www.amtechuk.com
gareth.jenkins@amtechuk.com

Process Intensification of Industrial Biocatalysis

or how to scale up without huge reactor volumes

Gareth Jenkins, COO

www.amtechuk.com
gareth.jenkins@amtechuk.com

Outline

- Addressing sustainability through industrial biocatalysis
- Reaction engineering and reactor sizing for IB
- Coflore Tubular Reactors
- Example applications for IB

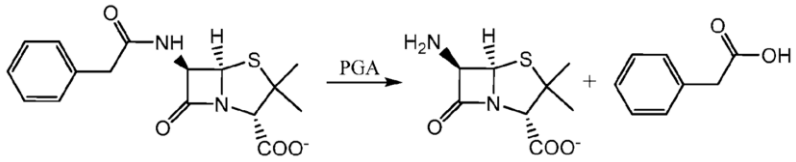
www.amtechuk.com
gareth.jenkins@amtechuk.com

Industrial Biocatalysis

www.amtechuk.com
gareth.jenkins@amtechuk.com

Example of the Sustainability of Enzyme-based Production Processes

6-Aminopenicillanic acid
Production (500 t)



Chemical Process

1000 t penicillin G

300 t dimethylchlorosilane

800 t N,N-dimethylaniline

600 t phosphopentachloride

160 t ammonia

4200 m^3 dichloromethane

4200 m^3 n-butanol

Enzyme Process

1000 t penicillin G

45 t ammonia

1 t immobilised enzyme

10000 m^3 water

www.amtechuk.com
gareth.jenkins@amtechuk.com

Advantages/Disadvantages compared to chemical catalysts

Advantages

- Stereo- and regio- selective
- Low temperature (0 – 110 °C)
- Low energy consumption
- Active at pH 2 – 12
- Less by-products
- Nontoxic when used correctly
- Can be reused
- Can be degraded biologically
- Can be produced in unlimited quantities

Disadvantages

- Cells and enzymes are
  - Unstable at high temperatures
  - Unstable at extreme pH
  - Unstable in aggressive solvents
  - Inhibited by some metal ions
  - Hydrolysed by petidases
- Some enzymes
  - Are still very expensive
  - Require costly cofactors
- When inhaled or ingested, are potential allergens
- Low substrate concentrations (nature operates at <0.01 M, can reach <1 M)

www.amtechuk.com
gareth.jenkins@amtechuk.com

Applications of new enzyme processes to meet sustainability challenges

- Production of optically pure fine chemicals
  - Especially where racemate is currently made and then resolved (50% waste)
- Synthesis of antibiotics
- Synthesis of pharmaceutical intermediates
- Paper production
- Oligosaccharide production for food or pharma
- Selective glycosylation of peptides/proteins and other biomolecular drugs
- Modification of lipids, fats and oils
- For environmental biotechnology
- Synthesis of biofuels from biomass
- Production of bulk products from biomass in biorefineries

www.amtechuk.com
gareth.jenkins@amtechuk.com

Characteristics of a Biotransformation

- Potential Issues
  - Substrate solubility
  - Substrate concentration
  - Substrate inhibition
  - Product solubility
  - Product concentration
  - Product inhibition
- Observed Reaction Rates
  - Slow kinetics
  - Limited ability to increase temp
  - Limited ability to increase conc
  - Mass transfer limited
  - Mixing sensitive

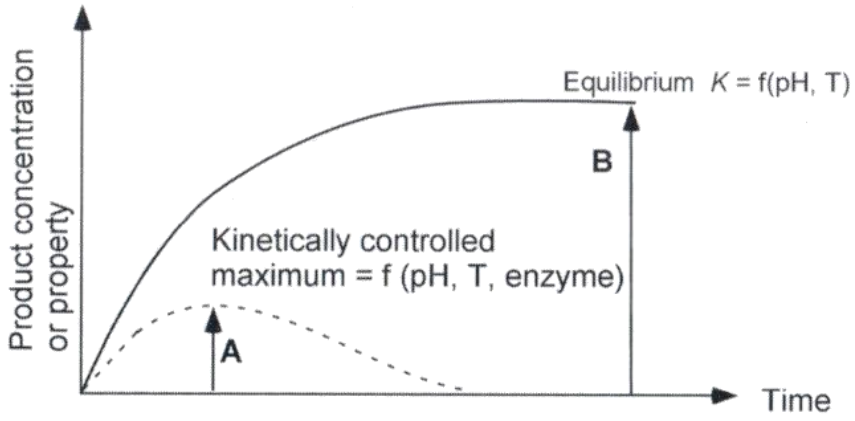
www.amtechuk.com
gareth.jenkins@amtechuk.com

Reaction Engineering

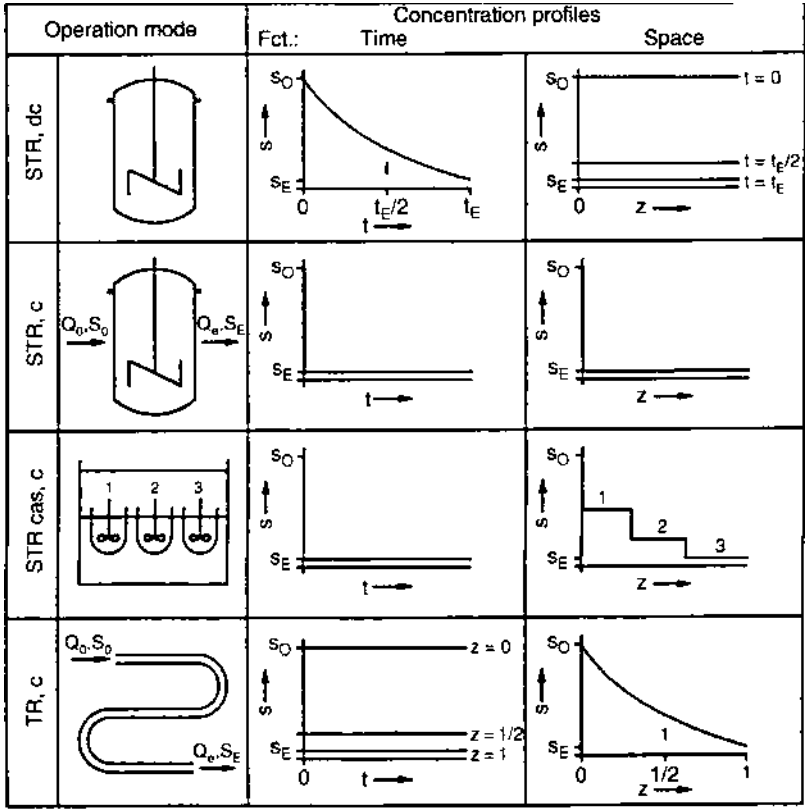
www.amtechuk.com
gareth.jenkins@amtechuk.com

