National University of Ireland, Galway
Ollscoil na hÉireann, Gaillimh

Enzymes and second generation ethanol

Dr. Maria Tuohy

Molecular Glycobiotechnology Group
Department of Biochemistry, NUI, Galway, & Eirzyme, Ireland

IEA Task 39 Workshop 2008
University College Cork

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Biomass to Bioenergy: Key Goals, Challenges & Barriers

CO_2

Global Energy targets
Environmental benefits
Sustainability
Biofuel crops
Technological challenges?
Barriers?

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Market Size and Targets: Bioethanol

Global Bioethanol Market:
2002: 17 mTon (€10,249 billion)
2020: Predicted 5-fold market increase

European Bioethanol market:
2004: 0.77 mTon (€367 million)
Annual growth rate of 72%

Irish Bioethanol market:
2005: 0 mTon
2010: 0.07 mTon
221 mL BioETOH (€34.1million)

1 hectare ≈ 8-10 tonnes wheat
≈ 2.4-3.0 ton bioethanol

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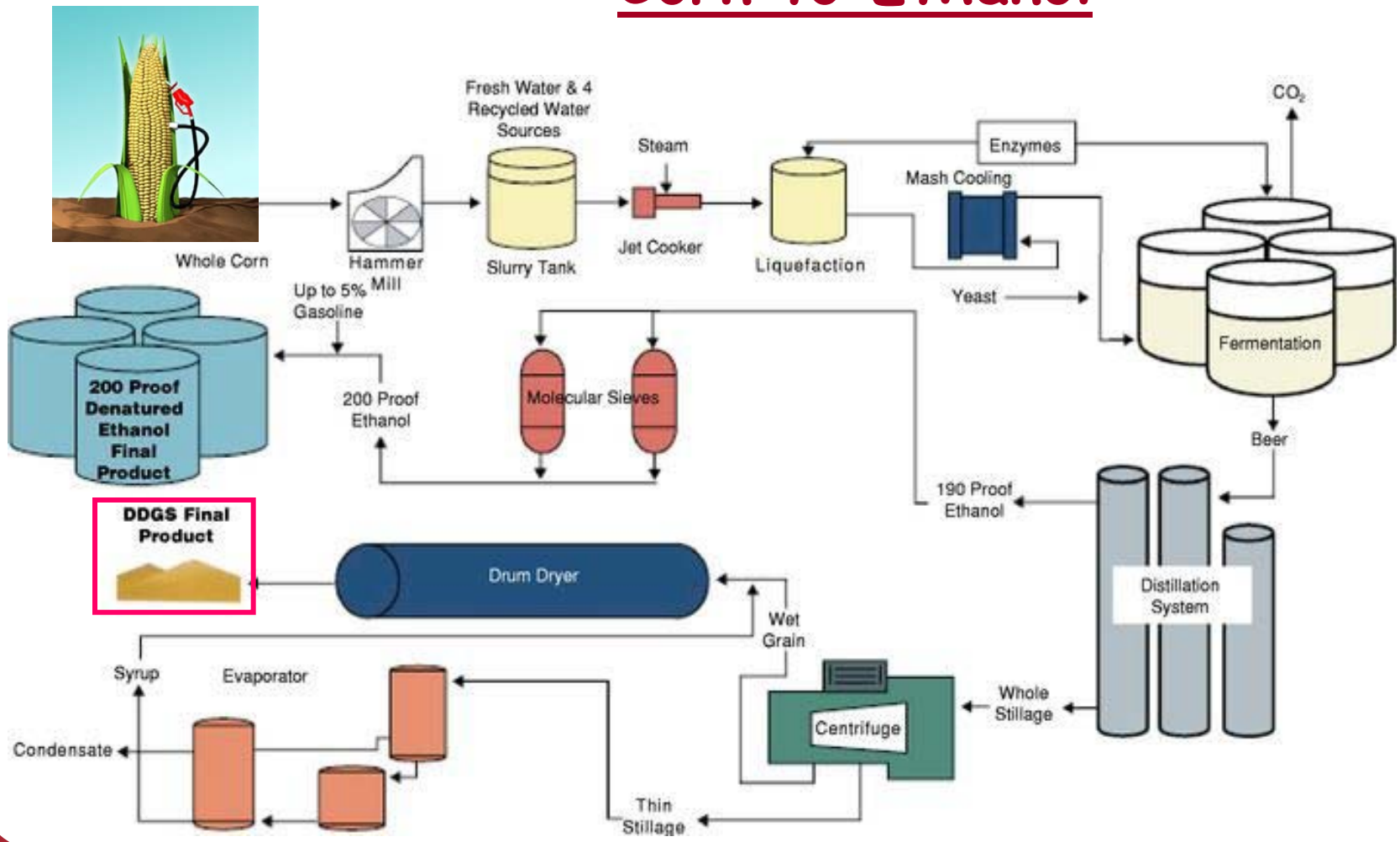
1st Generation Biofuel technology: Corn to Ethanol



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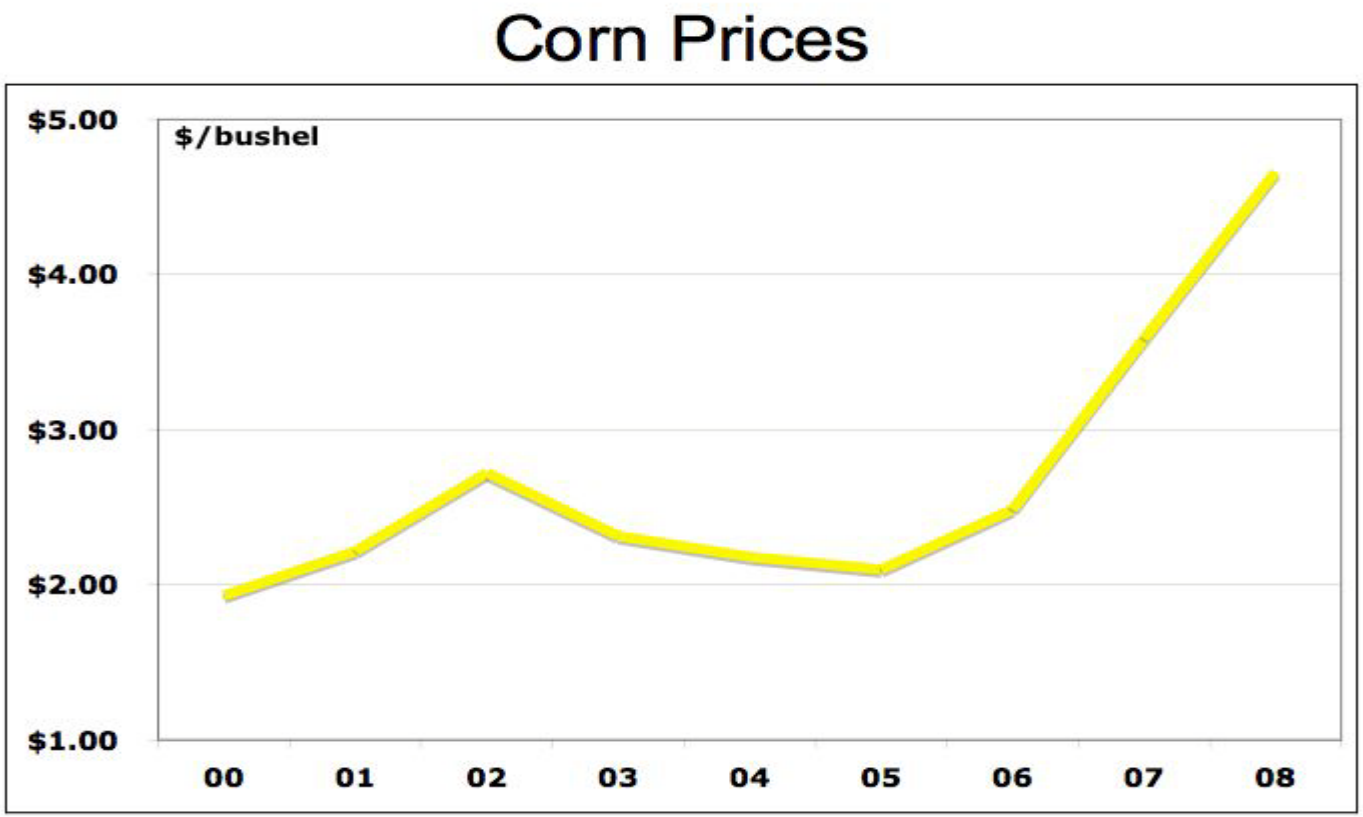
Food *vs* Biofuel!



