# Bioconversion of agricultural residues and bio-based waste streams

DST Science-meets-Industry Workshop: Organic waste
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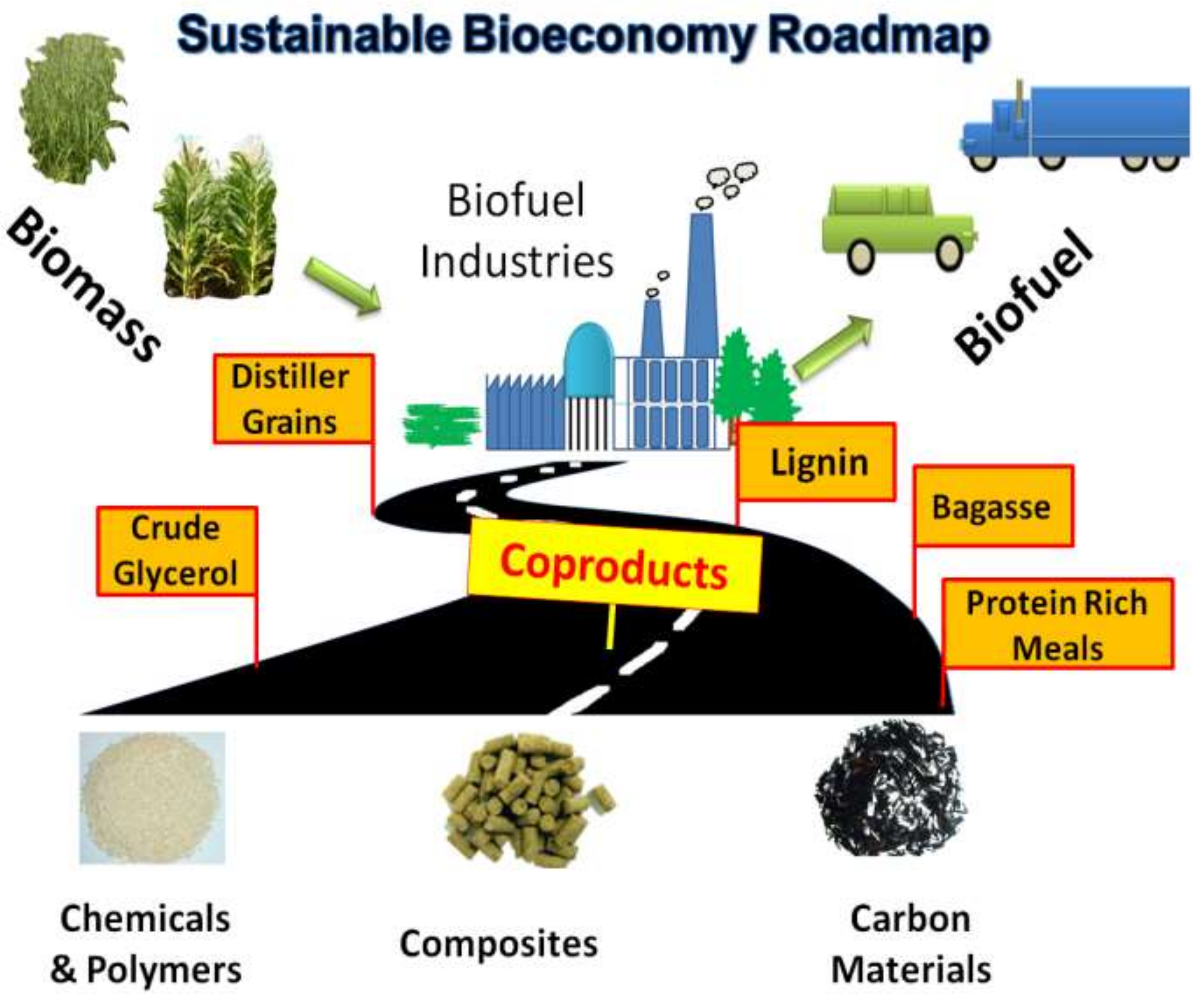
Rhodes University

