



# Biological Processing of Lignocellulose to Ethanol

Processing Lignocellulosic Biomass Conference

CPI, Wilton, Nov 8th

Dr Steven M Martin, Director

# 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

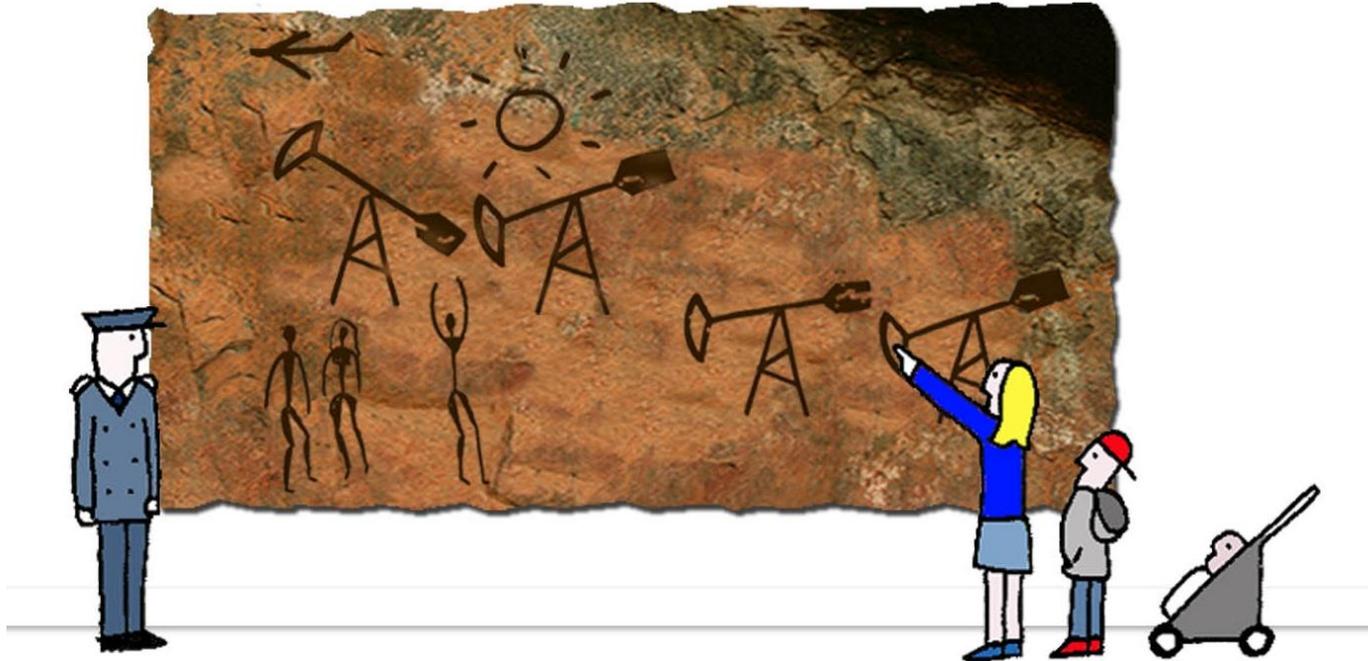
## TMO Corporate Introduction:

- 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

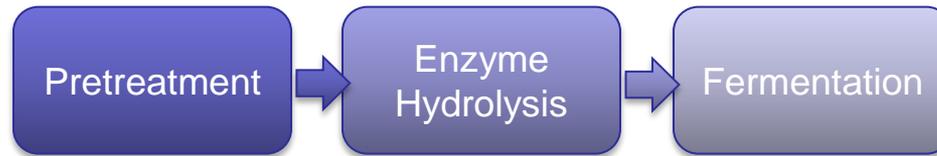


## There are many routes to ethanol from biomass

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

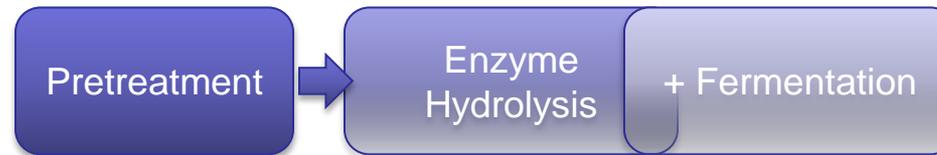


### Separate Hydrolysis & Fermentation (SHF)



TMO  
RENEWABLES

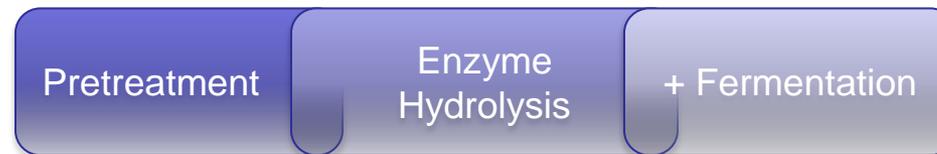
### Simultaneous Saccharification & Fermentation (SSF)



中糧  
COFCO  
自然之選 糧食之光

POET™

### Consolidated Bioprocessing (CBP)

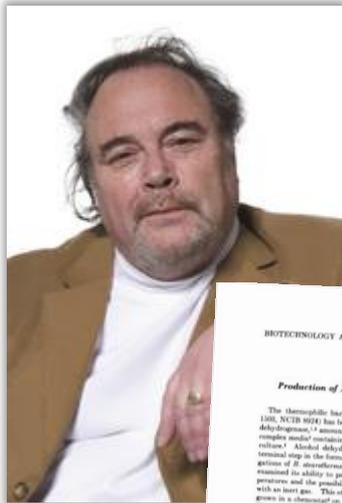


MASCOMA

# TMO's Story - How it Started

In 2002 the search for a talented strain...

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



Prof. Tony A.

