Bioethanol: Science and technology of fuel alcohol

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Preface

Research, development and industrialisation of renewable energy are currently moving at a rapid pace worldwide. Biofuels play significant roles in decarbonisation of our future energy needs and act to mitigate deleterious impacts of greenhouse gas emissions. Liquid transportation biofuels (bioethanol and biodiesel) now represent key contributors to the bioenergy portfolios in many countries. National governmental obligations and international directives are mandating the blending of biofuels in petrol (gasoline) and diesel and these are acting as great stimuli to this industrial sector. Bioethanol - fermentation-derived fuel alcohol - is the world's leading transportation biofuel and is mainly produced from starch (as in the US) and sugar (as in Brazil) feedstocks. However, the future lies with more sustainable fermentation substrates, including biowastes from agriculture and woody biomass. Lignocellulose-to-ethanol processes still pose many scientific and technological challenges, but we are now moving from demonstration pilot-plants to full scale industrial facilities.

This book provides a timely overview of biomass-to-bioethanol conversion technologies and is aimed mainly at advanced undergraduate students of biological and environmental sciences. I hope that readers will find it useful.

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1. Introduction

1.1 What is bioethanol?

Bioethanol is fermentation alcohol. That is, it refers to ethyl alcohol (ethanol – see Fig 1.1) produced by microbial fermentation processes, as opposed to synthetically produced ethanol from petrochemical sources. It is produced through distillation of the ethanolic wash emanating from fermentation of biomass-derived sugars. It can be utilised as a liquid fuel in internal combustion engines, either neat or in blends with petroleum (see Table 1.1).

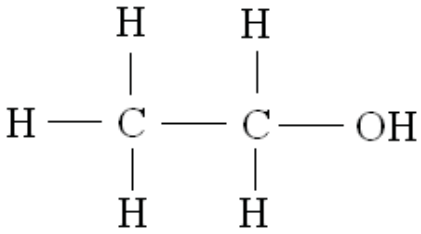

Fig 1.1 Ethanol: physico-chemical properties

Molecular formula C_2H_5OH
Molecular mass 46.07 g/mol
Appearance: colourless liquid (between -117 °C and 78 °C
Water solubility ∞ (miscible)
Density 0.789kg/l
Boiling temp. 78.5 °C (173 °F)
Freezing point -117 °C
Flash point: 12.8 °C (lowest temperature of ignition)
Ignition temp 425 °C
Explosion limits: lower 3.5% v/v; upper 19%v/v
Vapour pressure @38°C 50mmHg
Higher heating value (at 20°C) 29,800 kJ/kg
Lower heating value (at 20°C) 21,090 kJ/L
Specific heat, Kcal/Kg 60 °C
Acidity (pK_a) 15.9
Viscosity 1.200 mPa·s (20 °C)
Refractive index (n_D) 1.36 (25 °C)
Octane number 99

The *flash point* of ethanol is the lowest temperature (i.e. 12.8°C) where enough fluid can evaporate to form an ignitable concentration of vapour and characterises the temperature at which ethanol becomes flammable in air. The *ignition point* of ethanol is the minimum temperature at which it is able to burn independently (i.e 425°C). Ethanol has a high octane rating (99), which is a measure of a fuel's resistance to pre-ignition, meaning that internal combustion engines using ethanol can have a high compression ratio giving a higher power output per cycle. Regular petrol (gasoline) has an average octane rating of 88. Ethanol's higher octane rating increases resistance to engine knocking, but vehicles running on pure ethanol have fuel consumption (miles per gallon or kilometres per litre) 10-20% less than petrol (but with no loss in engine performance/acceleration).

In the 1920s, Henry Ford designed his famous Model T-Ford, the world's first mass-produced car, to run on ethanol.

Country	**Blend** (E=ethanol and number represents % in gasoline)	**Comments**
USA	E10	10% ethanol in gasoline is common (gasohol)
	E70-E85	Blend varies with State
Brazil	E25-E75	Higher blends possible via flex-fuel vehicles
	E100	
Europe	E5	Common in unleaded petrols
	E85	Relatively uncommon at present

Table 1.1 some typical bioethanol-gasoline blends employed in different countries.

Table 1.2 compares the energy content of bioethanol with conventional fossil fuels used for road and aviation transportation. In Brazil >20% of cars (and some light aircraft) are able to use E100 (100% ethanol) as fuel, which includes ethanol-only engines and flex-fuel vehicles which are able to run with either neat ethanol, neat gasoline, or any mixture of both.

Fuel	Energy content, MJ/L
E100	23.5
E85	25.2
E10	33.7
Gasoline (regular)	34.8
Gasoline (aviation)	33.5
Diesel	38.6
Autogas (LPG)	26.8

Table 1.2 Energy content of bioethanol compared with fossil fuels

Bioethanol can also be used in ethanol gels (domestic cooking), fuel for electric power, in fuel cells (thermo-chemical action), in flueless fires (eg. http://www.kost-alcohol.com/flueless.html) and in power co-generation systems. Anhydrous bioethanol has additional applications as a progenitor for other chemical commodities such as ETBE (ethyl tertiary butyl ether, a gasoline additive) and polyethylene terephthalate, PET (packaging, bottles).

> Bioethanol represents the largest volumetric production of any microbially-produced biofuel, with current annual worldwide production around 100billion litres (Renewable Fuel Association). The global leaders in bioethanol are USA with current production approaching ~50billion litres (from maize) and Brazil with ~35billion litres (from sugarcane).

Bioethanol is an example of a renewable transportation fuel, the other major one being biodiesel from plant oils or animal fat (not covered further in this book). Table 1.3 outlines the pros and cons of ethanol as a biofuel.

Pros	Cons
CO_2 neutral Reduced dependence on oil Allows agricultural diversification Clean burning, low toxicity Higher flash points (better fire safety) Better biodegradability Co-generation of electricity Low GHG emissions (~65% less than petrol)	Food-to-fuel is unethical Economics driven by oil price, which is dynamic Un-sustainability of some biomass sources Unfavourable energy balances Inefficiency of fermenting microbes Hydroscopic nature of liquid Higher fuel consumption (c.f. petrol) Some residues, emissions may be harmful

Table 1.3 Some pros and cons of ethanol as a biofuel

The main advantages of bioethanol are that the fuel is renewable and that is not a net contributor to greenhouse gas emissions (unlike fossil fuels). This is due to the fact that the biomass cultivated for bioethanol is able to re-fix (by photosynthesis) the carbon dioxide produced during bioethanol production and combustion.

Drawbacks include the fact that agricultural land may be used for biomass production for biofuel and this may impact adversely on food security. In addition, the use of genetically-modified organisms has a perceived detrimental environmental impact from the general publics' perspective. However, as will be outlined later in this book, these disadvantages can be ameliorated by using "second generation" feedstocks (eg. from waste lignocellulosic material) together with modern chemical technology and biotechnology. It has also been recently reported that future biofuel production in the EU can be secured without increasing the overall land area used for food crops (see www.biofuelsnow.co.uk).

The predominant microorganism responsible for ethanolic fermentations is the yeast species, *Saccharomyces cerevisiae*, but other yeasts and certain bacteria have future potential (see Chapter 4).

Yeasts like *S. cerevisiae* are described as ethanologenic, in that they have a propensity to convert sugars (via a metabolic pathway known as glycolysis) to pyruvate and thence by fermentation to ethanol, carbon dioxide, and numerous other secondary fermentation products.

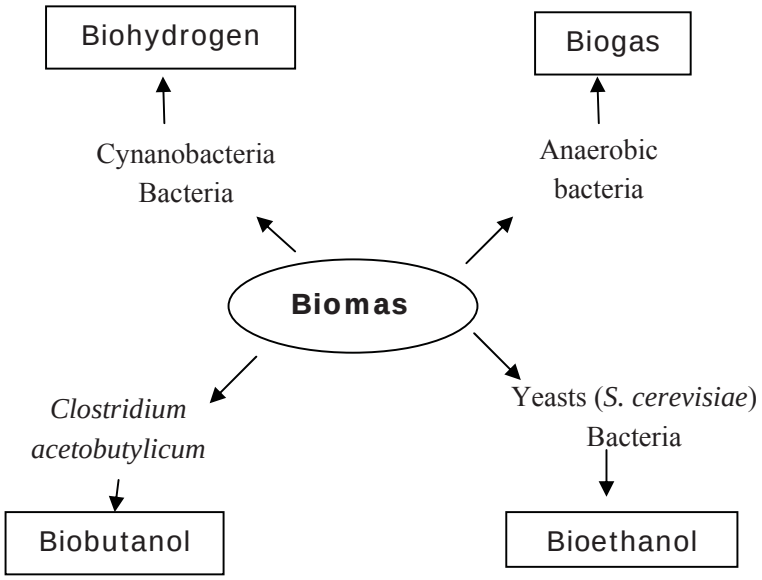

Fig 1.2 Microbial conversion of biomass to biofuels

Other microbial biofuels are biogas (methane from bacterial anaerobic digestion), biobutanol (a re-emerging technology using *Clostridium* spp. of bacteria) and biohydrogen (future potential), and are summarised in Fig 1.2. Recent research (eg. Steen *et al*, 2008) has also shown that *S. cerevisiae* can be genetically engineered (using *Clostridium* spp. genes) to produce n-butanol, and several companies are developing butanol (and isobutanol) production processes form yeast (eg. Gevo Inc - http://www.gevo.com/; Butalco – www.butalco.com). It is important to note that butanol exhibits several advantages over ethanol as a fuel, not least its better combustibility, amenability to storage and transportation and miscibility with diesel.

1.2 Economic aspects

The cost of bioethanol production is variable depending on the source of biomass (Table 1.4). If we assume that production costs for gasoline are 0.25 Euro/L, then this emphasizes the need to have governmental tax rebates in closing the price gap between biofuel and fossil fuels. Economic drivers for the production and consumption of all biofuels are inextricably linked to the global price of oil. This is obviously a dynamic situation (with increasing oil prices improving the case for biofuels) but Table 1.4 provides examples of bioethanol produced from various feedstocks and compares their production costs. It is apparent that for first-generation bioethanol feedstocks, Brazilian sugarcane represents one of the cheapest.

For bioethanol to be economically competitive with fossil fuels, production costs should be no greater than ~0.2€/litre compared with gasoline.

Biomass source	Production costs [€/litre]
Gasoline	0.25
US corn	0.42
Corn stover	0.45-0.58
EU wheat	0.27-0.43
EU sugarbeet	0.32-0.54
Brazil sugarcane	0.16-0.28
Molasses (China)	0.24
Sweet sorghum (China)	0.22
Corn fibre (US)	0.41
Wheat straw (US)	0.44
Spruce (softwood)	0.44-0.63
Salix (hardwood)	0.48-0.71
Lignocellulose (biowaste)	0.11-0.32

Table 1.4 Estimated bioethanol production costs (Euros) compared with gasoline

[Information from (www.eubia.org; Sassner *et al*, 2008; Abbas, personal communication; Gnansounou, 2008]

The figures presented in Table 1.4 are approximations due to fluctuating raw material costs. For example, the US corn ethanol production costs are based on $4 bushel corn (32 lbs of starch and 2.8 gals of ethanol). The 2010 cost of sugar cane is at a historial high and current ethanol production costs from this feedstock are estimated at around $0.35 per litre. Lignocellulosic biomass costs are highly feedstock dependent (eg. waste wood and paper costs will vary widely depending on locality and transport costs). Lignocellulose-to-ethanol production costs would be expected to become lower in the future as new technology improves the overall conversion processes (see Chapters 3 and 4).

Biomass feedstock costs represent the predominant expenditure in bioethanol production, with first-generation feedstocks generally 50-80% of total costs, whilst for lignocellulose bioethanol processes, the feedstock costs are only ~40% of total costs (Petrou and Pappis, 2009). The total value of second-generation bioethanol in the US is estimated to grow from 380 million Euro in 2010 to over 13,000 million Euro by 2020.

Fuel ethanol prices are negotiated between the buyer and seller and those prices are not publicly reported. Information on historical price data can be obtained from: www.usda.gov; www.opisnet.com; www.platts.com; www.dtnethanolcenter.com; www.jordan-associates.com; www.kingsman.com; www.argusmediagroup.com.

1.3 Energy balances

Biofuel production and consumption requires a positive net energy ratio (NER) to maintain environmental sustainability. It may be expressed as the energy from produced ethanol per energy used for its production, or (from Coombs, 1986):

$$\text{NER} = \frac{\text{Energy in ethanol expressed as higher heating value}}{\text{Energy content of all non-biological inputs}}$$

Bioethanol produced from lignocellulosic biomass and other biowaste materials generally result in very favourable (i.e positive) NER values.

A similar useful parameter in this regard is the Net Energy Balance (NEB), which is the ratio of the ethanol energy produced to the total energy consumed (in biomass growth, processing and biofuel production). Table 1.3 summarises energy balances from the production of bioethanol from sugarcane, maize and lignocellulose, and it is apparent that of the first-generation biomass sources, sugar cane represents the most favourable feedstock with respect to energy balance.

Feedstock	Energy balance
Sugar cane	6.5-9.5
Sugar beet	1.1-2.3
Sweet sorghum	0.9-1.1
Maize	1-2
Lignocellulose	Highly dependent on feedstock, but generally highly positive
[Gasoline (Gulf of Mexico oil)	6 for comparison]

Table 1.3 Energy balances for bioethanol production from different feedstocks

Energy balance values <1 mean that bioethanol production is unfeasible from energetic standpoint and is indicative of excess of fossil energy used to produce bioethanol. For maize (corn) ethanol processes in North America (USA), typical values are 1-2:1, whilst for sugarcane ethanol processes in South America (Brazil) typical values are 5-10:1. Figures are variable due to different geographic, climatic and agricultural reasons, but for Brazilian sugarcane ethanol operations, a typical energy balance of 8 (i.e. 8 times energy production in comparison to inputs) and GHG reductions of 90% (compared to only 30% for ethanol from corn) are achievable (Amorim, Basso & Lopes, 2009; Basso and Rosa, 2010). Brazilian bioethanol plants that combust residual bagasse to steam for electricity generation have very favourable energy balances. Brazil is thus considered to be a sustainable biofuel producer (see: http://bioenergytrade.org/downloads/sustainabilityofbrazilianbioethanol.pdf and http://english.unica.com.br/).

Calculations of energy balances in bioethanol production depend on several factors, for example, whether or not fossil fuel usage in agronomic practices and co-generation of energy from by-products are included. Nevertheless, there is scope to reduce energy inputs from the bioprocessing (rather than biomass cultivation) perspective, particularly through adoption of modern biotechnology. Mousdale (2008) has discussed energy balances in bioethanol production – see 9. Further Reading.

1.4 Main drivers for bioethanol

Current drivers for production of all biofuels may be summarised in Fig 1.4 and these depend on individual countries economic, environmental and political perspectives.

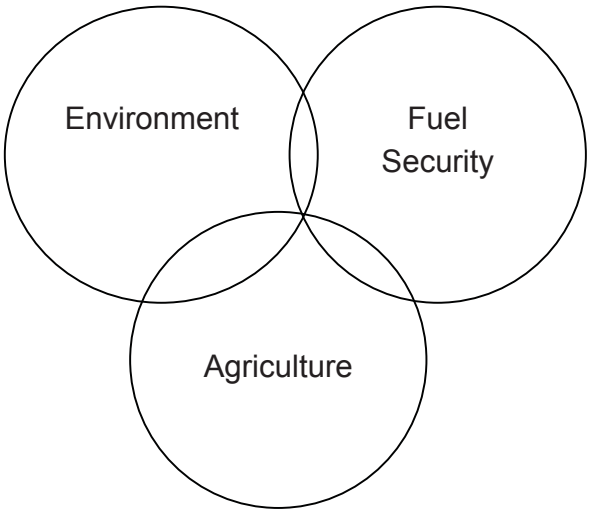

Fig 1.4. Principal drivers for biofuels

There is unprecedented potential for bioethanol production mainly due to factors such as:

- Significant variation in world crude oil prices (but generally an upward trend)
- International security concerns in regions containing crude oil resources (Middle East, Russia, Central America and Nigeria)
- Desire to improve farm incomes (in both developed and developing nations) and generally boost rural economies
- Environmental concerns (Kyoto and Bali Agreements) and potential to mitigate climate change through greenhouse gas emission reductions
- Potential for energy access in underserved areas – urban poor, rural off-grid communities
- Potential to improve trade balances

A US Report (see http:/www.bio.org/EconomicImpactAdvancedBiofuels.pdf) has analyzed how growth of an advanced biofuel industry impacts on job creation, economic output, energy security and investment opportunities. For example, biofuels industry could create 29,000 new jobs and $5.5 billion in economic growth over the next three years and could ultimately create 800,000 new jobs by 2022 with a positive effect on output of $148.7 billion. It is estimated that in the US, the cumulative total of avoided petroleum imports over the period 2010–2022 would exceed $350 billion. To stimulate the further development of US bioethanol, regulators should approve the deployment of E15 (15% ethanol, 85% gasoline) and to extend the tax credit for all ethanol feedstocks. Both public and private investment will be needed to commercialise global advanced biofuels and in Europe, development of second generation biofuels will be supported by the European Industrial BioEnergy Inititative (see http://www.biofuelstp.eu/eibi.html).

For the UK, climate change issues, together with agricultural diversification and security of fuel supply are the primary driving forces for bioethanol. Additionally, the UK government's Renewable Transport Fuel Obligation (RTFO) has set challenging targets for biofuel production (see Chapter 2).

One of the key challenges for the 21st century is to reduce dependence on finite supplies of oil, coal and gas, and move to renewable bioenergy sources. The main drivers for augmenting production of renewable transportation fuels like bioethanol are: maintenance of future fuel security; enhancement of the rural economy; and safeguarding the environment/reducing greenhouse gas emissions (see Chapter 8).

Crops grown specifically for biofuels, may provide part of the solution. Nevertheless, there are emerging concerns about environmental sustainability, biodiversity and the competition with food production (see Chapter 7).

> Industry is increasingly turning to residual biomass as a source of biofuel, and there is interest to utilize biowastes which are currently not exploited. *Second-generation* bioethanol refers to alcohol produced from fermentation of non-food biomass sources, such as lignocellulosic hydrolysates, and this topic is covered further in Chapters 3 and 4.

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2. Global production of bioethanol

2.1 Statistics

Global ethanol production in 2008 was 65.7 billion litres and will soon exceed 100 billion litres (Fig 2.1), with the largest increases in the US and Brazil. Production statistics are available from FO Licht (2007), Pilgrim (2009), USDA-ERS (2008) and Renewable Fuel Association (http://www.ethanolrfa.org/industry/statistics/)

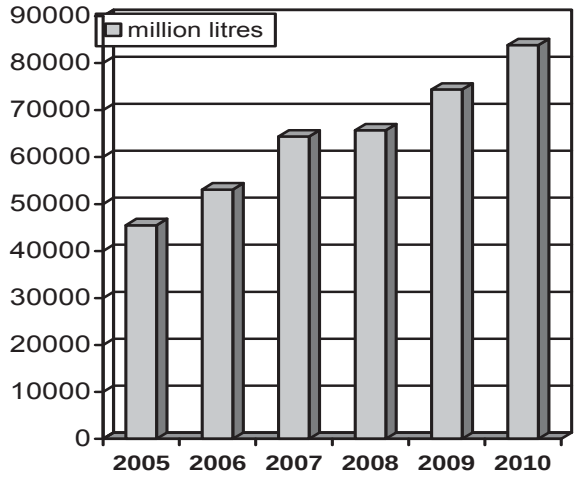

Fig 2.1 World fuel ethanol production (2005-2010) in million litres

Fig 2.2 summarises total global bioethanol production volumes and it is apparent that Brazil and the US are the dominant industrial players, accounting for 87% of global biofuel production (2008), driven by government support (see: 'Global Biofuel Market Analysis' http://www.marketresearch.com).

Brazil was the first country to embrace large-scale bioethanol production, via their government's *Proalcool* programme that was initiated in 1975 to exploit sugar cane fuel alcohol as a gasoline substitute in response to rising oil prices. Brazil is now the world's second biggest producer with around 30 billion litres/annum (2008) from sugar cane and is the world's biggest exporter of fuel ethanol. The number of sugarcane bioethanol plants in Brazil will increase to over 400 in the next few years and production is expected to reach 37 billion litres/year (from 728million tons of sugar cane) by 2012-2013 (Amorim, Basso and Lopes, 2009; Basso and Rosa, 2010).

Brazilian bioethanol

In Brazil, ethanol blends are mandatory (E20 to E25) and anhydrous ethanol (E100) is also available from thousands of filling stations. In addition, there are 6 million flex-fuel vehicles in Brazil and 3 million able to run on E100. Bioethanol now accounts for ~50% of the Brazilian transport fuel market, where gasoline may now be regarded as the “alternative” fuel.

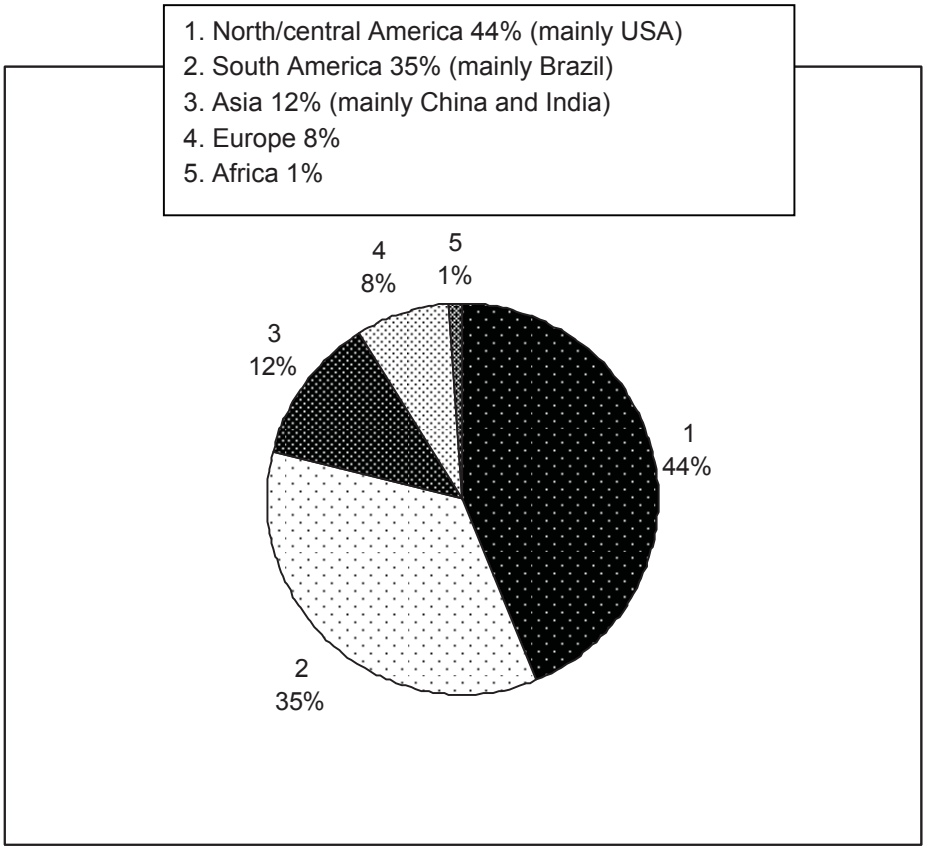

Fig 2.2 World bioethanol production (2007) Global total = 62.2billion litres

(Renewable Fuels Association, 2008)

The USA is the world's largest bioethanol producer (Fig 2.1). In late 2008, the production capacity of fuel alcohol from 180 US biorefineries was 13.6 billion US gallons (51.5 billion litres), with 31 billion litres in construction or expansion and set to commence production in 2009 (Ingeldew, Austin, Kelsall & Kluhspies, 2009). Fig 2.3 provides some bioethanol statistics from US production up to 2007 and this demonstrates the very rapid rise over recent years. For example, The Energy Information Administration (EIA) have shown that US bioethanol production increased 29% between 2009 and 2010 for the Jan/May period (Biofuel and Industrial News Issue 39 - 19 Aug 2010 www.hgca.com).

The predominant bioethanol feedstock in the US is maize (corn). If the annual corn crop (currently ~12 billion bushels) was all (i.e. starch and cellulose) processed to ethanol the total biofuel obtainable would be ~120 billion litres (at 7 gallons/bushel). The US Department of Energy Roadmap requires 40 billion gallons (~150 billion litres) of bioethanol by 2030. However, total replacement of liquid fossil fuels would require 200 billion gallons of biofuels (Abbas, 2010).

