# Online EtOH measurement in shake flask

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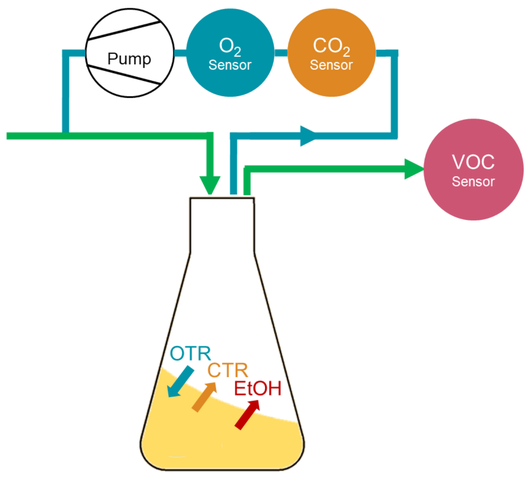
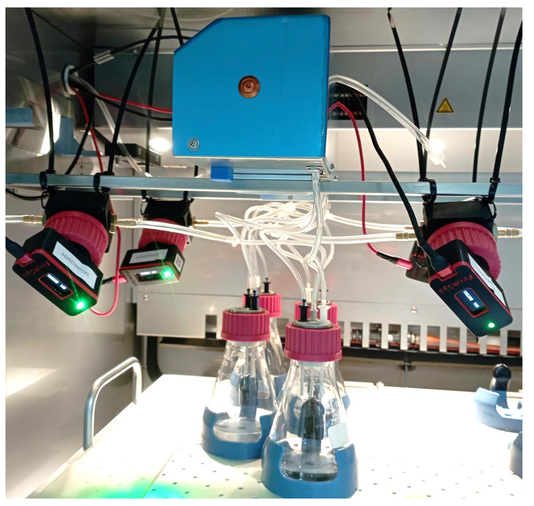
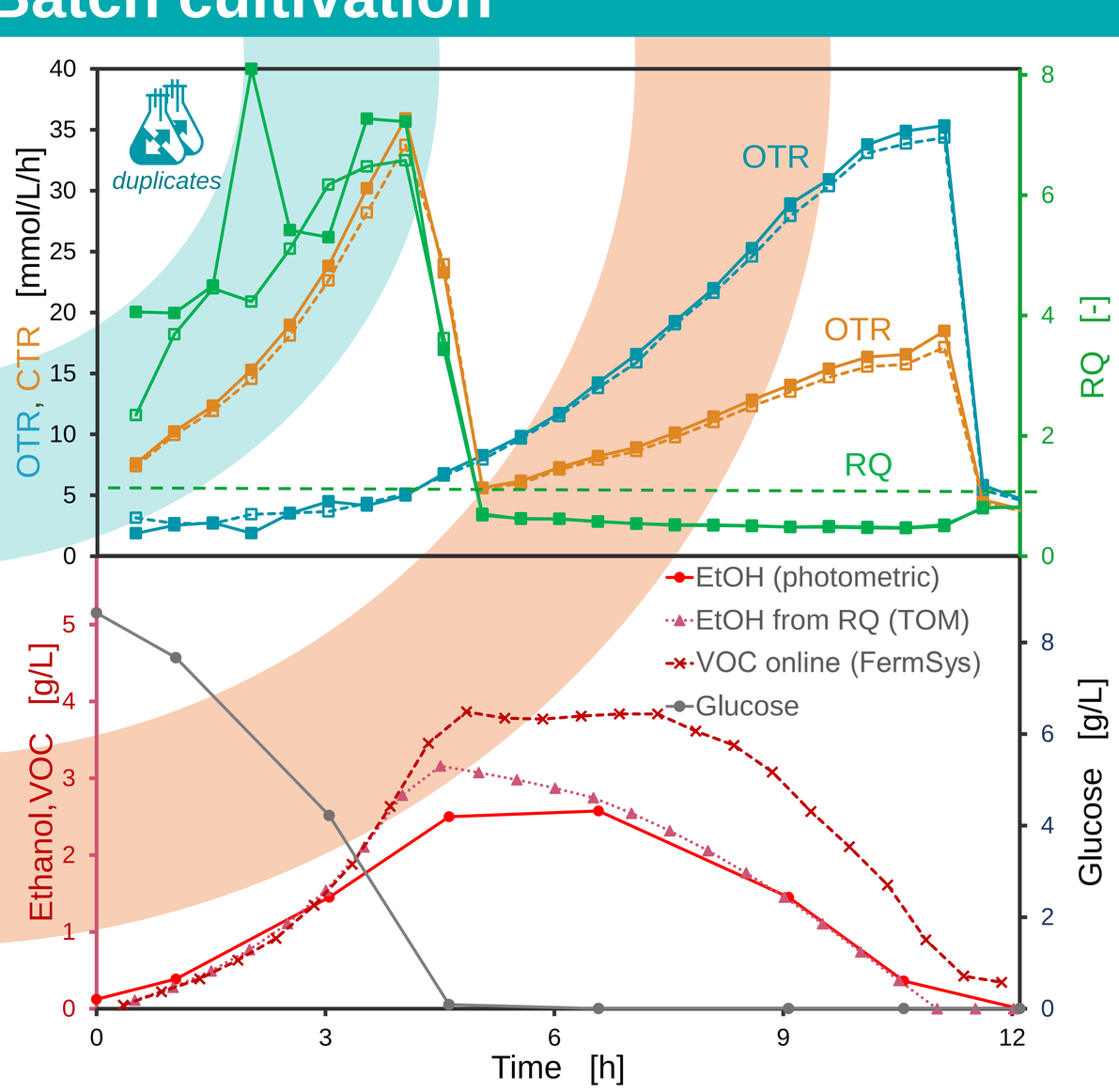
## Introduction

