State of the Art and Future Trends of Bioethanol Production

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ABSTRACT

With efforts to reduce global reliance on fossil fuels and lower the greenhouse gas emission, an increasing search for renewably sourced materials, which can be used as feedstock for biofuel production, is ongoing in the past few decades. At the present, ethanol is the most common alternate fuel and is already produced on a fair scale, representing a sustainable substitute for gasoline in passenger cars. Basically, in Brazil ethanol is produced through the fermentation of sugar cane molasses. In the United States ethanol is produced by fermenting starch crops that have been converted into simple sugars, and the major feedstock for this fuel is corn. Various countries have been increasing their ethanol production as well, such as India (using sugar cane), Thailand (cassava), France (sugar beet), China (corn) and Canada (wheat), among others. Though these agricultural commodities are major issues for both food and fuel economies, they are likely to be insufficient in the near future, presenting great challenges for food processors and biofuels producers in the 21st century. Alternatively, the conversion of cellulosic material into ethanol is relatively low up to date, compared to sugar or starch crops, leading the need to develop fermentation processes that can convert energy crops, such as grasses, and agricultural by-products, such as straw and corn stover, into bioethanol, allowing high conversion of both hexoses and the difficult to ferment pentoses into ethanol at high yields. Therefore, the search for technological breakthrough is on the high, aiming to develop technologies for effectively converting agricultural and forestry lignocellulosic residues to fermentable sugars.

CONTENTS

INTRODUCTION

In the past, essentially fossil fuels were used for transportation, relying on the abundant and less expensive petroleum supply by then, but in last few decades due to the increasingly expensive petroleum supply there has been considerable interest in the development of fuels generated from renewable resources, that is to say biofuels.

The term biofuel is attributed to any alternative fuel that derives from organic material, such as energy crops (corn, wheat, sugar cane, sugar beet, cassava, among others), crop residues (e.g. rice straw, rice husk, corn stover, corn cobs) or waste biomass (for instance, food waste, livestock waste, paper waste, construction-derived wood residues and others).

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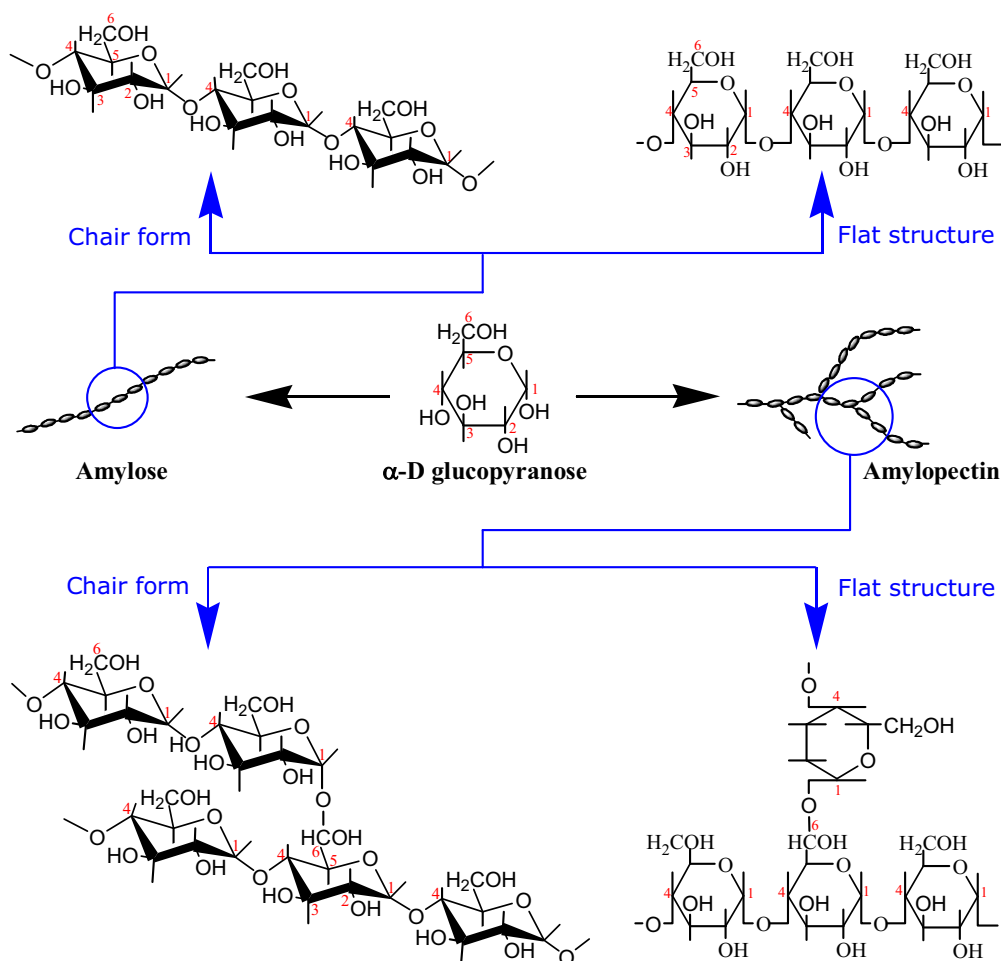
Fig. 1 Scheme representing the linear and branched starch building polymers.

Of all biofuels, ethanol has been trusted as an alternate fuel for the future and is already produced on a fair scale (about 14-26 million tons) worldwide. The bulk of the production is located in Brazil (16 billion liters produced in 2005) (ANP 2007), and the USA (10.6 billion liters in 2003) (Hamelinck *et al*. 2005). In this sense, bioethanol is expected to be one of the dominating renewable biofuels in the transport sector within the coming 20 years (Hägerdal *et al*. 2006).

Ethanol is a flexible transportation fuel that can be used in anhydrous form at 99.6 Gay Lussac (GL) as a blending agent in ethanol-gasoline blends, either directly or indirectly (i.e. in the form of ethyl tert-buthyl ether - ETBE), or as a primary fuel in neat hydrous form (95.5 GL). Ethanol makes an excellent motor fuel: it has a research octane number (RON) of 109 and a motor octane number (MON) of 90, which are higher compared to gasoline, which has a RON of 91 to 98 and a MON of 83 to 90. Ethanol also has a lower vapor pressure than gasoline (its Reid vapor pressure is 16 KPa versus 71 for typical gasoline), resulting in less evaporative emissions. Ethanol's flammability in air (1.3 to 7.6% v/v) is also much lower compared to gasoline (3.5 to 19% v/v), reducing the number and severity of vehicle fires (Goldemberg *et al*. 1993). On the other hand, when used as a neat fuel, ethanol has a lower energy density than gasoline (ethanol has lower and higher heating values of 21.2 and 23.4 MJ/L, respectively; for gasoline the values are 30.1 and 34.9 MJ/L) and cold-start problems exist as well (McMillan 1997).

A thorough review of technologies already available and future trends of bioethanol production is presented, as a source of information for those learning about the ethanol industry, as well as for those who are more deeply involved in this field, and for the policymakers while evaluating the role of bioethanol in the future of our society.
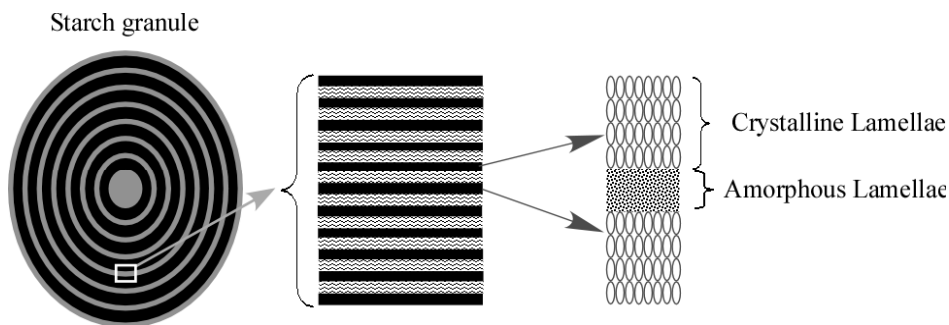
STARCH HYDROLYSIS FOR ETHANOL PRODUCTION

Starch is the major dietary source of carbohydrates, and the most abundant storage polysaccharide in plants, occurring as granules of size 1 to 100 μm (Phillips and Williams 2000). It is composed of a mixture of two kinds of polyglucans, namely amylose and amylopectin. Amylose is a linear component mostly comprised of α-1,4-linkages, with an average degree of polymerization (DP) up to 6,000 and molecular mass of 105 to 106 g/mol. Depending on the botanical source, the amylose content varies from 0 to 70% (Viswanathan 1999). Amylopectin (107 to 109 g/mol) is a highly branched polymer, consisting of short α-1,4-oligomers linked by α-1,6-bonds, with average DP of 2 million, making it one of the largest molecules in nature (Sajilata *et al*. 2006). The basic glucose building block is a ring-shaped molecule with six atoms in the ring (**Fig. 1**) (Hla 2002).

The most important origins of starch are maize, potato, wheat, tapioca and rice. The cereal crops maize, waxy maize and wheat are grown in America and Europe whilst potato is largely derived from the cooler climes of northern Europe. Tapioca starch is sourced from Brazil, Thailand and Indonesia, while rice originates mainly from Asia (Phillips and Williams 2000). Native starch granules are known to have semi-crystalline regions (**Fig. 2**) formed by amylopectin and less ordered amorphous regions, essentially amylose (Zhang *et al*. 2006).

The use of energy crops for ethanol production is a common practice. Most recently, crops residues and industrial by-products have been used as potential substrate for fermentation as well (Neves 2006). The typical composition and kinetic parameters during fermentation of wheat products are summarized in **Table 1**.

