Bioethanol: challenges and opportunities

Graeme Walker

UNIVERSITY of ABERTAY DUNDEE

www.abertay.ac.uk

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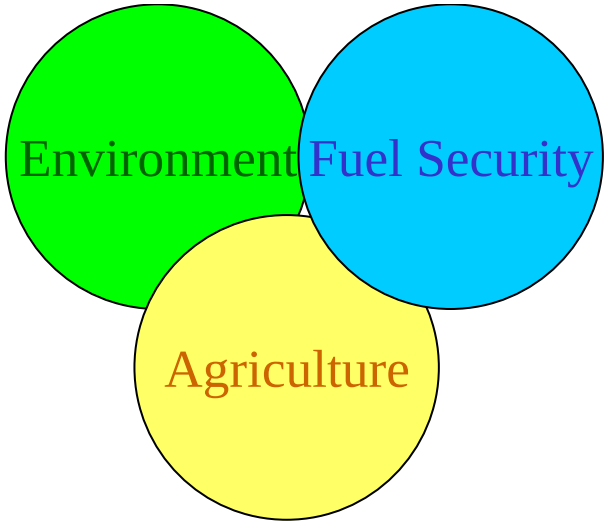
Outline

- Bioethanol: pros & cons
- Bioethanol: substrates, organisms & existing processes
- Bioethanol: challenges & opportunities
- Bioethanol from lignocellulose
- Conclusions & future prospects

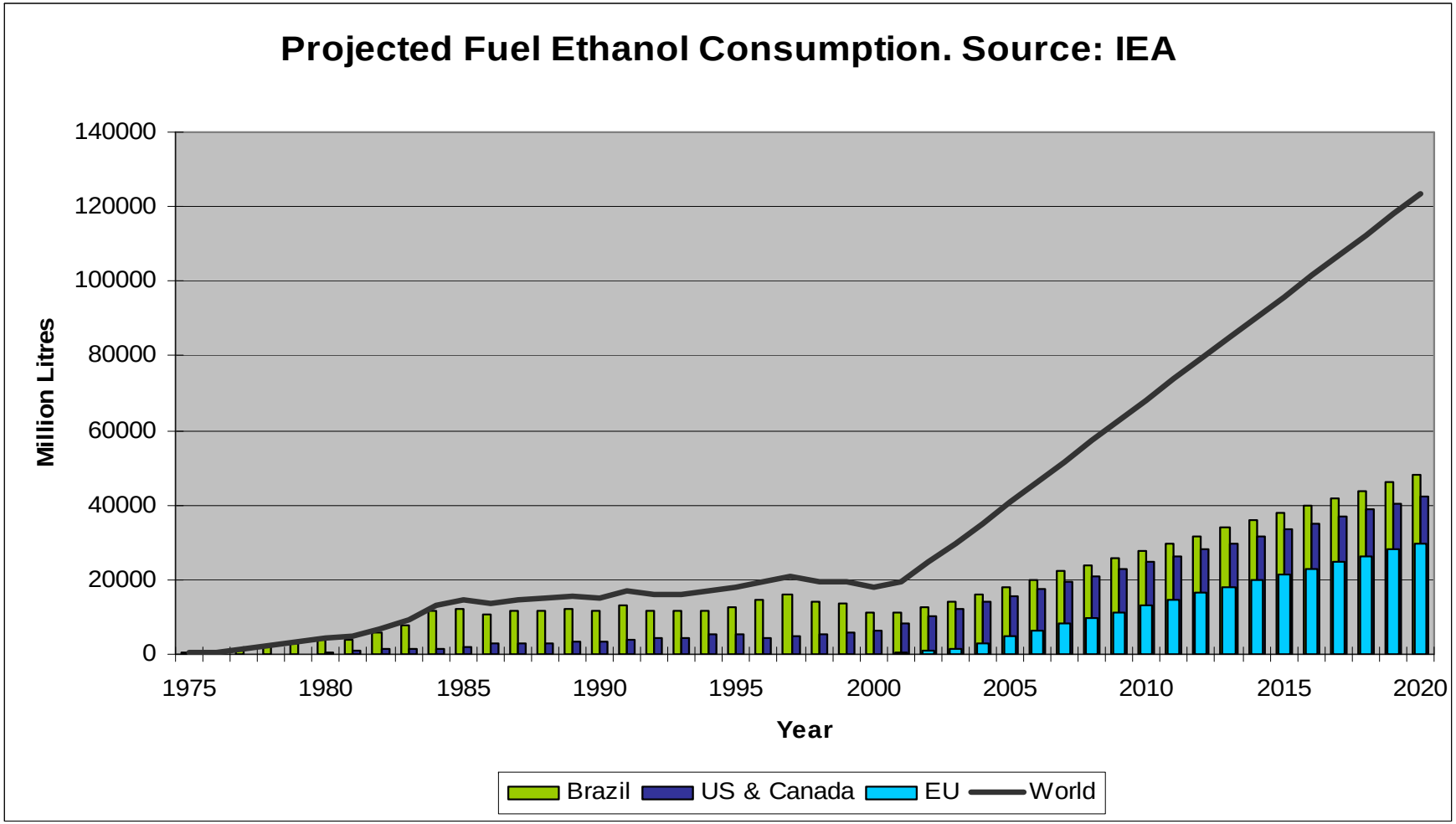
- Biogas (Anaerobic Bacteria)
- Biohydrogen (Cyanobacteria, Clostridia, PNS bacteria)
- Bio-oil (*Botryococcus braunii*)
- Biobutanol (*C.butylicum, C.acetobutylicum*)
- Biodiesel (*Brassica napus*, Palm oil, Animal/veg oils, algae etc)
- Bioethanol (Yeasts)

Why Bioethanol?



Pros	Cons
CO_2 neutral	Food-to-fuel
Reduced dependence on oil	Lower oil price
Agricultural diversification	Sustainability
Clean burning, low toxicity	Energy balance
Less GHG emissions (~65% less)	Residues, emissions
Higher flash points (better fire safety)	Inefficient microbes
Better biodegradability	Hydroscopic
Co-generation of electricity	Less mpg

