Flow cytometry-based viability staining for bioprocess monitoring of *Sulfolobus acidocaldarius*

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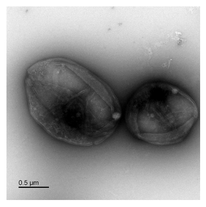
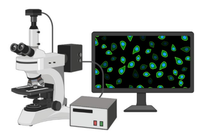
MOTIVATION

- No industrial process has been yet established with Sulfolobales
- Bioprocess development: viability necessary to monitor impact of process parameters
- Current state-of-the-art → plating assay, which is time consuming, material-intensive task and provide only time-lagged results

Goal → find suitable stains
→ faster bioprocess monitoring of viability
→ Flow cytometry- based viability staining





METHOD DEVELOPMENT

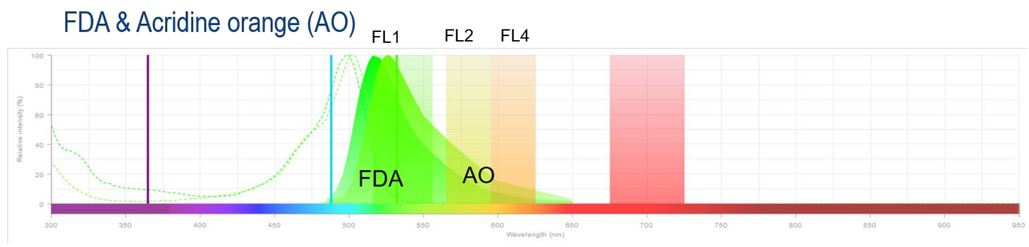
Screening of fluorescent dyes:

- Purple & blue marked dyes showed fluorescent under the microscope
- Blue marked also showed fluorescence in flow cytometry (FCM)

Dye	Ex. λ_{max} [nm]	Em. λ_{max} [nm]	Fluorescence colour	Permeability *	Mode of interaction	For detection of
Acridine orange AO	500	526	green	permeable	DNA/RNA	living and dead cells
SYTO	485	500	green	permeable	DNA/RNA	living and dead cells
Hoechst 33342	350	461	blue	permeable	DNA, A-T rich regions	living and dead cells
RH414	532	716	red	permeable	cell membrane	living and dead cells
Con A – Rhodamine	545	570	red	impermeable	Cell membrane	living and dead cells
Fluorescein diacetate FDA	485	520	green	permeable	enzymatic fluorophore generation	living cells
$DiBAC_4(3)$	493	516	green	impermeable	positively charged or hydrophobic regions	dead cells
Propidium iodide PI	535	617	red	impermeable	DNA/RNA	dead cells
7-AAD	546	647	red	impermeable	DNA, G-C rich regions, RNA	dead cells

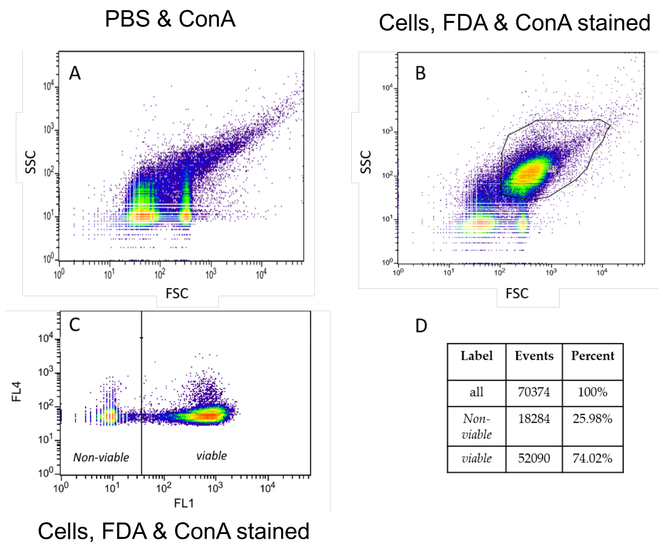
Flow cytometry (FCM)

- Fluorescein diacetate and Acridine orange overlapping spectra
- Fluorescein diacetate and Con A –rhodamine are distinguishable from another in the FCM



GATE DEFINITION

… for viability evaluation of *S. acidocaldarius*.



