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# Energy Metabolism and Survival of the Infective Juveniles of *Steinernema carpocapsae* under Oxygen-Deficient Conditions[1]

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*Abstract:* Energy metabolism and its relation to survival of the infective juveniles (IJ) of *S. carpocapsae* under anaerobic and oxygen-deficient conditions were studied by monitoring changes in survival rate, levels of key energy reserve materials, oxygen consumption, and respiratory quotient (RQ). The effects of various factors on the survival of IJ under anaerobic conditions were also investigated. Under anaerobic conditions, the IJ were inactivated but could survive for several days in an immobile state, using the carbohydrate reserves glycogen and trehalose for energy supply. The survival time of IJ was mainly dependent on the availability of energy supply, which, in turn, was influenced by factors such as temperature and metabolic by-products. Surviving, anaerobically incubated IJ fully recovered upon return to aerobic conditions. Recovering IJ were characterized by regaining mobility and restoration of carbohydrate reserves consumed during the anaerobic period. Carbohydrate reserves were restored by conversion from lipid reserves and possibly from anaerobic metabolic by-products. The infectivity of IJ recovered from the anaerobic state was not affected. At 1% oxygen level, IJ were also immobile and mainly depended on carbohydrate reserves for energy supply and the RQ was greater than 1. However, some oxygen was consumed; the survival time of these IJ was shorter than those kept in natural air but longer than those under anaerobic conditions. When IJ were incubated at oxygen levels of 3% to 21%, the RQs were maintained at 0.7 to 0.8. Oxygen consumption rates and the reduction in both mean dry weight and lipid levels were proportional to oxygen levels while the survival time of IJ was inversely proportional to oxygen levels.

*Key words:* anaerobic metabolism, energy reserve, entomopathogenic nematodes, glycerol, glycogen, lipids, nematode, oxygen deficiency, physiology, proteins, *Steinernema carpocapsae,* survival, trehalose.

The infective juveniles (IJ) of entomopathogenic nematodes (EN) naturally live in soils that are characterized by periodic low oxygen or anaerobic conditions. Van Gundy et al. (1968) found that immediately after irrigation, there was no detectable oxygen in the soil at a depth of 61 cm. Oxygen levels returned to normal after more than 12 hours and about 7 days in soils at the depth of 15 and 61 cm, respectively. Presumably, every heavy rain brings similar oxygen-level changes in soils. Sierra and Renaut (1998) showed that factors, such as soil porosity, water content, temperature, ground-water depth, and microbial respiration, all affect the oxygen level in soil.

Oxygen levels inside nematodes are mainly determined by ambient oxygen levels and body size because nematodes possess no respiratory and circulatory systems. Rogers (1962) calculated that at about 2% oxygen, diffusion of oxygen into nematodes became a limiting factor for those having a diameter greater than 50 μm.

The ability of nematodes to survive under anaerobic conditions varies dramatically among species and among different stages of the same species (Nicholas, 1984; Womersley et al., 1998; Föll et al., 1999). *Caenorhabditis briggsae* juveniles survived for 24 hours, but not 48 hours, in pure nitrogen, while eggs of this species survived for at least 3 days (Nicholas, 1966). The larvae of *Tylenchorhynchus martini* survived 18 days under nitrogen, while a species of *Dorylaimus* from African swamps survived for about 3 months under the same conditions (Banage, 1966).

Various studies on the oxygen requirement of EN during both development and storage have been reported. Wang (1993) investigated the effect of oxygen level (1 to 21%) on the population development of *S. carpocapsae* in liquid cultures and showed

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that the population sizes of IJ did not increase at 1% whereas at oxygen levels from 2 to 21%, population size increased significantly and proportionally to oxygen levels. Popiel et al. (1988) and Grewal (1998) found that partially dehydrated IJ consumed much less oxygen than untreated IJ. Burman and Pye (1980) examined the oxygen consumption of the IJ of *S. carpocapsae* at various temperatures and demonstrated that oxygen consumption was highly temperature-dependent and affected by the temperatures at which IJ had been produced. *S. carpocapsae* IJ survived at oxygen levels of 0.5% of saturation for at least 43 days at 20 °C.

This study examines the energy metabolism of the IJ of *S. carpocapsae* under anaerobic and oxygen-deficient conditions and its relationship to the ability of the IJ to survive under such conditions.

## Materials and Methods

*Nematode:* Infective juveniles of *S. carpocapsae* Agriotos used in this study were produced on a semi-solid medium containing mainly egg, corn flour, oil, and yeast distributed on crumbled polyether polyurethane sponge and inoculated with phase one *Xenorhabdus nematophilus* 4 days before the inoculation of nematodes, in flasks or in self-aerating trays (Bedding, 1984; Bedding et al., 1991). Pure IJs were obtained by incubating the freshly harvested nematodes in 0.4% (v/v) formalin solution for 2 hours to kill most of the non-infective stage nematodes and then having the surviving IJs migrate through a milk filter (W., R. & D. Wells, Melbourne, Australia).