Future Ethanol Consumption



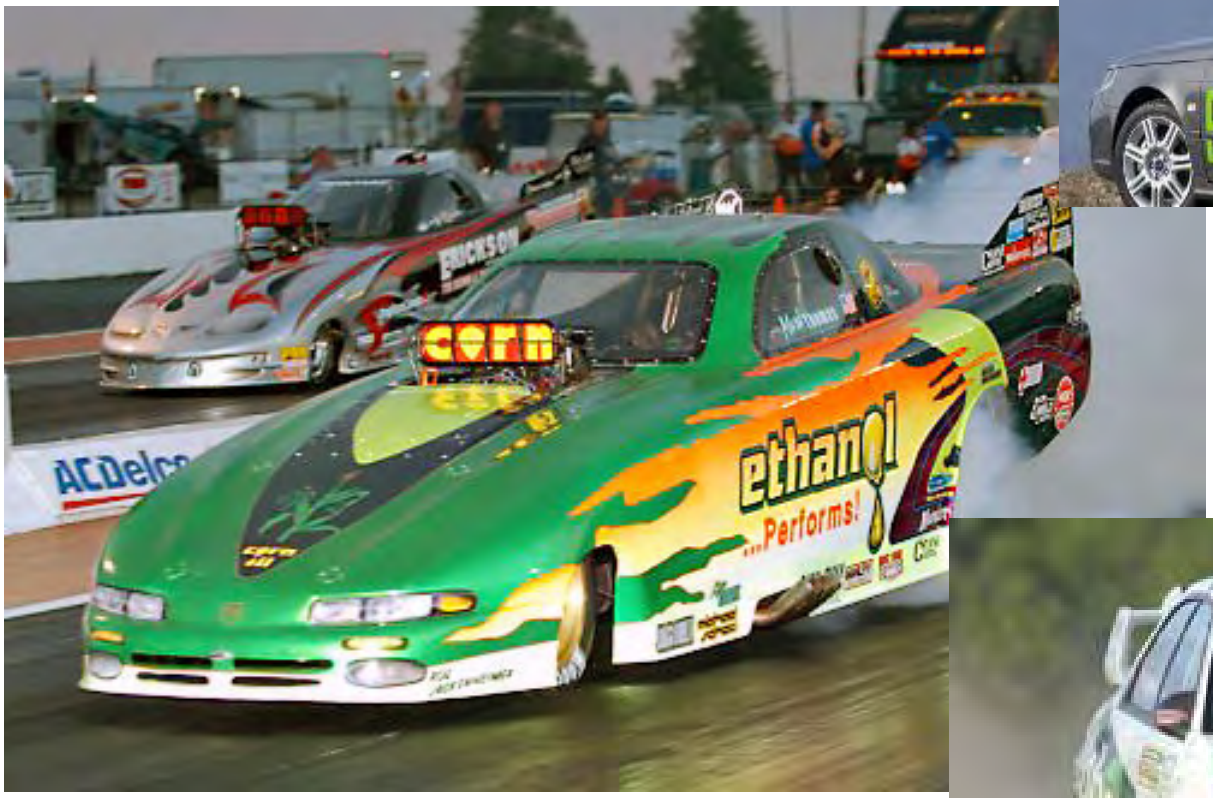
Some bioethanol-based motor fuels

[As volume extender/complete fuel/octane enhancer]

Fuel	Ethanol % v/v
Alcool, Brazil	
E20	20
E100	96
E85, USA & Europe	85
E10, USA/Canada (Gasahol)	10
Oxygenated fuel, USA	7.6
99 Octane, UK (e.g. Tesco)	5.0

- Tesco launched 99 Octane and Global Diesel in partnership with Green*ergy* Ltd
- 185 stores sell petrol with 5% bioethanol blend: 99 Octane

- Morrisons retailing Harvest Bioethanol E85 in partnership with Saab
- Morrisons have 274 petrol stations



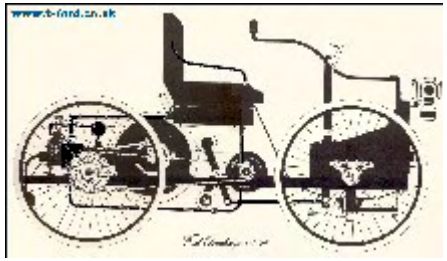
- ✓ 4000 bhp
- ✓ 0.25 mile in 5.3 sec
- ✓ Runs on bioethanol made from maize

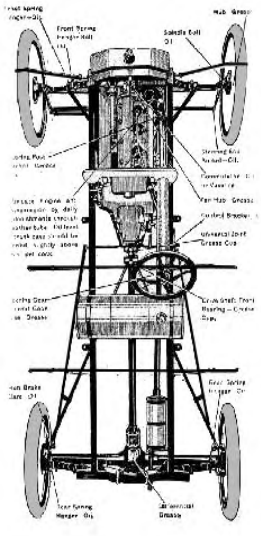
Saab Flexi-fuel

WRC team which promotes new
Tesco 99 OCTANE fuel
with 5% of bioethanol (Subaru Impreza N12)

Bioethanol is not new!

US Bioethanol? Since 1908 (**Model T Ford: 1908-1927 ~15 million** cars)

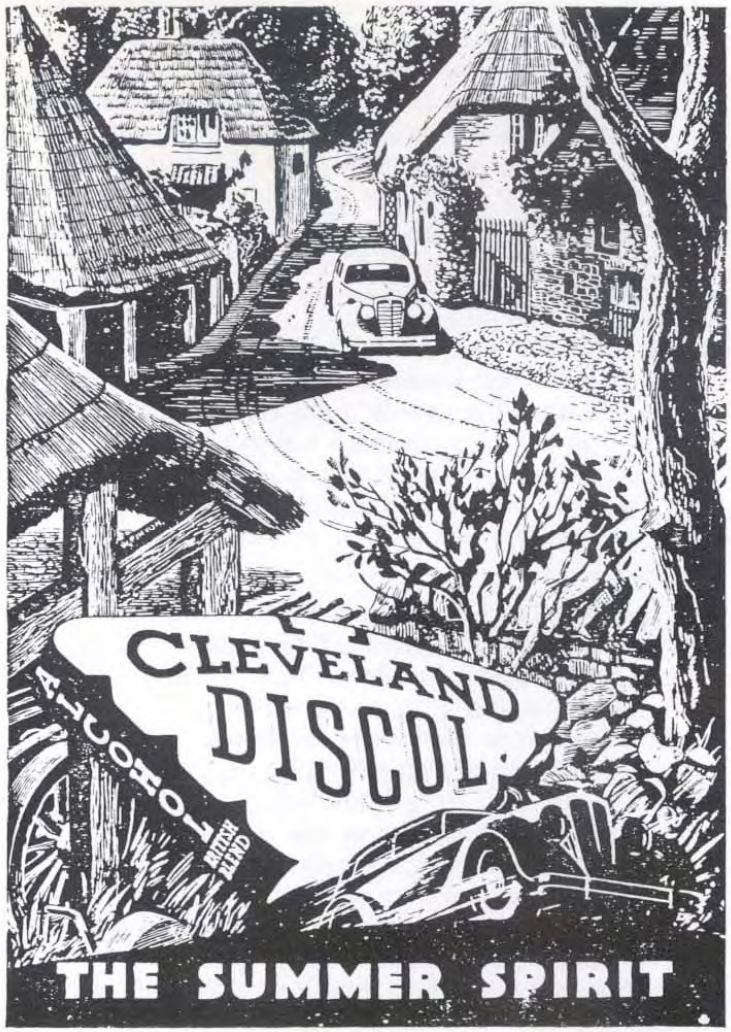


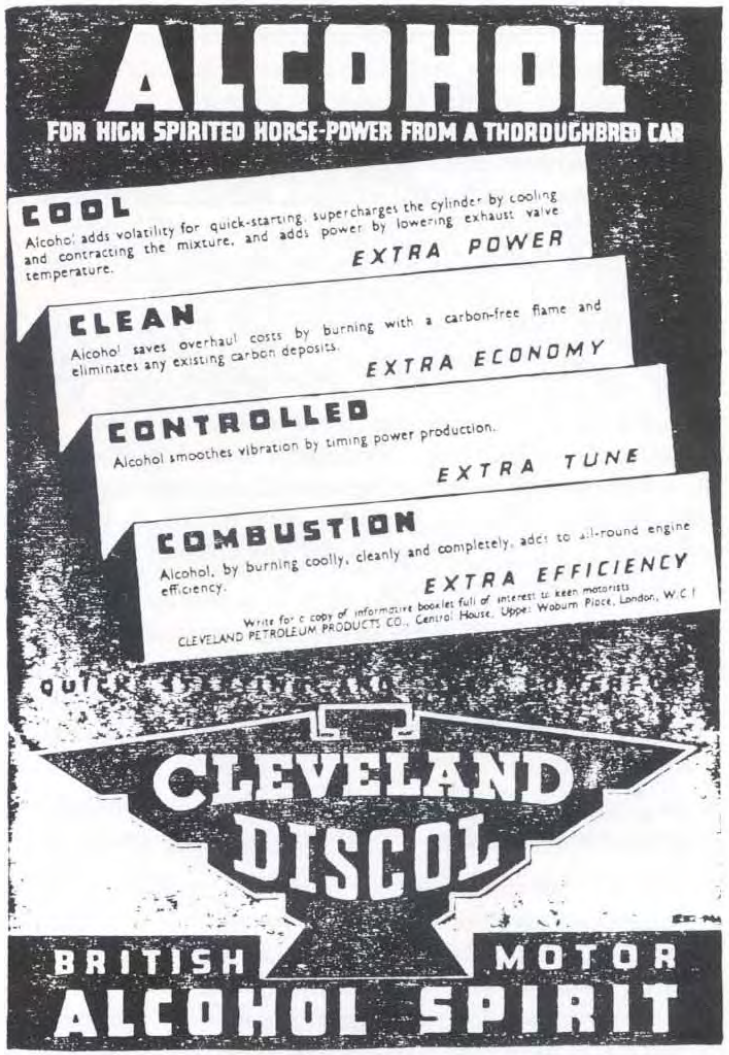


UK Bioethanol? Since 1930's (eg. Cleveland Discol)

