

2 chemical reactions

fermentation & respiration (colony size governs the switch)

- In *Saccharomyces cerevisiae*, fermentation → major pathway for energy production (even under aerobic conditions)
- but when glucose scarce, ethanol produced during fermentation → used as carbon source, requiring a shift to respiration (glucose + oxygen → CO₂ & ATP)

Key results:

***overall maximum CO₂ production rate ($V_{\max}$) → not related to the maximum CO₂ production rate per cell**

***highly significant correlation between $V_{\max}$ & maximum population size observed in all three media**

***human selection targeted → efficiency of cellular reproduction rather than metabolic efficiency**

Fermentation — glucose remains in cytosol → converted to CO₂ & ethanol

results from interaction numerous genetic, metabolic, and environmental parameters

- high rate of fermentation can be reached in two ways: enhancing the rate of fermentation per cell (i.e., high CO₂ rate per cell) and/or enhancing the number of cells per population
- relationship between fermentation kinetics & population dynamics
- focused on AF kinetics & main products of AF quantified at end of process
- for population level — measured “life history traits” → reflect population kinetics and have been found to be related to yeast evolution
- analyze the relationships between AF kinetics parameters, metabolic traits, and life history traits during alcoholic fermentation;

*nitrogen is the most important macronutrient for yeast after sugar

3 synthetic fermentative media were used—> differed in their levels of **sugar and nitrogen**, pH, osmotic pressure, and anaerobic growth factors

Fermentation Kinetics

*CO₂ released was determined by automatic measurement of glass reactor weight loss every 20 min

Alcohol Fermentation Time

—> The lag-phase time —> the time between inoculation and the beginning of CO₂ release (CO₂ production rate higher than 0.05 g liter⁻¹ h⁻¹)

—> End of fermentation —> when the CO₂ production rate dropped below 0.05 g liter⁻¹ h⁻¹.

- allowing measurement to AF time necessary to ferment sugar in the medium
- $V_{\max}$ (g liter⁻¹ h⁻¹) = maximal CO₂ production rate
- CO₂tot was the total amount of CO₂ released at the end of the fermentation (g liter⁻¹)

Population

- population growth and the cell size were monitored (more than 20 samples per fermentation taken from time of inoculation until carrying capacity K reached)

$$N_t = \frac{K N_0 e^{rt}}{K + N_0(e^{rt} - 1)}$$

N_t is the population size at time t , K is the carrying capacity (cells per ml), N_0 is the initial population size, and r is the intrinsic growth rate (number of divisions per hour).

- growth recovery (%) (parameter is usually assimilated as an indirect measure of cell viability in microbiology (62)).

- cell size measured using particle counter

CO₂-specific flux (the CO₂ production rate per cell)

ethanol concentration

residual glucose

acetic acid production

For the analyses of variance (ANOVAs), we considered a derived variable, ethanol/glucose ratio (in mol/mol), to compare ethanol yields.

External glycerol (g liter^{-1}) and residual nitrogen (g liter^{-1}) were assayed by the enzymatic method

residual nitrogen was used to determine nitrogen consumption (%).

During alcoholic fermentation, the principal carbon flux goes from the assimilable carbon source (glucose in this work) to ethanol.

Thus, to investigate glucose allocation to the main fermentation products, we expressed ethanol, acetic acid, and external glycerol in glucose equivalents (mol liter^{-1}), taking into account that 1 mol of glucose produced 2 mol of ethanol, 2 mol of acetic acid, or 2 mol of glycerol.