



Biological Processing of Lignocellulose to Ethanol

Processing Lignocellulosic Biomass Conference

CPI, Wilton, Nov 8th

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Why am I here...?

Personal Introduction:

- Formerly R&D Director at TMO – team of 30 scientists
- Microbial physiologist – expertise in fermentation
- Background in pharmaceutical industry
- Joined TMO in 2005 – 6 staff, an office and an idea

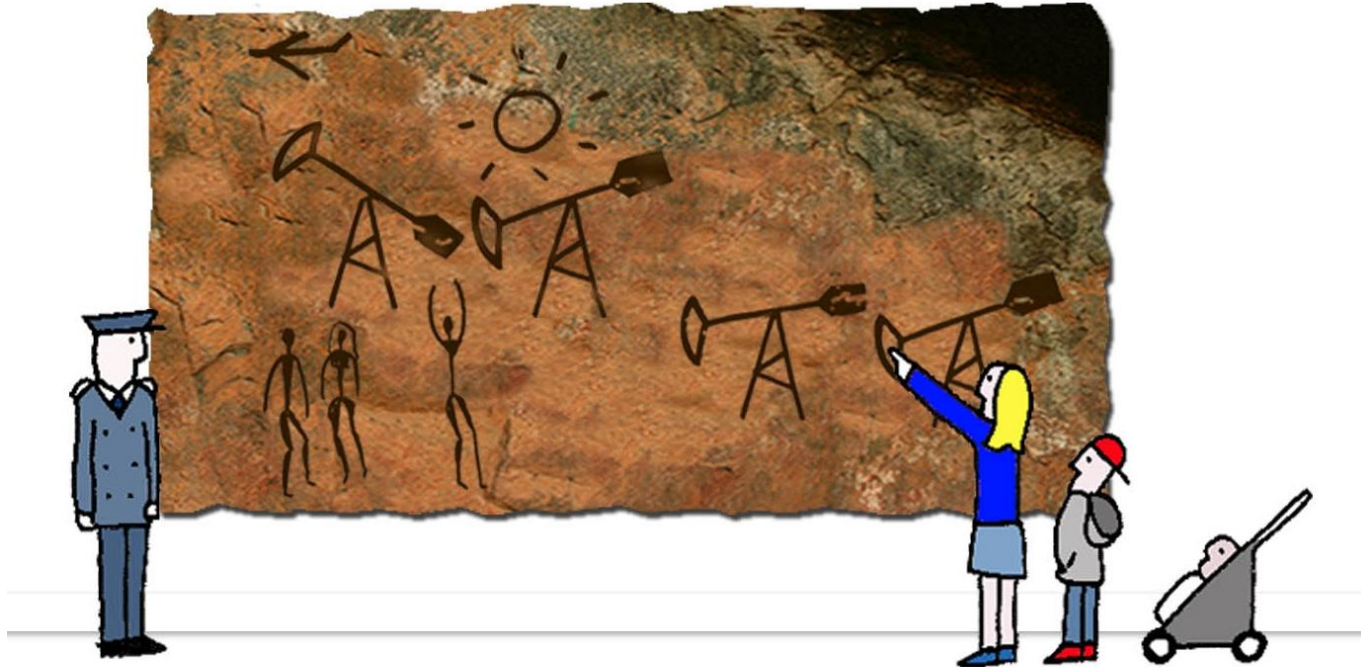
TMO Corporate Introduction:

- Technology to convert waste into useful products
- Use microbes that grow at high temperatures “thermophiles”
- Raised £55M to date
- Guildford-based global business – two UK sites
- Projects in EU, US and China
- It’s not just about the bug!
 - *Biomass to sugar platform – Argonaut Process*



A Shared Vision...

...to consign reliance on fossil fuels to the stone age



We need to develop sustainable processes and products that can

- Replace those based on fossil fuels
- Address global energy issues and mitigate climate change
- Avoid use of food or feed crops

Biological Routes to Ethanol

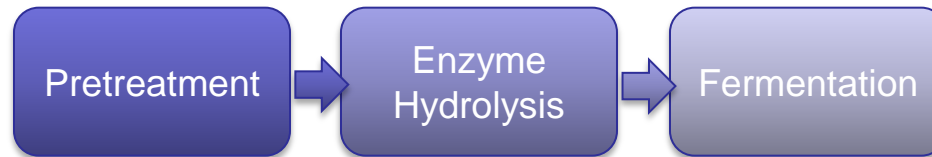


There are many routes to ethanol from biomass

- All rely on delivering fermentable sugars to a suitable microorganism
- All have pros and cons – all are in commercial development

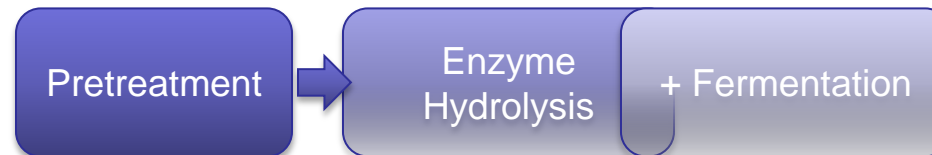


Separate Hydrolysis & Fermentation (SHF)



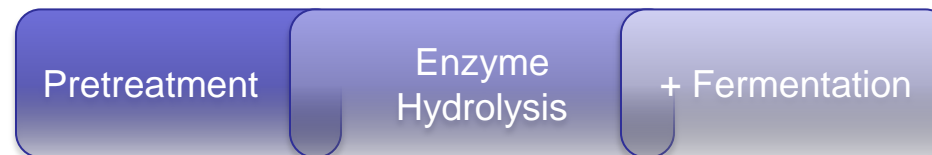
 TMO
RENEWABLES

Simultaneous Saccharification & Fermentation (SSF)



 中粮
COFCO
自然之道 和谐共赢
POET™

Consolidated Bioprocessing (CBP)

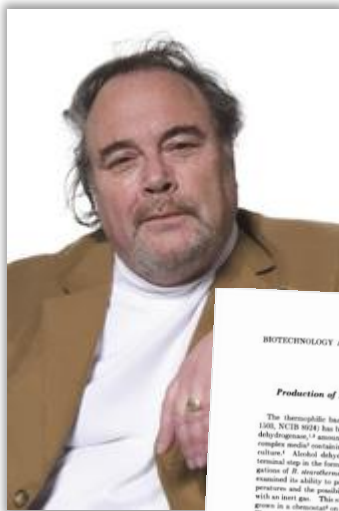


 MASCOMA

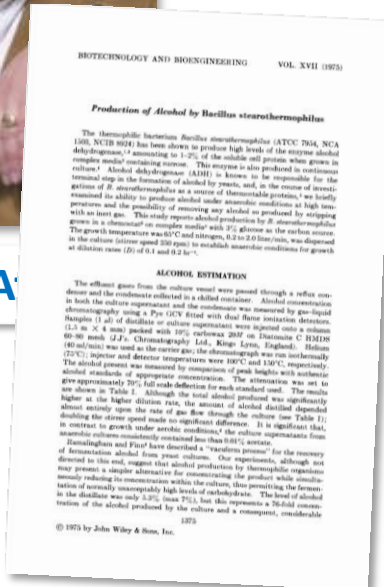
TMO's Story - How it Started

In 2002 the search for
a talented strain...

