Off-gas analysis of *Komagataella phaffii* on different carbon sources

Kuhner SHAKER

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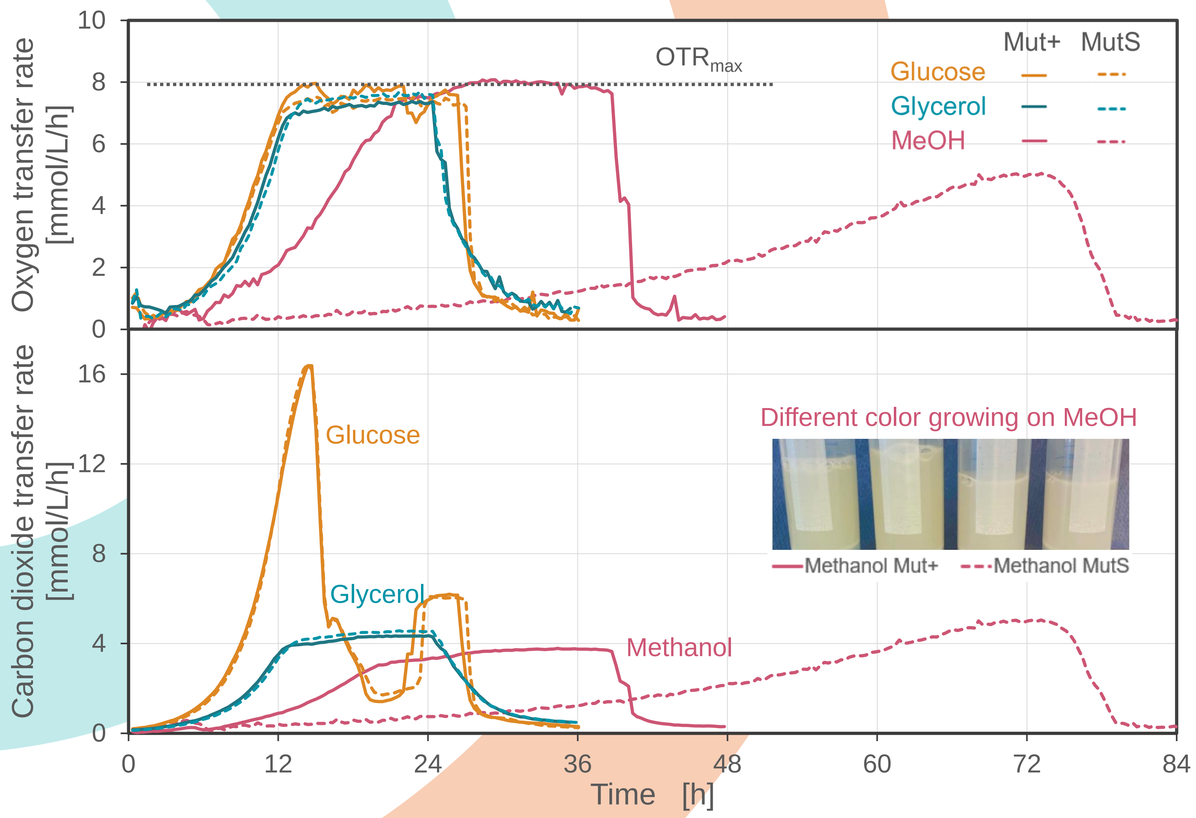
Introduction / Abstract

Two different *K. phaffii* strains were grown in batch culture on glucose, glycerol and methanol as sole carbon source (1%w/v) in shake flasks. Two of the key enzymes of the methanol utilization pathway (Mut) are the alcohol oxidases 1 and 2. In this study, a Mut$^+$ strain expressing both, *AOX1* and *AOX2*, and a Mut^S (Methanol utilization slow) strain only expressing *AOX2* were compared [A]. Growth physiology was analyzed with a Kuhner TOM System, measuring oxygen transfer rate (OTR), carbon dioxide transfer rate (CTR) and respiratory quotient (RQ).

