THE EFFECT OF HIGH SPEED BLENDING UPON PENICILLIUM CHRYSOGENUM

H.L. Packer*, M.D. Lilly
Advanced Centre for Biochemical Engineering, Department of Chemical and Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, U.K.

* Present address: Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OXI 3QU, U.K.

C.R. Thomas
BBSRC Centre for Biochemical Engineering, School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

SUMMARY
During high speed blending of two strains of Penicillium chrysogenum, fragmentation of the mycelia was accompanied by considerable (strain dependent) biomass loss. The mean clump size decreased significantly, and the freely-dispersed hyphae became shorter and less branched on average. The homogeneity of the samples increased with duration of blending. The more recent Panlabs PI strain was significantly more resistant to blending with respect to biomass loss than NRRL 1951.

INTRODUCTION
The use of high speed blenders for breaking fungal mycelia into fragments for the production of inocula was assessed by Savage and Vander Brook (1946), who determined that 2 min was the optimum duration of blending at 10000 rpm to produce seed cultures of hyphal fragments of Penicillium chrysogenum NRRL 1951, with a mean of 4 hyphal compartments per fragment. It was determined that the fragmentation had occurred primarily at the septa and in only approximately 20% of the fragments was breakage due to the actual fracture of the cell wall. A correspondingly high viability of the fragments was observed. On the other hand, Murase and Kendrick (1986), using the same technique, found that for Chaetomium cellulyticum five seconds of blending was enough to reduce cell growth due to hyphal damage.

The intention of this study was to determine the effects of blending on the disruption and morphology of fungal hyphae, particularly mycelial clumps (Packer and Thomas, 1990; Tucker et al., 1992) using image analysis to characterize morphology.

MATERIALS AND METHODS
Penicillium chrysogenum NRRL 1951 and Panlabs PI were grown in a medium containing cornsteep liquor (Savage and Vander Brook, 1946). Spore inocula of each strain were used to inoculate duplicate 2 L shakeflasks, each containing 300 mL of medium, to a concentration of 10^6 spores mL^-1. The shakeflasks were incubated at 26°C in an orbital shaker at 300rpm, 2" throw. After 72h, 130mL samples were removed for blending in a Waring Blender (model 31BL44, Blender 8011) at high (17000rpm) or low (14000rpm) speed, for 0.5, 1, 2, 3, 4, 5 and 10 min. After blending each sample was allowed to stand (in ice) for 5 min to allow foam to subside and was then gently shaken before withdrawal of 5mL samples, two for dry weight determination and one for morphological analysis. For dry weight analysis, 5 mL of broth were filtered through a prebaked and preweighed glass microfibre filter (Whatman grade GF/A, Whatman Int. Ltd., Maidstone, U.K.). The sample was washed with 10 mL of distilled water before oven drying at 105°C to a constant weight.
Samples for morphological analysis were prepared as described by Packer and Thomas (1990), using a dilution in water of 1 in 4 for NRRL 1951 and 1 in 6 for P1. An image analyser (Magiscan MD, Joyce Loeb Ltd., Gateshead, U.K.) attached to a Polyvar microscope (Reichert Jung, Optische Werke AG, Wien, Austria) was used for morphological characterisation by the image analysis technique of Packer and Thomas (1990). Additional measurements of the sizes (detected area) of aggregates, described as clumps by Packer and Thomas (1990), were also made. The image analyser was used semi-automatically, with the analyser automatically moving the motorised microscope stage from field of view to field of view to make measurements, but with manual selection of the microorganisms to be analysed in each field of view (by touching them with a light-pen on the screen). This methodology was chosen to ensure that all the blended fragments not classified as clumps were measured, as many were very small and likely to be rejected by a fully automatic method as not being of typical fungal morphology. A magnification of 100 times was used for the measurement of P1 samples and of 125 times for the NRRL 1951 strain. For each sample 40 fields of view were analysed.

RESULTS
At the end of growth, the morphological form of P. chrysogenum NRRL 1951 was pellets whereas the P1 mycelia were dispersed. Figure 1 shows that in both cases dry cell weight per unit volume of the samples decreased rapidly with the duration of blending until a final value was reached which was no longer affected by further blending. The speed of blending only affected the period required to reach the final dry cell weight. Strain NRRL 1951 lost 78% of its initial biomass in 2 min of high speed blending whereas the P1 strain lost only 30%.

The size of individual clumps also decreased with duration of blending until a minimum size was obtained which was not affected by further blending (Figure 2). Even after 2 min the reduction in size was considerable. The pellets in strain NRRL 1951 had quickly broken down into clumps and fragments, whilst strain P1 showed a 58% reduction in clump size. The percentage of clumps in the samples is shown in Tables 1 and 2. For strain NRRL 1951, after the pellets had broken down, the maximum subsequent reduction in the percentage of clumps was only 15%.

*P. chrysogenum NRRL 1951 was pelleted before blending