Although corn is the predominant bioethanol crop in North America, the US Environmental Protection Agency (EPA) has designated that sugar cane ethanol is an "Advanced Renewable Fuel" and it is anticipated that by 2022 around 15billion gallons (~57billion litres) of American bioethanol will be sugar cane-derived.

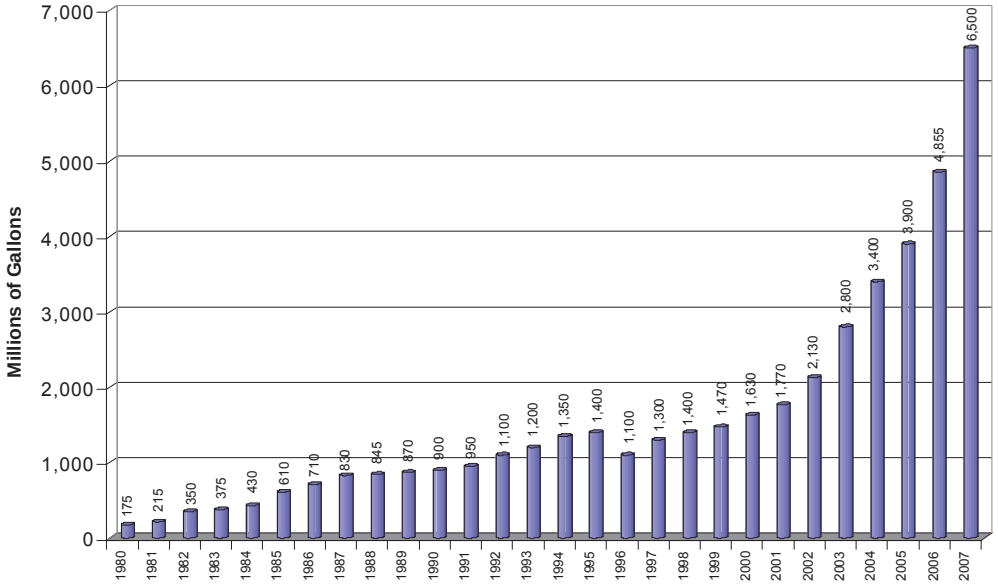

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Fig 2.3 US Fuel Ethanol Production to 2007

(6 Billion Gallons = 22.71 Billion liters. From Abbas, 2010)

Table 2.1 summarises some international bioethanol production developments. Worldwide bioethanol production has been predicted to increase at 5% Compound Annual Growth Rate (CAGR) from 2009-2018, with significant growth potential for biofuels in India and China. This prediction is reinforced by the OECD (Organisation for Economic Co-operation and Development, see: http://www.oecd.org) and UN FAO food agency, which forecast that global bioethanol production would double between 2007-2017 reaching 125 billion litres.

Further information on global bioethanol industrial developments can be obtained from various websites and e-newsletters (eg. Biofuel & Industrial News from www.hcga.com; www.ethanolproducer.com; http://domesticfuel.com; News@All-Energy; bio@smartbrief.com; www.biofuelreview.com; www.distill.com; http://www.best-europe.org). Pilgrim (2009) has reviewed bioethanol production statistics in various countries.

Country	Bioethanol developments
China	China is already the world's third largest producer of ethanol (90% from corn) and has ambitious future growth targets for bioethanol from second generation waste biomass. Current Chinese targets for bioethanol (10million tons by 2020) are considered conservative (Yan *et al,* 2010). Current bioethanol plants in China employ corn, wheat and cassava, but sweet sorghum and sugar cane have future potential. Regarding second generation feedstocks, COFCO (China National Cereals, Oils and Foodstuffs Corporation) is investing 50 million Yuan (U.S.$6.5 million) to build a cellulosic ethanol pilot plant in Zhaodong, in the northeastern province of Heilongjiang, with an annual capacity of 5,000 tonnes. Another cellulosic ethanol pilot plant with a production capacity of 10,000 tonnes is being planned in the Yucheng area of Shandong (see: http://www.biofuels.apec.org).
India	India accounts for around 4% of global bioethanol production (2m kilo litres in 2006) from sugar cane and has plans to expand its production, especially using cellulosic substrates (for example, see http://www.praj.net and http://www.rellife.com/biofuels.html). In February 2009, India and the US exchanged a memorandum for cooperation on biofuels development, covering the production, utilization, distribution and marketing of biofuels in India (see: http://www.indiaembassy.org/newsite/press_release/2009/Feb/1.asp)
Russia	In Russia, information on bioethanol production is provided by the Russian National Biofuels Association (see: http://www.biofuels.ru).
Nigeria	In Nigeria, a recent analysis of sugarcane and sweet sorghum as bioethanol feedstocks has concluded that the latter crop is better suited in terms of its adaptability to harsh climatic and cultivation conditions (Nasidi *et al*, 2010).
Australia	Information about bioethanol production in Australia is available from the Biofuels Association of Australia (see: http://www.biofuelsassociation.com.au).
Colombia	In Colombia, sugar cane, rather than maize, has been identified as the most promising feedstock to boost their domestic bioethanol production based on environmental and economical considerations (Quintero *et al*, 2008).
Japan/Asia Pacific	Regarding Japan and Asia Pacific, in comparison to Brazil, the US and Europe bioethanol production industry in these countries is in its infancy (see: http://www.biofuels.apec.org; http://www.biofuels.apec.org/me_japan.html; ISSAAS, 2007). In fact, Japan is the second-largest importer of ethanol (to meets its E10 mandates) as it lacks the conditions for large scale bioethanol production. [Walter *et al*, 2008]

Table 2.1 Selected international bioethanol production

In Europe, bioethanol production is on a steep increase (see Fig 2.4) and the main producers of bioethanol are France, Germany and Spain (Fig 2.5) using predominant feedstocks of cereals (mainly wheat) and sugarbeet. Figures from eBIO, the EU ethanol industry body, show EU fuel ethanol production increased from 2.8bn litres in 2008 to 3.7bn litres in 2009, a rise of 31%. France (1.25bn litres) and Germany (750M litres) were the largest producers with Spain third (465M litres), seeing increases in annual production of 25%, 32% and 46% respectively. EU bioethanol 2010 plant capacity was 7.7bn litres in 2010, and projections (F.O.Licht, 2007) for 2011 show an increase to 8.3bn litres as new plants come on line, particularly in Spain and Germany.

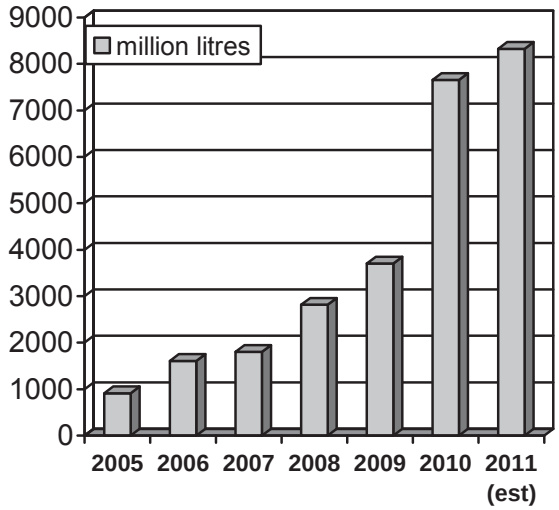

Fig 2.4 European fuel ethanol production (2005-2011) in million litres

(Infromation from: Biofuel and Industrial News Issue 39; 19 Aug 2010 www.hgca.com; eBIO, the EU ethanol industry body; FO Licht)

General information on bioethanol in Europe is available from The European Bioethanol Fuel Association (www.ebio.org) and The European Union of Ethanol Producers (www.uepa.be).

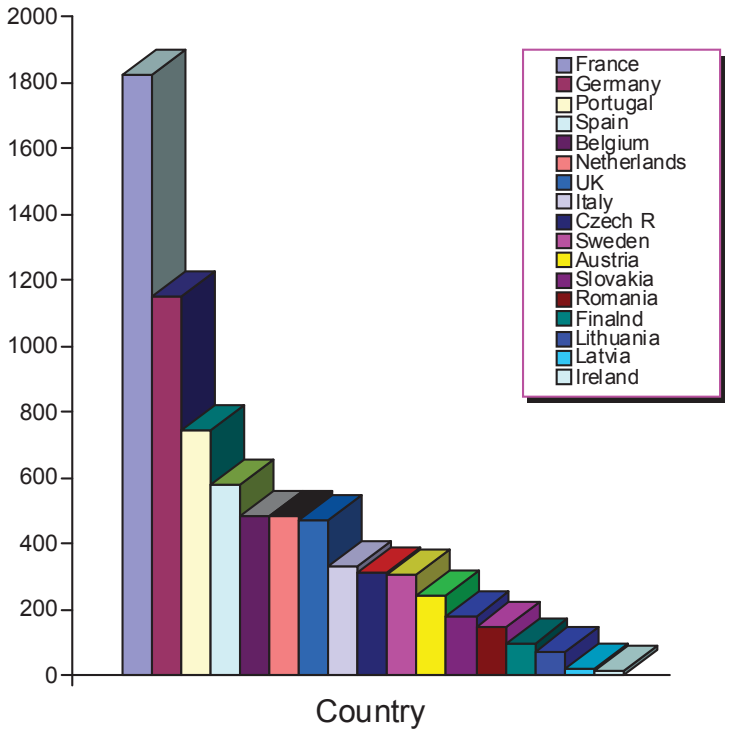

Fig 2.5 Main European fuel ethanol producers (2010) in million litres

(Information from: Biofuel & Industrial News, Aug 19, 2010 www.hgca.com)

The UK saw a small decline in bioethanol production from 75M litres in 2008 to 70M litres in 2009, although much higher figures are expected for 2010 as a major bioethanol plants (see Table 2.2) come on stream. Most UK domestic demand for bioethanol currently depends on imports but these will lessen as the UK bioethanol industry sector matures. A recent (August 2010) report from the UK Renewable Fuel Agency (RFA) indicated that in the first month of the 2010/11 Renewable Transport Fuel Obligation (RTFO) period, 141 million litres of biofuel were supplied (representing only ~1.6% of total road transport fuel, against a UK annual target of 3.5%). More biodiesel (65%) was supplied than bioethanol (35%). The most widely reported source of bioethanol used in UK transport fuels was sugarcane from Brazil (39% of bioethanol supplied). Future UK capacity is predicted to grow rapidly from 70M litres in 2009 to 470M litres in 2010 and 890M litres in 2011. (Further information from F.O.Licht (2007); www.britishbioethanol.co.uk; www.adas.co.uk)**.**

Company & location	Summary of production
Ensus (Wilton)	1.2m tons wheat/400m litres bioethanol/350,000t protein feed. On stream, March 2010. Europe's largest wheat refinery
British Sugar (Wissington)	75m litres bioethanol from sugar beet
Vivergo (Hull) http://vivergofuels.com	420m litres bioethanol (& potentially biobutanol) from 1.15mt wheat (with BP/British Sugar/Du Pont), plus ~0.5 Mt high-protein animal feed. Operational by 2011.
TMO Renewables (Guildford)	Cellulosic ethanol process demonstration unit
Future Fuels (NE England)	Under development (200m litres bioethanol/175,000t protein feed)
Vireol (Grimsby and Teeside)	370 million litres a year of bioethanol from ~ 1mt wheat
Bioethanol Ltd (Immingham)	120m litres bioethanol from 0.325 mt wheat (planning pending)
Abengoa (Stallingborough)	500m litres bioethanol from 1.3 mt wheat (planning pending)
Green Spirits Fuels (Henstridge)	Planning permission granted (2006) to convert 350,000 tonnes of locally grown wheat per year into 130 million litres of ethanol.
Green Spirits Fuels (Humberside)	250m litres bioethanol from 0.65 mt wheat (planning pending)
Roquette (Corby)	125m litres bioethanol from 0.3 mt wheat (planning pending)

Table 2.2 Some UK industrial bioethanol developments

The first tanker of UK bioethanol (sold to Shell) left the Ensus wheat-bioethanol plant at Wilton in Teesside, England in March, 2010. The Renewable Energy Association (REA) have reported (2009) that the UK has potential to deliver up to 80% of the biofuels needed to fulfil European obligations in a sustainable way without increasing overall land used for arable crops. The EU's Renewable Energy Directive (for which the UK is a signatory) states that 10% of road transport fuels must come from renewable sources by 2020, and the UK intends to "increase biofuels steadily from 2010 up to the level required in 2020" (RAE). [biofuelsnow.co.uk 22/10/09].

2.2 National and international directives

Various national governmental obligations and international directives on biofuel usage are acting as stimuli for the bioethanol industrial sector.

In the US, The American Energy Policy Act of 2005 created a Renewable Fuel Standard (RFS) that required refiners to "*use an increasing percentage of renewable fuels such as ethanol and biodiesel in their fuel mix, as well as creating new incentives for ethanol production from sugar, cellulose and other non-traditional feedstocks*". Subsequently, in 2009 the USA consumed around 42 bn litres (11.1 billion gallons) of ethanol, and that amount is expected to rise significantly in future years due to US federal mandates. The Renewable Fuel Standard was expanded when the US Congress passed the Energy Independence and Security Act of 2007, requiring the use of 9 billion gallons of renewable fuel in 2008, growing to more than 15 billion gallons in 2012 and 36 billion gallons (136.2 billion litres) by 2022. Importantly, a ceiling of 15 billion gallons (56.8 billion litres) has been set for the amount that can be produced from corn starch (see US EIA, 2008). Additional targets have been set for 80 billion litres of biofuels from other conventional feedstocks (such as sugar cane) as well as non-conventional cellulosic feedstocks.

In Brazil, bioethanol is now the preferred road (and potentially aviation) transportation fuel. Bioethanol production is also accelerating in other South American countries and information is available covering statistics, production, sustainability, feedstocks, governmental policy and other information for bioethanol in Latin America (see http://www.top-biofuel.org).

In Europe, volumetric output of ethanol is increasing year-by-year, primarily in response to governmental obligations (eg. the UK's Renewable Transport Fuels Obligation, RTFO see http://www.renewablefuelsagency.gov.uk) and European Commission directives. A European Directive (#2003/30/EG) from May, 2003 imposed on European Union member states an objective to have 5.75% biofuel substitution of fossil fuels by the end of 2010. On February 4, 2009, a European Parliament resolution entitled: "*2050: The future begins today – Recommendations for the EU's future integrated policy on climate change*" (2008/2105(INI)) - established a range of measures to reduce greenhouse gas emissions by 25-40% by 2020 and a reduction of at least 80% by 2050. The report advocates that the EU Members States should invest in research on advanced biofuels, among other renewable technologies.

In July 2008, the European Parliament's Committee on the Environment, Public Health and Food Safety recommended (July 2008) that biofuel targets for the year 2020 to be 8% of fossil fuel useage. The previous target, outlined in the European Commission's January 2007 "Renewable Energy Roadmap" was 10%. Current EU policy on biofuels must be viewed in a global perspective, with growing competition for productive land alongside an increasing need for renewable transportation fuels.

In the UK, the RTFO applies to road transport across the whole of the UK and *"requires suppliers of fossil fuels to ensure that a specified percentage of the road fuels they supply in the UK is made up of renewable fuels. The target for 2009/10 is 3.25% by volume."* (Renewables Fuel Agency). Following The Gallacher Review (2008), in April 2009, The RTFO Amendment Order (2009) amended the targets as follows: *"the level of the renewable transport fuel obligation by slowing the rate of increase (from 2.5% to 5% of total fuel supplied) in the amount of renewable transport fuel for which evidence of supply in the United Kingdom"*.

The Gallagher Review has stated that the EU's biofuels target for 2010 of 10% by energy "*is unlikely to be met sustainably and the introduction of biofuels should therefore be slowed while we improve our understanding of indirect land-use change and effective systems are implemented to manage risks*". The Renewable Fuels Agency has therefore proposed that (UK) targets higher than 5% should only be implemented beyond 2013/14 if biofuels are shown "to be demonstrably sustainable (including avoiding indirect indirect land-use change)".

Proposed rates of increase in UK biofuel-fossil fuel blends will rise to a maximum of 5% by 2013/14. (see Table 2.2). There will be a further review of UK biofuel targets in 2011/12 to coincide with the EU's review of member states' progress on biofuel targets. Those obligated by the RTFO include refiners, importers and any others who supply >450,000 litres/year of relevant hydrocarbon oil for UK road transport. Biofuels pertinent to the RTFO include bioethanol, biodiesel, pure plant oil, biogas (methane), biobutanol, bio-ETBE and HVO (hydrogenated vegetable oil, also referred to as renewable diesel).

	2008	2009	2010	2011	2013	2013
RTFO original targets (% volume)	2.5	3.75	5.0			
Gallacher Review targets (% volume)	2.5	3.0	3.5	4.0	4.5	5.0

Table 2.2 UK's Renewable Transport Fuel Obligation (RTFO) targets

2.3 Current and emerging status

Shortly after September 11, 2001 then President George Bush announced that the US would break its "addiction to oil", meaning that American national security was now linked to energy security. By 2009, increasing concerns about climate change provided additional momentum to develop sustainable and secure alternatives to oil (see: http://domesticfuel.com/2009/12/31/the-ethanol-decade/).

According to the Renewable Fuels Association (see http://ethanolrfa.org), biofuels came of age in the 2000s. This is exemplified by bioethanol production increments year-on-year – for example: the US produced 5.3 billion litres (1.4 bn gal) of bioethanol in 1999 in 54 plants, rising to over 40 bn litres (10.6 bn gal) in 2009 in more than 200 plants and predicted to reach 136 bn litres by 2022. Today, bioethanol is blended in more that 80% of US motor fuels. Importantly, the US bioethanol industry supports nearly 500,000 jobs and in 2008 generated an estimated $12 billion in federal tax revenues and $9 billion in state and local revenues. In addition, American oil imports from OPEC have been reduced by more than 300 million barrels a year.

Nevertheless, in the US, there is a need to break through the 10% blend wall and eventually move on to blends of 12%, 13%, 15% and beyond, while expanding the vehicle fleet and infrastructure for E85.
In the EU, a European Biofuels Technology Platform (see: http://biofuelstp.eu) has been established to contribute to:

- the development of cost-competitive world-class biofuels value chains,
- the creation of a healthy biofuels industry, and
- the acceleration of the sustainable deployment of biofuels in the EU through a process of guidance, prioritisation and promotion of research, technology development and demonstration.

International trade in ethanol is expected to grow rapidly over the next decade, mainly with exports from Brazil to the US and the EU.

Opportunities exist for exploiting second-generation (non-food) bioethanol substrates based on lignocellulosic biowastes generated from agriculture, industry and forestry activities but these approaches are fraught with key scientific and technological constraints (see sections 3.2 and 4.5). Current and emerging trends in bioethanol production from lignocellulosic materials in various countries (Korea, China, Canada, Brazil, India, Malaysia and Europe) have been discussed in a recent Special Issue of Bioreource Technology on lignocellulosic bioethanol edited by Pandey (2010).

> Bio-based transportation fuels offer many developing countries new economic opportunities, and will lessen their dependence on energy imports. Importantly, however, biofuel production must be sustainable and must not threaten biodiversity or directly compete with food production. Future biofuel policies should set clear sustainability criteria and promote development of second-generation bioethanol.

Further issues regarding future trends in global bioethanol production, and the scientific and technological challenges still to be overcome, are discussed in Chapter 8.

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3. Bioethanol feedstocks

3.1 First generation feedstocks (starch and sugar-based)

In general, bioethanol can be extracted from every sort of carbohydrate material that has the typical formula of $(CH_2O)_N$. These can be divided in three main groups: sugary, starchy and lignocellulosic biomass.

First-generation feedstocks for bioethanol production primarily refer to plant biomass (or phytomass) sources that are also sources of human and animal nutrition, namely: cereal starches and sugar crops. Table 3.11 summarises both first and second generation resources for bioethanol and Fig 3.11 summarises first generation crops for bioethanol. Further information is available from Pasha and Rao (2009) and Monceaux (2009).

Sugary materials	Starchy materials	Cellulosic materials
Sugarcane Sugarbeet Sweet sorghum Cheese whey Fruits (surplus) Confectionery industrial waste	Grains (maize, wheat, triticale) Root crops (potato, cassava) Inulin (polyfructan) root crops (chicory, artichoke)	Wood Agricultural residues (straws, stover) Municipal solid waste Waste paper, paper pulp

Table 3.11 Major resources for bioethanol production

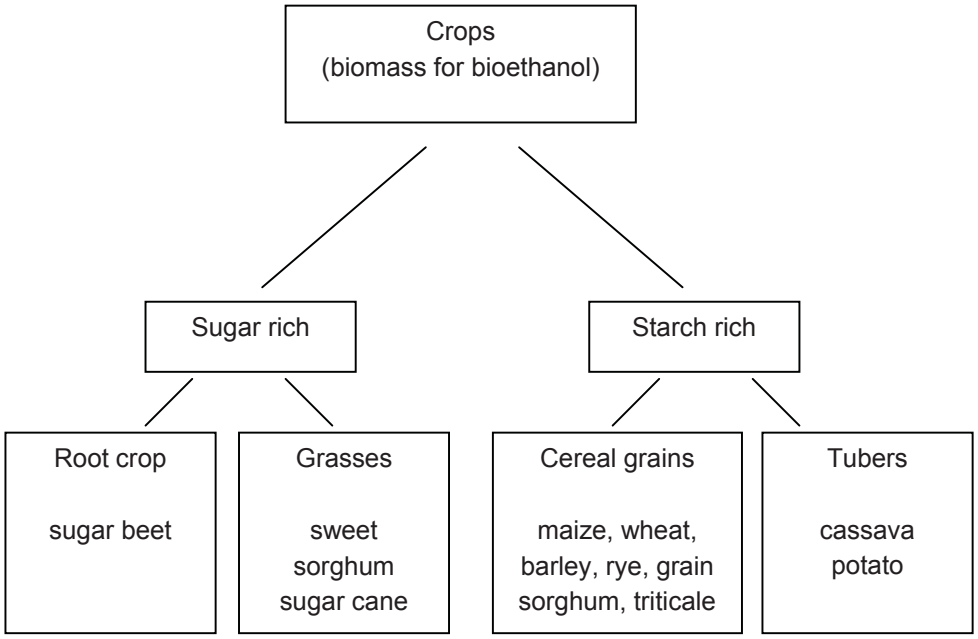

Fig 3.11 First generation crops for bioethanol

Sucrose-based materials are predominantly derived from sugar cane (*Saccharum* sp.) and sugar beet (*Beta vulgaris* L.), whilst starch-based materials are predominantly derived from cereal crops such as maize, wheat and other cereals. Simple-sugar based feedstocks for bioethanol production include sugar cane, sugar beet and sweet sorghum and these crops represent a readily fermentable sugar source (comprising mainly sucrose, fructose and glucose) whilst cereal starches require pre-hydrolysis to obtain sugars that can be fermented by yeast. Thus, fermentation can be carried out without accomplishment of prior hydrolysis or other pre-treatments because the sugar is available in disaccharides (containing one molecule of glucose and one molecule of fructose) which can be metabolised directly by enzymes present in yeast. For this reason, the conversion of sucrose-containing feedstocks is the easiest and most efficient compared with other feedstocks and the costs of the process are relatively low compared to the commodity price.

Another simple-sugar containing material is whey, a by-product of cheesemaking. Whey comprises around 5% w/v lactose which is a disaccharide of glucose and galactose. *S. cerevisiae* cannot directly ferment lactose (due to a lack of β-galactosidase and other lactose-utilising enzymes) unless lactose is hydrolysed to its component monosacchrides or the yeast is genetically modified. Some natural lactose-fermenting yeasts do exist, notably *Kluyveromyces marxianus*, but to date they have only been employed on a large scale for potable ethanol fermentations (eg. in Ireland, New Zealand and USA (California and Minnesota).

Table 3.12 shows the main macromolecular constituents of major starchy crops. The main crop for bioethanol production in North America is *Zea mays* (maize, or corn), whilst in Europe it is wheat. Most US (>80%) corn is cultivated in the mid-west states (mainly Iowa, Illinois, Minnesota, Nebraska and Indiana – see NCGA, 2010). Such crops are high in starch which is described as an alpha-polysaccahride comprising D-glucose monomers existing in two forms: amylase and amylopectin (see Fig 3.43).