*Effects of various factors on the survival of the IJ under anaerobic conditions:* Freshly harvested IJ were suspended in tap water or M9 buffer ($KH_2PO_4$ 0.3g, $Na_2HPO_4$ 0.6g, NaCl 0.5g, $MgSO_27H_2O$ 0.0025g in 1 liter distilled water, pH 7.0, Brenner, 1974) at a designated density. The nematode suspensions were then added to 500-ml reagent bottles sealed with Quickfit caps (Bibby Sterilin, England). Pure nitrogen (BOC Gases, Sydney, Australia) was bubbled vigorously into the bottle via a long needle plugged into the bottle through the septum on the Quickfit until no oxygen could be detected with a gas partitioner (Fisher Scientific, Model 1200, Pittsburgh, PA, USA). The bottles were then incubated under the various conditions described below. Aliquots of about 0.1 ml were taken at designated times using a syringe with a long needle and added to about 1 ml M9 buffer in tissue culture wells and then incubated at room temperature for the nematodes to recover. The survival rates were recorded as percentage of surviving IJ over the total IJ after nematodes were fully recovered, unless otherwise stated. Survival of IJ was judged by their mobility or response to a hair probe.

*Effects of temperature and anaerobic by-products on IJ survival:* These effects were determined by suspending IJ in M9 buffer at a density of $10^5$ IJ/ml. One hundred milliliters of nematode suspension was added to each reagent bottle filled with pure nitrogen using the method described above. The bottles were then incubated on shakers at 100 rpm at 5 °C, 23 °C, and 28 °C. To investigate the effect of volatile anaerobic by-products, such as carbon dioxide and volatile organic acids, on the survival of IJ, another 200 ml of the IJ suspension was added to a 500-ml reagent bottle and incubated at 23 °C. Pure nitrogen was bubbled into the suspension continuously at a rate fast enough to maintain the IJ in suspension. The survival of the bubbled IJ was compared to that in a sealed bottle filled with pure nitrogen. To examine the effect of anaerobic by-products on the recovery and survival of the IJ, 100 ml of IJ suspension in M9 buffer at a density of $10^5$/ml, which had been incubated in a sealed bottle filled with nitrogen for 6 days, was transferred to a 500-ml flask and then incubated aerobically at 23 °C on a shaker for another 8 days. Survival of the IJ was monitored over time.

*Effects of nematode density and buffer on IJ survival:* These effects were determined by suspending IJ in M9 buffer at densities of $10^5$/ml, $10^4$/ml, or $10^3$/ml, and in tap water at a density of $10^5$/ml or $10^3$/ml. One hundred milliliters of each of these suspensions

was added to a 500-ml reagent bottle. The bottles were then sealed, filled with pure nitrogen, and incubated at 23 °C on a rotary shaker at 100 rpm. Survival of the IJ was monitored over time. On the fourth and seventh day, 3 ml of suspension was taken from each treatment and the pH of the suspension was measured with a pH meter (Kent Eil, Model 7015, England).

*Effects of oxygen level on IJ survival:* Aseptically harvested and purified IJ were suspended in sterile M9 buffer containing 0.1% formalin (v/v) at a density of $10^3$ IJ/ml. One hundred milliliters of the suspension was added to each of five sterile reagent bottles. Then gases, with either 1%, 3%, 6% or 8.5% oxygen balanced with nitrogen (BOC Gases, Sydney, Australia) or air, were sterilized by passing through filters (Millex-FG, 0.2 µm, Sydney, Australia) and then bubbled vigorously into each of the bottles through a long needle for about 5 minutes. The bottles were incubated at 28 °C on a shaker at 100 rpm. This process was repeated once a week for the first 4 weeks (twice a week for 1% oxygen) and then once every 14 days until the completion of the experiment to maintain the variation of the oxygen levels within 10% of the set levels. A 5-ml sample was taken from each bottle to estimate the density of the IJ at designated times. Survival rates were estimated by comparing the density of the surviving IJ to the initial density.

*Changes in key energy reserve compounds of IJ under anaerobic or oxygen-deficient conditions:* About 300 ml of IJ suspension in M9 buffer ($10^5$ IJ/ml) was added to each of six 500-ml reagent bottles equipped with a Quickfit cap. Pure nitrogen, 1%, 3%, 6%, or 8.5% oxygen balanced with nitrogen or air, was bubbled into a bottle through a long needle (1.25 × 230 mm) inserted into the bottle via the septum on the Quickfit cap at a rate fast enough to maintain the nematodes in suspension. Another needle (0.90 × 38 mm) was inserted into the bottle via the septum to allow the exit of gas. The devices were maintained at 23 °C for the duration of the experiment. After 6 days, 100 ml of suspension was sampled from the bottle bubbled with pure nitrogen and added to a 500-ml flask. The flask was then incubated aerobically on a rotary shaker at 100 rpm at 23 °C for another 3 days to examine the changes in key energy reserve compounds of IJ during recovery from anaerobic conditions. Samples were taken for analysis of biochemical composition and assessment of survival and mean dry weight (MDW) at designated time intervals.

