

Microscale Downstream Processing Toolbox for Screening and Process Development

Project acronym: MICROTOOLS

Project no: EIB.12.061

Nicolas Szita, University College London

ERA-IB-2 final conference, Berlin, 16./17.02.2016

Project Partners



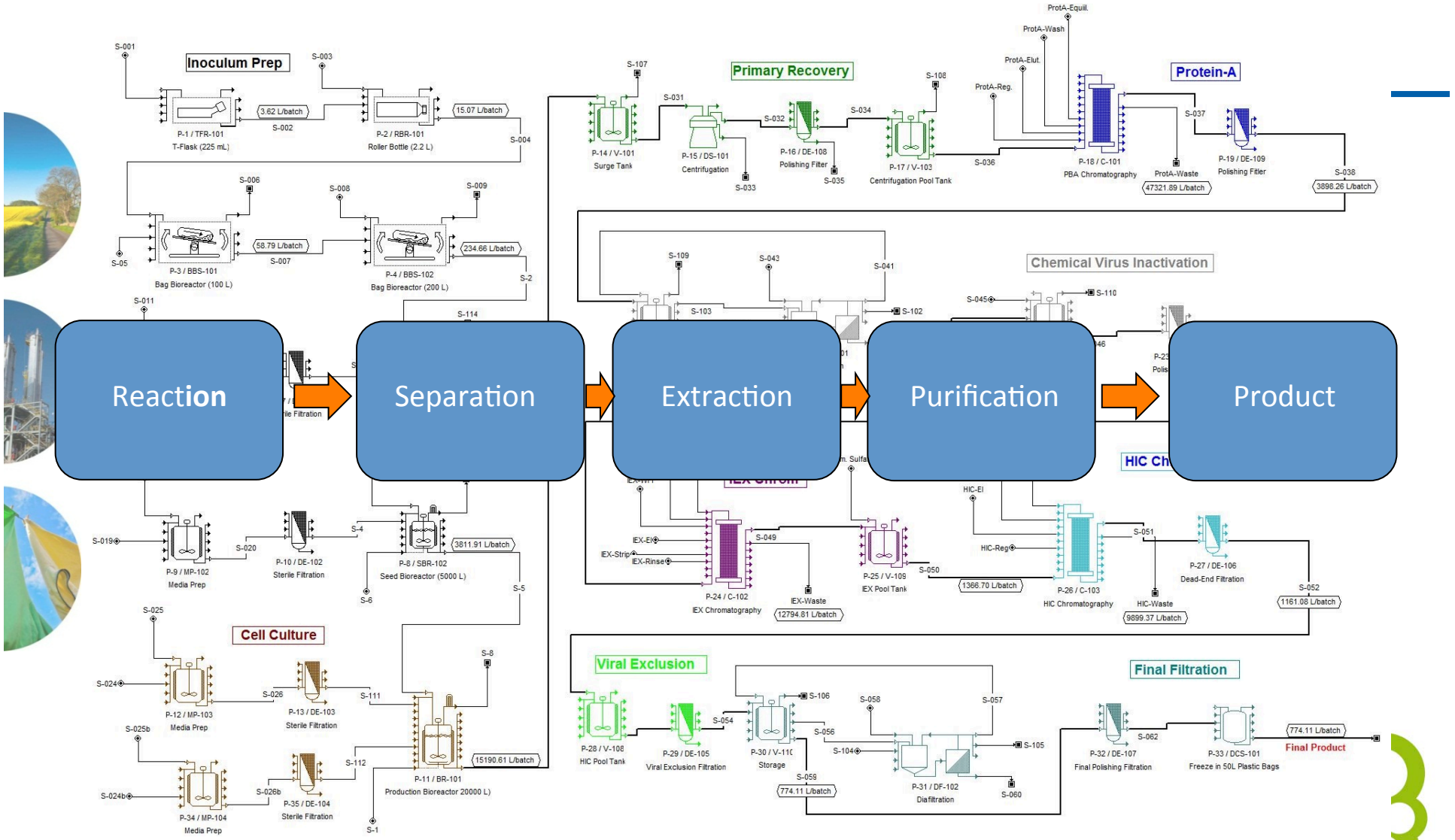
- *Department of Chemical and Biochemical Engineering, Technical University of Denmark (DTU); Denmark*
- *Svanholm.com; Denmark*
- *Chemical Engineering and Environmental Protection, "Gheorghe Asachi" Technical University of Iasi (TUI); Romania*
- *Medical Bioengineering, "Grigore T.Popa" University of Medicine and Pharmacy Iasi (UMPI); Romania*