Source: U.S. Department of Agriculture

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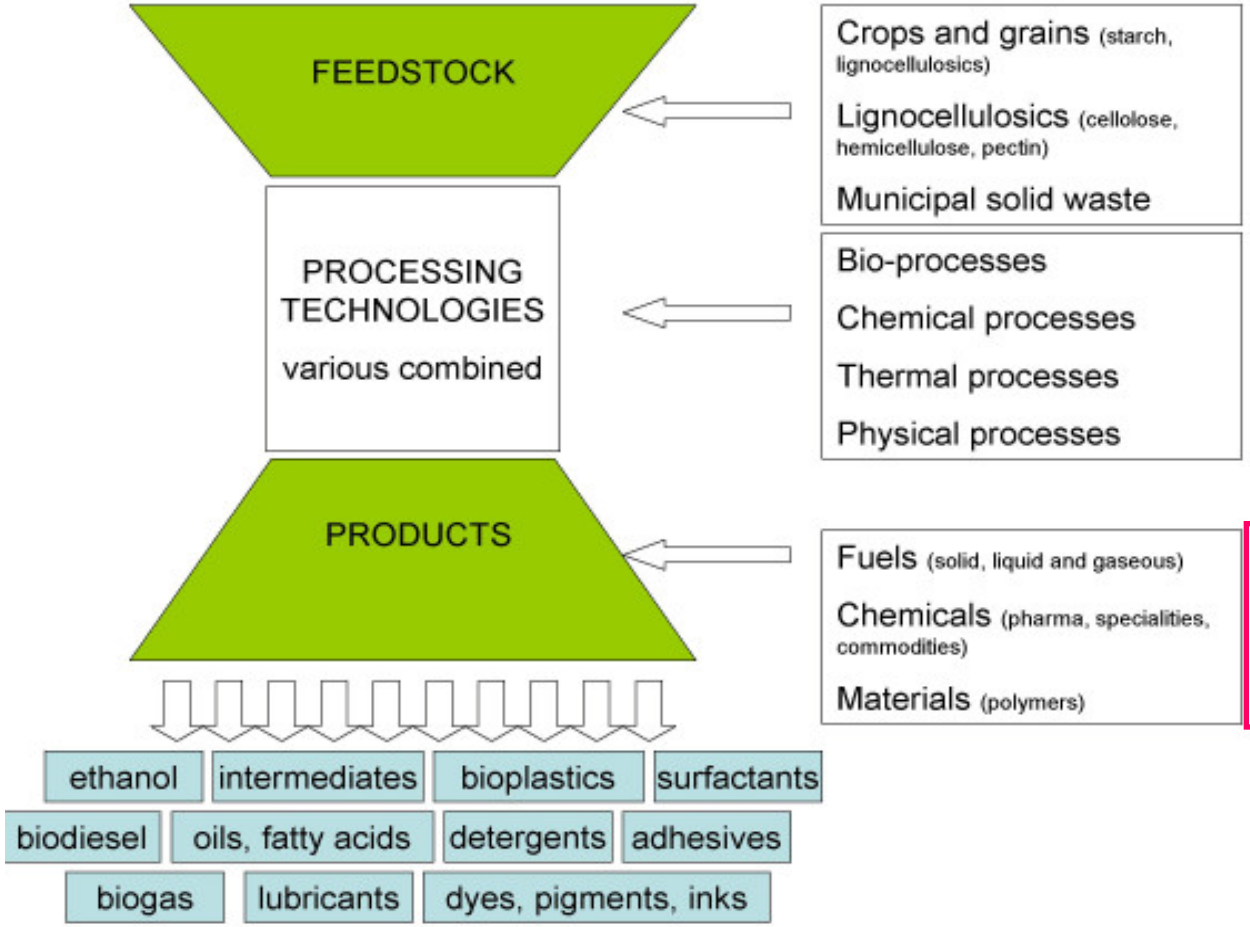
2nd Generation Biofuels:Technologies & Goals



Non-food crops & wastes
Novel crops, incl. transgenic crops

Better pretreatments
Novel low-cost Enyzmes/ Enzyme systems
Heat stable & improved specificity

Improved fermentation yields
Overcoming the C5 problem
Novel microorganisms
Alternative biofuels

Recovery & Exploitation of Co-products

Turner *et al. Microbial Cell Factories* 2007 **6**:9

The 'Biorefinery' Concept

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Molecular Glycobiotechnology Group, NUI, Galway

Research Team

Senior scientist, Post-doc &
PhD Students
Erasmus students, national
& international summer students

BioEnzTech Team (Pilot plant)

Project Manager
Research Assistants
Fermentation scientist
M.Sc. student

Spin-out Company: Eirzyme

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2nd Generation Biofuels: Technology development

Molecular Glycobiotechnology Core Facilities:

- Molecular Biochemistry, Cell Biology & Genetics Laboratory – enzyme biodiscovery, functional characterization & co-product analysis

- BioEnzTech Fermentation Technology Unit (100 L Enzyme production capacity) – enzyme scale-up

- Eirzyme Product & Process Development laboratory; Development of Large-scale enzyme production facility