Reaction Engineering

Kinetic or Thermodynamic control



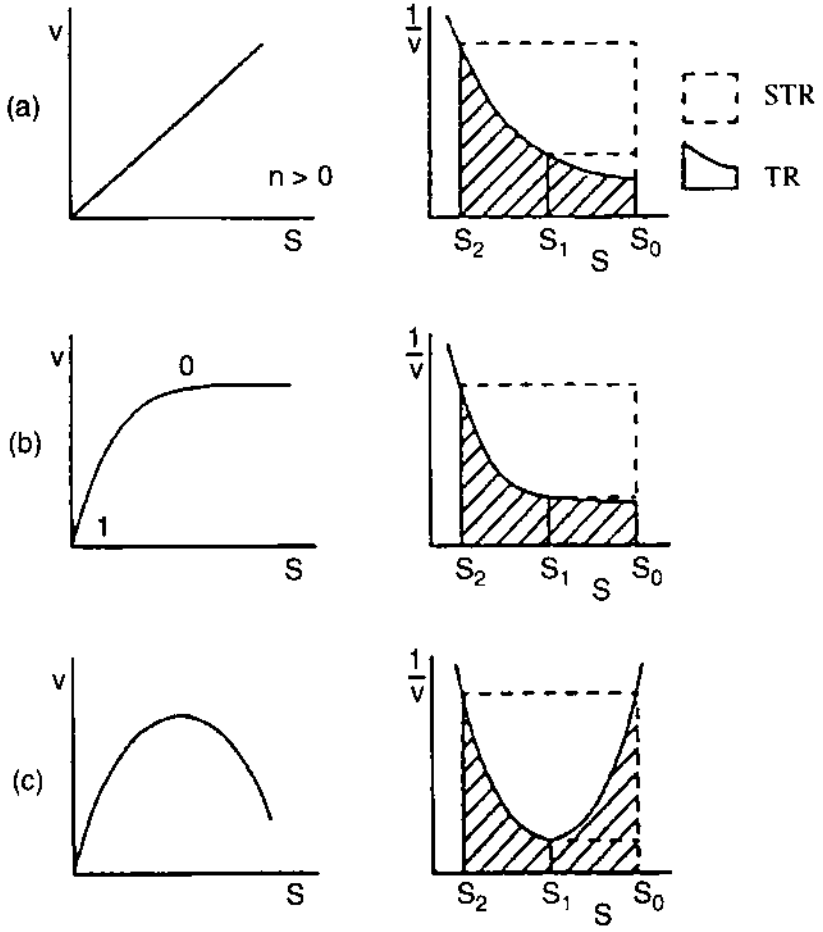
Stirred Tank or Tubular Reactor



www.amtechuk.com
gareth.jenkins@amtechuk.com

Sizing the Reactor

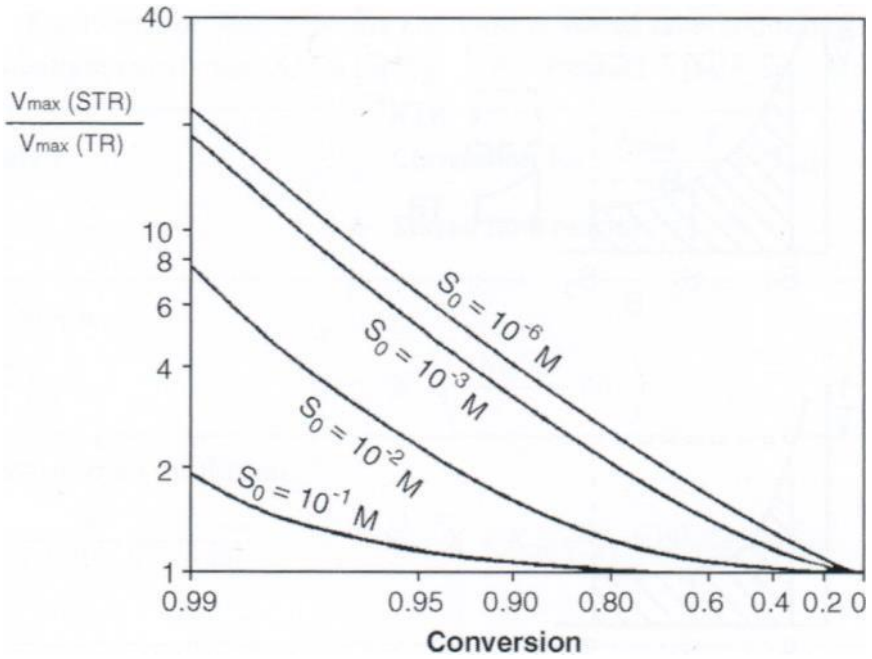
- For reactions with rate order >0
  - Tube reactor significantly smaller that an STR, especially as conversion increases
- For Michaelis-Menton kinetics
  - During zero-order phase, TR and STR are same size (ie $[S]_E > K_m$) but if higher conversions needed, TR will be smaller that STR
- Where substrate inhibition occurs, the choice of reactor depends on conversion required
  - Low conversion (S_1) = STR
  - High converstion (S_2) = TR



www.amtechuk.com
gareth.jenkins@amtechuk.com

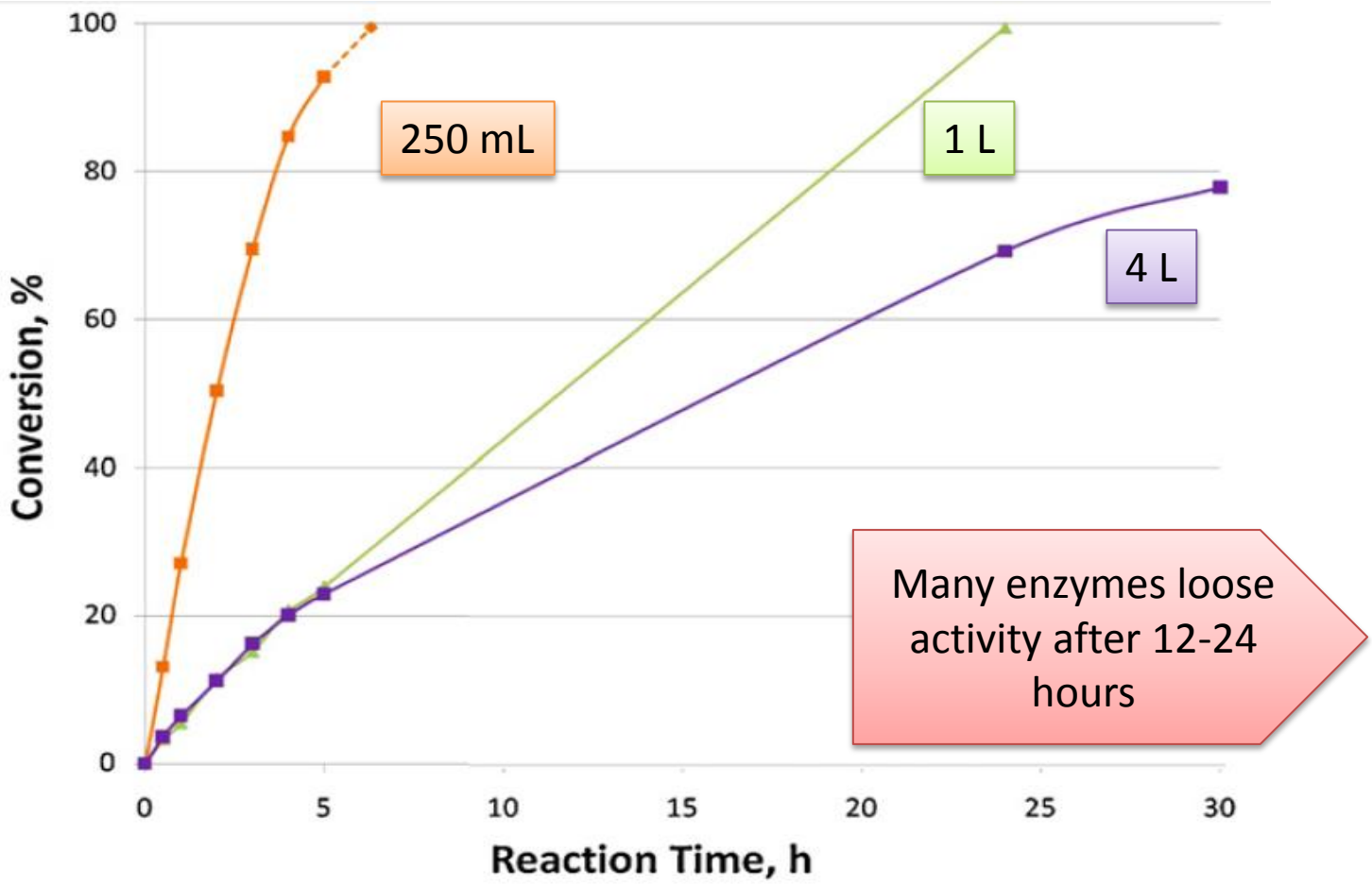
Influence of initial substrate concentration on reactor size

