Retting of Flax by *Aspergillus niger*

F. P. DE FRANÇA, J. A. ROSEMBERG, AND A. M. DE JESUS

Laboratory of Industrial Microbiology, School of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Received for publication 21 October 1968

In this study, retting was carried out by *Aspergillus niger*. The pH, galacturonic acid (GA), and total reducing sugar were determined; the end point was identified by the classic empirical processes and by the maximal GA content of the retting water. The process gave clear and resistant fibers, and the retting time was similar to that of current industrial processes with bacterial enzymes. Control of total acidity was not required, since the pH remained close to neutrality throughout the entire process.

The pectic enzymes produced by molds have been studied by Schroeder and Mueller-Stoll (8), Arima, Yamasaki, and Yasui (3), and Akira (1). The present investigation was motivated by the idea of using the pectic enzymes of fungi, rather than bacteria, in the retting process to produce linen. The mold *Aspergillus niger*, whose enzyme system we had previously studied, was chosen from our culture collection.

MATERIALS AND METHODS

Microorganism. *A. niger* strain EQ-29 was used.

Preparation of stalks. For each of the three experiments, 30 g of linen straw (*Linum usitatissimum*), middle type, was used. The roots were first removed, and the straws were cut to a length of 310 mm. The cut straws were then weighed and sterilized at 1 atm for 30 min. The sterile straws were placed in sterilized Pyrex trays (330 by 210 by 40 mm) and were washed in an aseptic chamber with 1 liter of sterile water for 90 min. The wash water was then replaced with 740 ml of sterile water.

Inoculum. Test tubes (220 by 22 mm) with 6.8 g of sterile straws, 160 mm in length, were inoculated with 10 ml of a spore suspension of *A. niger* prepared from growth on Czapek agar at 25 C for 8 days. The straws were permitted to over-ret and the organism was permitted to sporulate. Spores were suspended in 20 ml of sterile water, and the suspension was used to inoculate the straws that had been placed in the trays. Incubation was at 25 C.

Control procedures. Every 24 hr, a 25-ml sample of retting water was taken from each tray. The initial volume was kept constant by the addition of 25 ml of sterile water. Samples were clarified by filtration in the presence of activated charcoal, and the following measurements were made. To determine free acidity, we used 1 ml of 0.1 N NaOH per 100 ml of retting water with phenolphthalein as the indicator. For pH determination, a type PHM-22p potentiometer, radiometer was used. To determine the galacturonic acid (GA) content (mg) per 100 ml of retting water, we used the Kapp (5) method (see Fig. 1). Total reducing sugar (TRS) plus GA was measured as milligrams per 100 ml of retting water (see Fig. 2). The amount of TRS (mg) per 100 ml of retting water was determined by the Somogyi method (9). Since GA has reducing properties and is used in the determination of TRS, the quantity of TRS present in the retting water was obtained by subtraction (see Fig. 3). Retting observations were made in accordance with the empirical test for fiber discharge determination, with the Greenhill and Conchman method (4).

RESULTS AND DISCUSSION

There was no increase in the total acidity of the retting water throughout the entire process, with the pH remaining essentially constant at 7.0. This observation contrasts with an increase in total acidity obtained during bacterial retting.

The increase in GA began within 24 hr and reached a maximum at 72 hr (Fig. 1); at this time, the empirical test showed the fibers to be free and strong. After 72 hr, when retting was complete, GA began to be utilized by the microorganism, indicating that the pectin was completely hydrolyzed. In 96 hr, the fibers were weak and over-retted; but GA continued to be utilized until 168 hr, the end of the observation period. This increase in GA paralleled the accumulation of TRS in the retting water until 48 hr, when the level of TRS started to decrease and reached a minimum in 72 hr (Fig. 3). It should be noted that there was no difference in the rate of utilization of pectin and TRS during the under-retted phase. At approximately 96 hr, the concentration of TRS increased, probably due to the hydrolysis of polysaccharides. The mold, at this time, continued, to utilize GA. At some point between 96 and 168 hr,

---

1 Fellow of Research Council, Federal University of Rio de Janeiro, Brazil.
both of these substances were utilized equally; but TRS was depleted faster than GA, as indicated by the 168-hr determination. Retting was complete in the three experiments in about 72 hr, as determined by empirical tests and by the GA content (7). This retting time is similar to that of the industrial processes now in use with bacteria and takes 24 hr less than the natural process. Clear, clean, strong fibers were obtained.

This study shows the presence of pectic enzymes in A. niger and confirms the findings of Arima, Yamasaki, and Yasui (3), who found these enzymes when A. niger was grown on a potato substrate. This investigation also supports the work of Allen (2), who studied the microbiology of dew retting and considered Rhizopus, Aspergillus, Penicillium, and Cladosporium to be the responsible organisms.

It is possible that the industrial production of linen fibers could be accomplished by means of an aerobic retting process with A. niger. It should be noted that Marroquin (6) has assumed that, in the natural aerobic process with molds, inferior fibers would be produced. In our experiments, this was not true. The principal commercial process uses Bacillus comessi (Rossi method), and operational steps must be used to prevent increased acidity during retting. Other industrial methods, i.e. those employing B. macerans, present the disadvantage of producing high acidity, which requires subsequent neutralization. Our proposed method with A. niger is conducted at a neutral pH throughout and does not require the control of total acidity.

LITERATURE CITED
