COMUNICAÇÃO CIENTÍFICA

THE EFFECTS OF AERATION ON MYCELIUM PRODUCTION BY THE ENTOMOPATHOGENIC FUNGUS *Metarhizium anisopliae* (METSCH.) SOR. IN LIQUID FERMENTATION

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RESUMO

Efeito da Aeração sobre a Produção de Micélio pelo Fungo Entomopatogênico *Metarhizium anisopliae* (Metsch.) Sor. em Fermentação Líquida

O micélio seco de *Metarhizium anisopliae* (Metsch.) Sor.. foi produzido em meio líquido sob diferentes condições de aeração. A maior concentração de massa micelial foi produzida utilizando fluxo de ar de 1.5 V.V.M. (= volume de ar por volume de meio por minuto) e agitação de 300 rpm. A redução na concentração inicial de sacarose de 40 para 20 g/l apresentou uma leve redução na produção de massa micelial.

PALAVRAS-CHAVE: Insecta, patógenos, controle biológico, micélio seco.

The utilization of entomopathogenic microorganisms, mainly fungi, as biological control agents, have been intensively studied in the last decade as reviewed by McCoy & Tigano-Milani (1992). This strategy is more advantageous than chemical control because it is more specific, cheaper and less detrimental to the environment (Whipps *et al.* 1989, Roberts 1989).

The use of dry mycelium preparations of entomopathogenic fungi in biological control has been regarded as more advantageous than the use of conidial formulations (Pereira & Roberts 1990). These preparations have been used in the field to control insect pests in the Philippines (Rombach 1986) and in the United States (Wraignt *et al.* 1986). They seem to be more resistant to environmental changes than conidial formulations. Nevertheless, the majority of entomopathogenic fungi currently used in biological control programs are produced as conidia in solid substrates, a time consuming process.

The study of parameters such as inoculum volume, cultivation age, aeration and agitation conditions is very important in dry mycelium production process (Pereira 1987). Growth of

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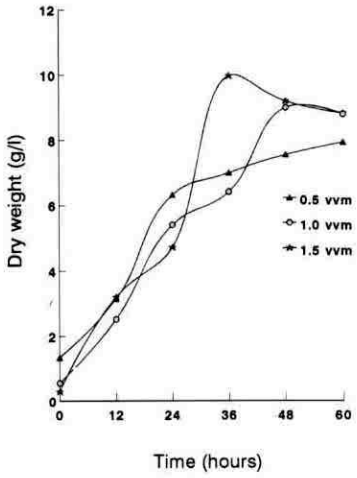

Figure 1. The effect of air flow rates (V.V.M. = volume of air per volume of medium per minute) on mycelical production (dry weight) of *Metarhizium anisopliae* in the fermenter at different air flow rates.

fungi usually increases with increasing aeration (Garraway & Evan 1984). However, our knowledge in this area has been restricted to a few examples of plant pathogenic fungi (Woodhead & Walker 1975, Garraway & Evans 1984). In this research, we report on the effects of aeration on dry mycelium production by *M. anisopliae* as well as on the consumption of sugar during this process.

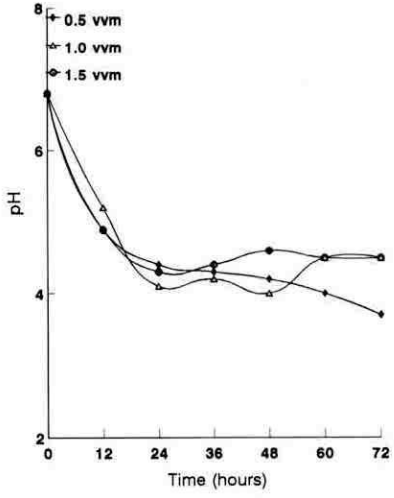

Figure 2. Evolution of pH values of *Metarhizium anisopliae* culture at different air flow rates (V.V.M. = volume of air per volume of medium per minute).