- The more dilute the initial substrate concentration, the more pronounced the difference in reactor size becomes
  - Low substrate concentration results in STRs that are 20x larger than PFRs
  - At high substrate concentration, STRs are still double the size of a PFR



www.amtechuk.com
gareth.jenkins@amtechuk.com

The Batch Scale-up Effect: Larger Scale Means Longer Reaction Times

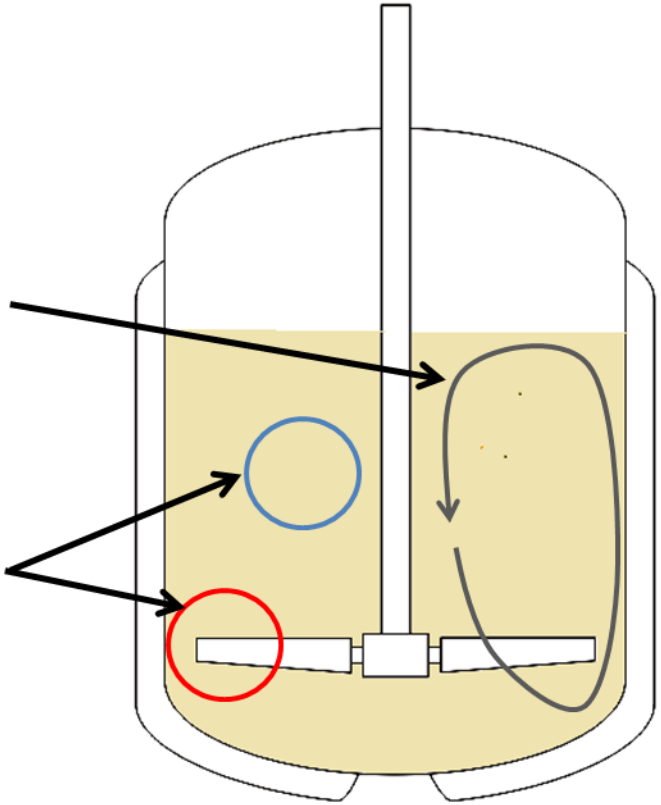


www.amtechuk.com
gareth.jenkins@amtechuk.com

Multi-phase systems and Mass Transfer

Batch reactor

- As a rule of thumb, the blending time in a batch reactor is the time it takes the fluid to travel 5 times around the mixing path. Since $P/V \propto V^2$, mixing times get slower due to mechanical limitations of the agitator.
- Shear (for overcoming mass transfer limitations) varies widely according to location within the vessel (O high to O low)



www.amtechuk.com
gareth.jenkins@amtechuk.com

Coflore Tubular Reactors

www.amtechuk.com
gareth.jenkins@amtechuk.com

Flow Reactor Market

The current flow reactor market is dominated by two types of flow reactor

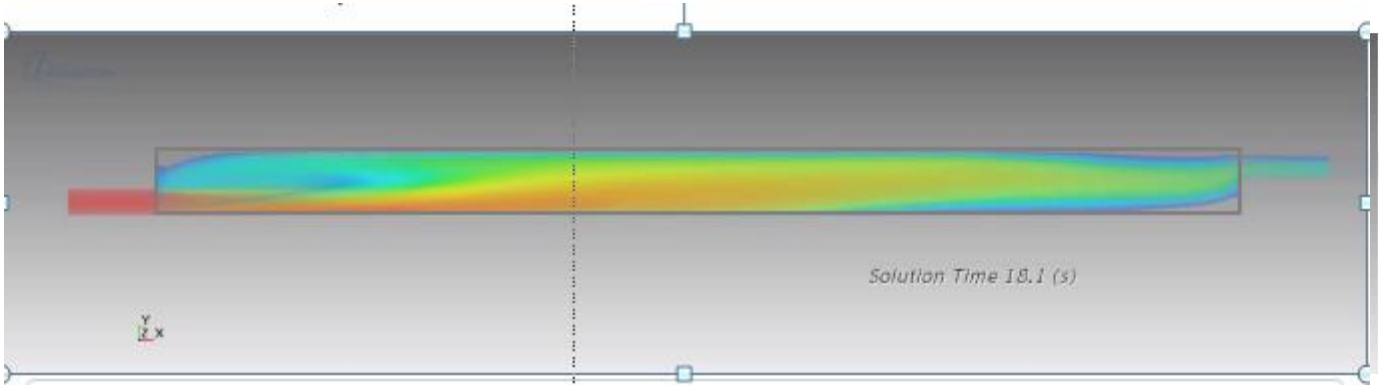
Micro reactors (Uniqsis, Syrris, Vapourtec, Ehrfeld, etc)

These are research machines and too small for industrial use.

Passively mixed flow reactors (Corning, Ehrfeld, ESK, Alfa Laval)

The are a variety of solutions based on static mixing and turbulent flow in small tubes. These are limited to short reaction times and generally clean fluids.

The limitation of passively mixed reactors is that fluids do not mix well at low velocities



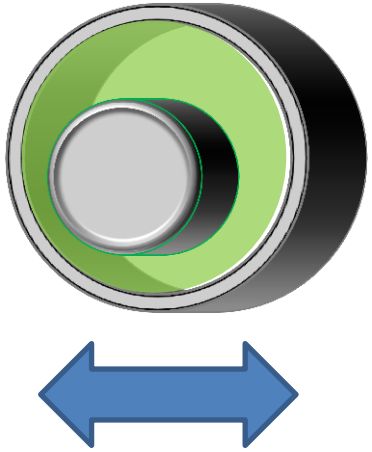
Courtesy of CD-adapco™

www.amtechuk.com
gareth.jenkins@amtechuk.com

Colfore Reactors

Coflore reactors use mechanical movement of the reactor body combined with free moving agitators to generate mixing. This is an inherently simpler and better way of mixing in flow systems.