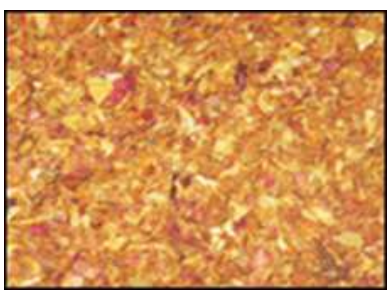
# Biobased economy, Bioeconomy, Biotechonomy, Biorefinery: producing VAPs




Technical applications of extremozymes in industrial processes

Skander Elleuche, Carola Schröder, Kerstin Sahm, Garabed Antranikian

# SECOND GENERATION BIOFUEL PRODUCTION



Agricultural residues

Bagasse

Hardwoods-
Eucalyptus

Softwoods-
Spruce

**Pretreatment**

**Hydrolytic enzymes**

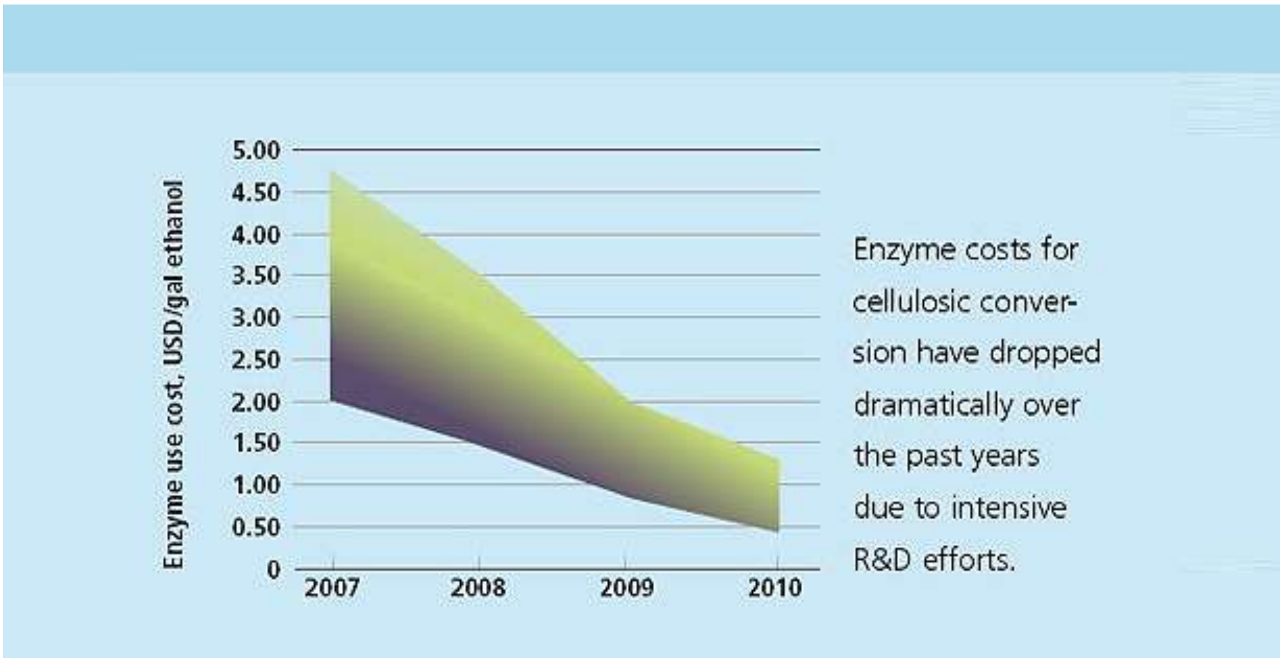
- <u>**Glucose**</u>
- **Mannose**
- **Galactose**
- **Xylose**
- **Arabinose**