We have reported on the kinetics of bioethanol production from wheat milling by-products using *Zymomonas mobilis* in batch fermentation (Neves *et al*. 2007). The

Starch granule

Fig. 2 Schematic diagram of crystalline and amorphous layer structure of starch granules.

Crystalline Lamellae

Amorphous Lamellae

Table 1 Major components and fermentation kinetic parameters of wheat products used for ethanol production[a].

Component / Parameter	Wheat product		
	Low-grade Flour	Wheat Bran	Wheat Flour
Components in starch slurry [b]			
Moisture (wt%)	14.0	12.2	13.1
Starch (wt%)	15.6	11.7	62.0
Ash (wt%)	2.7	5.6	0.6
Protein (wt%)	15.0	13.3	10.4
Fiber (wt%)	0.8	10.8	0.2
Other (pentosans) (wt%)	51.9	46.4	13.7
Kinetic parameters			
Ethanol production (g/L)	51.4	18.1	68.1
Ethanol yield (g/g-dry wt)	0.17	0.02	0.30
Overall volumetric productivity (g/L·h)	2.72	1.09	3.64

[a] Source: Neves 2006
[b] Initial concentration: 200 g/L; batch fermentation for 24 h in a small-scale bioreactor

ethanol production obtained from low-grade wheat flour (51.4 g/L) was comparable to that of wheat flour (68.1 g/L), and considerably superior to the ethanol production from wheat bran (18.1 g/L).

CELLULOSIC ETHANOL

An alternative to the use of energy crops as feedstock for ethanol production is to utilize rougher and woodier parts of plants for producing ethanol, the so-called "cellulosic ethanol". This field has gained attention in the latest decades, as lignocellulosic biomass is a potential source for ethanol that is not directly linked to food production (Chum and Overend 2001). The conversion of lignocellulosic material to ethanol is generally more complex, compared to starch hydrolysis and fermentation. In case of cellulose, more drastic hydrolysis steps are necessary for achievement of high conversion yields, due to the presence of various

amounts of other sugars, such as xylose and arabinose. **Table 2** summarizes the carbohydrate composition of some potential biomass resources for ethanol production.

The most expensive part of making ethanol from lignocellulosic feedstock is pre-treating the biomass to make it accessible to the enzymes that will then cut the sugars from the polymers so that they can be fermented (Sanderson 2006). Pretreatment basically refers to the mechanical and physical actions to clean and size the biomass, and destroy its cell structure to make it more accessible to further chemical or biological treatment. Lignocellulosic materials consist primarily of three components, namely cellulose (40-50%), hemicelluloses (20-30%) and lignin (20-30%) (Ehara and Saka 2002). The potential conversion of these components into bioethanol is briefly illustrated in **Fig. 3**. This generic example depicts the potential use of lignin for electricity cogeneration. The soluble sugar products are primarily xylose, and further mannose, arabinose and galactose. A small portion of the cellulose may already be converted to glucose. However, the cellulose bulk will be converted in a separate step. The product is filtered and pressed, solids (cellulose + lignin) go to cellulose hydrolysis, and liquids (containing the sugars) go to the fermentation step. The choice of a pretreatment technology heavily influences cost and performance in subsequent hydrolysis and fermentation. The present production costs of ethanol show a broad range: Projected cellulosic ethanol production costs in Europe lie between $34 and $45/gigajoule, and in the US between $15 and $19/gigajoule (Hamelinck *et al.* 2003).

Some of the most commonly used chemical, physical and biological pretreatment methods are discussed in detail:

Chemical pretreatment

Common chemical pretreatment methods comprise dilute acid, alkaline, ammonia, organic solvent, SO_2, CO_2 or other chemicals. Acid catalyzed pretreatment of biomass prior to fermentation provide a near-term technology for production of fuel-grade ethanol from cellulosic biomass, but the rela-

Table 2 Composition of potential biomass species for bioethanol production[a].

Biomass type	Composition (wt%, dry basis)						Klason Lignin	Total Carbohydrate
	Hexosan			Pentosan				
	Glucan	Galactan	Mannan	Xylan	Arabinan	Acetyl		
Hardwoods								
Silver naple	45.9	0	1.2	17.1	0.7	3.9	20.8	64.9
Sycamore	44.0	0	0.9	16.3	0.6	3.6	22.8	61.8
Black locust	49.4	0	1.0	16.2	0.4	3.8	21.5	67.0
Poplar Hybrid NE388	48.6	0.3	0.5	14.6	0.3	2.2	21.8	64.3
Poplar Hybrid N11	51.8	0.7	0.3	11.3	0.3	1.9	22.5	64.4
Sweet gum	49.5	0.3	0.4	17.5	0.4	2.3	21.8	68.1
Herbaceous sp.								
Switchgrass	36.6	1.2	0	16.1	2.2	1.1	21.9	56.1
Weeping lovegrass	36.7	1.7	0	17.6	2.6	1.1	21.2	58.6
Sericea lespedeza	31.5	0.9	0	14.5	1.6	1.3	31.6	48.5
Reed canary grass	26.0	0.1	0	9.8	2.4	0.9	15.6	38.3
Flatpea hay	28.9	1.5	0.1	7.4	2.0	1.4	24.5	39.9
Agricultural residues								
Corn snobs	39.4	1.1	0	28.4	3.6	1.9	17.5	72.5
Corn stover	40.9	1.0	0	21.5	1.8	1.9	16.7	65.2

[a]Adapted from Hägerdal *et al.* (2006) and Himmel *et al.* (1997)

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┌─────────────────────────────────┐
│      Lignocellulosic feedstock   │
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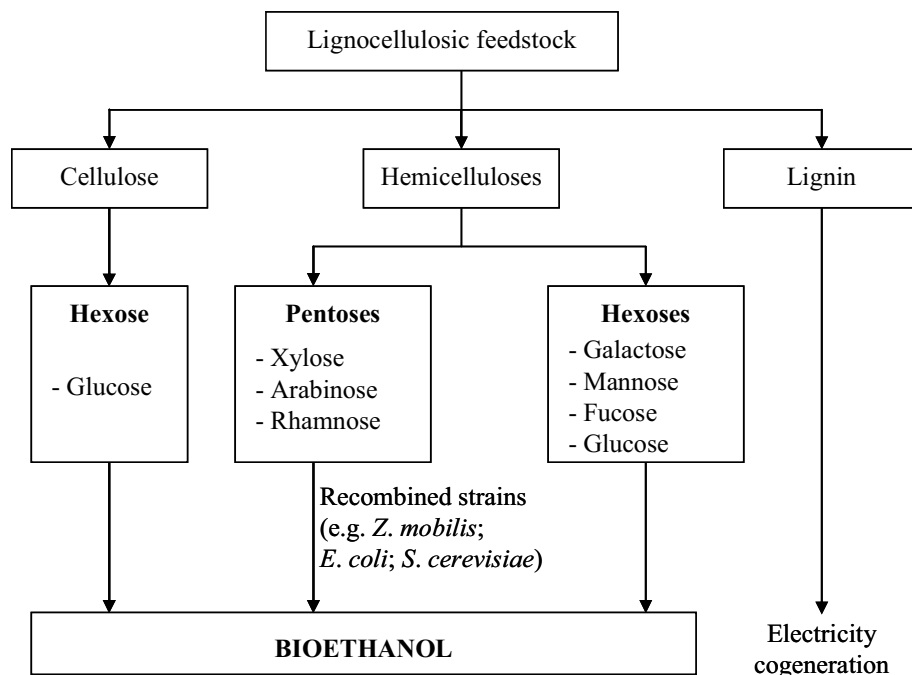
Fig. 3 Major sugars involved in the conversion of lignocellulosic feedstock into ethanol.

| Cellulose | Hemicelluloses | Lignin |

Hexose
- Glucose

Pentoses
- Xylose
- Arabinose
- Rhamnose

Hexoses
- Galactose
- Mannose
- Fucose
- Glucose

Recombined strains
(e.g. *Z. mobilis*;
E. coli; *S. cerevisiae*)

BIOETHANOL

Electricity
cogeneration

tively low yields of sugars from cellulose and hemicellulose (*c.a.* 50% to 60% of the theoretical yield) typical of dilute acid systems still have to be increased somehow, in order to be competitive with existing fuel options in a free market economy (Wyman *et al*. 1993). Concentrated acid or halogen acids achieve high yields (essentially, 100% of theoretical). However, because low-cost acids (such as H_2SO_4) must be used in large amounts while more potent halogen acids are relatively expensive, recycling of acid by efficient, low-cost recovery operations is essential to achieve economic operation (Wyman *et al*. 1993).

Alkaline processes use bases as NaOH or $Ca(OH)_2$. All lignin and part of the hemicellulose are removed. Cellulose reactivity is sufficiently increased and the reactor costs are lower than those for acid technologies (Hamelinck *et al*. 2003). Alkaline-based methods are generally more effective at solubilising a greater fraction of lignin, while leaving behing much of the hemicellulose in an insoluble, polymeric form (DOE 2007).

Physical pretreatment

Uncatalysed processes generally use steam explosion or Liquid Hot Water. Steam explosion is one of the most promising methods to make biomass more accessible to cellulase attack (Szengyel 2000). Basically, the method consists of heating the material using high-pressure steam (20-50 bar, 210 to 290ºC) for a few minutes; the reaction is then stopped by sudden decompression to atmospheric pressure. Using this method, xylose sugar recoveries between 45 and 64% were reported by Hamelinck *et al*. (2003), revealing steam-explosion pretreatment as economically attractive treatment.

