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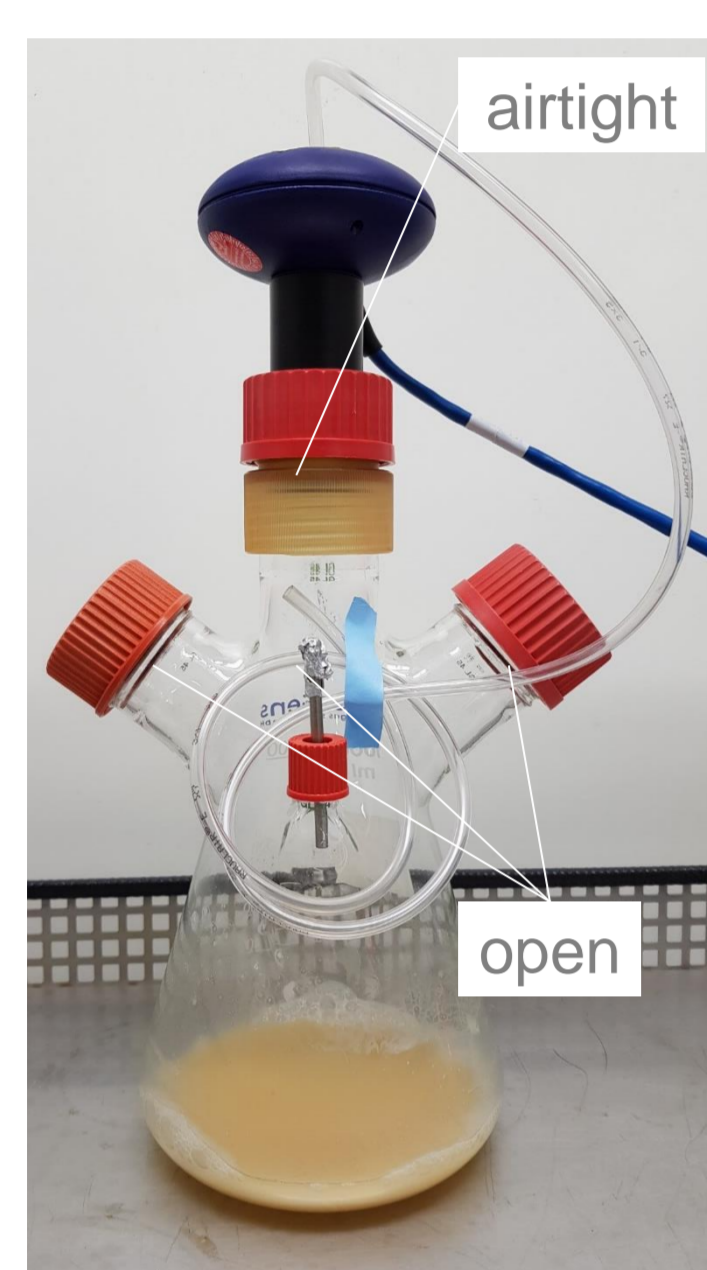
## Introduction

- Some biofuels like FAME show reduced storability due to increased microbial degradability [1].
- Microbes degrade fuels to CO<sub>2</sub>, so the measurement of CO<sub>2</sub> allows simple and sensitive detection of microbial activity.
- To assess the storability of fuels we use the BCP-CO<sub>2</sub> sensor of BlueSens Gas Sensor GmbH.

## Methods

- The off-gas sensor measures absorption of infrared light by CO<sub>2</sub> gas.
- The sensor is insensitive to the test system and can be applied, e.g., on single- and two-phase systems.
- At high microbial degradation rates, hence high CO<sub>2</sub> evolution rates, an aerated reactor can be used.

## Results and Discussion



Open single-phase reactor

- Under optimal growth conditions bacteria and yeasts produce CO<sub>2</sub> so fast that the open system (with air exchange) can be applied.
- Samples for off-line analytics like classical growth measurements can be taken.
- CO<sub>2</sub> evolution can be correlated with the growth of microbes (Figure 1).

