

Mass Culture of Axenic Nematodes Using Continuous Aeration¹

EDWARD J. BUECHER² AND EDER L. HANSEN³

Biochemical and physiological studies of nematodes often require sufficient material for analysis (5, 6). Two important limitations of axenic culture methods are the need for proteinaceous components (1, 8) and proper gas exchange (9). Proteinaceous supplements are tedious to prepare. Suitable gas exchange is obtained by using thin layers of medium (9), necessitating glassware of considerable size if large populations are desired.

The plant-parasitic nematode *Aphelenchus avenae* can withstand vigorous aeration in buffer (3). When air was continuously bubbled through a culture of *A. avenae* in 20 ml of a defined medium supplemented with fresh chick embryo extract and serum (7), the population increased from 400 to 11,000 per ml in 4 weeks. This aeration system was therefore applied to mass culture of other nematodes.

The free-living nematodes *Caenorhabditis elegans*, *Turbatrix aceti*, and *Panagrellus redivivus*, and the insect-parasitic nematodes *Neoaplectana glaseri* and *N. carpocapsae* (DD136 strain) were tested for growth under continuous aeration. Sufficient air flow was bubbled through the medium to continuously mix the nematodes. Air was sterilized by passage through a Millipore DA gas filter. Gas washing bottles (Kontes Glass Co., Vineland, New Jersey) of 125, 250, or 500

ml with open-end dispersion tubes were used as the culture vessels. The basal medium consisted of 3% soy peptone, 3% yeast extract (4), and 0.7% dextrose; it was autoclaved for 20 min at 121 C. MEM vitamins[®] 100 × solution (Grand Island Biological Co., Grand Island, New York), 5 ml per 100 ml of medium, and autoclaved antifoam emulsion Y-30[®] (Dow Corning Corp., Midland, Michigan), 0.2 ml per 200 ml of medium, were added aseptically. Filtered preparations of heated liver extract (10) or yeast extract (1) were added as supplements of 5 to 10 mg per ml; medium containing yeast extract was adjusted to pH 3.8 with glacial acetic acid. When either sodium caseinate (Nutritional Biochemicals Corp., Cleveland, Ohio) (W. Hieb, personal communication) or glycogen (Fisher Scientific Co., New York, New York) was used as the supplement, it was added at 10 mg per ml and autoclaved with the basal medium. Filtered hemin chloride (2) was then added at 10 µg per ml.

The inoculum size varied from 30 per ml for *N. carpocapsae* to 2,000 per ml for *T. aceti*; cultures were incubated at room temperature (20 to 25 C). Growth was determined from nematode counts; the final count was made after 3 weeks when no further increase in population was observed. The resulting populations are summarized in Table 1. Each species went through one or two generations. Counts were up to 10 times greater than in control test tube cultures, and increased up to 600-fold over the inoculum. Weights of lyophilized nematodes from the 170-ml liver-supplemented cultures were 500 to 720 mg. The 500-ml culture of *C. elegans*

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² Present address: Department of Biology, Temple Buell College, Denver, Colorado 80220.

³ Clinical Pharmacology Research Institute, 2030 Haste Street, Berkeley, California 94704. The authors wish to thank Dr. E. A. Yarwood and Mrs. G. Perez-Mendez for assistance.

TABLE 1. Populations of nematodes after 3 weeks in complex media under continuous aeration.

Species	Supplement	Medium volume	Final count per ml $\times 10^2$	Adults %	Increase over inoculum
<i>P. redivivus</i>	Liver	170	420	19	210 \times
<i>T. aceti</i>	Yeast	170	1,020	11	50 \times
<i>N. carpocapsae</i>	Liver	90	140	36	470 \times
<i>N. glaseri</i>	Liver	100	2	67	2 \times
<i>C. elegans</i>	Liver	170	2,090	7	210 \times
<i>C. elegans</i>	Liver	500	1,080	5	180 \times
<i>C. elegans</i>	Glycogen	170	1,870	5	620 \times
<i>C. elegans</i>	Sodium caseinate	170	1,770	18	590 \times

yielded a wet weight of 5 g and a lyophilized weight of 1 g of nematode tissue.

Stoll obtained high populations of *N. glaseri* with shaking of 3.5 mm-deep cultures (11). In a 100-ml culture with constant aeration we obtained a twofold increase in population (Table 1). The protein used, 5 mg per ml, was lower than optimum for this species.

Constant aeration appears to remove ammonia and thus retard a rise in pH and subsequent death noted in test tube cultures. In mass cultures less than 1% of the nematodes were dead at harvest compared to 50% in test tube controls. Exceptions were cultures of *C. elegans* in sodium caseinate medium, in which 10% were dead, and *T. aceti* in yeast medium, in which 4% were dead.

The tedious task of preparing protein extracts can be eliminated by using glycogen or sodium caseinate. Sodium caseinate is particularly useful in that it is an autoclavable, inexpensive, commercially available supplement that supports high nematode populations. It also supported maturation of *C. briggsae*, *C. elegans*, *P. redivivus*, *T. aceti* and *N. carpocapsae* in test tube cultures.

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