). Therefore, it appears that the physical structure of the environment has a significant effect on nematodes and differences in the sizes of nematodes may reflect differences in productivity of the environment and the effects of a physically more restrictive environment. Since our understanding of the effects of nematodes on nutrient cycling in the rhizosphere may be influenced by their size, we felt a culturing technique which more closely simulated the soil environment was necessary in order to extrapolate from laboratory results to natural systems. Our technique uses glass microbeads as a physically restrictive substrate which more closely models the soil system.

Glass microbeads have previously been used to culture microorganisms (4, 6); Parr and Norman (6) emphasized the value of microbeads as a defined porous medium and the similarity to some soil properties. Myers et al. (5) used microbeads to separate nematodes into length classes. Robbins and Barker (8) used glass beads of various sizes as a growth medium to test particle size/nematode relationships. The technique we developed uses glass microbeads to

simulate the physical environment of the soil. Changes in nematode populations are monitored by measuring the respiratory response of the culture unit. This system was then compared with soil and agar cultures containing similar nutrients.

MATERIALS AND METHODS

The culture apparatus consisted of four components: the train for absorption of carbon dioxide, a valve, the culture units, and alkali traps (Fig. 1). Air from a laboratory supply was bubbled through a series of three Erlenmeyer flasks containing 6 N NaOH as a CO₂ absorbent. The air was dispersed into small bubbles via a multi-perforated piece of tygon tubing attached at the end of a glass tube and placed below the level of the absorbent. This process produced CO₂-free air, which was then bubbled through a water bath to ensure high air humidity.

After the CO₂ was removed, the air flow was divided into five streams by a 5-way aquarium valve. The air flow at each of the outlets could be checked with a manometer and the valves adjusted so that the flow rate was equal at all outlets.

The culture unit consisted of a glass tube or thistle tube (inner diam 15 mm) with openings at the top and bottom. It was held in a vertical position and packed with 35 gm of glass microbeads (Fisher Scientific Co., Pittsburgh, Pa.) 0.1 to 0.2 mm in diam. Since the diameters of the beads were unequal, and beads were sometimes fused, pore spaces of different sizes were formed in a manner similar to the unequal pore size distribution in the soil. Rubber stoppers with small glass tubes were inserted in the open ends of the thistle tubes. Each small opening in the culture units was then plugged with cotton, and the units were sterilized by autoclaving. The beads were coated with a nutrient film

Received for publication 24 January 1977.

¹This paper reports on work supported in part by the National Science Foundation Grant BMS75-13468 for "System Controls of Anabiotic and Catabiotic Phenomena in the Belowground Ecosystem."

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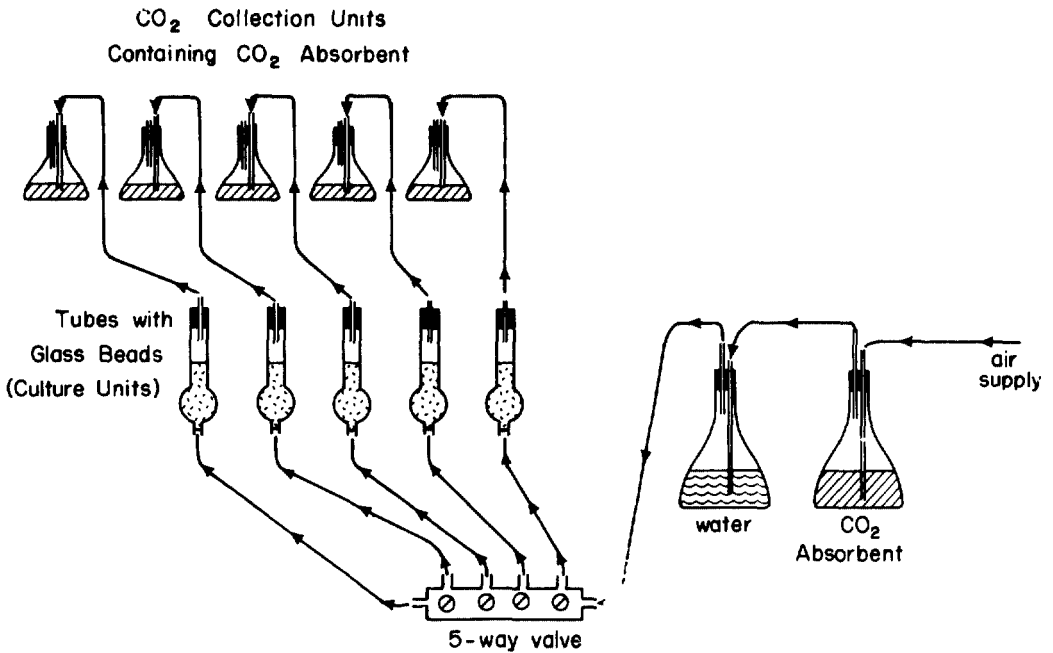


FIG. 1. Diagram of the culture apparatus showing the basic components and direction of air flow.