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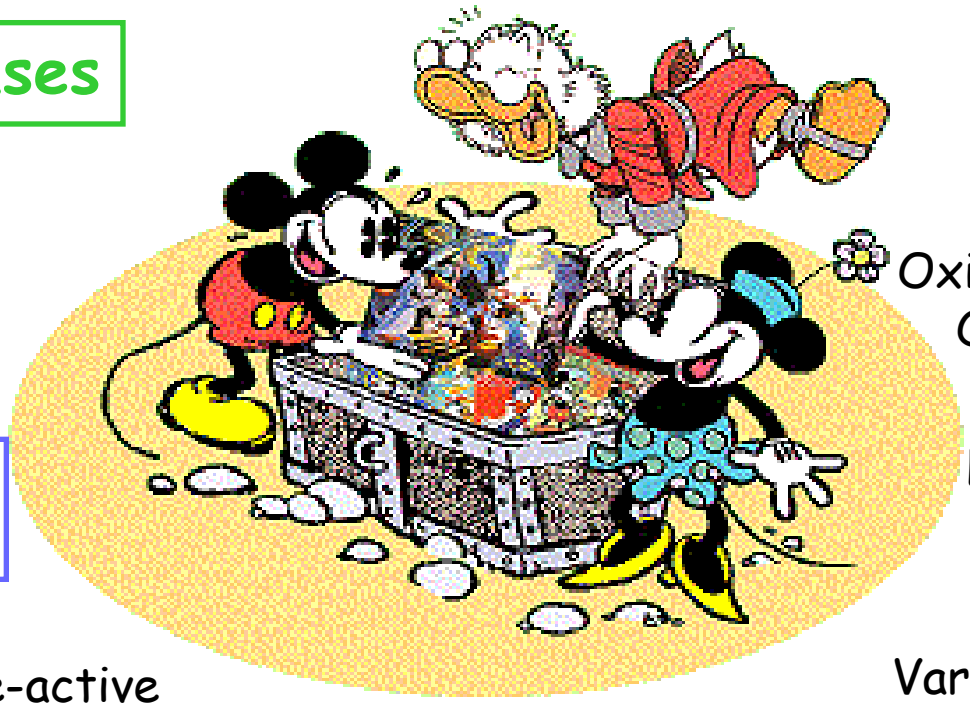
T. emersonii - a treasure trove of Biotechnological 'goodies'!!

Cellulases

Pectin-modifying enzymes

Starch-modifying enzymes

Novel carbohydrate-active enzymes



Peptidases

Oxidases/Peroxidases
Oxidoreductases

Lignin-modifying enzymes

Various esterases

Hemicellulases

Key Advantages of NUI,Galway Enzyme Technology

- Derived from a 'Generally regarded as Safe' (GRAS) Source'
- Unique & large portfolio of enzyme preparations
- Multiple applications, different industrial sectors
- Readily optimized for target applications

- Characterized by Thermostability
  - higher reactivity and efficiency,
  - shorter reaction times,
  - pasteurization of feedstock,
  - greater product yield

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NUI,Galway Enzyme Technology

Source Microorganism

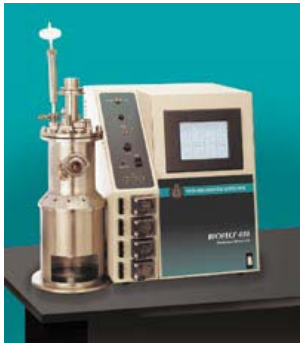
Enzyme production

Metabolism & Cell Biology

Genetics & gene regulation

Proteomics

Genomics



Optimized Enzyme Cocktails

Each Cocktail – biochemical 'fingerprint'

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Target substrates: A wide range of Carbohydrate-rich raw materials

Primary sources

Secondary sources

Terrestrial & Marine plants,
Fruits to woody tissues

Processing by-products
Various Wastestreams

NUI, Galway Platform
Technology

Value-added products

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Biofuel/Bioenergy Strategies

Substrate

+ **Thermozymes** → Residue (lignin) + **Simple sugars**

Biogas

Bioethanol

Flavour & Aroma precursors
Antioxidants
Biopharmaceuticals

Value-added products

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Overview of Biorefinery approach

Any substrate rich in carbohydrates

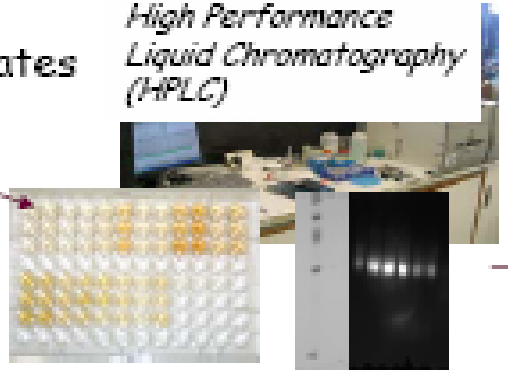
Biofuel production – lab-scale studies

APPLIED RESEARCH

T. emersonii

Thermophilic fungus

Specific Enzymatic cocktails



High Performance Liquid Chromatography (HPLC)

BioEnzTech (Fermentation Unit)

Extraction of Molecules with Commercial interest

Characterisation of Molecules with Commercial interest

Pilot Scale (1 Patent)

Patents

Patents

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Biomass conversion, Bioenergy & Biorefinery applications

Selected Examples

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Terrestrial Plants

- Woody materials & Residues
- Agricultural crops and residues, e.g. Sugar beet, Cereals, Novel crops
- Cellulose-rich fractions of commercial wastes

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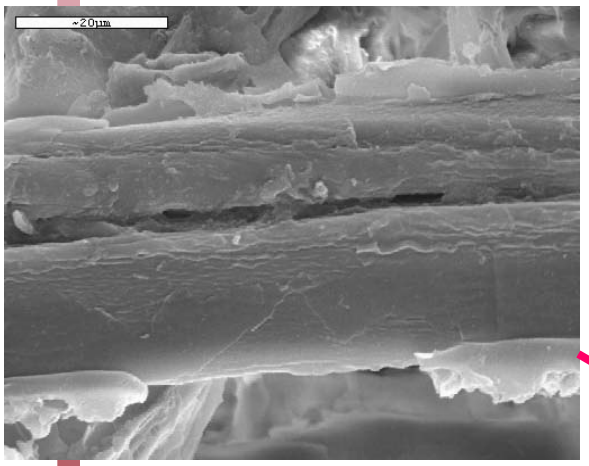
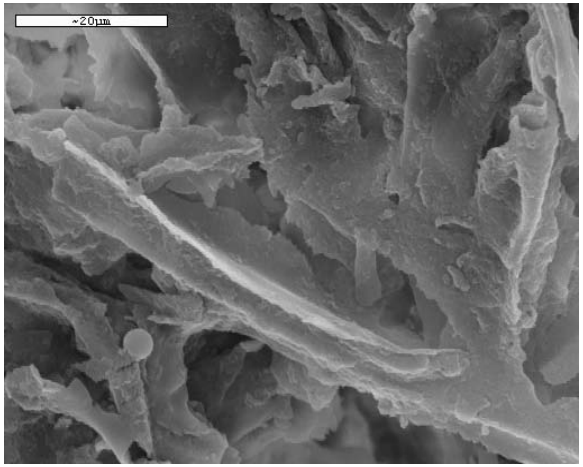
Low-cost Enzyme cocktail for conversion of Woody residues