...and some novel metabolic
engineering in partnership...



Prof. Tony A.

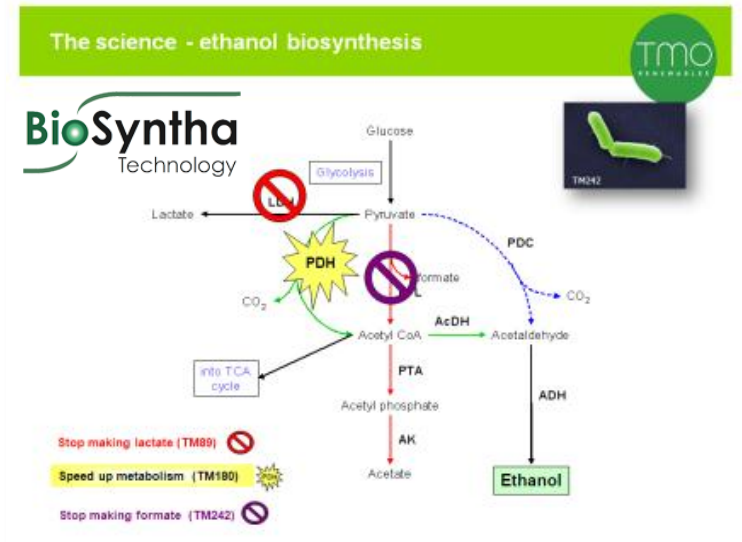


An early observation (1975)

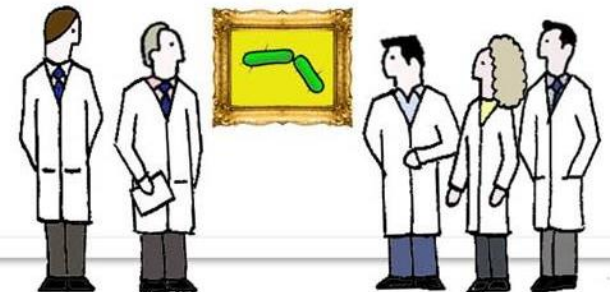
Wide substrate range...

- ✓ Glucose
- ✓ Xylose
- ✓ Arabinose
- ✓ Lactose
- ✓ Mannose
- ✓ Cellobiose
- ✓ Sucrose
- ✓ Starch
- ✓ Xylan
- ✓ Cellulose

...from nature



...which delivers TM242



Success! We are all going to be rich!

We had what everyone said they wanted...

- A strain that could convert sugars from waste into ethanol
- We went to sell the strain...

...but it wasn't enough

The market wanted the whole “engineered solution”

- Want to buy a complete package - feedstock to fuel
- Full design package – full mass & energy balances and full economic model
- Also need data - demonstrate at a meaningful scale
- *Biotechnology is not enough – need engineering!*
- Need to raise more money... build something bigger

There were dark clouds appearing on the horizon...

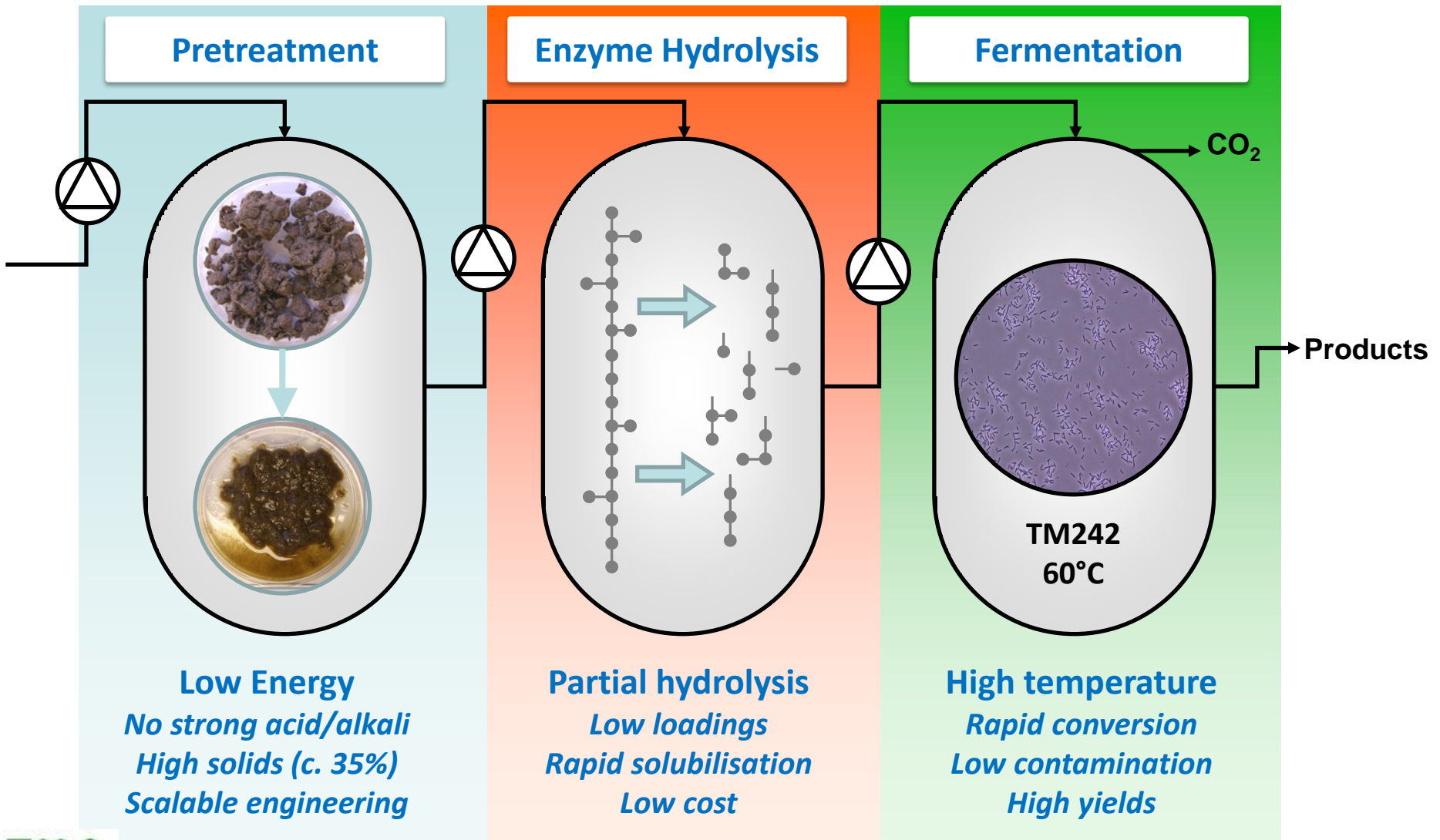
- The economic and political situation was changing
- Banking crisis and the “*Fuel vs Food*” debate



The Evolution of TMO...