(Difco nutrient broth) and inoculated with *Pseudomonas* sp. (1×10^7 bacteria per ml) by pouring the broth on the top of the beads in the tube and applying suction at the bottom. This negative pressure was maintained until suction was felt at the top of the tube (to ensure adequate aeration in our studies). The tubes and contents were then weighed to determine the amount of medium in the glass beads. Nutrient medium was added or removed as needed until a uniform amount was distributed in each of the culture units. Each culture unit received 5.25 gm (approximately 5 ml) which was 15% moisture by weight.

Used as the bacterial grazer was the nematode *Mesodiplogaster lheritieri* (Maupas, 1919) Goodey 1963 which was isolated from soil obtained at the Pawnee Site near Nunn, Colorado (field research facility of the Natural Resource Ecology Laboratory, Colorado State University, located on the USDA Agricultural Research Service Central Plains Experimental Range). The nematodes were surface-sterilized with merthiolate, 1:1,000 dilution, and placed on an antibiotic agar (2%) plate for 1 day before inoculation. The antibiotic agar plates were prepared by applying a thin film of an antibiotic solution (25 mg streptomycin, 25 mg tetracycline, and 10 mg

penicillin in 500 ml of distilled water) to the surface of the agar. Sterility checks of this technique, made by using Difco nutrient agar and Difco potato dextrose agar, showed the nematodes to be free of both surface and gut flora. Six nematodes/gram of glass beads were added to the units with a sterile pipette.

Air passing through culture units inoculated with organisms contained respired CO₂, which was collected in alkali traps (20 ml of 1 N NaOH) to determine the CO₂ output from each culture unit. A non-inoculated and ungrazed control was run in each series of five units. Thus we could monitor the effectiveness of the CO₂-scrubbing train, the amount of initial CO₂ in the system, and the CO₂ produced by the bacteria without the effects of the grazers.

With the soil samples and agar plates, three replicates were used for each determination of nematode numbers. Since the maximum number of glass-bead culture units was five, only one unit was sampled at each time interval. Noninoculated controls were not run during determination of nematode population responses. The experiment was repeated three times, however, with samples taken at the same time intervals. The points for nematode numbers in

glass beads in Figure 3, therefore, represent means determined from three observations.

Soil and agar cultures were prepared for a comparison of culture techniques. Each soil sample contained 10 gm of soil, an Ascalon sandy loam, from the Pawnee Site. The soil was sterilized with a propylene oxide technique (3) and brought to the same moisture content, 15% by weight, by adding nutrient broth. As with the glass beads, six nematodes/gram of soil were added. Agar plates were prepared by adding agar to 5 ml of nutrient broth per plate. The agar plates were inoculated with five gravid females. The agar plates and soil samples were placed in screw-cap jars with alkali traps for CO₂ collection.

Nematodes were extracted from the glass beads and soil by a modification of the Baermann technique. A 7.5-cm diam screen was placed just inside the top of a long-stem funnel. The screen supported a disc, 10 cm in diam, cut from a double layer of Kimwipes. After a sample was thoroughly mixed, 10 gm of soil or glass beads were distributed evenly over this disc, and tap water was added to the funnel until the water level was just above the glass beads or soil. After 48 h, 50 ml of water were removed from the bottom of the funnel, and the nematodes in this solution were counted by placing 1 ml on a counting slide. A minimum of three slides were examined for each sample. The microbeads were saved for reuse after acid washing. Nematodes on the agar plates were counted by removing and compressing agar plugs (9).