The Liquid Hot Water method uses compressed hot liquid water (at pressure above saturation point) to hydrolyse the hemicellulose. Xylose recovery is relatively high (88-98%), and no acid or chemical catalyst is required, which makes it environmentally attractive and economically interesting.

The use of microwave oven for pretreatment of lignocellulosic has also been reported to make the substrate more susceptible to the subsequent enzymatic hydrolysis (Adrados *et al*. 2004, 2005).

A comparison between various physico-chemical pretreatment methods is summarized in **Table 3**.

Enzymatic hydrolysis

One of the most extensively investigated pretreatment processes is the enzymatic hydrolysis (EH), in which fungal cellulolytic enzymes are used to convert the cellulose onto of the biomass to glucose, which is then fermented to ethanol (Szengyel 2000; Varga *et al*. 2002).

Basically, three major classes of enzymes may be used for EH pretreatment of lignocellulosic biomass for bioethanol production, as follows:

1. The endo-1,4-β- glucanases or 1,4-β-D-glucan 4-glucanohydrollases (EC 3.2.1.4), which act randomly on soluble and insoluble 1,4-β-glucan substrates and are commonly measured from carboxymethylcellulose (CMC).
2. The exo-1,4-β-D-glucanases, including both the 1,4-β-D-glucan glucohydrolases (EC 3.2.1.74), which liberate D-glucose from 1,4-β-D-glucans and hydrolyze D-cellobiose slowly, and 1,4-β-D-glucan cellobiohydrolase

Table 3 Comparison of various physico-chemical pretreatment (lignin removal and hemicellulose hydrolysis) options[a].

Pretreatment method	Chemicals	Temperature/pressure	Time	Xylose yield	Downstream enzymatic effect[b]	Costs[c]	Available
Dilute acid hydrolysis	Acid	>160ºC	2-10 min	75-90%	<85%	+	now
Alkaline hydrolysis	Base			60-75%	55%	++	now
Uncatalysed steam explosion	-	160-260ºC	2 min	45-65%	90%	-	2-5 years[d]
Acid catalysed steam explosion	Acid	160-220ºC		88% (2 steps)		-	2-5 years[d]
Liquid Hot Water	None	190-230ºC p>psat	45 s to 4 min	88-98%	>90%	-	5-10 years[d]
Ammonia fiber explosion	Ammonia	90ºC	30 min	50-90% (2 steps)			
CO_2 explosion	CO_2	56.2 bar			75%		

[a] Source: Hamelinck *et al*. 2003
[b] This is the efficiency of the downstream enzymatic hydrolysis of cellulose to glucose, usually in 24 h
[c] + indicates that the effect is advantageous (less expensive)   [d] Forecast

(EC 3.2.1.91), which liberates D-cellobiose from 1,4-β-D-glucans.

3. The "β-D-glucosidases" or β-D-glucoside glucohydrolases (EC 3.2.1.21), which act to release D-glucose units from cellobiose and soluble cellodextrins, as well as an array of glycosides (Sheehan and Himmel 1999).

These three types of enzymes have been recognized for EH of lignocellulosic material, as they work together synergistically in a complex interplay, resulting in efficient decrystallization and hydrolysis of native cellulose.

The EH yield is governed by many factors, which include:

1. *Type of substrate pretreatment*: Cellulosic biomass is naturally resistant to enzymatic attack. A pretreatment step is required to overcome this resistance, if the enzyme-catalyzed hydrolysis process is to proceed at reasonable rate with the high yields vital for economic viability. The pretreatment step must facilitate conversion of both the cellulose and hemicellulose fractions into ethanol, while minimizing the degradation of these fractions into compounds that cannot be fermented into ethanol (Wyman *et al*. 1993). Several options for biomass pretreatment using chemical or physical methods were considered above, including dilute acid or alkaline treatment, steam-explosion, liquid hot water, among others. Currently, most industrial processes are based on dilute acid hydrolysis, making use of the electricity co-produced from the non-fermentable lignin (Hamelinck 2005). In this process, about 0.5% H_2SO_4 is added to the milled feedstock, and the mixture is heated to 140-160ºC for 5-20 minutes. Under these conditions, most of the hemicellulose is hydrolyzed to form xylose, which is then removed in solution, leaving a porous material of primarily cellulose and lignin that is more accessible to enzymatic attack (Wyman *et al*. 1993). Nonetheless, the most successful method, which has been evaluated for various lignocellulosic materials, is the steam pretreatment (Szengyel 2000).

2. *Inhibition of enzymatic activity by the end-products of the biodegradation*: The overall activity of cellulases is contributed to by the efficiency of the active site, susceptibility to end-product inhibition and to nonspecific or dead-end binding to the substrate, and ability to decrystallize cellulose. The net effect of reducing end product inhibition and nonproductive binding is to increase available sites for substrate hydrolysis (Sheehan and Himmel 1999). An early report (Szengyel 2000) evidenced that enzyme solutions produced on steam-pretreated spruce showed less sensitivity towards toxic compounds formed during steam pretreatment for bioethanol production from wood.

3. *Thermostability of enzymes and effect of medium pH*: In general, most enzymatic reactions benefit from the Arrhenius relationship, and thus, higher operating temperatures mean a benefit from increased diffusion and thermodynamics of catalysis. Nevertheless, at the present, the extent of the benefits obtained from enhancing the temperature tolerance, as well as cellulose decrystallization, of saccharifying cellulases is to some extent unclear.

One of the major problems related to the EH of lignocellulosic biomass for bioethanol production is the different optimal conditions, mainly pH and temperature, for the hydrolysis of cellulose and fermentation. Cellulases work in an optimal way at 40-50ºC and pH of 4-5 whereas the fermentation of hexoses with *S. cerevisiae* is carried out at 30ºC and pH 4-5, and fermentation of pentoses is optimally performed at 30-70ºC and pH 5-7 (Cardona and Sánchez 2007).

Some other factors of relevance while using EH as pretreatment for bioethanol production may be considered as well, such as: enzyme concentration and adsorption on the substrate, duration of the hydrolysis, substrate concentration in the medium and rate of agitation of the medium.

The main difficulty in solving the problem of enzymatic de-polymerization of the lignocellulosic materials is used to their complexity. Cellulose fibrils are embedded in an amorphous matrix of lignin and hemicelluloses, which render the plant tissue resistant to microorganisms and their enzymes (Szczodrak *et al*. 1996). In this sense, developing accurate methods for measurement of enzymatic cellulose digestibility is crucial for evaluating the efficiency of lignocellulosic pretreatment technologies (Zhang *et al*. 2007).

Enzyme-catalyzed processes offer several advantages. They achieve high yields under mild conditions with relatively low amounts of catalyst. Moreover, enzymes are biodegradable and thus environment friendly (Wyman *et al*. 1993). Another advantage of EH is that corrosion-related problems can be neglected, compared to other chemical processes, such as dilute acid pretreatment. Nonetheless, the cost associated to enzymatic production is relatively high, unlike chemical or physical methods. In recent years, this cost is decreasing gradually due to the development of new engineered enzyme systems (Ehara and Saka 2002). On the other hand, a common disadvantage of the enzymatic hydrolysis is the end-product inhibition of the enzymes used to hydrolyze the cellulose and the remaining hemicellulose. This problem was reportedly minimized by performing the saccharification and fermentation processes simultaneously (as detailed in the section SSF below) (Öhgren *et al*. 2006).

Alternatively, the utilization of immobilized enzymes and hollow-fiber membrane reactor has been recently revealed as a promising alternative for hydrolysis of lignocellulosic biomass (Cardona and Sánchez 2007). In this type of system, the enzymes are confined inside the reactor allowing the separation of substrate and hydrolysis products (e.g. glucose, arabinose, xylose, among others) enabling the reutilization of the enzymes while preserving their activity as free catalysts. For instance, an increase of 53% in substrate conversion was attained by Gan *et al*. (2002) using commercial *Trichoderma reesei* for saccharification of lignocellulosic feedstock, compared to 35% conversion in the case of traditional batch hydrolysis. This increased efficiency was likely due to the reduction in the inhibition effect of formed sugars on cellulases, and to the increase in productivity during continuous operation.

Ultimately, significant cost reduction for EH of lignocellulose to fermentable sugars can be forecasted, should the scientific community keep on track with development of new engineered enzyme systems. Most likely, these systems should include inhibitor-tolerant pentose-fermenting industrial yeast strains.

CURRENT BIOETHANOL PRODUCTION PROCESSES

Ethanol has been produced by anaerobic yeast fermentation of simple sugars since early recorded history. These fermentations used the natural yeast found on fruits and the sugars of these fruits to produce wines. Beer fermentations made use of the amylases of germinating grain to hydrolyze the grain starches to ferment sugars. Current practices utilize bacterial and fungal amylases to efficiently hydrolyze grain or tuber starch to glucose for fermentation to ethanol (Klass *et al*. 1981).