*Effect of oxygen level on IJ recovery from anaerobic state:* Infective juveniles were incubated in M9 buffer at a density of $10^5$ IJ/ml at 23 °C for 5 days under anaerobic conditions, which was achieved by continuously bubbling pure nitrogen through the suspension. Five milliliters of suspension was transferred into each of five 500-ml reagent bottles sealed with Quickfit caps and filled with gases containing 1, 3, 6, or 8.5% oxygen balanced with nitrogen or air, using a syringe. The bottles were then incubated at 23 °C on a shaker. The trehalose levels and activities of the IJ were examined after incubation for 24 hours.

*Effect of oxygen level on IJ oxygen consumption rate and RQ:* Aseptically harvested IJ were suspended in sterile tap water with 0.1% (v/v) formalin at a density of $10^5$ IJ/ml. One hundred milliliters of the suspension was added into each of six sterile reagent bottles. The bottles were then sealed with Quickfit caps and filled with air or gases with 1, 3, 8, or 14% oxygen balanced with nitrogen sterilized by passage through a filter. The bottles were then incubated at 23 °C on a shaker at 100 rpm, and oxygen and carbon dioxide levels were measured over time using a gas partitioner. The RQ, oxygen consumption rate (OCR), and carbon dioxide synthesis rate (CDSR) were calculated with the following formulae:

$$RQ = (\text{Final } \%CO_2 - \text{Start } \%CO_2)/(\text{Start } \%O_2 - \text{Final } \%O_2);$$

$$OCR\ (\text{ml } O_2/\text{million IJ/day}) = 24/\text{time sealed (hours)}/\text{No. of IJ (millions)}*(\text{Start } \%O_2 - \%O_2)/100*(\text{Vol. of container} - \text{Vol. of IJ suspension});$$

CDSR (ml $CO_2$/million IJs/day) = 24/ time sealed (hours)/No. of IJ (millions)*(Final $\%Co_2$ – Start $\%CO_2$)/100*(Vol. of container – Vol. of IJ suspension).

To assess the effect of microorganisms on the results, at the fourth day, 0.2 ml of suspension without nematodes was sampled from each bottle and spread on NA (DIFCO, Detroit, USA) plates. Developing colonies were counted after incubation at 28 °C for 2 days.

*Effect of anaerobic incubation on IJ infectivity:* Infectivity of IJ incubated under anaerobic conditions (achieved by bubbling pure nitrogen through continuously for 4 days followed by revival under aerobic conditions at 23 °C for 24 hours) was compared to infectivity of IJ incubated aerobically for 5 days at the same temperature. The infectivity test was carried out using a method described previously (Bedding et al., 1993), in which 50 mealworm (*Tenebrio molitor*) larvae ca. 20 mm long were exposed to 5,000 IJ in moist peat moss at 23 °C for 4 days and then assessed for mortality. Three replicates were made for each IJ sample.

*Mean dry weight measurement and biochemical analysis:* The mean dry weight of IJ was estimated by filtering 6 ml of IJ suspension on a pre-dried and weighed Whatman No. 1 filter paper under vacuum and then drying in an oven at 75 °C for 3 hours. The lipid levels of the IJs were determined by methanolysis of hydrolysable fat together with heptadecanoic acid as an internal standard, then analysis of the fatty acid methyl esters using gas chromatography (Alltech Econocap carbowax, 30 -m × 0.32 -mm i.d., phase thickness 0.25 µm × 0.25 µm). Temperature program for the chromatogram was as follows: injection of 0.5 µl at 60 °C followed by temperature programming at 50 °C/min to 150 °C, then 8 °C/min to 22 °C, and then held at 22 °C for 10 minutes. Trehalose and glycerol levels were determined by direct trimethylsilylation of dried nematode together with sucrose as an internal standard and then analysis by gas chromatography (Alltech Econocap SE-54, 30-m × 0.32-mm i.d., phase thickness 0.25 µm). Glycogen and proteins were determined colorimetrically using anthrone (Mokrasch, 1954) and Commassie brilliant blue (Bradford, 1976), respectively, with pure glycogen (BDH, England) and BSA (Sigma, Sydney, Australia) as standards. Three replicates were made for each sample in both mean dry weight measurement and biochemical analysis.

*Statistical analysis:* The data on changes in mean dry weight and food reserve compounds of IJ over time were subjected to one-way analysis of variance (ANOVA) followed by Student-Newman-Keules test if the difference of ANOVA is significant ($P < 0.05$). The probit analysis was used to determine the difference of the time/survival lines of IJ under various conditions. All statistical analyses were performed using the statistical software SAS V6.12 (SAS Institute, Cary, NC, USA).