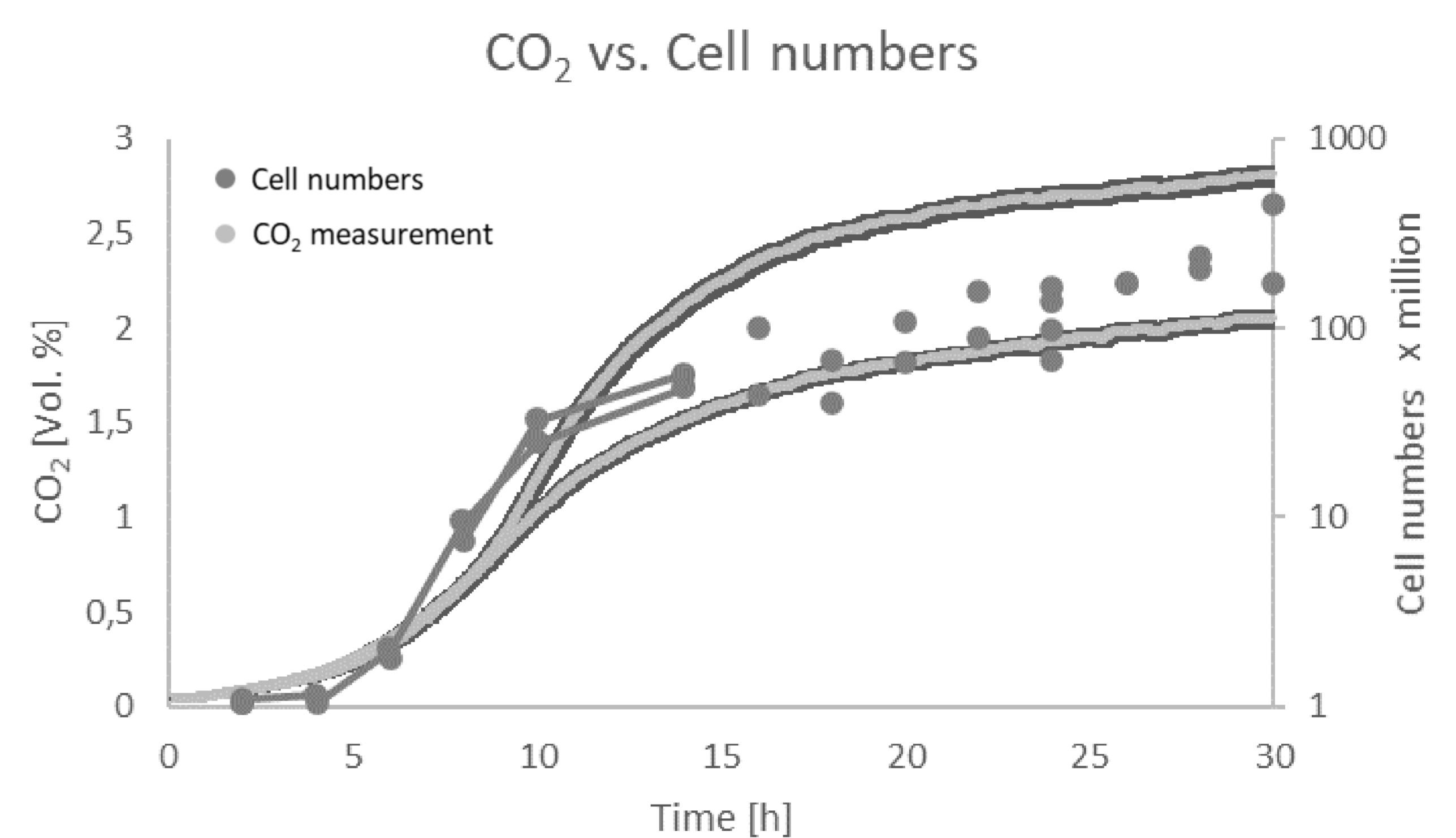


Figure 1: Correlation between growth and CO<sub>2</sub> production of *Y. lipolytica* in an open single-phase system. Growth on YEP medium is shown. Black edges represent the standard deviation of the CO<sub>2</sub> measurement.