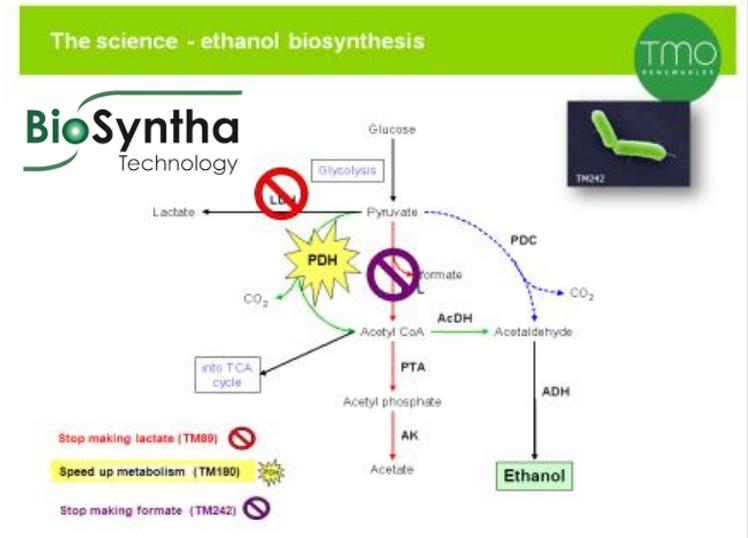


An early observation (1975)

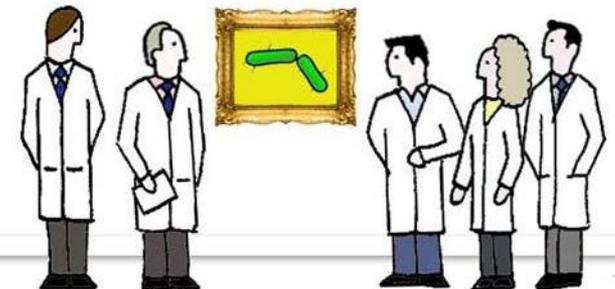
Wide substrate range...

- ✓ 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”

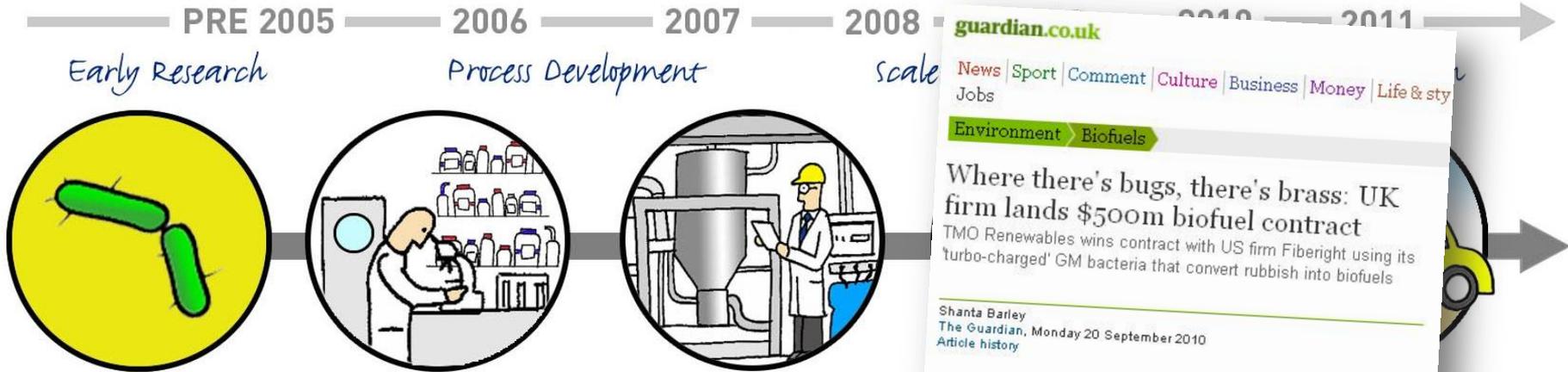
- Want to buy a complete package - feedstock to fuel
- Full design package – full mass & energy balances and full economic model
- Also need data - demonstrate at a meaningful scale
- *Biotechnology is not enough – need engineering!*
- Need to raise more money... build something bigger

## 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...

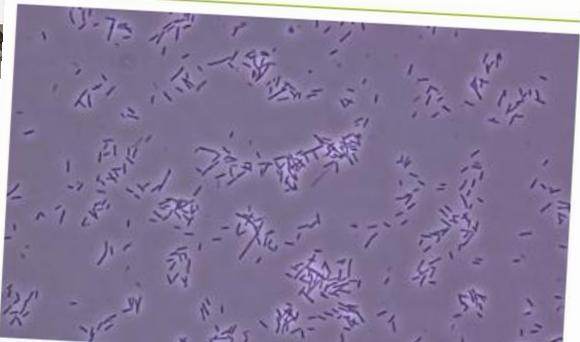


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Environment > Biofuels

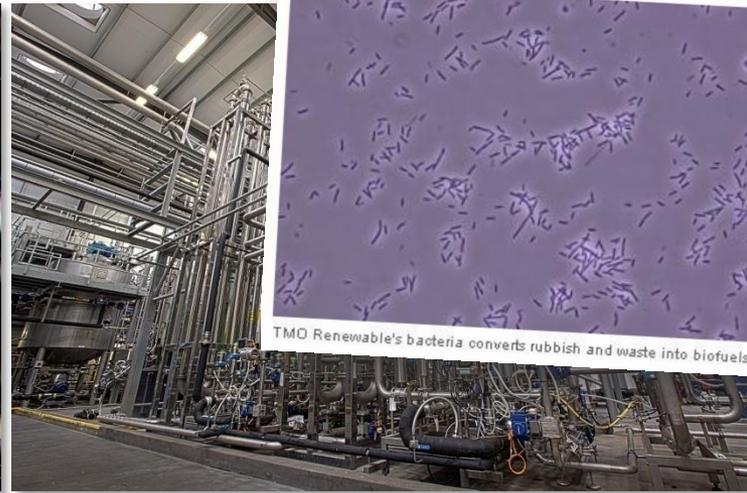
### Where there's bugs, there's brass: UK firm lands \$500m biofuel contract

TMO Renewables wins contract with US firm Fiberright using its 'turbo-charged' GM bacteria that convert rubbish into biofuels