Brazil Bioethanol? Since 1975 (Proalcool programme)

British bioethanol (1936)

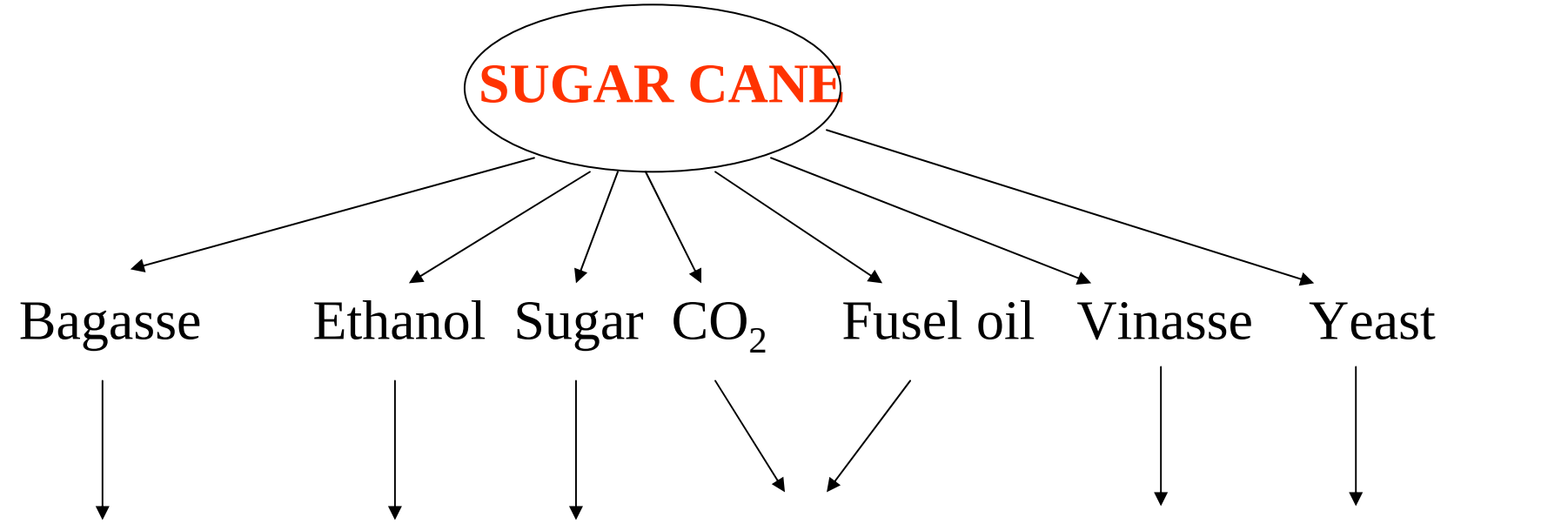




Bioethanol:

current substrates?

Brazilian ethanol programme (Proalcool*)



*~380 Distilleries in Brazil
->24bn litres ethanol/yr
[40% of transport fuel]
-700,000 jobs created
-$2bn savings pa.

USA Bioethanol processes (Gasahol)

CORN (maize)

