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An investigation of the possiblities of scaling-up and mass-producing entomopathogenic nematodes (EPNs)

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Abstract

The aim of this study was to design a bioreactor system for the cultivation of entomopathogenic nematodes (EPNs). These are worm-like living invertebrate organisms that can be used as bio-pesticides. The *Heterorhabditis indica* nematode from Ireland and the South African strain *Heterorhabditis Bacteriaphora* were used in the reseach. Both of these species feed on the *Photorhabdus luminescens* bacteria, and the experiments were carried out using *Photorhabdus luminescens* and these EPNs. We cultured *Photorhabdus luminescens* bacteria in both shake flasks and bioreactors in order to determine the optimal conditions for bacterial growth, and found that both the volume of the initial bacteria inoculum and the length of the period during which the bacteria were cultured in nutrient broth were important factors. The doubling time of *Photorhabdus luminescens* bacteria was estimated to be about 3–4 minutes in the batch reactors and about 20 minutes in the shake flasks.

In the scale-up experiments we conducted, the slower growth of bacteria in the shake flask was thought to offer a better match for the growth rate of the EPNs, in that it provided the EPNs with sufficient bacteria to feed off. In the experiments using reactor systems (a fermenter and an external recycle reactor), it proved difficult to match the growth rate of *Photorhabdus luminescens* bacteria and the EPNs. Because the reactor systems are supplied with adequate air, the bacterial growth rate increased: the bacteria multiplied quickly, reached their peak population density and then declined in number as the nutrients in the growth medium depleted. Since the growth rate of the bacteria and that of the EPNs did not match, the EPNs in the reactor did not have sufficient bacteria to feed on, and therefore died.

For this reason we proposed that the bacteria-rich medium (fed batch) should be replenished in the reactor order to ensure that the growth of bacteria is continuous, and that the life cycle of the EPNs progresses at the faster rate that the reactor offers. To ensure that the growth rate of the bacteria and EPNs match, we recommend that in future a double reactor setup is used and that the bacteria-rich

medium is transferred at a specific feed rate into the reactor in which the EPNs are being cultivated.