Constituent (%w/w)	Maize	Wheat	Barley	Sorghum	Rye	Cassava	Potato
Starch	65-72	57-70	52-64	72-75	55-65	65-82	14-24
Sugar	2.2	-	-	-	-	0.25	1.5
Protein	9-12	12-14	10-11	11-12	10-15	2-3	0.6-3.5
Fat	4.5	3	2.5-3	3.6	2-3	0.8	0.1
Cell wall material	9.6	11.4	14	-	-	-	2
Fibre	-	-	-	-	-	4.6	-
Ash	1.5	2	2.3	1.7	2	2-5	0.6-1.1

Table 3.12 Main constituents of starch-based feedstocks for bioethanol

[From Monceaux, 2009]

Bioethanol production from cereal grains comprises the following main stages: milling, starch hydrolysis, yeast fermentation, distillation (to ~95% ethanol) and water removal from ethanol (to 99.9% or absolute ethanol). It is possible to produce 1L anhydrous ethanol from ~3kg wheat. Table 3.13 compares the potential ethanol yields from typical starch and sugar crops, wheat and sugarbeet, respectively. It is apparent in this case that wheat yields a greater level of ethanol when compared to sugar beet on a weight basis, but that on an acerage basis, sugar beet is more productive.

Parameter	Wheat	Sugar beet
Moisture content (%)	20	76
Starch/sucrose content (%)	76	69
Starch /sucrose content /t (kg)	608	166
Ethanol yield (L/t)	374	100
Energy yield (GJ/t)	7.85	2.19
Crop yield (t/ha)	8.4	55
Ethanol yield (L/ha)	3,141	5,500
Energy per hectare (GJ/ha)	66	116.6
Cost of feedstock €/t	100	50
Cost of feedstock €/L of ethanol	0.267	0.50

Table 3.13 Key parameters for bioethanol production from starch and sugar

Other main starchy crops include *Hordeum vulgare* (barley), *Sorghum bicolor*, *Triticale* (a hybrid of wheat (*Triticum*) and rye (*Secale*) first bred in Scottish and Swedish laboratories during the late 19th century) and Cassava. "*Sugarcorn*", a hybrid cross between sugar cane and maize is under development by a US company, Targeted Growth Inc (www.ethanolproducer.com, January 2009).

Inulin-rich root crops such as Jerusalem artichoke have also been considered as potential bioethanol feedstocks as they can be grown in nutrient-poor soils. Inulin is a polyfructan (polymer of β-2,1 linked fructose monomers) that can be hydroysed by inulinases to fermentable fructose, or directly fermented by certain yeasts (eg. *Kluyveromyces marxianus*).

3.2 Second generation feedstocks (cellulose-based)

The use of first generation feedstocks to meet growing demands for future biofuel production is ultimately unsustainable and there are severe limitations to starch and sugar-based ethanol production. For example, if the US was to replace all gasoline with 10% ethanol, around 46% of the current maize crop would be required and this is obviously untenable. Non-food, or second generation, feedstocks for bioethanol are therefore the future due to abundance, ethical considerations and favourable economics.

Second generation raw materials for bioethanol production typically refer to non-food biomass sources, mainly lignocellulosic biomass. This represents the most abundant form of carbon on Earth (estimated annual production at 10^{10} MT, Sanchez and Cardona, 2008), and encompasses 2 main categories of feedstocks:

1. Waste materials (straws, corn residues (stover, fibres and cobs), woody wastes/chippings, forestry residues, old paper/cardboard, bagasse, spent grains, municipal solid waste (MSW), agricultural residues (oilseed pulp, sugar beet pulp).

2. Energy crops such as SRC (short rotation coppice, eg. basket willow *Salix viminalis*) and energy grasses *Miscanthus gigantum*, switchgrass (*Panicum vigratum*), reed canary grass (*Phalaris arundinaceae*), giant reed (*Arundo donax*), ryegrass, etc) growing on inferior agricultural land and contaminated industrial land.

Table 3.21 summarises some key parameters for major lignocellulosic feedstocks for bioethanol production.

Feedstock	Primary locations	Global availability (est)	Potential bioethanol yield (est)
Corn stover	Asia, Europe, N. America	409.5 (million T/year)	274.4 litres/ton
Wheat straw	Asia, Australia, N. America, Europe	702.9 (million T/year)	257.4 litres/ton
Sugar cane bagasse	Asia, S. America	564.4 (million T/year)	314.2 litres/ton
Municipal solid waste	Worldwide (173 countries) [reported by Shi *et al* (2009) that 82.9 billion litres ethanol possible]	500-1500 (million T/year)	170-486 litres/ton

Table 3.21 Some lignocellulosic feedstocks for bioethanol

(information from www.bioenergy.novozymes.com ; Shi, *et al*. 2009 and www.dialogue4s.de/_media/Prince_**Bioethanol**_Preparation_from_Organic_Waste_Residues.pdf)

Residues from maize (corn) processing include corn stover which comprises the maize stalk and leaves. For every kg of maize cropped, almost the same amount of stover is left in the field. This can be utilised in agricultural practices to prevent soil erosion, but simultaneous saccharification and fermentation (SSF) processes (see Chapter 4) can be used to produce bioethanol from stover. However, the amount of non-utilisable lignin in stover is high and varies between 17-26% dry wt. Other problems in converting cellulosic biomass to ethanol include: collection, pretreatment and conversion.

Energy crops

These are fast growing plants that can be exploited for bioethanol production and which are not utilised as food sources. Examples include:

- Switchgrass (*Pancium virgatum*) is a perennial C4 plant grown in USA currently as fodder crop or for soil conservation but can be de-lignified for bioethanol production.
- Reed canary grass (*Phalaris arundinacea L*) is a perennial grass that grows widely. Its stem components (dry wt) comprise: hexoses (38-45%); pentoses (22-25%); lignin (18-21%)
- Alfalfa (*Medicago sativa L*) comprises mainly cellulose, hemicellulose, lignin, pectin and protein.
- *Miscanthus x giganteus* (hybrid of *M. sinensis* and *M. sacchariflorus*) is a perennial grass with a low need for fertilzers and pesticides with a broad temperature growth range. Previously used as an ornamental landscaping, but now an attractive biomass source for biofuels For example, potential ethanol from miscanthus is around twice that from corn on an acreage basis

(From Arshadi & Sellstedt, 2008; Long, 2006; Grooms, 2008; Pilgrim, 2009; Pyter *et al*, 2009)

Lignocellulose: cellulose, hemicellulose and lignin

Woody biomass comprises major components of cellulose, hemicellulose (that can both be hydrolysed to fermentable sugars) and lignin (that cannot be converted to fermentable sugars). Fig 3.21 provides the basic structure of these components and Table 3.22 summarises lignocellulose composition from major biomass sources. In the context of bioethanol production, “biomass” refers to phytomass (trees, plants) that has a mole ratio formula of the main elements: $CH_{1.4}O_{0.6}$, whilst the chemical impirical formulae of biomass main constituents are: Cellulose $C_6H_{10}O_5$; Hemicellulose $C_5H_8O_4$; Lignin $C_6H_{11}O_2$.

Cellulose is described as a beta-polysaccharide of glucose (in β-(1,4)-linkages) with an average molecular mass of ~100,000Da and hemicellulose a complex (highly branched) polymer with an average molecular mass of 30,000Da consisting of xylose and arabinose (pentose sugars) and glucose, mannose and galactose (hexose sugars). In softwoods, the hemicellulose sugar backbone is mannose with glucose and galactose side-chains; whilst in hardwoods and grasses, the backbone is xylan with side chains of arabinose and glucuronic acid. In hardwood species (eg. *Salix*) some of the xylose units are acetylated (OH groups replaced by O-acetyl groups) and during pre-treatment (see 3.4) this can give rise to high levels of acetic acid that can inhibit subsequent yeast fermentations.

Biomass or waste	Cellulose	Hemicellulose	Lignin
Trees			
Poplar	45-50	17-19	18-26
Eucalyptus	50	13	28
Pine (spruce)	44	23	28
Salix (hardwood)	43	22	26
Grasses			
Switchgrass	31-45	20-30	12-18
Bermuda grass	25	36	6
Rye grasses	25-40	35-50	10-30
Paper			
Office paper	69-99	0-12	0-15
Newspaper	40-55	25-40	18-30
Paper pulp	60-70	10-20	5-10
Food/agriculture wastes			
Corn cobs	45	35	15
Corn stover	38-40	22-28	18-23
Corn fibre	14	17	8
Wheat straw	30-38	21-50	15-23
Rice husk	24	27	13
Bagasse	38	27	20
Nut shells	25-30	25-30	30-40
Leaves	15-30	80-85	0
Cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
Other wastes			
Sorted refuse	60	20	20
Primary wastewater solids	8-15	NA	24-29
Municipal solid Waste (MSW)	33	9	17
MSW paper pulp	62	5	11

Table 3.22 Composition of some lignocellulose sources (% dry weight)

[Information from Sun and Cheng, 2002 and Mosier *et al*, 2005; Zhang *et al*, 2009; Goyal *et al*, 2008; Sassner *et al*, 2008; bio-process.com/wp-content/uploads/2009/12/MSW.pdf]

Xylose and arabinose are polymerised in the form of xylan and arabinan, respectively to form arabinoxylan (a complex heteropolysaccharide – see Fig 3.22) and Table 3.23 provides the proportional composition of these polymers in different feedstocks.

Feedstock	% Xylan	% Arabinan
Ryegrass	16	5
Corn stover	19	3
Wheat bran	19	15
Wheat straw	21	3.4
Barley husks	20	9
Hardwood	15	1
Softwood	5	2
Bagasse	26	1.5
Newspaper	4.3	0.8

Table 3.23 Xylan and arabinan composition of selected lignocellulose sources

(Some information from Esterbauer, 1986)

Lignin (see Fig 3.21) is the secondary plant cell wall material which is a very tough, recalcitrant material comprising a 3-D network of di- and mono-methoxylated, and non-methoxylated phenylpropanoid units (derived from the corresponding *p*-hydroxycinamyl alcohols). Following acid hydrolysis of lignocellulosic biomass, acid-insoluble lignin remains, but a portion of it (i.e. acid-soluble lignin) may be released into the hydrolysis liquor. For bioethanol production processes, some adverse impacts of acid-soluble lignin components include cellulase inhibition and fermentation inhibition (due to formation of pre-treatment derived phenolic degradation products – see Chapter 4).

In addition to the principal components of lignocellulose (i.e. cellulose, hemicellulose and lignin) of various biomass sources provided in Table 3.22, other minor components such as ash (inorganic minerals), pectins (highly-branched polysaccharides of galacturonic acid and its methyl esters), acids and extractives (extracellular, non-cell wall material) will be present.

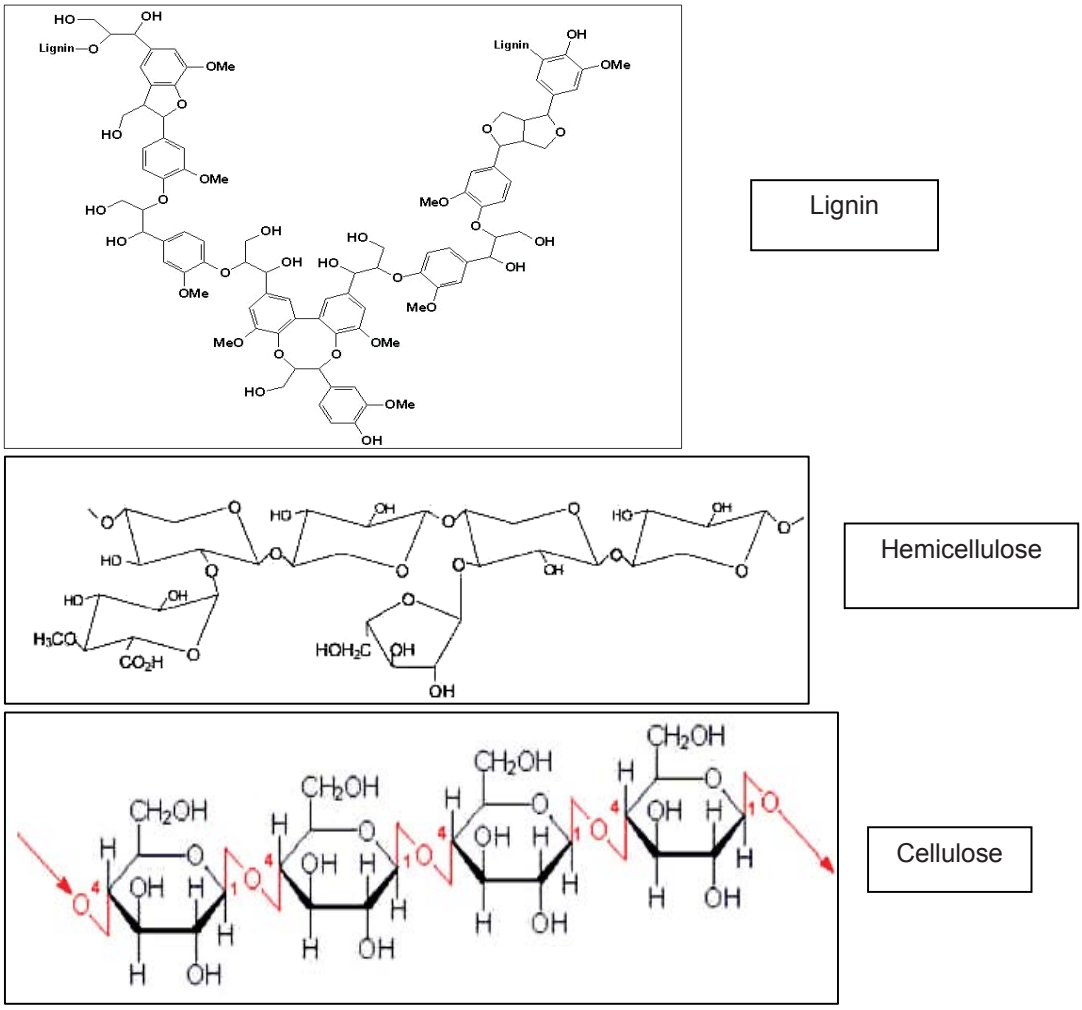

Fig 3.21 Basic structures of lignocellulose components: lignin, hemicellulose and cellulose

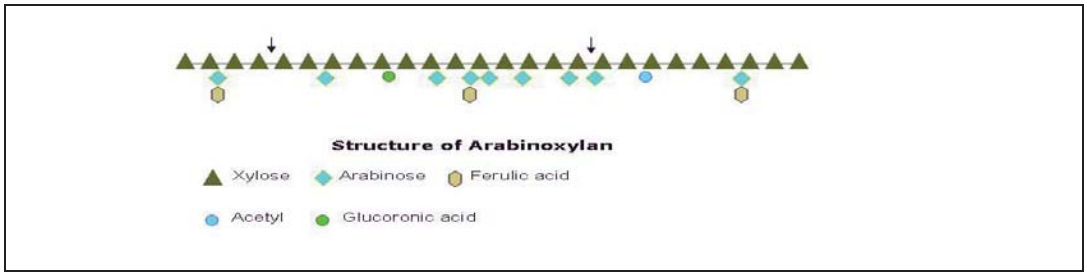

Fig 3.22 Basic structure of arabinoxylan (arrows indicate sites of xylanase enzyme attack)

Other cellulosic raw materials/feed stocks include food wastes and food/beverage processing residues (Kim and Dale, 2004). For example, spent grains (SG), the residue remaining after extraction of wort, are a major by-product of brewing and distilling, and provide lignocelluose-rich biomass as a potential source of sugars for fuel ethanol fermentations. White *et al* (2008) have shown that dilute acid and enzyme treatments can convert the hemicellulose and cellulose fractions to glucose, xylose and arabinose. Fermentation of this hydrolysate by non-*Saccharomyces* yeasts such as *Pichia stipitis* and *Kluyveromyces marxianus* results in favourable ethanol conversion yields (g ethanol/g substrate).

> Biotechnology and chemical technology research holds the key to enhancement of future developments in sustainable bioethanol production from lignocellulosic biomass.

3.3 Bioethanol feedstocks with future potential

Marine macroalgae (seaweeds) demand minimal use of agriculture areas and fresh water for cultivation, and represent an interesting biomass resource for bioethanol (Horn *et al*, 2000a; 2000b). Their attraction as biomass sources for biofuels stems from the fact that seaweeds have growth rates and primary production rates far in excess than those of terrestrial plants. As low-input, high-yielding biomass, seaweeds may represent an example of *third generation* feedstocks for bioethanol production. Brown seaweeds (Phaeophyta) in particular contain storage polysaccharides which are substrates for microbial degradation. They contain high amounts of carbohydrates such as alginic acid (structural) and laminaran and mannitol (storage) that can potentially be fermented to ethanol. Alginate typically makes up 30-40% of the dry weight in giant brown seaweeds (kelp). Laminarin is a linear polysaccharide of 1,3–β-D-glucopyanose and can relatively easily be hydrolysed to fermentable glucose. Unlike lignocellulosic biomass, they have low levels of lignin and cellulose making them more amenable for bioconversion to energy fuels than terrestrial plants. Fermentations of hydroysates derived from the fast-growing *Macrocystis* spp and *Laminaria* spp.hold the greatest potential for marine macroalgal bioethanol (eg. see www.ba-lab.com).

Another substance present in great quantities in the sea is chitin which is a polysaccharide consisting of N-acetyl glucosamine monomers. Chitin is a very hard, semi-transparent substance found naturally in the exoskeletons of crabs, lobsters and shrimps. Its structure resembles that of cellulose except one hydroxyl group is replaced by an acetyl amine group. It has been described as “cellulose of the sea”and has potential for bioconversion into chemical commodities, including ethanol.

Limited production of bioethanol is also possible by processing “waste” alcoholic beverages such as beer (eg. Merrick & Co., Colorado, USA – www.ethanolproducer.com June 6, 2008). Waste baked foods (eg. bread) also have potential for bioconversion to fuel alcohol (eg. Finnish company St1 Biofuels, www.st1.eu).

A major co-product of biodiesel production is glycerol, which has the potential to be converted to ethanol by certain bacteria (see Dharmadi *et al*, 2006) and yeasts such as *Candida magnoliae, Zygosaccharomyces rouxii* and *Pachysolen tannophilus* (see www.glyfinery.net).

Municipal Solid Waste

Other forms of potentially fermentable waste materials include municipal solid waste (MSW) which is collected for disposal by urban communites in developed countries. MSW represents one of the lowest cost feedstock sources for cellulosic bioethanol production. It comprises: paper/cardboard, kitchen and vegetation organic waste and possesses a higher heating value of 12.7 MJ/dry kg. There are therefore opportunities for combined disposal and energy recovery from MSW. It has been reported (Shi et al, 2009) that >80 billion litres of MSW paper-derived cellulosic ethanol can be produced worldwide. This would result in replacing over 5% of global gasoline consumption.

Several pilot facilities are developing new routes to bioethanol from both commercial waste and biodegradeable municipal solid waste (BMSW) (see: www.biofuelstp.eu/bioethanol; www.biofuelstp.eu/spm2/pdfs/poster_PERSEO.pdf; Li and Khraisheh, 2009) and a Canadian company (GreenField Ethanol Inc., Edmonton, Alberta) is developing one of the first MSW-to-ethanol facilities. [Ethanol Producer Magazine, 1/7/08].

3.4 Feedstock processing

Important considerations for processing bioethanol feedstocks include pre-processing, pre-treatment; hydrolysis, and microbial contamination control. The following section covers salient processing features for sugar cane, maize and lignocellulose feedstocks.

3.41 Sugar crop processing

Sugar cane contains ~15% sucrose and once this is pressed from the canes following chopping and shredding is readily fermented by *Saccharomyces* yeasts. The juice can be processed either into crystalline sugar or directly fermented to ethanol, as per many industrial plants in Brazil (see Fig 3.41).

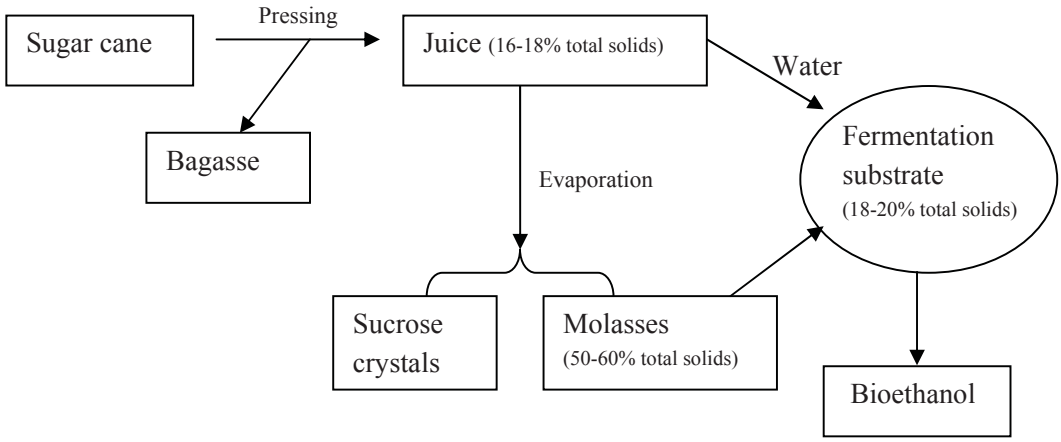

Fig 3.41 Sugar cane processing for Brazilian bioethanol fermentations

For sugar production, the juice is clarified with lime and evaporated to form crystals that are centrifuged, leaving a syrupy brown liquid by-product known as molasses. Molasses represents an almost complete fermentation medium as it comprises sugars (sucrose, glucose, fructose), minerals, vitamins, fatty acids, organic acids etc. (see Table 3.41). Additional nitrogen in the form of di-ammonium phosphate is commonly added. The more sucrose from sugarcane stalks that is removed for crystalline sugar production, the poorer the quality of molasses and some molasses contains excess levels of salts and inhibitors produced during heat treatments (furfurals, formic acid and browning reaction products). For bioethanol fermentations, molasses is diluted to 20-25% total sugar (measured in ^{o}Brix) treated with sulphuric acid and heated to 90°C for impurity removal prior to cooling, centrifugation, pH adjustment and addition of yeast.

Sugar cane juice can either be directly fermented, clarified following heat (105°C) treatment, or mixed with molasses in different proportions. Constituents in molasses that are important for bioethanol production include: sugar content: sugar % (w/w) and degrees Brix (^{o}Brix), colour, total solids, specific gravity, crude protein, free amino nitrogen, total fat, fibre, minerals, vitamins and substances toxic to yeast.

The yeast *S. cerevisiae* is the predominant microorganism employed in industrial molasses fermentations, but another yeast, *Kluyveromyces marxianus* and a bacterium, *Zymomonas mobilis*, have potential in this regard (see Senthilkumar and Gunasekaran, 2008).

Composition	Sugar cane juice g/L	Sugar cane molasses g/Kg	Sugar beet molasses g/Kg
Total solids	140-190	735-875	759-854
Total sugars	105-175	447-587	477-530
Sucrose	98-167	157-469	443-530
Reducing sugars	6-11	97-399	1.2-10
Raffinose	-	-	4.7-21
Nitrogen	0.08-0.3	0.25-1.5	1.3-2.3
Phosphorus	0.02-0.1	0.3-0.7	0.15-0.52
Potassium	0.7-1.5	19-54	15-52
Calcium	0.1-0.5	6-12	0.75-3.8
Magnesium	0.1-0.5	4-11	0.1-2.7

Table 3.41 Composition of simple-sugar based feedstocks for bioethanol production

3.42 Cereal crop processing

For processing of starch-based materials, cereal cooking, starch liquefaction and amylolysis are the main stages prior to fermentation. In North America, 2 major maize processes are differentiated: dry and wet milling (see Fig 3.42). In wet milling, maize kernels are soaked in water (or dilute acid) to separate the cereal into starch, gluten, protein, oil and fibre prior to starch conversion to ethanol. In dry milling, from which most US bioethanol is made, maize kernels are finely ground and processed without fractionation into component parts. Recent developments in both dry and wet milling have been discussed by O'Brien and Woolverton (2009).

Starch-bioethanol (from US maize) currently dominates global fuel alcohol production, but the projected use of maize for ethanol production is expected to level-off (at around 6 billion bushels) unless "idle" land can be used to grow more cereal for production of biofuels (Abbas, 2010).