↓

Crude starch

Microbial amylases →

↓

Syrup conversion

↓ ← Yeast

Fermentation

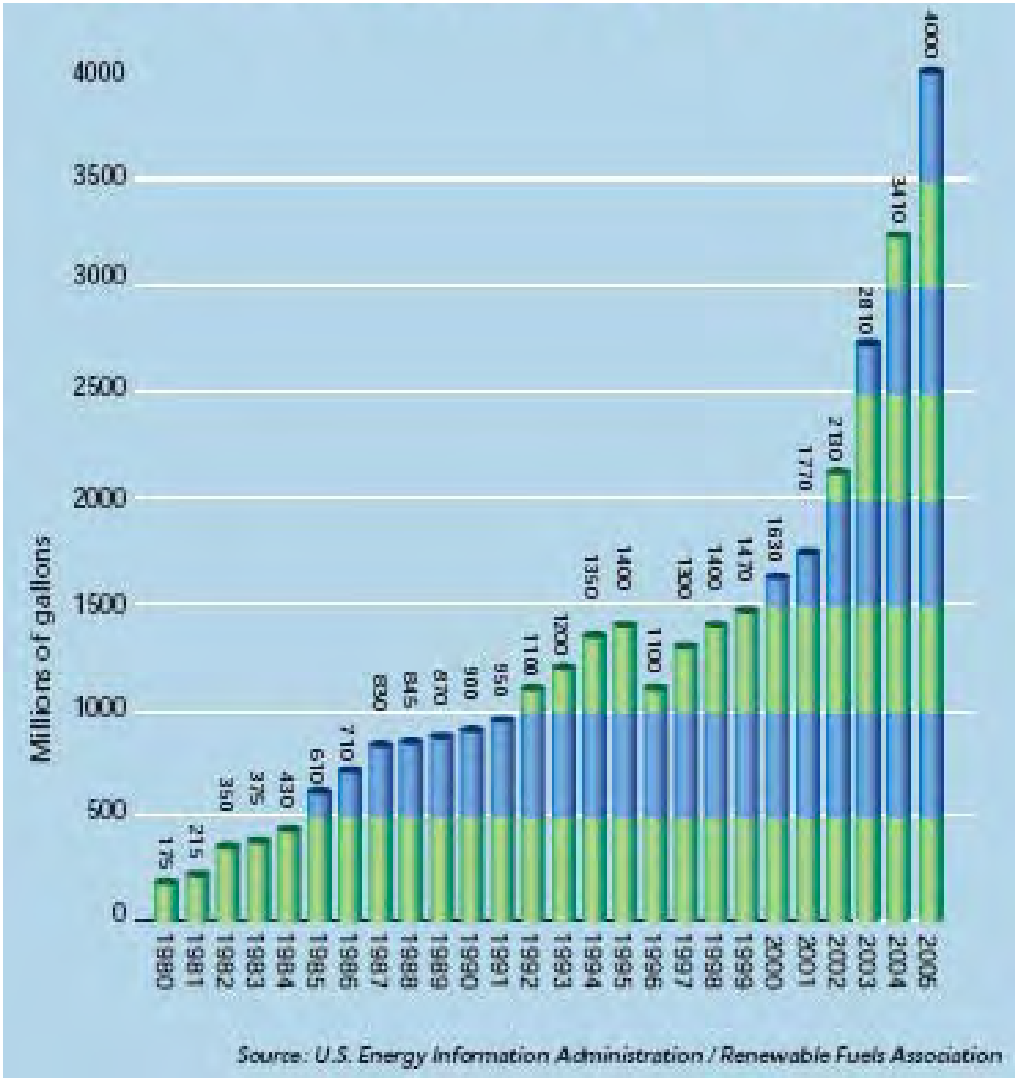
↓

Distillation

↓

GASAHOL

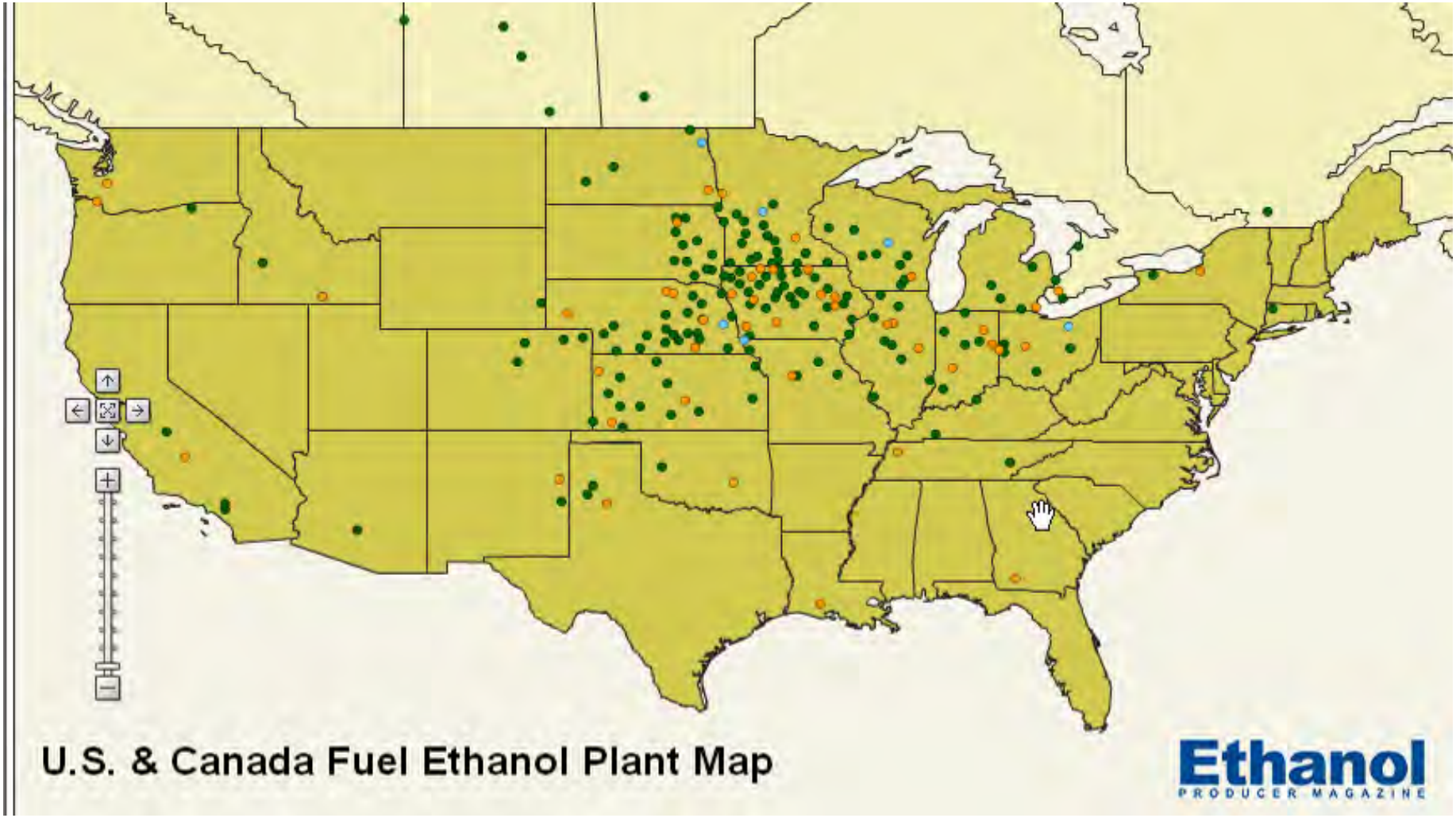
US Bioethanol



U.S. Fuel Ethanol Production

By the end of 2007, U.S. will have processed 3 billion bushels of corn for ethanol = 30.28 billion liters

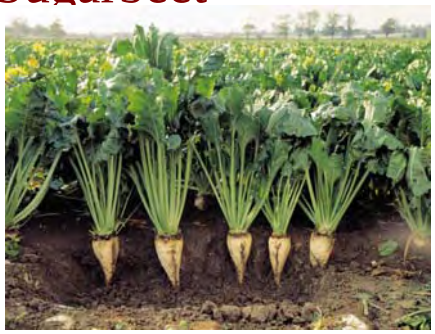
North American bioethanol production plants



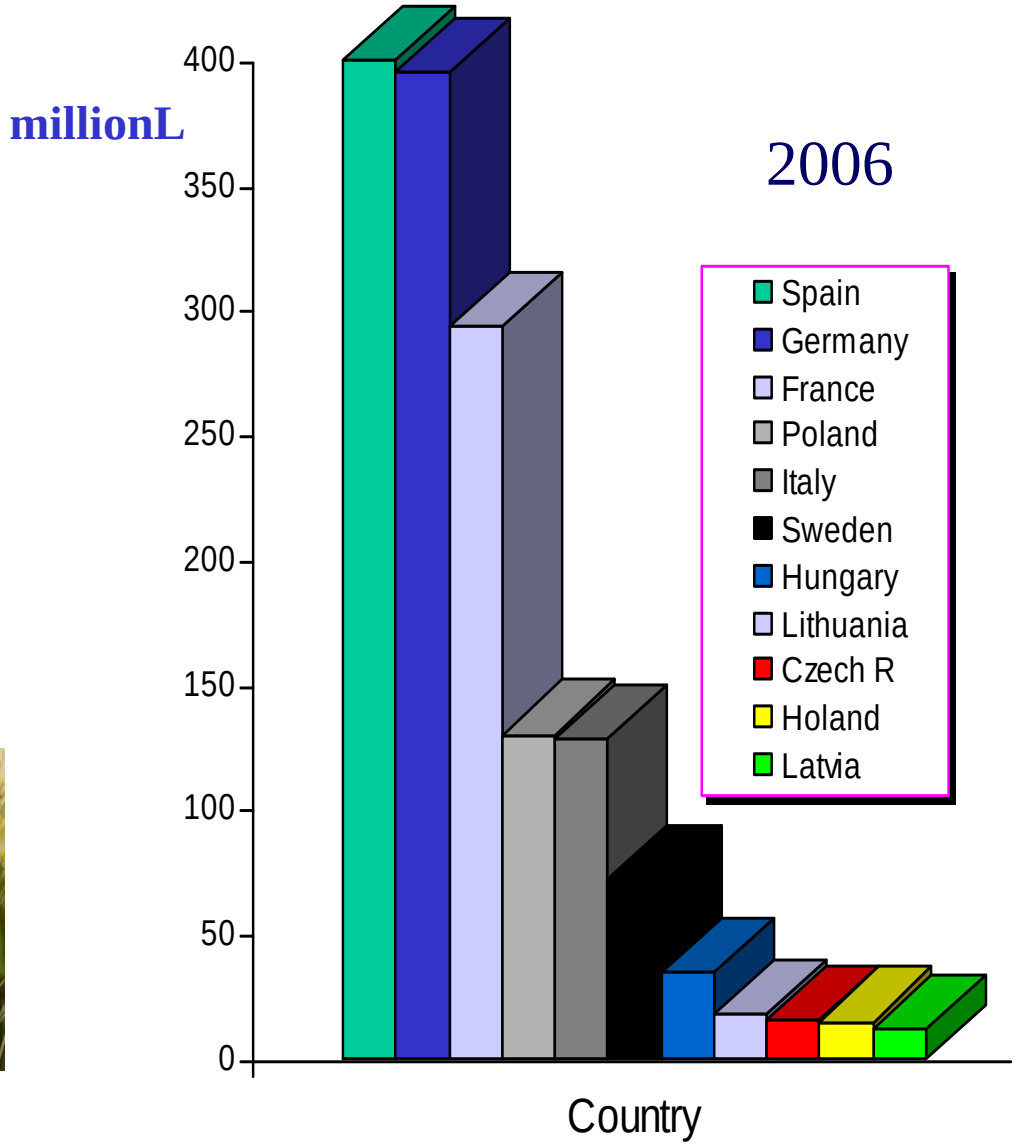
European bioethanol?

