Droplet-based microfluidics and automation to overcome challenges in the screening of secondary metabolites during bioprocessing

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1. Motivation   2. Theory

Technology	Highlight	Culture	Scope	Ref.
Biolector	lipids	*Y. lipolytica*	online BODIPY fluorescence	Back et al 2016
Biolector	enzyme e.g. cutinase	*C. glutamicum*	Lab scale green fluorescence GFP	Müller et al 2021
Biolector	polypeptide	*S. lividians*	Nano LC- MS / ms	Koepff et al 2017
DBM	denaturalization T° enzyme	*Y. lipolytica*	fluorescence	Beneyton et al 2017
DBM	enconding enzyme	C. glutamicum	fluorescence microscopy	Balasubramanian et al 2021
DBM	promotor	S. lividians	FAD	Tu et al 2021

(Adapted from : Loayza Mora 2025)

Fig 1 3D printing technology for production of chip substrates, as well as automation for handling of metallic fluids. INBM TUB Freiberg Germany

3. Methods

Fig. 2 Large scale fermentation process Versuchs- und Lehranstaltfür Brauerei in Berlin (VLB) e.V.

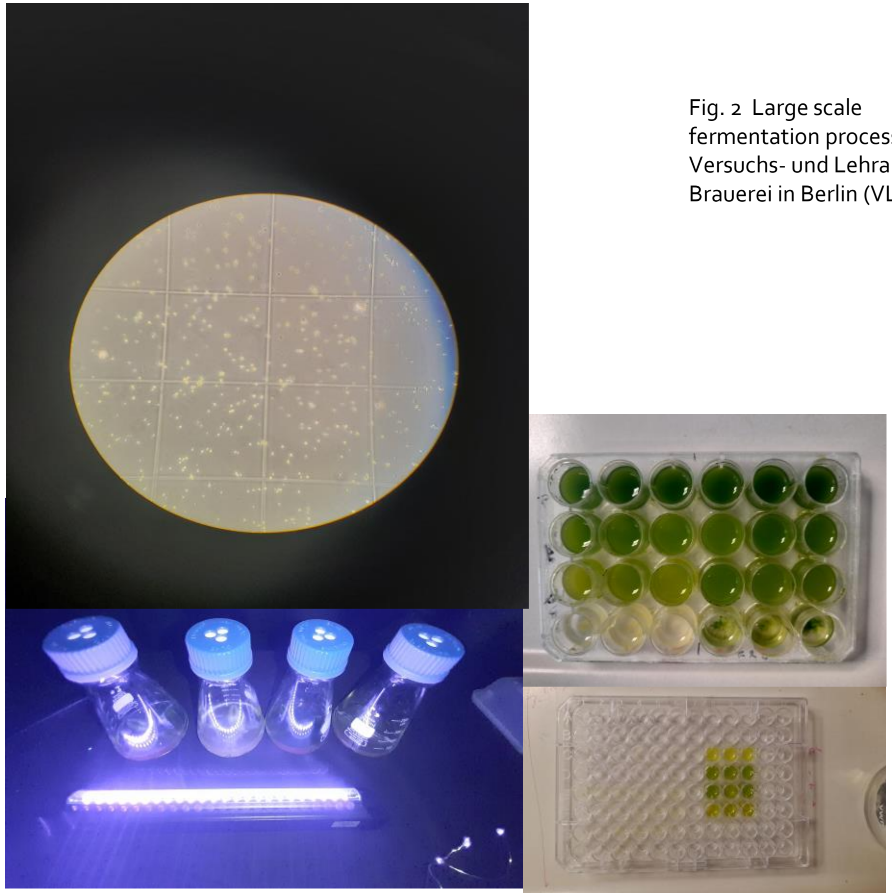

Fig. 3 Semi HTP bioprocess for microalgae cultivation at the scale-down level, addressing challenges such as desiccation. INBM TUB Freiberg

upscale

Photos : Loayza Mora, E. F

Fig. 4 Cell culture technology, including engineering as well as cytometry. TU Berlin Germany

