# Kuhner TOM for off-gas analysis in shake flasks

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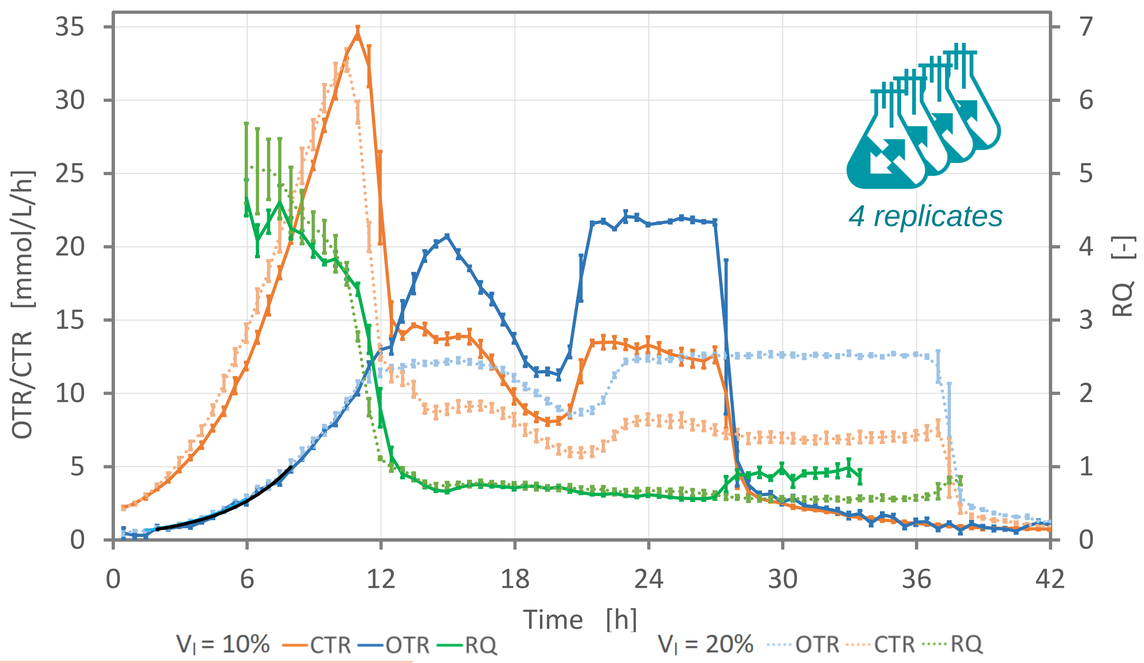
## Introduction / Abstract

To facilitate and speed up **process- and media development**, online measurement techniques for shake flasks accompany manual sampling for maximum information output per cultivation. Off-gas analysis gives oxygen transfer rate (**OTR**), carbon dioxide transfer rate (**CTR**) and respiratory quotient (**RQ**) as quantitative measures of the physiological state of the culture. On shake flask scale, multiple cultivations are usually run in parallel. Off-gas analysis should therefore be cost effective, easy to handle and versatile to match various applications.
Therefore, we developed a shake flask off-gas analysis system for **non-invasive** online determination of OTR, CTR and RQ. TOM (Transfer rate Online Measurement) is built **modular** for off-gas analysis in **4, 8, 12** or **16 individual shake flasks**. The new Kuhner TOM can be applied to **various shake flask sizes and types** (baffled, plastic, glass) enabling the user to get information about their existing cultivation procedures.