The Integrated TMO Process



Identifying the Major Hurdles

Feedstocks:

- Supply chain not fully developed
- Biomass cost (competition for biomass)

Use captive feedstocks

Multifeedstock capability

Capex/Opex:

- Multiple vessels - exotic alloys
- Unusual widgets – scalability
- *Energy, water and waste*
- Enzyme costs
- Yields – C5 and C6 sugars

Simplify process
No acid/base catalyst

Detailed enzyme investigations
& partner with suppliers

Let nature do the work &/or
Engineer new strains

Others:

- New technology – proof at scale
- *Market volatility*
- *Scale of operation*

Build and operate Demo facility

Feedstock Considerations

Supply Chain, Processing & Cost

- Many supply chains are still not established
- Seasonal supply – storage issues
- Use captive or cheap feedstocks
- There is competition developing
- Costs: -\$20 (waste) to >\$100 (energy crops)
- Cost is a large factor in overall economics

Composition & Productivity

- Ethanol productivity determined primarily by sugar composition – this varies greatly
- The composition of a particular feedstock can also vary significantly – see *DDGS or MSW*

Upstream Processing

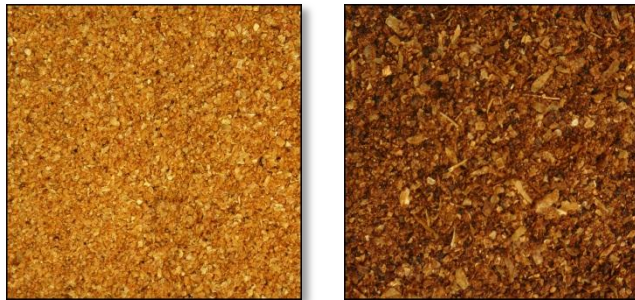
- Seasonal supply – storage issues
- Milling expensive – ideally avoid
- May need sorting or fractionation

Feedstock Description	TMO	Total sugar (% Dry Matter)	Max Productivity (L/Tonne)	Target Productivity (L/Tonne)
Dried Cassave Root		106	685	484
Cassava Stalk (core only)		78	506	357
MSW Fibre (High)		78	505	357
Miscanthus		73	469	331
Cane Bagasse		72	466	329
Corn Stover		72	464	327
Corn Fibre		71	462	326
Cassava Stalk		71	457	323
Switchgrass		68	441	311
Recycled Paper Fiber		67	435	307
High Sugar Grass #2		62	402	284
MSW Fibre (Medium)		61	393	277
Cassava Residue		52	337	238
High Sugar Grass #1		50	321	227
Wet Cake - Corn (High)		47	302	213
Cassava Residue		46	299	211
Wet Cake - Corn (Medium)		44	282	199
DDGS - Corn (High)		43	279	197
Wet Cake - Corn (Low)		40	258	182
DDGS - Corn (Medium)		38	244	173
MSW Fibre (Low)		34	219	155
DDGS - Corn (Low)		32	206	145
Paper Sludge Residue		18	114	80
MSW (Poor)		16	103	72

Feedstocks are Different

There are few rules that apply consistently...

- Even those that are the same can be quite different...



- DDGS: Free fatty acids from thermal degradation of corn oil - TMO developed a resistant strain – 40x more resistant to oleic acid
- The challenge is often not the sugars – but the non-sugars components
- Problems may be inherent in feedstock or consequence of processing

Cassava
Residue

Corn Fibre

DG Wet
Cake

Municipal

Waste

Sisal

Paper

Sludge

Miscanthus

Wheat

Corn Stover

Straw

High Sugar

Grass

DDGS

Spent
Germ

Switchgrass

Short Fibre

Pulp

Bagasse

Brewer's

Spent Grains

Cassava

Stalk



Pretreatment

Preparation of biomass prior to hydrolysis – numerous options

- **Strong acid hydrolysis**
 - solubilise C5 sugars – leave a cellulose rich cake – inhibitors, C5 yield loss.
- **Ammonia Fibre Expansion (AFEX)**
 - excellent results in the lab but challenging to scale-up
- **Steam explosion, steam cooking** (120°C to 240°C, 5 to 25 mins)
 - simple, scalable but not always effective without additions
- **Dilute ammonia, dilute acid** – promising but need proving at commercial scale
- **Biological pretreatments** – early stages and yet to see any convincing data

Regarded as most capitally intense step

- Pressure vessels – difficult and expensive at scale
- Acid/base - expensive alloys, inhibitors, waste streams, more complexity
- Mixing/mass transfer at high viscosity (1M cP) and large scale (>100m³) very challenging
- Integration with hydrolysis step essential – need to be optimised together
 - TMO did a fantastic job on this – “**Argonaut Process**”

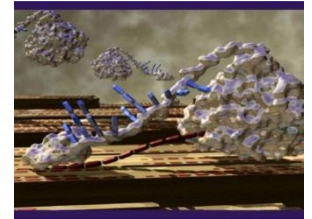


“The only thing more expensive than pretreatment... is no pretreatment”

Enzyme Hydrolysis – *it's all about cost*

Enzymes – still one of the most costly elements

- A typical commercial cellulase will cost \$4 to \$10 per kg
- Processing time will be 72 to 96 hours at 50°C and pH 5.0
- Typical enzyme loadings will range from 1% to 5% w/w cellulose
 - Less effective at high solids – yields decline, costs increase
 - Need good high solids model system – early on!
- Typical glucose yields will be about 50% to 70% at high solids (>20% w/w)
 - Significant advantages if you can use oligomeric sugars
- For economically viability - low end of both enzyme cost and enzyme loadings



