Electrochemistry in Biotechnology

John Collins
C-Tech Innovation Ltd

www.ctechinnovation.com
• Introduction to C-Tech
• A brief diversion into none electrochemistry
• Electrochemistry
  – Introduction
  – Examples
• **Electro-dialysis**
  – Introduction
  – Examples
• Conclusions
• Acknowledgements
• Origins in ‘ECRC Capenhurst’, a research centre for the electricity industry. Independent since 2001
• 65 people with around 40 degree qualified engineers and scientists.
• Core technical strengths are:
  1. Electrochemical and chemical engineering
  2. Advanced Thermal Processes (Industrial RF, Microwave and Ohmic)
  3. Pilot Rig Design & Build
Microwave Flow Chemistry

- **Faster** reactions, more throughput – e.g. 8h reduced to 3min
- **Cleaner** - no hot oil or fouling of surfaces
- **Greener** - higher yields (increased 35 to 70%), less by products, less waste, less energy (up to 90%)
- **Safer** - lower chemical inventories, easy temperature control

Temp up to 250°C
Pressure up to 25 Bar
Throughput up to 1000 kg/day
Applications

Very wide range of reactions possible; these include

- Synthesis of Ionic Liquids
- Esterification Reactions
- Suzuki couplings
Pilot Rig Design & Build

- Full mechanical and electrical design
- Build and commissioning.
- Modelling supported design (FEA, CFD, Multi-physics).
- Specialities: ATEX, Pressure, Vacuum, Hazardous Chemicals, **Supercritical processes**, Ultrasonic processing, RF/Microwave, Electrochemistry, Controlled atmospheres, Food processing.
It is chemistry performed by **electron exchange reactions** between the electrode surfaces and redox active chemical species.

**Oxidation** at the anode (loss of electrons)

**Reduction** at the cathode (gain of electrons)

Clean or reagent-less chemistry?

Electrons are cheap and controllable.
Organic electro-synthesis

• Direct oxidation or reduction on the electrodes e.g. L-cysteine by cathodic reduction of L-cystine.

• Mediated reactions – TEMPO, Cl, Ag, Co, ............. e.g. oxidation of cellulose fibres with a TEMPO mediator.

![Diagram showing electro-synthesis process](image)
Aqueous

- Electrochemical synthesis and waste water treatment.
- Metal recovery down to <ppm.
- Nuclear industry.
- Flow batteries.
- Industrial scale synthesis of high purity VRFB electrolyte.
- Hybrid gas / liquid fuel cells
- In situ gas generation (oxygen, hydrogen)
- Enzymes immobilised on electrodes
- Oxidant generation (feedstock breakdown, processing & cleaning)
- Metal addition
- Sensors

Non-Aqueous Systems

- Ionic liquid electrochemistry scaled to >500l scale.
- Both traditional ionic liquids & deep eutectic solvents
- Examples: aluminium electroplating, hard chrome plating, leaching / electro-winning
What is Electrodialysis?

- An ion exchange membrane based separation system
- Involves movement of charged species in an electric field
- Use of different sequences of membranes (anion, cation or bipolar) allows concentration, separation and salt splitting.

Before ED

ACID

FEED

BASE

After ED

ACID = 50mM HCl
BASE = 50mM NaOH
FEED = Reaction Mix

- Anions
- Neutral molecules
- Cations
Industrial Applications of Electro-dialysis

• **Reduce electrolyte content**
  – Potable from brackish water
  – Food products: whey, milk, soy sauce etc.
  – Nitrate from drinking water
  – Demineralisation of sugar and molasses

• **Recover electrolytes**
  – Pure NaCl from seawater
  – Salts of organic acids from fermentation
  – Amino acids from protein hydrolysates
  – Acids from metal pickling baths and rinse

**Scales of Operation**
Typical examples are at large (or sometimes very large scale).

These systems are not so accessible for the laboratory based chemist or biologist working from <1ml volume reactions and upwards.
Laboratory ED stack and system

**RED** = Enzyme reaction

**ORANGE** = Acid

**BLUE** = Base

**GREEN** = Electrode Rinse
Objectives -

- Make ED available as a research and process development tool for conventional lab scale bio-catalysis.
- Provide a route to pilot and medium production scale working with a supplier and process developer who can customise the system for individual applications.

Approach -

- Design and build equipment which can accommodate modest reaction volumes - a few litres for bench scale fermentations, down to sub 50 ml reaction volumes for biocatalysis/ biotransformations.
- Demonstrate in situ product recovery and downstream processing options using ED (mixed acid fermentation, transaminase, removal of inhibitory acetate in reactions).
- Make the systems more accessible (integrated units incorporating process monitoring & control).
- Optimise ED systems for biotechnology applications and develop new IP.
Objectives -

• Using ED to remove acids and optimise Succinic acid production from a mixed acid fermentation.