## Results

*Survival of IJ under anaerobic conditions:* The effects of temperature and anaerobic byproducts on the survival of IJ under anaerobic conditions are summarized in Figure 1. When IJ were incubated in M9 buffer at 23 °C under anaerobic conditions, they became straight and fully inactivated in about

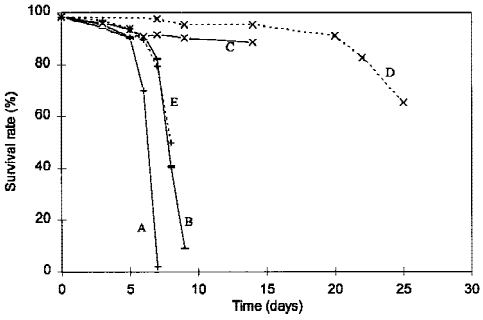


Fig. 1. Changes in survival of infective juveniles of *Steinemema carpocapsae* incubated under anaerobic conditions at various conditions (mean of 3 replicates, the maximum and minimum SE of the means are 1.9 and 0.3%, respectively). Treatments: A) 28 °C, bubbled with nitrogen for 10 minutes, then sealed; B) 23 °C, bubbled with nitrogen for 10 minutes, then sealed; C) 23 °C, bubbled with nitrogen for 10 minutes, then sealed for 6 days followed by aerobic incubation for 8 days; D) 5 °C, bubbled with nitrogen for 10 minutes, then sealed; E) 23 °C, continuously bubbled with nitrogen.

16 hours. The surviving IJ revived when they were returned to aerobic conditions. The time needed for recovery was proportional to the time under anaerobic conditions, varying from several minutes to more than 24 hours for those under anaerobic conditions for 7 days. There were significant differences in the survival of IJ incubated at 5 °C, 23 °C, and 28 °C under the anaerobic conditions achieved by bubbling with nitrogen for 10 minutes and then sealing the bottle (Fig. 1, lines A, B, and D; probit analysis, equality: $P < 0.001$). The calculated 90% survival time and 95% confidence interval of these IJ were 20 (17, 22), 7 (6, 8) and 5 (4, 6) days, respectively. IJ incubated anaerobically at 23 °C for 6 days recovered and maintained a high survival rate for more than 2 weeks when returned to aerobic conditions at 23 °C. There was no significant difference in the survival time of IJ incubated in M9 buffer at 23 °C under anaerobic conditions achieved by continuous bubbling of nitrogen or by bubbling nitrogen vigorously through the suspension for 10 minutes and then sealing the bottle (Fig. 1, lines B and E; probit analysis, equality: $P = 0.28$).

The pH of the tap water with an IJ density of $10^5$/ml and $10^3$/ml dropped from initial levels of 6.9 and 6.8 to 5.2 and 6.4, respectively, at the fourth day, and 5.0 and 6.2 at the seventh day, respectively. The pH of the IJ suspensions in M9 buffer remained at 7.0 during the experimental period regardless of IJ density. Both buffer and nematode density significantly affected IJ survival in anaerobic conditions (Fig. 2, lines A, B, D, and F; probit analysis, equality: $P < 0.01$). The calculated 90% survival time and 95% confidence interval of the IJ in tap water at a density of $10^5$/ml was 5 (3, 6) days, compared to 8 (6, 9) days for those at a density of $10^3$/ml in tap water and 6 (5, 7) and 7 (5, 8) days for those in M9 buffer at a density of $10^5$/ml and $10^3$/ml, respectively. However, the difference in the survival of the IJ in M9 buffer at a density of $10^5$/ml and $10^4$/ml was not significant (Fig. 2 lines C and D, probit analysis, equality: $P = 0.16$). The effect of nematode density on the survival of the IJ was much greater in tap water than in M9 buffer.

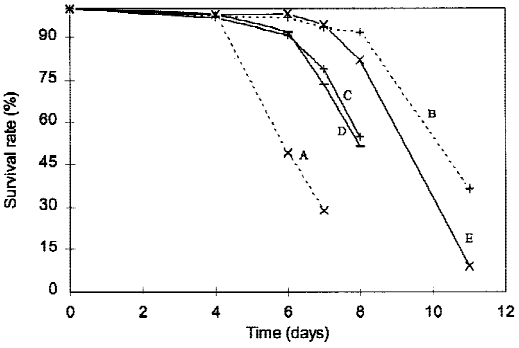


FIG. 2. Effect of buffer and density of nematodes on the survival of infective juveniles of *Steinernema carpocapsae* incubated under anaerobic conditions at 23 °C (mean of 3 replicates, the maximum and minimum SE of the means are 2.3 and 0.3, respectively). Treatments: A) in tap water at $10^5$ IJ/ml; B) in tap water at $10^3$ IJ/ml; C) in M9 buffer at $10^4$ IJ/ml; D) in M9 buffer at $10^5$ IJ/ml; E) in M9 buffer at $10^3$ IJ/ml.