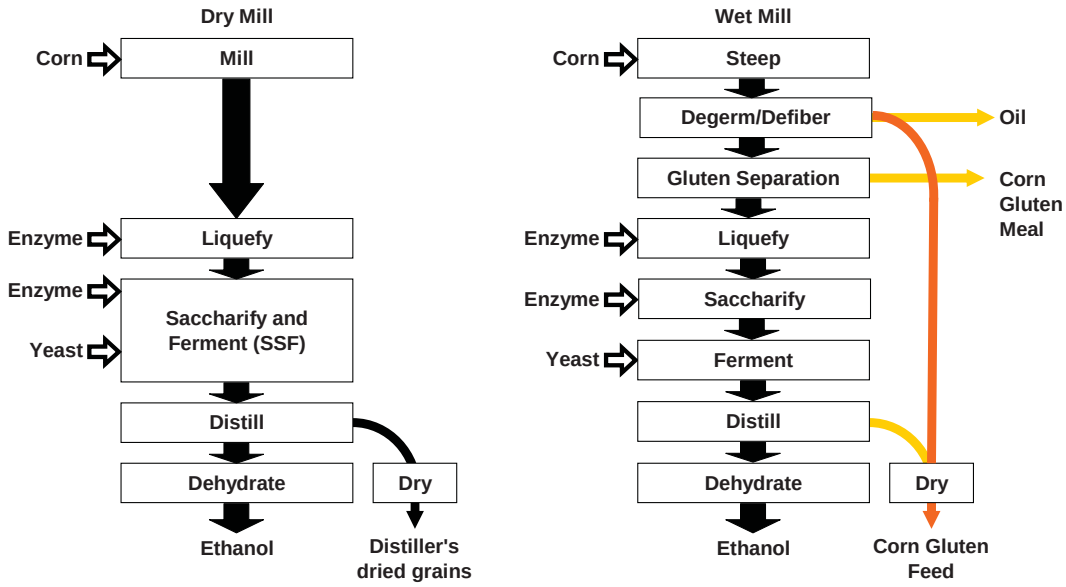

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Fig 3.42 Dry and wet milling corn processes for bioethanol

(From Abbas, 2010 reproduced with permission)

The principal stages in dry mill bioethanol processes encompass:

1. Milling (maize kernels ground to a fine powder or meal)
2. Liquefaction (water is added to the maize meal and temperature increased in the mash to solubilize starch)
3. Saccharification (enyzymatic hydrolysis of starch liberates simple sugars, mainly glucose)
4. Fermentation (starch hydrolysate is fermented by yeast to ethanol, CO_2 and secondary metabolites)
5. Distillation (the fermented wash, or beer, at around 10%v/v ethanol is distilled to ~96% v/v ethanol with the solid residues processed into animal feed)
6. Dehydration (water remaining in the ethanolic distillate is removed by molecular sieves to produce anhydrous ethanol)

Starch hydrolysis

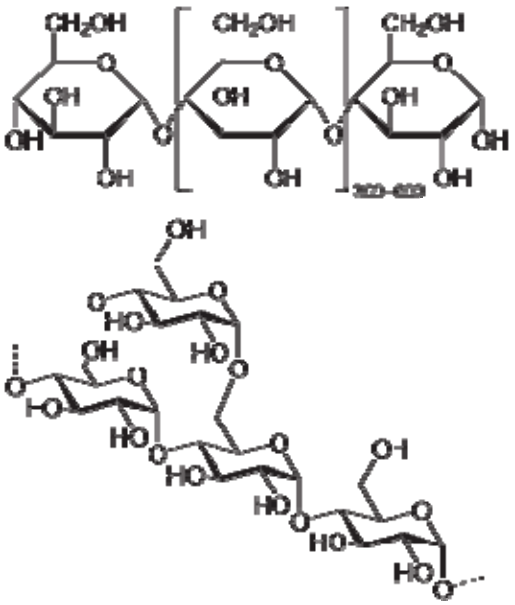
In order for starch to be converted to ethanol by yeast (*S. cerevisiae*) it has to be de-polymerised to constituent saccharides such as glucose and maltose. In traditional beverage fermentation industries such as brewing, this is partially accomplished using endogenous enzymes, mainly α- and β-amylases, present in malted barley. However, for bioethanol production, more complete starch hydrolysis is required (see Fig 3.44) and this is conducted using exogenous (microbially-derived) amylolytic enzymes including de-branching enzymes such as amyloglucosidase (or glucoamylase).

Starch is an alpha-polysaccahride comprising D-glucose monomers arranged in two basic formats: amylose and amylopectin (see Fig 3.43) and plant starches generally contain 10 to 25% amylose and 75 to 90% amylopectin (depending on the biomass source).

Industrial enzymes used as processing aids in starch-to-ethanol bioconversions (see Table 3.42) are produced by microorganisms (bacteria such as *Bacillus* spp. and fungi such as *Aspergillus* spp.) grown in closed fermentation tanks by specialist companies (eg. Novozymes, Genencor). The industrial production and purification of amylolytic enzymes for bioethanol production have been discussed by Nair *et al* (2008).

Application	Enzyme types
Liquefaction	α-and β- amylases
Saccharification	Amyloglucosidases (Glucoamylases)
Viscosity reduction	Glucanases Cellulases Xylanases

Table 3.42 Enzymes employed for starch-to-ethanol conversions



Amylose
(Glucose linked in straight-chain alpha 1,4- glycosidic bonds and enzymatically hydrolysed by α- and β-amylases to mainly maltose and glucose which are fermented by yeast)

Amylopectin
(Glucose linked in straight-chain alpha 1,4- and branched chain 1,6 glycosidic bonds enzymatically hydrolysed by α- and β-amylases and amyloglucosidase to mainly glucose which is fermented by yeast)

Fig 3.43 Structure of amylose and amylopectin

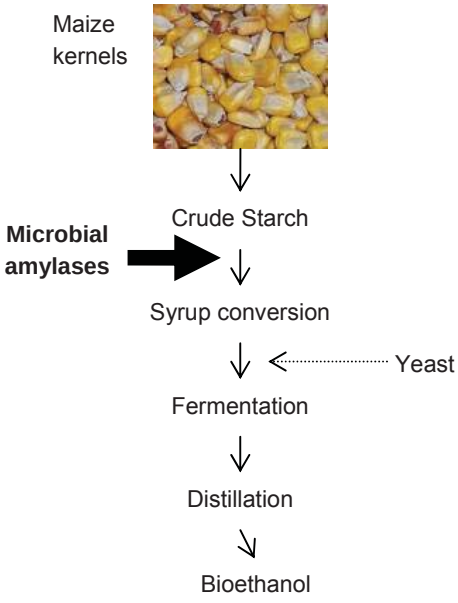


Fig 3.43 Use of amylolytic enzymes in maize bioethanol processes

Wheat-to-ethanol processes share similarities with the maize processes described above and Fig 3.44 summarises main stages taking place in a major wheat biorefinery.

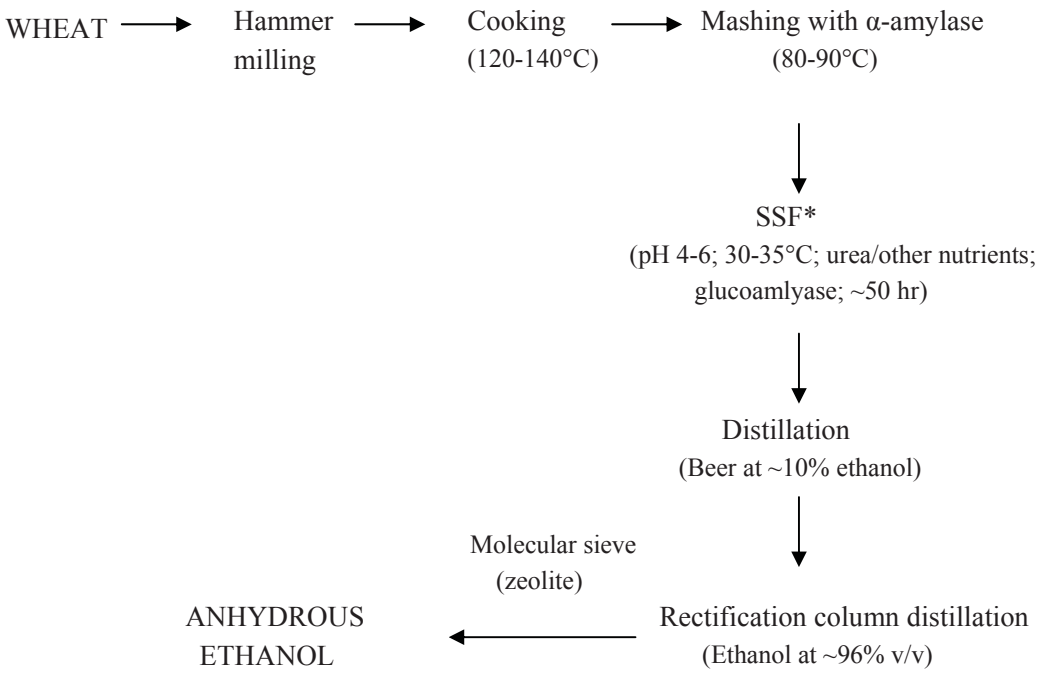


Fig 3.44 Flow diagram of a typical wheat bioethanol process

3.42 Lignocellulose processing

3.421 Lignocellulose pretreatments

For lignocellulose-based material processing for bioethanol production, more complex and demanding technology is required due to the tough, recalcitrant nature of the material compared with sugar and starch based biomass. Cellulose crystallinity, and its sheathing by hemicellulose, together with the lignin "sealant" all contribute to the recalcitrance of lignocellulosic material. After all Nature designed this material for a purpose!

The following represent the principal stages in lignocellulose-to-ethanol processes:

1. Pre-processing by mechanical removal of dirt, debris and shredding (eg. stover, straw, grasses) into smaller particles (Sokhansanj and Hess, 2009)
2. Pre-treatment (see Table 3.43, and note that a single pre-treatment method does not exist for all biomass forms)
3. Solid-liquid separation (hemicellulose sugars are separated from solid fibrous material comprising cellulose and lignin)
4. Cellulose hydrolysis (cellulase attack on crystalline cellulose to liberate glucose)
5. Fermentation (ideally of all C5 pentoses and C6 hexoses to ethanol)
6. Distillation (the fermented wash, or beer, is distilled to ~96% v/v ethanol with the solid residues comprising lignin and dead yeast combusted for energy or converted to co-products for animal feed or agronomical use)
7. Dehydration (water remaining in the ethanolic distillate is removed by molecular sieves to produce anhydrous ethanol)

Fig 3.45 outlines the basic features of lignocellulosic pre-treatment processes. Detailed consideration of pre-treatment technologies has been provided by Pandey (2010), Laxman and Lachke (2009), Alvira *et al* (2009) and Mosier *et al* (2005).

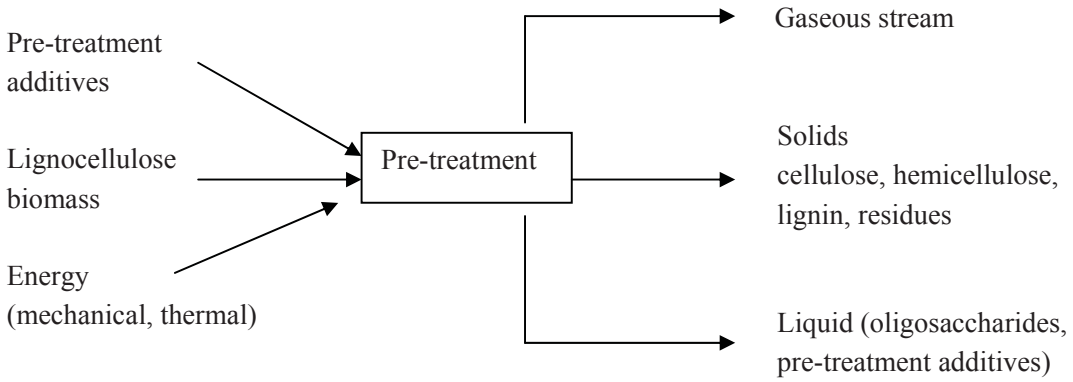


Fig 3.45 Basic features of lignocellulose pre-treatments

The following criteria are characteristics of an effective pre-treatment method:

- preservation of pentose sugars from the hemicellulosic fraction,
- limitation of lignin degradation products,
- minimisation of energy input and
- employment of low cost materials and methods.

Pre-treatments employed can be divided into physical, chemical and biological methods (see Table 3.43), but there is a strong inter-dependence of these processes. There is not a perfect pre-treatment method employed and remaining bottlenecks include generation of inhibitory chemicals (acids, furans, phenols), high particle load, high energy input and efficient separation of soluble sugars from solid residues. Specific pre-treatment conditions are required for individual feedstocks and mechanistic models can help in the rational design of such processes (Zhang *et al*, 2009). It is especially important to optimise lignocellulose pre-treatment methods because they are one of the most expensive steps in the overall conversion to bioethanol. For example, Mosier *et al* (2005) reported that pre-treatment accounts for ~30 UScents/gallon of cellulosic ethanol produced.

Fundamentally, pre-treatment methods should render cellulose more amenable to enzymolysis by disrupting its crystalline structure and in order to do this, the lignin "seal" needs to be broken.

Pre-treatment methods	Examples
Physical	Milling (mechanical comminution), microwave irradiation, ultrasound, thermal processes (pyrolysis, steam explosion), thermochemical processes (weak acid, high temperature), extrusion
Chemical	Alkali-pretreatment, ammonia fibre expansion (AFEX) technologies, organosolv (ACOS), liming, sulphur dioxide, wet oxidation, CO_2 explosion, SO_2 explosion, ozonolysis, H_2O_2 delignification, supercritical fluid and ionic liquid pre-treatments.
Biological	Microbial (eg. white-rot fungi such as *Phanerochaete chrysosporium, Trametes versicolor*) and enzymatic (eg. peroxidase and laccase) pretreatments (de-lignification).

Table 3.43 Lignocellulose pre-treatment and fractionation technologies

(Further information from Moesier *et al*, 2005 and Alvira *et al*, 2009)

By cominuting the material by physical pre-treatment the surface is enlarged proportionately to its volume and makes it more accessible for enzymes or chemicals which hydrolyze the substrate. However, this process is energy-intensive and expensive and may not always be feasible (Alvira *et al*, 2009).

Pyrolysis involves treating lignocellulosic materials to >300°C to produce gaseous product and residual char.

Ultrasound (eg. 36 KHz frequency) can be employed to pretreat wastepaper to enhance subsequent cellulolytic ability of enzymes (Ingram and Wood, 2001).

Steam explosion (or hydro-thermolysis, or autohydrolysis) is commonly employed and this involves treatment of biomass with high pressure steam (160-260°C, 0.69-4.83 MPa pressure) followed by rapid decompression to degrade hemicellulose and transform lignin, increasing cellulose hydrolysis potential.

Liquid hot water (LHW) treatments and wet oxidation (hot water plus oxygen) also involve high temperatures (eg. 200°C), but lower energy input technologies such as AFEX (ammonia fibre/freeze explosion involving impregnation with high-pressure ammonia followed by decompression – see Balan *et al*, 2009), ARP (ammonia recycling percolation – see Kim *et al*, 2009) and ACOS (acid catalyzed organosolv saccharification (cooking in aqueous alcohols, with an acid catalyst –see http://acos-biomass-refining.com/) processes are attractive.

Lime pre-treatments using calcium hydroxide with high temperature and pressure (see Mosier *et al*, 2005) selectively reduce lignin contents of biomass without affecting carbohydrate content.

Combined physico-chemical approaches include the use of concentrated hydrochloric acid (CHAP) or dilute sulphuric acid at 200°C.

Ionic liquids (eg. *n*-butyl-methy-lilidazolium chloride) which are stable liquid salts up to 300°C can solubilise cellulose within a few hours.

Other pre-treatment methods include ozonization which has been used effectively to improve enzymolysis of straws (ozone, a powerful oxidant, degrades lignin and slightly solubilises hemicellulose – see Garcia-Cubero *et al* (2009).

3.422 Lignocellulose hydrolysis and saccharification

To render lignocellulose amenable to fermentation, pretreated lignocellulose requires hydrolysis and saccharification to liberate fermentable sugars.

To maximise sugar release from pretreated lignocellulosic material, the hemicellulose fraction is subjected to mild acid hydrolysis followed by cellulolysis with enzymes. The following is an example of dilute sulphuric acid hydrolysis (for softwoods):

1. 0.7% H_2SO_4 at 190°C for 3minutes (to recover pentose sugars)
2. 0.4% H_2SO_4 at 215°C for 3minutes (for more acid-resistant cellulose)

More concentrated acid treatments (eg. 30-70% H_2SO_4) can be employed at lower temperatures (40°C), but they are more time-consuming (2-6 hours). Different approaches for acid hydrolysing lignocellulose have been discussed by Mousdale (2008) and Anish and Rao (2008). Acid hydrolysis of lignocellulose has a major disadvantage in that chemicals inhibitory to yeast in the subsequent fermentation stages are produced following degradation of sugars (see 4.6). For example, hydroxymethylfurfural (HMF) from glucose and furfural and acetic acid from xylose.

Cellulolysis using enzymes usually occurs at pH 4.8 and 45-50°C) and the enzymes are produced (by specialist enzyme companies such as Novozymes, Genencor, DSM) from bacteria (eg. *Cellulomonas fimi, Clostridium thermocellum, Bacteriodes cellulosolvens*) or fungi (eg. *Trichoderma reesei*). The following stages are involved in enzymatic degradation of cellulose:

- Adsorption of the enzyme to the cellulose surface
- Enzymatic hydrolysis of cellulose to liberate sugars
- Desoprtion of cellulose

Cellulases degrade the β-1,4-D-glucan bonds in cellulose to yield predominantly glucose, and also some cellobiose (glucose disaccharide) and cello-oligosaccharides (see Sukumaran, 2008). "Cellulase" is a collective term for 3 major types of celluloytic enzyme activity:

1. Endo-β-1,4-glucanase (expose reducing and non-reducing ends within cellulose)
2. Exoglucanases (acting on reducing and non-reducing ends of cellulose)
   - Cellodextrinases (liberating glucose)
   - Cellobiohydrolases (liberating cellobiose and cello-oligosaccharides)
3. β-Glucosidases (liberates glucose from cellobiose)

The activity of cellulase decreases during hydrolysis and this may be mitigated using surfactants (eg. Tween 80, polyoxyethylene glycol) to modify cellulose surface properties and minimise irreversible binding of the enzyme. Recycling of enzymes can increase cellulolysis and decrease costs. Cellulase activities are end-product inhibited by cellobiose and glucose and this may be reduced by: using high enzyme concentrations; supplementary β-glucosidases; ultrafiltration to remove produced sugars and SSF (see below).

Depending on the source of lignocellulose, the following enzymolysis approaches can be employed:

- SHF separate hydrolysis and fermentation (biomass pretreated with cellulase)
- SSF (simultaneous saccharification and fermentation)
- DMC (direct microbial conversion) where the fermenting microbe also produces cellulose

SSF using, for example, the fungus *Trichoderma reesei* together with the yeast *S. cerevisiae*, involve simultaneous yeast fermentation of sugars produced by fungal cellulose hydrolysis. Compromise temperatures of ~38°C are employed (between the optima for hydrolysis, 45-50°C, and fermentation, 30°C). Compared with 2-stage SHF processes, SSF has the following advantages: increased hydrolytic rates by yeast sugar utilisation to minimise cellulase inhibition; lower requirement for enzyme; lower sterility requirements; shorter times for bioprocessing and lower reactor volume since a single reactor is used. However, drawbacks include: inappropriate temperature for hydrolysis and fermentation and ethanol inhibition of enzymes (Sun & Cheng, 2002; Rudolf *et al*, 2009).

DMC approaches may encompass cellulase production, cellulose hydrolysis and fermentation in a single integrated step that has been termed *consolidated bioprocessing* (CBP – see Lynd *et al*, 2005). Chapter 4 covers lignocellulosic bioethanol fermentation aspects in more detail.

3.5 Alternative routes to ethanol

Biofuel production from lignocellulosic biomass may be achieved via two main routes:

1. Biological (as discussed in Chapters 3 and 4)
2. Thermochemical

Biomass-to-liquid (BTL) processes involving thermochemical conversions utilise pyrolysis/gasification technologies to produce "syngas" ($CO + H_2$) which acts as a progenitor for a wide range of biofuels, including bioethanol (eg. www.lanzarech.co.nz; Syntec Biofuel; Enerkem, Range Fuels; Gulf Coast Energy). For example, a Canadian facility (Enerkem Inc) has been reported to produce ethanol 360L of ethanol from woody waste using thermochemical gasification and catalytic conversion (see Ethanol Producer Magazine, January, 2009) and Range Fuels (http://www.rangefuels.com/) have reported (August 2010) production of cellulosic methanol using non-food biomass in Georgia, USA in the first phase of an operation to ultimately produce ~230 million litres of ethanol. Some anaerobic bacteria (eg. *Clostridium spp.*) can produce ethanol from syngas (eg. BRI Energy, Arkansas, USA).

Such technologies have a high demand for fossil fuel energy compared with biochemical routes to ethanol. These have been discussed in greater detail by Klass (1998) and Goyal, *et al*, 2008) and are not the focus of this book.

Regarding ethanol production from non-biomass sources, "synthetic" ethanol from petrochemical sources is well-established (eg. Pasha & Rao, 2009). For example, indirect ethylene hydration to ethanol involves a three-step process using sulphuric acid:

1. The hydrocarbon feedstock containing 35–95% ethylene is exposed to 95–98% sulphuric acid in a column reactor to form mono- and diethyl sulphate.

$CH_2=CH_2 + H_2SO_4 \rightarrow CH_3CH_2OSO_3H$

$2(CH_2=CH_2)2SO_2 + H_2SO_4 \rightarrow (CH_3CH_2O)2SO_2$

2. This is subsequently hydrolyzed with enough water to give 50–60% aqueous sulphuric acid solution:
$CH_3CH_2OSO_3H + H_2O \rightarrow CH_3CH_2OH + H_2SO_4$

3. The ethanol is then separated from the dilute sulphuric acid in a stripper column. The last step of this process is to concentrate the sulphuric acid and recycle to the process.

Direct hydration of ethylene was commercialized in 1947. In this process, an ethylene-rich gas is combined with water and passes through a fixed-bed catalyst reactor, in which ethanol is formed according to the following reaction.

$CH_2=CH_2 + H_2O \rightarrow CH_3CH_2OH$

The ethanol is then recovered in a distillation system.

Both direct and indirect hydration of ethylene gives rise to undesirable by-products such as diethyl ether, which reduce the quality of ethanol. Other processes to make ethanol synthetically are not commercially important.

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4. Fermentation aspects

4.1 Microbes for fermentation

The yeast, *Saccharomyces cerevisiae*, is the predominant industrial microorganism responsible for alcoholic fermentations. This organism, also known as baker's or brewer's yeast, is a unicellular microfungus that plays important roles in industry, the environment and medical science. It has been exploited for millennia in food and beverage fermentations and is the main "cell factory" in modern bioethanol production processes.

In addition to *S. cerevisiae*, non-*Saccharomyces* yeasts have potential in bioethanol fermentation processes and these are summarized in Table 4.1. Table 4.2 summarises some ethanologenic bacterial for use in bioethanol fermentations.

Yeast species	Characteristics	Disadvantages
Saccharomyces cerevisiae	Predominant bioethanol microbe capable of fermenting the main sugars derived from first-generation feedstocks (eg. glucose, fructose, sucrose, maltose) under large-scale industrial production conditions.	Incapable (unless genetically modified) of fermenting pentose sugars (eg. xylose, arabinose) derived from second generation lignocellulose feedstocks. Ethanol productivities of GM strains fermenting xylose are quite low 0.23-0.34 g/g sugar)
Pichia stipitis, Candida shehatae, Kluyveromyces marxianus, Pachysolen tannophilus	Non-*Saccharomyces* yeasts capable of fermenting pentose sugars (eg. xylose, arabinose) derived from second generation lignocellulose feedstocks.	Not particularly ethanol-tolerant yeasts and await exploitation for large-sclae industrial fermentation processes.
Hansenula polymorpha	High temperature xylose fermentations (Ishchuk *et al*, 2008)	Un-tested on industrial scale
Dekkera bruxellensis	"Wild" yeast found in distillery fermentations that may be capable of ethanol production under stressful conditions.	Not yet fully commercialized and awaits further research prior to industrial exploitation.
Candida krusei	Ethanolgenic yeast producing low levels of secondary fermentation metabolites such as succinic acid.	As with *D. bruxellensis*

Table 4.1 Yeasts for bioethanol fermentations

Strains	Characteristics	Typical ethanol productivity (g/g sugar)
non-GM Strains	Numerous ethanologenic bacteria are known, some of which (eg. *Zymomonas mobilis*) produce ethanol more effectively than yeast. *Klebsiella oxytoca* also has potential. May not survive the stressful environment in large-scale bioethanol plants, and ethanol productivities are generally quite low.	*Z. mobilis* 0.46 *K. oxytoca* 0.34-0.42
GM Strains (for lignocellulose hydrolysates)	*Geobacillus stearothermophilus* is a thermophile which ferments C5 and C6 sugars including short polymers at temperatures in excess of 60oC with yields ~80% theoretical maximum. It has been genetically modified to produce ethanol rather than lactate and formate (see www.tmo-group.com). Not particularly ethanol tolerant (~5% v/v). Attributes discussed by Candy (2009) *Escherichia coli* (with *Z. mobilis* genes encoding pyruvate decarboxylase and alcohol dehydrogenase) and *Erwinia chrysanthemi* (with pyruvate decarboxylase genes) also have potential	*G. stearothermophilus* 0.40 *E. coli* 0.41 *E. chrysanthemi* 0.45

Table 4.2 Candidate bacteria for bioethanol fermentations

Ethanologenic microorganisms possess the key fermentative enzyme, pyruvate decorboxylase (see 4.2), and many yeasts, but few bacteria, express this activity. *Zymomonas* spp. (*Z. mobilis* and *Z. palmae*) are the only bacteria naturally (i.e. without genetic engineering) producing ethanol as the main fermentation product under anaerobic conditions.

