

# **Sampling-free investigation of microbial carbon source preferences on renewable feedstocks via online monitoring of oxygen transfer rate**

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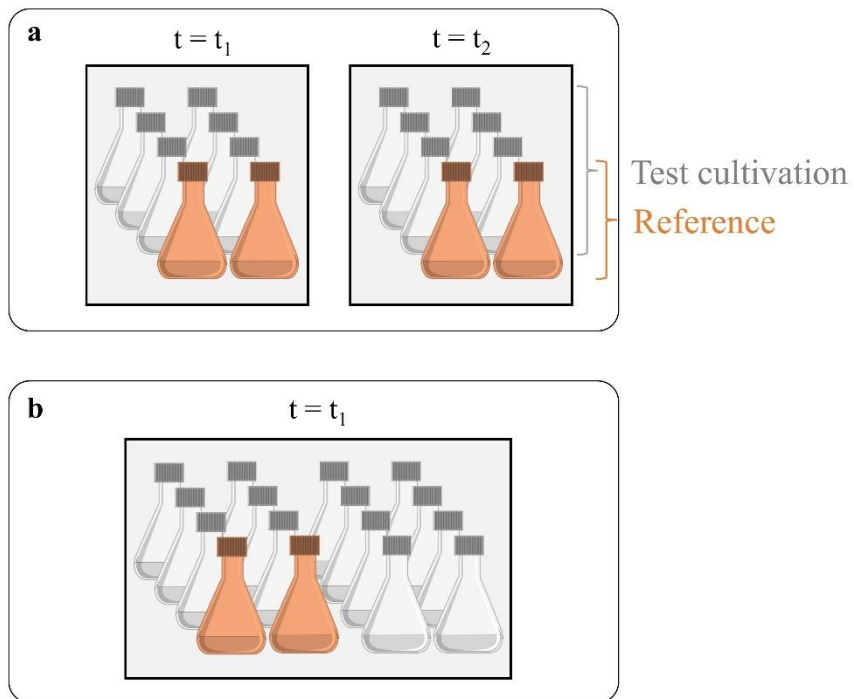
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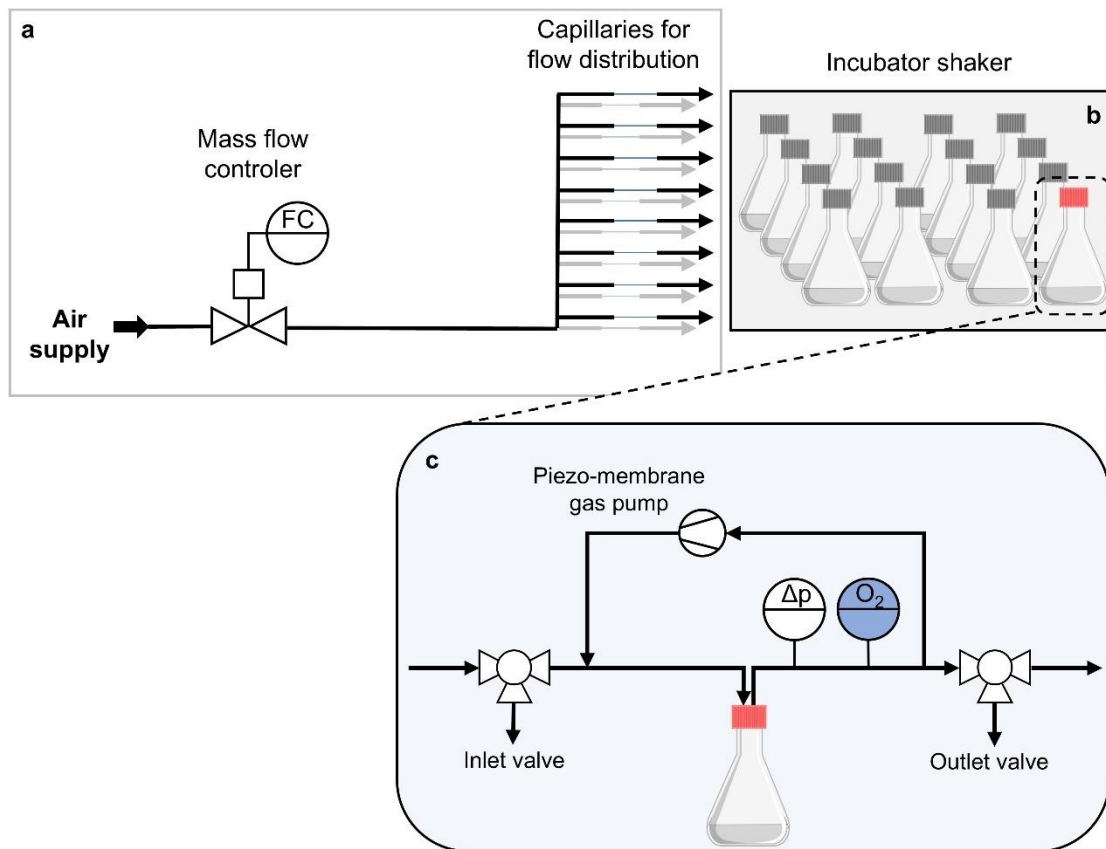
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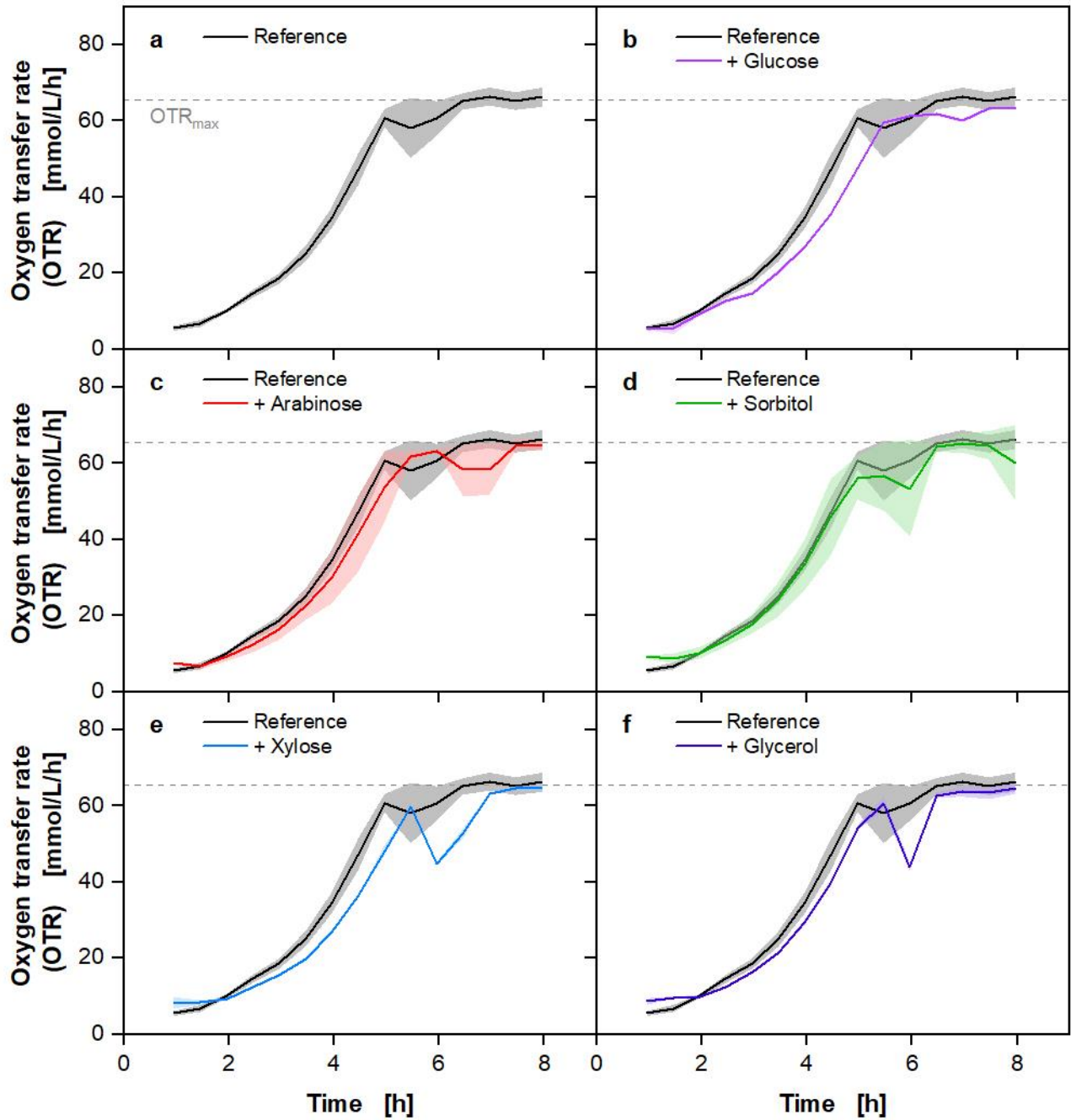
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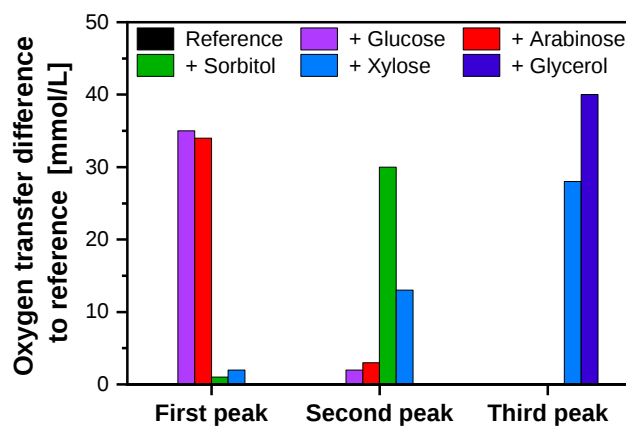
**Fig. S1:** Schematic illustration of the advantages of the 16-flask RAMOS device compared to the standard 8-flask RAMOS device. **a** Consecutive investigation of six carbon sources. **b** Simultaneous investigation of seven carbon sources