- *Coordinator: Department of Biochemical Engineering, University College London (UCL); London, UK*
- *Total project budget: ~ €1.8M*

Introduction



- *Bio-based processes are suitable to achieve our goal of a greener economy.*
- *Economic feasibility is key to achieve this goal, but bio-based processes are non-trivial; separation/ extraction/purification steps can be costly.*
- *How then can we best improve existing processes and establish new ones in an efficient manner?*



Petrides et al (2014) Bioengineering 2014, 1(4), 154-187

Introduction



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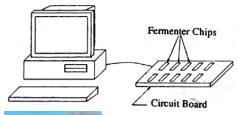
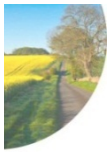
- *Economic feasibility is key to achieve this goal, but bio-based processes are non-trivial; separation/ extraction/purification steps can be costly.*
- *How then can we best improve existing processes and establish new ones in an efficient manner?*
- *Small (or micro) systems allow testing of separation/ purification steps with reduced amount of resources.*
- *Can we use such micro systems to obtain valuable information rapidly (in order to accelerate process design and implementation)?*

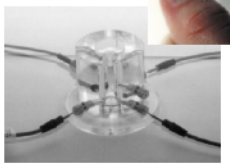
Miniaturisation of Reaction Step

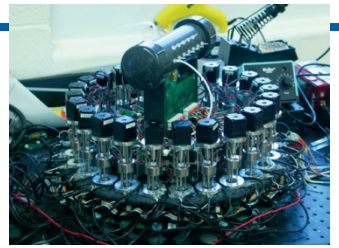
Kim, 1998
(Drexel/Hyundai)





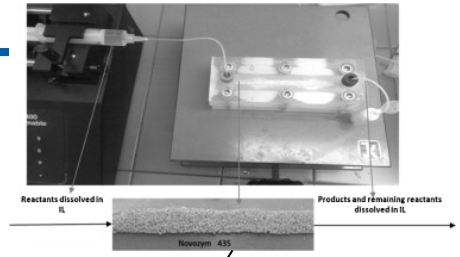
Lamping, 2003
(UC London)



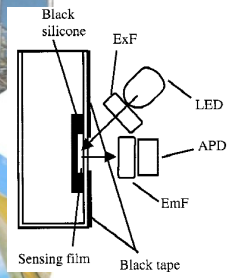


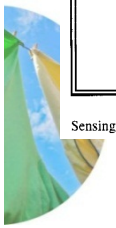
Harms, 2006
(U Maryland BC)

Cvjetko, 2012
(U Ljubljana)

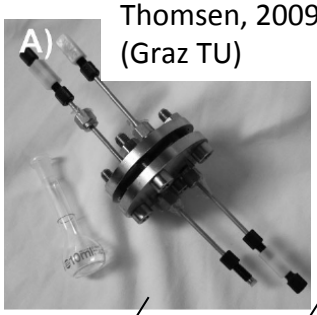


Kostov, 2001
(U Maryland BC)

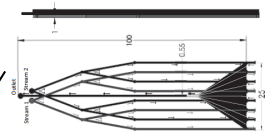




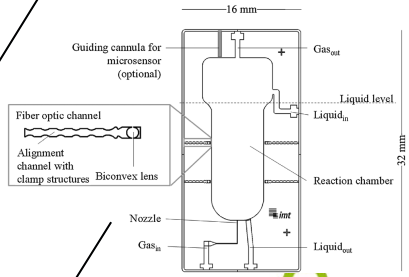
Thomsen, 2009
(Graz TU)



Bodla, 2013
(DTU)

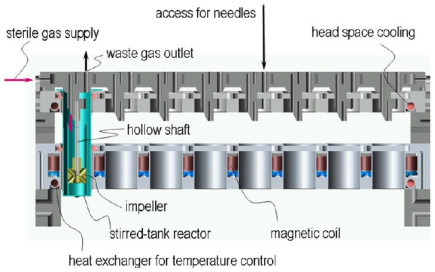


Peterat, 2014
(TU Braunschweig)