*Energy metabolism of IJ under anaerobic conditions:* The survival of IJ decreased from 99% to 92% by day 6 under anaerobic conditions, and the survival of IJ maintained fairly constant after return to aerobic conditions (Fig. 3). The mean dry weight of IJ decreased from 71 to 68 ng/IJ by the end of anaerobic incubation at day 6, and then dropped sharply to 58 ng/IJ after returning to aerobic conditions for 3 days. During the anaerobic period, glycogen and trehalose levels declined rapidly over time from initial

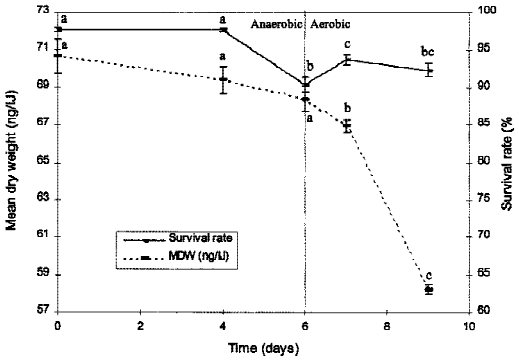


FIG. 3. Changes in survival and mean dry weight of infective juveniles (IJ) of *Steinernema carpocapsae* incubated anaerobically for 6 days, then aerobically for another 3 days at 23 °C. Bars (mean ± SE, $n = 3$) with the same letters indicate that the treatments were not significantly different at $P \leq 0.05$.

levels of about 4.5 and 1.4 ng/IJ to 1.3 and 0.3 ng/IJ at day 6, respectively (Fig. 4A). The lipid levels were not significantly changed, and protein levels increased slightly (Fig. 4B). When anaerobically incubated nematodes were returned to an aerobic environment, both glycogen and trehalose levels increased while lipid level decreased sharply.

*Effects of oxygen level on IJ survival:* Oxygen level significantly affected the survival of the IJ (Fig. 5, probit analysis, equality: $P < 0.001$). Except at 1% oxygen, the survival time of the IJ was inversely proportional to oxygen levels. The calculated 90% survival time and 95% confidence interval varied from about 4 (3, 5) weeks for IJ in 21% oxygen to 7 (5, 8), 10 (9, 12), and 13 (11, 15) weeks for IJ in 8.5%, 6%, and 3% oxygen, respectively.

*Energy metabolism of IJ under oxygen deficient conditions:* The reduction in IJ mean dry weight was proportional to the oxygen level, varying from about 3 ng/IJ for IJ incubated at 1% oxygen to 12 ng/IJ for those incubated at 21% oxygen (Table 1). When IJ were incubated at oxygen levels from 3% to 21%, the trehalose, glycogen, and protein levels, as percent dry weight, hardly changed, but lipid levels dropped significantly in inverse proportion to oxygen levels (Table 1). The changes in energy reserve compounds of IJ incubated at 1% oxygen differed from those incubated at a oxygen level from 3% to 21%, with a sharp drop in trehalose and glycogen levels but no significant change in lipid and protein levels.

*Effect of oxygen level on the recovery of IJ from anoxybiosis:* Infective juveniles that had been incubated under anaerobic conditions for 5 days were fully inactivated. When these IJ were incubated in an atmosphere with an oxygen level higher than 3% for 24 hours, they fully recovered and appeared to be more active than freshly harvested IJ when observed under a microscope. There was no observable morphological or behavioral difference between IJ recovered at oxygen levels from 3% to 21% for 24 hours. Nematodes recovered from 1% oxygen for 24 hours were still in a straight and inactive state, but they recovered in about 3 hours after incubation in air compared to at least 8 hours required for those incubated under

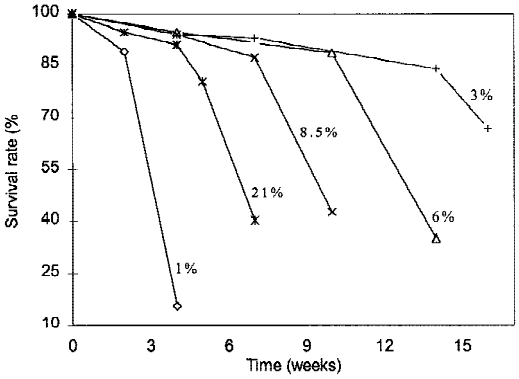


FIG. 5. Effect of oxygen level (1 to 21%) on survival of infective juveniles of *Steinernema carpocapsae* incubated in M9 buffer at a density of 1,000 IJ/ml at 28 °C on a shaker. Lines are the mean of 3 replicates. The maximum and minimum SE of the means are 1.9 and 0.3, respectively.

