



*Some Observations on Fermentations by Yeasts in the  
Presence of Azide\**

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W INZLER (1944) has shown that the theoretical yield of 2 mols of carbon dioxide per mol of glucose was obtained when *Saccharomyces cerevisiae* fermented glucose in the presence of  $10^{-3}$  M. sodium azide. In the absence of sodium azide variable yields of 70–90 per cent are normally obtained (Fales and Baumberger, 1948). The mechanism of the action of sodium azide has been discussed by Reiner and Spiegelman (1947).

The present communication deals with similar observations on *S. bayanus* (NRRL 966), *S. carlsbergensis* (NRRL 379), and *S. fragilis*. These organisms were selected because they are used in this laboratory in a differential fermentation method for the quantitative estimation of glucose-galactose-lactose mixture.

EXPERIMENTAL

*S. BAYANUS* WAS GROWN in the following medium: 3 gm. Difco yeast extract, 5 gm. Difco peptone, 3 gm. Difco malt extract, 40 gm. glucose, 10 mg. inositol, water to 1 liter.

*S. carlsbergensis* was grown in a broth of similar composition with omission of inositol and substitution of galactose for glucose.

*S. fragilis* was grown in the following medium: 1 gm. Difco yeast ex-

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tract, 2 gm.  $(\text{NH}_4)_2\text{SO}_4$ , 1 gm.  $\text{KH}_2\text{PO}_4$ , 0.3 gm.  $\text{MgSO}_4$ , 0.1 gm.  $\text{CaCl}_2$ , 40 gm. lactose, water to 1 liter.

The yeasts used for the fermentation experiments were 24–48 hour cultures grown in 250 ml. centrifuge bottles. After centrifuging and washing three times with distilled water (centrifuging each time), the organisms were suspended in a small amount of distilled water. The concentration of yeast was estimated by measuring the turbidity of the suspension with a Klett photoelectric colorimeter (No. 42 filter).

The sugars used in the fermentation experiments were commercial C.P. samples checked for purity by measuring the optical rotation and recrystallized where necessary.

Fermentations were done in the Warburg manometric apparatus at 30° C. Into the body of the flasks were introduced 1.0 ml. of a solution containing 0.5 mg. sugar, 1.0 ml. of 0.15 M. monosodium succinate, and 0.5 ml.  $6 \times 10^{-3}$  M. sodium azide. Into the side arm was pipetted 0.5 ml. of a suspension containing 50 mg. yeast per milliliter. After equilibration with nitrogen, and after thermal equilibrium was attained, the contents of the side arms were tipped into the body of the flasks and readings of  $\text{CO}_2$  evolution taken at appropriate intervals until fermentation ceased. The volumes of  $\text{CO}_2$  recorded in Table 1 have been corrected for the controls without sugar representing the endogenous respiration of the yeast.

Table 1.—Fermentation of sugars in the presence of azide by *Saccharomyces* spp.

Organism	Sugar	$\text{CO}_2$ <i>micro liters</i>	Percentage of theoretical	Min.*
<i>S. bayanus</i> .....	glucose	128	103	45
	galactose		not fermented	
	lactose		not fermented	
<i>S. carlsbergensis</i> .....	glucose	127	102	14
	galactose	122	98	210
	lactose		not fermented	
<i>S. fragilis</i> .....	glucose	131	106	145
	galactose	35	28	120
	lactose	96	78	210

\* Duration of time at which  $\text{CO}_2$  evolution ceased.

#### DISCUSSION AND SUMMARY

THE RESULTS PRESENTED in Table 1 indicate:

a) All three strains of yeast tested produced quantitative yields of CO<sub>2</sub> from glucose fermentation in the presence of azide but the time required was appreciably different in each case.

b) *Saccharomyces carlsbergensis* could be used in a manometric determination of galactose.

c) Azide appears to inhibit the fermentation of galactose, and of lactose to a lesser extent, by *S. fragilis*. Both of these sugars are fermented completely in 48 hours in the absence of azide.

Result (a) might have been anticipated from the fermentation rates in absence of sodium azide and from the work of Spiegelman, Reiner, and Cohnberg, 1947. Result (b) might also have been anticipated (Spiegelman, Reiner, and Cohnberg, 1947, and Spiegelman and Reiner, 1947) although it has not been explicitly stated previously. Result (c), however, was somewhat unexpected and again emphasizes the singularity of lactose-fermenting organisms. A further intensive exploration of this phenomenon might help to elucidate the details of lactose fermentation in yeasts.

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