Microbes for lignocellulosic hydrolysate fermentations are subject to intense research activity. For example, Mousdale (2008) has listed over 30 US patents taken out in recent years for genetically engineered yeasts and bacteria that can produce ethanol from such feedstocks.

Genetic manipulation strategies with bioethanol yeasts are designed to:

- expand metabolic pathways and alleviate of metabolic blocks (expand substrate use by gene cloning, address issue of redox imbalances, eliminate/reduce or down-regulate feedback inhibition reactions, redirecting C flux through pathways to improve efficiency)
- circumvent sugar transport limitations (eg. glucose repression, new sugar transport permeases)
- overcome lignocellulosic hydrolysate toxicity
- reduce recycling of process water in fermentation make up (high gravity fermentations)

Note that some non-GM approaches to improve the fermentation performance of bioethanol yeasts exist, including:

- use of hexose and pentose-fermenting co-cultures (*S.cerevisiae* + *Pichia stipitis*)
- immobilisation technology (yeast and enzyme (eg. xylose isomerase) immobilisation)
- selection of indigenous (distillery resident) robust yeast strains
- mineral preconditioning of yeast (Mg, Zn enrichment)
- sterol pre-enrichment (pre-oxygenation, mild aeration)
- improving ethanol tolerance (by nutrient (ethanol) adapatation in chemostats)

For existing and emerging industrial bioethanol fermentations, Fig 4.1 represents the main desirable characteristics of the fermenting microbe, and most of these are currently met by the yeast, *S. cerevisiae*.

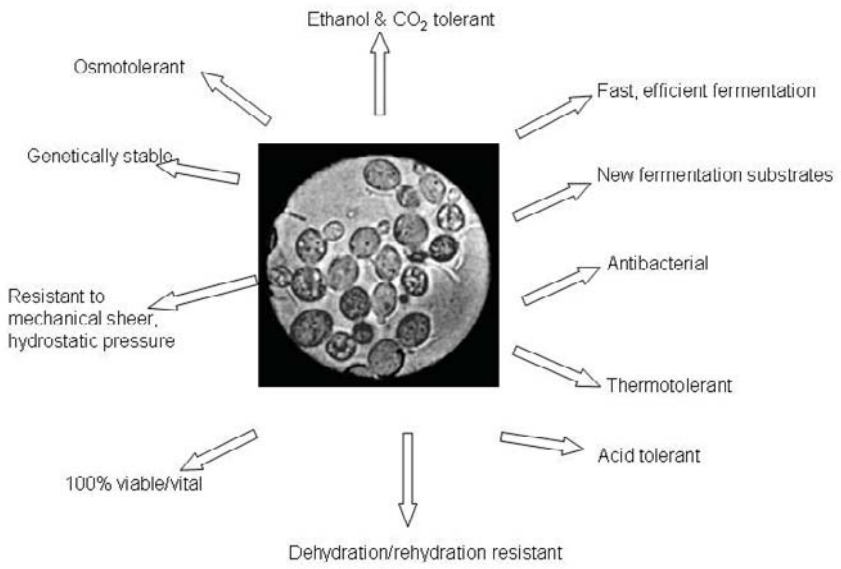


Fig 4.1 Desired attributes for bioethanol yeasts

4.2 Fermentation – theoretical aspects

Yeasts, in particular strains of *S. cerevisiae*, are the premier organisms for bioethanol production and the following section focuses on aspects of yeast physiology (nutrition, growth and metabolism) as they pertain to alcohol fermentations. (See Walker, 1998; 2009; 2010 for more information on yeasts).

In order to grow and ferment, yeast cells require a range of essential nutrients. These can be categorized as:

- macronutrients (sources of carbon, nitrogen, oxygen, sulphur, phosphorus, potassium, and magnesium) required at the millimolar level in growth media;
- micronutrients (sources of trace elements such as Ca, Cu, Fe, Mn, Zn) required at the micromolar level.

Most yeasts grow quite well in simple nutritional media, which supplies carbon and nitrogen-backbone compounds together with inorganic ions and a few growth factors. The latter are organic compounds required in very low concentrations for specific catalytic or structural roles in yeast, but are not used as energy sources. Growth factors for yeast include vitamins, which serve vital functions as components of coenzymes; purines and pyrimidines; nucleosides and nucleotides; amino acids; fatty acids; sterols; and other miscellaneous compounds (e.g., polyamines and choline).

Most yeasts thrive in warm, dilute, sugary, acidic, and aerobic environments. Industrial *S. cerevisiae* strains grow best from 20-30ºC and between pH 4.5 and 5.5. Concerning oxygen requirements, *S. cerevisiae* is not, strictly speaking, a facultative anaerobe and is generally unable to grow well under completely anaerobic conditions. This is because oxygen is needed as a growth factor for membrane biosynthesis, specifically for fatty acid (e.g., oleic acid) and sterol (e.g., ergosterol) biosynthesis.

S. cerevisiae species reproduces asexually by budding and sexually following the conjugation of cells of the opposite mating type. It is ellipsoid in shape with a large diameter of 5-10 μm and a small diameter of 1-7 μm. It is a eukaryotic microorganism that portrays the ultrastructural features similar to that of higher eukaryotic cells and possesses a nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, vacuoles, microbodies, and secretory vesicles. The growth of budding yeasts such as *S. cerevisiae* is concerned with how cells transport and assimilate nutrients and then integrate numerous component functions in the cell in order to increase in mass and eventually divide. Budding coincides with the onset of DNA synthesis, followed by localized weakening of the cell wall to allow the extrusion of the cytoplasm in an area bounded by new cell-wall material. In *S. cerevisiae*, multilateral budding is common in which daughter buds emanate from different locations on the mother cell surface. In *S. cerevisiae*, cell size at division is asymmetrical, with buds being smaller than mother cells when they separate. Under ideal (laboratory-optimised) conditions, *S. cerevisiae* can reproduce approximately every 90min, but in industrial fermenters the budding cycle takes considerably longer due to the stressful physico-chemical environment.

For alcoholic fermentations conducted by *S. cerevisiae*, the principal fermentable sugars derived from first-generation feedstocks are: sucrose, glucose and fructose (in sugarcane juice and in molasses), glucose and maltose (in cereal starch hydrolysates) and those derived from second generation feedstocks are glucose, xylose and arabinose (in lignocellulose hydrolysates). Note, however, that *S. cerevisiae* does not readily ferment the pentose (5-carbon) sugars xylose and arabinose and various microbiological and molecular genetic approaches have been adopted to enable efficient fermentation of these compounds (see 4.5).

The metabolic pathway of glucose to pyruvate is called glycolysis (or, the Embden Meyerhof Parnas pathway) and may be summarised as follows:

$$\text{Glucose} + 2\text{ADP} + 2\text{Pi}+ + 2\text{NAD}+ \rightarrow 2\text{Pyruvate} + 2\text{ATP} + 2\text{NADH}+ + 2\text{H}+$$

Where ATP = adenosine tri phosphate (biological energy)

NAD = nicotinamide adenine dinucleotide (a co-enzyme involved in biological oxidations and reductions; NAD+ is the oxidised form and NADH is the reduced form)

Pi = inorganic phosphate

In terms of chemical stoichiometry, the theoretical conversion to ethanol from glucose is as follows:

$C_6H_{12}O_6$		$2C_2H_5OH$ + $2CO_2$
Glucose	⟶	Ethanol Carbon dioxide
180kg		92kg 88kg

For each kilogram of glucose fermented, around 470g of ethanol can be produced (i.e. <50%) representing a yield of 92% of theoretical maximum. However, in industrial fermentation practice, the best yields obtainable are only around 90% of this theoretical conversion (eg. using sugarcane molasses as feedstock). This is because fermentable carbon is diverted to new yeast biomass and minor fermentation metabolites (organic acids, esters, aldehydes, fusel oils etc).

The overall glucose-to-ethanol pathway, involving fermentative enzymes, is summarised in Fig 4.2. Individual glycolytic enzyme-catalysed reactions have been omitted for clarity. In more biochemical detail, it can be stated that fermentative yeasts are able to use sugars in the absence of oxygen as electron donor, electron acceptor, and carbon source. In doing so, *S. cerevisiae* reoxidizes the reduced co-enzyme NADH to NAD+ in terminal step reactions emanating from pyruvate.

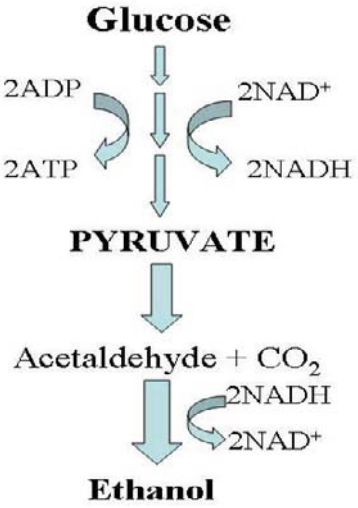
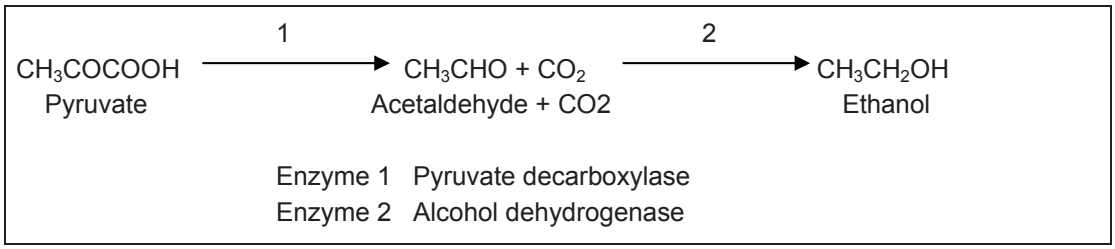


Fig 4.2 Summary of glycolysis and fermentation pathways converting glucose to ethanol

In the first of the terminal fermentative reactions, pyruvate is decarboxylated to acetaldehyde (catalyzed by pyruvate decarboxylase), which is finally reduced by alcohol dehydrogenase to ethanol as follows:



The regeneration of NAD maintains the redox balance and keeps glycolysis proceeding. In essence, the glycolytic pathway may be summarised as:

- Enzymatic oxidations/phosphorylations of glucose to yield two molecules of pyruvate (i.e. a 6 carbon sugar is split into 2 pieces of a 3 carbon compound)
- Energy is generated (2ATP)
- Oxidative processes generate reduced co-enzyme (NADH)
- NAD+ is re-generated by the terminal fermentative enzyme, alcohol dehydrogenase

Saccharomyces yeasts *Zymomonas* bacteria both convert sugars to ethanol via *homoethanol* pathways, but by different routes. *S. cerevisiae* employs the Embden-Meyerhof-Parnas pathway, whilst *Z. mobilis* emplys the *Etner-Doudoroff* pathway (Jarboe, Shanmugam and Ingram, 2009).

Sugars that are fermented by yeast are converted to ethanol and carbon dioxide as the principal metabolic products, but during alcohol fermentations (e.g., of beer, wine, distilled spirits and fuel alcohol), other fermentation metabolites, in addition to ethanol and carbon dioxide, are produced by yeast. For beverage production, these are important in the development of flavour, but for bioethanol production, their production by yeast is undesirable (due to loss of ethanol yield). These metabolites include: fusel alcohols (e.g., isoamyl alcohol); polyols (e.g., glycerol); esters (e.g., ethyl acetate); organic acids (e.g., succinate); vicinyl diketones (e.g., diacetyl); and aldehydes (e.g., acetaldehyde).

The undesirability of production of secondary fermentation metabolites (notably glycerol) in bioethanol plants leads to potential loss of ethanol and efforts are made to dissipate this. For example, simultaneous saccharification and fermentation (SSF) processes – see 4.2) prevent osmostress and this limits the production of glycerol as an undesired by-product. Construction of yeast strains with reduced glycerol production is also possible (eg. Guo *et al*, 2009). Ebert (2009) has calculated that reducing glycerol concentration at the end of fermentation by as little as 0.1% (from 1.4 to 1.3%) would produce an additional 163,600 gallons of ethanol in a 40MMGY distillery.

4.3 Fermentation – applied aspects

4.31 Fermentation systems

Regarding industrial bioethanol fermentation processes, several systems may be adopted, including batch, continuous, semi-continuous and immobilised (as summarised in Fig 4.31).

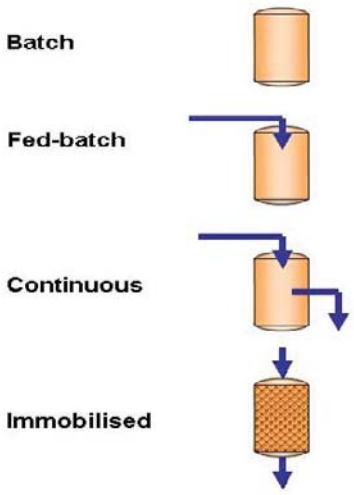


Fig 4.31 Diversity of fermentation systems for bioethanol production (idealised)

Table 4.31 outlines some of the pros and cons of the various fermentation systems available to bioethanol producers.

Fermentation system (brief description)	Advantages	Disadvantages
Batch (microbial inoculum introduced into fermentation medium and left until complete)	Large capacity. Simple, robust, traditional (eg. brewing). Ease of sterilisation and cleaning. Complete substrate conversion.	μmax (short). Unbalanced, asynchronous. Low productivity Low cell densities. Labour intensive.
Fed-batch (nutrient fed incrementally, or batch-wise, to a growing yeast culture)	Traditional (baker's yeast) and modern (therapeutic proteins). Extends exponential phase (high cell densities). Complete substrate conversion	Low μ. Unbalanced (growth rate). Labour intensive.
Continuous (nutrient fed into a growing yeast culture at a rate equal to removal of culture broth)	Steady-state system. Growth rate controlled by dilution rate [D=μ]. High productivity. Nutrient balanced (chemostat). Low labour costs and good utilisation of thereactor. Valuable research tool (eg. adaptive evolution).	Costly interruptions due to contamination and mutation of productions yeast strains.
Immobilised (cells entrapped in a polymeric matrix or immobilised on the surface of an inert support material)	High yeast concentration 10^8 - 10^9 cells/ml. Cheap support materials (eg. wood chips). Continuous operation	Un-tested on a large scale for bioethanol production.

Table 4.31 Advantages and disadvantages of different fermentation systems for bioethanol

Variations of the systems outlined in Table 4.31 are possible and one example of a semi-continuous operation is the modified Melle-Boinot system adopted in many Brazilian fuel ethanol plants (Amorim, Basso and Lopes, 2009 and section 4.5). Other systems include:

- simultaneous saccharification and fermentation (SSF)
- direct microbial conversion technologies (DMC)
- very high gravity fermentations (VHG)

4.32 Fermentation monitoring

Irrespective of the fermentation system employed, bioethanol producers seek to achieve fast and efficient conversion of available sugars to ethanol. Typical parameters monitored during fermentation include: changes in yeast cell density, sugar consumption, pH, temperature, degree of foaming and alcohol. To ensure consistency of fermentation performance, distilleries not only monitor, but also control several of these parameters, notably temperature and pH. Of particular importance are spirit yield calculations, conversion efficiencies (of sugar to ethanol) and the relationship between initial sugar concentration and final yield of ethanol. Table 4.32 provides some typical data concerning expected ethanol yields from different feedstocks.

Sugar	Ethanol Yield in Defined Media	Ethanol Yield in Corn Stover Hydrolysate	Specific Ethanol Productivity in Rich medium	Ethanol Concentration from Hydrolysate
Glucose (and other hexoses)	>90%	<80%	~2 g/g/hr	4% or less
Xylose	80%	~25%	0.2-0.5 g/g/hr	
Arabinose	60%	Unknown	~0.07 g/g/hr	

Table 4.32 Ethanol yields from sugars derived from different feedstocks

(Information from Abbas, 2007)

The predicted spirit yield (PSY) predicts how many litres of alcohol can be expected to be made from 1 tonne of cereal. It is calculated from the extract and fermentability of the cereal. This has been traditionally measured in the potable alcohol industry by lab scale process of milling, mashing, fermentation and distillation, but more recently near infra-red analysers can provide rapid predictions. Typical values for wheat would be 385-400 litres/tonne. From a more agronomical viewpoint, bioethanol feedstocks may also be ranked according to potential ethanol yields per hectare of cultivable land and the following provides some examples (Annon, 1986; Gatel & Cormack, 1986):

Sweet sorghum	4.0-6.5 tonnes ethanol/ha
Wheat	4.8 tonnes ethanol/ha
Jerusalem artichoke	4.0-4.7 tonnes ethanol/ha
Sugar beet	3.3-3.8 tonnes ethanol/ha
Chicory	2.0-3.0 tonnes ethanol/ha
Potato	2.0-2.9 tonnes ethanol/ha

4.33 Fermentation: microbiological issues

In order to maximise ethanol fermentation efficiency for bioethanol production, it is important to ensure yeast is of good viability and vitality and also to minimise levels of contaminant bacteria. Poor yeast quality and the presence of wild yeasts and lactic acid bacteria (mainly *Lactobacillus* spp.) in the fermenting medium can subtract significantly from ethanol yield.

> Every molecule of lactic acid made in a fermenter by Lactobacilli that compete with yeast for sugars means the loss of a molecule of ethanol. The importance of sterilization of fermenters, yeast mixing vessels and associated pipe-work should not be underestimated in an effort to control bacteria.

Lactic acid bacteria are sensitive to acids and many distilleries acid-wash (eg. H_2SO_4) their yeast slurries to reduce bacterial contamination. Major concerns for bioethanol plant operators relate to unwanted microbes and additional contamination control measures centre on good plant hygiene and cleanliness involving:

- use of preventative antibiotics (although many countries now have strict measures on such applications)
- chemical cleaners, sanitisers, sterilants (eg. chlorine dioxide, ammonium bifluoride, potassium bisulphite, hydrogen peroxide, hop acids), some as alternatives to antibiotics in fermenters
- heat sterilisation (of raw materials, air, water, vessels).

Regarding the nature of yeast for bioethanol fermentations, it is crucial to employ the correct strain for specific applications and to maintain the strain as a pure culture free from wild yeast and bacterial contaminants. Different distilling strains of *S. cerevisiae* are available from yeast manufacturers (eg. Fermentis, Lallemand Ethanol Technology, AB Mauri) as cream, compressed (cake) and dried preparations.

Commercially available strains of *S. cerevisiae* have now been developed that can produce ethanol at relatively high concentrations (>10%v/v), can ferment effectively in high (>20%) solids and are generally stable and robust to survive the rigours of industrial fermentations (Fig 4.32). Through careful attention to yeast nutritional physiology, it is now possible to produce over 20%v/v ethanol in high gravity wheat fermentations (Thomas and Ingledew, 1992).

Some distilleries operate yeast recycling and this circumvents the need to regularly purchase new batches of yeast from commercial suppliers. Other plants conduct their own (fairly rudimentary) yeast propagation in order to boost biomass required as starter cultures for fermentation. These propagators employ vigourous aeration to stimulate yeast growth.

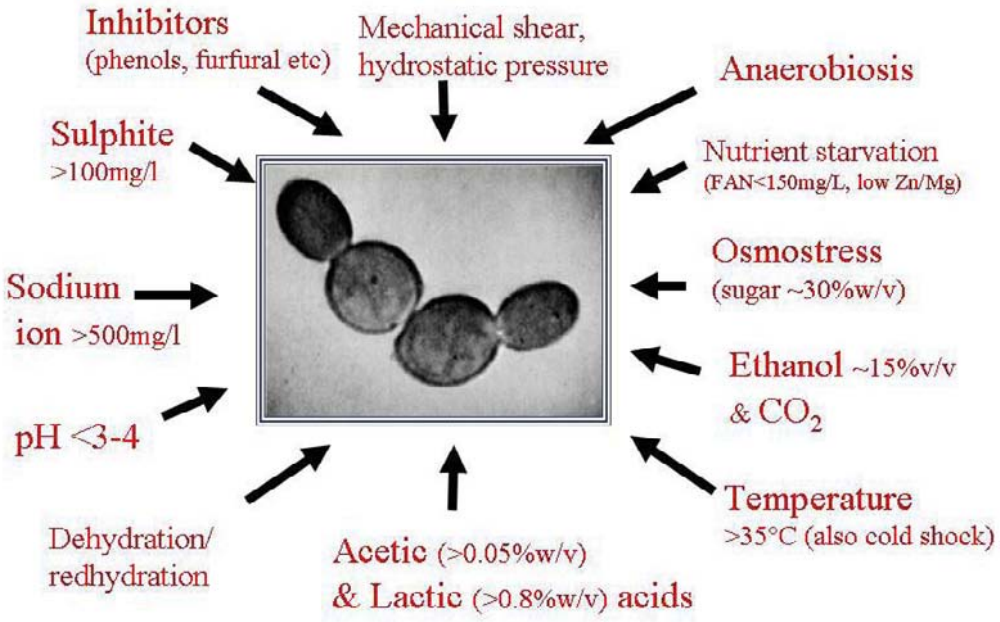


Fig 4.32 Environmental stresses experienced by bioethanol yeasts

There is a need to further develop stress-resistance in industrial yeast strains, particularly with regard to temperature and ethanol tolerance, and the ability to withstand chemical inhibitors such as those found in lignocellulose hydrolysates (acids, phenols, furans etc – see 4.6). These are topics of intense research and development, often involving genetic engineering to enable yeast cells to withstand environmental stress and overcome substrate toxicity (eg.Bettiga *et al* 2008).

4.4 Sucrose fermentations

Simple-sugar feedstocks in the form of sugar cane, sugar beet and sweet sorghum provide sugars in the form of sucrose, glucose and fructose that can be directly fermented by yeast and these crops account for around half of bioethanol produced globally. No extraneous enzymes are required to liberate sugars for yeast fermentation, as *S. cerevisiae* produces the enzyme invertase to hydrolyse sucrose into readily-fermentable glucose and fructose.

For sugar juices, ethanol yields are improved following heat treatment and clarification to reduce impurities and bacterial and wild yeast contaminants. Mixing clarified juice with molasses improves yeast nutrition and fermentation performance. In sugar cane and sugar beet refineries, a dark brown, syrupy liquid known as molasses is generated following sucrose crystallisation/centrifugation. The more sucrose that is removed, the poorer the molasses quality for alcohol fermentations, but generally speaking, molasses represents a nutritional medium for yeast (see section 3.1). Nevertheless, molasses does contain some compounds that are formed during sugar processing that can inhibit yeast activity during fermentation (potassium salts, browning reaction compounds, furfurals, formic acid etc).

Some fuel ethanol plants employ diluted molasses (20-25% total sugar) that is treated with sulphuric acid (to pH 4.5) and heated to 90°C prior to decanting for impurity removal.

Two basic fermentation systems are employed for sugar-based bioethanol production:

1. Fed-batch addition of substrate with yeast propagation
2. Fed-batch addition of substrate with yeast recycle

These systems have been described by Amorim *et al* (2009) and Monceaux (2009). In the first system, each fermenter is pitched with freshly grown yeast (to minimise bacterial contamination) followed by controlled addition of sugar substrate. In the second system, recycled acid-washed yeast is introduced to sugar-rich substrate every 12 hours or so to achieve very rapid fermentations and minimum yeast growth. The yeast recycling consists of treatment with sulphuric acid (to pH 2.2) to minimise bacterial contamination. Ethanol concentrations achievable in the latter system are 8-10% v/v. Basso *et al* (2008) have discussed the behaviour of yeasts in Brazilian fuel alcohol plants employing yeast recycling. Distillery-resident strains of yeast in such systems exhibit higher tolerances to stress compared with cultured strains and have potential as selected starter cultures for Brazilian bioethanol processes.