Spruce fibres – no enzyme

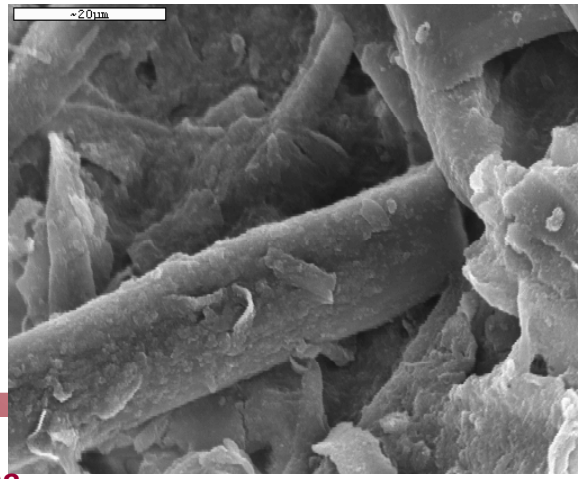


Best, optimized Commercial enzyme preparation (40°C)



Complete loss of enzyme activity at higher temperatures

T. emersonii enzyme cocktail (40°C)



Complete loss of fibre structure at higher temperatures

Enzyme stable

Extensive cellulose degradation

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Other Terrestrial Biomass Substrates

- **Project title**: 'Thermozyme technology for production of high-value products from Sugar beet'
- **Project title**: 'A sustainable strategy for Bioethanol production in Ireland' - A wide range of Cereals, Cereal Residues & Grasses
- Others: Sugarcane, various Agri-residues, Food processing residues.
- **Results:** Enzyme cocktails – highly efficient release of simple, fermentable sugars (68-89% conversion)

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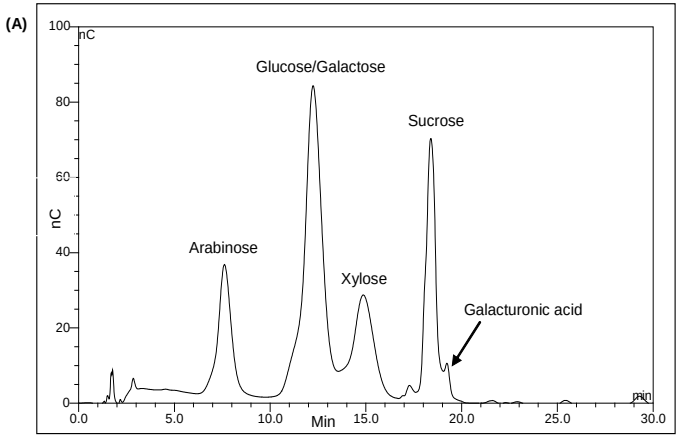
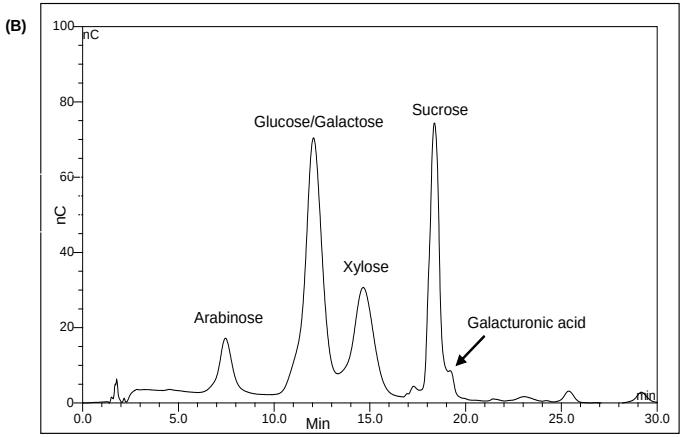
Enzyme technology for the bioconversion of Cereals & Sugar beet





Simple sugars released from sugar beet by cocktails 1 and 5

Sugar Beet - no chemical pretreatment
Cocktail 1: Glc (47.6%), Gal (8.8%), Xylose (20.2%), Ara (19.3%) & GalUA (4.1%)
Cocktail 5: Glc (49.3%), Gal (5.6%), Xyl (26.0%), Ara (9.3%) & GalUA (9.9%)

Cereals & cereal Grains: mechanical discruption
oat straw, oat grain, wheat bran and mixed retail flour: 70-92% conversion of polymeric carbohydrates to simple sugars
~65-72% are monosaccharides, with Glc(42-51%), Gal/Man (6-8%), Xyl (17-23%), Ara (6.5-21%)

In progress: Distillers grains (with Teagasc)

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Marine Biomass to Energy

Collaborative Algal Research with AER Ltd.

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Macroalgae & Microalgae

Simple Sugars → Biofuel

+ Value-added co-products

Key Goals:

- Develop Thermozyme technology to harness the potential of Algae
- Investigate a wide range of species across different phyla
- Utilise a renewable and valuable plant resource – 2nd generation feedstock

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Macroalgae: rich in Polymeric Carbohydrates

Brown

35% to 70%
Dry Weight

Red

25% to 50%
Dry Weight

Green

20% to 46%
Dry Weight

9 Brown algae	6 Red algae	2 Green algae

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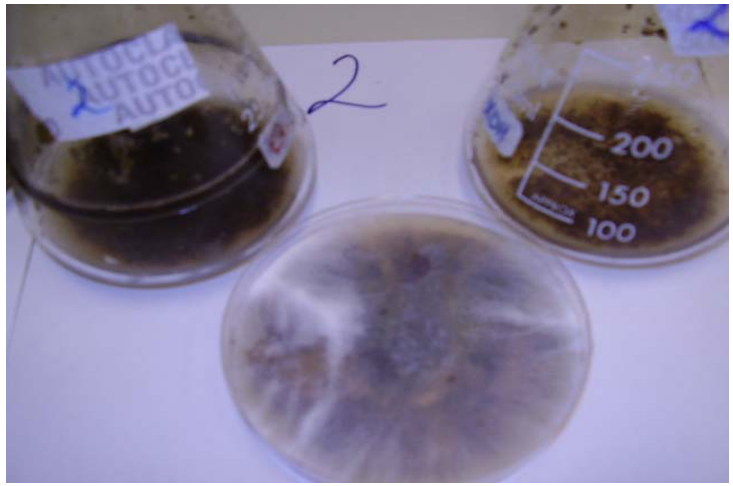
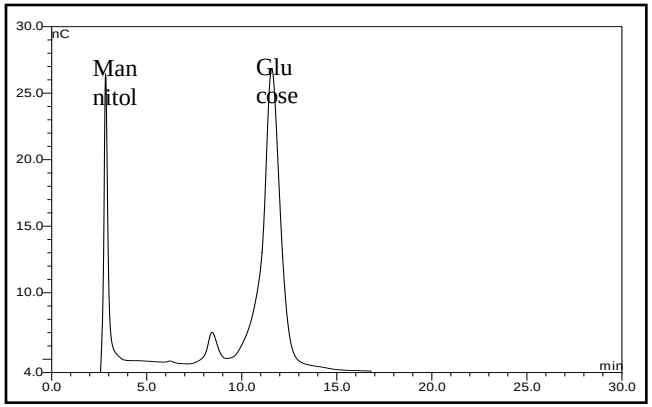
Example: Brown Algae