**Fig. S2:** Schematic illustration of the 16-flask RAMOS. **a** Air supply controlled by thermal mass flow controller and capillaries to ensure even gas distribution to all 16 flasks. **b** Temperature-controlled shaker, containing 16 measurement flasks. **c** Measurement loop of one shake flask with in- and outlet valve, piezo-membrane gas pump, pressure sensor, and oxygen sensor. The measurement technique and calculation of OTR are described in detail by Anderlei et al. [1, 2]. The setup was adapted from Finger et al. [3]



**Fig. S3:** Initial trial of polyauxic growth characterization via OTR on five carbon sources with *Escherichia coli* BL23(DE3). The reference medium contained 5 g/L each of the carbon sources glucose, arabinose, glycerol, xylose, and sorbitol. Additional carbon source addressed by legend was supplied with additional 5 g/L, resulting in a total concentration of 10 g/L. Lines represent the averages of biological duplicates and shadows indicate the minimum and maximum values of these duplicates. Cultivation conditions: Wilm-MOPS medium, 250 mL shake flasks,  $V_L = 10$  mL,  $T = 37$  °C,  $n = 350$  rpm,  $d_0 = 50$  mm,  $OD_{600, Start} = 0.5$  [-]



**Fig. S4:** Graphical presentation of the consumed oxygen, derived from the OTR integral, in the three OTR peaks. To enable a clearer comparison, the OTR integrals are shown as the difference to the reference. The corresponding cultivation data can be found in Fig. 2.

**Table S1:** Time shift of graphs in regard to the reference in Fig. 4 and Fig. 5

| Investigated carbon source                                                                                                      | Timeshift [h] |
|---------------------------------------------------------------------------------------------------------------------------------|---------------|
| <b>Fig. 4:</b> Demonstration of versatility with <i>U. trichophora</i>                                                          |               |
| Glucose                                                                                                                         | 2.0           |
| Galacturonic acid                                                                                                               | 1.5           |
| Xylose                                                                                                                          | 2.0           |
| Glycerol                                                                                                                        | 2.5           |
| Rhamnose                                                                                                                        | 0.0           |
| Lactic acid                                                                                                                     | 0.0           |
| Sorbitol                                                                                                                        | 1.0           |
| <b>Fig. 5:</b> Application on the crude substrate corn leaf hydrolysate with <i>U. maydis</i> MB215 $\Delta$ cyp1 $\Delta$ emt1 |               |
| Glucose                                                                                                                         | 2.0           |
| Sucrose                                                                                                                         | 0.0           |
| Arabinose                                                                                                                       | 0.0           |
| Xylose                                                                                                                          | 2.0           |
| Galactose                                                                                                                       | 2.0           |

## References

1. Anderlei T, Büchs J (2001) Device for sterile online measurement of the oxygen transfer rate in shaking flasks. *Biochemical Engineering Journal* 7:157–162. [https://doi.org/10.1016/S1369-703X\(00\)00116-9](https://doi.org/10.1016/S1369-703X(00)00116-9)
2. Anderlei T, Zang W, Papaspyrou M et al. (2004) Online respiration activity measurement (OTR, CTR, RQ) in shake flasks. *Biochemical Engineering Journal* 17:187–194. [https://doi.org/10.1016/S1369-703X\(03\)00181-5](https://doi.org/10.1016/S1369-703X(03)00181-5)
3. Finger M, Schröder E, Berg C et al. (2023) Toward standardized solid medium cultivations: Online microbial monitoring based on respiration activity. *Biotechnol J* 18:e2200627. <https://doi.org/10.1002/biot.202200627>