RENÉ HANKE[1],
DAVID WOLLBORN[1],
YOSHIHIRO OJIMA[2],
JOCHEN BÜCHS[1*]

Moderate increases in viscosity improve the oxygen supply in shake flask cultures

Observation

The viscosity of many microbial production processes increases over time. This affects the oxygen supply of aerobic organisms.

- ► Degradation of viscous substrates
- ► Production of viscous substances such as γ-PGA
- ► Changing morphology of filamentous organisms

We observed that moderate viscosities can improve the oxygen supply in shake flask cultures (**Figure 1**). This phenomenon constitutes a contrast to stirred tank reactors, where elevated viscosity results in a decreased oxygen supply and must be considered during bioprocess development and scale-up[3].

Microbial cultures secreting moderate amounts of viscous γ-PGA improve their own oxygen supply

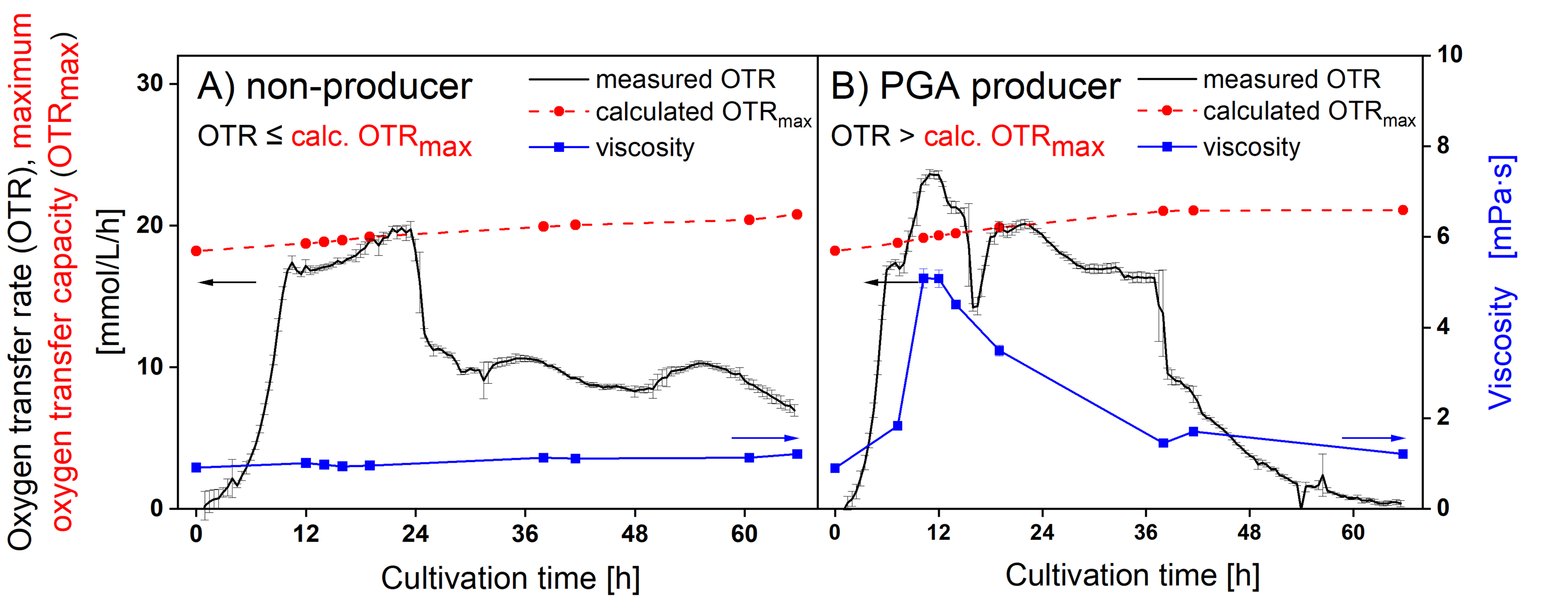


Figure 1. ***Bacillus*** **cultivations with and without production of the viscosifying agent γ-poly-glutamic acid (γ-PGA). (A)** *B. subtilis*, does not produce γ-PGA, **(B)** *B. licheniformis*, produces γ-PGA. V3b Medium, T = 37°C, V_L = 30 mL, 250mL RAMOS-flasks, n = 250 rpm, d_0 = 50 mm, N = 3. The theoretical maximum oxygen transfer capacity for water-like viscosities (OTR_{max}) is calculated based on culture conditions and measured osmolality according to Meier *et al.* (2016)[4].

Investigation

Shaking conditions influence viscosity range where oxygen supply is improved or impaired