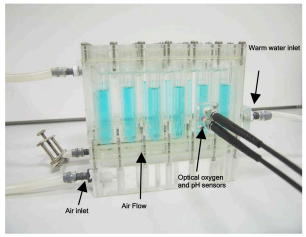


Szita, 2005
(MIT)

Weuster-Botz, 2005
(TU München)



Doig, 2005
(UC London)



Reichen, 2013
(UC London)

Today

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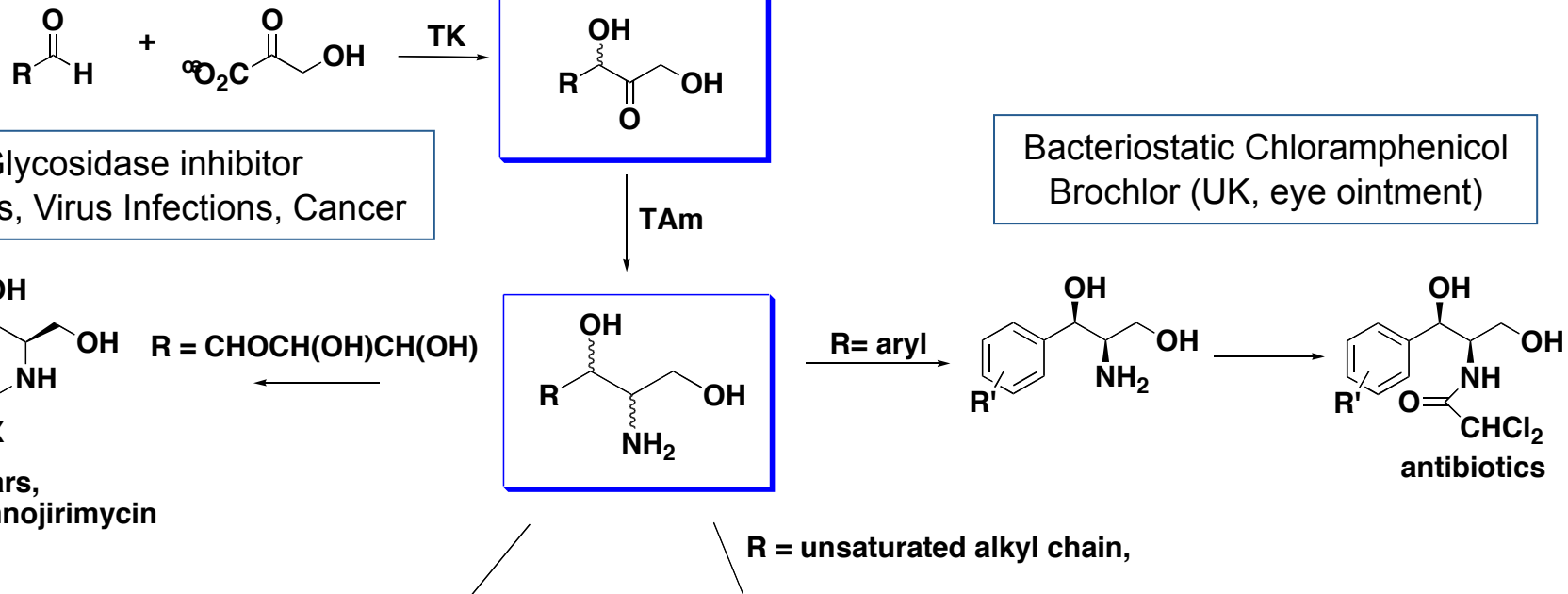
Biocatalytic Approaches to Keto/Amino-Diols



Glycosidase inhibitor
Diabetes, Virus Infections, Cancer

Bacteriostatic Chloramphenicol
Brochlor (UK, eye ointment)

Chiral synthon to make other synthons (asymmetric synthesis)