The fungus utilized in this study was *Metarhizium anisopliae* (Metsch.) Sor., isolate CG-46, originally obtained from the spittlebug *Deois flavopicta* in Brazil. It was grown at 27°C during seven days in Sabouraud-dextrose-agar (SDAY) (2% dextrose, 1% neopeptone, 1% yeast extract and 2% agar-agar) titrated to pH 6.5 with NaOH. The inoculum for the fermentation assays was precultured in shaken flasks (250 ml) with 150 ml of medium, at 125

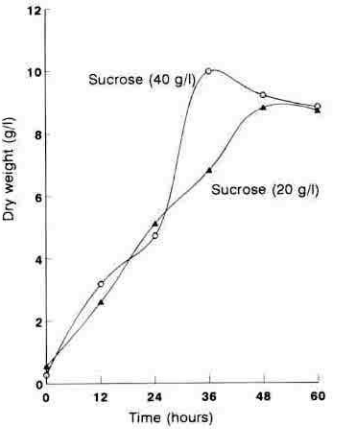

Figure 3. The effect of sucrose concentration on mycelial production by *Metarhizium anisopliae* in fermenter.

rpm and 27°C during 48 h. The fungus was cultured in sabouraud-dextrose (4% dextrose, 1% neoptone and 1% yeast extract; titrated to pH 6.8 with NaOH). The conidium concentration in the flasks was 10^7/ml. The inoculum concentration in the fermenter was 5% (V/V). In the fermenter, other liquid media (2 or 4% sucrose, 1% yeast extract; titrated to pH 6.8 with NaOH) were used. All assays were performed on a Microferm New Brunswick Fermenter (New Jersey,

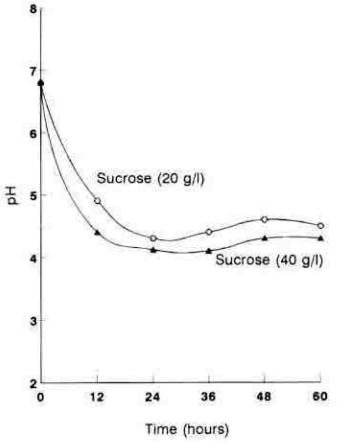

Figure 4. The effect of sucrose concentration on the evolution of pH during growth of *Metarhizium anisopliae* in fermenter.

USA) using 5 l as working volume at 27°C during 120 h. The air flow rates studied were 0.5, 1.0 and 1.5 V.V.M. (= volume of air per volume of medium per minute) at 300 rpm internal pressure of 1.0 atm, and 26 ± 2°C. Sucrose (4%) was used as carbon source.

In a second set of experiments, the sucrose concentration was tested at 2% in order to reduce the production costs. Agitation and aeration flow rate were 300 rpm and 1.0 V.V.M. Samples were collected at 0, 12, 24, 36, 48, and 60 h to determine dry cell mass at 70°C, reducing sugar content in the filtrate, and pH. The reducing sugar content was measured using the dinitrosalicilic acid method (Miller 1959).

The results from mycelial mass production assays using different aeration flow rates indicate 1.5 V.V.M. as the best flow rate with a mycelial mass concentration of 10 g/l 36 h post-inoculation (Fig. 1). The air flow rate of 1.5 V.V.M. also induced an early maximum mycelial concentration in culture. However, even this mycelial mass concentration is considered low

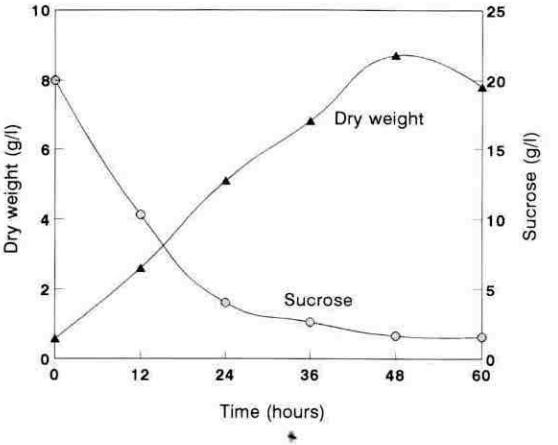

Figure 5. The consumption of sucrose and mycelial production by *Metarhizium anisopliae* during fermentation at 300 rpm and 1.5 V.V.M. (= volume of air per volume of medium per minute).

as also found by Magalhães *et al.* (1994) working at 1 V.V.M. with *M. anisopliae*. This may indicate a high demand for oxygen by *M. anisopliae*. The pH evolution during mycelial growth followed a similar pattern at the different flow rates tested (Fig. 2).

The reduction of sucrose concentration by half did not cause a significant decrease in the mycelial mass production (Fig. 3), and did not interfere with pH variation (Fig. 4). This indicates that sucrose may not be a limiting factor for *M. anisopliae* growth. Nevertheless, other studies including suppression or reduction of other medium ingredients should be conducted in order to optimize the culture medium. The consumption of sucrose 24 h post-inoculation was approximately 80% (Fig. 5) and at 60 h of cultivation the whole consumption was 99.2% with a biomass yield factor (Y.x/s) of 0.44 g of mycelium/g sucrose.

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