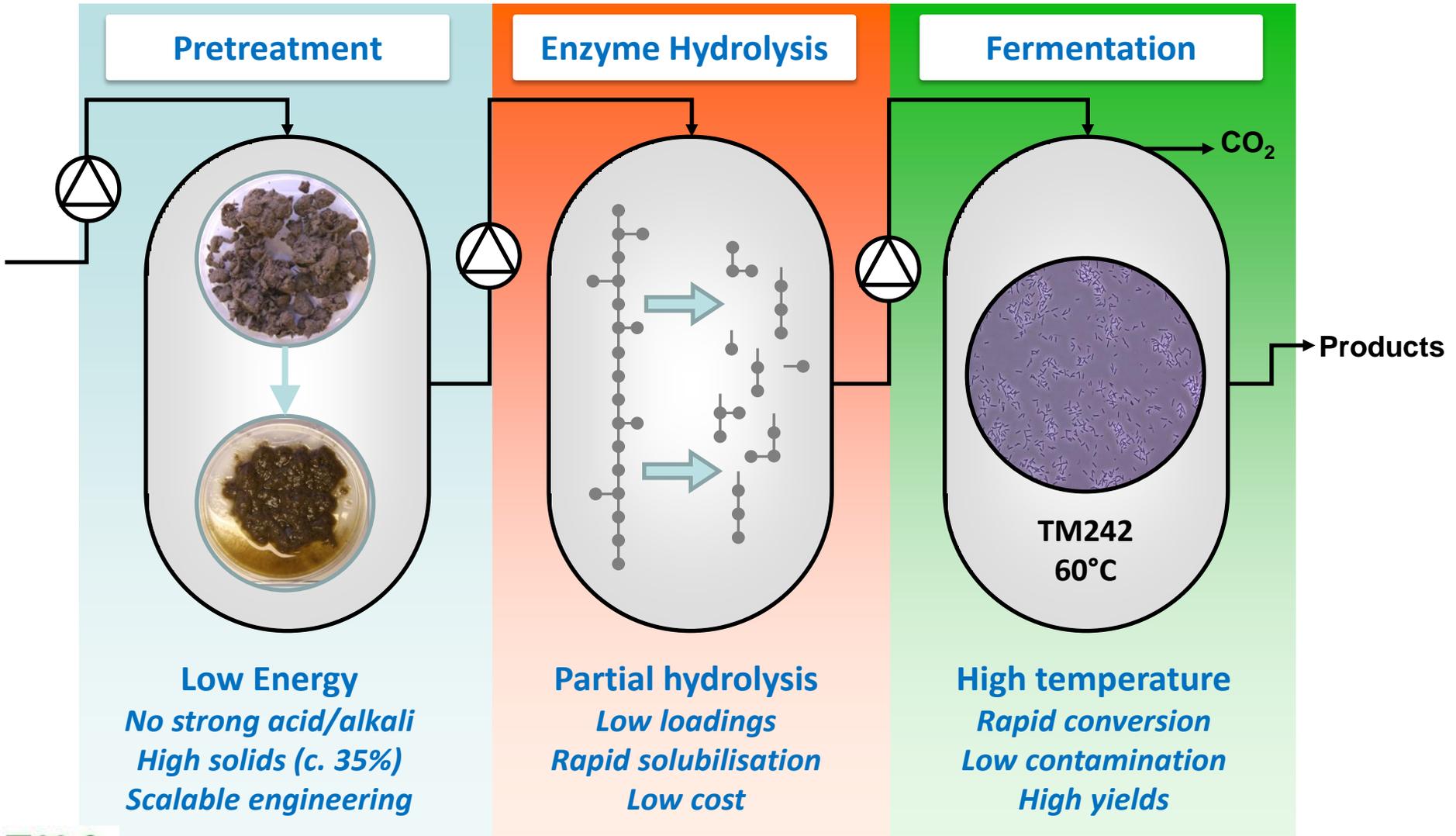
Shanta Barley  
The Guardian, Monday 20 September 2010  
Article history



TMO Renewable's bacteria converts rubbish and waste into biofuels



# 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

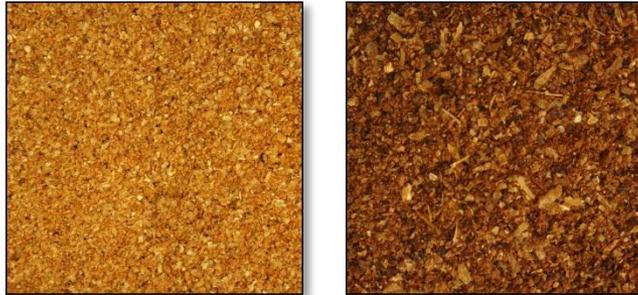
- Seasonal supply – storage issues
- Milling expensive – ideally avoid
- May need sorting or fractionation

| Feedstock Description            | TMO | Total sugar (% Dry Matter) | Max Productivity (L/Tonne) | Target Productivity (L/Tonne) |
|----------------------------------|-----|----------------------------|----------------------------|-------------------------------|
| <i>Dried Cassave Root</i>        |     | 106                        | 685                        | 484                           |
| <i>Cassava Stalk (core only)</i> |     | 78                         | 506                        | 357                           |
| <i>MSW Fibre (High)</i>          |     | 78                         | 505                        | 357                           |
| <i>Miscanthus</i>                |     | 73                         | 469                        | 331                           |
| <i>Cane Bagasse</i>              |     | 72                         | 466                        | 329                           |
| <i>Corn Stover</i>               |     | 72                         | 464                        | 327                           |
| <i>Corn Fibre</i>                |     | 71                         | 462                        | 326                           |
| <i>Cassava Stalk</i>             |     | 71                         | 457                        | 323                           |
| <i>Switchgrass</i>               |     | 68                         | 441                        | 311                           |
| <i>Recycled Paper Fiber</i>      |     | 67                         | 435                        | 307                           |
| <i>High Sugar Grass #2</i>       |     | 62                         | 402                        | 284                           |
| <i>MSW Fibre (Medium)</i>        |     | 61                         | 393                        | 277                           |
| <i>Cassava Residue</i>           |     | 52                         | 337                        | 238                           |
| <i>High Sugar Grass #1</i>       |     | 50                         | 321                        | 227                           |
| <i>Wet Cake - Corn (High)</i>    |     | 47                         | 302                        | 213                           |
| <i>Cassava Residue</i>           |     | 46                         | 299                        | 211                           |
| <i>Wet Cake - Corn (Medium)</i>  |     | 44                         | 282                        | 199                           |
| <i>DDGS - Corn (High)</i>        |     | 43                         | 279                        | 197                           |
| <i>Wet Cake - Corn (Low)</i>     |     | 40                         | 258                        | 182                           |
| <i>DDGS - Corn (Medium)</i>      |     | 38                         | 244                        | 173                           |
| <i>MSW Fibre (Low)</i>           |     | 34                         | 219                        | 155                           |
| <i>DDGS - Corn (Low)</i>         |     | 32                         | 206                        | 145                           |
| <i>Paper Sludge Residue</i>      |     | 18                         | 114                        | 80                            |
| <i>MSW (Poor)</i>                |     | 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    |
| Municipal Waste       | DG Wet Cake   |
| Paper Sludge          | Sisal         |
| Corn Stover           | Miscanthus    |
| High Sugar            | Wheat Straw   |
| 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 (>100m<sup>3</sup>) 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