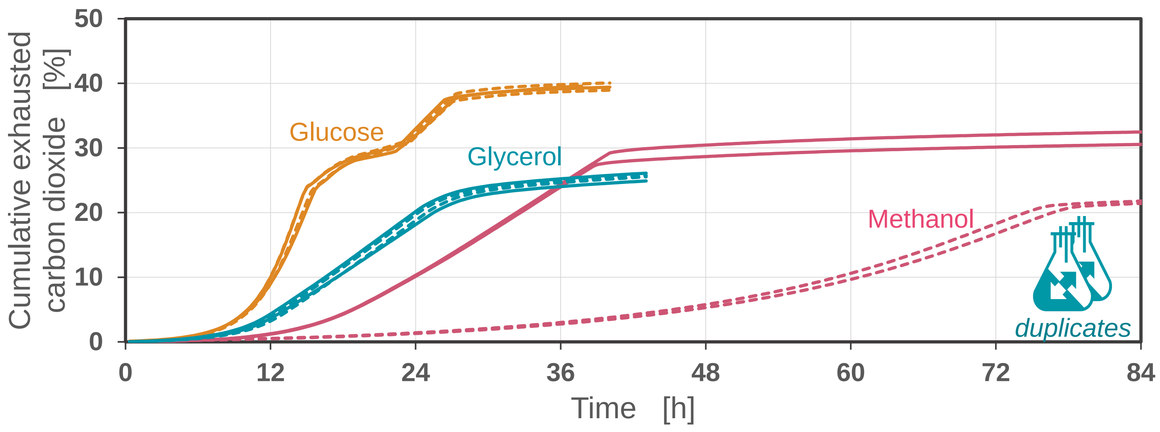
Batch growth on different C-sources



K. phaffii, minimal medium (1% w/v carbon source), n = 110 rpm, V_{flask} = 250 mL (baffled), V_L = 50 mL, d_0 = 25 mm, T = 28°C, biological duplicate measurements shown as average

Growing on **glucose** and **glycerol**, no obvious difference in respiratory activity can be found between Mut$^+$ and Mut^S strains.
Also, the OTR pattern is similar for both carbon sources as the cultures become oxygen limited after approx. 12h. Small differences in OTR$_{max}$ may occure due to different oxygen solubility. However, CTR indicated several phases of overflow or byproduct formation and consumption, e.g. ethanol, when growing on glucose as carbon source. On glycerol, no different physiological states were obvious.
Growth on **methanol** exhibited differences in growth rate and total cultivation duration. Expressing both *AOX1* and *AOX2*, the Mut$^+$ strain showed a higher growth rate and even entered oxygen limitation after 20h.

Carbon source utilization



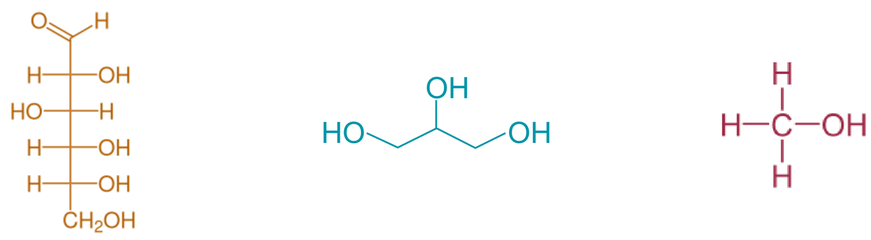
All cultivations started with a similar concentration (w/v) of carbon source. The final OD$_{600}$ was higher on glycerol than on glucose. Hence, more carbon was used for biomass formation, agreeing well with less carbon being exhausted. The theoretical methanol carbon balance may vary due to possible methanol evaporation [B]. Therefore, the slow growing Mut^S ended up at the lowest cumulative exhausted carbon.