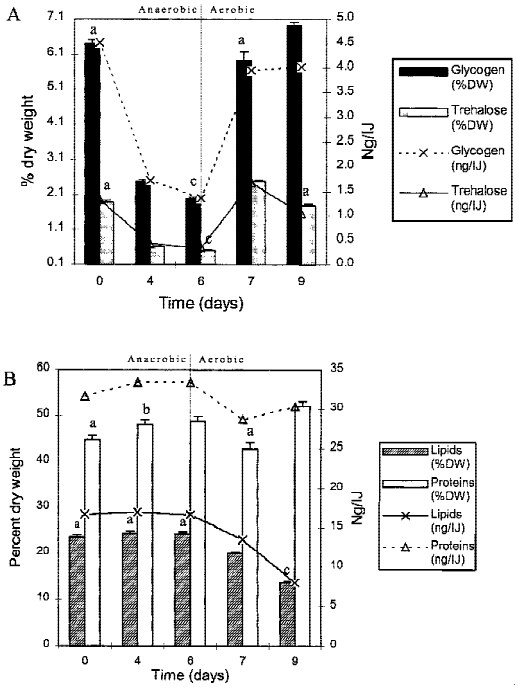


FIG. 4. Changes in food reserve compounds of infective juveniles (IJ) of *Steinernema carpocapsae* incubated anaerobically for 6 days, then aerobically for another 3 days at 23 °C. A) glycogen and trehalose levels; B) protein and lipid levels. Bars (mean ± SE, $n = 3$) with the same letters indicate that the treatments were not significantly different at $P \leq 0.05$.

TABLE 1. Mean dry weight (MDW) and energy reserve compounds of infective juveniles (IJ) of *Steinernema carpocapsae* incubated in M9 buffer at various oxygen levels at 23 °C for 7 days.

| Energy reserve compounds | Initial levels | Oxygen level (%) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 3 | 6 | 8.5 | 21 |
| MDW (ng/IJ) | 71 ± 1.0a* | 68 ± 0.6b | 65 ± 0.4c | 64 ± 0.3c | 62 ± 0.3d | 58 ± 0.3e |
| As percent MDW | | | | | | |
| Lipids | 26 ± 0.5a | 25 ± 0.4a | 22 ± 0.1b | 20 ± 0.2c | 20 ± 0.1c | 15 ± 0.1d |
| Glycogen | 6.7 ± 0.27a | 2.4 ± 0.2b | 6.8 ± 0.1a | 7.1 ± 0.1c | 7.3 ± 0.3c | 7.2 ± 0.3c |
| Trehalose | 1.9 ± 0.1a | 1.1 ± 0.0b | 2.9 ± 0.0c | 2.9 ± 0.0c | 2.9 ± 0.1c | 2.3 ± 0.1d |
| Proteins | 48 ± 2.0a | 52 ± 1.9a | 51 ± 1.2a | 55 ± 1.5b | 52 ± 1.5a | 55 ± 0.9b |
| Weight (ng/IJ) | | | | | | |
| Lipids | 18.2 | 17.2 | 14.2 | 12.6 | 11.8 | 8.98 |
| Glycogen | 4.73 | 1.61 | 4.44 | 4.51 | 4.49 | 4.18 |
| Trehalose | 1.35 | 0.77 | 1.85 | 1.84 | 1.79 | 1.31 |
| Proteins | 33.9 | 35.6 | 33.3 | 34.8 | 31.7 | 31.9 |

* Mean ±SE, $n = 3$, numbers labeled with the same letters indicate that they were not significantly different at $P \leq 0.05$.

anaerobic conditions for 5 days and then recovered in air directly. Anaerobic IJ incubated for 24 hours in oxygen levels higher than 3% had their trehalose levels restored, while for those at 1% oxygen, trehalose level increased only slightly (Fig. 6).

*Effect of oxygen level on IJ respiration:* Except for one colony of the symbiotic bacterium *X. nematophilus* found on an NA plate from the treatment of 14% oxygen, no other bacterial or fungal colonies were found, demonstrating that microorganisms had negligible effects on the results. Under anaerobic conditions, even with IJ fully inactivated after 12 hours, the $CO_2$ level increased gradually to about 1% after 72 hours (Table 2). The rate of $CO_2$ synthesis dropped sharply from 0.53 ml/million IJ/day in the first 12 hours to 0.02 ml/million IJ/day on the fourth day. When IJ were incubated at $O_2$ levels varying from 1% to 21%, the RQ of the IJ was related to the initial $O_2$ level. When the oxygen level was higher than 3%, the RQ was maintained at 0.7 to 0.8; when it was lower than 3%, the RQ was greater than 1 (Table 2).

*Effect of anaerobic incubation on IJ infectivity:* The infectivities toward mealworms of IJ incubated aerobically at 23 °C for 5 days and IJ incubated under anaerobic conditions for 4 days and then revived under aerobic conditions for 24 hours were 89% ± 1.3 and 87% ± 1.8 (mean ± SE, $n = 3$), respectively, and did not differ significantly (paired t-test, $P = 0.18$).

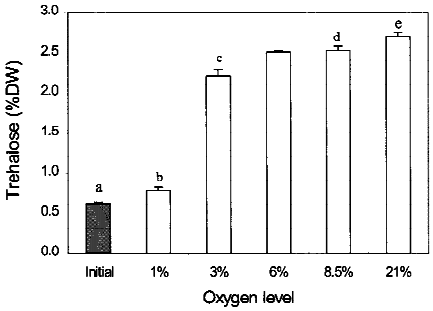


FIG. 6. Trehalose levels (as percent dry weight [DW]) of infective juveniles of *Steinernema carpocapsae* incubated at 23 °C under anaerobic conditions for 5 days, followed by incubation at various oxygen levels for another 24 hours. Bars (mean ± SE, $n = 3$) with the same letters indicate that the treatments were not significantly different at $P \leq 0.05$.

