Two new methods to study anaerobic microbial metabolism & kinetics

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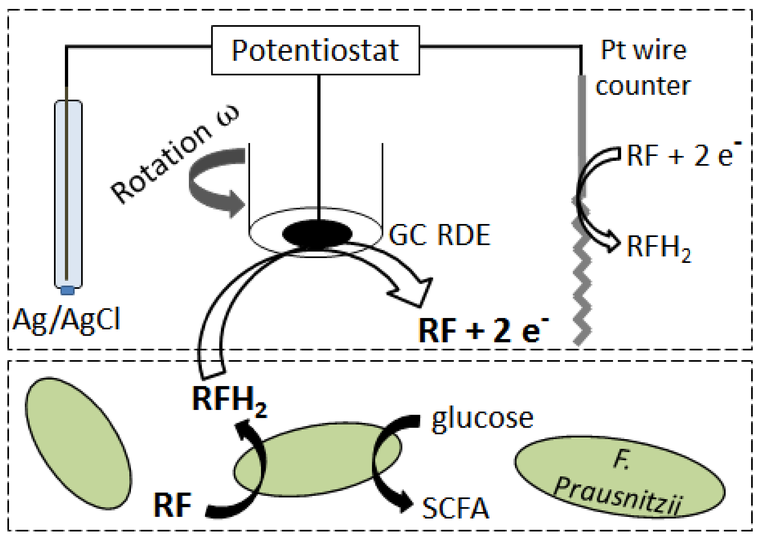
Methods to on-line monitor microbial metabolism and kinetics are important for industrial biotechnology and fundamental studies. We present (1) a novel, highly sensitive **electrochemical** approach based on a rotating disc electrode (RDE) and (2) a micro-titer plate based **spectrophotometric** assay to **accurately** monitor the **kinetics** of **anaerobic** planktonic cells in a non-growing state.

1. Electrochemical *Principle* *2. Spectrophotometric*

Model organism: Faecalibacterium prausnitzii A2-165
- anaerobic butyrate-producing gut bacterium
- metabolizes glucose / reduces riboflavin (RF/RFH_2)
- 37 °C, anaerobic incubation, stationary phase, non growing



$[RFH_2]$ monitoring with RDE

Bacterial metabolism

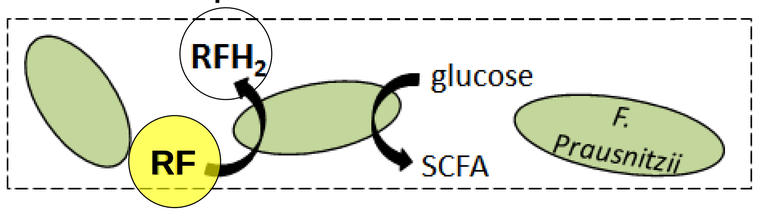
GC RDE: glassy carbon rotating disc electrode

$j = f(t) \xrightarrow{Levich} [RFH_2] = f(t) \xrightarrow{+\ cell\ count}$ kinetics

2 options:
- Platereader in anaerobic chamber
- Plate sealed with petroleum jelly

[RF] removal monitoring at OD_{450nm}

transparent



Bacterial metabolism

yellow

$OD_{450\ nm}$ decrease = f(t) $\xrightarrow{+\ cell\ count}$ kinetics

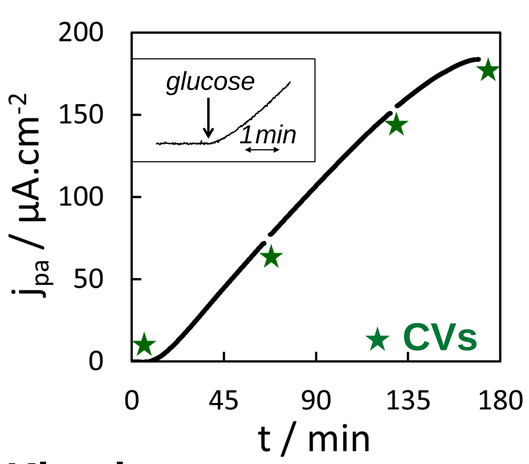
1. *Monitoring kinetics* *2.*

Metabolic reaction rate for RF:

$$r = \frac{d[RFH_2]}{dt} = K_1 \times \frac{dj_{pa}}{dt}$$

Tangent slope of chronoamperometry (CA)