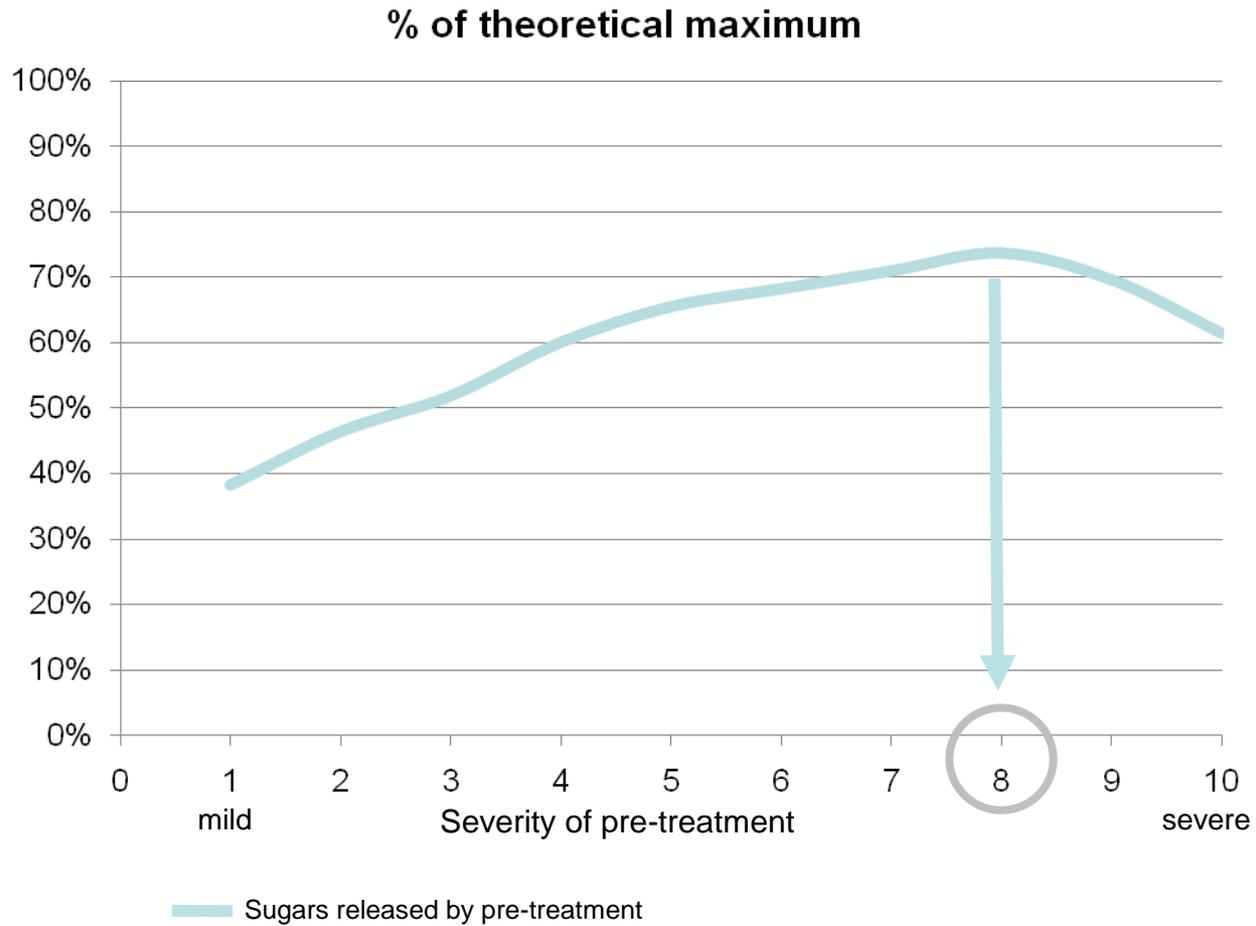
## Enzyme suppliers have limited bandwidth