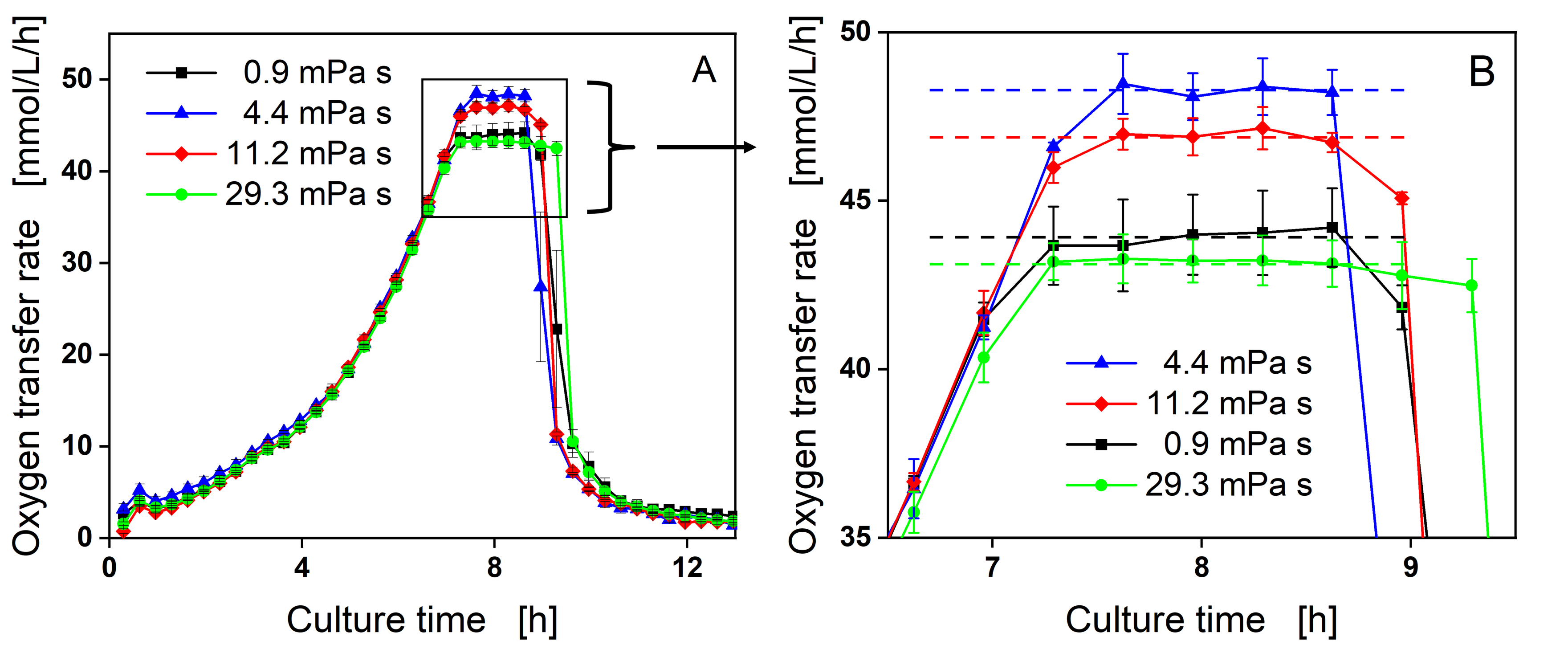