**?**

**Fermentation**

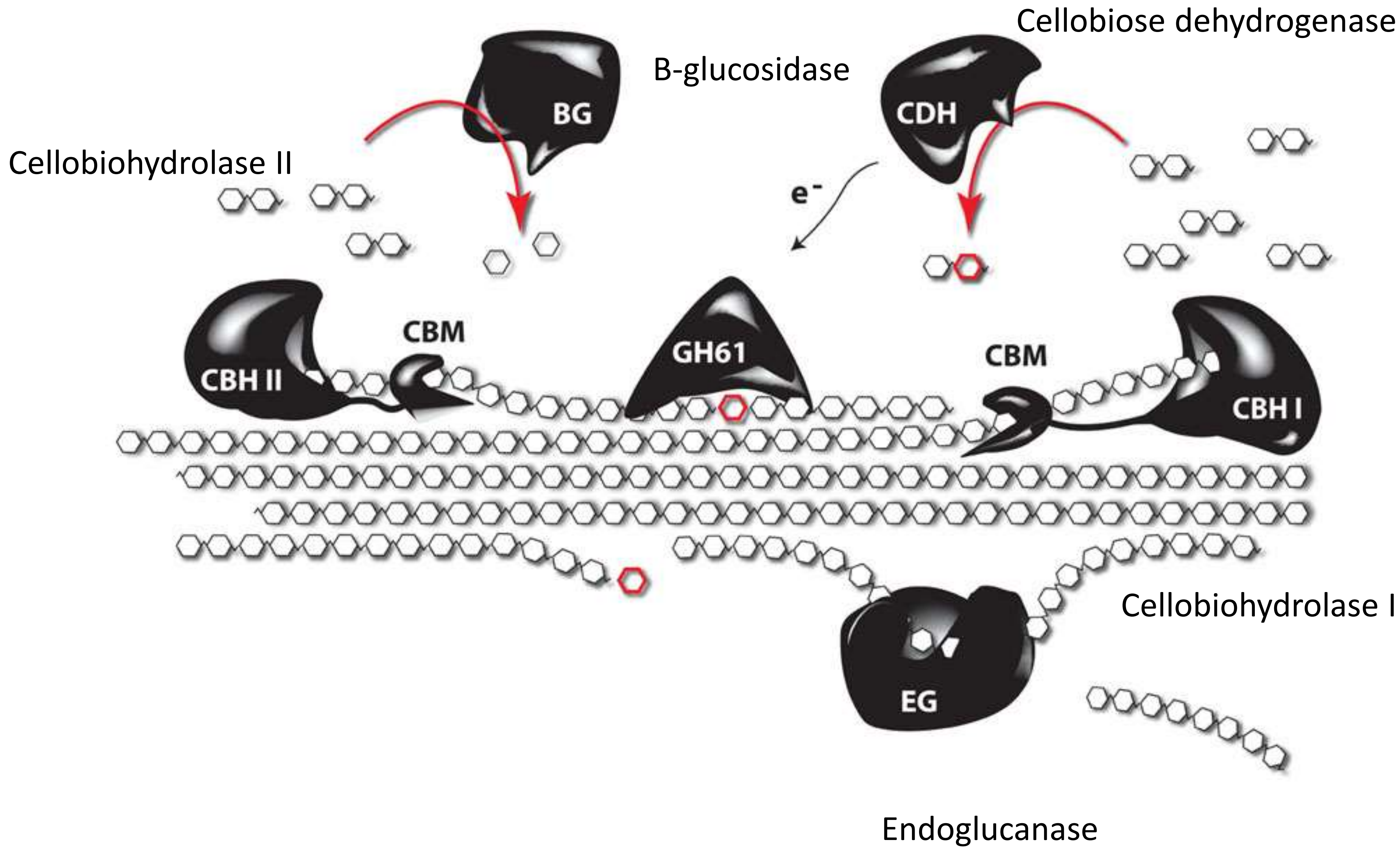
# Problem: Bottleneck in biomass conversion