Substrates:

- Sugarbeet

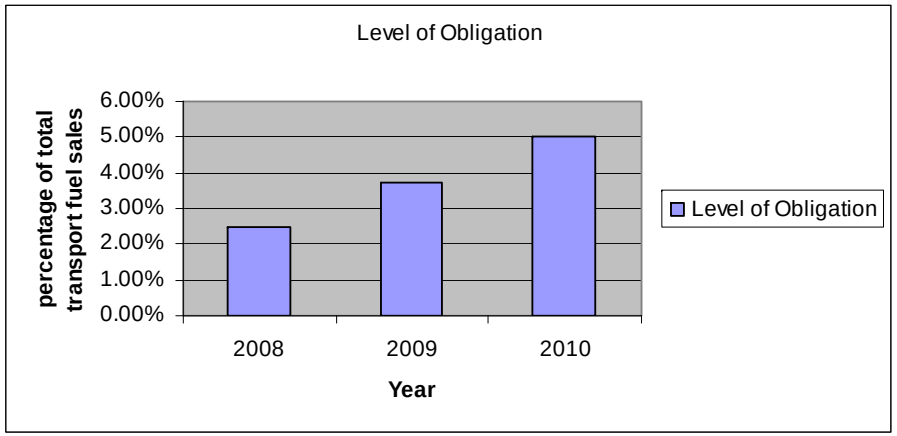


- Wheat



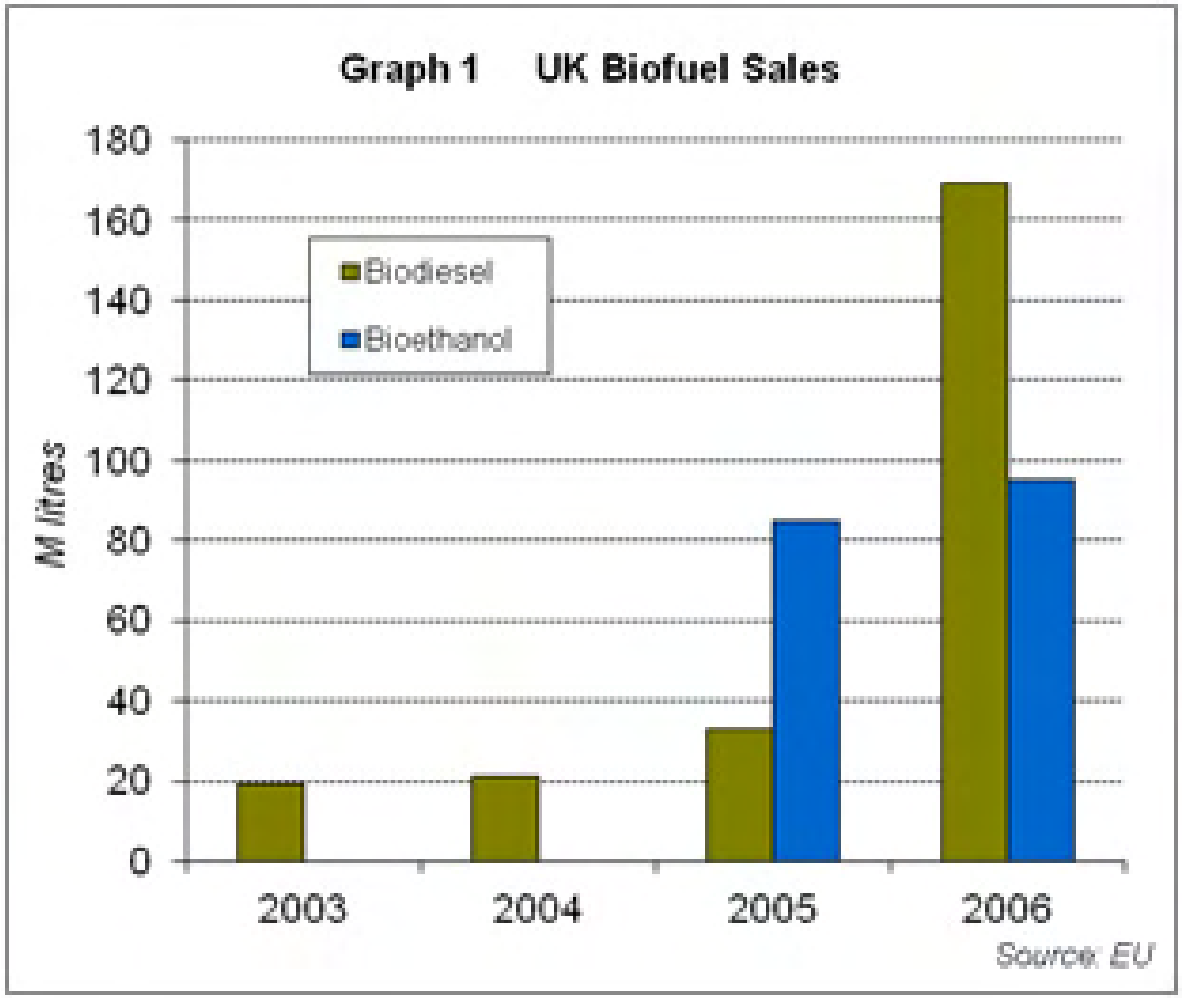
UK's Renewable transport fuel obligation (RTFO)

- UK transport fuels need to have following levels of biofuels:
  - 2.5% by 2008 (15/4/08)
  - 3.5% by 2009
  - 5.0% by 2010
  - [EU 2009 Directive 10% by 2020]



- Current levels are ~0.5%
- Legislation in place!

RTFO signed by Minister 25/10/07; legislation 14/4/08.



Bioethanol in the UK

(HGCA Newsletter 2/4/08)

- So far, UK biofuel production is much less than the amount required to fulfil the RTFO target (5% by 2010),
- Currently biofuels only account for a small percentage of the fuel market, a mere 0.70%.
- There is presently only one ethanol plant in operation (British Sugar, production capacity of 55,000t).
- This would suggest that total UK biofuel production for 2007 was around a quarter of a million tonnes.

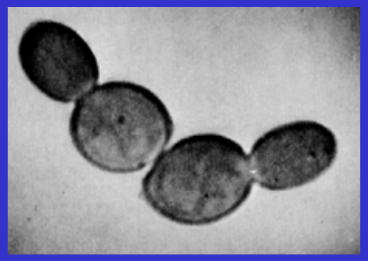
Bioethanol: challenges & opportunities

Ethanologenic yeasts

Feedstock	Fermentable substrate	Yeasts*
-Wheat, Maize, Barley	Starch hydrolysate (maltose, glucose)	*Saccharomyces*
-Sugarcane/Beet	Sucrose	*Saccharomyces*
-Potato, Rice	Starch hydrolysate (maltose, glucose)	*Saccharomyces*
-Agave/Artichoke	Inulin hydrolysate (fructose)	*Kluyveromyces*
-Cheese whey	Lactose	*Kluyveromyces*
-Raw starch	Starch	*Schwanniomyces*
-Seaweed	Laminarin	*Pichia angophorae*
-Paper, sawdust, straw, wood, spent grains, cornsteep liquor, paper, rapeseed residues, MSW, bagasse, corn fibre	Xylose, arabinose, glucose, cellobiose	*Pichia*, *Candida*, *Pachysolen*, *Kluyveromyces* GM *S.cerevisiae*

*Other microbes? *Zymomonas, E. coli (GM), Klebsiella (GM), Bacillus (GM), Thermoanaerobacterium saccharolyticum*

Is *Saccharomyces cerevisiae* the best yeast?