- Efficient radial mixing
- No baffles (self baffling), no centrifugal effects
- No seals or magnetic couplings
- No shaft stability problems



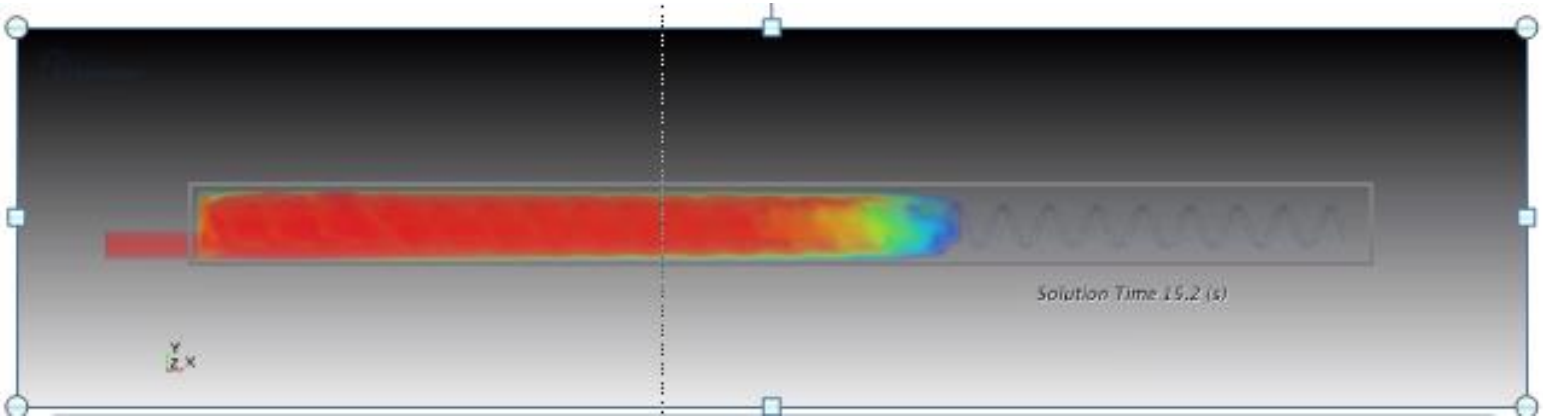
Lab Scale Unit
10 x 10ml = 100 ml

10 Tube Based System
10 x 100ml = 1L

www.amtechuk.com
gareth.jenkins@amtechuk.com

Coflore agitation – dynamic active mixing in large diameter tubes

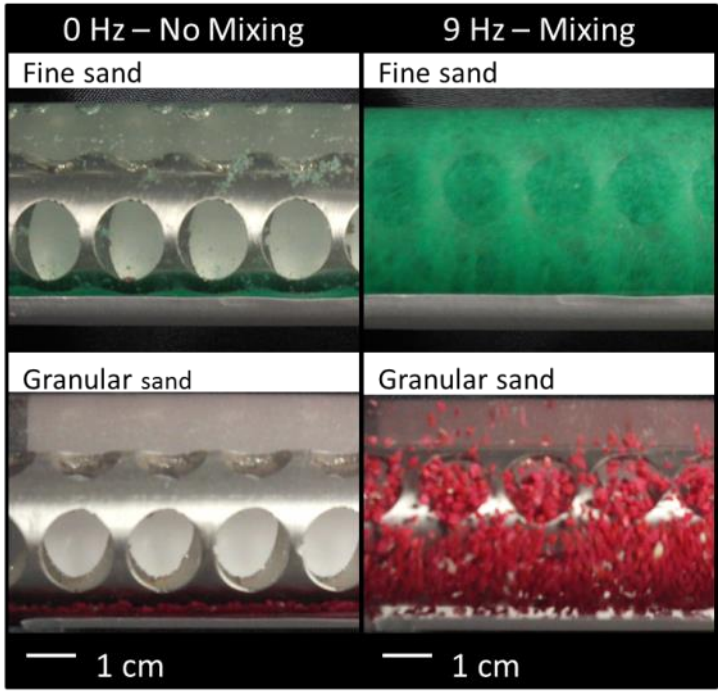
- CFD animation of tracer dye injected into Coflore mixed 22mm diameter tube at fluid velocity of 0.1 m/s with 5 Hz shaking



Courtesy of CD-Adapco™

www.amtechuk.com
gareth.jenkins@amtechuk.com

COFLORE Processing Advantages



- Mixed phases:
  - Liquid-liquid
  - Liquid-solid
  - Liquid-gas
  - Liquid-solid-gas
- Applications:
  - Heterogeneous catalysis
  - Biocatalysis
- Mass transfer limited processes
- Slow kinetic or thermodynamic limited processes
- Active mixing decouples flow rate and tube length from mixing
  - Shorter reactor tubes
  - Much lower pressure drop
  - Less start up / shutdown waste

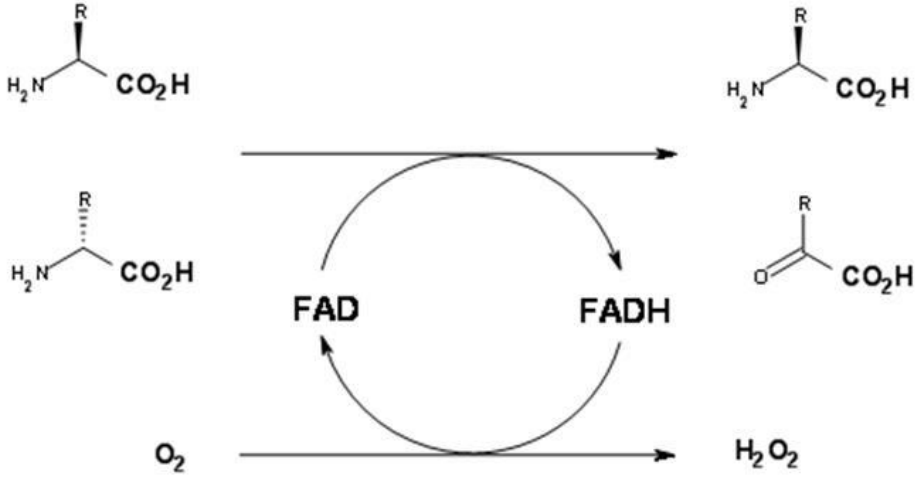
www.amtechuk.com
gareth.jenkins@amtechuk.com

Example applications with IB

www.amtechuk.com
gareth.jenkins@amtechuk.com

Study #1 - Biocatalytic oxidase SCALABILITY

- DL – amino acid resolution:



- Production of L – amino acids and α – keto acid.
- Move away from using a batch process towards a continuous system.
- G/L/S system.
- > 24 hours reaction time
- Enzyme presented as freeze-dried whole cells.

ingenza

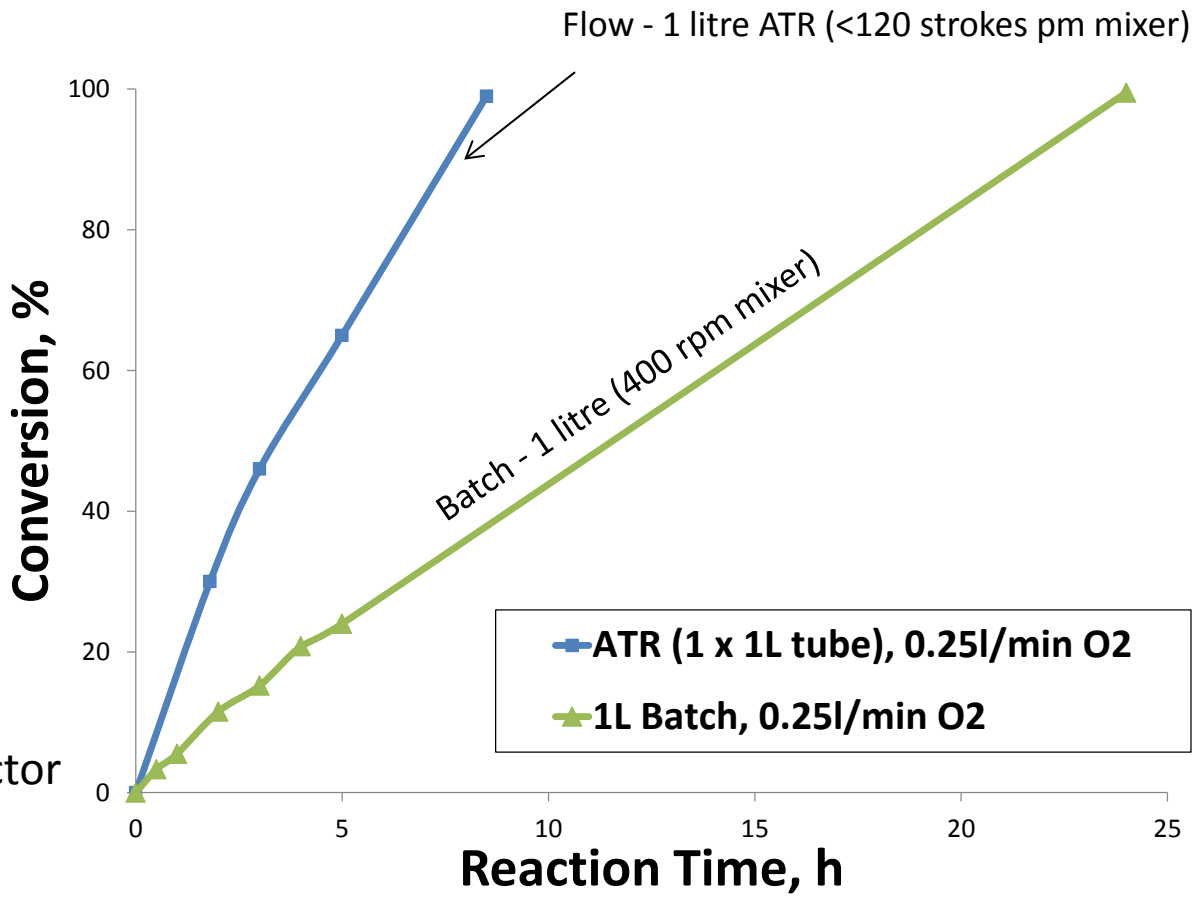
C-Tech INNOVATION

BIOCHEMIST

www.amtechuk.com
gareth.jenkins@amtechuk.com

Biocatalytic oxidase – Scale Up

1-10 litre ATR flow reactor

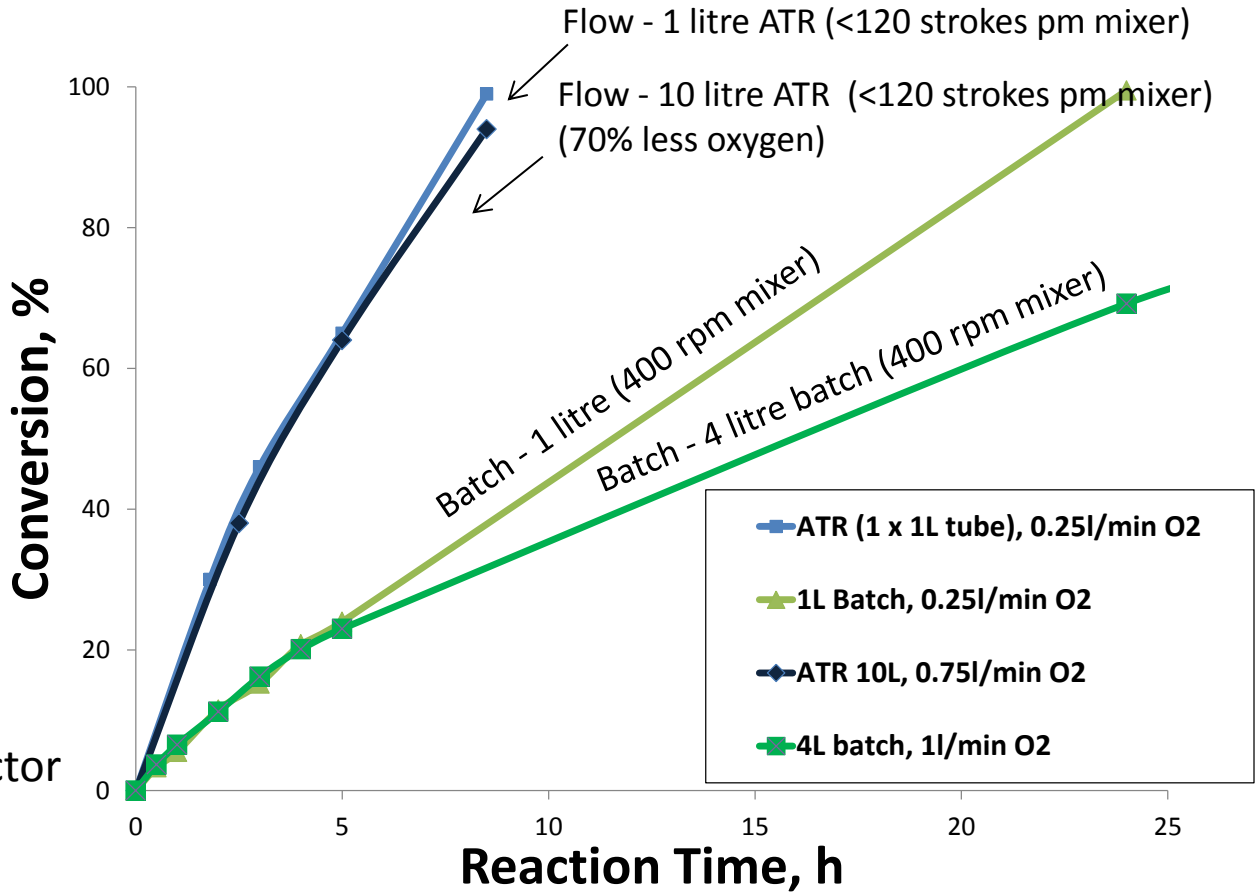


BIOCHEMIST

www.amtechuk.com
gareth.jenkins@amtechuk.com

Biocatalytic oxidase – Scale Up

1-10 litre ATR flow reactor



BIOCHEMIST

www.amtechuk.com
gareth.jenkins@amtechuk.com

Biocatalytic oxidase – Scale Up

1-10 litre ATR flow reactor

Continuous makes this process scalable

LCA data: 10 L continuous vs 10 1L batch cycles

- 88% reduction in kWh/L consumption
- 90% reduction in CO2 production

Energy consumption and CO2 production increase more slowly in continuous than batch