In yeast fermentations, ethanol formation is a common phenomenon. Depending on the process it is seen as desired product or undesired overflow metabolite or byproduct. Crabtree negative yeasts (e.g. *P. pastoris, H. polymorpha*) usually form ethanol under oxygen limiting cultivation conditions while crabtree positive yeasts (e.g. *S. cerevisae)* can also form ethanol when sufficient carbon source is present in the culture medium. In this study we online-monitored ethanol formation in *S. cerevisiae* shake flask cultivations via off-gas analysis (Kuhner TOM) and online VOC sensors (FermSYS).



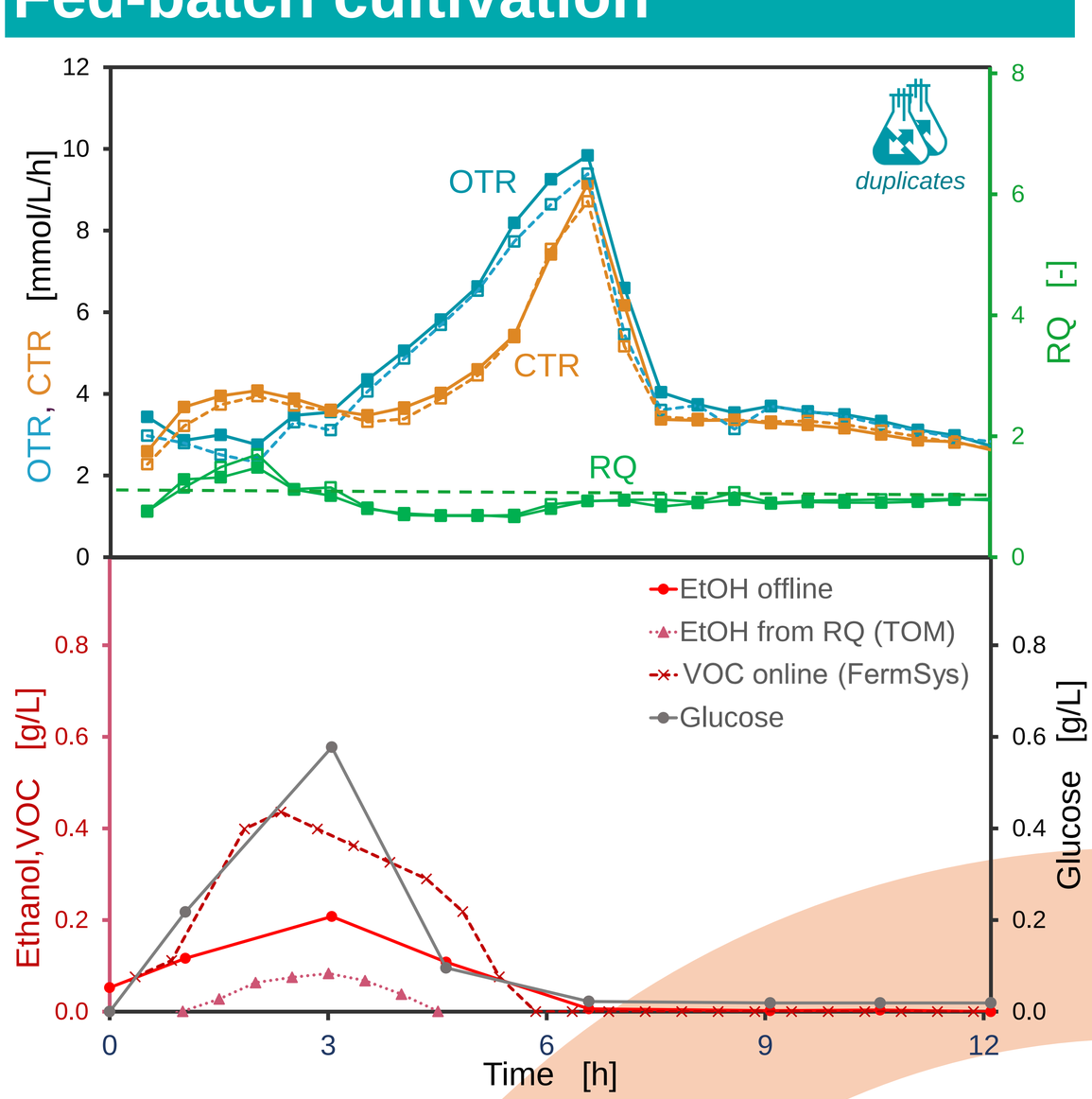
## Batch cultivation




Batch cultivation, *S. cerevisiae*, YNB-medium (10 g/L glucose), 250 mL flask, 25 mL filling volume, 30°C, 320 rpm, 50 mm shaking diameter

Batch cultivations were performed with 10 g/L initial glucose concetration. Excess of glucose leads to formation of EtOH as observed via online VOC sensors (**v**olatile **o**rganic **c**ompounds), stoichiometrical analysis and an offline photometrical method. Both online methods are yielding comparable but slightly higher EtOH titres, indicating that (A) organic compounds other than EtOH may have been formed (e.g. acetate) that have an effect on sensor output and carbon balance or (B) sensor calibration and stoichiometrical model assumptions may need to be revised.

## Fed-batch cultivation




Fed-batch cultivation, *S. cerevisiae*, YNB-medium (without glucose), 250 mL flask, 25 mL filling volume 30°C, 320 rpm, 50 mm shaking diameter, 2 x FeedBead (high release, 12mm)