Brain lipids
- Vehicle to deliver drugs
- Biologically active

Reaction Cascading



Microfluidic Approaches 1

- *Reduced use of resources: expensive medium (such as isotopic labeled substrates, e.g. ^{13}C, or growth factors), scarce cells/biomolecules, also less waste*
- *High control over culture/process environment*
- *In-situ and real-time monitoring (pH, T, DO, OD, nutrient concentrations): relevant kinetic data (investigating and determining operating space, aka 'windows of operation')*

Microfluidic Approaches 2



- *Reduced footprint*
- *Fewer mechanical manipulations (set-up effort)*
- *Ease of integration with information technology*
- *Automation with robotics, for example for sample loading*

Capability to validate results must be maintained (effectively establish that results obtained are 'representative')

Miniaturised Downstream Unit Operations

- Less developed than micro(bio)reactors
- Development driven by the necessity for rapid and cost-effective process characterisation
- Enable optimisation of process parameters

Challenges

- Geometrical complexity of the industrial scale equipment
- Defining critical process criteria
- Integrated approach (train of unit operations)
- Yield appropriate material (including to test later operation performance)
- Modelling of individual unit operations (and complete processes)

Titchener-Hooker et al (2008) Biotechnology and Bioengineering, 100, 473-487

Priority Research Challenges for Bioprocessing



As identified by the BRIC (Bioprocessing Research Industry Club) in the UK

High-throughput process technologies

- automated ultra-scale down techniques
- predictive models

Effective modeling

- prediction of large scale

Improved Tools for Bioprocessing

Analytical methodologies

- improved analytical methods and tools for the design, analysis and control of bioprocessing
- measurement of critical parameters

Improved downstream processing

Technical Overview : Case Study

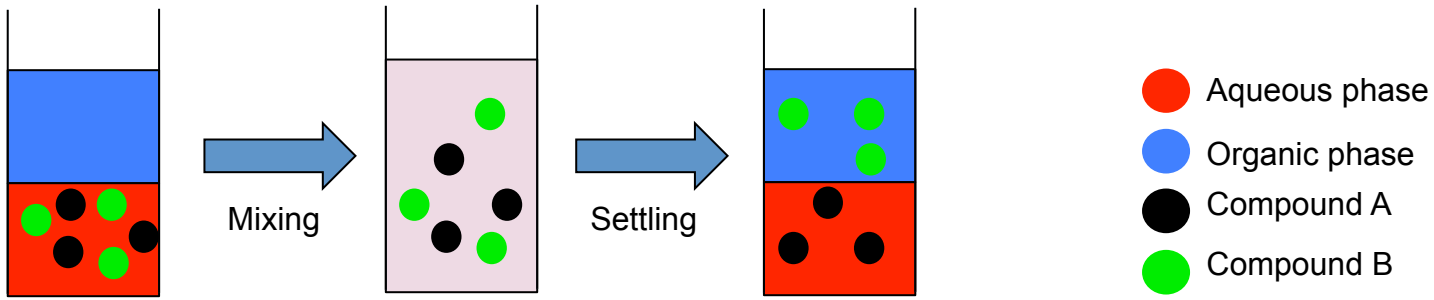


Liquid-Liquid Extraction Unit

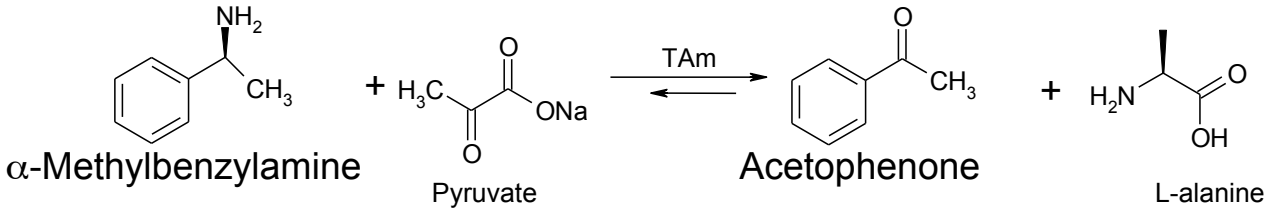
Extraction Mechanism



"Separation of compounds present in a mixture based on the solubility between two immiscible solvents"