D

Label	Events	Percent
all	70374	100%
Non-viable	18284	25.98%
viable	52090	74.02%

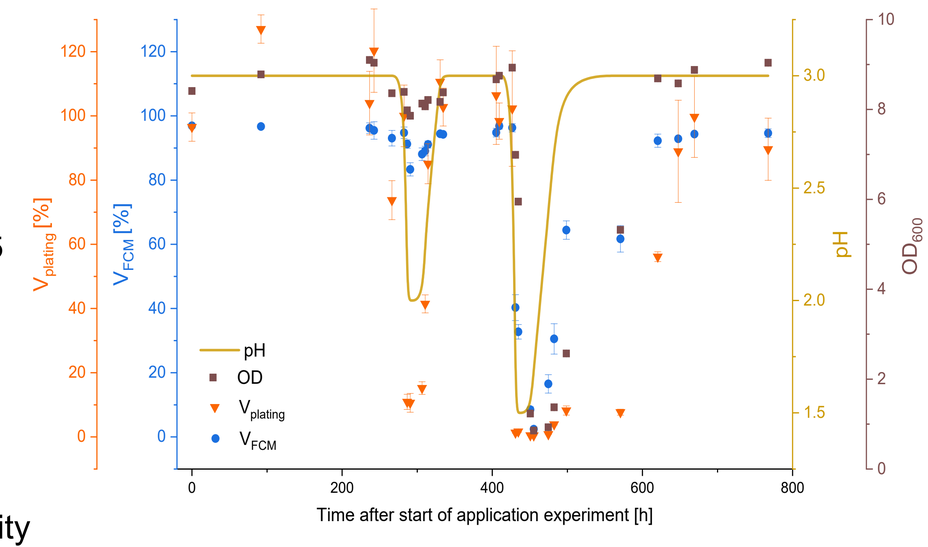
Gate definition for viability evaluation of *Sulfolobus acidocaldarius*. FL1 (536/40 nm bandpass) & FL4 (610/30 nm bandpass)

APPLICABILITY TEST

2L bioreactor in continuous cultivation – *S. acidocaldarius*

75 ºC & d=0.03 h^{-1}

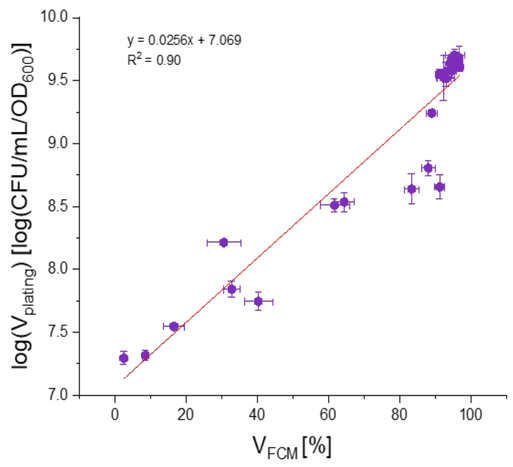
- Change of pH value
  - From pH optimum of 3.0 to pH 1.5
- Monitor viability via FCM (V_{FCM})
- Monitor viability via plating assay ($V_{plating}$)
- Change in pH → change in viability according to FCM and plating assay



Comparison of viability measurements of *Sulfolobus acidocaldarius* in response to the shift in pH value, observed over time [h].

SUMMARY

- Found suitable dyes for this archaeon
  - FDA & Con A - rhodamine
- Bioprocess monitoring is possible
  - Monitor impact of bioprocess parameters →Temp, stirrer, pH, dilution rate, …



Logarithmic trend of V_{FCM} [%] versus log ($V_{plating}$) with a correlation factor of R^2=0.90.

Comparison between FCM and plating assay

- Logarithmic trend between V_{FCM} and $V_{plating}$

Why logarithmic correlation?

➢ V_{FCM} and $V_{plating}$: assess viability differently

Metabolic activity versus proliferation

References
1. Rastädter, K., Tramontano, A., Wurm, D.J. *et al.* Flow cytometry-based viability staining: an at-line tool for bioprocess monitoring of *Sulfolobus acidocaldarius*. *AMB Expr* **12,** 107 (2022). https://doi.org/10.1186/s13568-022-01447-1

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