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

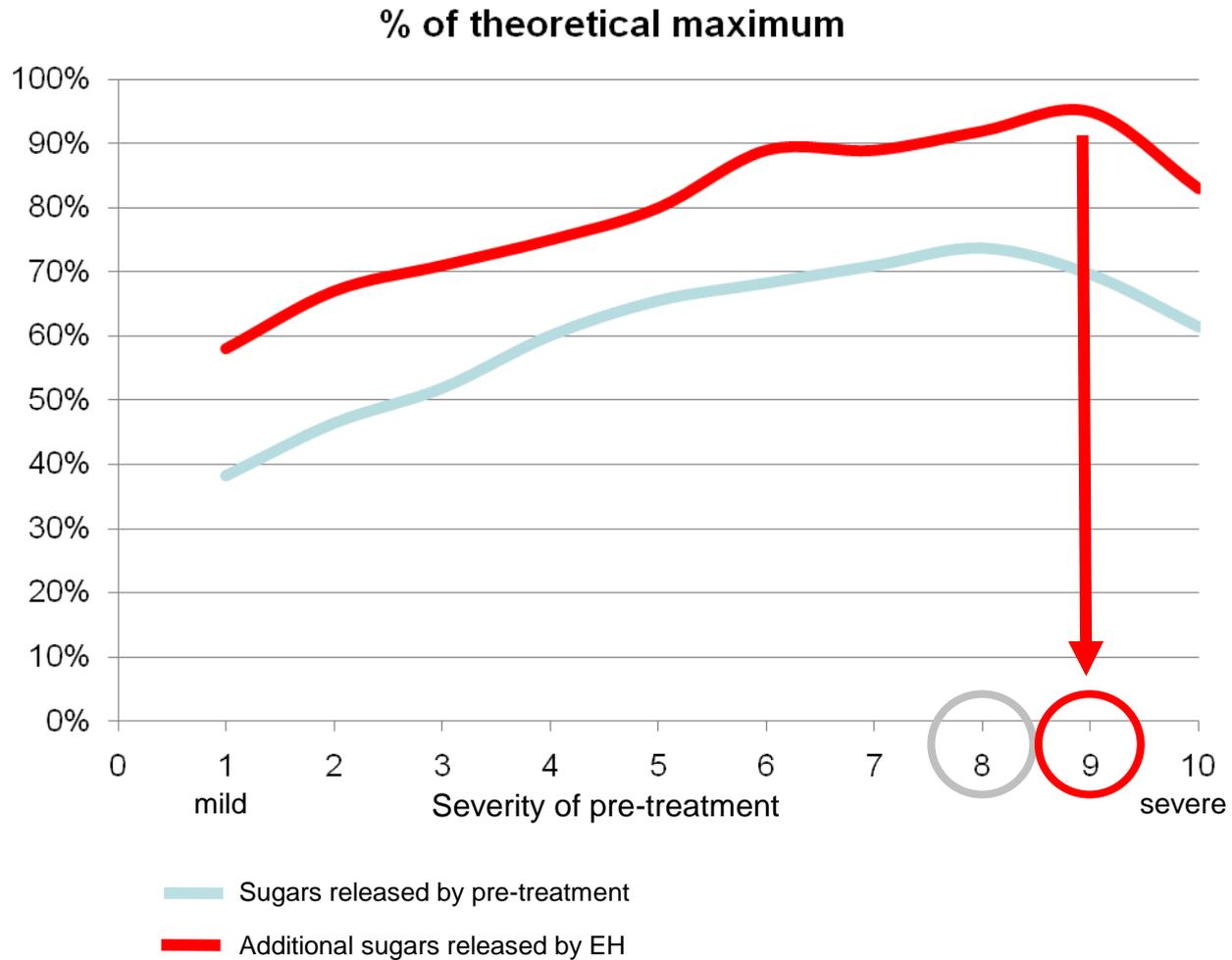
## Single cellulase may be insufficient to saccharify biomass