RESULTS AND DISCUSSION

The agar cultures produced more CO₂ than either the soil or glass-bead cultures (Fig. 2). With both the latter techniques, the cultures containing nematodes had a rapid increase in CO₂ production which was followed by a leveling off after about 7 days. The cumulative CO₂ produced in the grazed systems, except for the last three sampling dates in the soil system, was higher than that in the nematode-free control. This increased output with grazers may reflect a faster turnover of essential nutrients which allows more rapid bacterial growth and consequently more CO₂ production in the grazed system. Differences in cumulative CO₂ output between the bac-

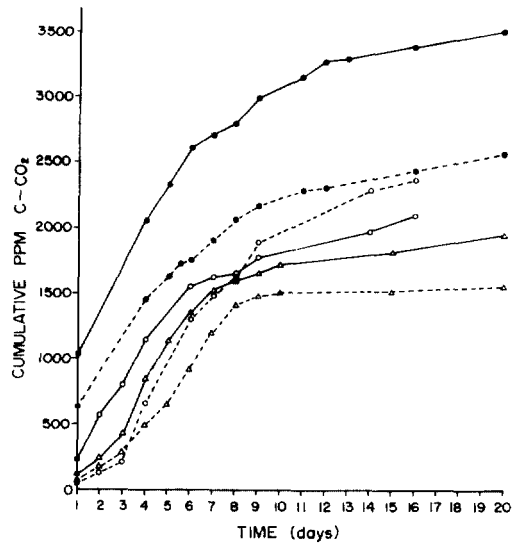


FIG. 2. A comparison of the cumulative CO₂ output in agar, soil, and glass-bead culture systems. Nematodes and bacteria —●—, bacteria only —○—, ● on nutrient agar, ○ in soil, △ in glass beads.

terial and grazed cultures were significant (*t*-test, $P < 0.05$, was used for all significance testing) for the first four and last two sampling dates in the soil and glass-bead cultures. After the first three sampling dates, there was no significant difference in CO₂ output between the soil and glass beads. The slightly higher CO₂ output in the soil system was probably due to a greater amount of available carbon from some soil organic matter.

The numbers of nematodes produced and the growth responses were similar in the soil and glass beads (Fig. 3). The

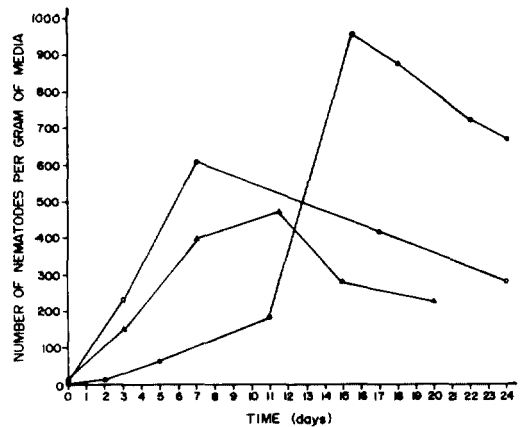


FIG. 3. The numbers of nematodes produced per gram of substrate in agar, soil, and glass bead systems. On nutrient agar ●, in soil ○, in glass beads △.

TABLE 1. Body dimensions and standard deviations for dauer larvae and adult females of *Mesodiplogaster lheritieri* in various culture media.

Media	No. of measurements	Length		Width		L/W ratio	
		(mm)	SD	(mm)	SD	Ratio	SD
Dauer larvae							
Agar	40	0.266	0.038	0.019	0.003	14.55	2.57
Soil	20	0.273	0.006	0.020	0.002	14.39	1.56
Glass beads	20	0.276	0.014	0.021	0.003	14.75	2.13
Adult females							
Agar	40	1.148	0.081	0.062	0.012	19.31	7.30
Soil	20	0.987	0.010	0.041	0.006	24.84	3.67
Glass beads	20	0.997	0.026	0.043	0.005	23.36	2.98

nematodes in the agar cultures again responded differently, with the population peaking later and at a higher density. The larger numbers in the agar cultures were probably the result of increased bacterial numbers and of more habitable space. The nematodes were unrestricted and moved over and through the agar. In the soil and glass-bead systems, nematode movement was restricted to pore spaces of adequate size; thus there was less habitable area, both spatially and in relation to food resources, in these systems. With all culture techniques, the nematode populations were primarily dauer larvae at their peak and entirely dauer larvae by the end of the experiment.

A comparison of nematode lengths and widths gives further evidence of the physical limitations of beads and soil versus agar (Table 1). Adult females were longer and wider in agar than in either soil or glass beads. The more confined physical environment yielded thinner nematodes which would have greater ease of movement in small spaces. This thinner condition may have reflected the inaccessibility or reduced productivity of the bacterial food supply, greater physical restrictions, or possibly a combination of both. Interestingly, the dauer larvae themselves did not differ significantly in any of the culture techniques; only in the later developmental stages does there appear to be a response to the restricted environment.

In conclusion, the glass microbeads tended to elicit nematode responses similar to those in soil. Agar cultures generally deviated significantly in nematode responses from soil or glass beads, which were generally not significantly different. The

glass microbeads have many of the advantages of agar, however, since the nutrient content of the system can be well defined, and glass beads are easily sterilized and reused.

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