Hydrolysis/Utilization of brown alga by the Source organism

Alga + Thermozyme → Sugar-rich stock Upto 92% conversion of polysaccharides to glucose and mannose fermentable sugars

Optimum reaction conditions

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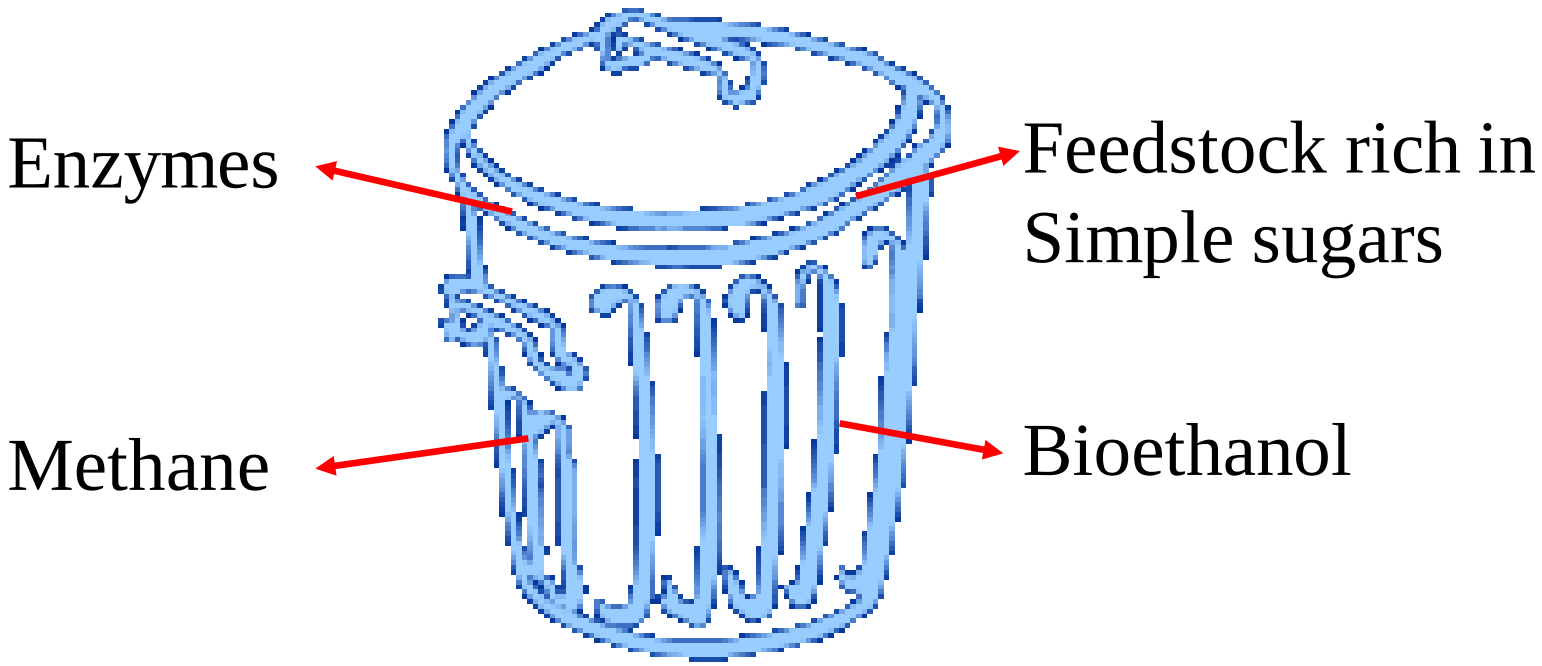
Waste Valorization & Management



Environmentally friendly Bio-Technology

WASTE MANAGEMENT
Volume reduction

RESOURCE RECOVERY
Energy & Value-added
By-products

A wide variety of Wastes: Cellulose-rich Clinical Wastes, MSW, Food wastes, Print & card wastes

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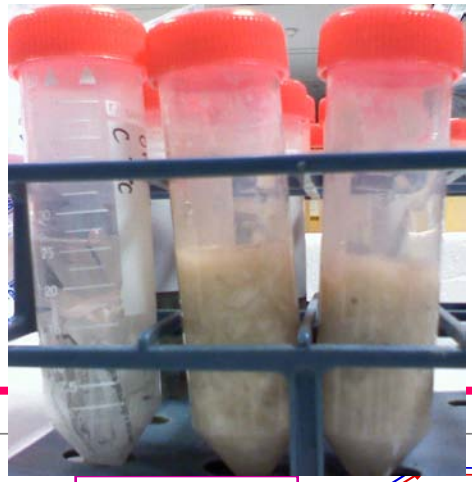
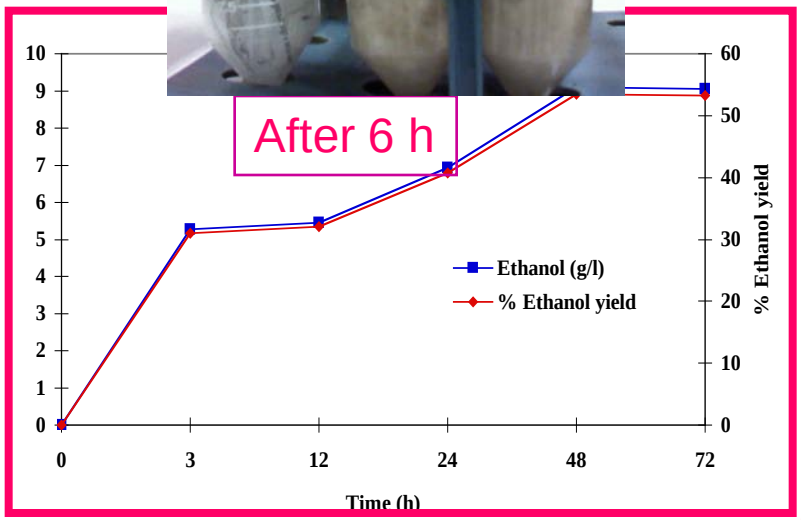
NUI, Galway Thermozyme cocktail: Bioconversion of Cellulose-rich PAPER wastes