## DISCUSSION

Changes in key energy reserve compounds, RQ, oxygen consumption rate, and carbon dioxide synthesis rate of IJ incubated under anaerobic or oxygen-deficient conditions indicate that ambient oxygen level significantly affected energy metabolism. Under anaerobic conditions, IJ were immobile and their glycogen and trehalose contents decreased sharply while lipid and protein contents did not change substantially. Carbon dioxide was produced but at a low level compared to the amount of carbohydrate consumed. Thus, under anaerobic condi-

TABLE 2. Changes in levels of oxygen and carbon dioxide, respiratory quotient, oxygen consumption rate, and carbon dioxide synthesis rate of the infective juveniles (IJ) of *Steinernema carpocapsae* incubated in sterile tap water under various initial oxygen levels.

| | Initial $O_2$ level (%) | Time interval (hours) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0–12 | 12–24 | 24–36 | 36–48 | 48–72 | 72–96 |
| Oxygen level (%) | 1 | 1.0[a] | 0.47 | 0.24 | 0.14 | 0.00 | 0.00 |
| | 3 | 2.8 | 1.6 | 0.49 | 0.26 | 0.18 | 0.00 |
| | 8 | 7.9 | 4.1 | 1.7 | 0.79 | 0.36 | 0.00 |
| | 14 | 14 | 8.8 | 4.3 | 2.6 | 1.4 | 0.91 |
| | 21 | 21 | 15 | 10 | 6.2 | 3.6 | 1.6 |
| Carbon dioxide level (%) | 0 | 0[a] | 0.47 | 0.65 | 0.78 | 0.89 | 1.0 |
| | 1 | 0 | 0.75 | 1.2 | 1.4 | 1.6 | 1.8 |
| | 3 | 0 | 1.2 | 2.0 | 2.6 | 2.6 | 2.8 |
| | 8 | 0 | 2.6 | 4.3 | 5.0 | 5.6 | 6.0 |
| | 14 | 0 | 3.8 | 6.8 | 8.1 | 8.8 | 9.5 |
| | 21 | 0 | 4.4 | 7.8 | 11 | 13 | 14 |
| Respiratory quotient | 1 | 1.4[b] | 1.9 | 2.5 | 1.0 | – | – |
| | 3 | 1.0 | 1.0 | 1.6 | 0.13 | 0.93 | – |
| | 8 | 0.68 | 0.69 | 0.76 | 1.3 | 1.3 | – |
| | 14 | 0.78 | 0.66 | 0.9 | 0.69 | 1.3 | 3.7 |
| | 21 | 0.81 | 0.67 | 0.73 | 0.70 | 0.70 | 1.5 |
| Oxygen consumption rate (ml/million IJ/day) | 1 | 0.54[b] | 0.19 | 0.08 | 0.12 | 0.00 | 0.00 |
| | 3 | 1.6 | 0.61 | 0.19 | 0.06 | 0.07 | 0.00 |
| | 8 | 4.0 | 2.0 | 0.72 | 0.34 | 0.14 | 0.00 |
| | 14 | 4.5 | 3.6 | 1.2 | 0.88 | 0.21 | 0.04 |
| | 21 | 5.1 | 4.1 | 3.3 | 2.1 | 0.78 | 0.15 |
| Carbon dioxide synthesis rate (ml/million IJ/day) | 0 | 0.53[b] | 0.15 | 0.05 | 0.08 | 0.04 | 0.02 |
| | 1 | 0.75 | 0.36 | 0.20 | 0.12 | 0.08 | 0.05 |
| | 3 | 1.7 | 0.61 | 0.31 | 0.01 | 0.07 | 0.12 |
| | 8 | 2.7 | 1.4 | 0.55 | 0.43 | 0.18 | 0.10 |
| | 14 | 3.5 | 2.4 | 1.1 | 0.61 | 0.27 | 0.16 |
| | 21 | 4.1 | 2.7 | 2.4 | 1.5 | 0.55 | 0.23 |

[a] Initial level of the time interval.
[b] Average rate for the time interval.

tions, IJ of *S. carpocapsae,* like most other free-living nematodes, cannot use lipids and depend on anaerobic degradation of their carbohydrate reserves, mainly glycogen and trehalose, for energy supply (Tielens and Van den Bergh, 1995). Thompson et al. (1991), who detected succinic, acetic, propionic, and lactic acid in anaerobically incubated IJ of *S. carpocapsae* using NMR spectroscopy, found that succinic acid was discharged into the solution in which IJ were incubated. The decrease in pH of nematode suspensions incubated under anaerobic conditions is in line with this observation. Thus, carbohydrates are likely to be metabolized via a pathway similar to that found in the adults of *Ascaris suum* (Tielens and Van den Bergh, 1995). However, Föll et al. (1999) showed that *Caenorhabditis elegans* produced organic acids lactate, acetate, succinate, and propionate under anaerobic conditions. The profiles of the acids produced were temperature dependent and were mainly excreted. Shih et al. (1996) examined the activity and characteristics of several key enzymes involved in glycolysis in the IJ of *S. carpocapsae* and demonstrated that active phosphoenolpyruvate carboxykinase and pyruvate kinase, which determine the carbon flow at the PEP branch point in the glycolytic pathway, were present in the IJ.