- 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

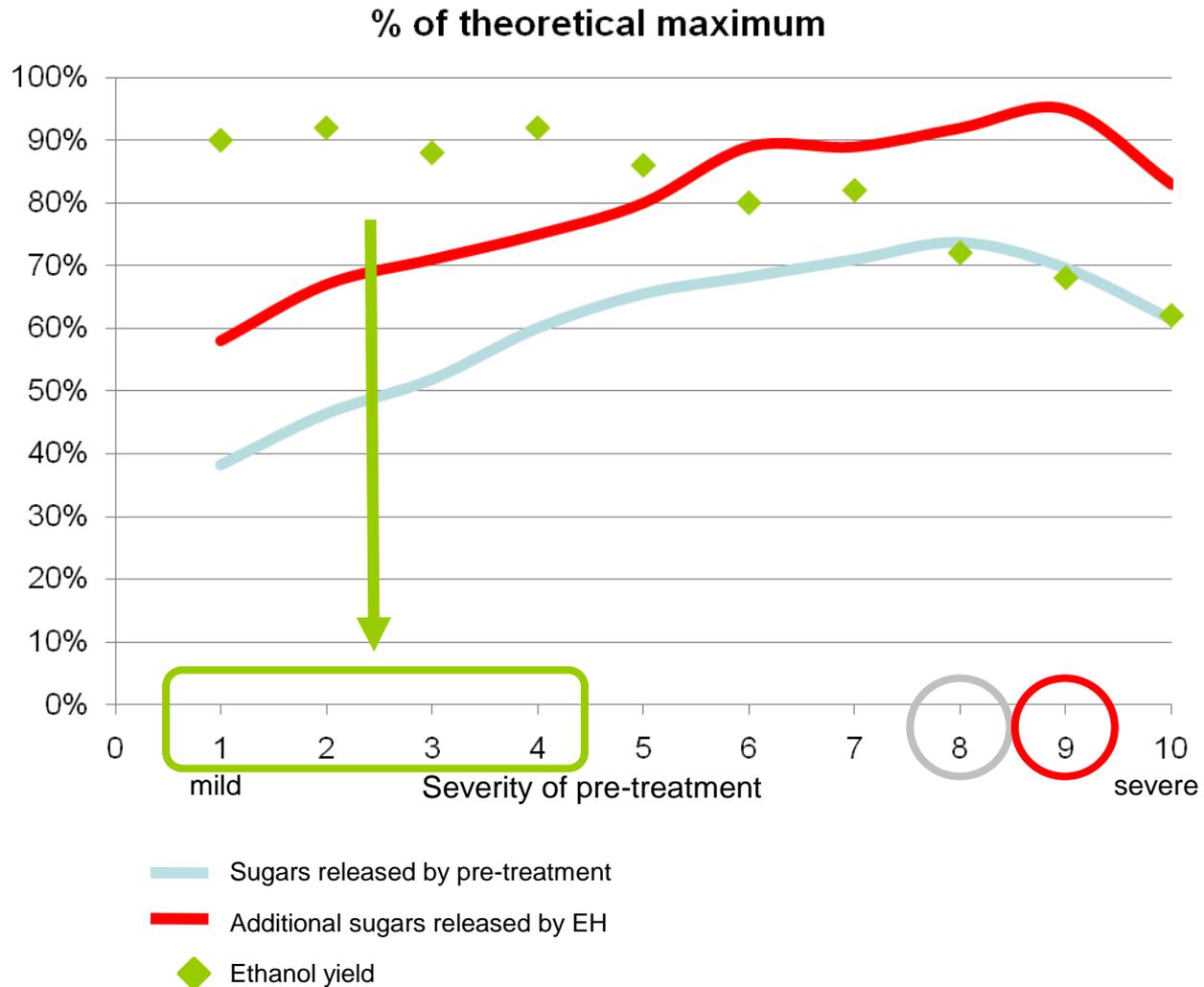
# Pretreatment Study – Corn Fibre



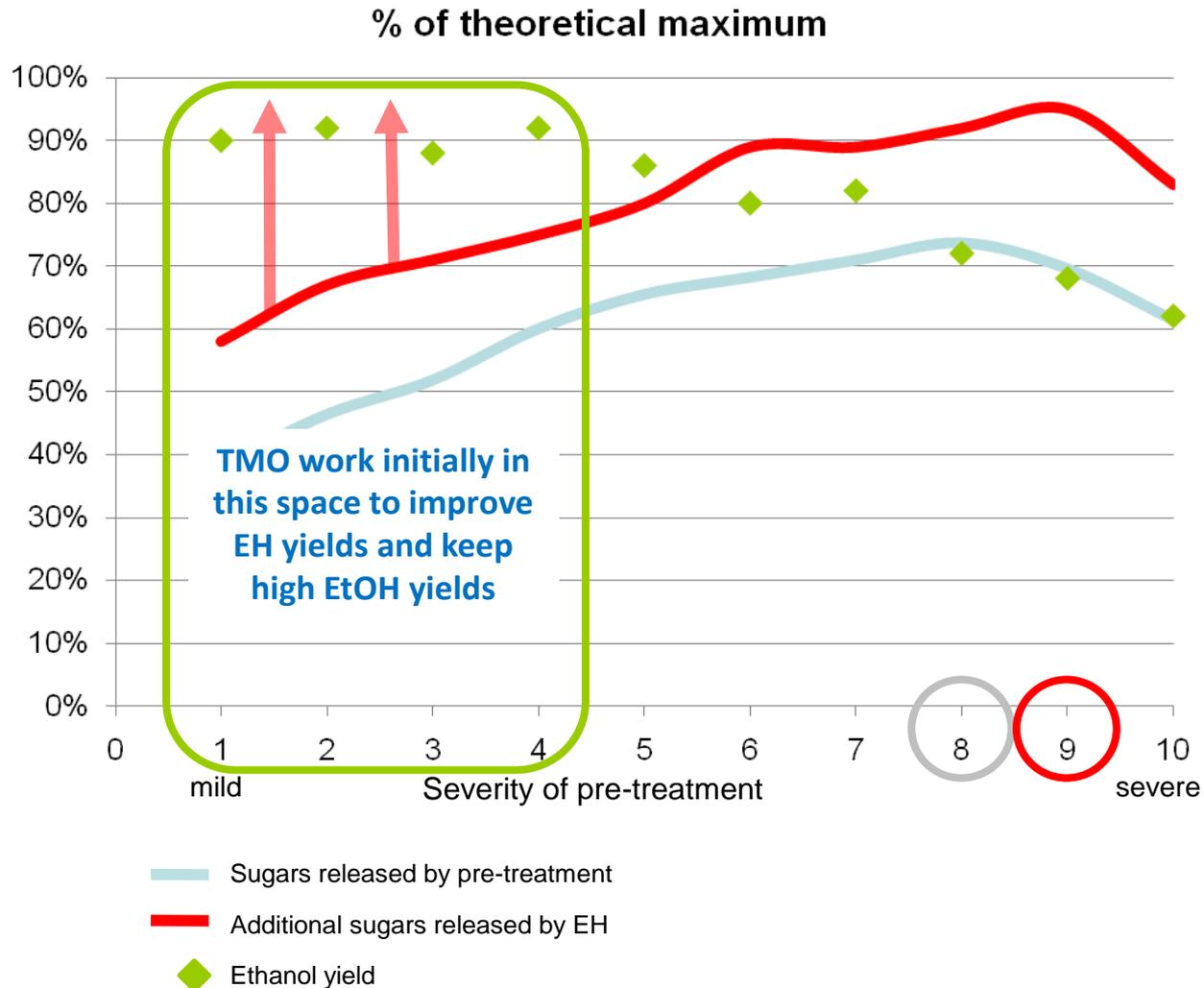
# Following Enzyme Hydrolysis



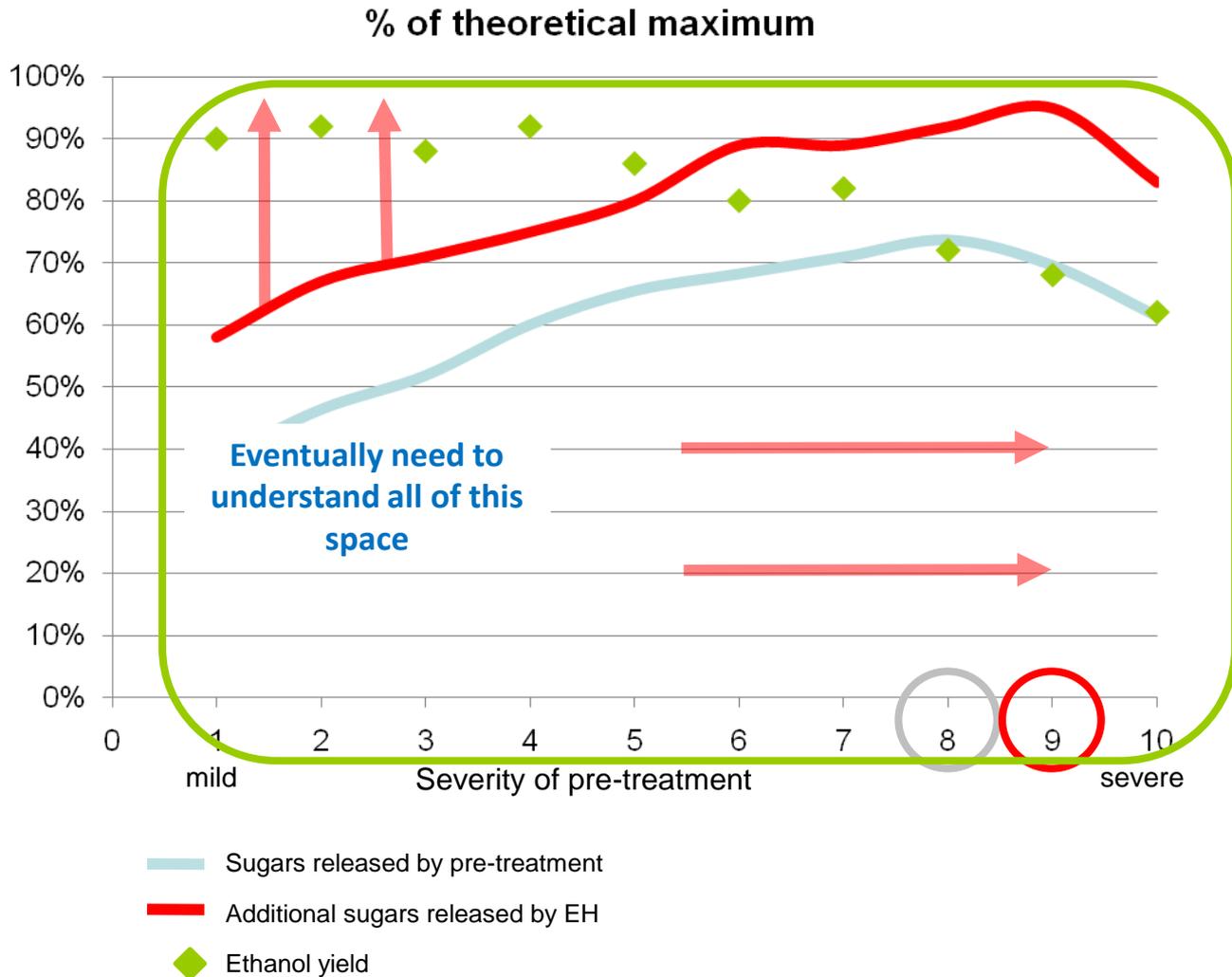
# After Fermentation



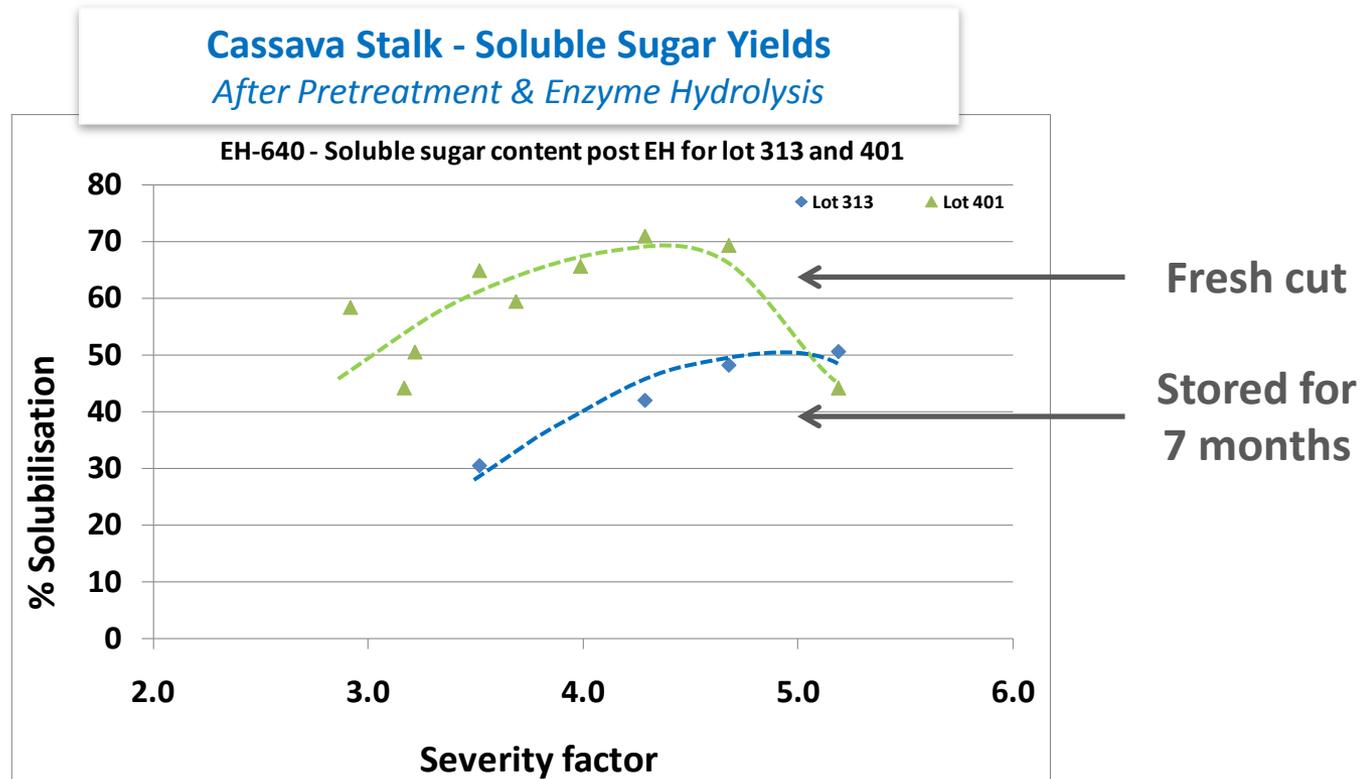
# After Fermentation



# After Fermentation



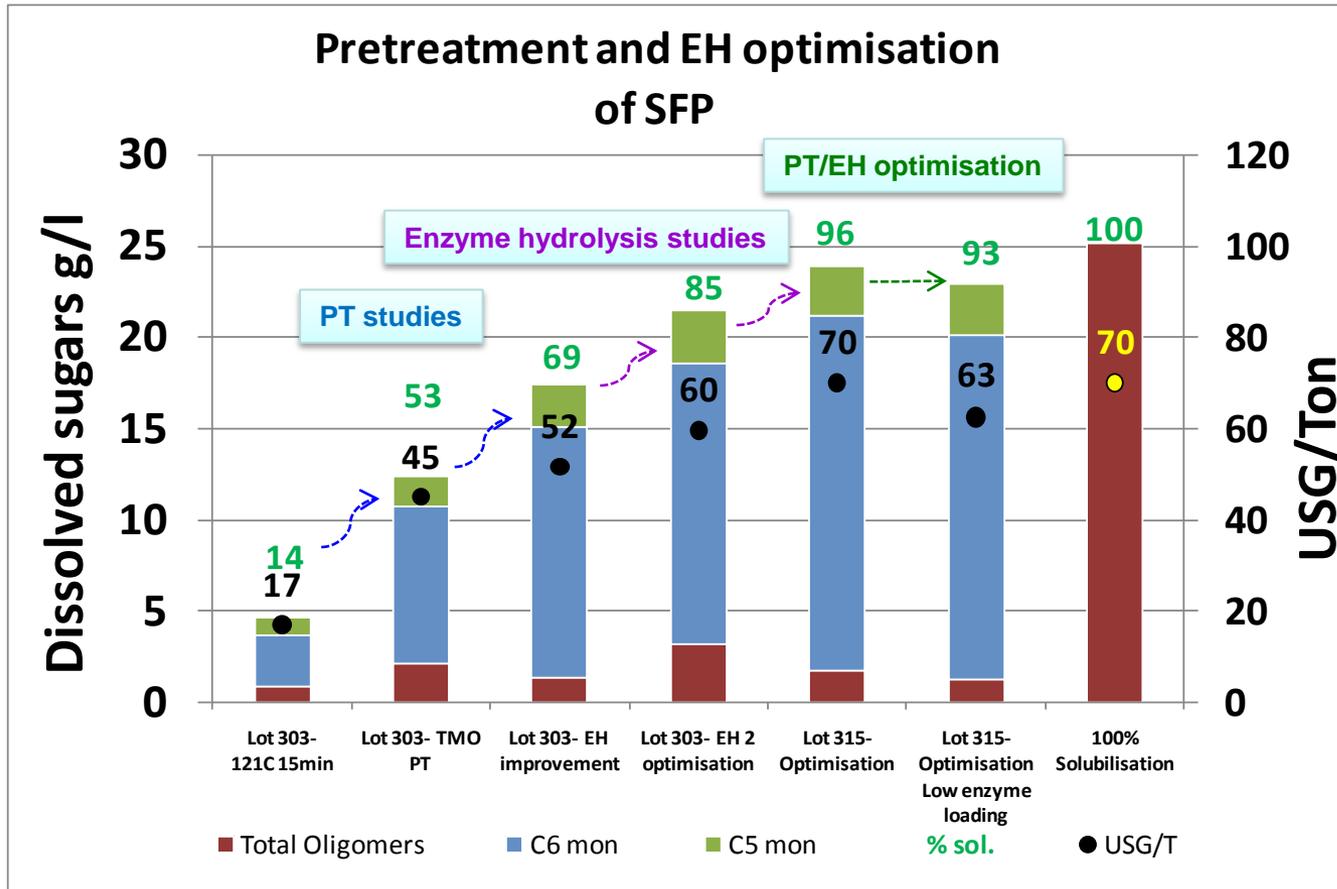