- Current cost of industrial enzymes = R2 per liter



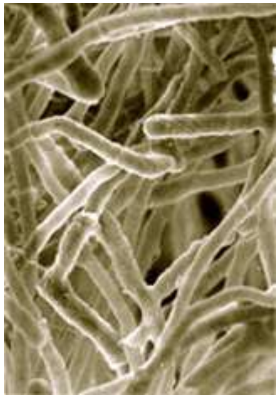

"The bottleneck in lignocellulose bioconversion lies in the enzymes required for hydrolysis, we need better enzymes and a better understanding of how they synergise most effectively"

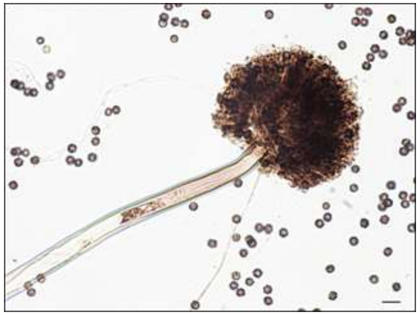
# Enzyme discovery, synergy and immobilisation are the answer: e.g. cellulases



# Where are the enzymes?

- **Microbes: Fungal cellulases and bacterial hemicellulases – we use a selective medium with cellulose, hemicellulose**





*<u>Aerobic fungi</u>*

*Trichoderma reesei*

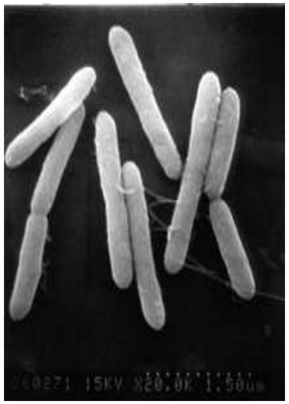
*Aspergillus niger*

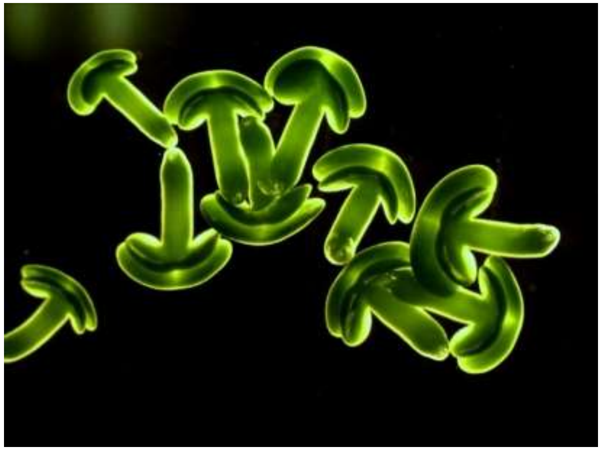
*<u>Anaerobic bacteria (one step)</u>*

*Clostridium thermocellum*

*Clostridium cellulovorans*

*Clostridium cellulolyticum*





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# Synergistic associations between *Clostridium cellulovorans* enzymes XynA, ManA and EngE against sugarcane bagasse

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$NH_4OH$ (0.114 M/g SCB/36 h/ 70$^{o}$C) and 75% XynA:

25% ManA effectively increased SCB digestibility 13.1 fold.

Highest activity and degree of synergy (2.85).

*Beukes and Pletschke (2011). Bioresource Technology 102 (2011) 5207–5213.*

# Fruit Production in S.A.

S.A. produces large quantities of fruit wastes (pomace) and waste water from the fruit juicing and canning industry

