

Descriptive – stages of fermentation:

Start of fermentation – inoculate at 10^6 cells/mL *Sacch. Cerevisiae*

(Wild yeasts *Klechera*, *Hanseniaspora*, *Candida*, *Pichia*, *Hansenula* may be present)

Yeast growth cycle 3 principle phases:

- Limited growth phase (2-5 days) $10^6 \rightarrow$ between 10^7 and 10^8 cell/mL
- Stationary phase for about 8 days where cell population remains more or less constant
- Death phase where viable population drops to 10^5 cell/mL, can last several weeks

Growth limited to 4-5 generations throughout. Stopping of growth not result of disappearance of energy nutrients (ie decrease in sugar). Death phase is always much longer than the growth phase. Fermentation speed is at its maximum (and practically constant) for about 10 days (corresponds to first 2 stages). Fermentation then progressively slows through the next few weeks, when the yeast population is in the survival phase. Stopping of fermentation is not simply result of insufficient yeast growth. Metabolic activity of non-proliferating cells can also be inhibited.

Where sugar is $<200\text{g/L}$, fermentation entirely or almost entirely complete after first 2 stages. Rapid and problem free.

Elevated sugar – yeast population is continuing fermentation while in death phase, where metabolic rate is decreasing. Fermenting to dryness under these conditions will depend on survival capacity of yeast population (stress factors will be a big influence here).

In fermentation mixtures of glucose and fructose, glucose will be consumed more quickly than fructose. This is thought to be due to differences in kinetic properties of the sugar transporters for the two different sugars. Becomes important towards the end of fermentation when all glucose is gone and only the (sweeter) fructose remains. Some yeasts utilise glucose and fructose at similar rates, (just glucose faster), some prefer to consume almost all the glucose before fructose begins to be utilised.

Lack of oxygen is not only reason that yeast catabolise glucose via fermentation rather than respiration – the sugar concentration is also a factor. At sugar $>2\text{g/L}$ fermentation is favoured

over respiration even in the presence of oxygen. This is thought to be due to a combination of reasons, respiration may require a greater range of enzymes to be efficient, and while respiration yields far greater energy (than fermentation) it also requires much greater inputs of energy. The Pasteur effect: inhibition of fermentation by oxygen. Oxygen availability will foster respiration over fermentation as an energy-generating pathway only if sugar levels are low and if some other nutrient such as nitrogen is limiting. Aeration of grape juice ferments will not result in respiration as sugar concentrations are too high, even in the stationary phase when nitrogen levels are low.

Aeration of grape juice will stimulate yeast growth but this is not due to respiration.

In addition, production of ethanol by fermentation favours *Saccharomyces* species as it is relatively ethanol tolerant. In the presence of air, *Saccharomyces* can also utilise ethanol as a carbon source which may become preferable in a low sugar environment.

Other end-products:

Glycerol, volatile and non-volatile organic acids:

Organic acids produced as a result of limited TCA cycle activity