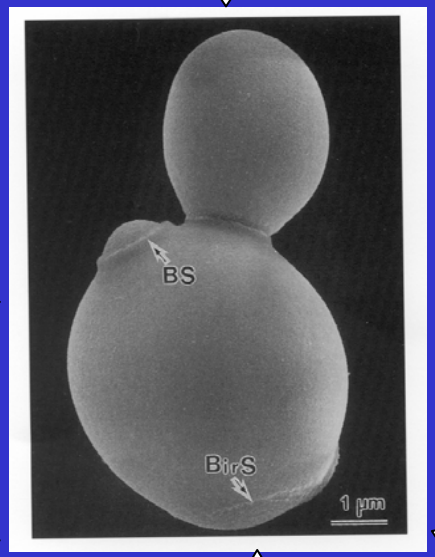
- Can’t ferment xylose, arabinose, cellobiose, lactose, maltodextrins……
- Variable stress tolerance (temp, ethanol, inhibitors etc)
- Secondary fermentation metabolites
- Non-anaerobic growth
- Unusual sugar uptake
- Crabtree positive, glucose repressible (low μ, low Y in fed-batch)
- Not antibacterial
- Cellular polymorphism
- Genetic instability
- Flocculence undesired

FLOCCULATION

Non-flocculant

Strongly-flocculant

Stress factors for bioethanol yeasts



Ethanol/CO_2

Osmostress

Mechanical sheer, hydrostatic pressure

Oxidative stress

Heat/cold shock

Nutrient starvation, anaerobiosis

Inhibitors (furfural, phenols etc)

Cell aging

Acids/pH shock

Dehydration/rehydration

The yeast message

- **Yeasts are extremely diverse physiologically, but yeast biodiversity virtually untapped**
- ***S.cerevisiae* is a rather exceptional yeast and may not be the best for bioethanol**
- **We need more knowledge of yeast cell physiology - Especially in *exotic non-Saccahromyces yeasts***
- **Yeast stress is a dilemma**
- **Bacteria are competitive**

Bioethanol from lignocellulose

Scientific & technological challenges

Second-generation bioethanol: research challenges

Lignocellulosic material

1 Pretreatment

Pre-hydrolysis
[physico-chemical]

Hydrolysis [biological]

Fermentation (hexose)

2

Fermentation (pentose)

4

Energy

Distillation

3

BIOETHANOL

Celluose-to-ethanol plants (USA)

- New York (wood chips – Mascoma)
- Tennessee (switchgrass – Mascoma)
- Maine (wood chips – RSE Pulp & Chemical)
- Kentucky (corncobs – Ecofin/Alltech Inc)
- Louisiana (bagasse – Celunol)
- Ohio (corn fibre – Genahol)

Selected others:

- Iogen (straw – **Canada**); Abengoa (straw – **Spain, US**); Etek (softwood – **Sweden**); Elsam (straw –**Denmark**); TMO (straw etc. – **UK**); Tavda (wood – **Russia**); NEDO (rice straw – **Japan**)

Lignocellulosic bioethanol

– key areas for improvement

- Cellulose hydrolysis
  - Steam explosion, acids, enzymes (cellulases, arabinozylanases, ligninases)
  - New pre-treatments: ozonolysis+ultrasound?
  - SSF (e.g. *Thermoanaerobacterium saccharolyticum*)
- Fermentation
  - Xylose fermenting yeasts or bacteria
  - Very high gravity wort (>20%v/v ethanol)
  - Thermotolerant/alcohol tolerant/inhibitor tolerant yeasts
  - Optimised nutrition (esp. metal ions like Mg, Zn)
- Distillation
  - Anhydrous ethanol (molecular sieves, azeotropes, membrane pervaproation)
  - Energy balances!

Bioethanol from wheat bran?

Whole Wheat

Wheat Bran

14-19% of the grain

Starch

Arabinoxylans

Cellulose

Beta-glucan

Protein

Lignin

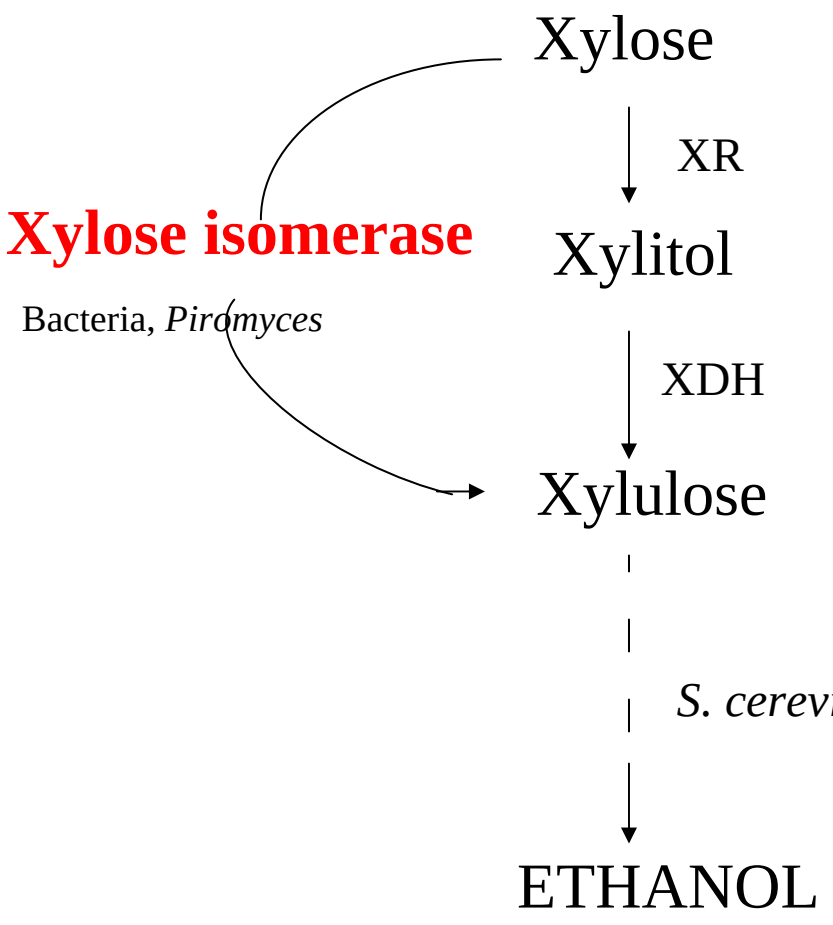
Ethanol ?

Saccharomyces cerevisiae strains cannot ferment pentose sugars as D-xylose and L-arabinose.

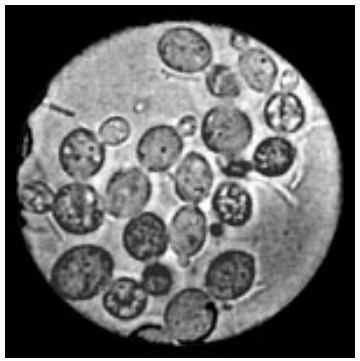
Too bad, because…

- **Grass** (16% xylan, 5% arabinan)
- **Corn stover** (19% xylan, 3% arabinan)
- **Wheat bran** (19% xylan, 15% arabinan)
- **Barley husks** (20% xylan, 9% arabinan)

Xylose fermentation pathway



- *Brettanomyces naardenensis*
- *Candida intermedia var intermedia*
- *Candida lyxosophila*
- *Candida shehatae var. lignosa*
- *Candida tenuis*
- *Cryptococcus albidus*
- *Kluyveromyces marxianus*
- *Pachysolen tannophilus*
- *Pichia stipitis*



Non-GM approaches to bioethanol-producing yeasts?

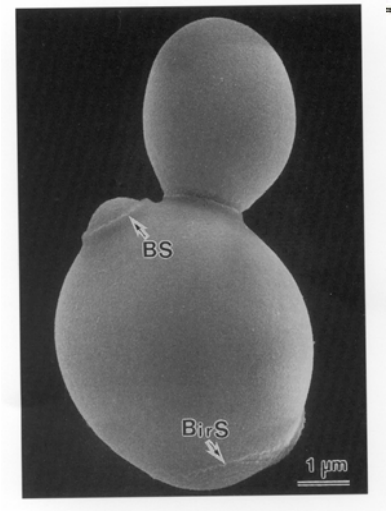
- Co-cultures (*S.cerevisiae* + *P. stipitis*)
- Immobilised yeast (+ xylose isomerase)
- “Indigenous” distillers strains (molasses distilleries)
- Physiological cell engineering
  - Mineral preconditioning (Mg, Zn enrichment)
  - Sterol pre-enrichment (pre-oxygenation, mild aeration)
  - Nutrient adaptation (ethanol tolerance in chemostats)
  - “Population genetics” (xylose-fermenting *S.cerevisiae*?)

Scottish bioethanol?