**Apples**

**Grapes**

**Citrus**

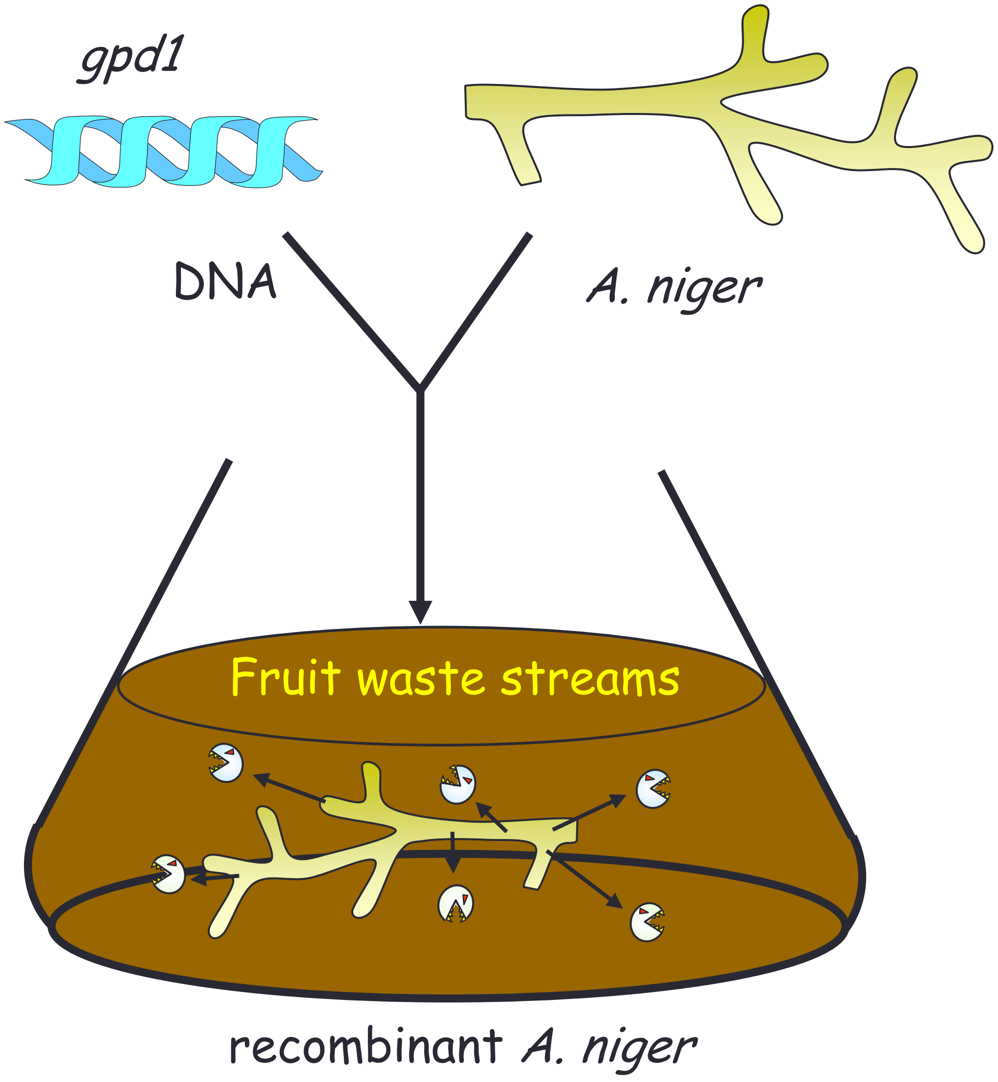
Juicing and canning

**Table 1: Production volumes for various fruit crops in South Africa, as well as volumes processed (DAFF, 2013). Terminology in brackets: Processed = canning and/or juicing; Dried = prepared as dried fruit; and Pressed = pressed for wine making**

| Fruit crop | Total production in tonnes (2011/2012) | Volume processed in tonnes |
|---|---|---|
| Citrus (oranges, lemons, limes, grapefruit and naartjies) | 2 102 618 | 441 899 |
| Grapes | 1 839 030 | 1 649 (processed), 151 628 (dried) 1 413 533 (pressed) |
| Apples | 790 636 | 244 469 (processed), 1 110 (dried) |
| Bananas | 371 385 | Not indicated |
| Pears | 346 642 | 120 811 (processed), 9 872 (dried) |
| Peaches | 190 531 | 125 706 (processed), 8 994 (dried) |
| Pineapples | 108 697 | 81 753 |
| Watermelons and melons | 93 277 | Not indicated |
| Avocados | 87 895 | Not indicated |
| Apricots | 66 762 | 48 792 (processed), 8 725 (dried) |
| Mangoes | 65 439 | Approx 50 000* |
| Plums | 60 925 | 1 712 |
| Guavas | 23 699 | 20 896 |
| Papayas | 12 565 | Not indicated |
| Litchis | 7 782 | Not indicated |
| Strawberries | 5 543 | 2 724 |
| Other berries | 5 073 | 3 914 |
| Prunes | 3 426 | Not indicated |
| Figs | 1 925 | 448 |
| Pomegranates** | 1 324 | 883 |
| Cherries*** | 775 | 83 |
| Granadillas | 484 | Not indicated |
| Quinces | 208 | Not indicated |