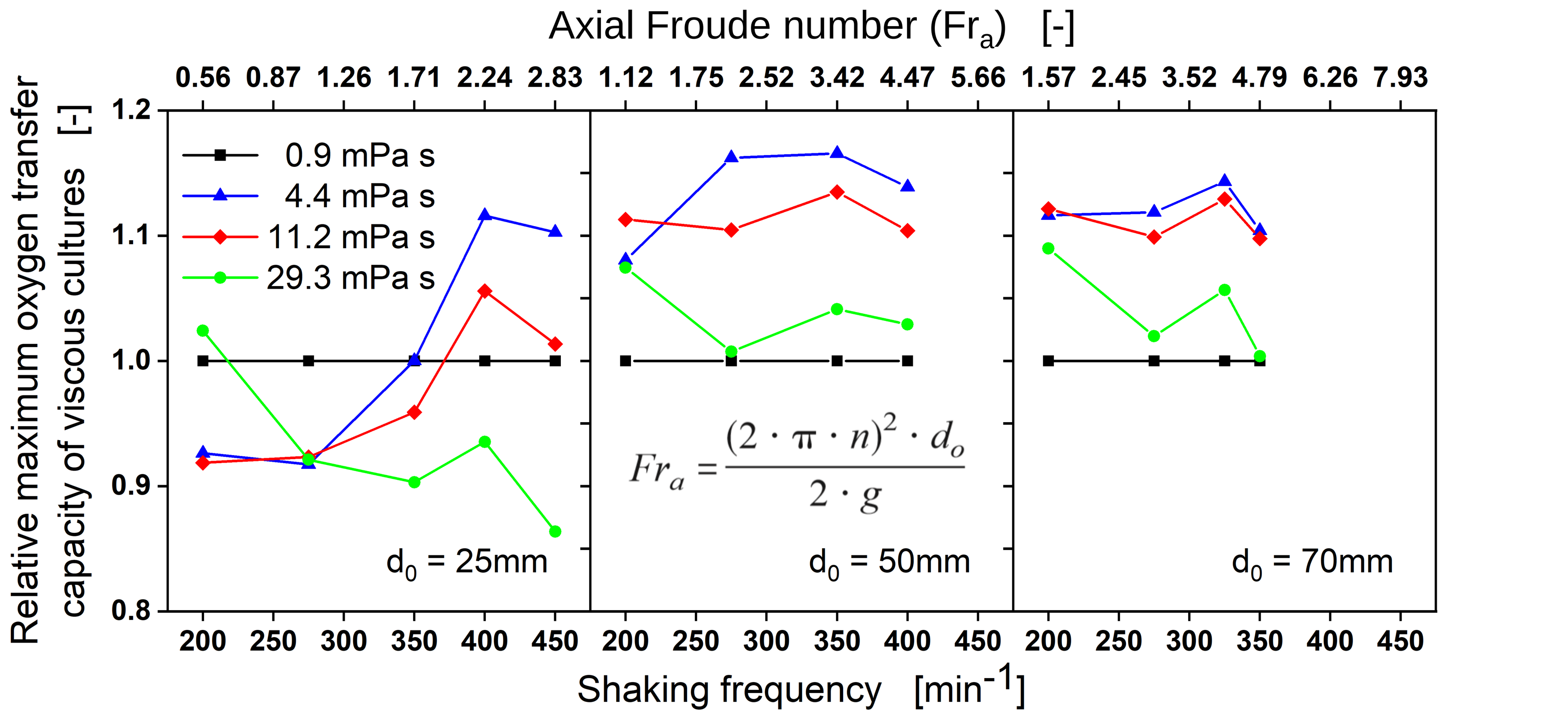