CO<sub>2</sub> Evolution below different heating oils

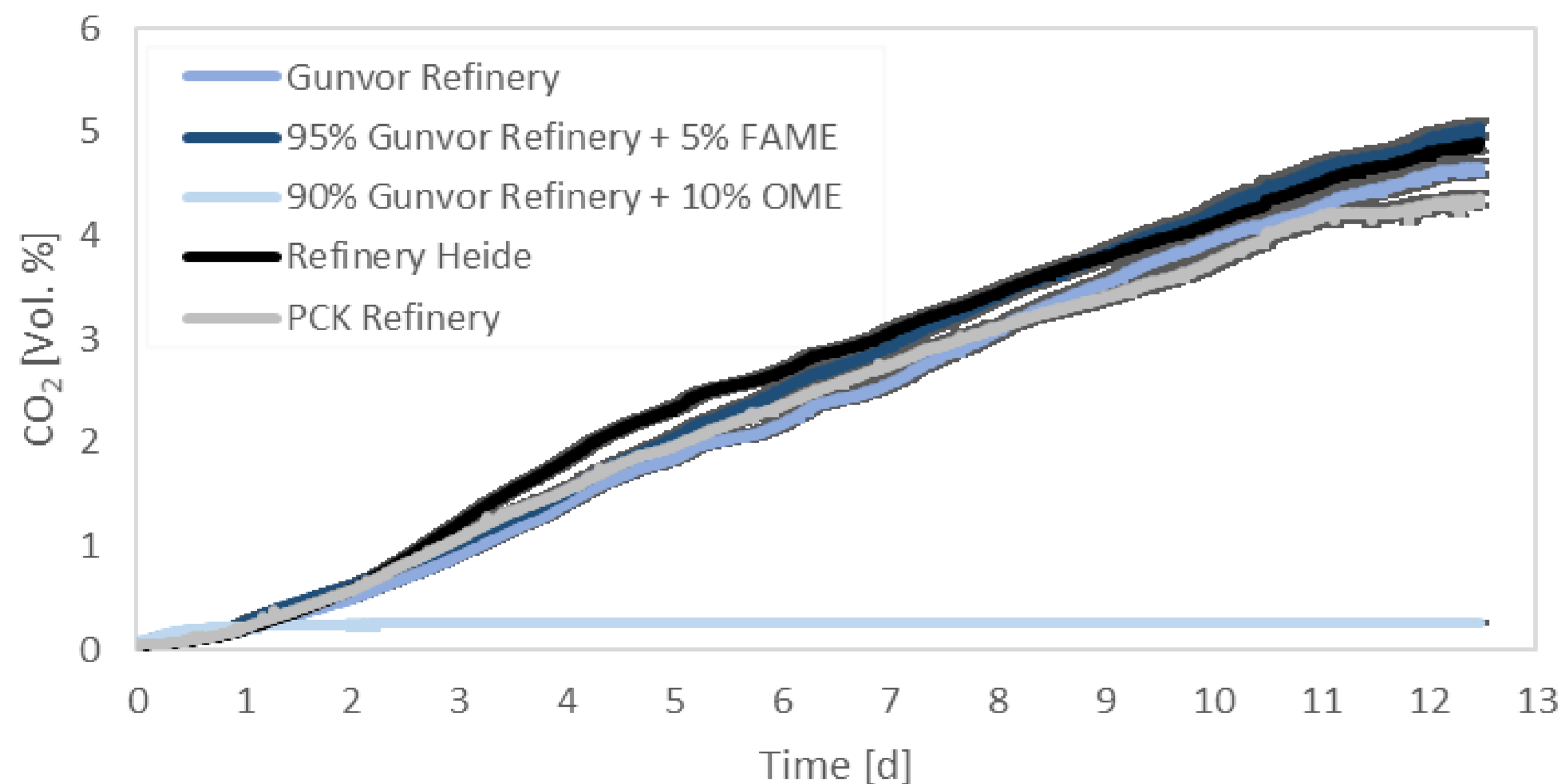
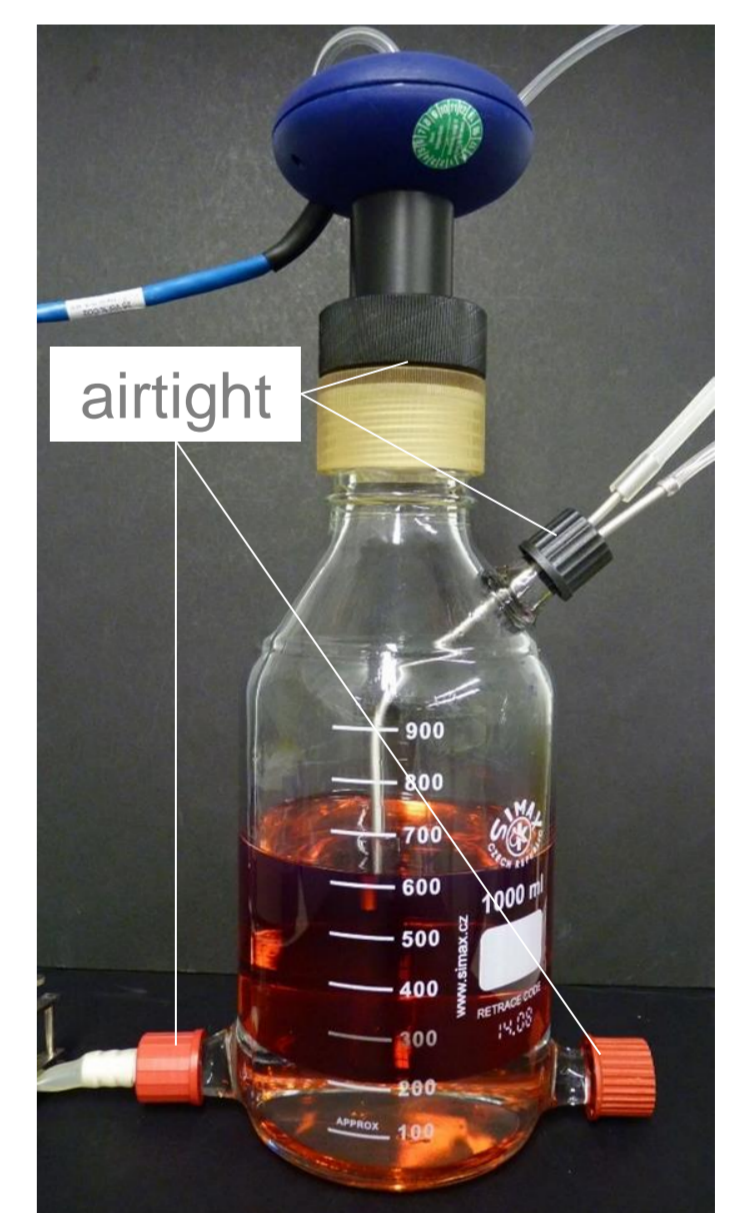