Ethanol can be produced by biologically catalyzed reactions. In much the same way that sugars are fermented into beverage ethanol by various organisms including yeast and bacteria, sugars can be extracted from sugar crops, such as sugar cane, and fermented into ethanol. For starch crops such as corn, starch is first broken down to simple glucose sugars by acids or enzymes, known as amylases. Acids or cellulase enzymes similarly catalyze the breakdown of cellulose into glucose, which can be then fermented to ethanol. The hemicellulose fraction of biomass is broken down into various sugars, e.g. xylose, in the presence of acids or
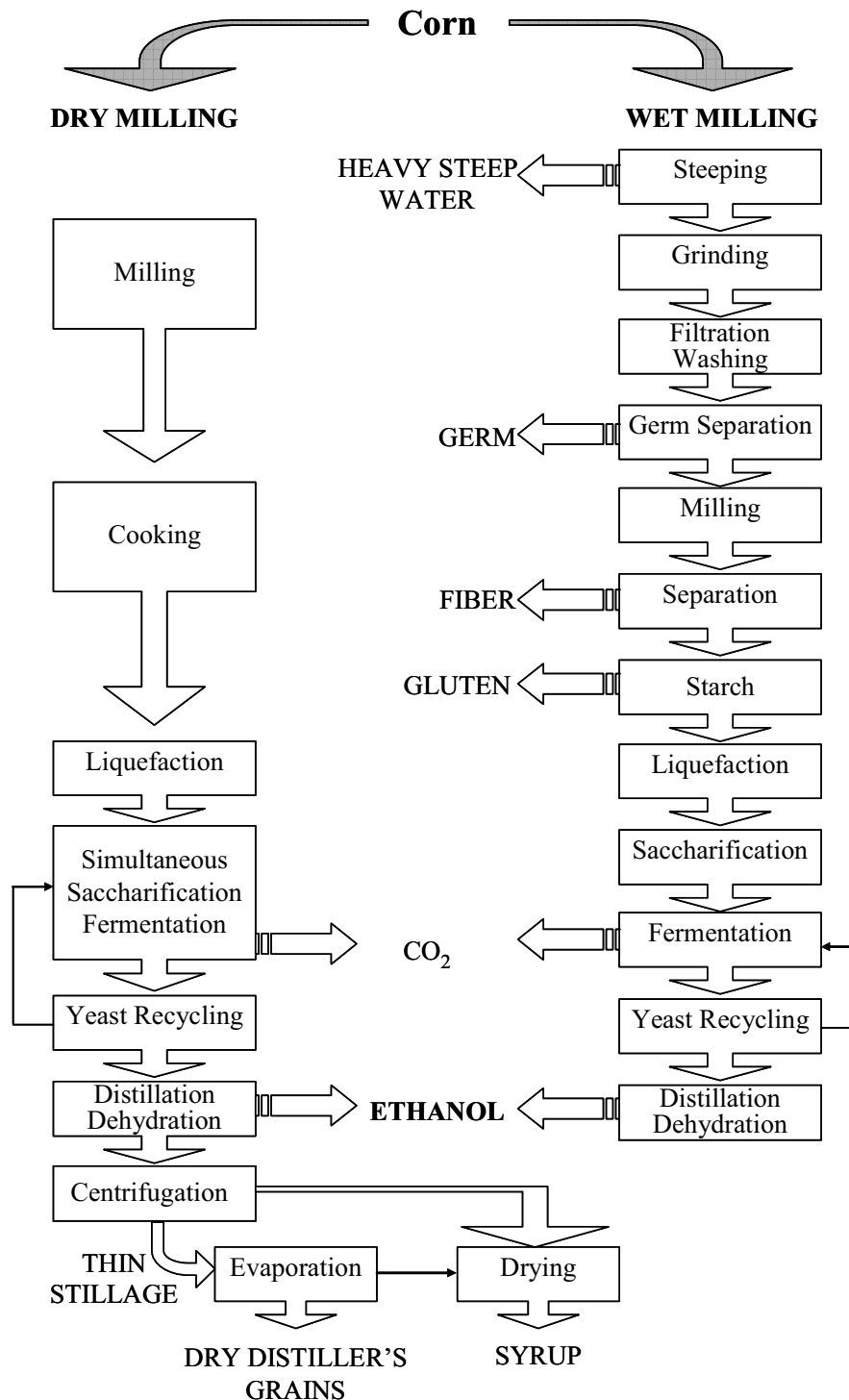
Corn

DRY MILLING   **WET MILLING**

HEAVY STEEP WATER ← Steeping

Grinding

Filtration Washing

Milling

GERM ← Germ Separation

Milling

Cooking

FIBER ← Separation

GLUTEN ← Starch

Liquefaction

Liquefaction

Simultaneous Saccharification Fermentation

Saccharification

CO_2 ← Fermentation

Yeast Recycling

Yeast Recycling

Distillation Dehydration → **ETHANOL** ← Distillation Dehydration

Centrifugation

THIN STILLAGE

Evaporation → Drying

DRY DISTILLER'S GRAINS

SYRUP

Fig. 4 Representation of dry milling and wet milling processes for bioethanol production. (Adapted from DOE 2007).

enzymes known as xylanases; conventional organisms cannot ferment many of the sugars derived from hemicellulose into ethanol with reasonable yields. However, recently new technologies capable of efficiently convert hemicelluloses into ethanol are under development.

Innumerous reports related to biomass conversion into ethanol have published recently, For instance, using starch crops such as wheat for bioethanol production resulted in considerable high ethanol concentration in reduced fermentation time (Montesinos and Navarro 2000a). In that case, slurries containing 300 g/L of raw wheat flour were initially liquefied using 0.02 g α-amylase/g starch at 95°C for 2 h, followed by saccharification using two different levels of amyloglucosidase activity (270 U/kg starch and 540 U/kg starch) and simultaneous fermentation by *Saccharomyces cerevisiae* at 35°C for 21h, reaching a final ethanol concen-

tration of 67 g/L. As for the hydrolysis of lignocellulosic biomass, various levels of enzyme load have been reported in the literature: Wooley *et al*. (1999) obtained bioethanol yield of *c.a.* 250 liter/ton by simultaneous saccharification and co-fermentation (SSCF) of Hardwood Yellow Poplar. The feedstock was pretreated with cellulase at 15 FPU/g of cellulose (FPU, Filter Paper Unit is the unit utilized to express cellulase activity) at 30ºC for 7 days. Öhgren *et al*. (2006) were able to reach final ethanol concentrations of about 25 g/L by simultaneous saccharification and fermentation (SSF) of corn stover pretreated by EH using cellulase at 65 FPU/g of cellulose at 40ºC for 4 days.

Basically, two different processes can be used to produce ethanol from starch crops: dry grind and wet milling, depicted in **Fig. 4**. In dry grind, the feed material is ground mechanically and cooked in water to gelatinize the starch.

Enzymes are then added to break down the starch to form glucose, which yeasts ferment to ethanol. In that case, a fixed amount of ethanol is produced, along with other feed products and carbon dioxide, and has almost no process flexibility. In wet milling, the insoluble protein, oil, fiber, and some solids are removed initially, remaining only the starch slurry fed to the ethanol production step. This process has the capability to produce various end products and considerable higher process flexibility, compared to the dry milling (Fernando *et al*. 2006). Currently, about 65% of the ethanol in the US is produced from dry grind corn processing plants (DOE 2007).

Biological processing offers a number of advantages for converting biomass into biofuels. First, the enzymes used in bio-processing are typically capable of catalyzing only one reaction, and so formation of unwanted degradation products and by-products is avoided (Schmidt 2002). Additionally, material not targeted for conversion can pass through the process unchanged and be used for other applications.

Although the individual steps for converting biomass into ethanol can be conveniently isolated, these can otherwise be combined in various ways in order to minimize the production cost (Johansson *et al*. 1993). Some of these integrated processes are described below.

Separate hydrolysis and fermentation

The Separate Hydrolysis and Fermentation (SHF) process uses distinct process steps for starch hydrolysis and glucose fermentation (as described in **Fig. 5**). The primary advantage of this configuration is that starch hydrolysis and sugar fermentation can be treated separately, thus minimizing the interactions between these steps. However, α-amylases are often inhibited by the accumulation of sugars, and considerable efforts is still needed to overcome this end-product inhibition, which impedes to achieve reasonable ethanol concentrations at high rates and with high yields even at high enzyme loadings (Borzani *et al*. 1998).

This two-step enzymatic hydrolysis was reported recently for the conversion of starch-containing material into ethanol, by enzymatic hydrolysis and fermentation using *Saccharomyces cerevisiae* (Mojović *et al*. 2006). Basically, the starch molecule is initially hydrolyzed by the action of amylolytic enzymes: α-amylase (for liquefaction) and glucoamylase (for saccharification). After complete hydrolysis, the fermentation is conducted as single step, in separate.
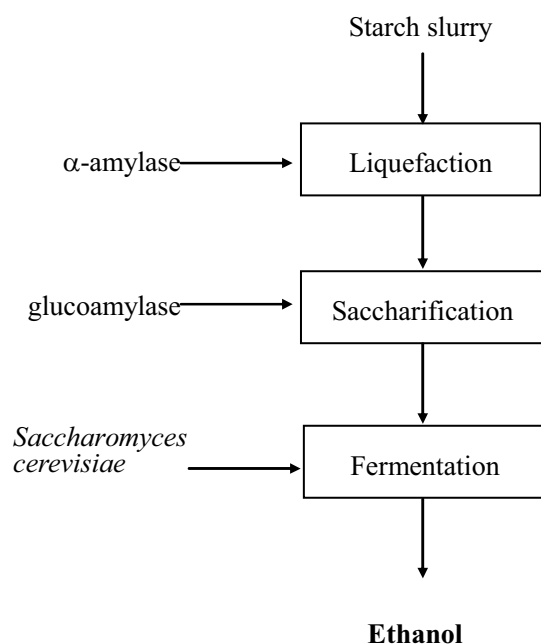
Those authors were able to achieve ethanol yield values of more than 80% (w/w) of the theoretical yield, and a considerable reduction of the fermentation time for 4 h was also observed.