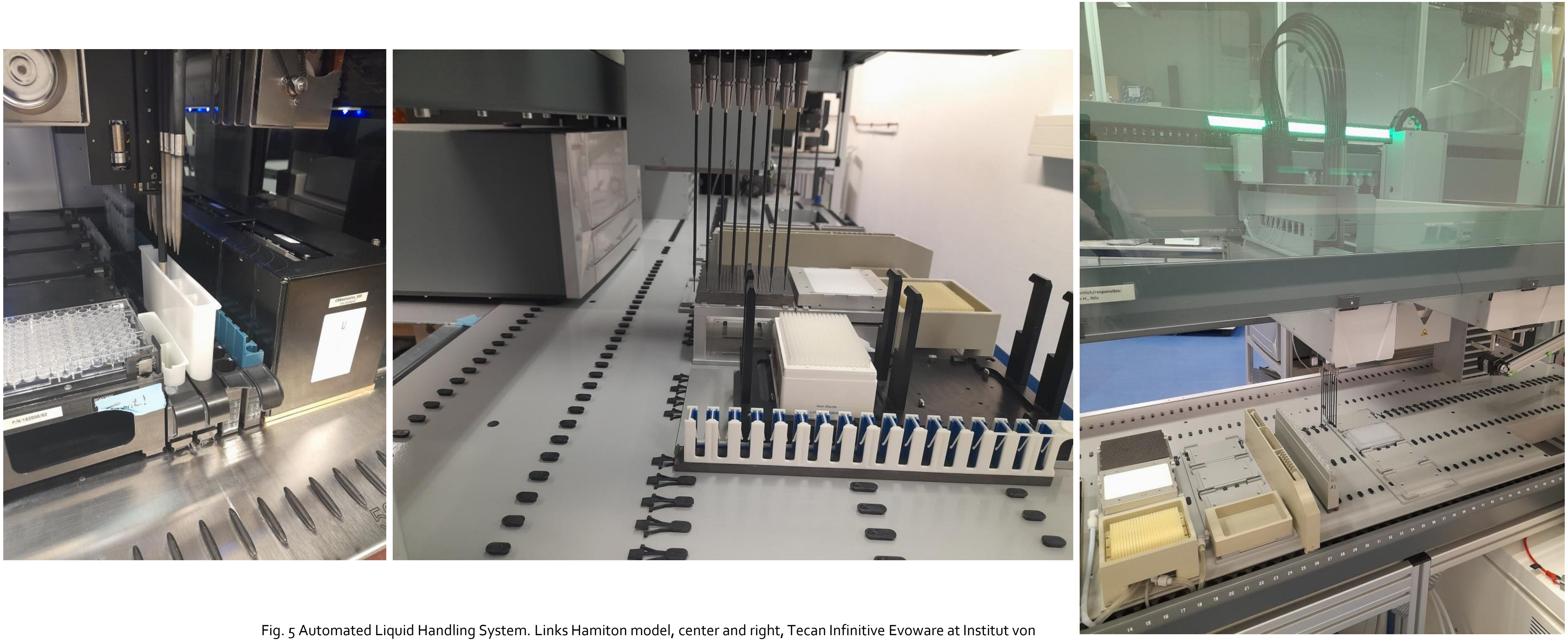

Fig. 5 Automated Liquid Handling System. Links Hamiton model, center and right, Tecan Infinitive Evoware at Institut von Bioverfahrenstechnik of Technische Universitat Berlin.

Photos : Loayza Mora, E. F

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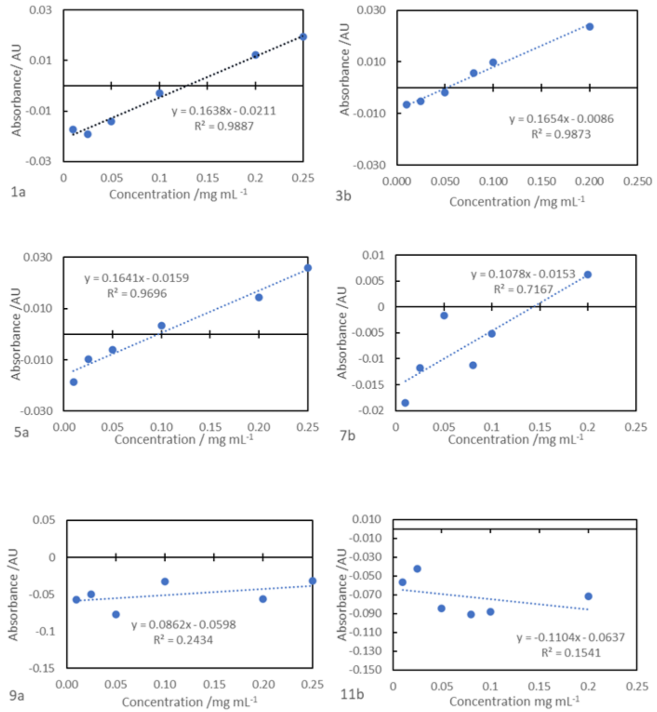


Fig. 6 Acetic acid determination by using 3 µL with acetate kinase. From: Master Thesis Loayza Mora TU Berlin 2023.

e.g. 3 mL

Fig.10 Typical chromatography equipment at INBM TUB Freiberg

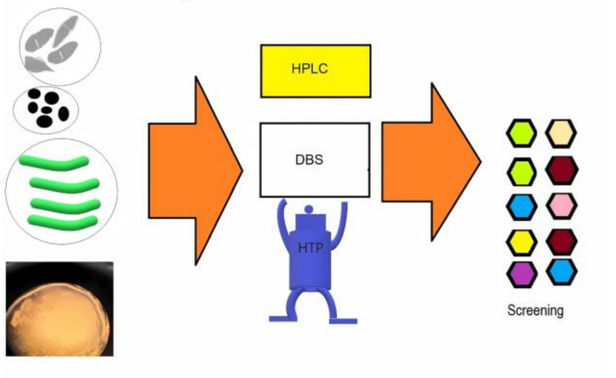
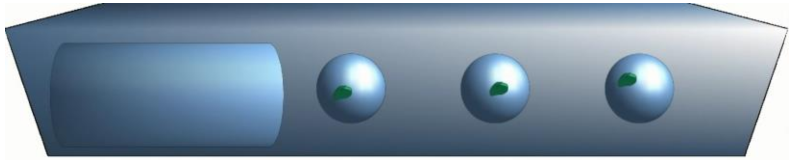


Fig.9 Schematic diagram of the proposed workflow-level automation technology combining miniaturization and DMB

Fig.7 Wafer 300 mm at INBM TUB Freiberg



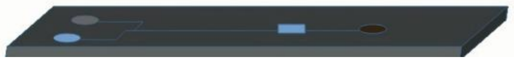


Fig. 8 Transferring volume a large number of samples from DBM, then collecting the samples at the chip's outlet to achieve volumes compatible with automated miniaturization technology (LHS), can resolve the bottleneck e.g. 3µL. Above 384 MTP format Greiner. Institut von Bioverfahrenstechnik of Technische Universitat Berlin.