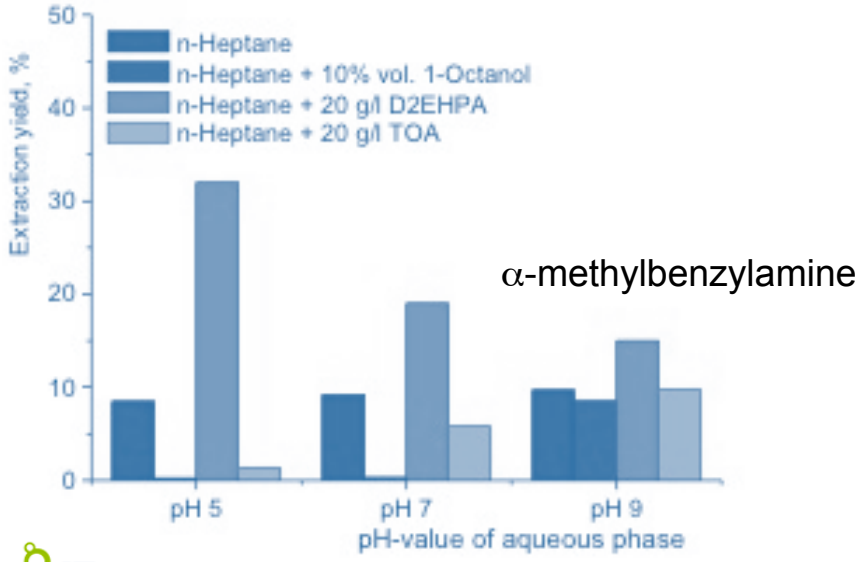


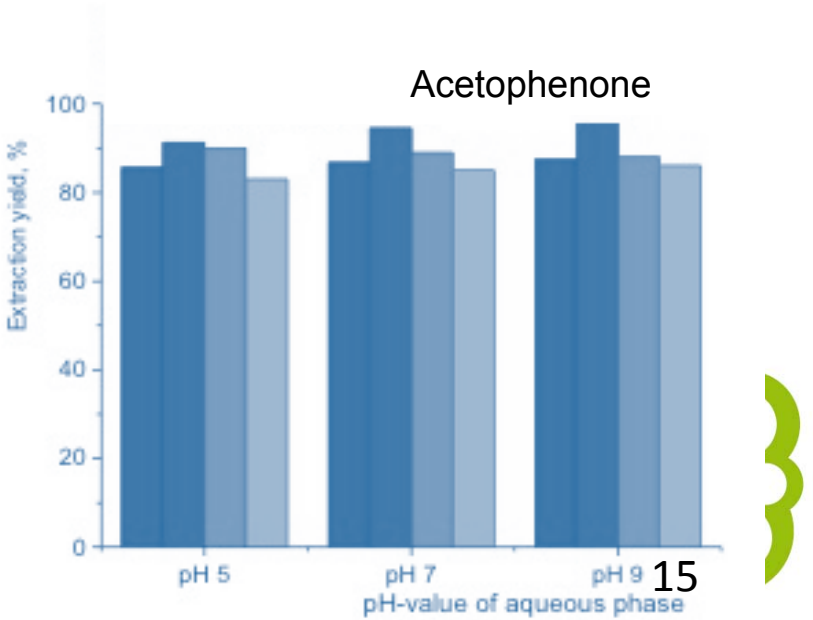
Extraction System (Bench-Scale)



- Building block in the pharmaceutical, chemical and agrochemical industries.