*Data obtained from South African Mango growers' association (www.mango.co.za)
**Data obtained from Pomegranate Association of South Africa (www.sapomegranate.co.za)
***Data obtained from South Africa cherry growers' association (www.cherries.co.za)

# Enzyme production with *Aspergillus*

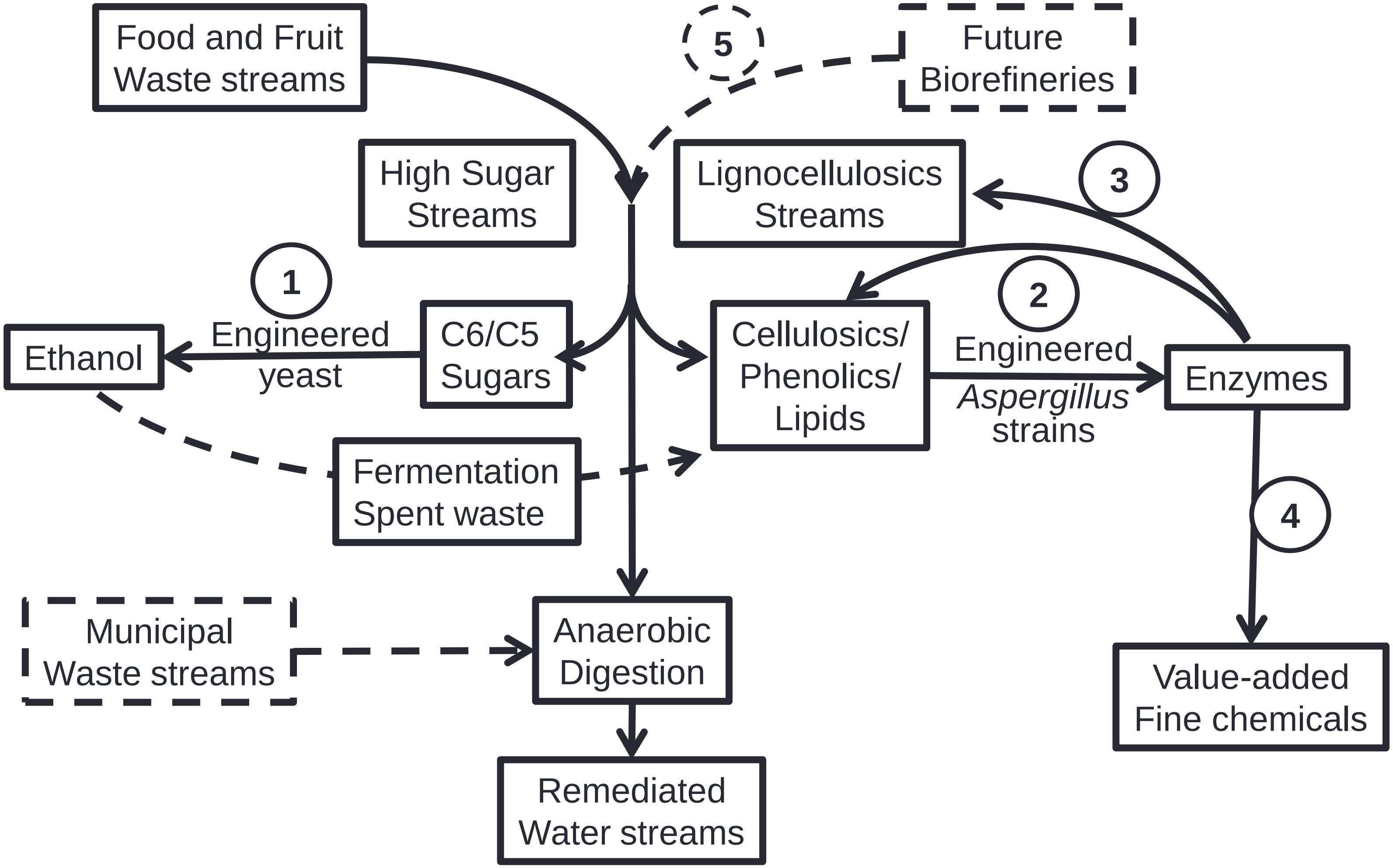


# Identification of target enzymes and strains

- *A. niger* strains had previously been constructed in-house and are known to produce high levels of extracellular enzyme activity.