→ even more benefits will be achieved at larger scale

BIOCHEMIST

www.amtechuk.com
gareth.jenkins@amtechuk.com

Conclusions Study #1

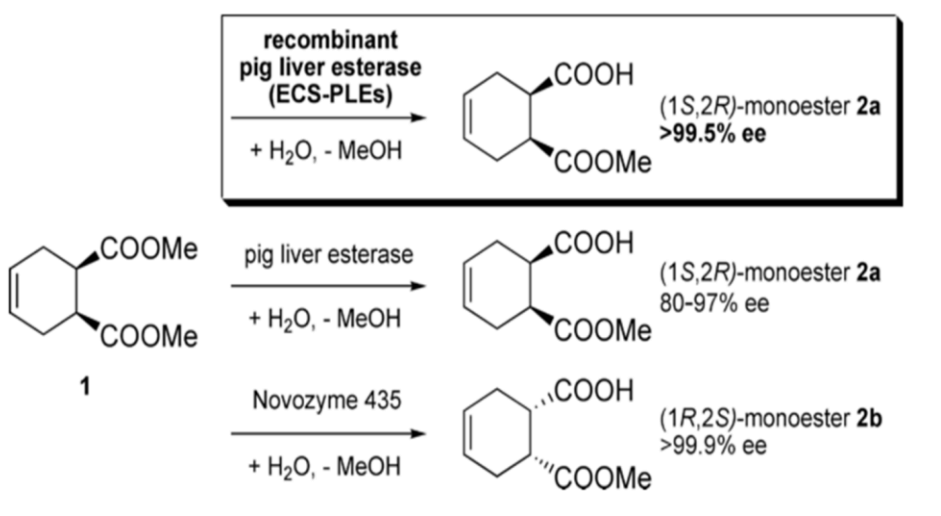
- 3-Fold increase in conversion compared to small stirred batch
- Almost identical, excellent conversion using 70% lower oxygen

www.amtechuk.com
gareth.jenkins@amtechuk.com

Study #2 - Biocatalytic Desymmetrisation

SPACE TIME YIELD IMPROVEMENTS

- Pig Liver esterase catalyse desymmetrization of dimethyl cyclohex-4-ene-cis-1,2-dicarboxylate
- Catalyst as a cell paste or its lyophilisate, dissolves in water
- Substrate is immiscible with water
- Simple buffer system (NaHCO3): Gas generation



Enzymical®
Enzymes | Chemicals | Solutions

Süss, P. et al, *Org. Process Res. Dev.*, **2014**, *18* (7), pp 897–903

Stirred tank (from the paper)

- 8.5L
- ECS-PLE06 = 140 U/g diester
- T = 40°C
- Full conversion in 4 h
- 82% yield, > 99.5% ee

www.amtechuk.com
gareth.jenkins@amtechuk.com

Biocatalytic esterification

Batch

- Large scale stirred tank (from the paper)
  - 8.5L
  - ECS-PLE06 = 5.5 U/mL
  - T = 40°C
  - Full conversion in 4 h
  - 82% yield, > 99.5% ee
- Small scale stirred tank
  - 10mL
  - ECS-PLE-06 = 5.5U/mL
  - T = 44°C
  - 14.03% conversion in 30 minutes

Initial Continuous Feasibility Study

- Inconsistent results
- Dimethyl cyclohex-4-ene-cis-1,2-dicarboxylate immiscible in water
- Unstable enzyme / buffer solution
- Full conversion in 50 min
- 38 experiments, 150 ml each

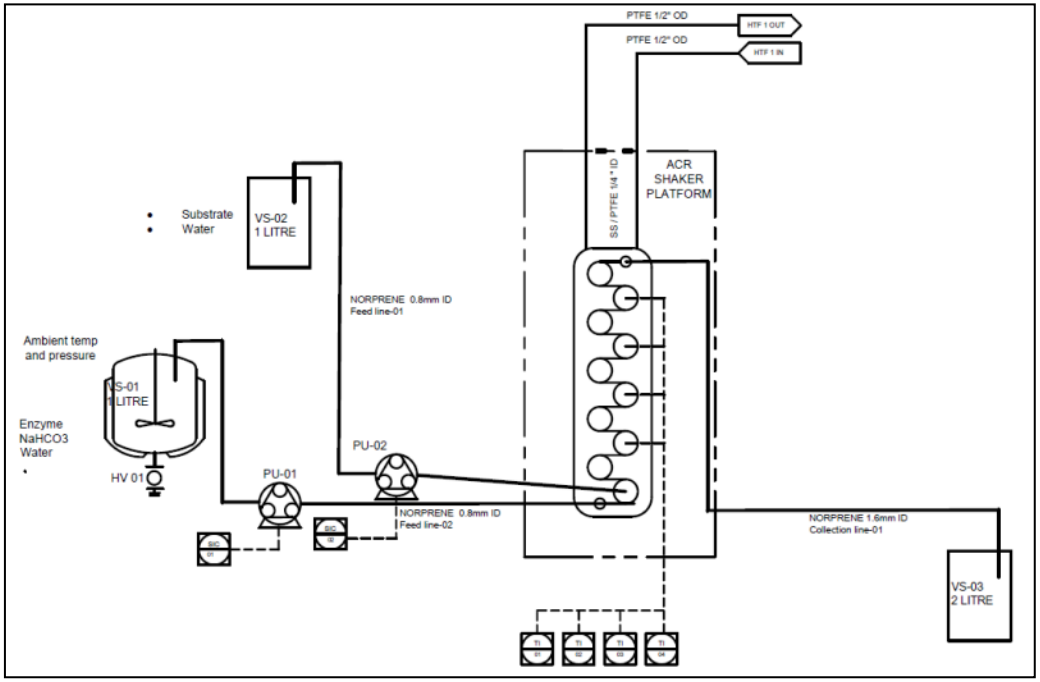
www.amtechuk.com
gareth.jenkins@amtechuk.com