CA: set potential → measure current



CA at – 0.25 V, 2000 rpm

-2.4×10^7 cells/mL
-140 µM RF
-11 mM glucose
} saturation

From 30 to 130 min: maximal rate r_{max}

Kinetics parameter:

r_{max} increases linearly for $10^5 \leq [\ bact.] \leq 10^8$ cells/mL

Analogy: Michaelis-Menten model:

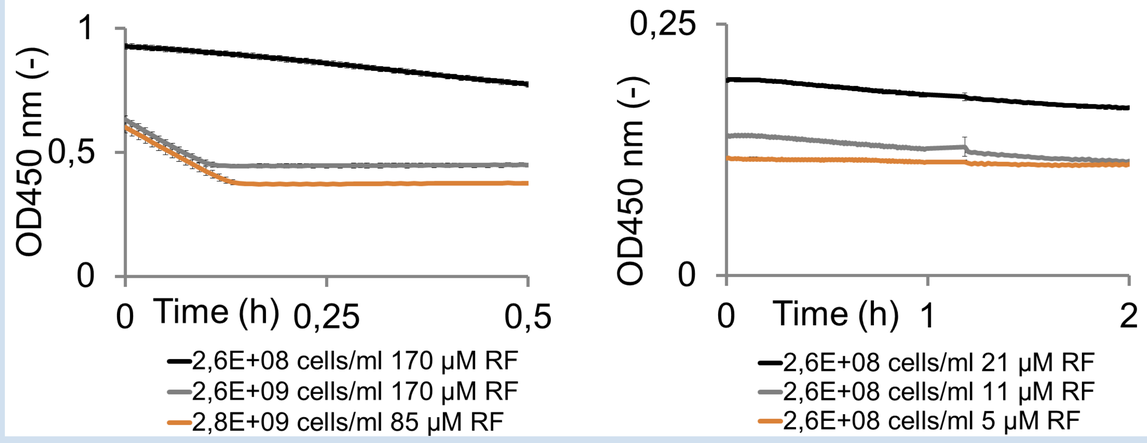
$$k_{cat} = \frac{r_{max}}{[bact]} = K_1 \frac{slope_{max}}{[bact]} = 5.3 \pm 1.3 \times 10^5\ s^{-1} \quad (n = 7)$$

Metabolic reaction rate for RF:

$$r = \frac{-d[RF]}{dt} = K_2 \times \frac{d\ OD_{450nm}}{dt}$$

Tangent slope of OD_{450nm}

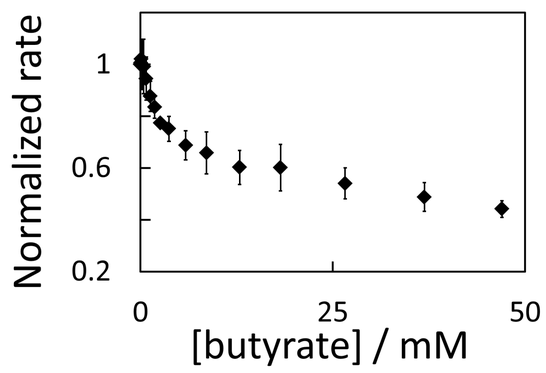
- 5 - 170 µM RF
- 11 mM glucose = saturation
- n=3



1. Electrochemical Applications

Simple and fast measurements for:
- Kinetics parameters in ≠ conditions
- Inhibition curves

Ex.: butyrate inhibition



- Study of bacterial synergy?...

1.		*2.*
✓ 1.6 × 10^4	RF turnover rate (s^{-1}) Determined for the same suspension	✓ 2.06 ± 0.76 × 10^4
✓ 8.6 × 10^4	Min. [*F. prausnitzii*] (cells.mL^{-1})	2.6 × 10^6
✓ 0.28	Min. initial [RF] required (µM)	4.8
✓ 5.3 × 10^{-9}	Min. RF consumption rate (M.min^{-1})	7.6 × 10^{-6}
✓ 0.5 - 2	Min. recording time (min.)	> 5
✓ 500	Max. linear RF removal (min.)	1 – 132
1 sample; multiple conditions		~20 conditions/samples in triplicate
e^- shuttle needed		colour changing e^- acceptor needed
Solids are no issue		Solids interfere

Further reading:
A. Prévoteau et al. (2015) Hydrodynamic chronoamperometry for probing kinetics of anaerobic microbial metabolism – case study of *Faecalibacterium prausnitzii*. Scientific Reports 5, 11484.

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