

Report of Short Term Scientific Mission (COST – 850)

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Name and Organisation of Host Institution:

Koppert Biological Systems
R&D Microbials
Veilingweg 17
P. O. Box 155
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The Netherlands

Duration of Mission: 20.02.2005 – 21.03.2005

Scientific report:

The aim of the study was to learn about nematode mass-production in fermentors and to extrapolate the results of the shake flasks cultures done in Poland to a process in a liquid bioreactor.

As aeration and mixing in a fermentor is fundamentally different from oxygen transfer in shake flask cultures the research performed at Koppert was divided in two parts:

- 1) First, various fermentations with different aeration rates and mixing intensities were done to determine the most optimal parameters. These fermentations were done with a relative low inoculum size. The recovery of DJ to hermaphrodites within 8 days was determined
- 2) Finally, one fermentation was done with the optimal aeration rate and stirring speed and optimal doses of DJ. In this fermentation besides the recovery also the final production of DJ was assessed.

Materials and methods

Monoxenic cultures of *Heterorhabditis megidis* with its symbiotic bacteria *Photorhabdus luminescens* were used. Shake flask cultures were used to produce inocula for the fermentations. Bioreactors with a working volume of 15 litres were used. Process conditions like aeration rate, stirring speed, oxygen levels, and pH were recorded with a data acquisition system and controlled.

Regularly samples were taken to assess the number and stage of nematodes (by microscopy) and the number of bacteria (plate counting).

Results:

In the first experiments various stirring speeds and aeration rates were tested to obtain the most optimal conditions for the nematode cultivations.

The recovery of DJ into hermaphrodites varied between 17% and 65%. In total 6 fermentations were done in the first three weeks of my short term mission.

With the most optimal conditions a new fermentation with the optimal dose of DJ found in my studies performed in Poland. In this reactor again a good recovery of DJ into hermaphrodites was obtained after 6-7 days. After 18 days of cultivation a reasonable yield of DJ was obtained.

Conclusion

I have learned how to use fermentors and what the key parameters for controlling a fermentation process are.

Extrapolation of shake flask culture to bioreactors proved:

1. The recovery of DJ into hermaphrodites in flask culture varied between 14 – 49%, therefore it was lower than in bioreactors (17 – 65%)
2. With the most optimal conditions in flask cultures the hermaphrodites were obtained after 3 – 7 days, while in bioreactors 6 – 7 days.
3. Although the recovery was good, the final yield of DJ was lower than expected. Apparently, the optimum in inoculum size found in shake flasks was less optimal in the bioreactors. Further optimizations might yield a commercial expectable yield.