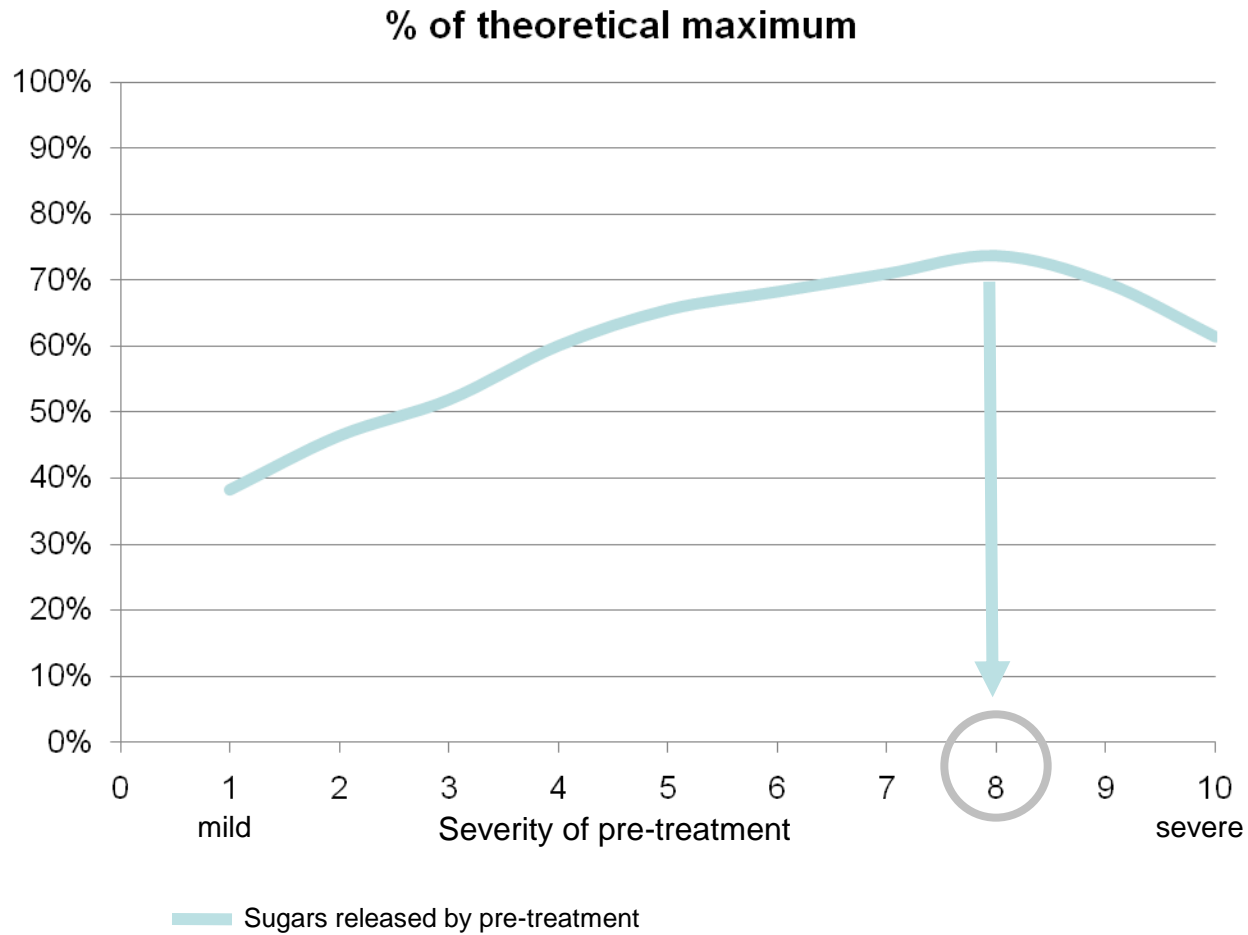
Enzyme suppliers have limited bandwidth

- Most focus on cellulases – generic approach - bespoke only for a few clients
- Generally the latest cellulases from Novozymes and Genencor are excellent

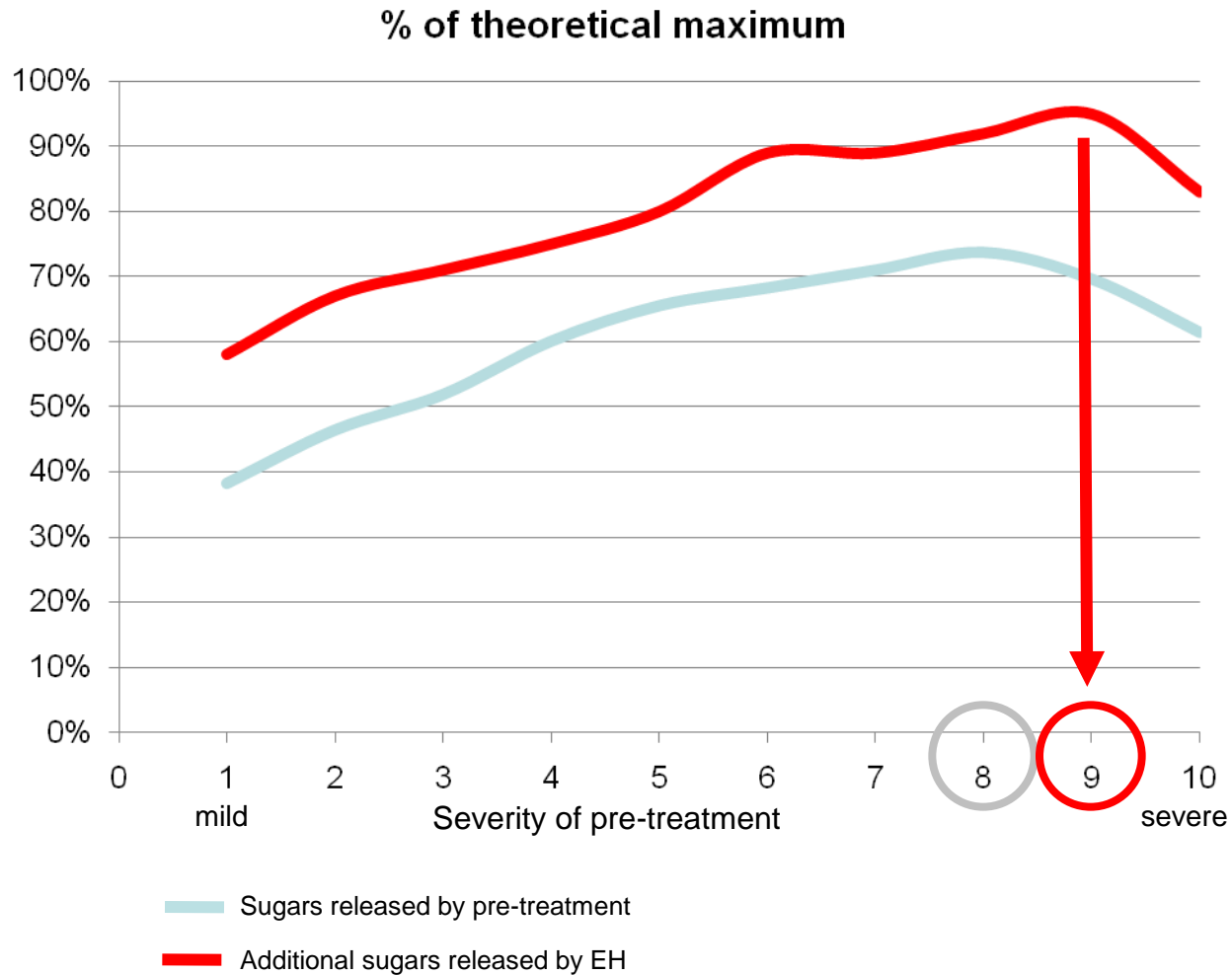
Single cellulase may be insufficient to saccharify biomass