# Feedstock Storage Example



## 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

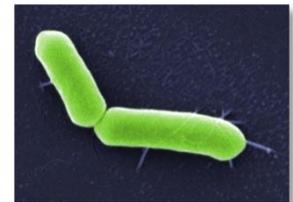
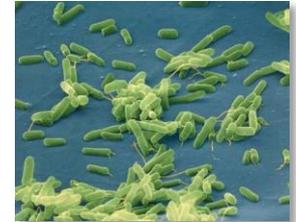
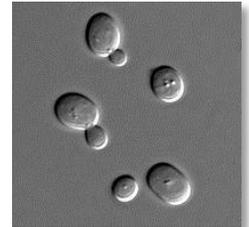
# Fermentation – where the magic happens

## 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

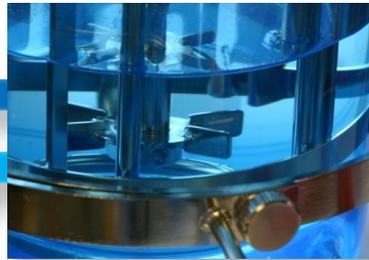
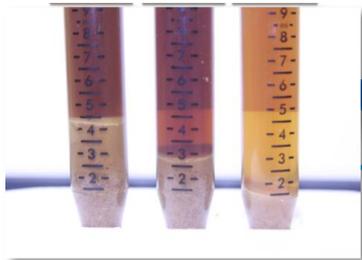
**Opportunities for multiple players**



# 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:

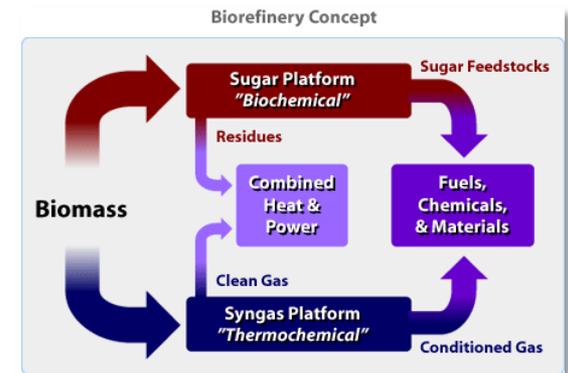
- Established, mature technology
- Some co-products (CO<sub>2</sub> and DDGS)
- Doesn't make money all the time
- Vulnerable to energy and food/feed prices
- How likely is it that 2G technologies in isolation will fare better?



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***