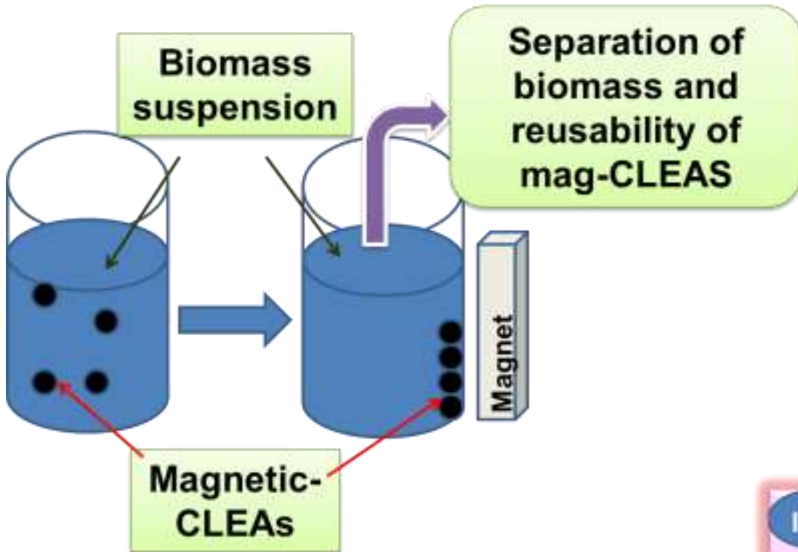
| Strain | Foreign gene | Activity | Substrate* |
|---|---|---|---|
| *A. niger* D15[pGT1] | none | | All |
| *A. niger* D15[eg2] | *Trichoderma reesei* egII | Endoglucanase | Lichenan |
| *A. niger* D15[man1] | *Aspergillus aculeatus* man1 | Endomannanase | Locust Bean Gum |
| *A. niger* D15[xynB] | *Trichoderma reesei* xynB | Endoxylanase | Beechwood xylan |

* Lichenan (Sigma); Locust bean gum (Sigma). Birchwood xylan has been discontinued and therefore beechwood xylan (Fluka) had to be used as substitute