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4. Discussion

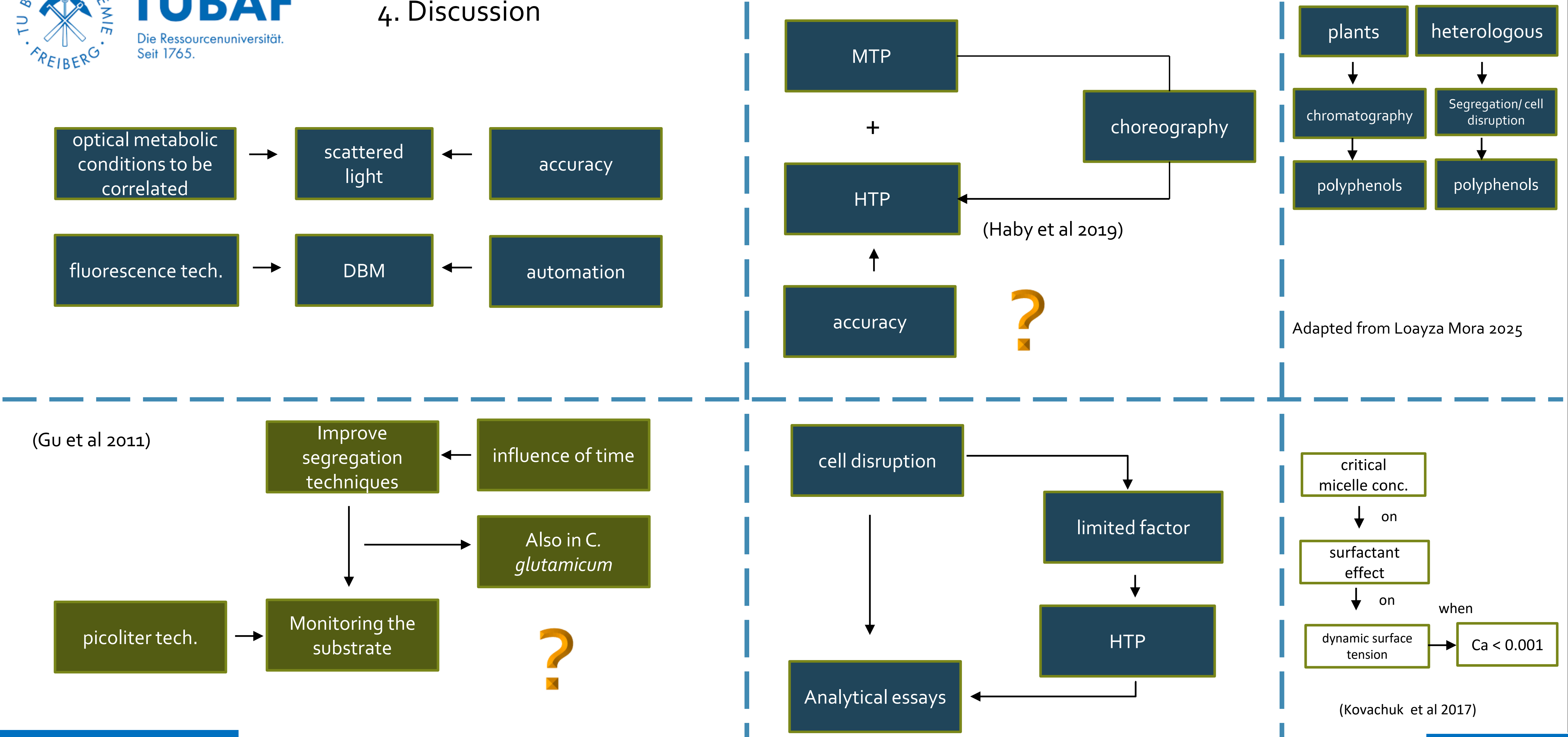
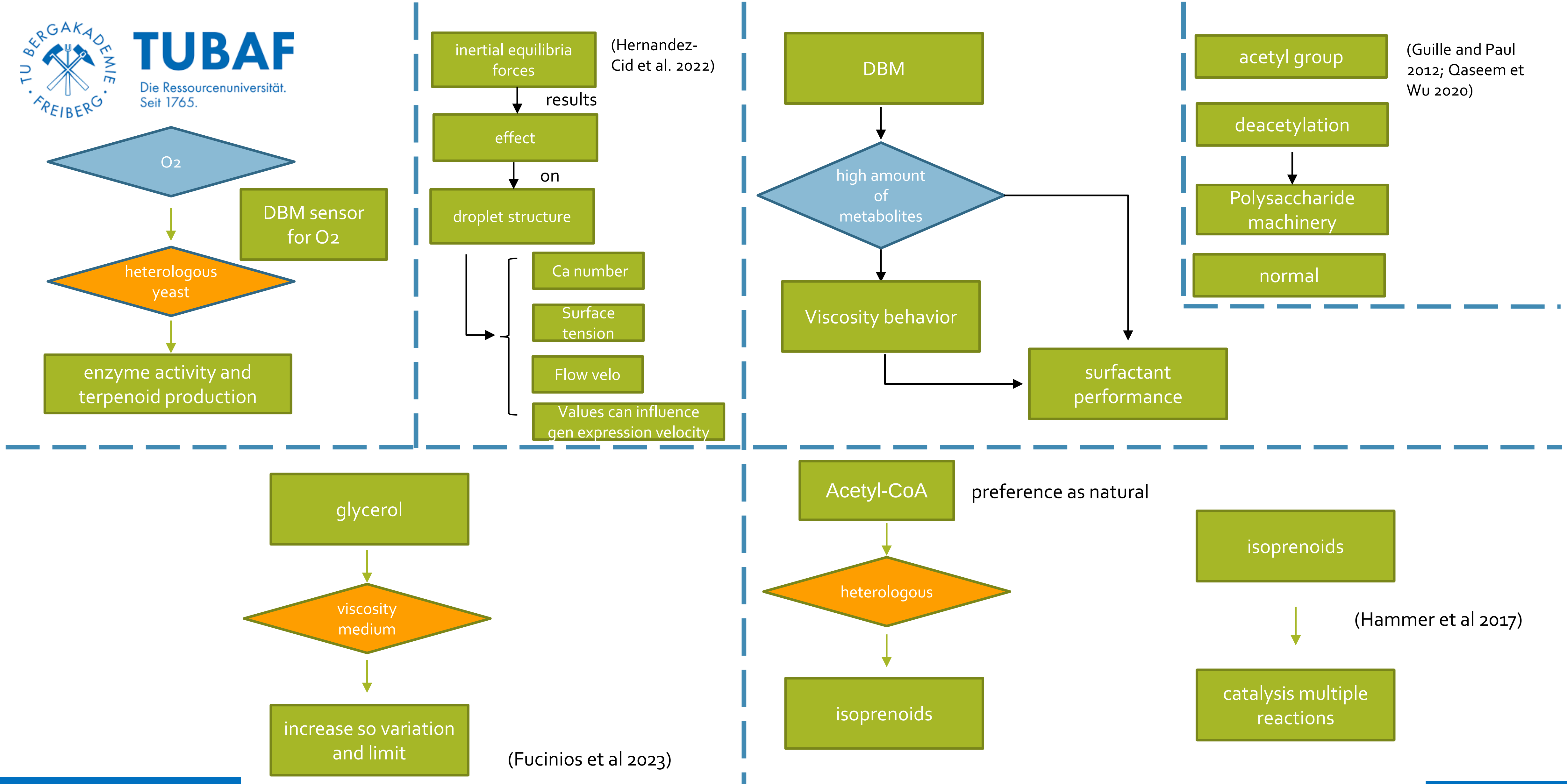