When IJ were incubated in 1% oxygen, they were immobile but could be revived when returned to air in a much shorter period compared to those under anaerobic conditions. The pattern of changes in key energy reserve materials of these IJ was similar to that under anaerobic conditions and the RQ was greater than 1, suggesting that at 1% oxygen, IJ mainly depend on anaerobic

degradation of carbohydrates for energy supply. However, the consumption of oxygen and the noticeably longer survival time of these IJ compared to those under anaerobic conditions suggests that an aerobic pathway may be at least partially functional. The amount of oxygen consumed and the changes in contents of energy reserve materials indicated that if aerobic energy metabolism is present, it is at a very low level and may be limited to certain parts of nematode body such as tissues close to the body surface where oxygen tensions are possibly higher. It is not clear from the data available whether aerobic metabolism, if present, is the result of lipid utilization or the aerobic metabolism of carbohydrates, or both. The more rapid recovery and longer survival time of the IJ at 1% oxygen than those under anaerobic conditions may occur because aerobic metabolism produces more energy than anaerobic metabolism and may also enable the IJ to maintain the activity of aerobic metabolic enzymes.

When IJ were incubated at oxygen levels from 3 to 21%, they were mobile, trehalose levels, and glycogen levels and RQ did not change substantially, but lipid levels decreased over time. The oxygen consumption rate and the reduction of lipids and IJ mean dry weight were proportional to oxygen level, while the life spans of the IJ were inversely proportional to oxygen level. This indicates that at oxygen levels from 3 to 21%, IJ can use any of these reserves for energy generation and, most likely, via the same pathways, but the metabolic rate was significantly affected by oxygen levels.

This study demonstrates that survival of *S. carpocapsae* IJ under anaerobic or oxygen-deficient conditions is similar to that under aerobic conditions, mainly depending on availability of energy supply. The effect of oxygen level on IJ survival time is a result of the influence of oxygen level on (i) what reserve materials can be used for energy generation, (ii) the metabolic pathway(s) used, and (iii) metabolic rate. Under anaerobic conditions, IJ can use only glycogen and trehalose but not the primary energy reserve-lipids. Glycogen and trehalose account for only about 10% dry weight of the IJ, and the efficiency of anaerobic metabolism is much lower than oxidative metabolism. Therefore, under anaerobic conditions, IJ survive a much shorter time even though they are fully inactivated. However, since previously anaerobic IJ can rapidly convert lipids into glycogen and trehalose with as little as 3% atmospheric oxygen and since their infectivity is not substantially reduced, IJ survival in the field, where anaerobic conditions are most likely to be periodic, will be greatly enhanced.

The effects of buffer and nematode density on IJ survival time under anaerobic conditions are a result of the effects of anaerobic by-products on their energy metabolism. The decrease in the pH of the IJ tap water suspension resulting from the production and excretion of organic acids when IJ are under anaerobic conditions may inhibit some key enzymes of the glycolytic pathway such as phosphofructokinase, and so block the only energy generation path under anaerobic conditions. M9 buffer may prevent or postpone the decline of pH and therefore extend the survival of the IJ, explaining why IJ in M9 buffer survive longer than those in tap water at a high IJ density. However, as M9 buffer is an ionic solution, IJ may have to expend extra energy, maintaining the ionic balance in the same way as IJ under aerobic conditions do (Qiu and Bedding, 2000). Thus, IJ in tap water at low density survived longer than those in M9 buffer. The similarity in survival of IJ incubated under anaerobic conditions, whether achieved by continuously bubbling nitrogen or bubbling nitrogen for 10 minutes and then sealing the bottle, suggests that $CO_2$ and volatile anaerobic metabolic by-products are not key factors affecting IJ survival. The survival of IJ incubated under anaerobic conditions for 6 days and then returned to aerobic conditions remained high for a long time, indicating that nonvolatile anaerobic by-products were not toxic to IJ under aerobic conditions.

The correlation between survival time, oxygen consumption rate, lipid consumption rate, and oxygen levels when IJ were

incubated at oxygen levels of 3 to 21% demonstrates that *S. carpocapsae* IJ are typical conformers, i.e., their metabolic rate correlates to oxygen level. This characteristic may be used to extend the shelf life of EN-biopesticide products, especially during periods of in-house storage when oxygen levels can be controlled readily.

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