NUI, Galway Thermozymes



Paper products before treatment

After 6 h



Ethanol production

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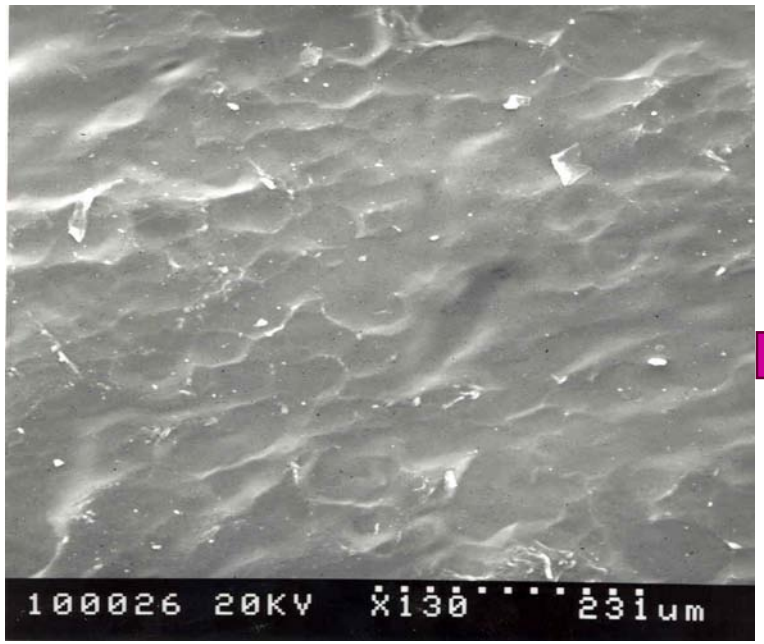
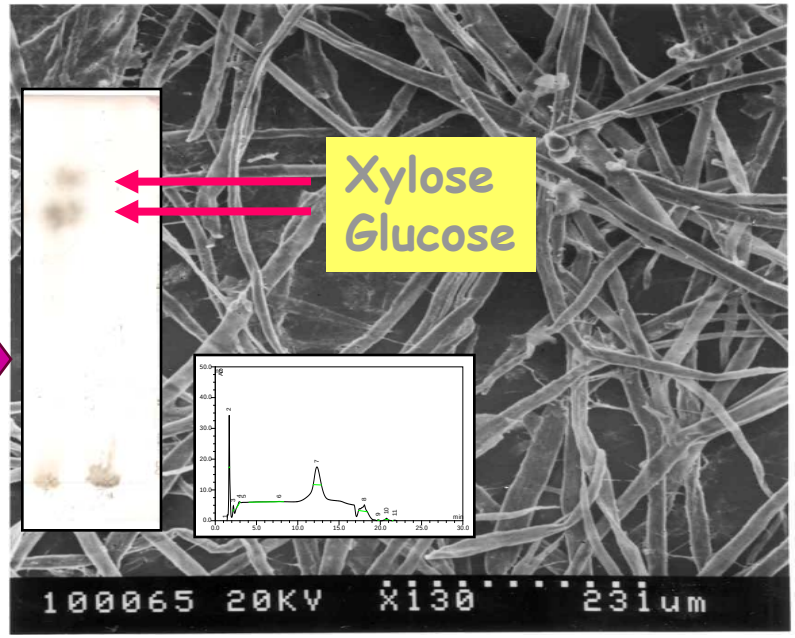
Visible changes in paper products



No Enzyme treatment



Enzyme treatment for 24 h

Reduced reaction time at 65-80°C
No chemical pre-treatment required – shredding or rough maceration enhances sugar yield

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Range of Paper products screened

- Newsprint
- Office Paper
- Brown paper & Card
- Coloured print & Card
- Glossy print
- Catering products
- Tissue products
- Packaging

Process parameters:
Pretreatment
Temperature
pH
Duration of reaction
Moisture content

Steam pretreatment > chemical pretreatment

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Feedstocks for Biogas production

Target Biomass Substrate

Thermozyme treatment

Sugar-rich hydrolyzate

Anaerobic Digestion

Upflow Anaerobic Hybrid Reactors

Biogas → ~45-65% Methane

Other gases

Shorter Retention times – potential for higher loading rates and reduced reactor size/costs

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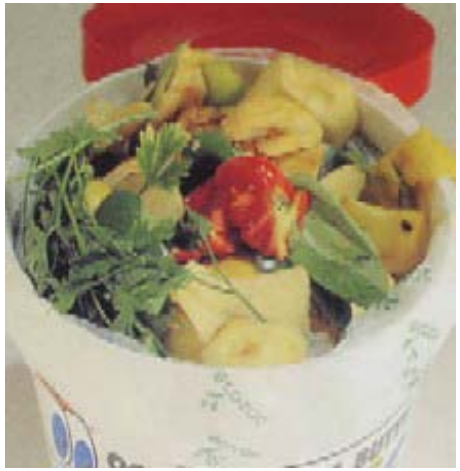
Sources of OFMSW (Food Waste – Non animal)