Fig. 11 Synthesis of discussion (Loayza Mora 2025)



(Back et al. 2016)

(Sidiqui et al 2012)

Fig. 12 Synthesis of discussion (Loayza Mora 2025)

Adapted from Loayza Mora 2025

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heterologous protein production

growth capacity

metabolic activity

gene expression

?

depleted oxygen availability

low pH

Adapted from Loayza Mora 2025

glycolysis

Route B

ubiquinone and terpenoids

(Kanehisa and Goto 2000, Kanehisa et al. 2019, Kanehisa et al. 2023)

antioxidant and enzyme

secondary carotenoids

(Bouzidi et al. 2022)

e.g. increasing substrate concentration

enzyme inhibition

in microalgae

DBM HTP

miniaturization HTP

Acetyl-CoA

Heterologous

Mevalonate pathway

Normal

Biosynthesis

enzyme, steps

production of terpenoids in Y. lipolytica

Fig. 13 Synthesis of discussion O2, pH glycolysis, terpenoid production (Loayza Mora 2025)

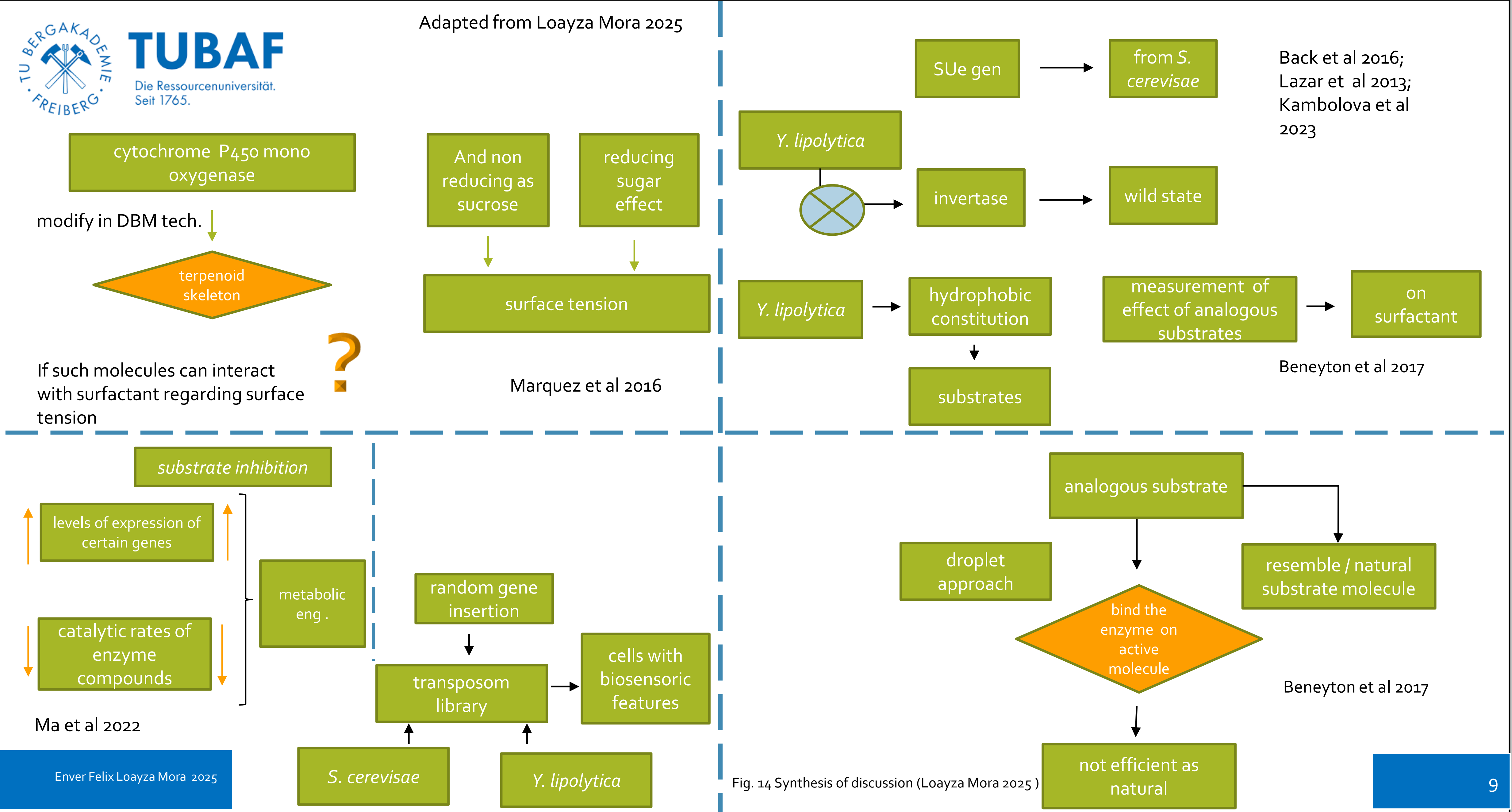

Fig. 14 Synthesis of discussion (Loayza Mora 2025)

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Adapted from Loayza Mora 2025