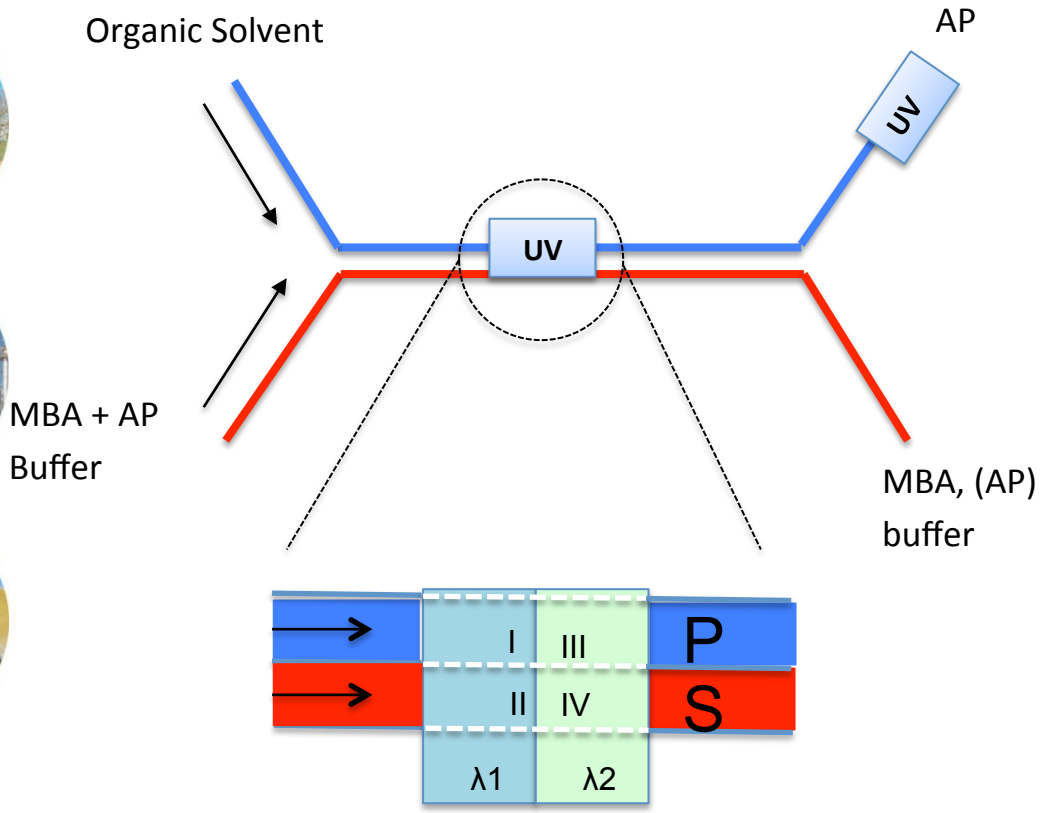
- raw material for the production of resins
- precursor of fragrances





Kloetzer et al. (in preparation)

The Overall Concept for the L-L Extraction

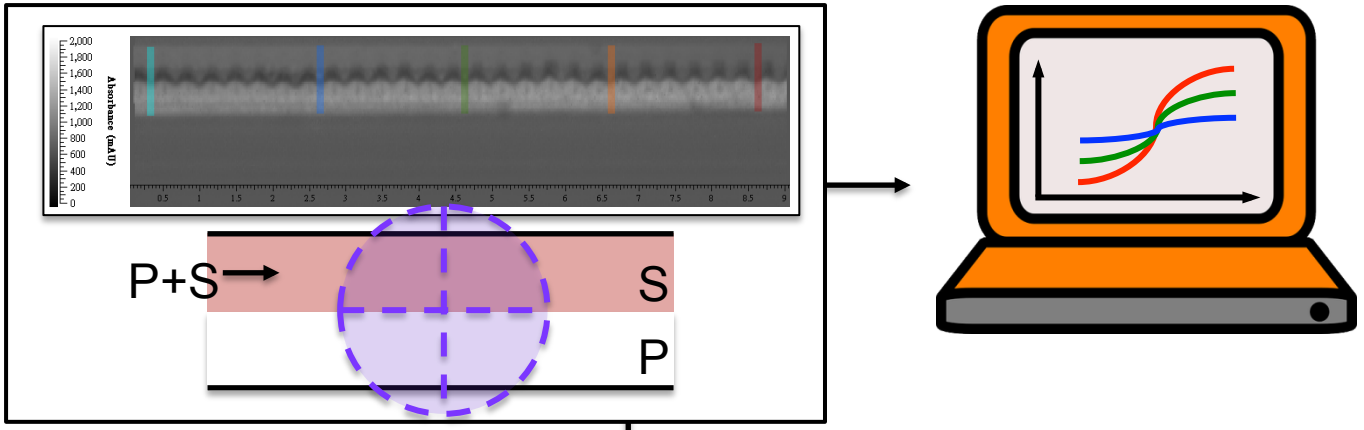


- Extraction yields are monitored in real time using UV-based absorbance spectroscopy
- Monitoring during extraction zone will be performed using a novel bespoke dual-wavelength detector system (Paraytec Ltd)