4.5 Starch hydrolysate fermentations

If we take bioethanol-from-maize (*Zea mays*) as an example of a starch-based process for fermentation, several key stages can be outlined. Fig 4.5 outlines a simplified production process of bioethanol from maize using the dry-grind process described in 3.1. Such processes are capable (in the US) of producing >400 litres of ethanol per tonne of maize (at 63% starch).

The following stages in this process can be summarised:

1. Maize grain milling (particle size reduction)
2. Mashing and cooking (milled maize mixed with water and heated to gelatinise starch)
3. Liquefaction (commercial α-amylase enzymes added to reduce viscosity and produce maltose and dextrins)
4. Saccharification (commercial glucoamylase added to liberate fermentable sugars from dextrins)
5. Fermentation (yeast conversion of sugars to ethanol and CO_2)
6. Distillation (ethanol concentrated to ~95%v/v)
7. Dehydration (near-anhydrous ethanol produced using molecular sieves)
8. Centrifugation (produces thin stillage and wet cake)
9. Evaporation (thin stillage concentrated to a syrup)
10. Drying (evaporated stillage and wet cake dried and mixed to 90% dry wt DDGS)

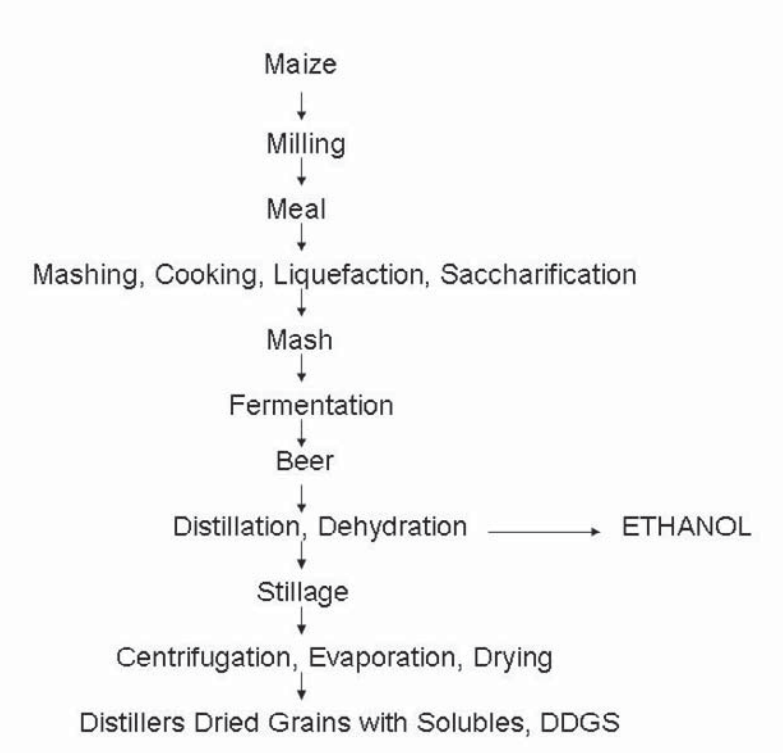


Fig 4.5 Ethanol production from maize (dry-grind process)

The maize wet-milling processes fractionate the cereal grains into starch, germ, gluten and fibre to yield a variety of products. The maize starch slurry is converted (following amylolysis using commercial enzymes) by yeast to ethanol and carbon dioxide. DDGS are also generated.

4.6 Lignocellulosic hydrolysate fermentations

Fig 4.51 outlines the general scheme for producing bioethanol from lignocellulose and Fig 4.52 summarises the SSF and SHF processes.

The following outlines the theoretical conversion of cellulose to glucose:

$$(C_6H_5O_5)n + nH_20 \longrightarrow nC_6H_{12}O_6 \longrightarrow 2n\,CH_3CH_2OH + 2nCO_2$$

Cellulose Water Glucose Ethanol Carbon dioxide

Ethanol yields (litres/dry metric ton) from the following lignocellulose sources are possible (Sassner *et al*, 2008):

Hardwood: 345 and 121 from hexose and pentose fermentation, respectively
Softwood: 426 and 59 from hexose and pentose fermentation, respectively
Corn stover: 302 and 191 from hexose and pentose fermentation, respectively
From wheat straw, yields of ~300 litres of bioethanol per ton would be expected.

In practice, such conversions are inefficient and improving the overall cellulose-to-ethanol process remains a technological challenge, for several reasons including those outlined below.

Lignocellulosic biomass from woody wastes, corn cobs/stover, switchgrass, spent grains, paper waste, municipal solid waste etc. can be pre-treated and hydrolysed (section 3.2; Dien and Bothast, 2009) to produce fermentable sugars, and cocktails of chemical inhibitors (see Fig 4.55). Such chemicals include breakdown products of sugars (furfural and hydroxymethyl furfural) as well as organic acids (namely, acetic acid from hemicellulose, formic and levulinic acid from sugar degradation) and lignin-degradation products (primarily phenolic compounds such as ferulic and coumaric acids). These compounds can act to suppress the activities of yeast (and bacteria) in converting hydrolysate sugars to ethanol. Further problems arise due to the presence of heterogeneous C5 and C6 sugar slurries derived from lignocellulosic feedstocks as these are not readily fermented by yeast (see below).

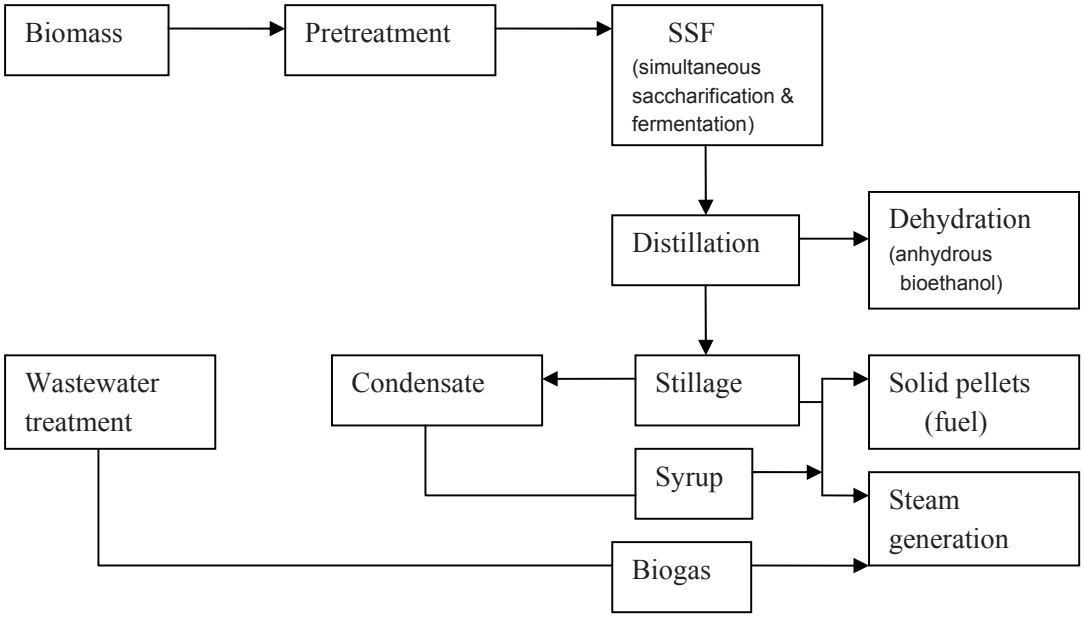


Fig 4.51 Schematic view of a generalised lignocellulose-to-bioethanol process

(adapted from Sassner *et al* 2008)

Several systems are employed in processing and fermenting lignocellulosic hudrolysates: batch, fed-batch, simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF), separate hydrolysis and fermentation (SHF), consolidated bioprocessing (CBP), drop-add, or continuous cascades. These processes frequently involve enzymatic (or microbial) hydrolysis comprising: production of cellulases and hemicellulases; hydrolysis of pre-treated biomass; fermentation of hexose (glucose, mannose, galactose) and pentose (xylose, arabinose) sugars. CBP processes, developed by Lynd and colleagues (Lynd et al 2002; 2005) permit these bioconversions to occur in a single step without the need for a production stage for cellulolytic enzymes.

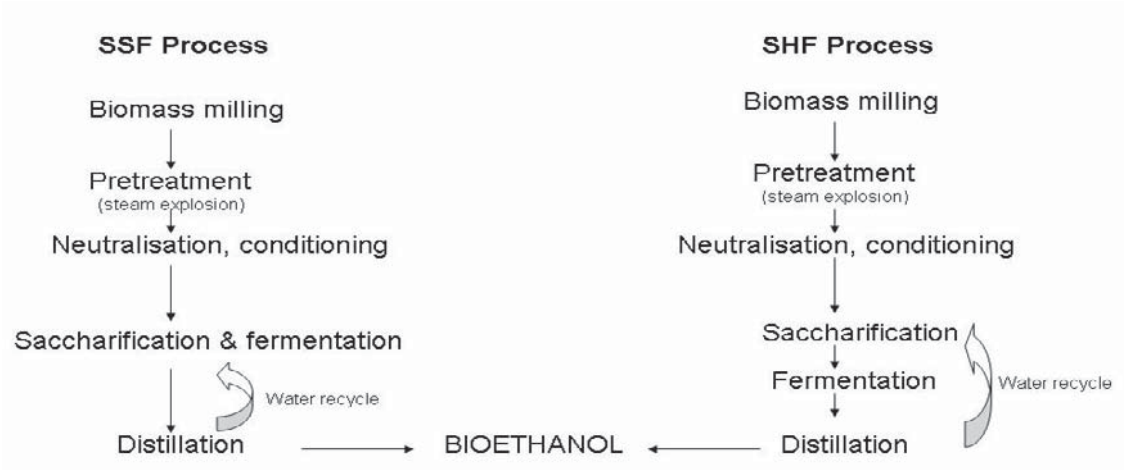


Fig 4.52 Outline of simultaneous sacchrification and fermentation (SSF) and separate hydrolysis and fermentation (SHF) processes for conversion of lignocellulose to bioethanol

When cellulose and hemicellulose polymers are hydrolysed, the resultant monomeric sugars represent a mixture of C5 (pentose) and C6 (hexose) sugars. Conventional yeasts like *Saccharomyces cerevisiae* are able to effectively ferment hexoses (mainly glucose), but are unable to metabolise pentose sugars such as xylose and arabinose. Fig 4.53 outlines the xylose fermentation pathway in microorganisms.

The XR-XDH pathway (some yeasts – see Table 4.5):

XR XDH XK
Xylose ----→ D-xylitol -----→ D-xylulose -----→ D-xylulose-5-P ---→ Ethanol

The XI pathway (bacteria and some fungi):

XI XK
Xylose ----→ D-xylulose ----→ D-xylulose-5-P -----→ Ethanol

XR=xylose reductase; XDH=xylitol dehydrogenase; XK=xylulose kinase; XI=xylose isomerase

Fig 4.53 Pathways for microbial xylose utlisation

Various approaches have been adopted to overcome this dilemma. These include:

- The use, either singularly or in co-fermentations with other C6-fermenting yeast species, of yeasts with pentose-fermenting ability. Examples of such yeasts include: *Pichia stipitis, Candida shehatae, Kluyveromyces marxianus* (see Table 4.51). These yeasts, however, are unable to ferment pentoses anaerobically;
- Genetic engineering of *S. cerevisiae* with the metabolic machinery to enable it to ferment xylose. Successful cloning of xylose isomerase genes from fungi (eg. *Piromyces*), other yeasts (eg. *Pichia stipitis)* and bacteria (eg. see Butalco GmbH, http://butalco.com/) into *S. cerevisiae* has been achieved enabling this yeast to effectively ferment xylose (see Fig 4.54 and van Maris *et al*, 2006);
- Use of genetically engineered bacteria, such as *E. coli, Zymononas, Klebsiella oxytoca, Thermoanaerobacetrium, Geobacillus* (with xylose-utilising genes).

Recombinant strains of *S.cerevisiae*
Brettanomyces naardenensis
Candida intermedia var intermedia
Candida lyxosophila
Candida shehatae var. lignosa
Candida tenuis
Cryptococcus albidus
Kluyveromyces marxianus
Pachysolen tannophilus
Pichia stipitis

Table 4.51 Xylose-fermenting yeasts

Fig 4.54 provides a schematic of the approach to engineer *S. cerevisiae* with foreign xylose isomerise (XI) genes. The expression of XI genes, rather than xylose reductase (XR) and xylitol dehydrogenase (XDH) avoids accumulation of xylitol and an imbalance of the co-factors NADPH and NAD.

Bettiga *et al* (2008); Brat *et al* (2009) and Kuyper *et al*, 2003) have discussed various molecular biological strategies for xylose fermentation of lignocellulosic hydrolysates. Several of these approaches have been successful and there are now some industrial-scale lignocellulosic bioethanol plants in operation. For example, in the US: Mascoma, Poet, Range Fuels, Verenium*, Celunol, DuPont; in Canada: Iogen and in Europe: DONG (Denmark), TMO (UK, The Netherlands).

* In August 2010, BP acquired Verenium's cellulosic biofuels business, including Jennings (San Diego, USA) facilities.

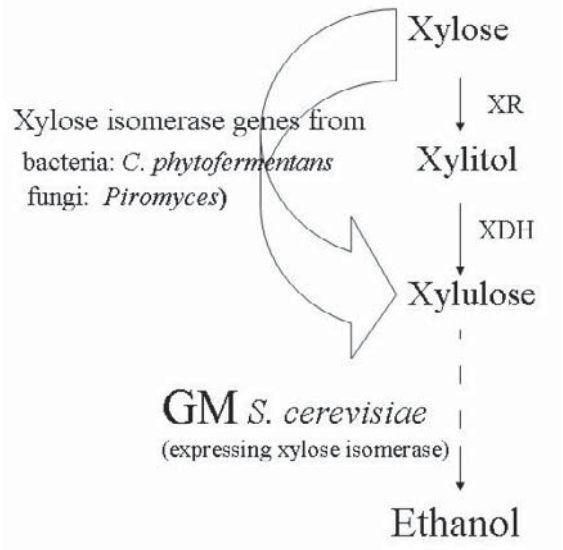


Fig 4.54 Xylose fermentation with GM *S. cerevisiae* expressing xylose isomerise

Some bacterial processes operate at high temperatures and a recombinant *Geobacillus* spp. can ferment straw hydrolysate at 70°C in a continuous fermentation (eg. www.tmo-group.com). For lignocellulosic ethanol, such thermophilic bacteria possess some key advantages over yeast-based processes, including continuous utilisation of all C5 and C6 sugars at high temperatures at a fast rate. However, some of these bacteria are not particularly ethanol tolerant (at levels >8%v/v). Table 4.52 shows some thermophilic bacteria with cellulolytic, ethanologenic properties.

Geobacillus thermoglucosidasius
Thermoanaerobacterium saccharolyticum
Thermoanaerobacter mathranii
Clostrdium thermocellum,
Clostridium thermohydrosulfuricum

Table 4.52 Some thermophilic bacteria with cellulolytic and ethanologenic characteristics

(More information from Taylor *et al* (2009); Lynd *et al* (2005); Lee *et al* (2008))

For yeast processes, significant challenges remain to engineer yeast strains for lignocellulosic hydrolysates. There are also major challenges presented in such hydrolysates due to the presence of chemicals that are toxic to the fermentative microorganisms (yeasts and bacteria). The sources of these chemicals are outlined in Fig 4.55.

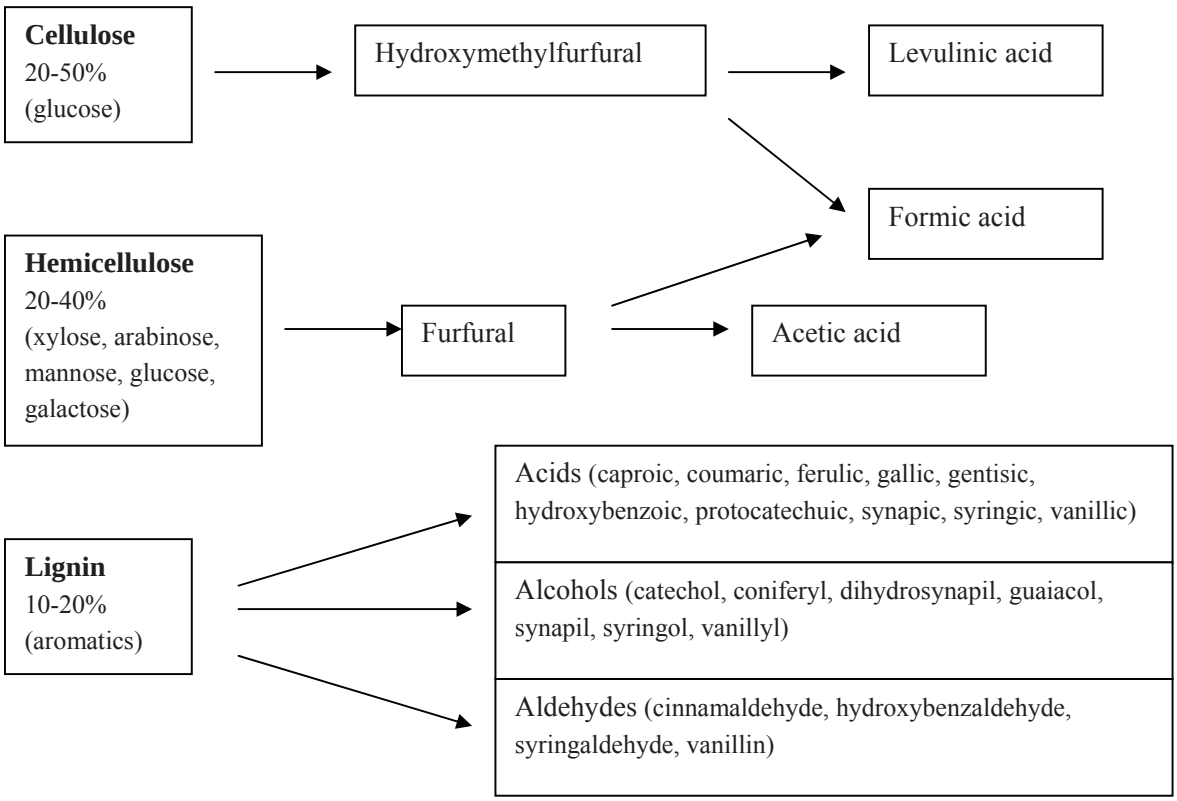


Fig 4.55 Sources of chemical inhibitors derived from acid-hydrolysis of lignocellulose

Rudolf *et al* (2009) have outlined various methods to alleviate the deleterious effect of chemical inhibitors in lignocellulosic hydrolysates. For example, "steam stripping" (see Zhu *et al*, 2009) or nanofiltration membranes (eg. Weng *et al*, 2010) or polymeric adsorbent materials (eg XAD-4 amberlite resin – see Wei *et al* 2002) can be used to selectively remove inhibitors from the soluble sugar fractions derived from biomass hydrolysates. Although modern scientific developments are undoubtedly assisting lignocellulose-to-ethanol bioconversions, it should be mentioned that fermentations of wood hydrolysates are not particularly new technologies, and there are some examples of industrial scale-plants in Europe and Siberia that have been operational for many years (eg. Borregaard in Norway - http://www.borregaard.com, a company established in 1918; and Tavda Hydroysis Plant in Russia since 1943 – see http://www.distill.com/woodhydrolysis/woodprocess.html).

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5. Distillation

5.1 Distillation technology – theoretical aspects

The recovery of ethanol from fermented media is predominantly performed by distillation. “Distillation” refers to separation of mixtures of two or more chemicals on basis of differences in their *volatility*, which is the ratio of the partial pressure to the mole fraction in the liquid. For alcohol:

$$\text{Relative Volatility } \alpha = \frac{\text{Volatility of alcohol}}{\text{Volatility of water}}$$

The detailed theory of distillation is outwith the scope of this book and the reader is referred to a classic text on the subject (Robinson and Gilliland, 1950). Basically, alcohol distillation therefore refers to the separation of ethanol from a binary alcohol-water mixture based on their different boiling points (see Fig 5.11), and there are some common fundamental principles that pertain to all alcohol distillation systems:

1. A dilute ethanolic solution is fed into the system (a column)
2. Heat (usually steam) directly enters the base of the column
3. The purified (“overhead”) product with the lower boiling point (i.e. ethanol) is vapourised
4. The higher boiling point product (i.e. dilute aqueous stillage) is received at the bottom of the column
5. A water-cooled heat exchanger condenses the alcohol vapour
6. The condensate is split into 2 streams – one is the desired product and the other is the reflux which is returned to the top of the column

The column (as in Fig 5.11) comprises a rectifying section (above the entry point for the fermented mixture) and a stripping section (below the entry point) and in this way a relatively pure ethanol overhead product and a “bottoms” stillage product can be produced. Fig 5.1 is a very simplified system and in practice the column comprises several internal structures called “trays” to permit intimate contact between rising ethanol vapour and descending liquid to facilitate their passage and separation.

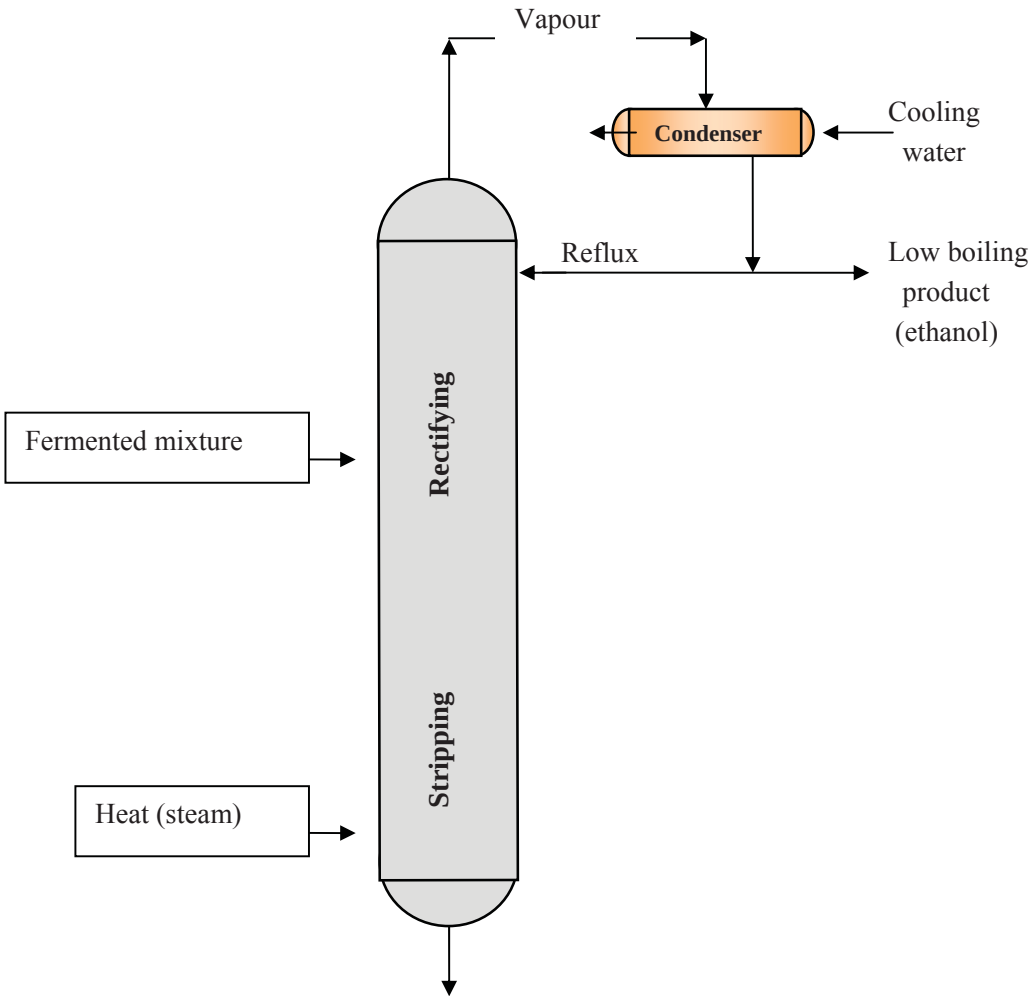

Fig 5.11 An idealised alcohol distillation column

Fig 5.12 shows the ethanol-water equilibrium at atmospheric pressure, where x is the ethanol concentration in liquid, and y in the vapour phase. The plot could also be made for mole percent ethanol (Masdon, 2009) and allows tower distillation units to be analysed by graphical techniques. For example, the 45° line (showing points at which the vapour concentration equals the liquid concentration) drawn on Fig 5.12 can be used to determine the ranges of compositions that can be separated and the distillation conditions where it is not possible to perform a separation. Where the equilibrium curve crosses the 45° line, the mixture forms an *azeotrope*. Fuel ethanol needs to be almost completely dry and simple single-stage distillation systems can never produce 100% (or *anhyrdrous*) ethanol due to the formation of constant boiling ethanol-water azeotropes. Standard distillation only produces around 96% v/v ethanol and additional approaches are needed to completely de-hydrate ethanol (see 5.3) for blending with petrol (gasoline).