- Complex arabinoxylan (e.g. corn) needs debranching enzymes - expensive
- Testing a range of enzymes useful - different feedstocks require different recipe
- Establish indicative test for yields and cost – the most efficient enzymes may be too expensive

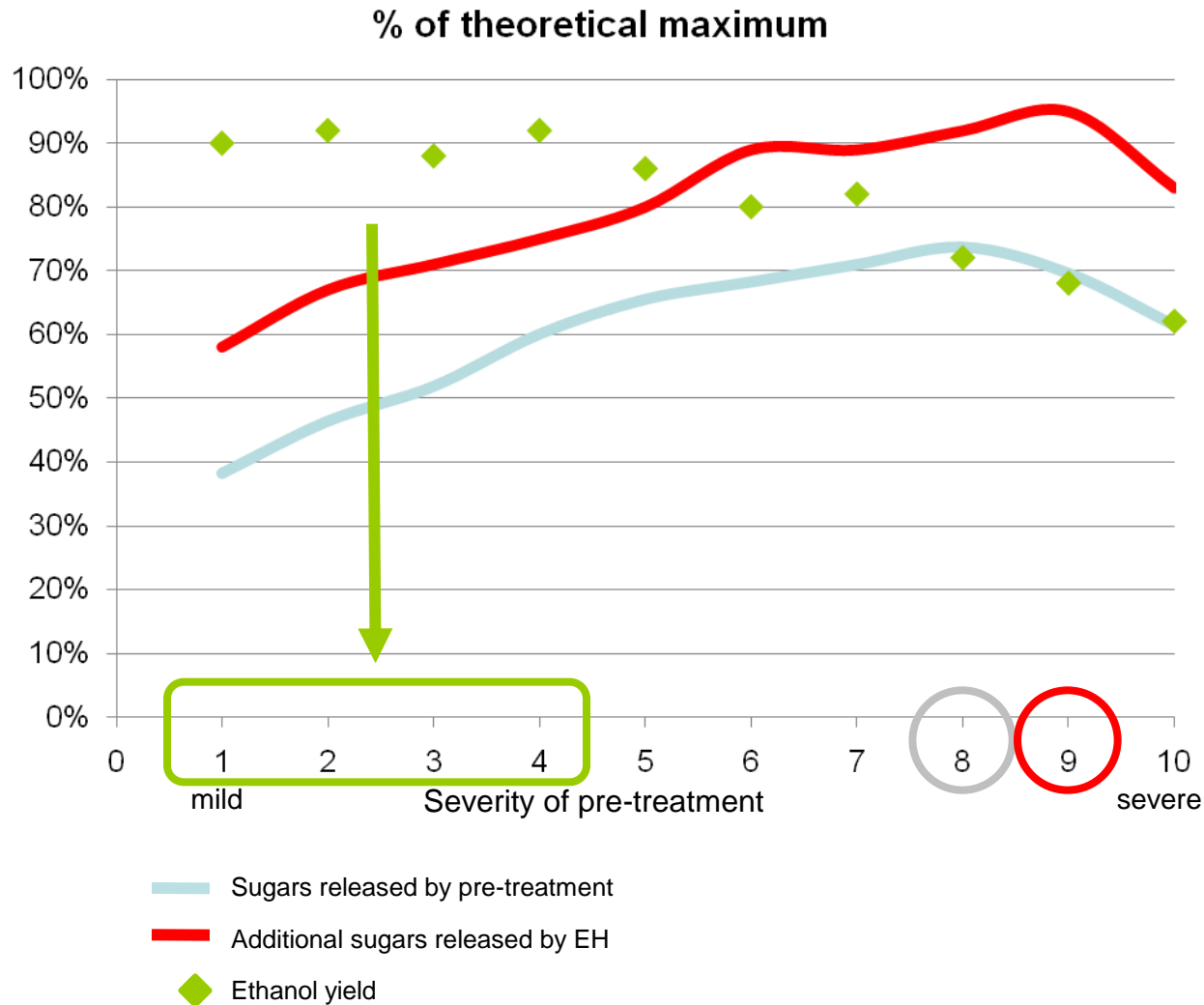