Fed-batch was realized using FeedBeads. FeedBeads are small silicon discs that releases glucose at a constant rate to imitate Fed-batch conditions on small scale. A short phase in the beginning of the cultivation is observed where minor amounts of glucose accumulate and ethanol is formed to a very low extent. RQ is close to 1 throughout the cultivation. EtOH formation and consumption shows the same trend for all three methods even at small titers.

## Reaction stoichiometry

Ethanol formation and consumption was estimated via measurement of comsumed oxygen and produced carbon dioxide

### Growth

$\mathrm{RQ} = 0.98 \quad Y_{X/S} = 0.4$ (for growth only)

$$1\ Glucose + 3.2\ O_2 + 0.46\ Amm. = 3.6\ Biomass + 4.6\ H_2O + 3.1\ CO_2$$

### Ethanol formation

$$1\ Glucose + 0\ O_2 = 2\ EtOH + 2\ CO_2$$

### Ethanol consumption

$\mathrm{RQ} = 0.55 \quad Y_{X/S} = 0.4$

$$1\ EtOH + 2.3\ O_2 + 0.46\ Amm. = 0.74\ Biomass + 2.6\ H_2O + 1.3\ CO_2$$

## Conclusion

Ethanol formation and consumption during *S. cerevisiae* cultivation on glucose as primary carbon source was monitored in shake flask experiments. Cultures were monitored online with a Kuhner TOM that was combined with FermSYS online VOC sensors.

Data from VOC online sensors and EtOH calculated from Kuhner TOM off-gas analysis via stoichiometrical correlations were compared with data from photometrically analyzed samples. Both online methods showed great potential to make manual sampling superfluous. For both methods (VOC sensors and balancing), other metabolites than ethanol being formed in large amount are posing a challenge.

1 von Stockar, U. Liu, J. Does microbial life always feed on negative entropy? DOI: 10.1016/s0005-2728(99)00065-1
2 Anderlei et al. Online respiration activity measurement (OTR, CTR, RQ) in shake flasks. DOI 10.1016/S1369-703X(03)00181-5

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