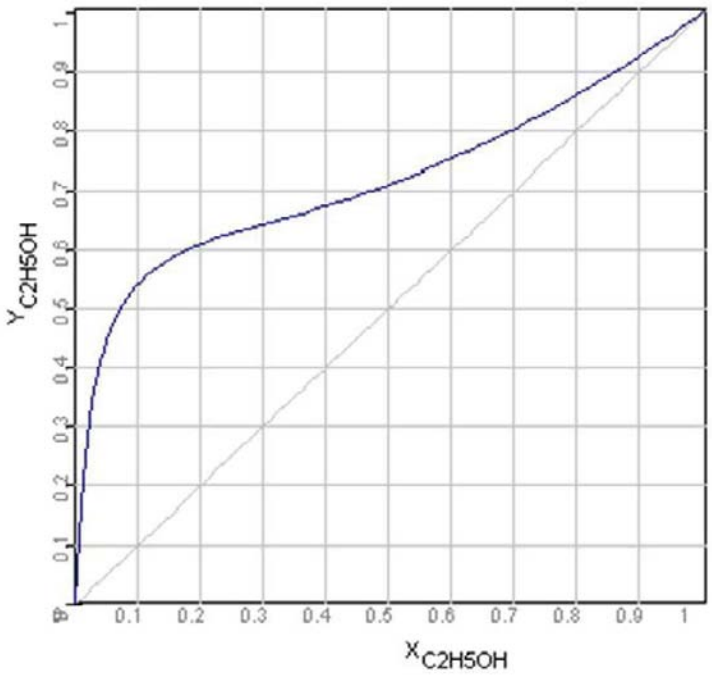

Fig 5.12 Ethanol-water equilibrium plot at atmospheric pressure

(The bold blue line represents the equilibrium curve)

(From: www.asther.de/en/help/examples/C2H5OH-H2O/index.html)

Of course, the liquid following fermentation does solely comprise ethanol and numerous yeast secondary fermentation metabolites and other compounds are also present that are distilled. Volatile chemicals present in distillates are collectively referred to as "congeners" by the potable spirits industry and comprise:

- Low volatility congeners. These are the higher alcohols which are often termed fusel oils (*e.g.* optically active amyl alcohol, iso amyl alcohol, isobutanol, propanol, 2-phenylethanol) and fatty acids (*e.g*. propionic, isobutyric, isovaleric, hexanoic, octanoic).
- Medium volatility congeners. These include esters (*e.g.* ethyl acetate, ethyl propionate, ethyl octanoate, phenylethyl acetate, ethyl palmitate)
- High volatility congeners. These include acetaldehyde, diacetyl, acetone, methanol and some sulphur compounds

The range and concentration of these volatiles will vary depending on the feedstock used for fermentation, the process conditions and the type of distillation columns employed, but Table 5.1 lists their concentrations in a typical fresh distillate of a fermented cereal mash.

Congener	Concentration (g/100L)	Volatility Range
Acetaldehyde	3.2	
Ethyl acetate	23.7	High
Diethyl acetal	1.7	
Methanol	5.1	
Propanol	40.8	
Iso-butanol	79.8	
Optically active amyl alcohol	47.7	Low
Iso-amyl alcohol	142.5	
Total higher alcohols	331.1	
Ethyl lactate	4.7	
Ethyl octanoate	1.6	
Furfural	3.3	
Ethyl decanoate	5.7	
β-phenylethyl acetate	5.7	Medium
Ethyl laurate	2.1	
β-phenylethanol	3.8	
Ethyl myristate	0.6	
Ethyl palmitate	2.7	
Ethyl palmitoleate	1.5	

Table 5.1 Analytical profile of volatile compounds in a typical cereal distillate

For both potable and fuel alcohol distillation processes, fusel oils (the higher alcohols) need to be processed to recover ethanol and a decanter can be used for their separation (and return for further rectification) from the water-alcohol stream. Fusel oil may be concentrated by a distillation column before feeding the decanter. The main fusel oil constituents (percentage by weight) are: iso-amyl alcohol 87.3%; iso-butyl alcohol 0.7%; and n-propanol 0.3%

5.2 Distillation technology – applied aspects

Various options are available for the design and optimisation of distillation systems for producing bioethanol and these have been discussed in detail by Madson (2009). Tried and tested systems are available for distilling ethanol from cereals, sugar cane juice and molasses and other fermentation feedstocks. Diverse systems incorporate various modes of batch and continuous distillation encompassing standard and multi-column stills.

The basic operation of a continuous alcohol distillation column system involves the following stages:

- Steam is sparged at the base of the column
- Dilute alcoholic liquid flows across a feed plate into the column
- Downcomer pipes permit the liquid to flow down through a series of sieve plates (trays)
- Holes in the sieve plates permit vapours to pass upwards through the column
- The stripping section below the feed plate separates the more volatile from the less volatile components
- The rectifying section above the sieve plates concentrates the more volatile components

Newer systems focus on water recycling, energy conservation and computer control systems for process optimisation. Modern technological developments for bioethanol production include vacuum distillation and pervaporation using membranes. Because distillation is the major energy-consuming stage in bioethanol production, such new technologies are sought to improve overall energy balances in modern biorefineries. Another approach is to increase the ethanol concentration in the final beer to be distilled, as exemplified by energy consumption figures in Table 5.2.

Ethanol (%v/v) in beer	Energy consumption (MJ/kg)	
	To azeotrope	To pure ethanol
5	8.5	
6	8.6	8.0
8	6.7	7.2
10	5.8	6.4

Table 5.2 Energy consumption for ethanol distillation

(Data from Morris, 1985)

5.3 Anhydrous ethanol methods

Although ethanol is completely miscible in petrol (gasoline), even small amounts of water can quickly lead to phase separation (when ethanol will absorb any water present in the system) and this in turn can lead to poor vehicle performance and potentially engine damage. Hydrated ethanol at ~96%v/v is obtained following distillation of the "beer" (fermented feedstock) is therefore de-hydrated (i.e. to produce anhydrous ethanol) for petrol-blending using various approaches outlined in Table 5.3.

Although various options are available for anhydrous ethanol recovery, the use of molecular sieves has proved successful on an industrial scale and this approach is commonplace in new bioethanol plants. The "sieving" involves the properties of synthetic zeolite resins with pore sizes small enough (0.3nm) that permit water molecules (0.28nm diameter) to penetrate, but not ethanol molecules (0.44 nm diameter). Swain (2003) has discussed operation of molecular sieves for ethanol dehydration.

Method	Description & comments
Azeotropic distillation	Addition of a solvent (eg. benzene, cyclohexane or monoethylene glycol) to break the ethanol-water azeotrope (see 5.1). When the additive is more volatile than water, separation is called azeotropic distillation, and when it is less volatile than water, it is called extractive distillation. Now seldom used due to solvent carcinogenicity/toxicity.
Molecular sieves	Examples include zeolite resins ("molsieves"), and synthetic zeolites (based on aluminium silicates) that act as desiccants to selectively adsorb water from aqueous ethanol streams Bibb Swain (2009).
Vacuum distillation	Anhydrous ethanol obtained under pressures of 10kPa.
Membrane pervaporation	The use of membranes to recover ethanol by "pervaporation" (ethanol removal by vacuum applied at the permeate side of a membrane) conserves energy by abolishing energy-expensive distillation. It is possible to concentrate ethanol from 80 to 99.5% by pervaporation ((Parisi, 1986). It can also reduce yeast ethanol (and inhibitor) toxicity problems if applied during fermentation.
Miscellaneous	eg. Liquid extraction, supercritical fluid extraction, Intermediate Heat pumps and Optimal Sidestream Return (IHOSR) technique using an inorganic salt (potassium acetate) as entrainer (see Serra *et al*, 1987)

Anhydrous bioethanol can also be used for the production of other fuel additives, such as the high-octane gasoline component bio-ETBE (1kg of which is composed of 0.4975kg ethanol and 0.5025 kg isobutylene).

5.4 Biorefinery concept

A *biorefinery* (term originally coined by Charles Abbas from ADM company in the US, and analogous to a petro-refinery) is a singular facility that produces multiple products from biomass and may be defined as follows: *"A biorefinery processes renewable agricultural feedstocks to higher value added products for use as food, feed, fuel, or fiber"* (Realff and Abbas, 2004). *Biorefining* has been defined by the International Energy Authority (IEA) as: "*The sustainable processing of biomass into a spectrum of bio-based products (food, feed, chemicals, materials) and bioenergy (biofuels, power and/or heat")*.

In the current context, a biorefinery comprises integrated biomass conversion technologies to produce, not only bioethanol, but other useful and valuable commodities including energy (see Fig 5.4). This has been discussed by Pilgrim and Wright (2009) and the range of value-added chemical commodities potentially obtainable from a bioethanol biorefinery include: cosmetics, neutraceuticals, bioplastics, solvents, herbicides etc. These represent high-value, but low-volume products (as opposed to high-volume, low-value bioethanol and DDGS). For example, corn stover residues from a corn bioethanol plant can be utilised in an additional fermentation step to produce polylactic acid (PLA), a valuable commodity in the manufacture of biodegradable films and fibres (Gruber, 2003).

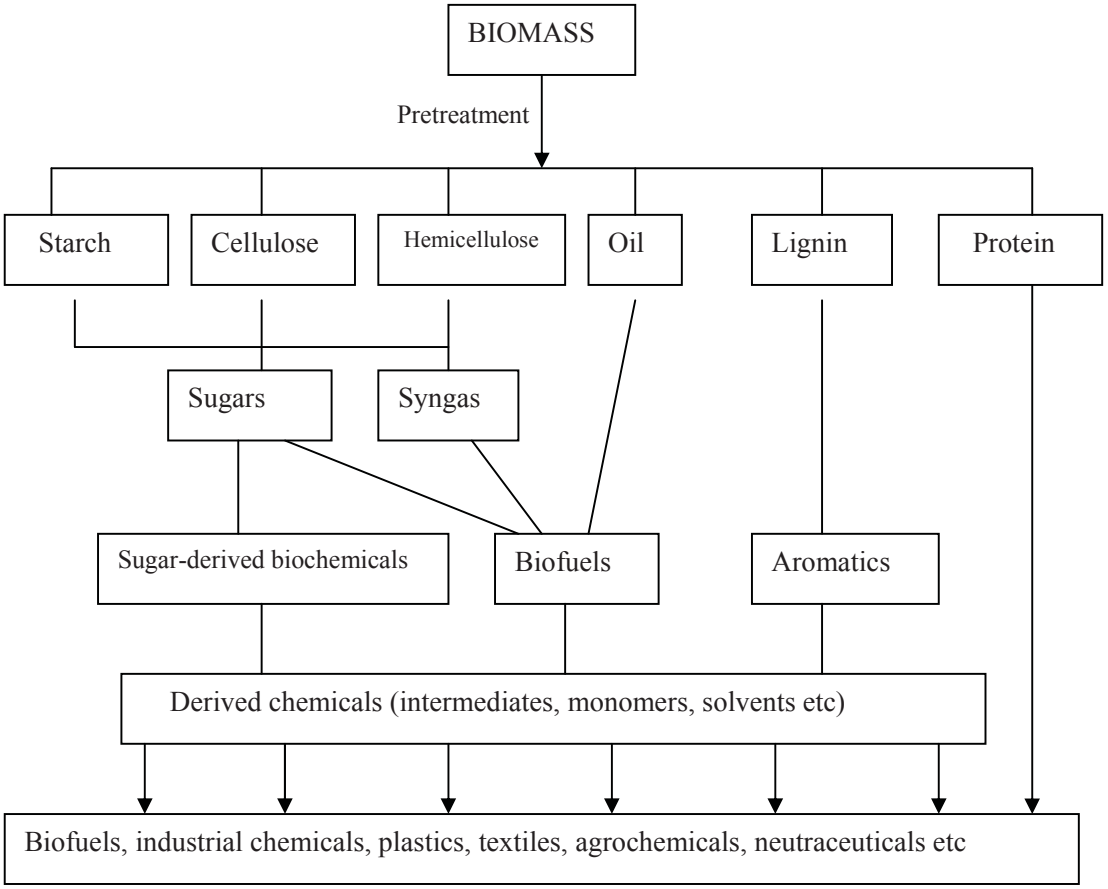

Fig 5.4 Flow diagram of a generalised biorefinery

In essence, the biorefinery concept is to exploit the whole biomass, rather than just a component of it, using chemical and biotechnologies in a sustainable manner that reduces waste and saves energy. The concept of "zero emissions" in biorefining has been discussed by Gravitis (2007).

5.5 References

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6. Bioethanol quality control

6.1 Quality parameters – process and product

Although the bioethanol industry is not regulated to the same extent as the food or pharmaceutical sectors, Ebert (2009) has discussed voluntary quality assurance models for fuel ethanol production plants, based on:

- ISO (International Organisation for Standardisation)
- HACCP (Hazard Analysis and Critical Control Points)
- USDA PVP (United States Department of Agriculture Process Verification Program)
- Six Sigma

Quality control monitoring for individual bioethanol plants, based on teamwork and accurate statistical analyses of process data, is essential to boost profitability and maintain competitiveness.

In addition to ensuring quality of bioethanol processes, quality parameters of the end product are also important. In the US, The American Society for Testing and Materials International (ASTM) approves analytical specifications for bioethanol transportation fuel performance quality (Davis, 2009). This includes the key parameters to be measured, their units of measurement and their influence on quality. For example, pH and water elimination are important parameters for internal combustion engines. The Renewable Fuels Association (RFA) recommend minimum testing frequencies and methods for bioethanol to ensure product quality and consistency and to meet ASTM standards. Table 6.1 provides an example of ASTM specification for denatured fuel ethanol and E85.

Additional aspects of bioethanol formulations and specifications for gasoline blends, are described in 6.2.

Quality parameter	**Limits for denatured fuel ethanol**	**Limits for E85**
Ethanol, %v/v min	92.1	74*
Methanol, %v/v max	0.5	0.5
Water, %v/v max	1.0	1.0
Acidity (as acetic acid), mass% (mg/L) max	0.007 (56)	0.005 (40)
pHe	6.5-9.0	6.5-9.0
Copper, mg/kg max	0.1	0.07
Inorganic chloride, mass ppm (mg/ l) max	40 (32)	1 (mg/kg)
Solvent-washed gum, mg/L max	5.0	5.0
Sulphur, mass ppm max	30	
Sulphate, mass ppm max	4	
Denaturant, %v/v	1.96 (min); 5.0 (max)	
Hydrocarbon/aliphatic ether, %v/v		17-26
Appearance	Clear and bright, visibly free of suspended or precipitated matter	Clear and bright, visibly free of suspended or precipitated matter
*plus higher alcohols		

Table 6.1 Standard ASTM specifications (2007) for denatured fuel ethanol and E85

6.2 Fuel alcohol specifications, denaturation requirements

ASTM publish standards with specifications for the following bioethanol products:

- ASTM D 4806-07 (Standard specification for denatured fuel ethanol for blending with gasolines for use as automotive spark-ignition engine fuel)
- ASTM D 5798-07 (Standard specification for fuel ethanol (Ed75-Ed85) for automotive spark-ignition engines)

These specifications are updated regularly (www.astm.org) and those for denatured bioethanol and E85 (for 2007) are presented in Table 6.1. The specification for denatured ethanol defines the acceptable and unacceptable hydrocarbon denaturants and these are also regulated by the US Alcohol and Tobacco Tax and Trade Bureau (TTB) to ensure bioethanol is unfit for human consumption. Gasoline (petrol) is an acceptable denaturant, and anhydrous ethanol may also be denatured with diethyl phthalate and isopropanol (Mansfield *et al* (1999).

In most countries, bioethanol is blended with gasoline at proportions of 2-10%, the current exception being Brazil where all gasoline used contains 20-25% ethanol (E20, E25). For blending with gasoline, ethanol requires to be anhydrous. For flex-fuel vehicles, FFVs (and those in Brazil that run on "neat" ethanol), hydrated ethanol is used.

6.3 References

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Mansfield *et al* (1999) US Patent # US005968535A).

7. Environmental aspects

7.1 Sustainability and climate change

The demand and utilisation of global energy has increased dramatically in recent times, particularly due to the rapid rate of industrialisation in developing countries (eg. India and China). Currently, this energy is being met primarily by combusting fossil fuels – over 80% of the 13TW of energy used globally. In turn, this has led to elevation of greenhouse gas (GHG) emissions (especially carbon dioxide) in the atmosphere which is causing global warming and resultant changes in our climate.

> "There is now clear scientific evidence that emissions from economic activity are causing changes to the Earth's climate" (Stern, 2007).

The production and use of biofuels such as bioethanol, at the expense of fossil fuels, contribute in a meaningful way to reducing GHG emissions. This is because the biomass feedstocks employed fix carbon dioxide photosynthetically during their growth and this leads to significant reductions in CO_2-equivalent GHG emissions compared to oil and gas combustion. Importantly in this context, the combustion of road transport fuel is currently responsible for around 20 % of GHG emissions.

The US Environmental Protection Agency (EPA) have stated that, relative to gasoline, utilisation of corn ethanol reduces GHG emissions by at least 20%, and sugar cane ethanol by an average of 61% (making this particular feedstock on a par with cellulosic ethanol emissions). In 2006, the combustion of 4.9 billion gallons of bioethanol saved ~8 million tons of CO_2, which equated to removal of 1.2 million automobiles (Pilgrim, 2009).

Significantly, cellulosic ethanol useage reduces emissions far in excess of 60% (Renewable Fuels Association, 2010). Regarding the latter, switchgrass-derived ethanol was determined by the EPA to reduce GHG emissions by 110%!

According to the Kyoto Agreement, Europe is committed to reduce carbon dioxide emissions by 8% from 2008-2012. The European Parilament's Directive 2009/30/EC provides some information on GHG emissions and savings (compared to fossil fuel combustion) of bioethanol (L 140/88 EN Official Journal of the European Union 5.6.2009). Table 7.1 provides a summary of such savings (assuming no net carbon emissions from land use change).

Under the EU Renewable Energy Directive, which was established in 2009, the 27-nation bloc was set the target of ensuring that 20% of its energy consumption came from renewable sources by 2020. The directive also required nations to ensure that renewables accounted for 10% of the energy used in the transport sector.

Biofuel production pathway	Typical greenhouse gas emission saving	Typical greenhouse gas emissions (gCO2eq/MJ)
Sugar beet ethanol	61 %	12-19 (33)*
Sugar cane ethanol	71%	14 (24)
Wheat ethanol (process fuel not specified)	32 %	23-32 (57)
Wheat ethanol (lignite as process fuel in CHP plant)	32 %	32 (57)
Wheat ethanol (natural gas as process fuel in conventional boiler)	45 %	21 (46)
Wheat ethanol (natural gas as process fuel in CHP plant)	53 %	14 (39)
Wheat ethanol (straw as process fuel in CHP plant)	69 %	1 (26)
Corn (maize) ethanol, Community produced (natural gas as process fuel in CHP plant)	56 %	15-20 (37)
Wheat straw ethanol	87%	11 (11)
Waste wood ethanol	80%	17 (17)
Farmed wood ethanol	76%	20 (20)

Table 7.1 Greenhouse gas emission savings from bioethanol usage

*Figures in parenthesis include total CO_2 emissions for cultivation, processing, transport and distribution

(Adapted from DIRECTIVE 2009/30/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 23 April 2009 amending Directive 98/70/EC)

Additional environmental and health benefits of bioethanol production include:

- Removal of toxic methyl tertiary-butyl ether (MTBE) as a gasoline oxygenate (especially in the US)
- Ethanol as an oxygenate reduces harmful exhaust pipe emissions due to more complete fuel combustion (ethanol contains 35% oxygen)
- Toxic and carcinogenic gasoline additives (eg. lead, benzene) are replaced by ethanol
- Ethanol is readily biodegradable

Although ethanol is less toxic when combusted compared with petrol or diesel (much less emissions of nitrous oxides and carbon monoxide gases and less volatile organic carbon compounds) and is more biodegradable in the environment, it should be mentioned that "clean and green" credentials of bioethanol have been questioned (eg. by the US Senate Hearing on The National Sustainable Fuels and Chemicals Act 1999). Journalists have even gone as far as describing corn ethanol production as the "Big green fuel lie" (Howden, 2007) and "Fields of dreams" (Girling, 2008).

> Products from ethanol combustion include formaldehyde and acetaldehyde (known carcinogens), may lead to increased levels of atmospheric peroxyacetylnitrate (PAN). Additionally, emissions of acetaldehyde may deleteriously affect air quality as this compound is an ozone precursor (Jacobsen et al, 2007).

Regarding the question of bioethanol being a *sustainable* industry, it is apparent that the increasing use of cereals and sugar beet crops for biofuel production is ultimately unsustainable (and unethical) due to deleterious impacts on human food security as agricultural land is diverted to biofuels. [The World Conservation Union in Switzerland have even suggested that the grain required to fill the tank of one vehicle with ethanol would be sufficient to feed one person per year.] A possible 1st-generation feedstock exception to this is sugar cane (www.iea.org), particularly in Brazil where this may be regarded as a sustainable crop.

European countries are being encouraged to set up certification schemes to ensure biofuels help cut emissions and do not threaten biodiversity. The European Palrilament and the Council of the European Union have decreed that "*Biofuel production should be sustainable*" and a relevant section in the legislation is presented in Table 7.2.

The increasing worldwide demand for biofuels, and the incentives for their use provided for in this Directive should not have the effect of encouraging the destruction of biodiverse lands. Those finite resources, recognised in various international instruments to be of value to all mankind, should be preserved. Consumers in the Community would, in addition, find it morally unacceptable that their increased use of biofuels could have the effect of destroying biodiverse lands. For these reasons, it is necessary to provide sustainability criteria ensuring that biofuels can qualify for the incentives only when it can be guaranteed that they do not originate in biodiverse areas or, in the case of areas designated for nature protection purposes or for the protection or rare, threatened or endangered ecosystems or species, the relevant competent authority demonstrates that the production of the raw material does not interfere with those purposes. The sustainability criteria should consider forest as biodiverse where it is a primary forest in accordance with the definition used by the Food and Agriculture Organisation of the United Nations (FAO) in its Global Forest Resource Assessment, which countries use worldwide to report on the extent of primary forest or where it is protected by national nature protection law. Areas where collection of non-wood forest products occurs should be included, provided the human impact is small. Other types of forests as defined by the FAO, such as modified natural forests, semi natural forests and plantations, should not be considered as primary forests. Having regard furthermore, to the highly biodiverse nature of certain grasslands, both temperate and tropical, including highly biodiverse savannahs, steppes, scrublands and prairies, biofuels made from raw materials originating in such lands should not qualify for the incentives provided for by this Directive. The Commission should establish appropriate criteria and geographical ranges to define such highly biodiverse grasslands in accordance with the best available scientific evidence and relevant international standards.

Table 7.2 Directive 2009/30/EC of the European Parliament (paragraph 11)

In August 2010, EU Energy Commissioner Gunther Oettinger announced the following 3 measures regarding biofuel sustainability:

- *'Sustainable biofuel certificates'* - governments, industry and NGOs are encouraged to establish "voluntary schemes". In order for the schemes to be recognised by the European Commision, they must be independently audited.
- *'Protecting untouched nature'* - the fuels must not be made from raw materials from tropical forests or recently deforested areas, drained peatlands, wetlands. For example, the Commission said the conversion of a forest to a palm oil plantation would not meet its sustainability criteria.
- *'Promote only biofuels with high greenhouse gas savings'* - biofuels have to deliver savings of at least 35% compared with fossil fuels, rising to 50% in 2017 and to 60% by 2018.

