Effects of Oxygen and Acetoin on Fermentation in Yeasts

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In contrast to the Pasteur effect, found in many yeasts, a negative Pasteur effect is observed in the fermentation of glucose by yeasts belonging to the genus *Brettanomyces* (Custers, 1940; Wikén et al., 1961).

The Pasteur effect is generally regarded as an inhibition of fermentation by respiration in consequence of a shortage of ADP.

The negative Pasteur effect, i.e. the inhibition of fermentation under anaerobic conditions, is attributed to a shortage of NAD (oxidized form), which is required for the oxidation of phosphoglyceraldehyde; this inhibition can be abolished by O_2, which oxidizes $NADH_2$ via the respiratory chain, or by certain carbonyl compounds, able to oxidize $NADH_2$ enzymatically (Scheffers, 1961). As soon as the initial shortage of NAD, which may be brought about by the activity of various redox systems in the cell after addition of glucose, is overcome by the action of O_2 or a carbonyl compound, dehydrogenation of phosphoglyceraldehyde can take place and consequently acetaldehyde is produced, which reoxidizes the $NADH_2$ formed in the dehydrogenation of phosphoglyceraldehyde.

In Warburg experiments with *Brettanomyces* spp., addition of the carbonyl compound acetoin (1 mM) stimulates the anaerobic fermentation of glucose to rates beyond those of the aerobic fermentation (in air). Also, in presence of low concentrations of O_2 (ca. 0.1%), the rate of fermentation may surpass the rate in air. Apparently, at certain low O_2 tensions, the anaerobic inhibition of fermentation is overcome by oxidation of $NADH_2$ via the respiratory chain, whereas the depressing effect of respiration on fermentation, known as Pasteur effect, does not yet manifest itself to the full extent. It is concluded, that in *Brettanomyces* spp. the normal Pasteur effect is obscured by the negative Pasteur effect, but may become evident after the anaerobic inhibition of fermentation is abolished by addition of O_2 or acetoin in low concentrations.

On the other hand, acetoin increases the rate of anaerobic fermentation in a number of yeasts showing a normal Pasteur effect, e.g. in species belonging to the genera *Endomycopsis*, *Pichia*, *Hansenula*, and *Torulopsis*. Low concentrations of O_2 may have a similar effect. It is concluded, that in these yeasts a partial inhibition of fermentation by anaerobic conditions is masked by the normal Pasteur effect.

Finally, in many yeasts, e.g. in species belonging to the genera *Saccharomyces*, *Hanseniaspora*, *Candida*, and *Kloeckera*, an initial inhibition of anaerobic fermentation is found, which is overcome after a short period of time even without addition of O_2 or acetoin. Apparently, in these cases the yeast itself is able to reoxidize $NADH_2$ via a side reaction. By addition of low concentrations of O_2 or acetoin, this lag phase in anaerobic fermentation is eliminated.

In summary, it is concluded that in one and the same yeast, fermentation may be inhibited, on the one hand, by higher O_2 tensions leading to a shortage of ADP as a consequence of respiratory chain phosphorylation; on the other hand, by anaerobic conditions leading to a shortage of NAD. The ratio between both inhibitions determines, whether a normal or a negative Pasteur effect is observed.

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Fixation of Molecular Nitrogen by an Aerobic *Vibrio* or *Spirillum*

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In studying the distribution and ecology of *Beijerinckia* in soils (Becking, 1959, 1961a and b), *Spirillum* and *Vibrio* forms were regularly observed in the "N-free" enrichment cultures. The organism was in all probability identical with Beijerinck's (1925) *Spirillum lipoferum* (also *Azotobacter spirillum* Beijerinck, 1922) for which N_2 fixation was at first claimed in partially pure culture, although this statement was later withdrawn. Schröder (1932), using single cell cultures, failed to find N_2 fixation.

In the present study, cells of an isolate exposed to an atmosphere of labelled N_2 gas showed significant N^{15} incorporation. With 43 atom % N_2^{15}, the cells contained 0.43 atom % N^{15}, with 65 atom % N_2^{15} the labelling of the cells increased to 1.04 atom % N^{15}.

Hydrogenase activity could not be detected with methylene blue ($E_0^1 = +0.011$ V) as H-acceptor; this activity was demonstrated only with benzyl ($E_0^1 = -0.359$ V) and methyl viologen ($E_0^1 = -0.446$ V). N^{15} experiments showed that H_2 gas inhibited, but did not entirely repress, N_2 fixation. With D_2 gas a rapid H-exchange occurred, revealing hydrogenase activity in the absence of exogenous artificial dyes. Measurements of D_2 exchange in A and N_2 atmospheres showed that N_2 inhibited H-exchange, which once again proved the close relationship between nitrogenase and hydrogenase.

Classification of the organism is difficult. In N-poor, sugar-rich media the slightly curved rods ($2 - 4 \times 1\ \mu$) are completely filled with poly-β-hydroxybutyrate. In broth and peptone media, however, the cells are elongated and spiral-shaped, lacking polymer inclusions. The cell has a single flagellum (characteristic of *Vibrio*) and is probably related more closely to *Desulfovibrio gigas* (personal communication by Le Gall) than to *Spirillum*. The organism is, however, strictly aerobic, although in deep liquid layers accumulation of the cells at some distance from the surface ("*Spirillum* respiration figure") was observed. N_2 fixation did not occur in N-free medium, but only in the presence of 0.01 — 0.005% Difco Yeast extract. The growth factor required was not vitamin B_{12}, biotin, or pyridoxine.

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