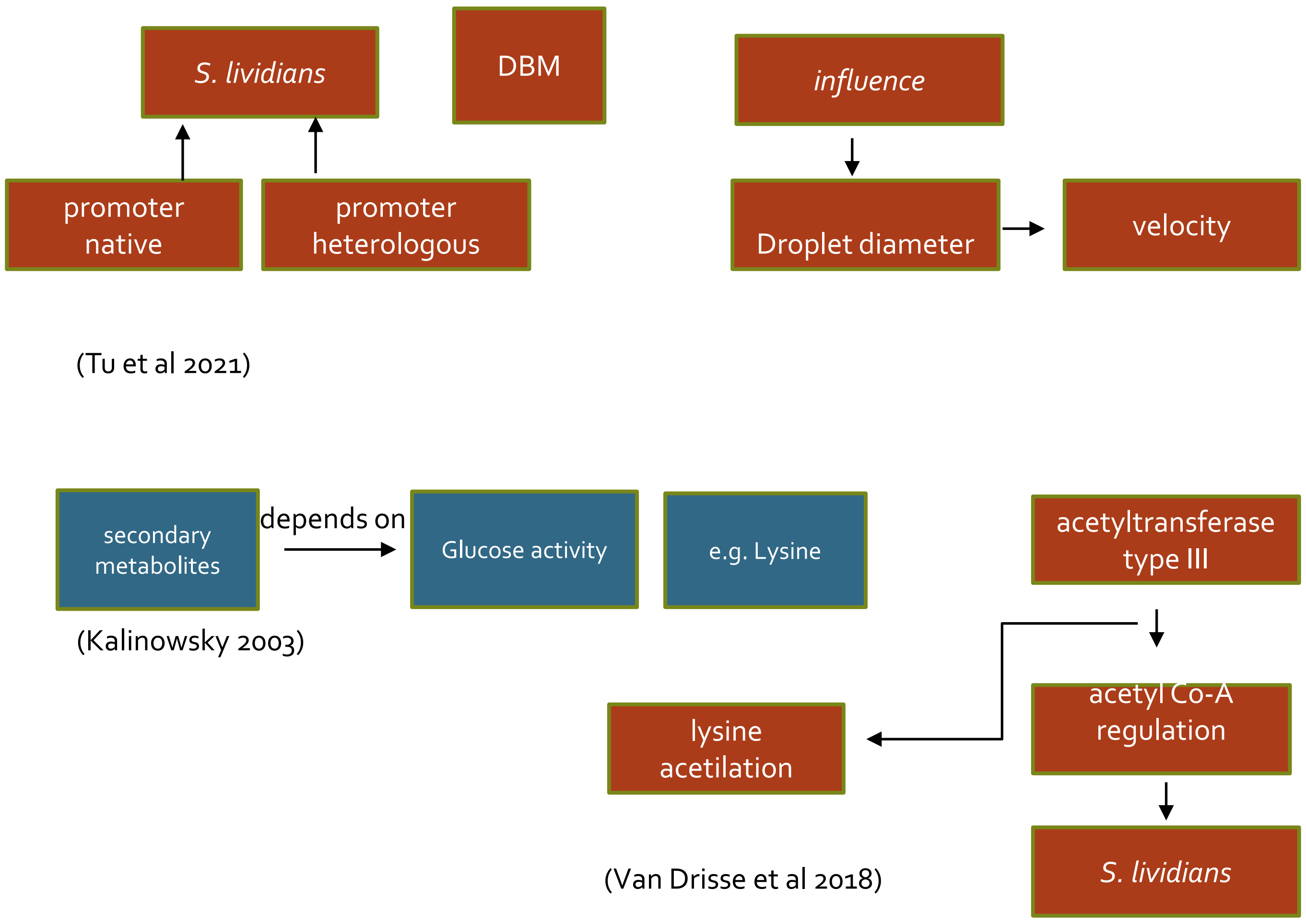

Fig. 15 Synthesis of discussion (Loayza Mora 2025)

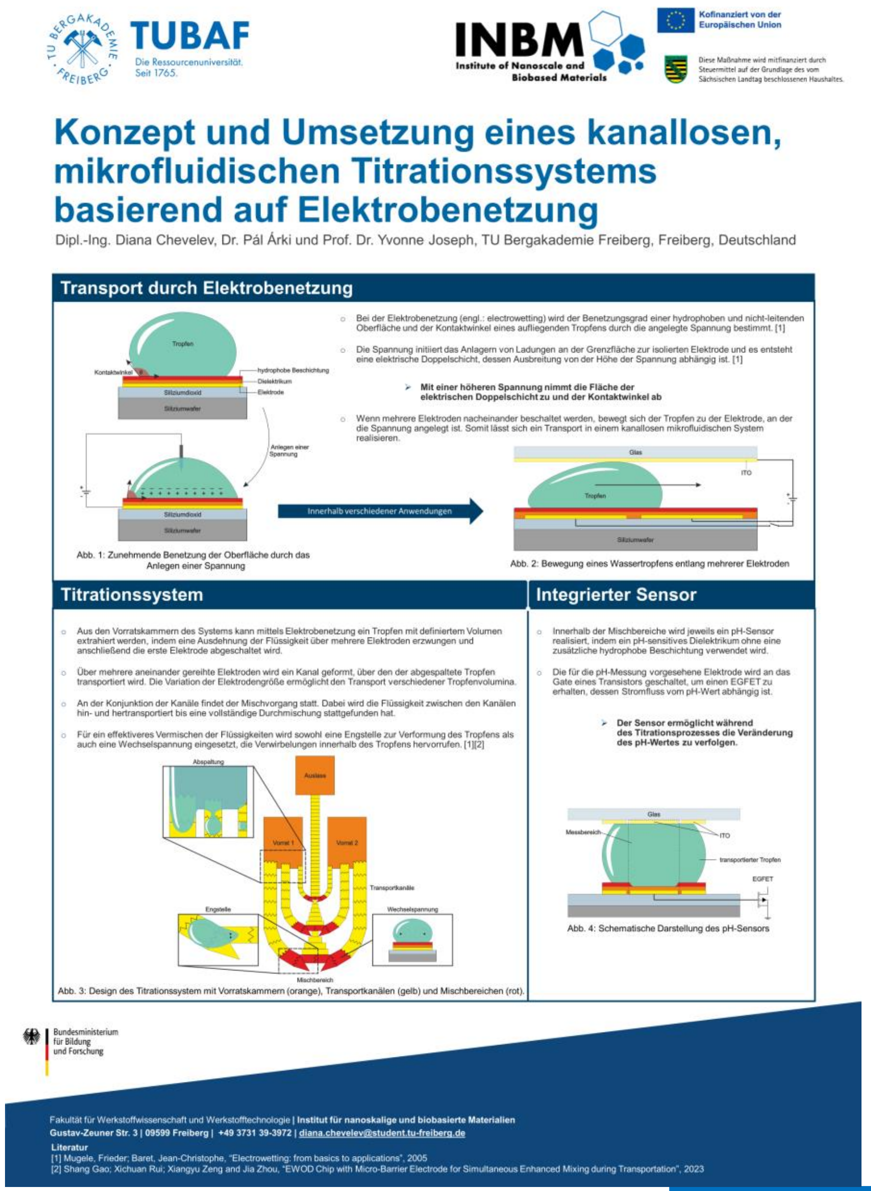

Fig. 16 *Titrationssystems* – at INBM TUB Freiberg Germany

Data monitoring → without cell lysis → secondary metabolites } **challenge**

C. glutamicum → segregation → enhanced → bioprocess optimization } Main advantage

Heterologous strain → Higher protein production > J0190

(Balasubramanian et al 2021)

Fig. 17 Synthesis of discussion (Loayza Mora 2025) C. glutamicum

Can segregated metabolites interact with antibiotics as a relevant effect during bioprocess?

Key :
Dependency -> protein acetylation -> glutamate production (overestimated)

(Nagano- Shoji et al 2025)

Adapted from Loayza Mora 2025

Challenges

Machines do not react so well to human intervention at the spontaneous and/or accidental way. They required follow their buckle or cycles.