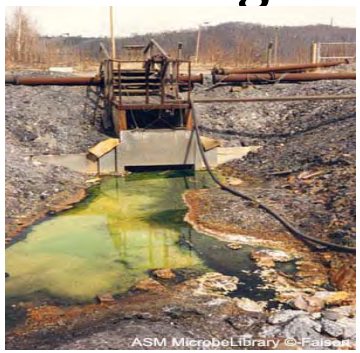
- **E**xpected substrates?
  - Wheat, sugarbeet, spent grain (16.8% cellulose; 20% xylan; 8.5% arabinan), straw, whey, potatoes
- **E**conomics?
  - A Scottish (70ML) plant would be “marginally competitive” with imports (ITI, 2006)
  - Scottish bioethanol from wheat could be made for ~30pence/L (ITI, 2006)
- **E**nergy? Energy in ethanol/Energy needed to obtain ethanol - current NEV (maize) = 1.34
- **E**nvironment?
  - ~70% less carbon emissions than petrol
  - If all UK petrol had 5%bioethanol, carbon saving equals 1million cars off the road
- **E**fficiency? Need >95% CE
- **E**thanol yields? >20%v/v?

Yeast research at Abertay University

Food/beverage industries

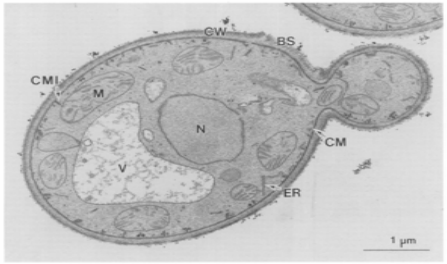


Environmental technologies

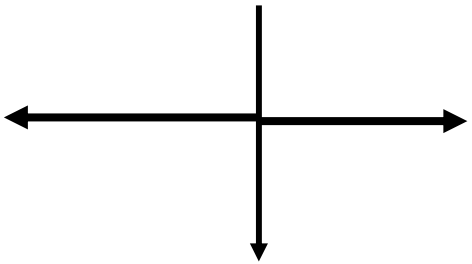


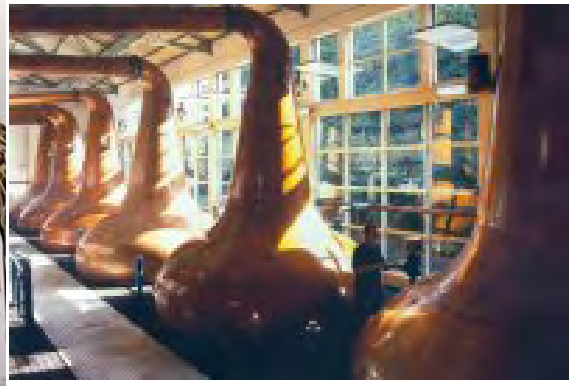
Chemicals
(bioethanol)

Basic research (cell physiology)



Bioethanol from spent grains?





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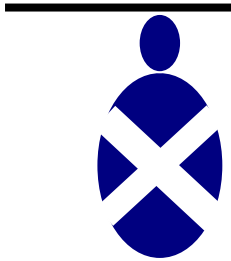


Spent grains are cereal residues from breweries and distilleries

Scottish grain and malt distilleries

2006 figures

	Whisky Production ($x10^6$ L p.a.)	Yield alcohol (L/t)	Residues (1000 t dry matter)
Grain (wheat or maize)	**273**	**490**	**209**
Malt (barley)	**170**	**531**	**135**

Lager brewery

85% malt
15% wheat

Grain distillery

86% maize
14% malt

Ale brewery

96% malt
4% roasted malt

Malt distillery

100% malt

Bioethanol from spent grain: Current Research

- **Ale** (malt)
- **Lager** (malt + wheat)
- **Whisky** (malt)
- **Spirit** (maize + malt)

Pretreatment

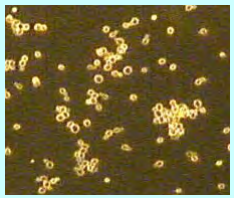
Pre-hydrolysis

↓

Enzyme Hydrolysis

1. Temp? Acids?
2. Novel pre-treatments?
3. Enzymes
4. Characterisation of sugars, inhibitors

Fermentation (Glucose, xylose, arabinose)



