Production of D-Mannitol by Conidia of
Aspergillus candidus

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Conidia of Aspergillus candidus converted glucose and other sugars to mannitol.
Low pH (ca. 3.0) apparently favored the percentage yield but decreased the fermentation rate.

This work was part of an investigation of the metabolic activities of microbial spores. Previous reports have concerned the saccharification of starch by Aspergillus wentii (2) and the inversion of sucrose by A. oryzae (1, 4). Smiley et al. (6), also of this laboratory, reported the formation of D-mannitol from D-glucose by A. candidus mycelium in shaken flasks and in 20-liter fermentors; they obtained a 53% yield in 7 to 9 days and 69% in 10 to 12 days. We have found that conidia of

A. candidus can produce mannitol from 1 or 2% (w/v) initial glucose in about 75% yield (based on sugar consumed) in 7 days.

Conidia of A. candidus NRRL 305 were grown on cracked corn. Approximately 3 x 10^11 spores per Fernbach flask were recovered after 1 to 2 weeks of incubation. Repeated washing and centrifuging (~8 washings) gave spore suspensions substantially free of nutrients. Most of the fermentations were carried out in indented 300-ml Erlenmeyer flasks containing 50 or 60 ml of glucose or other sugar in water or in 0.2 M phosphate buffer, with no other nutrients. Enough spore suspension was added to bring the spore concentration to the desired level (usually 5 x 10^6/mL). The flasks were incubated on a rotary shaker at 250 to 300 rev/min. Mannitol was estimated by the periodate oxidation method of Lambert and Neish (3) supplemented by thin-layer and gas-liquid chromatography. Reducing sugar was determined by Nelson’s arsenomolyb-

date modification of the copper-reduction method Somogyi (5, 7).

Table 1 exhibits the averaged results at 165 hr of two experiments with a series of 0.2 M phosphate buffers in the pH 2 to 8 range and 1% glucose at 28°C. Adjusting pH to the original levels was done with 2 M NaOH when necessary. Sugar consumption was most rapid at pH 6 where the supply was substantially exhausted at 144 hr. At pH 4, the rate was over half the maximum, but there was a sharp decline between pH 3.0 and 4.0. Even at pH 2.0, fermentation was not

![Fig. 1. Effect of temperature on mannitol formation with 2% initial glucose.](http://aem.asm.org/)

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**Table 1. Effect of pH on mannitol production**

<table>
<thead>
<tr>
<th>pH</th>
<th>Glucose consumption rate (mg per ml per hr)</th>
<th>Mannitol produced (mg/ml)</th>
<th>Yield (%)</th>
<th>Glucose consumption rate (mg per ml per hr)</th>
<th>Mannitol produced (mg/ml)</th>
<th>Yield (%)</th>
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</thead>
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</table>

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1 Presented at the 71st Annual Meeting of the American Society for Microbiology, Minneapolis, Minn., 2-7 May 1971.
completely inhibited, the rate being roughly one-tenth of the maximum.

The 24-hr samples were obtained on only one of the experiments, and the values are hence less reliable. However, they do show that the initial average glucose consumption rates were much higher than those at 165 hr. The decrease in yields at 165 hr is more marked at higher pH levels. This could mean that the mannitol-forming enzymes are less active at high pH as the sugar concentration declines or that mannitol is differentially utilized in a pH-dependent system.

Figure 1 shows the effect of temperature. Experiments at different temperatures were carried out in solutions containing 1 and 2% glucose; the 2% glucose series is shown in this figure. Mannitol production rates increased with temperature up to 45 C. However, sugar consumption ceased at 40 C in 5 days with half the sugar (1%) left; at 45 C, this stoppage occurred in 3 days. The cessation of sugar consumption was not due to the low pH since it has been shown to take place at higher pH in other experiments; evidently it was due to thermal inactivation of the spores. At 50 C sugar consumption ceased after the first day; mannitol formation ceased on the second day, as shown on the 50 C curve in Fig. 1.

Figure 2 represents an experiment with different initial glucose concentrations, from 4 to 80 mg/ml, run at 30 C (300 rev/min). This series was carried out in phosphate buffer (0.2 m, pH 6.8). Mannitol accumulation continued until the glucose was substantially exhausted. Average sugar consumption rates were nearly equal, regardless of the initial concentration. Mannitol percentage yields were low, having apparently fallen off when the sugar neared exhaustion. In the highest initial concentration, where ample sugar remained, the mannitol yield was 48%.

In experiments at lower pH (3.0 to 3.5) comparing 1% with 2% initial glucose (28 C, 250 rev/min), the mannitol formation rate with 2% sugar was higher at first than with 1%, but at 117 hr the production curves had become nearly parallel. The yields at 165 hr were 74 and 76%.

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LITERATURE CITED