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Adapted from Loayza Mora 2025

Automation in bioprocess

IMPACT
HTP
DBM
Parallelized bioreactors

Working at mL level
Integration of LHS
Optimization

Architecture
Required
Sensors

New approach
Integrate sensors to automation,
Specially to DBM.

e.g. biosensors and nanotech (quantum dots surface functionalization)

Fig. 18 Synthesis of discussion (Loayza Mora 2025).
Automation approach.

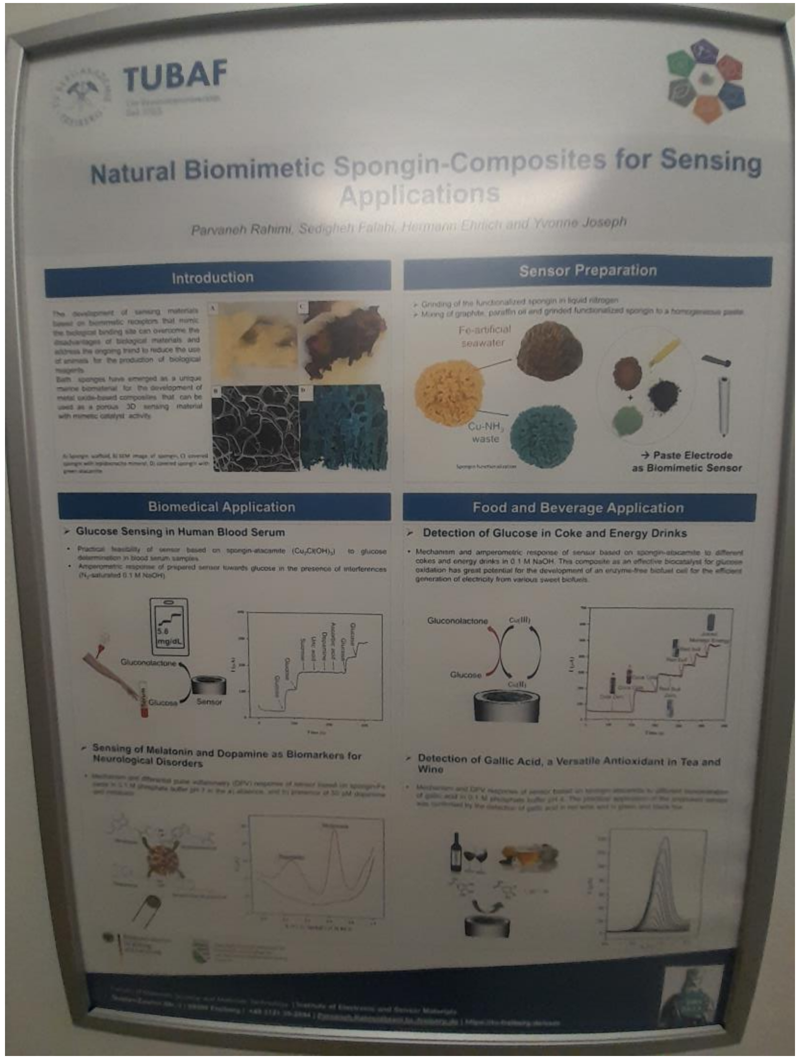
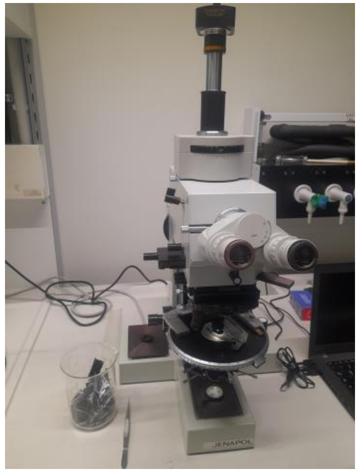
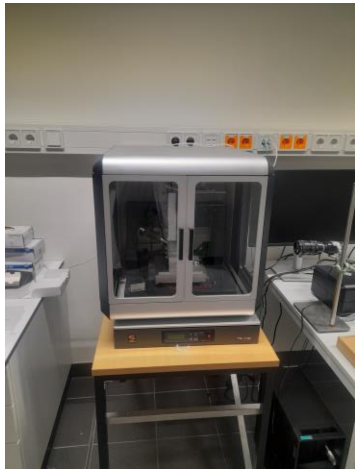


Fig.19 Sensing applications at INBM TUB Freiberg. Germany

5. Related projects





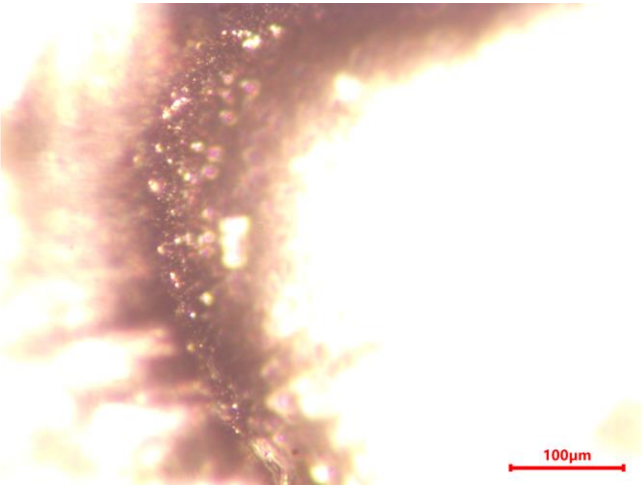
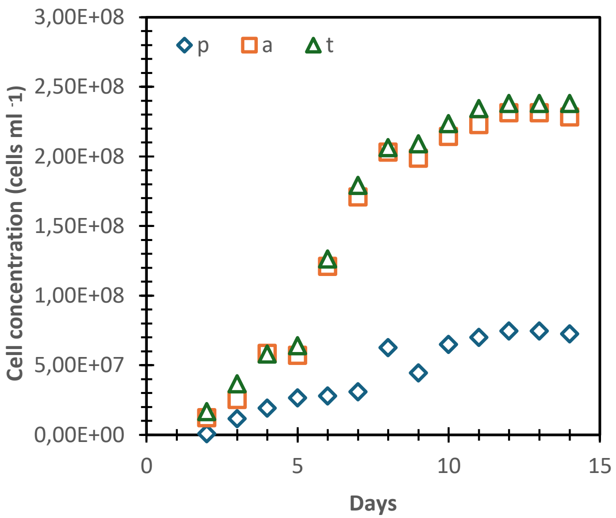