Simultaneous saccharification and fermentation

The concept of the process for enzymatic hydrolysis of cellulose and simultaneous fermentation, so-called Simultaneous Saccharification and Fermentation (SSF) was introduced by Gulf Oil Company, US and the University of Arkansas (Gauss *et al*. 1976; Huff *et al*. 1976). The sequence of steps for the SSF is virtually the same as for the separate process, except that saccharification and fermentation steps are combined in one vessel (as described in **Fig. 6**). The presence of yeast or bacteria along with enzymes minimizes the sugar accumulation in the vessel, and because the sugar produced during starch breakdown slows down α-amylase action, higher rates, yields and concentrations of ethanol are possible using SSF rather than SHF, at lower enzyme loading. Additionally, the presence of ethanol makes the mixture less vulnerable to contamination by unwanted microorganisms, which is a frequent burden in case of industrial processes (Montesinos and Navarro 2000a; Roble 2003).

In this process, the saccharification of sugars released during starch hydrolysis (mainly maltose) is conducted simultaneously with fermentation. Immediately after liquefaction by α-amylase, the enzyme glucoamylase is added to the slurry, concomitantly with yeasts, and the SSF is conducted in a single reactor (Montesinos and Navarro 2000b).

Various reports on bioethanol production have mentioned the superiority of ethanol yield and productivity using the SSF process, compared to the SHF process (Söderström *et al*. 2005; Neves *et al*. 2007).

Other promising integration alternative is the inclusion of pentose fermentation in the SSF, process known as Simultaneous Saccharification and Co-fermentation (SSCF) (Sheehan and Himmel 1999; Cardona *et al*. 2007). In this configuration, it is necessary that both fermenting microorganisms be compatible in terms of operating pH and temperature. A combination of *Candida shehatae* and *S. cerevisiae* was reported as suitable for the SSCF process (Cardona and Sánchez 2007).

Economic analysis

In recent years, numerous studies aimed at quantifying the true cist of ethanol production have been undertaken by many international organizations. Bioethanol production costs are calculated by dividing the total annual costs of a system by the produced amount of fuel. The total annual
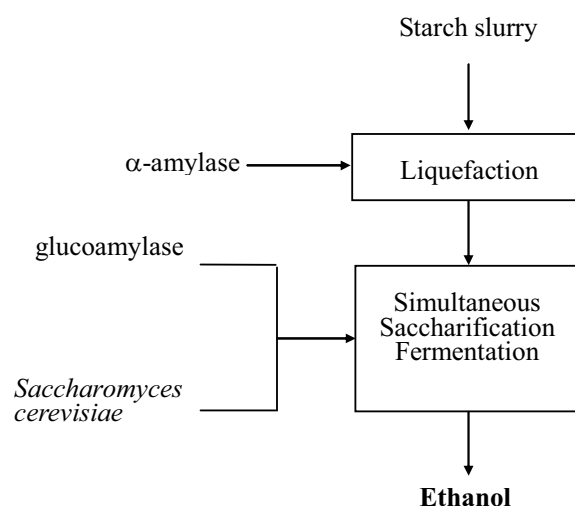

Fig. 5 Scheme of a generic Separate Hydrolysis and Fermentation process for bioethanol production from starch products.

Fig. 6 Scheme of a generic Simultaneous Saccharification and Fermentation process for bioethanol production from starch products.

Table 4 Average ethanol production costs in Brazil (1989 US $/liter)[a, b].

	Average
Direct costs	
Labor	0.006
Maintenance	0.004
Chemicals	0.002
Energy	0.002
Other	0.004
Interest on working capital and commercial costs	0.002
Cane[c]	0.127
Fixed costs	
Capital (milling, fermentation, distillery, storage, others)	
6%	0.030
12%	0.051
Other	0.011
Total	
6%	0.208
12%	0.229

[a] Source: Goldemberg *et al.* 1993
[b] Based on a sample of 50 mills. Data are for anhydrous ethanol; hydrated ethanol is 7 to 10% lower in cost
[c] Average correspond to 77.7 liters of ethanol per ton of sugar cane

costs consist of annual capital investments, operating and maintenance, biomass feedstock and electricity supply/demand (Hamelinck *et al.* 2003). As illustrative example, the average production costs for anhydrous ethanol are summarized in **Table 4**.

According to estimates made by the National Renewable Energy Laboratory, in the US bioethanol production could cost anywhere from $0.30 to $0.38/L, depending on the technology utilized and availability of low cost feedstocks for conversion to ethanol (Wooley 1999). In the case of Brazil, production of ethanol from sugar cane costs in average $0.22/L, from which about 60% is the raw material cost (Daishou 2004).

The production costs may decrease through time, due to various reasons. Process improvement (higher efficiency, cheaper installation) is masked by other factors such as a larger scale, and cheaper biomass feedstock. In fact, the price of raw material used for bioethanol production plays a major role on the total production costs (**Fig. 7**).

The seasonal production pattern due to the harvest period of various agricultural feedstocks used for bioethanol production is another important factor on the final price of the fuel on the market. For instance, In Brazil harvesting of sugar cane generally starts at the beginning of autumn (March), extending up to mid-summer (end of December). Generally, in that country biorefinery plants remain inoperable for about 4 months within harvests, in order to conduct thorough maintenance procedures, which amounts up to 5% of total income, in order to avoid major breakouts during the harvest season (Magalhães 2007). Often, very low stocks within harvest seasons cause considerable fluctuations on ethanol prices for the domestic market, as well as for those countries importing ethanol from Brazil (**Fig. 8**).

ADVANCEMENTS ON BIOETHANOL PRODUCTION IN VARIOUS COUNTRIES

Brazil is one of the world leaders in bioethanol production and use as biofuel, as well as in terms of potential for expansion and cost of production (Segall and Artz 2007). The bioethanol produced in the country is essentially sugarcane-based, and in the year 2005 the total ethyl alcohol production was c.a. 16 billion liters (**Fig. 9**), including hydrated alcohol (95 wt% ethyl alcohol, used directly as automotive fuel) and anhydrous alcohol (99.9 wt% ethyl alcohol, used for blending with gasoline). In addition, the bioethanol producing cost in the country (nearly US $0.20 per liter) is one of the lowest throughout the world (Daishou 2004).