On-line Monitoring (UV Absorbance)

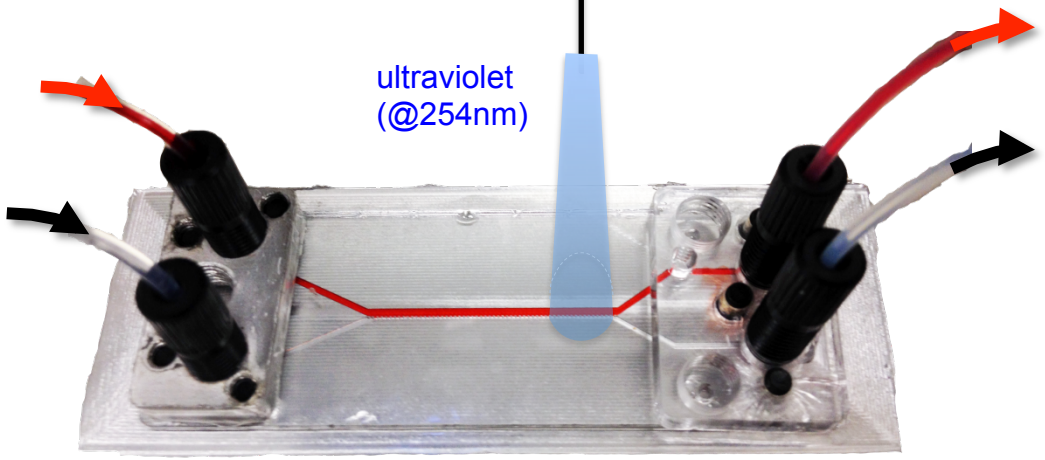


High interfacial area gives a suitable platform to investigate different interfacial phenomena



5 mM APH

n-Heptane



Summary



- *What was proposed*

Create a toolbox of microsystems that mimic unit operations of a biocatalytic process, with online monitoring, and standardised experimental protocol for scale translation

- *What was achieved*

L-L extraction unit, characterised, UV read-out, and mass transfer analysis; Filtration unit for enzyme separation/re-use, monitoring of trans-membrane pressure, addressing bio-fouling; resin-based ?

Summary

- *What are the plans for future (any follow-up projects?)*

We are still funded for most of 2016

- *Finalise case studies for TK and TAm-catalyzed reactions*

Put together a plug 'n' play system, i.e. not only provide a characterised toolbox of individual units, but also a platform technology (or at least better understanding the challenges)

Summary

- *What are the plans for future (any follow-up projects?)*

Still funded in 2016; follow-on projects, via new funding or mini-projects:

- *Interested in collaboration and apply our comprehensive microsystem technology platform for*
  - *Exemplify the versatility of our approach for one other enzyme system*
  - *Investigate scale translation, standardised experimental protocols*
  - *Explore/demonstrate potential to produce high-value/low-volume compounds/metabolites*
  - *Investigate process condition screening*

Project Outcomes

Design and fabrication of several microfluidic downstream devices prototypes , some for the first time (e.g Flocculation device)

Put together a plug 'n' play system, i.e. not only provide a characterised toolbox of individual units, but also a platform technology (or at least understanding the challenges in full)

Development of new monitoring system (in collaboration with Paraytec Ltd, UK)

Pushing the boundaries of NIR and Raman monitoring capabilities in microfluidics device)

Project Outcomes

So far:

1 article published

2 PhD thesis (partially) and 3 MSc thesis (more to come)

Several conference presentations

In the pipeline:

- *Pallipurath et al.,* Flocculation on chip: a novel approach to determine growth rates of single flocs
- *Chiang et al.*. Micropillar-based aqueous-organic continuous liquid-liquid extraction device for the extraction of pharmaceutical compounds
- *Lawrence et al.* Automated Microfluidic Filtration device

General Evaluation



- *Benefits of international collaboration; publications; exchange of researchers etc.*
  - *Exchange of researchers*
  - *Access to new technologies*
  - *Collaboration with industrial partners, Sigma-Aldrich*
  - *Collaboration with UK SMEs (Paraytec)*
  - *Collaboration with Foundries*
  - *Opportunities for new projects*
  - *Visiting Professor*
  - *Greater in-house synergies*