University Caterers

Domestic

Florists

Catering & Restaurants



Grain processors

Local Coffee shops

Bakery & Confectionery

Fruit & Vegetable Suppliers

Alcoholic beverage producers

Fruit processors

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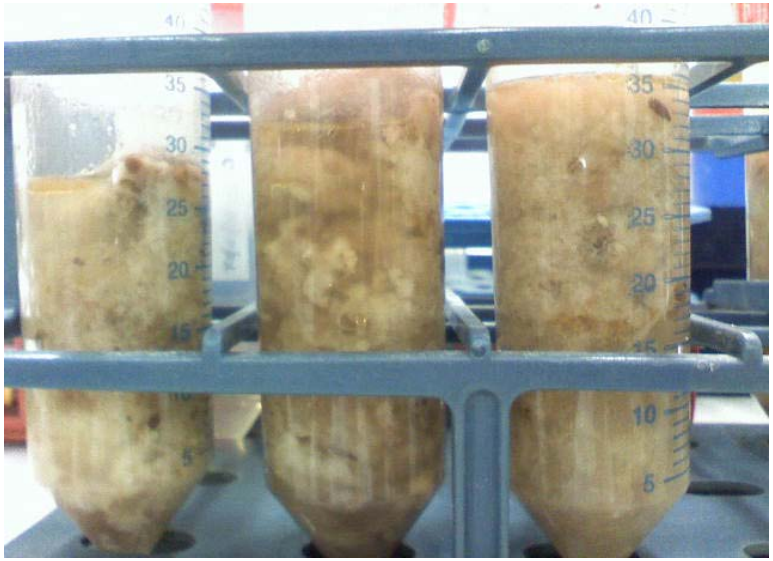
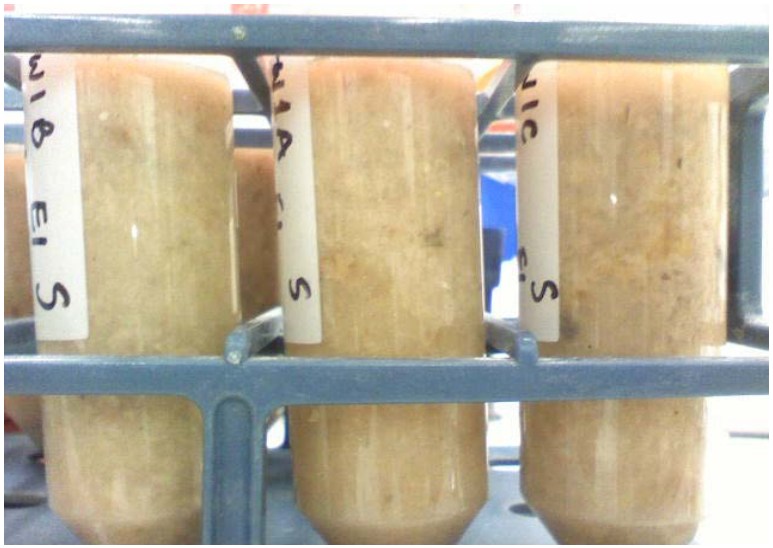
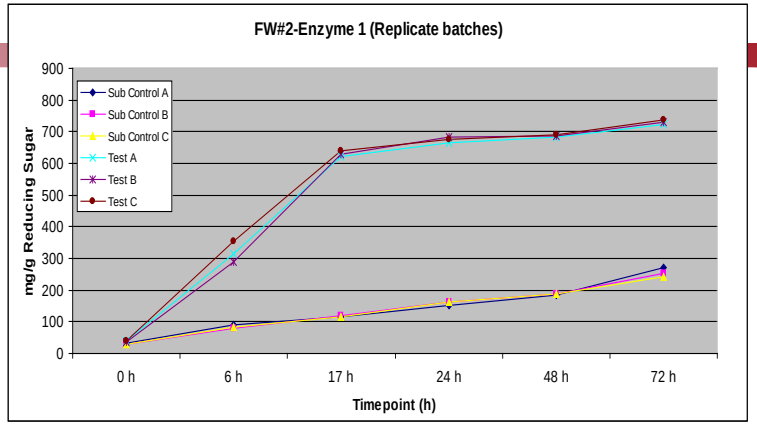
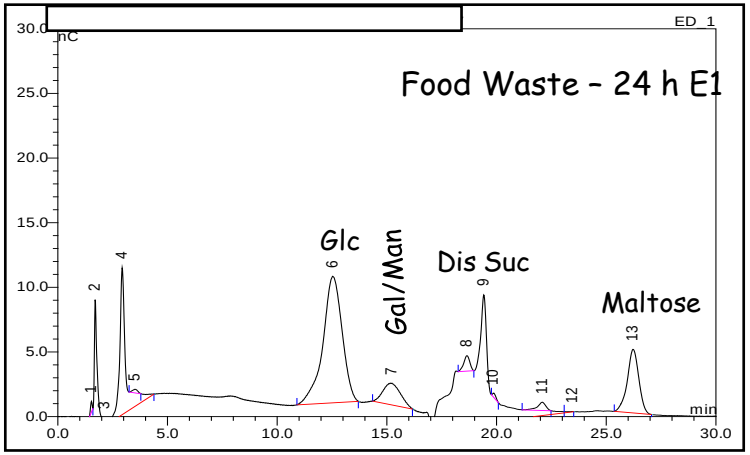
Mixed Food Waste









% Conversion of Food waste polysaccharides: 65.4-94% on a DW basis. In addition, 72.0-92.5% of the available carbohydrate was converted to reducing sugars at 24 h and 72.8-96.2% at 72 h. ~18-30% = monosaccharides; remainder = di & trisaccharides

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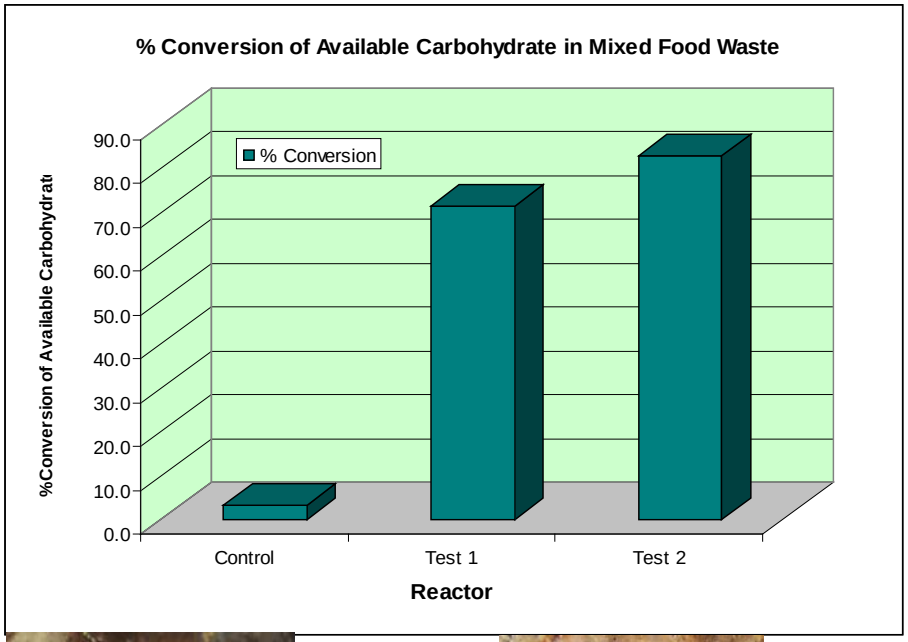
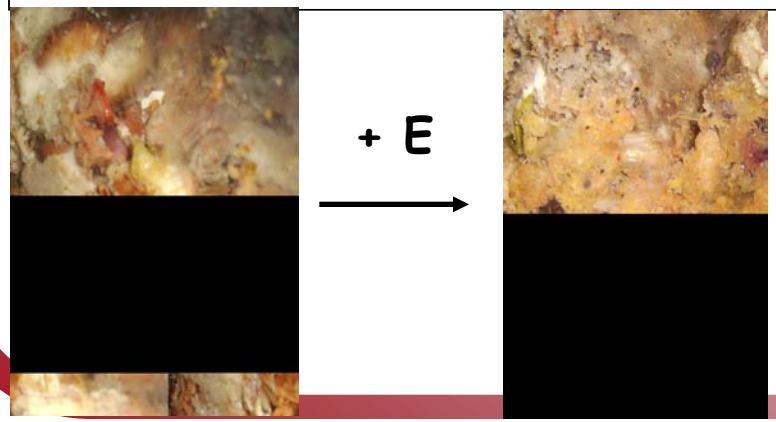
Mixed Food Waste





Food Waste

Thermozyme 40°C ↓ 3.5d/~40°C

71-83% conversion

↓

Fermentable sugars (leachate)

Biogas: 317 kWh/tonne
(Heat for 10.8-17.8 houses p.a.)