Based on statistics provided by the ANP - *Agência Nacional do Petróleo, Gás Natural e Biocombustíveis* (National Petroleum, Natural Gas and Bio-combustibles Agency, Brazil) (http://www.anp.gov.br), there are 243 bioethanol processing plants registered in the country, and nearly
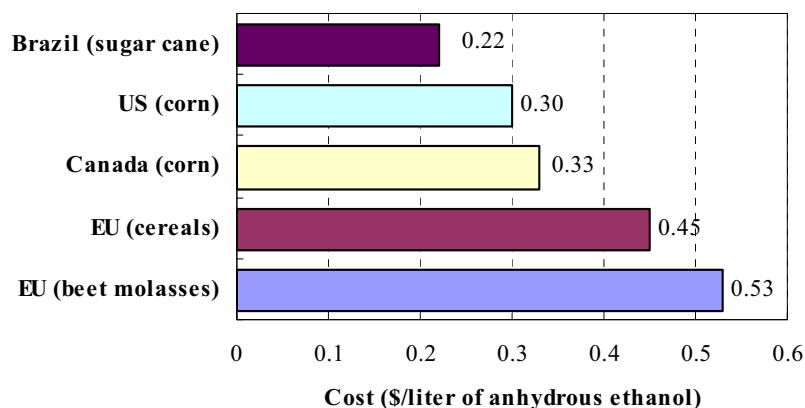
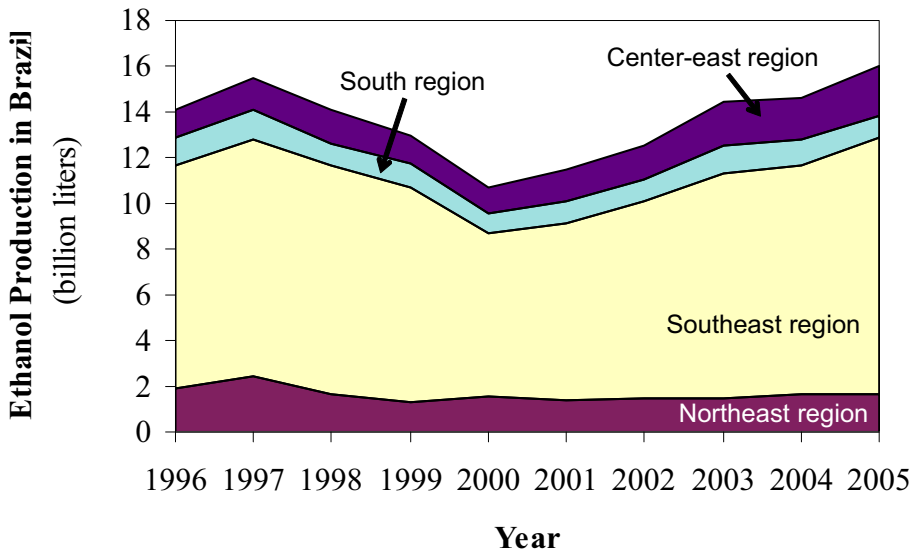**Fig. 7 Ethanol production costs in various countries (from raw material to final product) (Based on ICONE 2007).**
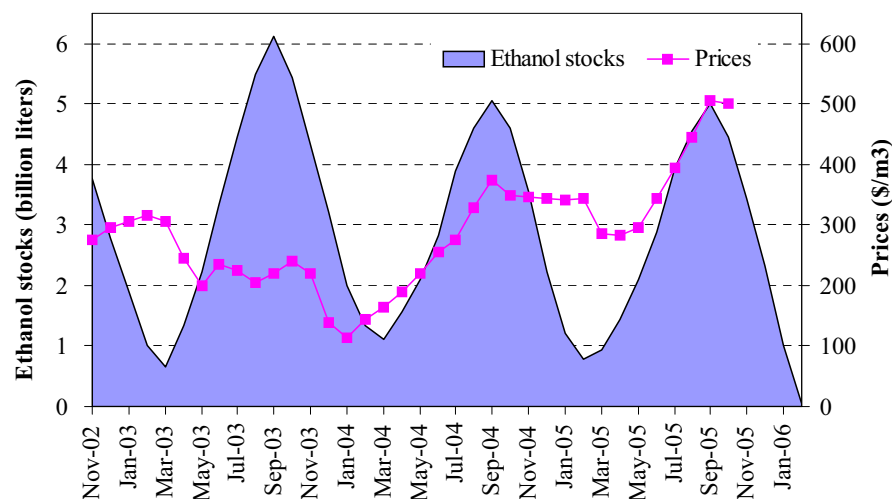
Fig. 8 Ethanol prices associated to stock movements in recent years in Brazil (Based on Messias 2006).
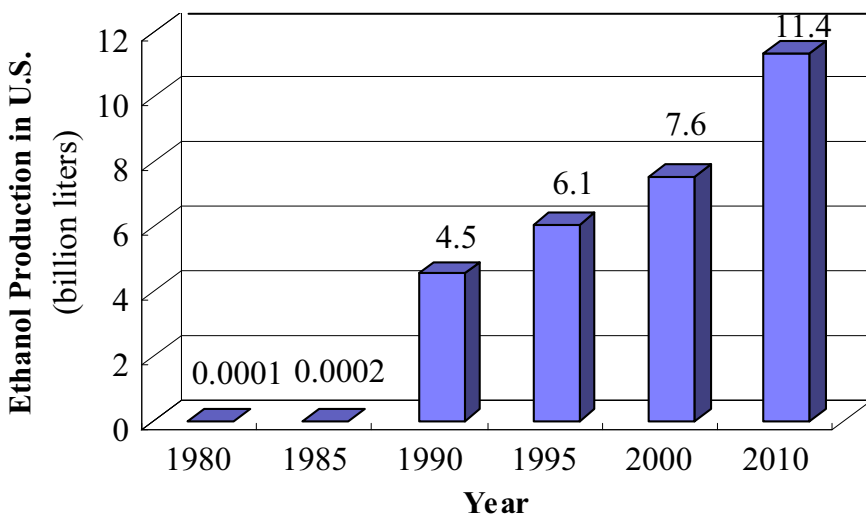
Fig. 9 Total ethanol production by region in Brazil (Based on ANP 2007). The Northern region was not included in this graphic due to the low magnitude of its ethanol production, compared to the other regions.
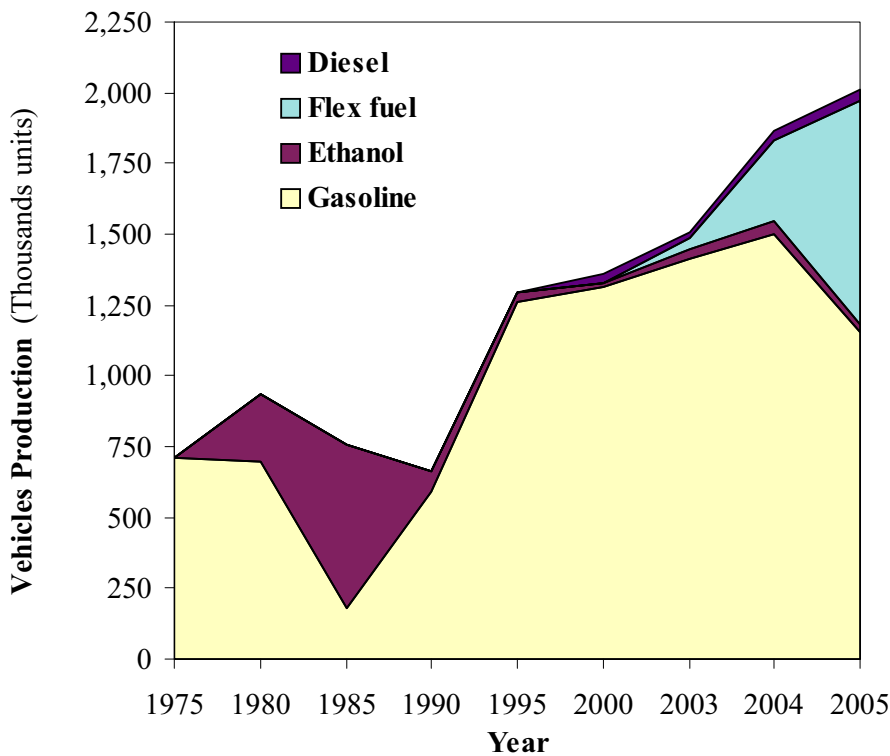
Fig. 10 Vehicles production by fuel type in Brazil - 1975/2005 (Based on ANFAVEA 2006).

Fig. 11 Ethanol production capacity in the U.S. (Based on DOE 2007). 2010 = forecast

33,620 gasoline service stations authorize for sale of bioethanol.

Most recently, with the development, production and booming sales of flex-fuel vehicles, the production of gaso-line-only-fueled cars has been decreasing drastically in Brazil (Delgado *et al*. 2007), as depicted in **Fig. 10**.

Brazilian ethanol exports have been growing significantly since 2002, and amounted to 2.6 billion liters in 2005.
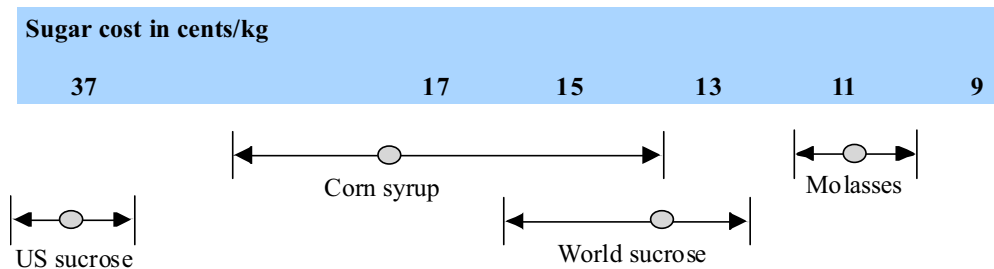
Fig. 12 Sugar production cost using various sources (Based on Biomass 2005).

For instance, in February 2007 the country's state-owned oil company Petrobras exported its first 20 million L shipment of anhydrous ethanol to the state-owned Nigerian National Petroleum Corporation, to use in E-10 fuel (a blend containing 10% ethanol in gasoline). According to a recent forecast from Petrobras, Brazilian ethanol exports should reach up to 3.5 billion L by the year 2010, from which nearly 90% should be exported to Japan (Spitz 2007).

In the US most fuel ethanol is produced from corn by either dry-grind (67%) or wet-mill (33%) process (Bothast 2005). Since 1980 US ethanol production has risen from an average of 1 million L a day to 40 million L a day, and a further doubling by 2012 has been called by federal mandates (Sanderson 2006). The continuous increase in bioethanol production recently in the US is depicted in **Fig. 11**, reaching the world leader position in the year 2006 (Jank 2007).

Since the early 1980's, production costs have dropped from an average of US $0.60/L to around US $0.27/L (Daishou 2004; Novozymes and BBI 2004). Most of this 55.5% cost reduction has been the result of the repetitive-ness of the design, the sharpening of the process, and the ability to negotiate for lower costs with suppliers because of the increasing volume. Still, the raw material actually used for bioethanol production in the country (essentially corn) plays a major role on the final production cost, considering that its cost is considerably high, compared to other sugar sources (**Fig. 12**). Broadening the range of substrates used for bioethanol production in the country, besides the ongoing lignocellulosic biomass-based research projects, a state grant amounting to $40,000 has been announced recently by the Wisconsin Bio Industry Alliance, for the development and implementation of a process for the conversion of whey – a cheese by-product – into bioethanol (http://www.wisconsinbioindustry.com).

Aiming to achieve the 28.5 billion L of renewable fuels mandated by the US government by 2012 (Biomass 2005), various bioethanol processing facilities are in operation in the country, and many other are under construction. As reported by the Renewable Fuels Association (http://www.ethanolrfa.org), there are currently 113 ethanol biorefineries in operation in the US, with the capacity to produce more than 21 billion L of ethanol. An additional 79 biorefineries are under construction and 7 expanding that will add more than 23.5 billion L of new capacity when complete. According to the Alternative Fuel Data Center (http://www.eere.energy.gov/afdc), there are actually 1073 ethanol stations in the country.