Carbon source	Final OD$_{600}$ [-]	Total carbon in medium [mM]	Exhausted carbon [mM]	μ (OTR) [h^{-1}]
Glucose Mut+	10.4	333	131	0.316
MutS	10.2		131	0.322
Glycerol Mut+	17.4	326	84	0.277
MutS	17.2		85	0.281
MeOH Mut+	4.9	312	100	0.177
MutS	5.0		71	0.047

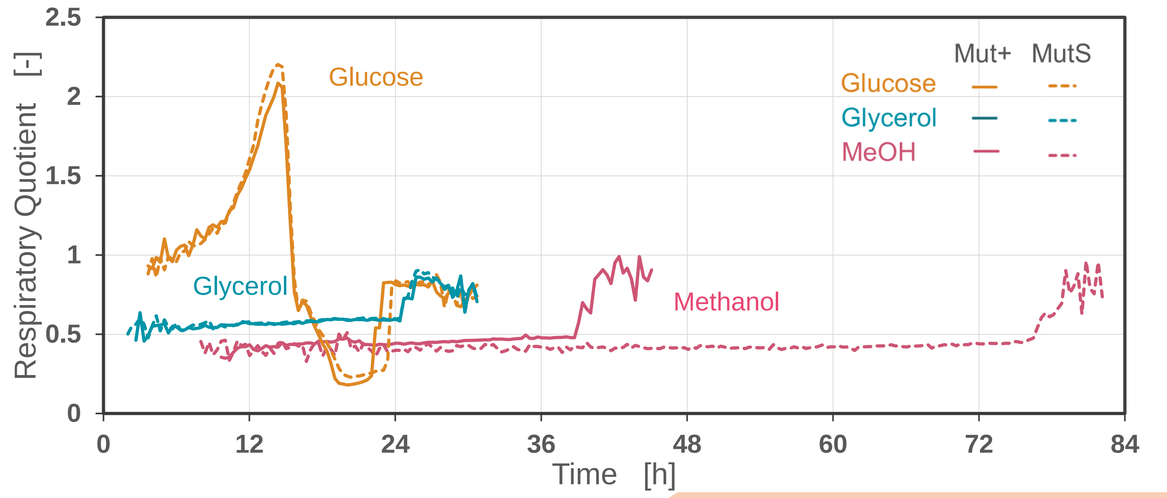
Reaction stoichiometry

The theoretical respiratory quotient (RQ) was calculated based on literature biomass yield coefficient and elemental biomass composition, whereas carbon, nitrogen, hydrogen and oxygen were balanced [C].
The measured and the theoretical RQ matched for glucose and glycerol consumption. In contrast, the measured RQ for MeOH consumption was significantly lower than the theoretical value, potentially indicating protein formation or a higher biomass yield. For methanol, RQ values below the theoretical value were also reported in literature before [D].

$$RQ = CTR/OTR$$



$$C\text{-}source + \alpha O_2 + \beta NH_3 \leftrightarrow \gamma CO_2 + \delta Biomass + \varepsilon Product + \zeta H_2O$$



Average respiratory quotient of biological duplicates. K. phaffii cultivated in minimal medium (1%w/v carbon source), n = 110 rpm, V_{flask} = 250 mL (baffled), V_L = 50 mL, d_0 = 25 mm, T = 28°C

Both strains showed similar growth patterns and respiratory quotients when grown on the same C-source.

Carbon source	Yx/s (literature) [g/g]	Calculated RQ (from Biomass yield) [-]	RQ in exponential growth [-]
Glucose	0.56 [B]	1	0.90-1.10
Glycerol	0.6 [C]	0.57	0.56-0.60
Methanol	0.14 [C]	0.62	0.41-0.48

Conclusion

With Kuhner TOM, different growth behaviour of *K. phaffii* on different carbon sources was investigated successfully. Respiration of duplicates was very reproducible and differences in RQ could be well resolved.
On glycerol and glucose, Mut+ and MutS strains behaved similar.
Glucose consumption seemed to lead to by-product formation (such as ethanol, acetate, …) and needs to be confirmed by HPLC.
On MeOH, MutS grew much slower than Mut+ and the measured RQ was lower than the theoretical value, maybe due to protein formation. Especially in Mut$^+$ strain AOX1&2 can make <30% of total soluble protein under solely methanol fed conditions. Possibly part of MeOH evaporated in Mut^S strain cultivation due to slow metabolism because lacking *AOX1* gene.

References:
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