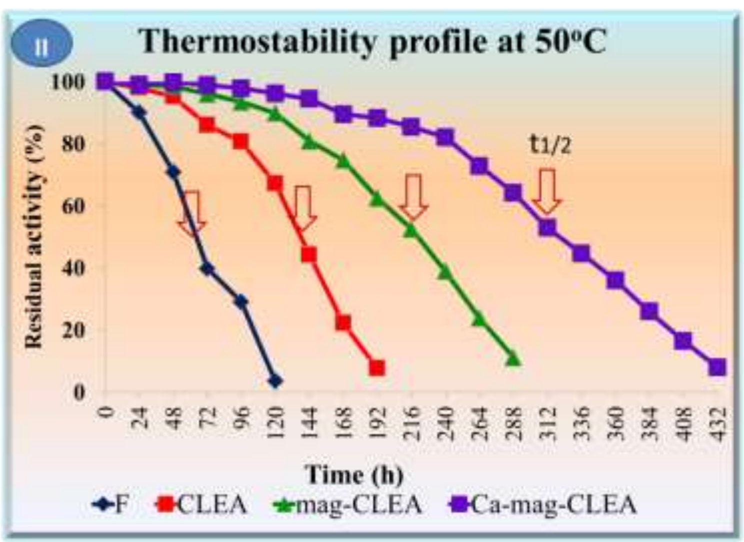


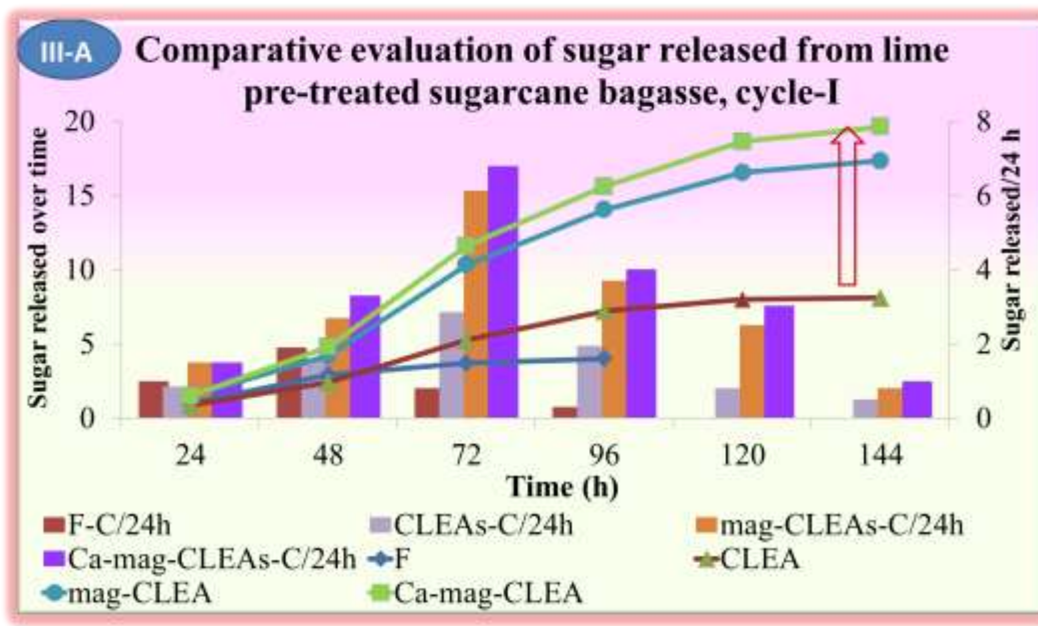

Integrated approach for remediation and beneficiation of fruit wastestreams (ReBenFruWaste). Wastestreams can be divided in sugar-rich streams for (1) ethanol production and cellulosic/phenolics/lipid rich streams for (2) enzyme production by *Aspergillus* strains. Enzymes can be used for bioconversion of (3) lignocellulosic streams or the (4) production of value-added fine chemicals. The process can also include (5) biorefinery waste streams of future bioeconomies

# Cellulase and hemicellulase immobilisation on to Mag-CLEAs (magnetic cross linked enzyme aggregates)



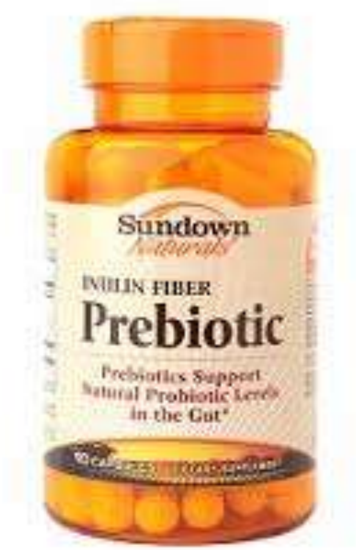

Abhishek Bhattacharya



# Expanding the bioeconomy value chain beyond biofuels

Produce key VAPs:
xylose, mannitol, xylitol, chitin-oligosaccharides (CHOS), xylo-oligosaccharides (XOS), enzymes

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“Fruit waste streams in South Africa and their potential role in developing a bio-economy”

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