Biocatalytic Desymmetrisation – Preliminary results in flow

- Benchmark of agitators: 50% volume vs. spring

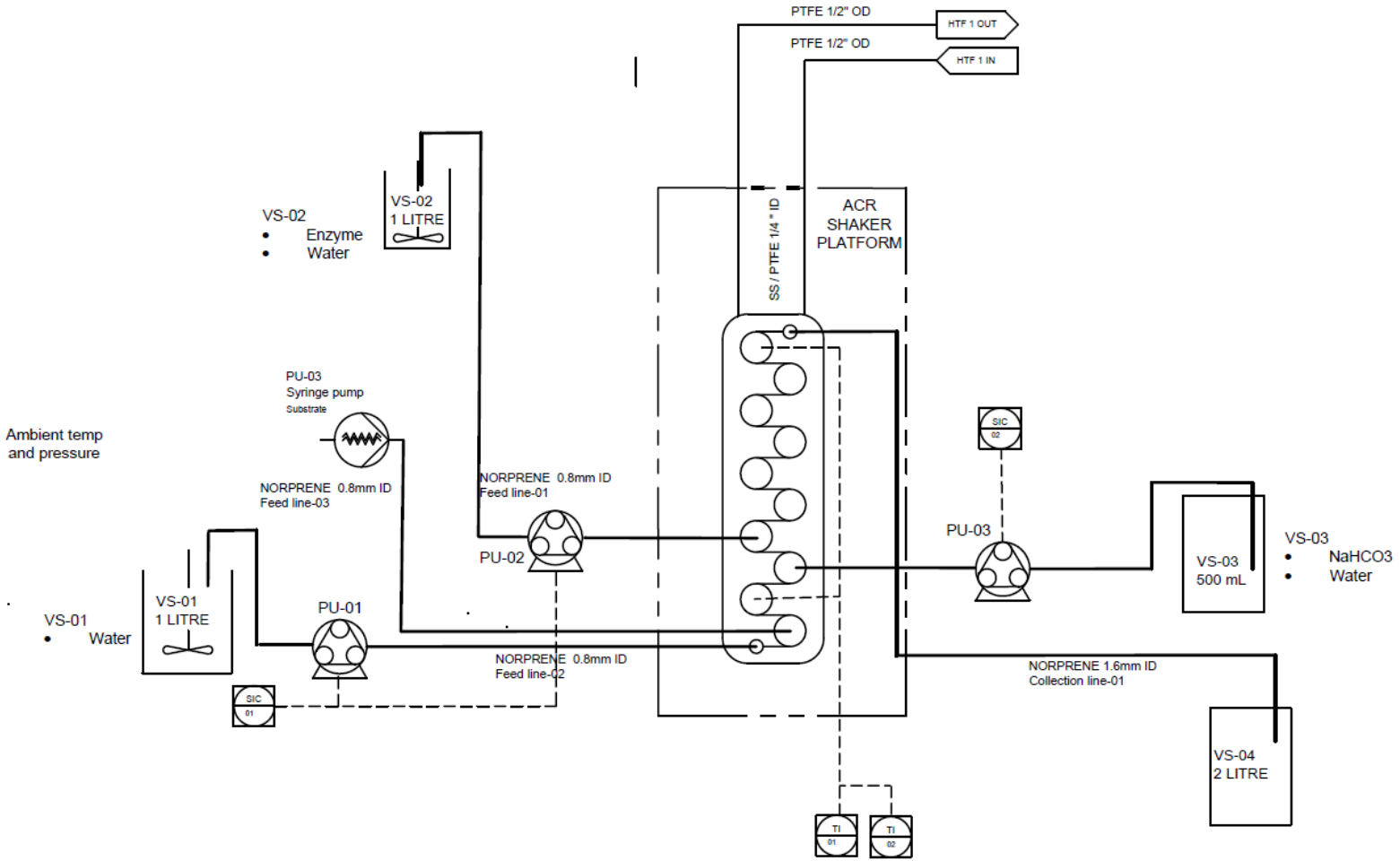
- Enzyme and buffer were dissolved in one solution which caused degradation of the enzyme in some cases

- This was overcome by modifying the setup …



www.amtechuk.com
gareth.jenkins@amtechuk.com

Modified Setup



www.amtechuk.com
gareth.jenkins@amtechuk.com

Modified Setup: Benchmark

Residence time	Conditions	Conversion (%) Cell 7	Conversion (%) Cell 10
25 min*	ACR, 50% vol agit.	31	66*
50 min	ACR, 50% vol agit.	95	96

* 14% conversion after 25 min (20 mL batch, in-house)

- The enzyme and the buffer solution are kept separate until they mix within the reactor
- Samples are collected at cell 7 and cell 10 to monitor the rate of conversion throughout the reaction

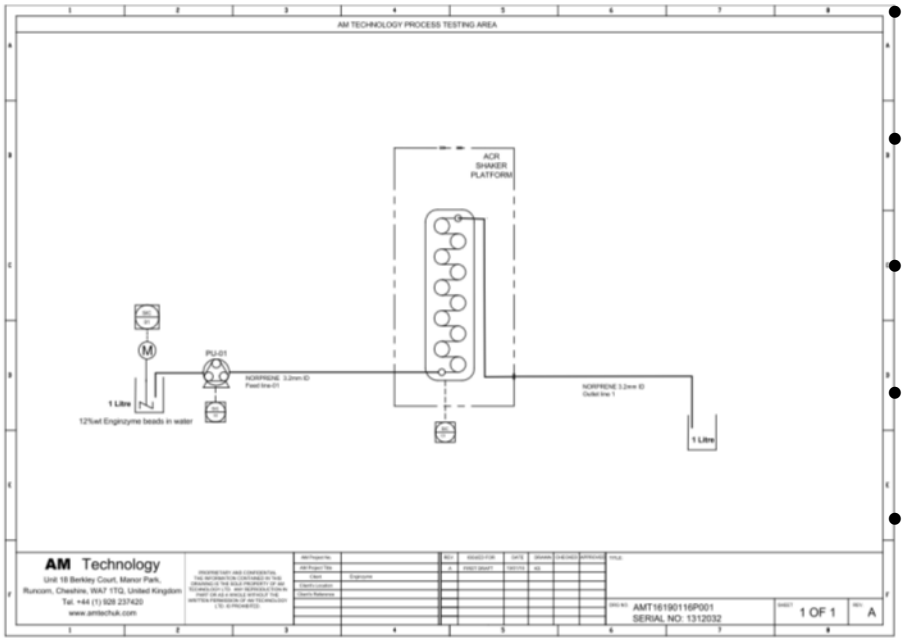
www.amtechuk.com
gareth.jenkins@amtechuk.com

Conclusions Study #2

- Faster reaction in flow
- Flexibility in equipment set up was essential for success
- Significant improvement in Space Time Yield demonstrated on moving to continuous processing:
  - 8.8L batch reactor STY 9.2 g L^{-1} h^{-1}
  - 100mL continuous reactor STY 44.7 g L^{-1} h^{-1}