- *C6 & C5 sugars*
- *P. stipitis, K. marxianus etc.*

Distillation

↓

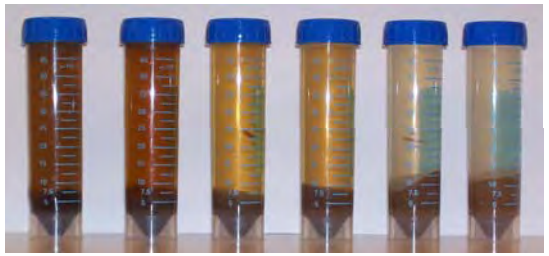
BIOETHANOL

Caledonian Brewery Spent Grains

(ale mash: 96% malted barley, 4% roasted); 77% moisture

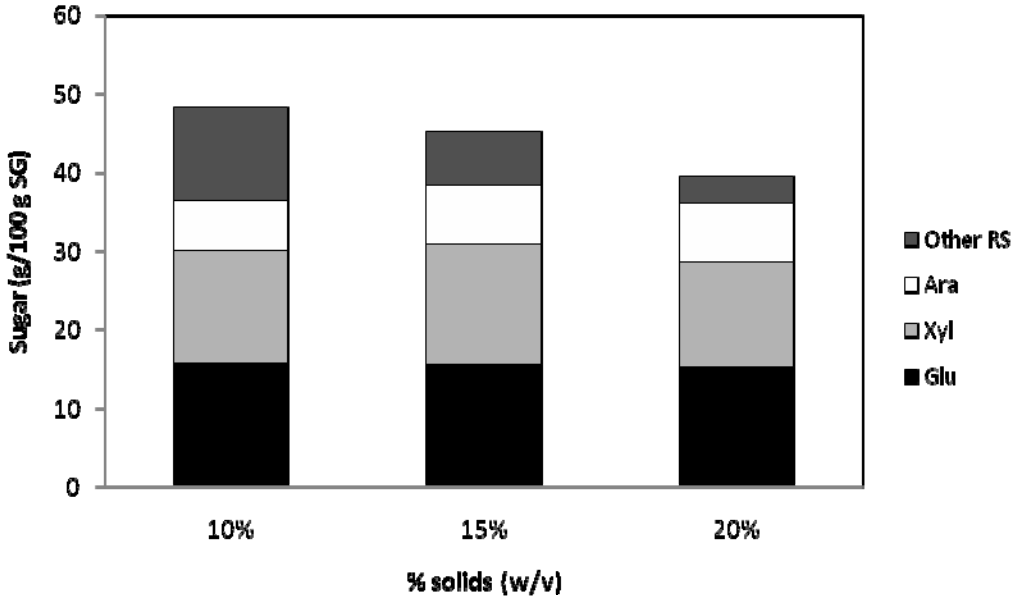
Wet

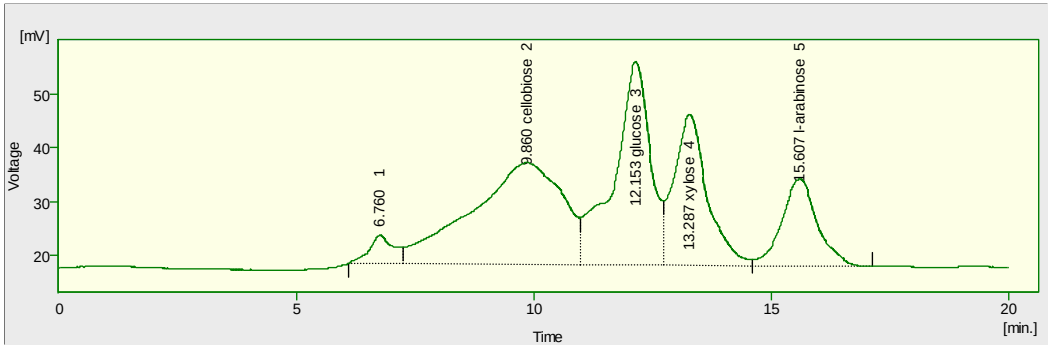
Milled



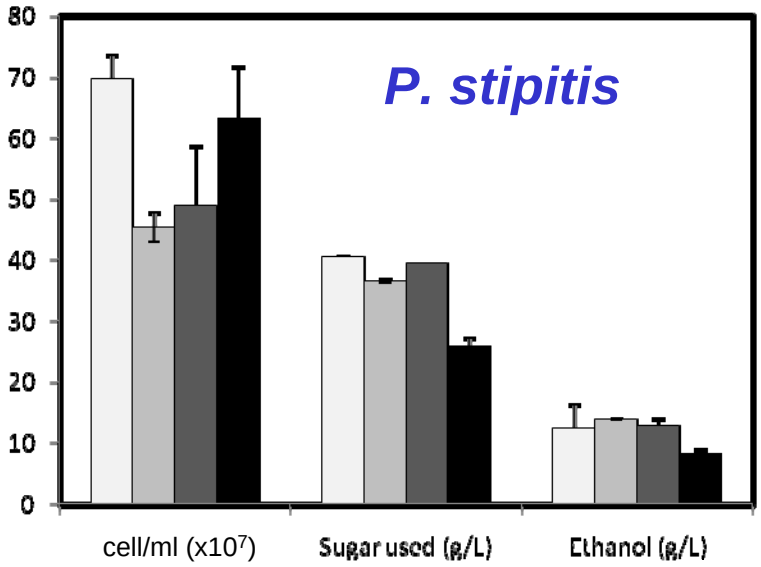
- Dilute acid pre-treatment (0.08N HCl or HNO_3 at 121°C)
- Enzyme hydrolysis (cellulase + β-glucosidase + xylanase)
- Higher solids loading? (10, 15, 20% SG)
- Typical hydrolysate –16.7 gL-1 xylose; 11.9 gL-1 arabinose; 27.9 gL-1 glucose
- Fermentation of hydrolysate and ethanol yields?

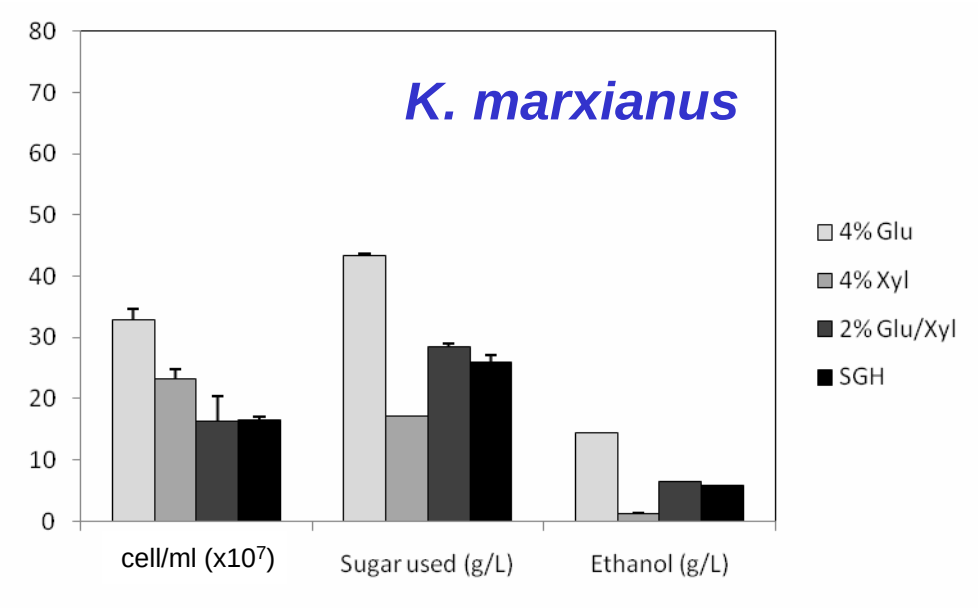
Brewer's spent grain hydrolysate:
composition of sugars





Fermentation of spent grain hydrolysate





Pichia stipitis

$Y_{P/S}$: 0.25 g ethanol (g sugar)$^{-1}$
% theoretical conversion: 48.1%
Yield: 4.2 g ethanol (100 g spent grain)

Kluyveromyces marxianus

$Y_{P/S}$: 0.18 g ethanol (g sugar)$^{-1}$
% theoretical conversion: 35.3%
Yield: 2.5 g ethanol (100 g spent grain)

“Potential” bioethanol from UK spent grains

British Breweries (2006)

- 210,974 tonnes SG
- Would yield:

  11.4M litres bioethanol

Scottish Distilleries (2006)

- 344,500 tonnes SG
- Would yield

  18.5M litres bioethanol

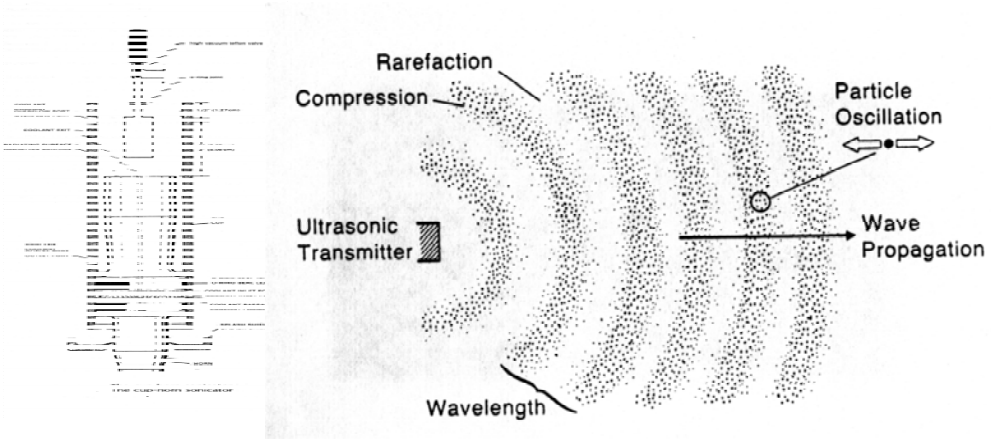
NOTE: Existing (lab-based) process is inefficient
(hydrolysis and *Pichia* fermentation ~20%CE)
- estimated potential ~100m litres/year

Novel lignocellulose pre-treatment technology?

- Ultrasound (20 kHz-1 MHz)

+

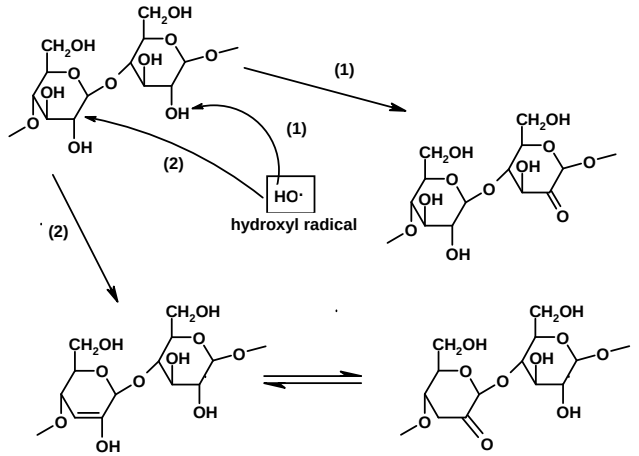
- Ozonolysis (O_3)



$O_3 \longrightarrow O_2 + O\cdot$ oxygen atom

$O\cdot + H_2O \longrightarrow 2HO\cdot$ hydroxyl radical

$2HO\cdot \longrightarrow H_2O_2$ hydrogen peroxide



Conclusions & future prospects

- Worldwide bioethanol increasing rapidly
- UK is lagging behind
- Yeast biodiversity is untapped for fermentation
- New lignocellulosic pre-treatments required
- Plentiful biowaste available (eg. spent grains, straw, wood, paper etc.)
- 2nd-generation bioethanol is the way forward!

Acknowledgements

- **Abertay University Yeast Group**

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- **Dr Carlos Harrison & SCI**

Thank you!