In order to regulate the blending of ethanol into gasoline in Japan, on August 2003 the Ministry of Industry and Economy introduced a law allowing for a 3% ethanol mixture in gasoline, a fuel so-called E3 (MOE 2003; Daishou 2004). Biomass Nippon Strategy was designed in the country to promote the production and use of biomass fuel. Besides reducing CO_2 emission, foreseeing prevention of global warming, this strategy also aims at reinforcing the concept of a recycling-oriented society (MAFF 2007). However, biofuel discussions in the country are still in the early stages. For example, the International Biofuel Conference took place on February 2007 in Tokyo, aiming to identify the challenges posed by the emerging biofuel industry, and to introduce a roadmap for economically viable biofuels utilization (http://www.ibc2007.jp/en/index.html). The

first bioethanol processing plant in commercial scale in Japan was launched in January, 2007. The facility owned by Bio-ethanol Japan (http://www.bio-ethanol.co.jp) is based in Osaka, and is expected to produce about 1.4 million L of bioethanol in the first year of operation, using waste wood and new lignocellulosic technology. In addition, other experimental ethanol processing plants are in operation in Hokkaido, using wheat and maize; in Yamagata, using sorghum; in Okinawa, using sugarcane; and a second waste wood facility planned in Okayama (Nishizaki 2006; Licht 2007). Most recently, the Japanese enterprise Mitsui Oil Co. signed an agreement with Petrobras, a state-owned oil company from Brazil, planning for the construction of 40 new biorefinery plants in Brazil, meant to produce and export bioethanol to the Japanese counterpart. The Japan Bank of International Cooperation (JBIC) is expected to fund the project and implementation of the refineries awaited to launch by 2011, with a total investment of the order of $8 billion. Besides producing bioethanol, the refineries should be designed to co-generate electricity from sugar cane bagasse as well (Spitz 2007).

In Indonesia, an E-10 mandate by the government would require 43 million L of bioethanol annually; in that country most of bioethanol available up to date is produced from cassava. In the Philippines an E-5 mandate is set up to start in January 2009, and cassava is the major feedstock for bioethanol production in the country as well (Roble 2003).

Concentrating efforts on the development of lignocellulose-to-ethanol technologies, the European Commission (EC) launched a 4-year integrated project so-called NILE (New Improvements for Lignocellulosic Ethanol), to design and investigate new solutions for an efficient and cost-effective conversion of lignocellulose to bioethanol (http://www.nile-bioethanol.org). In addition, in order to lessen the bioethanol dependency on energy crops, the EC sold recently a total of 63.2 million L of wine alcohol for fuel ethanol production (Licht 2007). Particularly, in France, since January 2007 the biofuel "Superéthanol E85" (a blend of ethanol and gasoline containing 85 wt% and 15 wt%, respectively) has been authorized by the Ministry of Economy, Finances and Industry, for sale as transport fuel. Most bioethanol produced in that country up to date is produced from sugar beet.

In Sweden, a fully integrated pilot plant for ethanol production from softwood, comprising both two-stage dilute acid hydrolysis and the enzymatic process, was taken into operation in mid 2004. This plant has a maximum capacity of 2 ton (dry matter) wood per day (Hägerdal *et al*. 2006).

TRENDS IN BIOETHANOL PRODUCTION DEVELOPMENT

In the last couple of years technological breakthrough has been enormously necessitated due to the lack of alternative feedstock and considerable shortage on agricultural land. In this sense, advances in metabolic pathway engineering and genetic engineering have led to the development of microorganisms capable of efficiently convert biomass sugars into ethanol. Generally, such development relies on broadening the substrate range to include other biomass sugars such as arabinose or xylose in strains that cannot ferment sugars other than glucose. Examples of such microorganisms include *Escherichia coli*, *Saccharomyces* sp. (Neves *et al*. 2006a; Öhgren *et al*. 2006; Plessas *et al*. 2007), and *Zymo-*
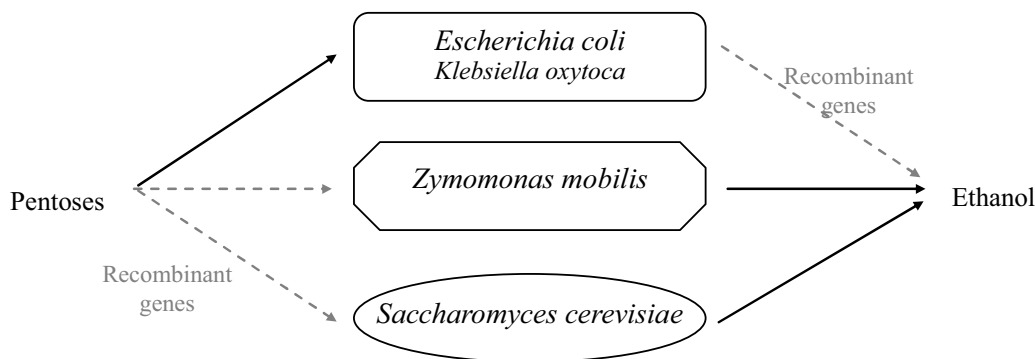
Fig. 13 Strains that can be metabolically engineered for ethanol production. (Adapted from Hägerdal *et al.* 2006).

monas mobilis (Torres and Baratti 1988; Saha and Woodward 1997; Davis *et al.* 2006; Neves *et al.* 2006b).

As for the cellulosic ethanol industry, aside from *Pichia stipitis*, a natural xylose-fermenting yeast, most efforts have concentrated on obtaining recombinant strains of bacteria and yeast able to ferment pentose sugars, such as xylose and arabinose. Basically the tail end, as in *E. coli* and *Klebsiella oxytoca*, or the front end of metabolism, as for *Saccharomyces cerevisiae* and *Zymomonas mobilis* can be recombined (**Fig. 13**).

Future directions for the development of lignocellulose-to-ethanol processes should necessarily include the efficient de-polymerization of cellulose and hemicellulose to soluble sugars. For instance, considering the SSF process, if pentose sugars present in the lignocellulosic material could be fermented at the same time as glucose, the ethanol concentration in the slurry after fermentation could be increased significantly, leading to considerable reduction on the energy demand in the distillation afterwards (Öhgren *et al.* 2006). Moreover, the genetic engineering of plants is another promising research field, which will most likely play a major role on the biofuel industry. The latest developments on hybrid varieties have enabled considerable increases in starch yield from energetic crops. Actually, a bushel of 25 kg of corn contains *c.a.* 15 kg of starch. In the near future, that same bushel may contain as much as 17 kg of starch through improved hybrid corn. This would result in a gain of nearly $2 million in annual revenue from processing the same bushel of corn in a 120 million L per year facility (DOE 2007).

Protein engineering using the "informational" approach offers powerful opportunities to enhance the efficiency of enzymatic hydrolysis. Simple modifications to the amino acid sequence of a protein can have dramatic impacts on performance (Sheehan and Himmel 1999).

Though ethanol is the major biofuel actually produced on commercial scale, its usage as transport fuel poses a few obstacles, such as: the tendency of ethyl alcohol to pick up water up endurances its transport, particularly in pipeline; in addition, it is corrosive, considerably volatile and its energy density is low compared to regular petrol. In order to overcome such disadvantages, another form of alcohol has been thought to replace ethanol, such as butanol, an alcohol with four carbon atoms in its molecule, which has been produced experimentally in the U.S. using sugar beet and cellulosic feedstock (Sanderson 2006).

The stability of e-diesel, a new biofuel under development has been investigated recently (Lapuerta *et al.* 2007). This biofuel is produced by direct blending of bioethanol and diesel fuel, and has a considerable potential to reduce particulate emissions, with low production cost. One drawback in this process is the fact that ethanol is ordinarily immiscible with diesel fuel, thus requiring in many cases the presence of surfactants. Most recently, Singh *et al.* (2007) reported that ultrasonic processing used in biodiesel production delivers a biodiesel yield in excess of 99% in five minutes or less, compared to one hour or more using conventional batch reactor systems. On this way, research and development of new strains allied to design of novel reactors and high yielding processes are essential, in order to

keep bioethanol competitive with other biofuels, such as biodiesel, in the future.

Bioreactor design

The ultimate ability to achieve the contacting and movement of liquid and solids effectively in a large-scale device is a major challenge and will likely determine the comercial success of the bioethanol industry (Saha and Woodward 1997). This said, the immobilization of cells is a technique that has proved increased ethanol productivity, operation stability and easier downstream processing, compared to processes using suspended cells (Tanaka *et al.* 1985; Jain *et al.* 1985a, 1985b; Ogbonna *et al.* 1996, 2001; Plessas *et al.* 2007). However, the specific advantages of immobilized cells depend on the type of cells, the reactor configuration and nature of the process. Entrapment of cells in natural polymers by ionic gelation (alginate) or by thermal precipitation (carrageenan and agar) are methods commonly used for cell immobilization (Ogbonna *et al.* 1991). Immobilization by passive adhesion to surfaces has great potential for industrial application since the immobilization method is relatively simple. The use of cheap carriers ensures that this method can be exploited with minimal increase in the overall production cost. Thus, one limiting factor of this technology is that it can only be adapted for practical industrial production if the expected increase in bioethanol productivity can overcome the increase in the production costs (cost of the carrier and immobilization) (Ogbonna *et al.* 1996).

The use flocculent microbial strains for bioethanol production has also been proved more efficient than the conventional strains used for industrial processes, as evidenced by the higher specific rates of glucose uptake and ethanol production, increased ethanol yields and higher volumetric productivities reported for flocculent strains of *Zymomonas mobilis*, compared to industrial ethanol-producing strains of *Saccharomyces cerevisiae* (Baratti *et al.* 1986; Davis *et al.* 2006).

The selection and definition of suitable process configurations are crucial while designing cost-effective processes for bioethanol production (Cardona and Sánchez 2007). As pointed out by Lin and Tanaka (2006), structured models should be used for optimization and control of ethanol fermentation. Nevertheless, these models are limited by the fact that they do not considerate simultaneously the substrate limitation, substrate inhibition, product inhibition and cell death, which are the four major factors affecting the resulting ethanol concentration. Traditional fuel ethanol producing plants consisted of a single step for ethanol fermentation, but it is necessary the analysis of other models applied to more complex processes like co-fermentation, SSF and SSCF. Efforts are currently underway in order to design and develop such bioreactor systems, as follows:

➢ External loop liquid-lift bioreactor: this spinning-sparger bioreactor was developed with the purpose of enhancing the production by simultaneous mass transfer and fermentation (Stang *et al.* 2001). Basically, oleic acid was used to produce circulatory fluid flow in the reactor, and to absorb ethanol for the aqueous fermenta-

tion media.