## Process understanding

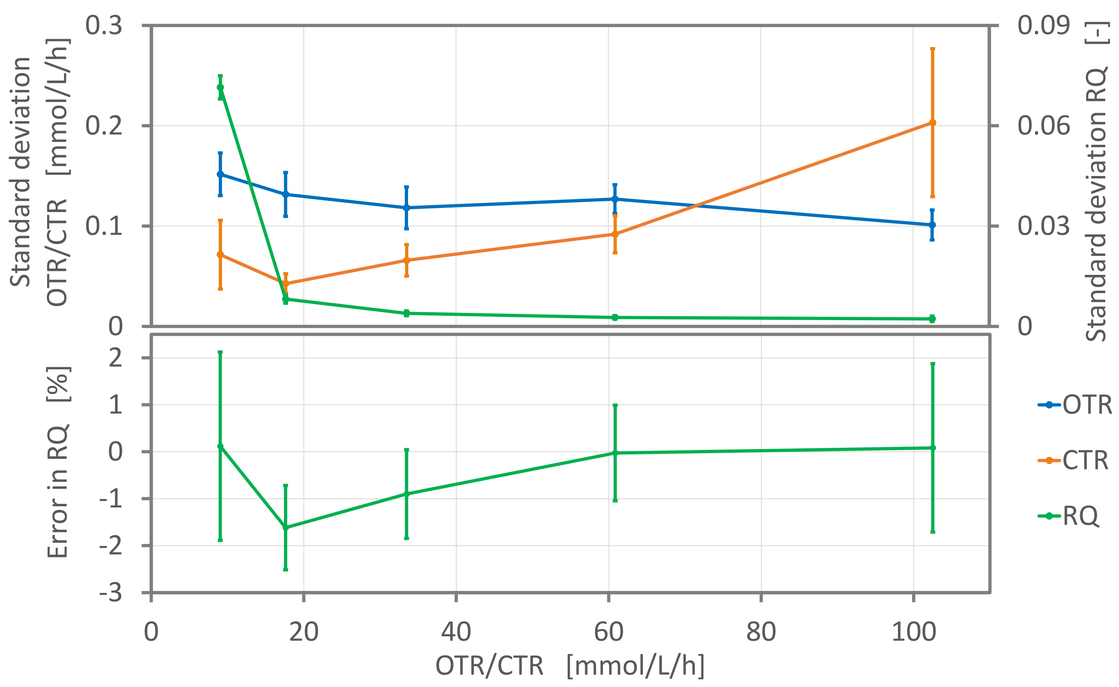



*S. cerevisiae*, YEP-medium (20 g/L glucose), n = 250 rpm, $V_{flask}$ = 250 mL, $d_0$ = 25 mm, T = 30°C, varying filling volumes (10%, 20%), Average values from 4 individual cultivations are presented with corresponding standard deviations.

Exponential growth occurs during the first 8 h at an RQ of 4-5, indicating production of a reduced product (e.g. ethanol). Depletion of glucose is indicated by the drop in CTR after approx. 10 h. This is accompanied by a metabolic switch to an RQ of approx. 0.65 indicating growth on a reduced substrate (e.g. ethanol). Both cultivations run into oxygen limitation after 22 h and 23.5 h. This allows for the calculation of $k_La$ values for the respective cultivation conditions.

| Filling volume | Maximum OTR [mmol/L/h] | $k_La$ [1/h] | $\mu_{max}$ [1/h] |
|---|---|---|---|
| 10% | 21.7 | 127 | 0.32 |
| 20% | 12.6 | 77 | 0.33 |