Figure 2: Monitoring of CO<sub>2</sub> evolution by a defined microbial mix [2] using heating oils as sole carbon and energy sources. A closed two-phase system was used. Black edges represent the standard deviation of the CO<sub>2</sub> measurement.

- Using the closed (airtight) two-phase system the oil tank situation can be simulated.
- A free water phase is inoculated with a defined mix of microbes which can be found routinely in regional oil tanks.
- An overlay of heating oil to be tested for storability is applied and the CO<sub>2</sub> production is monitored (Figure 2).



Closed two-phase system

## Conclusions

- The formation of CO<sub>2</sub> is a measure for the metabolic activity of present microbes. Allowing air exchange the currently measured CO<sub>2</sub> corresponds to currently present microbes and their metabolic activity. In the open system samples for growth measurement can be taken. The CO<sub>2</sub> evolution correlates with the development of microbial cell numbers under optimal conditions.
- The CO<sub>2</sub> sensor can be used under oil tank conditions, a two-phase system consisting of a contaminated free water phase below an excess of heating oil. For the inoculation of the free water phase a defined mix of microbes is used. This way, the extent of CO<sub>2</sub> evolution and the correlation with microbial growth, depends only on the applied heating oil and the nutritional conditions it offers to the microbes.
- This non-invasive measurement tool allows the evaluation of the storability of heating oils long before problematic signs of microbial contamination like biofilm formation or microbial induced corrosion take place.

## References

[1] Leuchtler, B. Mikrobiologische Kontamination von Heizöl - Ursachen und Auswirkungen auf Brennstoff und Tank [dissertation on the internet]. Aachen, Germany: RWTH Aachen University; 2015 [cited 2020, Apr 31].

[2] Leuchtler, B, et al. Defined inoculum for the investigation of microbial contaminations of liquid fuels. Int. Biodeterior. Biodegradation. 2018; 132: 84-93.