• Corn steep liquor fermented by Actinobacillus.

Approach -

• Organic acids were stripped from the whole fermentation broth as they were produced.

• Bio-fouling was controlled by polarity reversal techniques.

• The process automatically controlled the pH of the broth by varying the rate of removal of the acid by the ED system, so that the pH remained constant throughout the experiment thus avoiding acid inhibition.

• Modest scale - 2 litre volume bench top fermentation system.
Actinobacillus succinogenes fermentation

<table>
<thead>
<tr>
<th></th>
<th>Succinic</th>
<th>Acetic</th>
<th>Formic</th>
<th>Propionic</th>
<th>Pyruvic</th>
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</thead>
<tbody>
<tr>
<td>Typical final concentration (g/l, from literature)</td>
<td>67.2</td>
<td>12.0</td>
<td>8.7</td>
<td>2.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Formula weight</td>
<td>118</td>
<td>60</td>
<td>46</td>
<td>74</td>
<td>88</td>
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<tr>
<td>Molarity</td>
<td>0.57</td>
<td>0.2</td>
<td>0.19</td>
<td>0.03</td>
<td>0.05</td>
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<tr>
<td>pKa1</td>
<td>4.16</td>
<td>4.75</td>
<td>3.75</td>
<td>4.87</td>
<td>2.47</td>
</tr>
<tr>
<td>pKa2</td>
<td>5.61</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solubility (g/l, cold water)</td>
<td>76.9</td>
<td>Miscible</td>
<td>Miscible</td>
<td>Miscible</td>
<td>Miscible</td>
</tr>
</tbody>
</table>

- Enabled a fed batch process by the removal of the inhibitory Succinic acid.
- Conversion of glucose to succinate significantly improved.
- Volume controlled improved and no base addition required.
- 95% of total succinate could be removed from fermentation broths using ‘down stream’ ED processing
- > 90% reduction of other contaminating acids (chiefly acetate and propionate) in product stream by manipulation of the pH to just above the pKa of the target molecule.
- Process cost for recovery of succinate by this method estimated at 10p per kg succinate, or about 1.3 p per mole.
EXAMPLE 2 – Amino acid biotransformation for conversion of DL- or L-Serine to D-Serine with acetate removal by ED

- Demonstrated continuous removal of inhibitory acetate co-product.
- Turned a batch reaction into a fed batch reaction with increased yield and substrate conversion.

In process Acetate removal prior to a 2nd batch

Downstream Acetate removal after fermentation
EXAMPLE 3 - TRANSAMINASE
ED provides scope for process improvement

1. Equilibrium strongly favours starting materials, only a low % yield obtained without reaction engineering

2. Catalyst – inhibited by products, including strong inhibition by pyruvic acid

3. Needs expensive cofactor, buffer adds to cost

4. Amine donor – must be supplied in excess

5. Isolation of product can be difficult

6. Very dilute system – needs intensification

KYROBIO project collaboration with Wolfgang Kroutil, University of Graz. ED provides options for all the process issues identified.
• pH control via ED system therefore buffer can be eliminated from system.
• No PLP co-factor loss identified
• Simultaneous amine and acid removal from reaction stream (The 'Feed') into separate product streams.
• Both in situ product removal (ISPR) and end of process product removal were separately demonstrated.
• The pK$_a$ of a product can be used to selectively remove it from a solution e.g. isolation of organic acids from a mixed fermentation, isolation of amines.

• Control pH and facilitate continuous removal of product.

• Production of acid and/or base from a neutral salt.

• Reduce electrolyte content (desalination)/recover electrolytes.

• Can be applied from 50 ml reaction volume upwards using C-TECH ED system.
• Real (commercial) process challenges in projects to explore economics and scalability.

• Immobilised biocatalyst formats, continuous processes.

• Other challenges - cofactors, metal ions, multiple enzyme systems.

• C-TECH products are now available - flexible ED equipment and information toolkit available to researchers at appropriate scale (volume), with functions specified by end users.
Acknowledgements

John.Collins@ctechinnovation.com
www.ctechinnovation.com

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Technology Strategy Board

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C-Tech is Seeking -

• Partners with processes suitable for applications of electrodialysis (process development and/or product recovery)

• Hydrolase reactions particularly welcome!

• Partners with interest in redox chemistry, including enzyme mediated processes

For more information please visit our website or email me directly-

www.ctechinnovation.com          Kay.McClean@ctechinnovation.com