Figure 2. Procedure for empirical determination of maximum oxygen transfer capacities for different viscosities. *Corynebacterium glutamicum* DM1730 in 20 mL CGXII medium with 20 g/L glucose, supplemented with 0, 20, 40 and 60 g/L of polyvinylpyrrolidone (PVP) which resulted in viscosities of 0.91, 4.40, 11.17 and 29.30 mPa·s, respectively. Measurements were performed in a 250mL non-baffled shake flask at a 50 mm shaking diameter and at 350 rpm. The experiments were run in quadruplicates at 30°C using a RAMOS device[5,6]. **B** represents zoomed in display of **A**.



$$Fr_a = \frac{(2 \cdot \pi \cdot n)^2 \cdot d_0}{2 \cdot g}$$

Figure 3. Relative maximum oxygen transfer capacity for viscous cultures. Relative maximum oxygen transfer capacities are calculated by dividing the empirical relative maximum oxygen transfer capacity for viscous cultures by the empirical maximum oxygen transfer capacity for water-like viscosity (0.9 mPa s). Shaking frequencies were varied from 200 rpm to 350 rpm, 400 rpm or 450 rpm at shaking diameters (d_0) of 25 mm, 50 mm and 70 mm. Axial Froude number (Fr_a) is calculated according to Büchs et al. (2000)[7]. Measurements were performed as stated in **Figure 2**.

Implication

Previous studies by Meier et al. and Giese et al.:

- Liquid film on shake flask wall contributes to oxygen supply[4]
- Viscous film is thicker → absorbs more oxygen during rotation[8]

Our hypothesis: Higher shaking diameters increase this effect.

Observation:

- Oxygen enrichment for d_0 25mm → 50mm (**Figure 3**)
- No improvement for d_0 50mm → 70mm (**Figure 3**)

Explanation: position of bulk liquid and height of liquid film on shake flask wall show only slight differences for 50mm → 70mm.

Liquid distribution changes more drastically when switching from 25mm to 50mm shaking diameter