➢ Circulating loop bioreactor: this is a modification of an external loop air-lift bioreactor, constructed for simultaneous aerobic and anaerobic processes, and optimized for direct ethanol production from raw cassava starch, using immobilized cells of *Aspergillus awamori* and *Saccharomyces cerevisiae* (Roble *et al*. 2003).

Biorefineries

Biorefinery is a relatively new concept, based on the use of biomass as input to obtain multiple products by complex processing methods, an approach similar to e petroleum refinery where fossil fuels are used as input (Fernando *et al*. 2006). The ultimate goal of a biorefinery is to convert the biological materials consisting biomass (e.g. carbohydrates, lignin, proteins, fats and to a lesser extent various other chemicals such as dyes, vitamins and flavors) into value-added products using various technologies and processes.

On this way, the integration of ethanol production with combined heat and power plant, or with pulp and paper mill, could represent a further reduction in production costs, which is mandatory to accomplish the step from pilot- and demo-scale to competitive full-scale production (Hägerdal *et al*. 2006). This type of integration is also known as co-generation, and generally should result in increased process efficiencies, helping to survive unpredictable fluctuations in feedstock and fuel costs (Novozymes and BBI 2004).

OTHER FACTORS ASSOCIATED TO BIOETHANOL PRODUCTION

The booming increase on biofuels production and use in recent years is intimately related to various factors other than technological development. Among them, the energetic security, due to potential reduction of dependency on increasingly expensive and scarce imported oil, which price is forecasted to reach $100/barrel by the end of this decade (Bertelli 2007); the contribution of transport sector gas emissions to global warming; and supporting the sustainable development of small farmers, are some of the outstanding reasons for the recent demand on biofuels worldwide. Some of these factors are lined up below.

Environmental assessments

Carbon dioxide (CO_2) emmisions from the transport sector worldwide have been increasing steadly in the past few decades, mainly due to the increasing production from road transport, representing the main gas responsible for the greenhouse effect and consequently global warming. For example, CO_2 emissions from the European transport sector alone could increase by 50% between now and 2020 (http://www.nile-bioethanol.org). The development of cost-efficient biofuels production system should help to increase the share of biobased fuels in the transport sector and hence decrease the amount of CO_2 released. In Brazil alone, the use of bioethanol as neat-fuel or blended to gasoline, within the years 1975 and 2000, has avoided the emission of 403 million ton of CO_2 (Bertelli 2007).

The impact of bioethanol production in the global energy market is still very low, representing less than 1% of the fossil fuels production, in terms of energetic equivalent (Jank 2007), and less than 2% of world gasoline consumption (Bertelli 2007). Thus, there is enough room for the biofuel industry to grow widely based on renewable sources, which production can be boosted e.g. by increasing the cultivated area or crop productivity, unlike nonrenewable fossil fuels, which originates from the subsoil of a few countries.

In recent decades, discussions have centered on the use of biomass for biofuel production, and the fact that doing so would contribute to deforestation by creating new incentives for cutting down natural forests. But considering that it is not cost effective to gather biomass from a wide area and transport it to the refinery, affordable supplies must be available locally on a continuous basis, ensuring that biomass production should be carried out sustainably (Johansson *et al* 1993).

The production of bioethanol from various lignocellulosic feedstock such as forest and agricultural or industrial residues e.g. wheat milling by-products (Reith *et al*. 2002; Neves 2006), offers a set of advantages, but its development is often constrained by economic and technical obstacles. Nonetheless, further commitment of nations' leaders, making every possible effort to transpose these obstacles, is still needed for groundbreaking on these technologies.

Socio-economic aspects

Asides from being a major environmental issue, the expansion of bioethanol industry carries a definite load of socio-economic impact. The biofuel production in developing countries is closely related to small-to-medium farmers, and in many cases to familiar agricultural production where the harvest of the energy crops is done manually. In this way, the relatively new bioethanol industry, as well as the most recent biodiesel industry, has been the major – if not the only – source of income to many of these farmers.

Among these countries, probably Brazil is the best example of social impact caused by the biofuel industry. Since the Brazilian fuel-ethanol program (Pro-álcool) was created in 1975, overseeing to reduction in regional income differences, and an increase in job opportunities for both skilled and unskilled workers, the sugarcane agro-industry has generated more than 700,000 jobs in the country (Goldemberg *et al*. 1993). The booming increase on biofuels usage has provided major source of income for many small farmers, meanwhile contributing to the reduction of poverty in many developing countries.

In the near future, the socio-economic influence of biofuels industry is expected to play a major role on the development of many countries, mainly in South and Central America, Caribbean Islands and Africa.

Bioethanol versus food: an ongoing critical issue

There is a concern about the potential increase in prices of agricultural products, as consequence of ongoing plans to extend biofuels production worldwide. With that in mind, various governmental agencies are calling for careful monitoring of the effects of such plans on agricultural supplies and prices (ElAmin 2006). Additional efforts have been made in order to diversify the supply of agricultural products used for bioethanol production rather than relying in just one or two products, such as sugar and corn (the two major commodities directly related to bioethanol production in Brazil and the US, respectively, which represent together nearly 72% of the world production). For instance, in case of Brazil, nearly half of its sugarcane crop is currently used for sugar and the other half for ethanol production (**Fig. 14**). In the US, about 20% of corn produced in 2006 was used for bioethanol production, causing an increase in corn price of about 80% compared to the previous year (Jank 2007).

On the other hand, this shifting of agricultural production away from food chain might represent an opportunity for agricultural diversification and a better use of resources (Fletcher 2007). For example, in South Asian or South-East Asian countries the use of alternative agricultural products for biofuel production have been increasing recently, which is the case of cassava-based bioethanol production in the Philippines (Roble *et al*. 2003), or biodiesel obtained using palm oil or other non-edible seed oils, like Jatropha and Pongamia (Sarin *et al*. 2007).

On the meantime investing on design, technological advancement and implementation of the lignocellulose-to-ethanol industry might represent an alternative to release the immense cost pressure on food and feed industries.
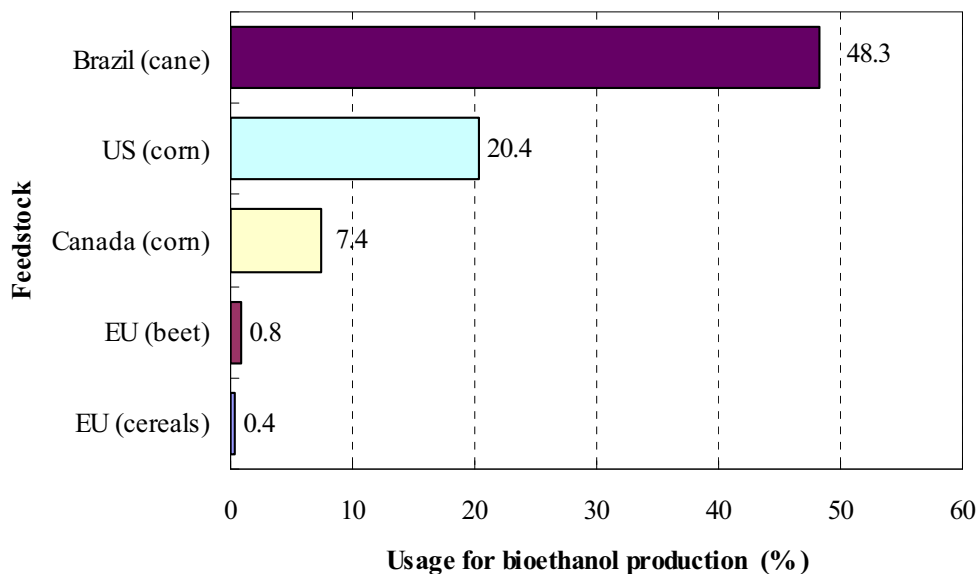
Fig. 14 Percentage of feedstock production used for bioethanol production (Based on ICONE 2007).

CONCLUSION

Biomass is an important contributor to the world economy. The biomass industry can produce additional ethanol by fermenting by-product sugar streams, looking towards the energy self-sufficiency. Development of technologies for effectively converting agricultural and forestry lignocellulosic residues as well as energy crops to fermentable sugars is of outstanding interest, and one of the greatest challenges for the society in the 21st century, envisaging improved environmental quality and sustainable energy production. For instance, a combined effect of higher hydrolysis-fermentation efficiency, lower specific capital investments, increase of scale and cheaper biomass feedstock costs, could contribute with significant reduction on ethanol production costs. The pace of recent developments, and often discussions about the "Four F" (Food, Feed, Fiber and Fuel) which synthesize the main destinations of agricultural products, suggests that accelerating the use of agricultural residues, as supplemental feedstock for bioethanol production in comercial scale, is a potential solution for reducing production costs, and most likely will come on line in the near future.

Actions towards the development in technology by ethanol producers, vendors of process technology, government and academic laboratories have been initiated in many countries, but asides the continuous need for technological development, the rapid increase of the biofuel industry urges governments to create and implement new standards for the transport sector based on liquid biofuels from renewable resources, in order to reduce the fossil fuels dependency, as well as to lower the contribution of petroleum derivatives to climate changes and air pollution.

Finally, we believe that providing reliable data on bioethanol production is essential for global socio-economic and environmental assessments, and for the design of new technologies, enabling the ample use of biofuels for our future generations.

ACKNOWLEDGEMENTS

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