

Fling, M., N. H. Horowitz, and H. Macleod. Large scale culture of *Neurospora*.

In order to obtain the large quantities of mycelium necessary for enzyme isolation, we are now growing *Neurospora* in a converted washing machine. The

machine is a top-loading automatic washer of the type which spins the water out through a single row of holes around the top of the inner tub (Westinghouse model LUC 27). The holes have been covered with a strip of stainless #30 wire mesh and the top of the inner tub fitted with a removable plexiglass cover containing two one-inch ports, one for an air line and one for introducing medium. The blades on the agitator have been cut down so that the center spindle is smooth and the blades at the bottom are about an inch high. Aeration is through a ring of heavy rubber tubing (15/16" OD, 3/8" ID) in which sixteen 0.07" holes have been drilled along the inner side. The ring rests on the bottom of the tub and fits closely to the sides of the tub. It is connected with two plastic T-tubes to two vertical lengths of rubber tubing which are secured to fittings on the underside of the plexiglass lid. This support enables the aerator to go through the spin cycle of the machine without damage. Aeration is through one of the vertical lengths of tubing which passes through one of the ports in the lid. (The air line is disconnected before spinning!) The other vertical length of tubing ends blindly.

Sterilization is accomplished by filling the tub half-full (ca. 25 liters) with hot (60°) tap-water and adding 1600 ml. of 5.25% sodium hypochlorite solution (ordinary household bleach), plus 100 ml. concentrated HCl. This brings the pH to about 7.5 and liberates chlorine gas. The solution is left in the machine for 30 minutes, with agitation. It is then spun out, the tube is rinsed once with 8 liters of sterile water and is then filled with 40 liters of sterile medium. (The medium is autoclaved in carboys and siphoned into the tub). Both aeration and agitation are used during growth. The machine's programmer is disconnected and is replaced by a timer which operates the agitator approximately half of the time (8 minutes on, 12 minutes off), thus preventing overheating of the bearings. A fan blowing across the motor prevents transfer of heat to the medium. Under these conditions, the temperature of the medium has been 22-25°. (The machine is not located in a constant-temperature room).

We have found the modified machine particularly useful for inducing tyrosinase synthesis in *Neurospora*. After 3 days' growth, we spin off the medium, wash the mycelium twice with sterile water and then re-suspend it in sterile phosphate buffer. Yield of mycelium after induction has been 300-500 grams wet weight. (The mycelium loses about 25% of its weight during induction). This work was supported by the National Science Foundation. ---Biology Division, California Institute of Technology, Pasadena, California, U.S.A.