Fig. 20 Microalgae cells located into hydrogel surround. INBM TUB Freiberg. Foto: Loayza Mora, J. Tiang



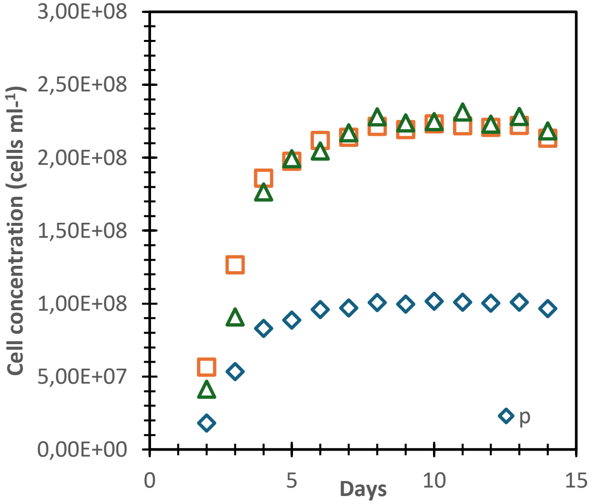


Fig. 21 Cell culture at HTP or semi HTP by using 200 µL during 2 weeks. Above 50 mL

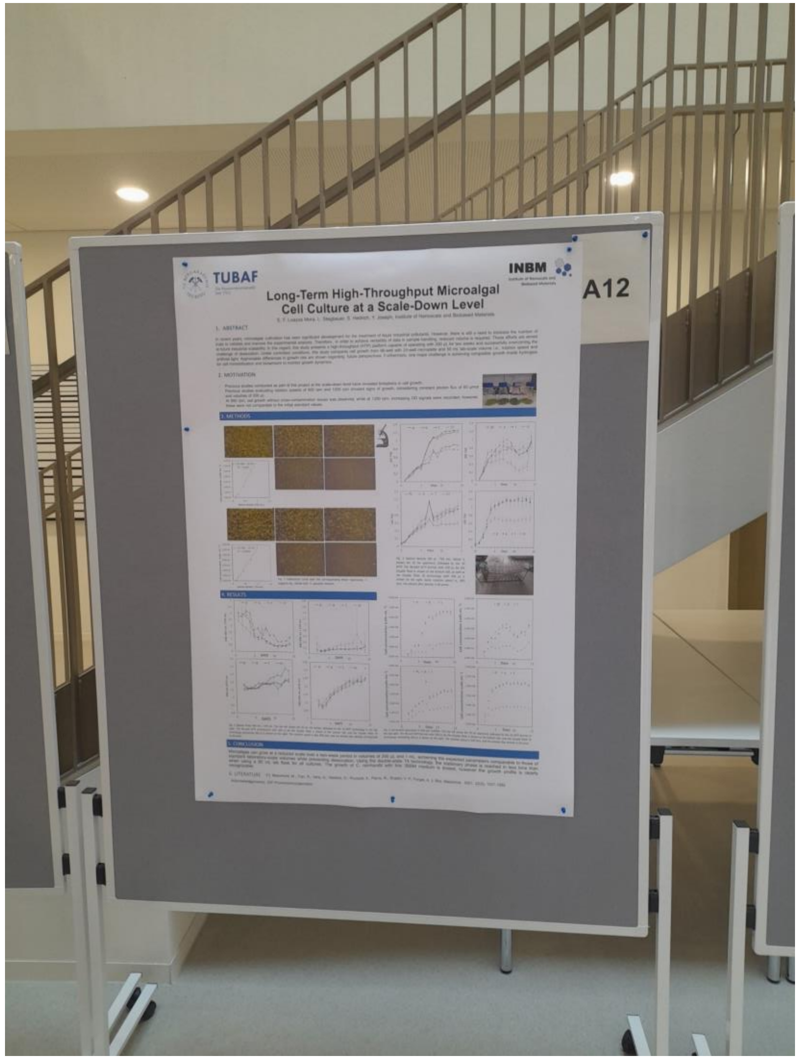


Fig. 22 Poster presentation Long term microalgal cell culture at HTP or semi HTP by using 200µL during 2 weeks

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- Institut von Bioverfahrenstechnik of Technische Universität Berlin
- Technische Fakultät - Universität Bielefeld
- Universidad Católica de Santa María


Keywords :
Bioproces, DBM, HTP, Automation, SERS-Raman

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