In the UK, the RTFO (Renewable Transport Fuel Obligation) specifies that 80% of biofuel (biodiesel and bioethanol) feedstocks should meet “environmental sustainability standards” in the year 2010/11. The RTFO’s “Sustainable Biofuel Meta-Standard” specifies certain environmental sustainability criteria under the following principles:

Principle 1 Carbon Conservation: *Biomass production will not destroy or damage large above or below ground carbon stocks*

Principle 2 Biodiversity Conservation: *Biomass production will not lead to the destruction or damage of high biodiversity areas*

Principle 3 Soil Conservation: *Biomass production does not lead to soil degradation*

Principle 4 Sustainable Water Use: *Biomass production does not lead to the contamination or depletion of water sources*

Principle 5 Air Quality: *Biomass production does not lead to air pollution*

The Meta-Standard also encompasses the following social principles:

- Biomass production does not adversely affect workers rights and working relationships
- Biomass production does not adversely affect existing land rights and community relations

However, there are currently limitations on the certification of sustainability standards for several feedstock/country combinations. For example, in the first 2010/11 RTFO reporting period, only 23% of biofuels in the UK met an environmental standard, compared to a target of 80% (Renewable Fuel Agency, August 2010).

Certified sustainable feedstocks will hopefully become more available in the future over time (as standards develop in response RTFO-led demand and general increasing concern about the sustainability of agricultural commodities).

Waste biomass and lignocellulosic materials – the second-generation feedstocks - represent the most sustainable and ethically acceptable sources for future bioethanol production. They also offer the greater cost reductions compared with starch and sugar crops for bioethanol (www.iea.org). The use of degraded/contaminated land for growth of energy crops (eg. switchgrass) for bioethanol biomass is particularly attractive in this regard and has been highlighted in the EU's Renewable Energy Directive 2009/28/EC. The use of E85 derived from switchgrass grown on abandoned/marginal cropland has even been deemed to be "carbon negative" whilst cellulosic ethanol in general leads to further GHG emissions compared with first generation feedstocks (Yan *et al*, 2010).

7.2 Energy and water conservation

The bioethanol industrial sector requires to be proactive on environmental issues, from both sociological (public perspectives) and regulatory (governmental) viewpoints (Delano, Kohl and Roddy, 2009). Particular issues relate to water and energy conservation and effective treatments of solid and liquid residues.

In addition to potentially deleterious impacts on food security, bioethanol production in certain areas of the world may compete with supplies of fresh water. For example, the US Department of Energy has estimated that for corn ethanol, 830L of fresh water is needed to produce 2.7 kg of corn from which 1L of bioethanol is produced.

Modern plants pay special attention to the principal energy and water consuming activities and employment of technologies to facilitate their reduction. Examples include:

- Biological waste treatment (eg. anaerobic digestion for biogas)
- Membrane filtration and reverse osmosis (for water recycling and removal of organics)
- Water re-use (eg. wastewater/condensate treatments to provide makeup water for fermentation)
- Hot water recovery systems (eg. from still condensers)
- Contaminated air emission controls (to reduce hazardous air pollutants)
- Electricity self-sufficiency (eg. combustion of residues and biogas)

According to a survey covering the 2001-2006 period (compiled by Argonne National Laboratories*, www.anl.gov) US bioethanol plants were able to reduce their water consumption by 26.6%, their use of electricity by 15.7%, and their total use of energy by 21.8%.

* A U.S. Department of Energy laboratory managed by University of Chicago Argonne, LLC.

Comprehensive life-cycle analyses are required to appraise the operations and environmental management of bioethanol plants. A key facet of biofuels versus fossil fuels centres on energetic favourability, and bioethanol production and consumption should be characterised by a positive energy balance (see section 1.3).

7.3 Co-products: generation and utilisation

The bioethanol industry generates a variety of so-called co-products (residues) during the processing of feedstock to ethanol (e.g. CO_2, fusel oils, cereal residues, bagasse, stillage, spent yeast etc). The main co-products from cereal (maize) bioethanol production are DDGS (distillers' dried grains with solubles) and DWG (distillers wet grains). In the US ~65% of maize residues for animal feed is DDGS and 35% is DWG. These products (in USA) are mainly used as components (up to 40%) in livestock feed (beef and dairy cattle, ~85% of consumption); but can also be incorporated in feeds for non-ruminants such as poultry (~5%) and swine (~10%). Distillers' dried grains without solubles may also be employed as animal feed, but such products are lower in protein that DDGS (Pilgrim, 2009; Corrigan and Mass, 2009). The nutritional composition of typical DDGS from a dry-grind bioethanol production process are summarized in Table 7.31.

Nutritional component	Concentration (% dry matter)
Dry matter	89
Crude protein (CP)	30
Carbohydrates	52
Fat	11
Acid hydrolysed fat	11
Fibre	7
Acid detergent fibre	14
Nitrogen-free extract	45
Ash	6
Total digestible nutrients	87

Table 7.31 Composition of DDGS (typical maize dry-grind ethanol process)

(Information from Monceaux & Kuehner, 2009)

Besides DDGS, there are a variety of other applications and potential application for co-products and other "residues" generated by bioethanol plants and these are summarised in Table 7.32.

Bioethanol feedstock	Co-product	Application/potential application
Cereals (maize, wheat)	Cereal residues (spent grains)	Animal feed (DDGS, DWG), drying and combustion, bioconversion to biofuels
	Backset (stillage) residues from distillation	Re-cycling options for mash preparation and supplements to fermentation media. Requires treatment prior to discharge to waste streams.
Sugar cane	Bagasse (sugar cane processing residues)	Combustible energy source (eg. for Brazilian bioethanol plant power, and surplus to electricity grid)
	Vinasse (stillage)	Vinasse also used as agricultural fertilizer
Suagr beet	Pulp (residue of milling process)	Fibre-rich animal feed component
Lignocellulose	Lignin (residue from lignocellulose bioconversion is~40% lignin)	Combustible energy source (formulated into dry pellets or thermally gasified to synthetic natural gas, SNG)
All	Fusel oil (higher alcohols fraction from distillation)	Chemical commodities (cosmetics, paints/inks)
	Carbon dioxide	Liquefied CO_2 for carbonated drinks, and use in greenhouses. Carbon sequestration technologies
	Spent yeast	Animal feeds (directly and incorporation with other co-products)

Table 7.32 Examples of co-product utilisation from bioethanol production processes

Regarding another major co-product from bioethanol production plants, namely carbon dioxide, this may be "scrubbed" (further purified) prior to liquefaction and sold for carbonated beverages or for use in greenhouses. Large-scale bioethanol facilities (eg. ADM, Decatur, USA) may employ carbon sequestration technology to mitigate CO_2 emissions. This involves injection of CO_2 into deep-drilled porous sandstone. Another application involves utilisation of fermentation-derived CO_2 in photosynthetic microalgal bioreactors for biodiesel production. Development of CO_2 sequestration technologies can be encouraged either by taxing C-pollution, or positive trading of C-credits (eg. providing a market value for CO_2).

7.4 Effluent treatment and control

Wastewaters from bioethanol plants with high biological oxygen demands (BOD) cannot be discharged directly into watercourses due to detrimental impacts on aquatic flora and fauna.

BOD tests measure the concentration of biodegradable organic matter in a sample of water and gauges water quality, or its polluting "power". COD is chemical oxygen demand and measures all the organic compounds in a water sample that can be oxidised to form CO_2 (expressed as mg/L) which indicates the mass of oxygen consumed per litre of water.

Both BOD and COD values for distillery wastewaters are required by local authorities and can be used to inform the design of discharge treatment facilities. Stillage (residue from distilling operations) is particularly polluting due to its: high BOD; dissolved solids content (5-10%); low pH (being acidic in nature). Evaporation of thin stillage concentrates it to a syrupy thick stillage that can be added to spent grains (as in DDGS) to augment protein content of animal feed. Evaporator condensate from this process is still high in BOD and requires further treatment, and together with other wastewater streams can be treated using biological and membrane systems. A detailed description of the science and technology of such systems is outwith the scope of this book, but AD processes for bioethanol wastewaters is summarised in Fig 7.5.

Biological wastewater treatment encompasses both aerobic and anaerobic microbiological strategies to reduce pollution. The latter process, anaerobic digestion (AD), also provides energy in the form of biogas (methane). For example, as well as reducing the soluble COD by >90% AD systems can also produce 0.35m^3 CH_4/kg soluble COD. Conventional slurry AD systems can take a significant amount of solids but are slow to operate (taking days or weeks). Additionally, 1/3 of original solids left as digestate (or biosolids) need to be disposed off. High-rate systems operate faster (hours and days) and require that suspended solids concentrations are <1000mg/l. These produce smaller amounts of biosolids.

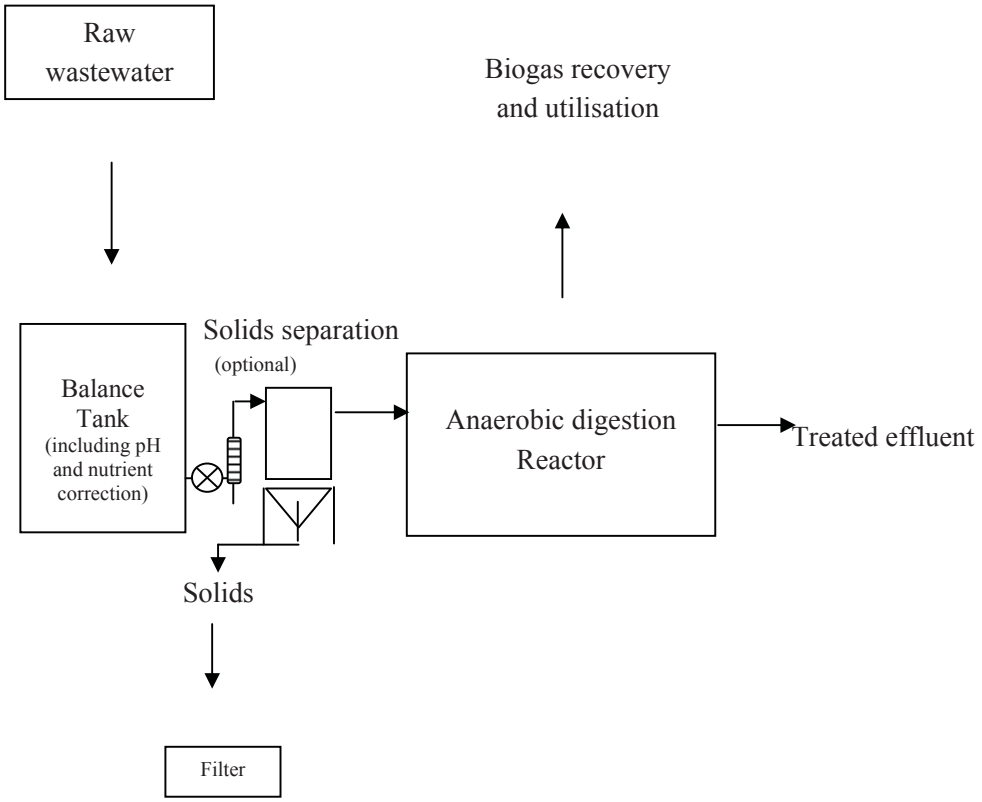

Fig 7.5 Basic flow diagram of anaerobic digestion processes to treat wastewater from bioethanol plants

(From J Akunna, personal communication)

The resultant biogas can be used as follows:

- Heating of anaerobic digester, useful in small weak influent systems, i.e. operating on spent lees and steep water
- Direct firing in existing boiler, modifications to burner required.
- Reciprocating gas engine, robust and site maintainable technology, production of electricity and heat
- Support fuel for biomass boiler
- Gas Turbine, external maintenance support generally required, electricity and useful heat in exhaust gases

Biogas may have relatively high concentrations of H_2S, hydrogen sulphide, which at levels >1% is toxic, corrosive and malodorous. H_2S levels can be controlled using iron sponge (which forms stable iron sulphide) or by chemically scrubbing with NaOH. Biological desulphurication treatments are also possible.

Depending on the receiving waters, more extensive treatments following anaerobic digestion may be required, particularly to control ammoniacal nitrogen which is toxic to aquatic life. For example, aerobic bacterial nitrification to convert ammonia to nitrite, followed by further oxidation to nitrate.

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8. Future prospects for Bioethanol

8.1 Global trends and issues

In 2008, global production of liquid biofuels (bioethanol and biodiesel) was ~87 gigalitres which equates to almost the total volume of liquid fuel consumed by Germany that year (Sommerville *et al*, 2010).

Looking to the future, the US Energy Information Administration (EIA) have forecast that renewable fuels may account for 8.5% of global energy use by 2030. Worldwide bioethanol production, research and development continues apace, with predictions (eg. Walter *et al*, 2008) of this particular biofuel replacing 20% of gasoline usage by 2030 (total 566 Gl). Current production is still heavily dominated by US and Brazilian bioethanol from maize and sugarcane, respectively, but Asian bioethanol is growing rapidly. However, increased production from starch and sugar feedstocks is unsustainable due to socio-economic factors including public awareness on issues such as food-to-fuel ethics, the rising cost of cereals and diminishing biodiversity.

The International Energy Agency (IEA) have stated that starch and sugar beet crops have limited ability to act as oil substitutes and climate change mitigators (www.iea.org and Sims et al, 2008). Additionally, it may be argued (with the exception of sugar cane processes in Brazil) that 1st-generation bioethanol is faced with severe economic and environmental constraints, including:

- contribution to higher food prices (by competing with food crops)
- production is not cost-effective (without government subsidies)
- limited GHG reduction benefits
- dubious sustainability criteria
- potential negative impacts on biodiversity
- competition for scarce water resources

It is therefore apparent that non-food/feed biomass (especially lignocellulosic residues and biowatses) needs to be exploited further to meet future rising global demand for fuel alcohol (eg. Royal Society of Chemistry, 2007; Sommerville *et al*, 2010; Pilgrim and Wright, 2009). Full industrial exploitation of cellulose-to-ethanol conversion technologies that are economically and energetically feasible are now a reality following successful operation of pilot scale/demonstration facilities, especially in the US – for example, Abengoa Bioenergy Biomass (Kansas); Ceres Inc (Califronia); BBI BioVentures (Colorado); Coskata (Florida); Mascoma (New York); Poet (Iowa and South Dakota); Range Fuels (Georgia); Verenium (Louisiana). Recent further developments in lignocellulosic biomass pre-treatment and fermentation will bring second generation bioethanol processes closer to commercial reality on a global scale (see http://biofuels.abc-energy.at/demoplants; Burkheisser, 2009). European industrial initiatives include Inbicon (Denmark); BioGasol (Denmark); Abengoa (Spain); TMO (UK and The Netherlands). Several European Commission funded collaborative research projects are focusing on novel bioprocesses for cellulose-to-ethanol conversions (see www.biofuelstp.eu/cell_ethanol/html).

Displacing 20% of gasoline by 2030 will necessitate significant increases in volumetric bioethanol production from lignocellulosic materials, as well as fostering biofuel technologies in developing countries and enhancing international biotrade. In the future, bioethanol plants that use cereal starch or sugar can be adapted to biorefineries that process the entire biomass, including lignocellulosic residues, by integrating both first and second generation ethanol technologies. Table 8.1 summarises projected (until the year 2020) bioethanol production from 1st and 2nd generation biomass feedstocks in different countries. The economic impact of increased bioethanol from lignocellulose is apparent from the US where this market is expected to grow from 125M Euros in 2010 to 13,000M Euros in 2020.

	Million litres			
	2009	2010	2015	2020
1st generation bioethanol				
USA Brazil Other Global	39743 28300 6319 74361	45420 31700 6729 83849	56775* 48700 9220 114695	56775 67041 12632 136448
2nd generation bioethanol				
USA		379	11355	39743

Table 8.1 Projected bioethanol production from 1st and 2nd generation feedstocks

*1st generation ethanol in the US has been capped at 56775 million litres per year from 2015 onwards

[Information from Renewable Fuels Association (RFA); US Department of Energy; www.i-sis.org.uk/BiofuelRepublicBrazil.php; Ethanol Producer Magazine and Chicago Board of Trade; USDA ERS (2008)]

Fig 8.1 provides some projected data for the year 2022 for bioethanol production from US maize (ultising both starch and lignocellulosic components). Data in Fig 8.1 have assumed that: corn residues can be effectively bioprocessed to ethanol; 50% stover can be collected; sugar-to-ethanol is close to theoretical conversion; and 25billion bushel corn crop (300 bushels/acre) can be harvested. It also compares whole corn ethanol with potential yields obtainable from energy crops.

Abbas (2010) has estimated that to replace all US transportation fuels with ethanol, over 800billion litres would be required and if this was to come from first-generation feedstocks (maize) then this would necessitate 500million acres of cultivable land. As this currently stands at 473 million acres, it is apparent that the future lies with second-generation feedstocks, and especially lignocellulosic wastes and energy crops. The Biotechnology Industry Organisation (BIO) in the US have predicted that cellulosic ethanol will reach "technology maturity" beyond 2020. Projecting even further ahead, it has been estimated that US and world cellulosic bioethanol production could reach 178 and 203 Gl, respectively, by 2030 (US Department of Energy and International Energy Authority).

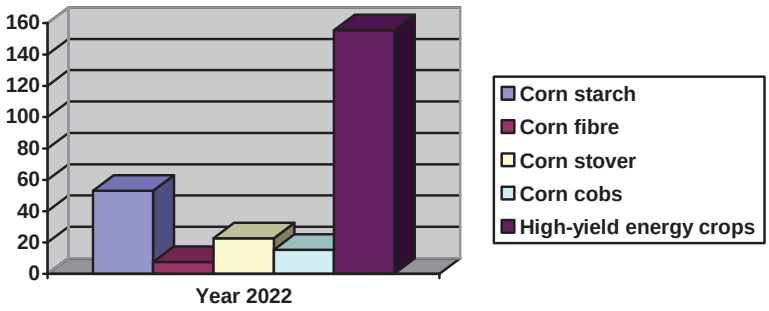

Fig 8.1 Year 2022 projections for bioethanol from US maize

(From C. Abbas, personal communication)

Somerville (2010) and his colleagues at the University of California at Berkeley have recently determined that if sugar cane bagasse-to-ethanol conversion technologies become more readily available, Brazil could potentially produce up to 750billion litres of bioethanol (by comparison current first-generation Brazilian sugar cane ethanol production is ~30billion litres). This represents a substantial proportion of global transportation fuels

In the US, it has been predicted that cultivating energy crops such as Miscanthus on less than half of the land currently under the Conservation Reserve Program would be sufficient to meet the 136billion litres of biofuels mandated in the US by 2022.

8.2 Future challenges

It is clear that bioethanol offers great benefits for safeguarding the environment, boosting the rural economy and ensuring fuel security. Nevertheless, there are significant scientific, technological, sociological and political challenges facing future bioethanol production. Increasing ethanol's worldwide share of gasoline consumption to ~20% by 2030 will require industrialisation of second-generation (cellulosic) technologies and the challenges to be faced in meeting such targets have been summarised in Table 8.2. Several of these challenges are being overcome and the pending implementation of improved lignocellulose-to-ethanol technologies will provide opportunities to use new biofuel feedstocks that reach beyond current crops - and avoid encroachment of agricultural land that is currently used for food.

Area presenting scientific challenges	**Potential solutions** (some examples)
Geo-political	Fostering production in developing countries and enhancing international ethanol trade
Biomass - high lignin composition	Genetic engineering to reduce lignin content. Ultratsructural and molecular-level understanding of plant cell walls
- cellulose depolymerisation	Basic understanding of cellulolysis (and role of cellulosomes). Novel low-energy pretreatments (eg. ultrasonics) and novel cellulase and arabinoxylanase enzymes
- enzyme expression	eg. GM maize expressing thermostable enzymes (eg. α-amylase genes from *Thermococcales* bacteria)
- stress-tolerance	Genetic engineering for drought-resistance in crops etc [See Padgette (2008); Moeller & Wang (2008) and Torney *et al* (2007) for further information]
Fermentation - incomplete conversion of available sugars	Xylose fermenting yeasts or bacteria Novel SSF (e.g. using *Thermoanaerobacterium saccharolyticum*) Modelling and omics analysis (strain/pathway engineering – see Nevoigt, 2008)
- new, robust yeasts	Yeast metabolic engineering (see below)* Alcohol fermentations of high-gravity mashes to consistently >20%v/v
Distillation (lowering energy input)	Imporved membrane pervaporation technologies
Effiecient biorefineries	Flexible lignocellulosic biofuel refineries (ethanol and butanol, together with high-value chemical commodities)

Table 8.2 Examples of second-generation bioethanol challenges

*Major advances in metabolic engineering of yeast cells have been made in recent years, particularly with regard to conferring the following properties on *S. cerevisiae* for bioethanol (and potentially also biobutanol) production:

- Expressing xylose (& arabinose) fermenting enzymes
- Expressing amylolytic & cellulolytic enzymes
- Reduced glucose-repression
- Reduced glycerol, xylitol & arabitol biosynthesis
- Acetic acid as electron acceptor (no glycerol, more ethanol – Medina *et al*, 2010)
- Stress & inhibitor tolerance (Alper *et al*, 2006; Nevoigt, 2008)
- Metabolic engineering for n-butanol fermentation (Steen *et al*, 2008)

Nevertheless, although such engineered strains perform well under laboratory conditions, successful scale-up to more stressful industrial conditions is fraught with difficulties, and further research is required regarding development of robust yeasts that can survive and thrive in large-scale fermenters.

Major challenges in efficiently converting lignocellulose to ethanol are being addressed globally by intensive research in academia and industry. In Europe, the EBTP (European Biofuels Technology Platform) is engaging with industrialists, researchers and policy makers to map out a strategic research agenda for deployment of sustainable biofuels in the EU (www.biofuelstp.eu). Recent EU Directives (Renewable Energy Directive 2009/28/EC) have specifically stipulated the usage of non-food cellulosic and lignocellulosic material for biofuel production and this awaits Member State Implementation into national legislation by December 2010. It is apparent that global production of cellulosic ethanol will not become fully commercialised without governmental support.

> In addition to considering large-scale industrial bioethanol production, the future may also incorporate small-scale production units, including “community bioferineries” and even “do-it-yourself” micro-refinery units. An example of the latter is the E-Fuel 100 Microfueler launched in the US by E-Fuel Corp in 2008. Such portable devices require yeast and a fermentable feedstock (sugar, waste beer, cellulosic waste) and following fermentation, ethanol is produced using solid-state distillation technology (see www.efuel100.com and Baker, 2010). In the US, federal law permits individuals to produce and use E100 in their own vehicles up to 10,000 gal per year.

In addition to scientific and technological challenges facing future bioethanol production, there are also important geo-political and ethical challenges to be overcome. Regarding the former, potential solutions lie in fostering production in developing countries and enhancing international ethanol trade.

The following represent the most important ethical challenges raised by increasing future bioethanol production:

- Economics (affordability)
- Food-to-fuel (changes in agricultural land use)
- Genetic engineering (employment of GM-feedstocks)
- Local environment (localisation/building of new biorefineries; demands on fresh water)
- Bio-business (potential monopolisation of bio-resources or patents)

The land use issue is a controversial one, and there is widespread misconception that the huge Brazilian bioethanol industry is leading to Amazonian rainforest destruction. However, the vast majority of Brazilian cultivation of sugarcane (and the bioethanol distilleries) are located in Sao Paulo state, using degraded pasture land. Therefore, sugar cane-derived ethanol in Brazil is not linked to deforestation and represents the most sustainable and perhaps the most ethical biofuel currently being produced. Globally, biofuels account for a small proportion (<3%) of total cultivated land (USDA-ERS, 2008).

Concerning the impacts of biofuels on human food security, and food-to-fuel ethics, **a** recent World Bank report *'Placing the 2006/08 Commodity Price Boom in Perspective'* (see Biofuel and Industrial News Issue 39 - 19 Aug 2010) concluded that the impact of biofuels on food prices was not as large as initially thought.

Nevertheless, emerging second-generation bioethanol (especially from biowastes) may be regarded as being the most ethically acceptable. Nowadays, biofuels have a bad press, meaning that scientists, industrialists and policy makers need to more clearly communicate benefits of renewable transport fuels, and openly discuss ethical issues, to wider audiences (including environmental pressure groups).

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9. Further reading

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10. Notes

1 hectare (ha) = 2.47105 acres

1 US gallon = 3.7854 liters

1 US gal of ethanol has 0.655 energy content of gasoline

1 metric ton= 39.4 million bushels

1 US ton = 0.907 tonne