Visiting Professor

Prof Ph Renaud, EPFL, Switzerland, @ UCL, April – June 2015

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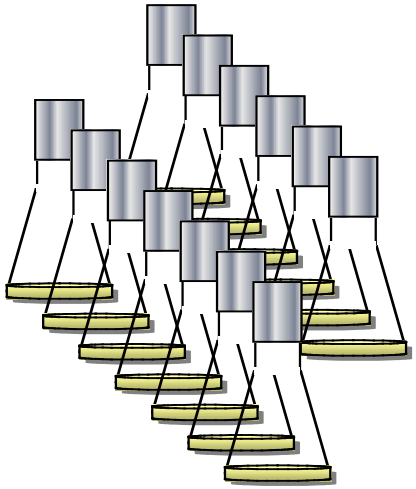
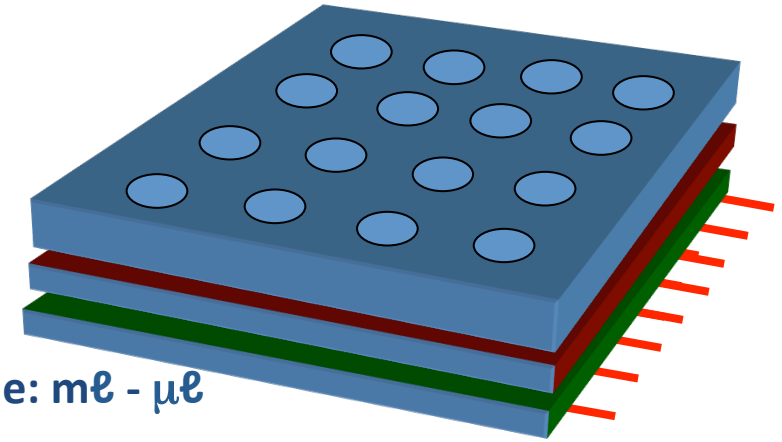
Enhanced In-house Synergies

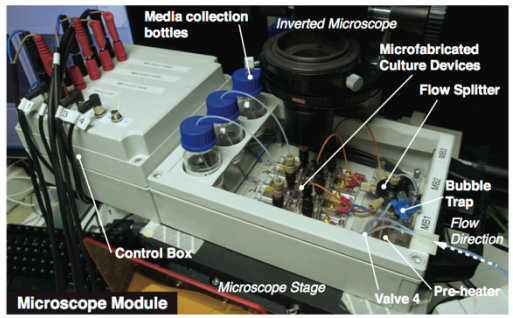




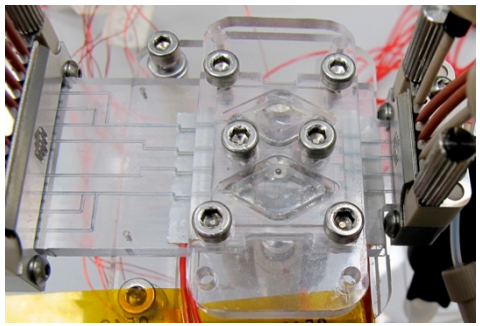
New laboratory scale: mℓ - μℓ

Development of novel culture vessels with system-wide integration of analytics to facilitate process understanding towards:

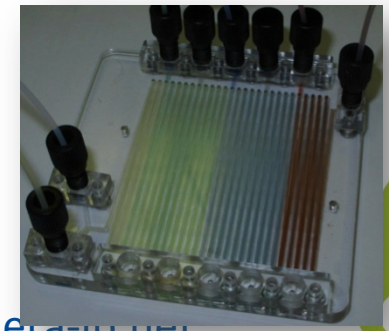
Production of Cells



Antibiotics



Small Molecule Drugs



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General Evaluation

- *Comments, feedback to ERA-IB*
  - *Fantastic programme with great opportunities*
  - *Partners should ideally start at the same time*
  - *Is there assistance in organising a topical workshop?*

  "Opportunities and pitfalls of microsystems for industrial biotechnology"

Contact Details



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