Pretreatment Study – Corn Fibre



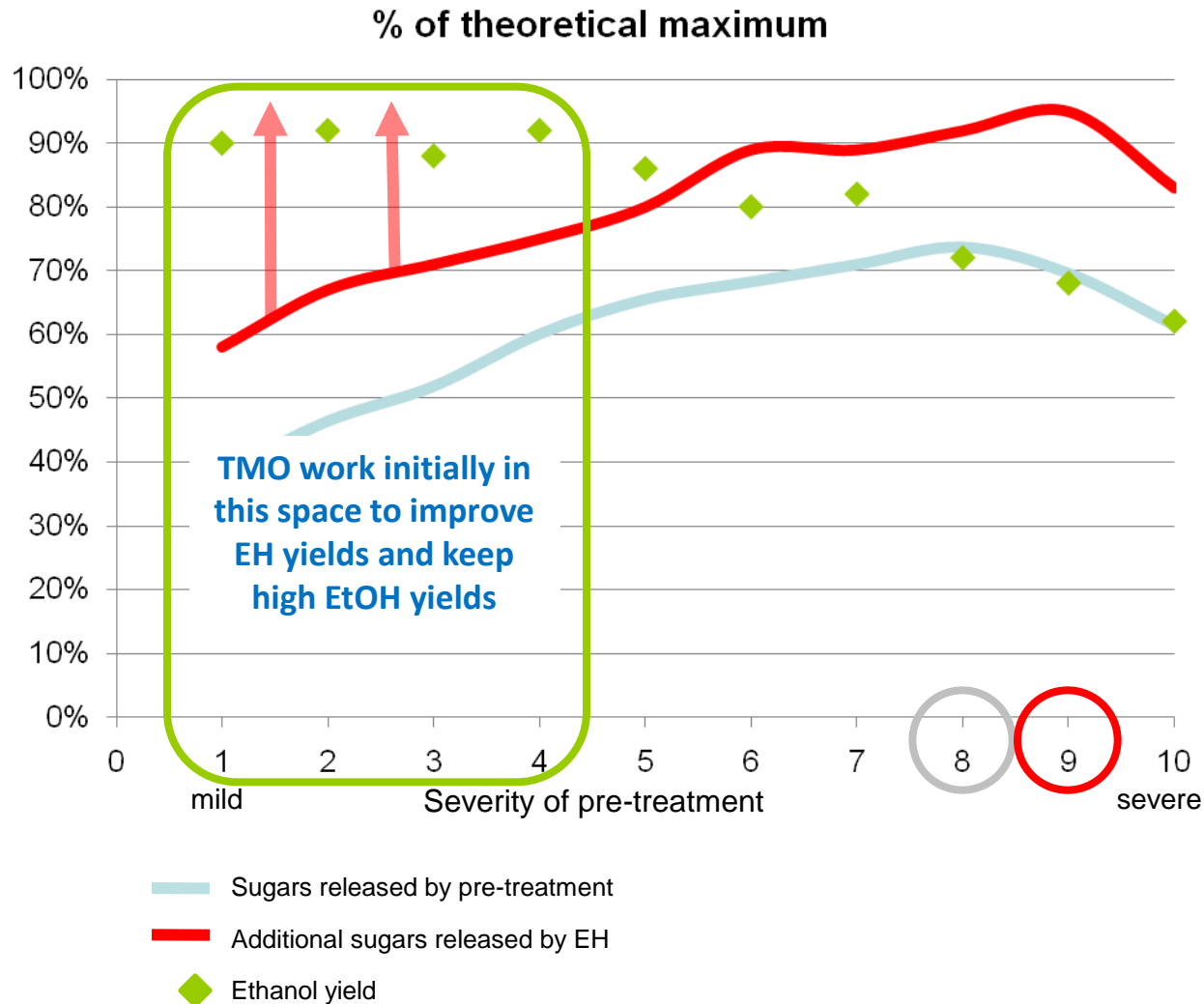
Following Enzyme Hydrolysis



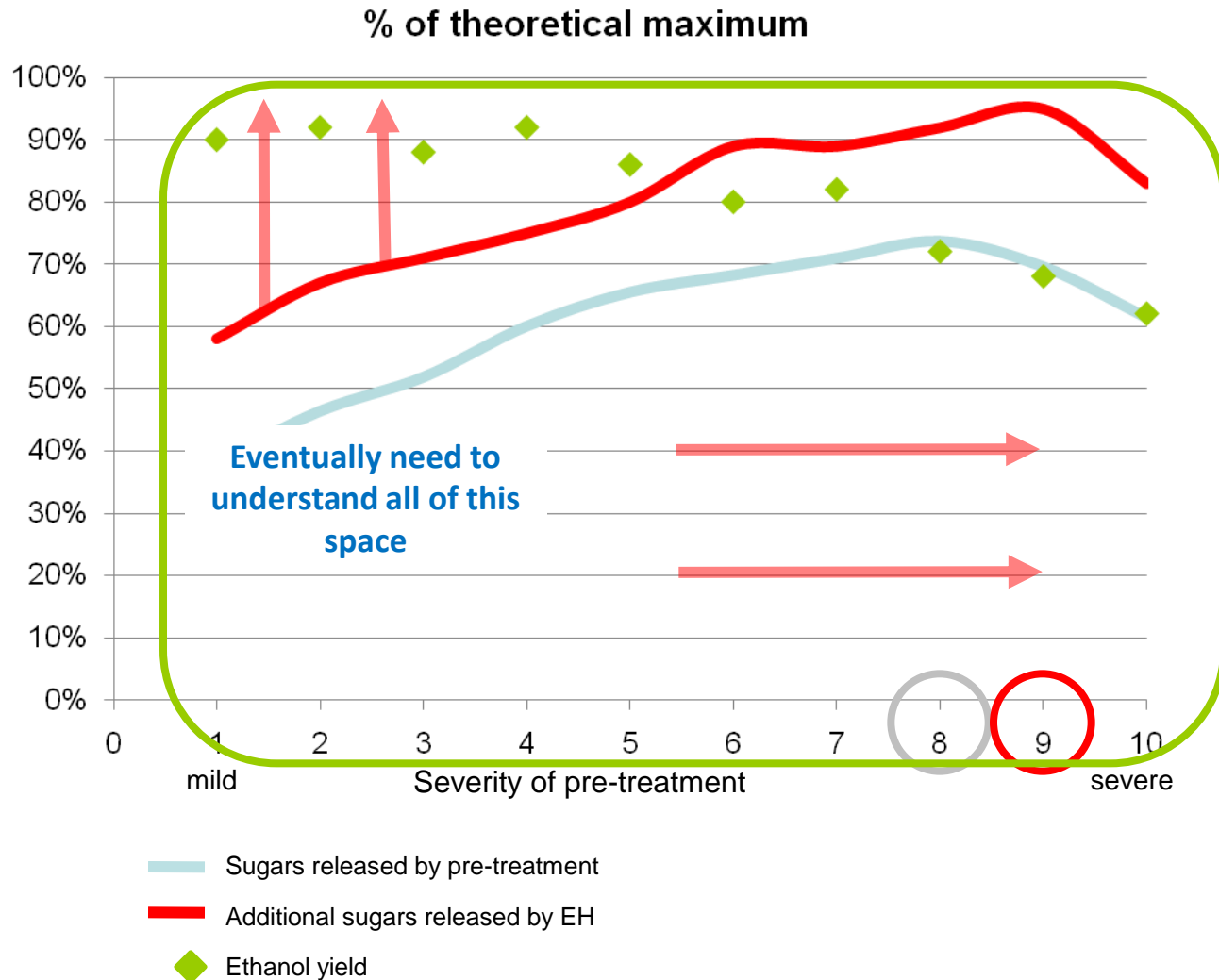
After Fermentation



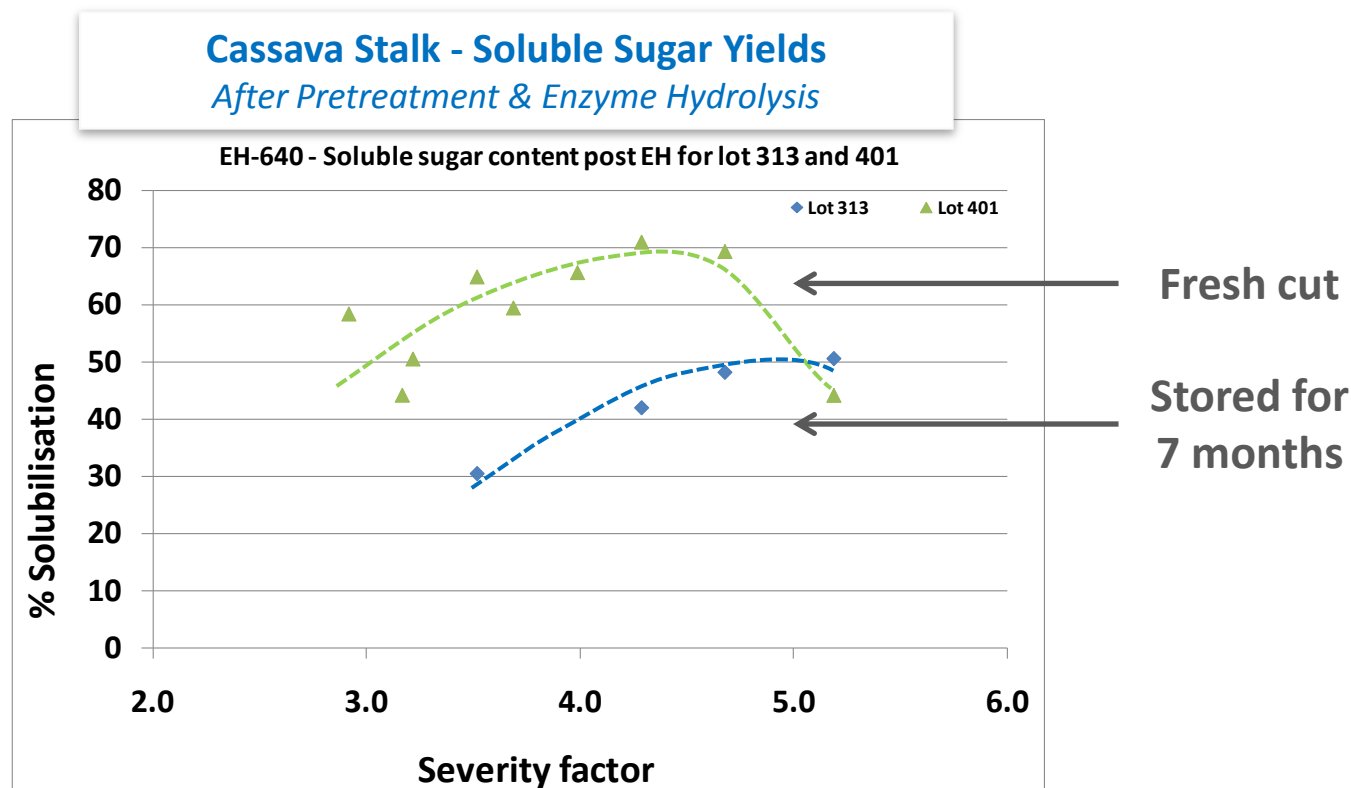
After Fermentation



After Fermentation



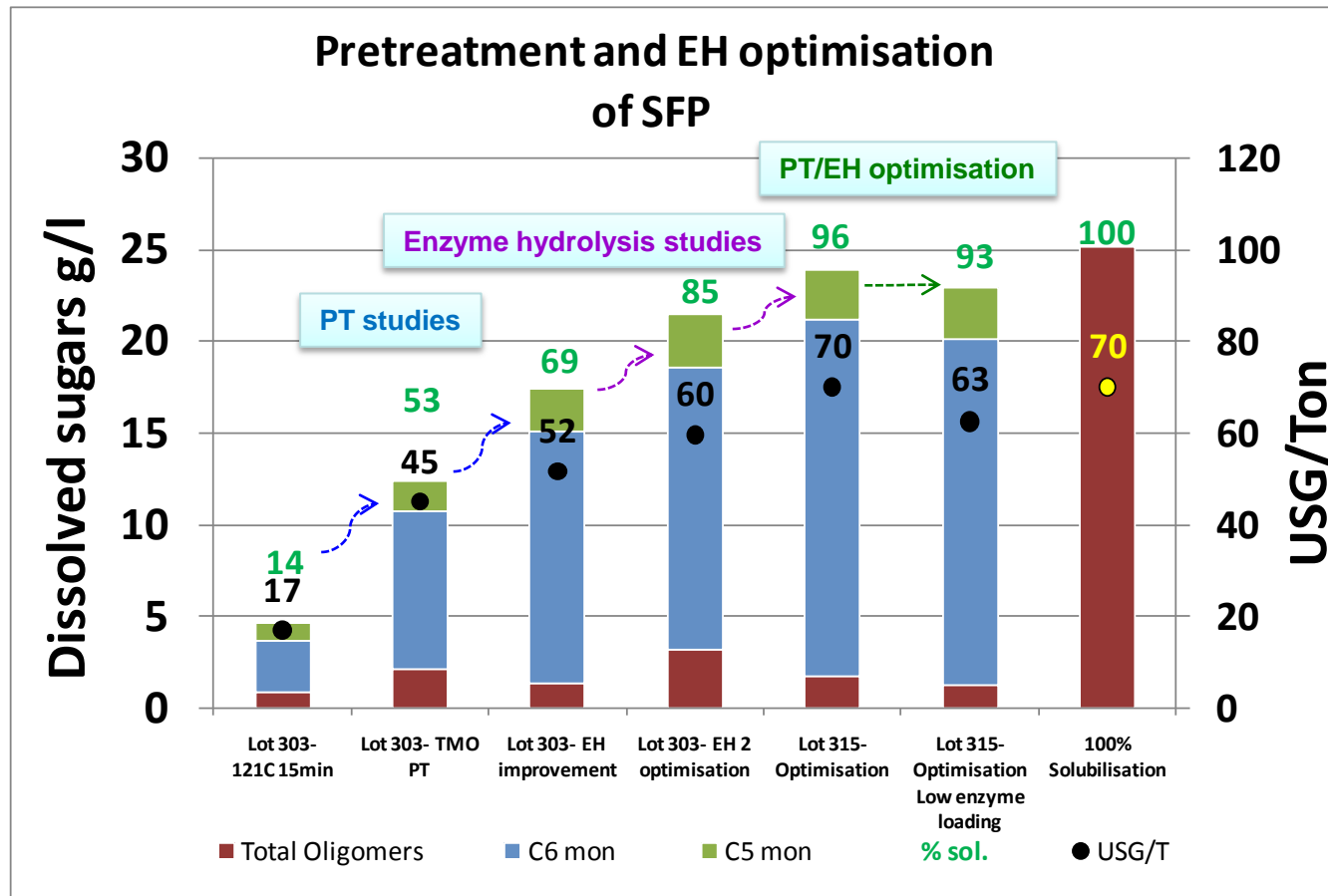
Feedstock Storage Example



Importance of testing different batches

- How biomass is stored has significant impact on performance
- Regional & seasonal changes, operational changes for captive feedstocks
- DDGS – TMO scientists could often tell how the 1G plant is running through quality of the material