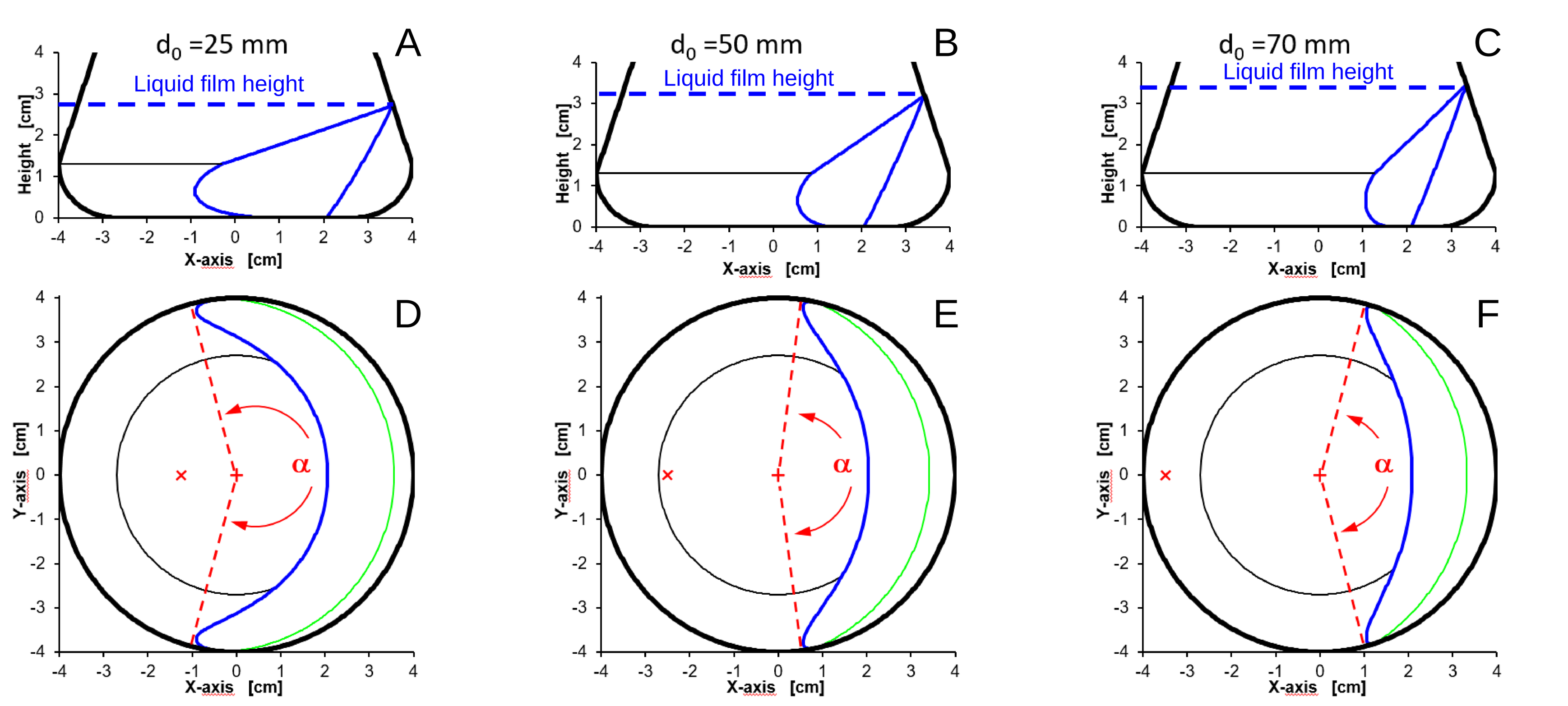


Figure 4. Simulated liquid distribution in shake flasks for varying shaking diameters (d_0). The liquid distribution in a shake flask was simulated for a liquid volume of 20 mL, a shaking frequency of 200 rpm and shaking diameters of 25 mm, 50 mm and 70 mm. **A-C** side view, **D-F** top view.

[1] RWTH Aachen University, AVT - Aachener Verfahrenstechnik, Chair of Biochemical Engineering, Forckenbeckstraße 51, 52074 Aachen, Germany

[2] Osaka City University, Department of Applied Chemistry and Bioengineering Graduate, School of Engineering, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585 Japan

[*] Corresponding author. Tel.: +49 241-80-24633. E-mail address: jochen.buechs@avt.rwth-aachen.de (J. Büchs).

[3] Kawase, Y., Moo-Young, M. (1988). Volumetric mass transfer coefficients in aerated stirred tank reactors with Newtonian and non-Newtonian media. Chem Eng Res Des 66:284–288

[4] Meier, K., Klöckner, W., Bonhage, B., Antonov, E., Regestein, L., Büchs, J. (2016). Correlation for the maximum oxygen transfer capacity in shake flasks for a wide range of operating conditions and for different culture media. Biochemical Engineering Journal, 109, 228-235.

[5] Anderlei T, Büchs J. 2001. Device for sterile online measurement of the oxygen transfer rate in shaking flasks. Biochem Eng J 7:157–162

[6] Anderlei T, ZangW, Papaspyrou M, Büchs J. 2004. Online respiration activity measurement (OTR, CTR, RQ) in shake flasks. Biochem Eng J 17(3):187–194.

[7] Büchs J., Maier U., Milbrandt C., Zoels B. (2000). Power Consumption in Shaking Flasks on Rotary Shaking Machines: II. Nondimensional Description of Specific Power Consumption and Flow Regimes in Unbaffled Flasks at Elevated Liquid Viscosity. Biotechnol Bioeng. 68(6):564-601.

[8] Giese H, Azizan A, Kümmel A, Liao A, Peter CP, Fonseca JA, Hermann R, Duarte TM, Büchs J. Liquid films on shake flask walls explain increasing maximum oxygen transfer capacities with elevating viscosity. Biotechnol Bioeng. 2014;111(2):295–308.