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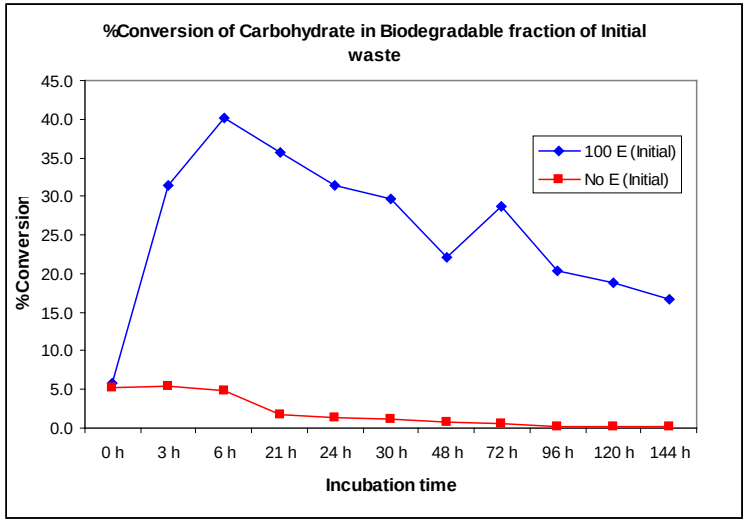
Unsorted MSW

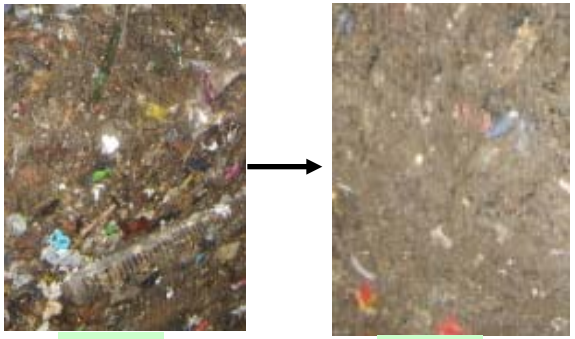


~40% of the Biodegradable fraction

Thermozyme ↓ **6 h**/~40°C

Fermentable sugars (leachate)

Methane: 40-60% of biogas



0 h → 6 h

Yield can be increased ~2-fold at 70°C
>80-85% is monosaccharide;
remainder = di- and higher oligo-saccharides.
Glc ~ 50-55%; Fru ~29-33%.

Steam pretreatment > chemical pretreatment

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Scale-up & Development of Enzyme Cocktails

Lab-Scale → 10 L → 100 L → 1000 -50,000L

QC & validation

- Profile of enzyme activities present
- 'Fingerprint' for each cocktail – chromatographic and proteomic analyses
- Biochemical characterization & functionality tests; Effect of reaction parameters
- Novel activities characterized/cloned – detailed characterization studies
- Thermostability and stability on storage

BioEnzTech Fermentation Facility

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MGBG: Technology Development to date

- Novel, low-cost approaches for production of target enzyme cocktails
- Increased enzyme yields ~3-5 fold at pilot-scale and production time by decreased 36-72 h **(COST SAVINGS)**
- **Retention of full biological activity from laboratory to pilot scale**

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Acknowledgements

Enterprise Ireland (Basic, Strategic, Applied Research, RIF & C-Plus awards; Innovation Partnership; IP protection; Start-up and IP protection); **DAF/FIRM and RSF** NDP HEA; European Union; EPA – **ERTDI**

MGBG Team, NUI, Galway
BioEnzTech team NUI, Galway; Prof. E. Colleran and Prof. V. O'Flaherty, Dept. of Microbiology, NUI, Galway
TTO, NUI, Galway

Teagasc Oakpark Research Centre, Carlow
International Collaborators:
VTT, Helsinki, Finland; KTH, Stockholm; RUGhent, Belgium

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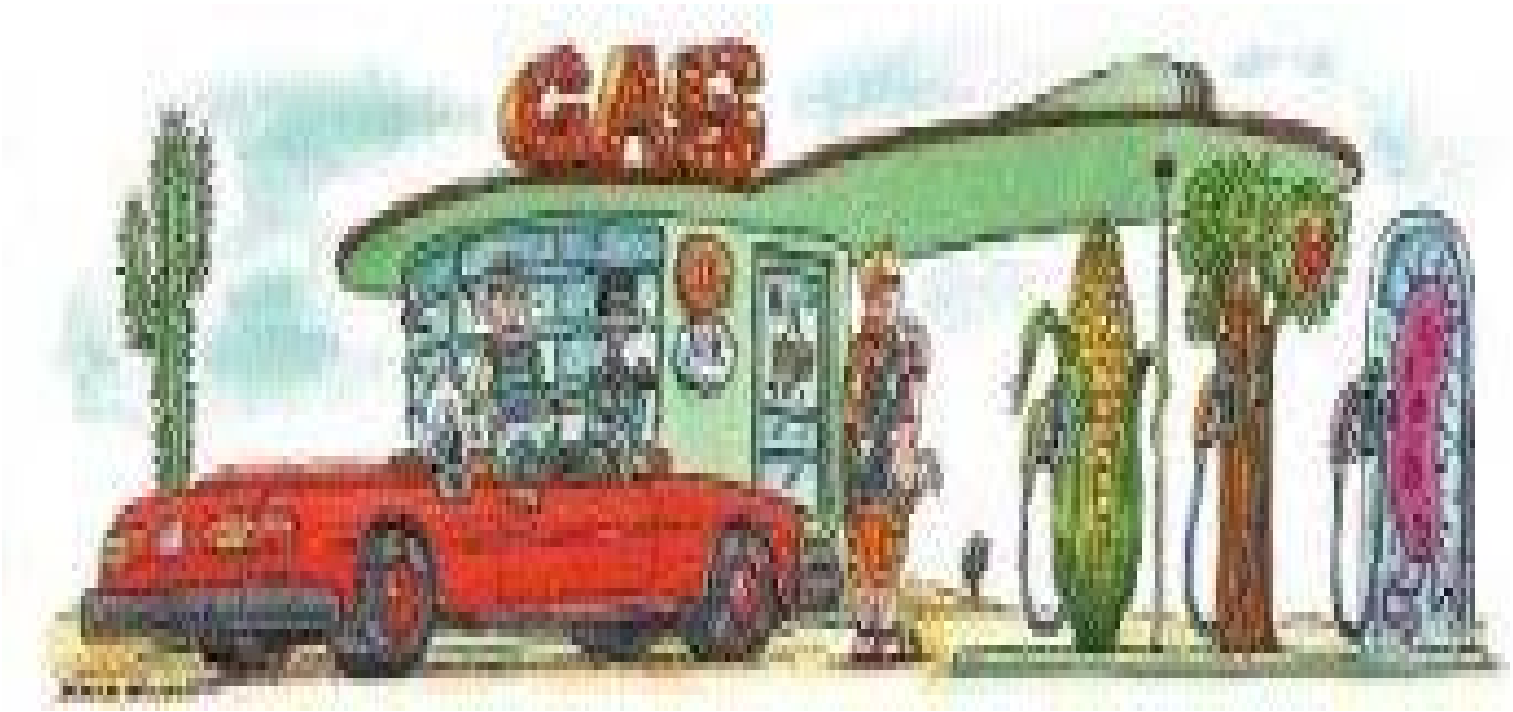
The Future: Power to the Plants!!



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