Process Improvement Example - Paper pulp

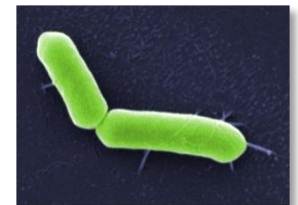
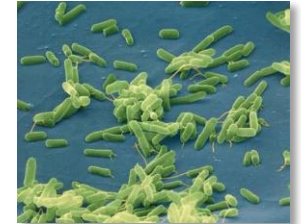
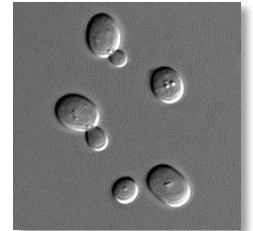


- Significant process improvement possible with standard enzyme loadings
- Significant know-how required to achieve this

Fermentation – where the magic happens

Many different approaches for microbial ethanologens

- **Standard brewing (C6) yeast**
 - **Pros:** well established, high ethanol titres, well understood, tolerant
 - **Cons:** monomeric glucose only - lower yields, contamination
- **Recombinant (C5 & C6) yeast**
 - **Pros:** yield improvement over C6 yeast, some tolerance
 - **Cons:** monomers only, C5 yields need improving, contamination, GMO
- **Assorted mesophilic bacteria (e.g. *E.coli*, *Zymomonas*, *Clostridium*)**
 - **Pros:** Genetics well developed, C5 utilisation, may use oligomers
 - **Cons:** Tolerance, robustness at industrial scale, contamination
- **Assorted thermophilic bacteria (e.g. *Geobacillus*, *Clostridium*)**
 - **Pros:** Quick conversion, C6, C5 & oligomer utilisation, less contamination
 - **Cons:** Genetics less developed, tolerance, less established



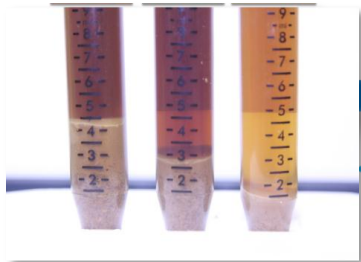
All the ethanologens have pros and cons
unlikely that a single option will work for every feedstock

Opportunities for multiple players

Building Confidence – Data, Data, Data...

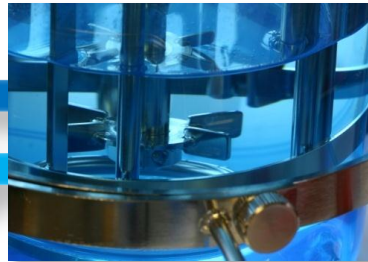
TMO developed a systematic feedstock testing program

- Phased approach – significant milestones and data packages
- Client involvement at each stage – build confidence and trust
- The data will ***always*** have the last word - clients want that data at pre-commercial scale



Phase 1: Initial Evaluation

- Detailed compositional analysis
- Performance at small lab scale (tubes, flasks)
- Low DS (<10%)
- Test wide range of PT conditions
- Test standard EH methods



Phase 2: Lab Fermenters

- Increase solids - 10% to 20% DS
- Dilute acid/base in PT
- Wider enzyme cocktail testing
- Assess PT/EH additives
- Early process definition
- Evaluate performance in lab fermenters (up to 10 litres)
- Assess toxicity issues



Phase 3: Pilot Scale

- Scale up to pilot system (100 litres)
- Confirm comparability
- Process improvements
- Support for PDU



Phase 4: Demo Scale

- Detailed process description
- Robust data package
- Full energy and mass balance
- Bespoke Aspen model
- Full economic modelling

Biomass to Ethanol is Just a Beginning

The 1G ethanol business can teach us something:

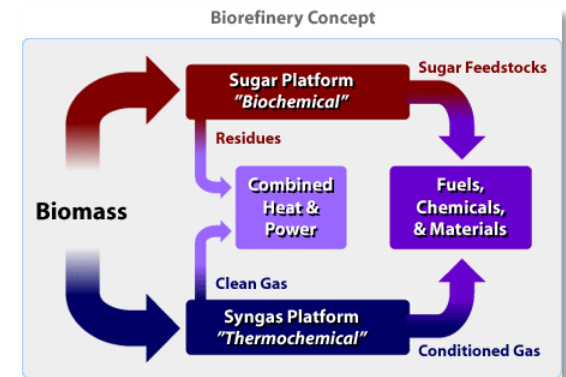
- Established, mature technology
- Some co-products (CO₂ and DDGS)
- Doesn't make money all the time
- Vulnerable to energy and food/feed prices
- How likely is it that 2G technologies in isolation will fare better?



Source: Poet

We need modern integrated biorefineries:

- Multiple feedstocks to provide a variety of sustainable products
- Hedge against volatility of a single product or feedstock
- Improve overall economics – may even enable the whole
- Reduce scale of operation & capital investment (i.e. risk)
- Enable regional instead of world-scale plants



Source: NREL

We need each other more than we realise...



The Key to Success is Partnership

A combination of complementary technologies

- A critical mass of business & research
- In partnership – academia, industry, BIS, TSB, RCUK, etc
- Companies like TMO and many other UK SME's need to engage actively
- Universities (Bath, Nottingham, Aberystwyth, Imperial and many others) can provide innovation and value-added modular technologies
- UK Centres of Excellence like CPI build expertise, value and integrate complementary components



There is a great opportunity for the UK to take a lead:

- Many stakeholders share this vision - strong political and social will
- A demonstrated path to market - a network of keen international customers
- The UK has labs, pilot and demo scale facilities and a growing expertise
- There is an opportunity – but not for long - we must **act now**

I have been impressed with the urgency of doing. Knowing is not enough; we must apply. Being willing is not enough, we must do.

Leonardo da Vinci