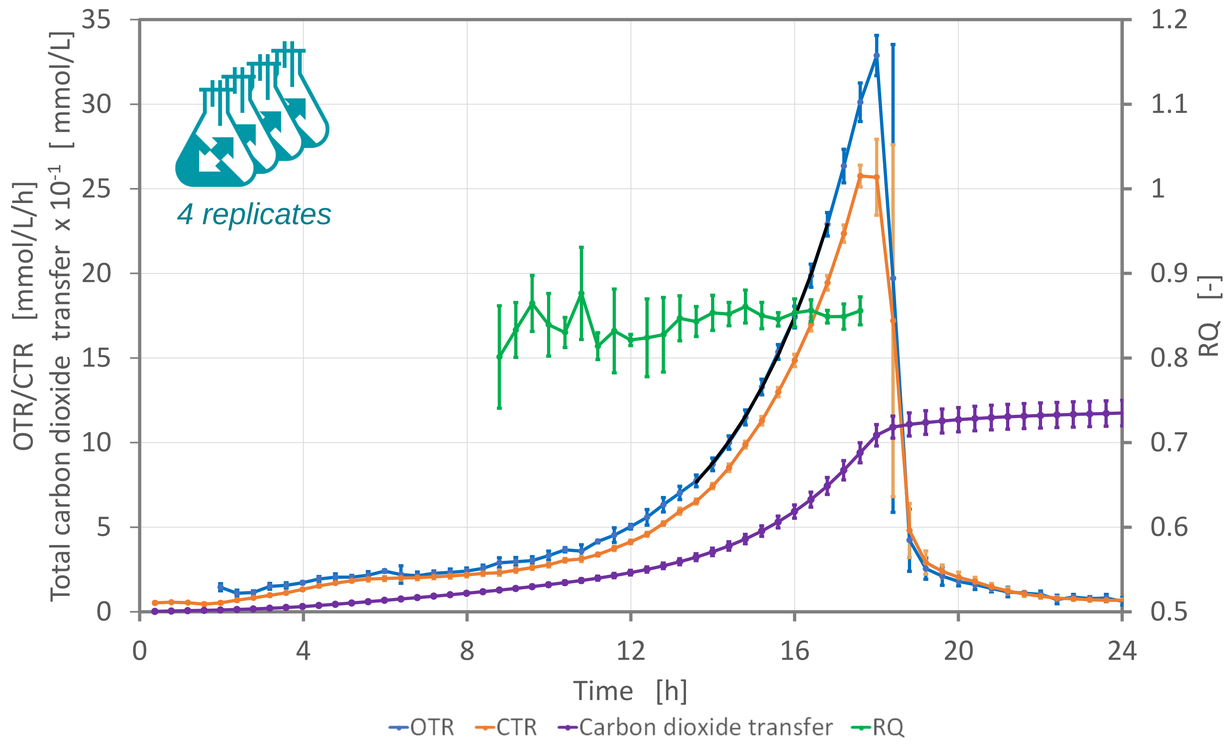
## Measurement precision




Average standard deviation in OTR, CTR and RQ measurement in 12 individual shake flasks, T = 30°C, n = 200 rpm, $d_0$ = 25 mm, 4% filling volume. Determined with model system at RQ = 1.

Precision of OTR and CTR measurement depends on filling volume and measurement frequency (here 3/h). It is slightly concentration-range dependent due to non-linearity of $CO_2$ sensor output.

## Reproducibility




*E. coli*, Biener medium (5 g/L glycerol, 5 g/L ribose), n = 300 rpm, $V_l$ = 4%, $V_{flask}$ = 500 mL, T = 30°C, $d_0$ = 25 mm. Average values from 4 individual cultivations are presented with corresponding standard deviations.

*E. coli* was cultivated in defined Biener medium containing 5 g/L glycerol and 5 g/L ribose to investigate the preferred C-source and respective growth rates. Shaking conditions were not oxygen-limiting as no saturation in OTR is visible. Surprisingly, no diauxic growth is visible, indicating that both glycerol and ribose are metabolized simultaneously. This is supplemented by the measured RQ which does not change significantly throughout the cultivation and well matches the theoretical RQ for simultaneous consumption of both C-sources.
RQ is calculated as quotient of measured CTR and OTR. Precision and significance is therefore a function of accuracy and noise of these measures. It is improving with higher respiration (see passage “measurement precision”).

| Total carbon dioxid transfer [mmol/L] | Yx/s [g/g] (from carbon balance) | $\mu_{max}$ [1/h] | Theoretical RQ [-] (for simultaneous consumption) |
|---|---|---|---|
| 117 | 0.55 | 0.34 | 0.833 |

## Conclusion

Off gas analysis on shake flask scale comes with various information that speed up media- and process development and scale-up:
**Growth rate, substrate consumption and limitation, pH and substrate inhibition, product formation** and **inhibition, balancing of carbon dioxide, oxygen limitation, $k_La$** and **out of phase operating** conditions can be derived from off-gas analysis data.
Kuhner TOM is a new versatile off gas analysis system that enables the user to collect this information with high precision OTR, CTR and RQ measurement.

References:
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**Acknowledgement**

Special thanks to Prof. Dr. Eibl from ZHAW Wädenswil who supported the *E. coli* cultivation in his laboratories.