SUPPRESSION OF PENETRATIVE HYphaE OF Rhizopus oligosporus By MEMBrANE FILTERs IN A MODEL SOLID-STATE FERMENTATION SYSTEM

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SUMMARY

Membrane filters overlaid on slabs of a model solid substrate enabled recovery of biomass of Rhizopus oligosporus. Although the presence of the membrane filter affects the growth of Rhizopus oligosporus it provides a useful tool for studying solid-state fermentation.

INTRODUCTION

A major obstacle to the study of fungal growth in solid-state fermentation (SSF) has been the inability to measure directly the biomass because of the close association between the mycelium and the substrate. Mitchell et al. (1986, 1988a) developed a model solid substrate consisting of cassava starch and other nutrients embedded in a kappa-carrageenan gel matrix. The model substrate could be removed by dissolving the gel matrix with heat and enzymatically digesting the starch, enabling the biomass to be recovered by filtration. However, the recovery procedure resulted in the loss of significant amounts of biomass (Mitchell et al., 1988a).

The present paper describes the use of membrane filters to suppress penetrative hyphae during growth of Rhizopus oligosporus on the model solid substrate, enabling complete and rapid biomass recovery.

MATERIALS AND METHODS

Microorganism, maintenance and inoculum preparation: These were performed as described by Mitchell et al. (1986).

Membrane filter culture: The model substrate was the MZ-2N model substrate of Mitchell et al. (1988b) but the starch concentration was varied. After gelatinization the substrate was pressed into plastic dishes of 4 cm diameter and 5 mm depth. Once set, the substrate was overlaid with a wet 47 mm diameter polycarbonate Nucleopore membrane filter. A wire loop was used to spread 0.1 ml inoculum over the filter surface. The dishes were incubated at 37 °C, within a 9 l airtight plastic box to prevent excessive moisture loss. Triplicate dishes were removed for analysis. Samples of known area were cut, the membrane filter was discarded and the sample was weighed. Protein was determined by the Folin reaction and starch by digestion with amylglucosidase, as described by Mitchell et al. (1986). Cross-sections of the slab were stained with iodine (0.3% iodine plus 3% potassium iodide) and observed microscopically at 100x magnification for the clearing of starch.
RESULTS AND DISCUSSION

Effect of membrane filter pore size

Low initial starch concentrations of 5 and 15 g starch per 100 ml water were used, compared with the concentration of 25 g starch per 100 ml water in the MZ-2N model substrate of Mitchell et al. (1988b). The aim was to make any limitations due to the membrane filters more obvious.

For both initial starch concentrations membrane filter pore sizes from 0.1 to 1.0 μm had no significant effect on either the depth to which starch was cleared or the fresh weight of biomass produced after 24 h of growth of \textit{R. oligosporus}. However, in both cases the depth of clearing of starch was significantly greater in the absence of a membrane filter. Biomass production could not be compared because, without a membrane filter, the fresh biomass could not be separated from the substrate. Note that at the lower initial starch concentration some hyphae managed to penetrate through the 0.8 and 1.0 μm pores. The presence of a membrane filter therefore decreases the starch-clearing activity of \textit{R. oligosporus}. This is not due to restricted diffusion of the amylolytic enzymes through the membrane filter since pore size had no effect. Penetrative hyphae must be important. They might serve to decrease the distance over which the amylases must diffuse to reach the starch. Alternatively, firm anchorage of the mycelium by the penetrative hyphae may promote growth.

Since pore size did not affect starch-clearing activity, membrane filters of 0.2 μm pore size were used in the subsequent studies to ensure no hyphae could penetrate into the substrate.

Growth in membrane filter culture

During the growth of \textit{R. oligosporus} in membrane filter culture the model substrate nearest the biomass became cloudy as opposed to the originally translucent appearance. Since this was obviously a consequence of the growth, the cloudy and translucent regions were partitioned into separate samples. The mycelium comprised a third sample. Whole samples containing all the substrate plus the mycelium were also analyzed.

Fig. 1 shows the fresh weights of the various samples during growth of \textit{R. oligosporus} in membrane filter culture on the model substrate (MZ-2N with 25 g starch per 100 ml water). The decrease in the weight of the whole samples is due largely to conversion of the substrate into carbon dioxide. During growth the influence of the fungus on the substrate increased - the cloudy region comprised an increasing proportion of the model substrate.

Analysis of whole samples (Fig. 2) showed starch utilization was initially undetectable but later quite rapid. The glucose concentration increased slowly. A protein content of 9.9 mg per gram of sample was achieved by 38 h.

The partitioned samples gave an insight into the starch utilization. Not only did the cloudy region of the substrate increase in size as shown in Fig. 1 but also the starch concentration in this region decreased rapidly (Fig. 3). This region contained significant glucose, a product of starch hydrolysis. The translucent region decreased in size (Fig. 1) but the starch concentration only began to fall at 38 h (Fig. 3). Therefore the translucent region consists of model substrate which the amylases excreted by the fungus have not yet reached. However, the change in substrate appearance from translucent to cloudy is not necessarily a direct consequence of amylase action. The slight decrease in starch concentration in the translucent region at 38 h indicates that