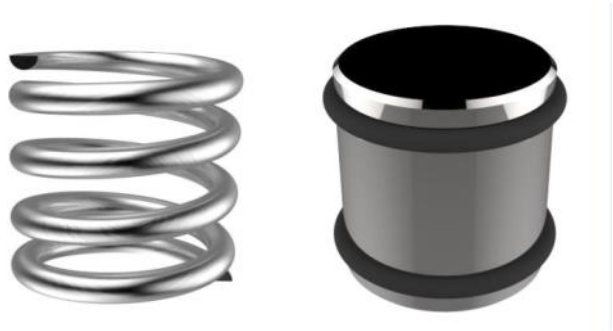
www.amtechuk.com
gareth.jenkins@amtechuk.com

Study #3 - Immobilised Biocatalysts



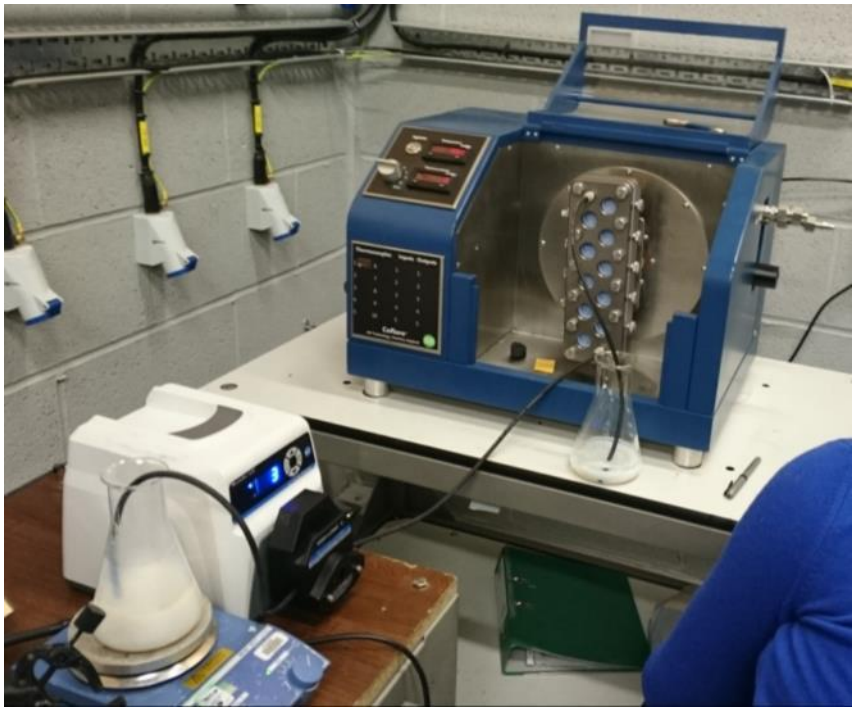
HANDLING SLURRIES

- Fixed slurry concentration at 12.5% w/v in water throughout
- Simple slurry feed – magnetically stirred flask
- Slurry pumped in from bottom to top (against gravity, worst case)
- Three residence times before sample taken
- Two different agitators



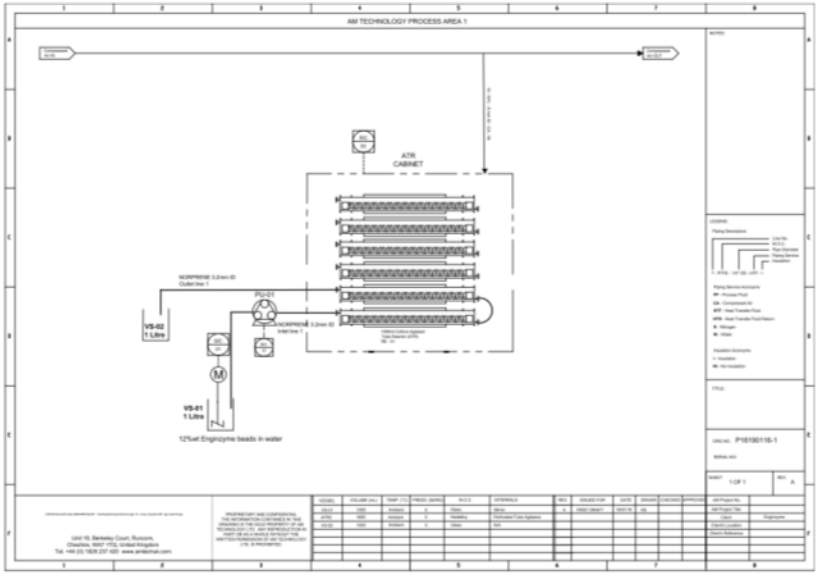
www.amtechuk.com
gareth.jenkins@amtechuk.com

ACR System As Set Up and In Use



www.amtechuk.com
gareth.jenkins@amtechuk.com

Pilot Agitated Tube Reactor (ATR) Set Up



- Single run
- Two tubes to check for interstage issues
- Feed in at lower tube so moving slurry against gravity

www.amtechuk.com
gareth.jenkins@amtechuk.com

ATR System As Set Up and In Use

www.amtechuk.com
gareth.jenkins@amtechuk.com

Conclusions Study #3

- Immobilised catalysts presented as slurries up to 12.5% w/v can be processed
- Early results look promising
  - Evaluating a wide range of typical solid supports, including glass beads
  - Evaluating reduction in attrition of solid support
  - Evaluating performance of a range of immobilised catalysts

www.amtechuk.com
gareth.jenkins@amtechuk.com

Coflore and bio-processes

Process	Enzyme	Partner	Summary of results
DL amino acid resolution by oxidation	Wild-type D-amino acid oxidase immobilised on whole cells	Ingenza and C-Tech Innovations	From 24h in 1L batch to 4h in ACR From 30+ h in 4L batch to 7h in ATR10 70% less oxygen
β-D glucose to gluconic acid	Gluzyme Mono	DTU	From 10h in 200ml batch to 1h in ACR
Reduction of 4-tertbutylcyclohexanone to cis-4-tertbutylcyclohexanol	CRED 161A	Almac	ACR 7 times faster than 500 ml batch reactor . In progress
Esterification of oleic acid to ethyl oleate	Lipozyme CalB L	Novozymes	From 4 h in batch to 2 min in ACR and ATR1
Desymmetrisation of dimethyl cyclohex-4-ene-cis-1,2-dicarboxylate	ECS-PLE06	Enzymicals	8.8L batch reactor STY 9.2 g L-1 h-1 100mL continuous reactor STY 44.7 g L-1 h-1
Continuous enzymatic processing of sugar beet pulp for pectin breakdown	TBC	UCL Industrial Biotechnology Research Group	In progress
Immobilised enzymes	TBC	GSK, AZ, Johnson Matthey	In progress

www.amtechuk.com
gareth.jenkins@amtechuk.com

Conclusions

- Flow processing has the potential to accelerate bioprocesses due to enhanced mass transfer
- Faster reaction times will make large scale production more economically feasible
- Faster reaction times and use of tubular continuous reactors will result in significantly smaller equipment
- More examples of lab and scale up applications are needed but the results are consistently promising.

- Many thanks to

ALMAC
C-Tech INNOVATION
DTU Technical University of Denmark
Enzymical Enzymes | chemicals | solutions
ingenza
novozymes Rethink Tomorrow
UCL ENGINEERING Change the world