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Advances in the Development of Bioethanol: A Review

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1. Introduction

Henry Ford, father of the modern automobile, constructed his Model T in the early years of the 20th century, when he planned to fuel it with ethanol obtained from cereals. Ford promoted the use of this fuel with such conviction that, by 1938, plants in Kansas were already producing 18 million gallons of ethanol a year (about 54,000 t/year). But interest in ethanol declined after the Second World War because of the enormous availability of natural gas and oil.

At the end of the Seventies, following the first oil crisis, various oil companies began to sell a petrol containing 10% of ethanol, called gasohol, taking advantage of the tax deductions granted on ethanol. Bioethanol did not immediately meet with the success it deserved, however, because it already had competitors on the market, such as methyl tert-butyl ether (MTBE), which was better than ethyl tert-butyl ether (ETBE) in both economic terms and performance. In subsequent years, MTBE proved to be heavily polluting, so it was banned and bioethanol returned to become one of the most attractive prospective solutions for reducing CO_2 emissions.

Another factor that helped to relaunch bioethanol was the growing awareness that we are nearing the so-called tipping point, i.e. the moment commonly indicated as the critical point of no return, when the curve of the demand for oil intersects the declining curve of its availability.

There is an ethical issue, however, that particularly concerns bioethanol, but also affects the other fuels of biological origin. Biofuels are obtained mainly from raw materials such as plants and cereals, that would otherwise be destined for the foodstuffs industry.

To deal with this problem, recent research has been concentrating on an inedible perennial herbaceous plant called Miscanthus giganteus that has a calorific value of approximately 4200 kcal/kg of dry matter. Using lignocellulose materials, municipal solid waste or the wheat wasted each year (around 5%, which would provide about 9.3 Gl of bioethanol) could also overcome the ethical obstacles.

Bioethanol can be used in various forms: added in proportions of 5-10% to the diesel oil in diesel engines; mixed in proportions of 10-85% in petrol for internal combustion engines, or to replace 0-100% of the petrol used in flexible fuel vehicles (FFV). The number of FFV on the roads is constantly increasing: in Brazil their sales now reach 400,000 vehicles/year and

there are approximately 1,500,000 of them (mainly public vehicles) circulating in the USA; in Europe, Sweden has around 15,000 vehicles of this type fueled with E85 (85% ethanol).
Research is also underway on improved engines fueled with bioethanol, and on fuel cells that use the internal reforming of bioethanol to obtain hydrogen.

1.1 Ethyl tertiary butyl ether

Ethyl tertiary butyl ether (ETBE) is a high-octane bioethanol product obtained mainly by making the ethanol react with isobutylene (a byproduct of oil refining) under the effect of heat and various catalysts. It is consequently considered as being partially renewable.
ETBE has technological and functional features that are very like and distinctly better than those of the alcohol it is obtained from. Moreover, it lacks the latter's problems of volatility or miscibility with petrol and it features a high octane number.
Being an ether, it contains oxygen in the molecule, and this enables it to help improve the vehicle's emissions of pollutants. A recent paper (Da Silva et al., 2005) conducted a study on the effects of the anti-detonating properties and Reid vapor pressure (RVP) of petrols mixed with various additives, concluding that adding ETBE improves the mixture's anti-detonating properties and reduces the vapor pressure without interfering with the volatility needed to start a cold engine.
ETBE obtained from bioethanol (also called bioETBE) offers the same benefits as bioethanol, i.e. a lower emission of pollutants, a higher octane number and a reduction in crude oil imports, without the technical and logistic problems posed by the alcoholic nature of bioethanol. BioETBE also contributes to the diffusion of biofuels in the transport sector.

1.2 Diesel and bioethanol mixtures (e-diesel)

The development and increasing use of diesel and bioethanol mixtures in diesel engines has been driven mainly by the European countries needing to comply with the European Union directive 2003/30/CE (which establishes that at least 5.75% of the fuels market must consist of biofuels by the year 2010), as well as the need to dispose of a petrol surplus in the refineries due to the greater demand for diesel vehicles. The drawbacks of the so-called e-diesel mainly concern a reduced viscosity and lubrication issues, a lower cetane number and injection capacity, a greater volatility (which can lead to an increase in the emissions of uncombusted hydrocarbons) and a lesser miscibility (Marek & Evanoff, 2001; Hansen et al., 2005; Lapuerta et al., 2007). In particular, Lapuerta et al. studied different diesel-bioethanol mixtures in different conditions of temperature, water content and additives, developing level maps that give a precise idea of the mixtures' areas of stability and of kinetic separation, that prompt the following conclusions:

- the presence of water in the mixture facilitates the separation of the ethanol phase;
- when its temperature increases, the mixture becomes more stable and the solubility of the ethanol in the diesel also increases;
- the mixture's sensitivity to the effects of water content and additives is higher, the higher the temperature of the mixture;
- mixtures with a bioethanol content up to 10% (v/v) can be used in diesel engines in regions where temperatures in winter rarely drop below -5°C;
- using stability-improving additives can increase the range of ethanol proportions in the mixtures, or the geographical extension of their applicability, enabling any phase separation to be avoided.

1.3 Research projects and bioethanol promotion

To succeed in demonstrating the feasibility of replacing petrol and diesel oil with bioethanol, the European Union developed the BEST project (BioEthanol for Sustainable Transportation) (European Union, 2011), involving six European countries (Sweden, the Netherlands, the United Kingdom, Ireland, Spain and Italy), and also Brazil and China: the global aims of the project are to introduce at least 10,500 FFV and 160 bioethanol-fueled buses, as well as to build 148 service stations, 135 to provide E85 and 13 to provide E95.

The NILE project (New Improvements for Lignocellulosic Ethanol) (Eurec, 2011) focuses instead on proposing the best processes for an economically effective production of bioethanol from lignocellulose biomass, suitable for use in internal combustion engines. The main goal of the NILE project is to reduce the cost of producing bioethanol from this type of raw material so as to make the technology commercially competitive. The NILE project brings together 21 industrial and research organizations from 11 member states, with complementary professional backgrounds and expertise so as to cover the whole cycle of bioethanol production and usage. On a technical level, the problems that remain to be solved concern reducing the cost of the enzymatic hydrolysis process by developing new artificial enzymatic systems, eliminating the current drawbacks intrinsic in converting fermentable sugars into ethanol, and validating the artificial enzymatic systems and yeast strains in a fully-integrated pilot plant.

Finally, there is the European LAMNET research program (Latin America Thematic Network on Bioenergy) (LAMNET, 2011), the main aim of which is to establish a trans-national forum to promote the sustainable use of biomass in Latin America and other emerging countries.

2. Raw materials

One of the great merits of bioethanol consists in the enormous variety of raw materials, and not only plants, from which it can be produced. The production methods vary depending on whether or not the raw material is rich in fiber.

The basic materials for producing biofuels must have certain features, including high carbon and hydrogen concentrations and low concentrations of oxygen, nitrogen and other organic components. The following is a brief description of some of the most important raw materials suitable for use in bioethanol production.

2.1 Alfalfa (medicago sativa)

This is a lucerne of the Fabaceae family that grows in cool subtropical and warm temperate regions. It demands no nitrogen-based fertilizers and its leaves are a precious source of protein in animal fodder. In a recent paper (Dien et al., 2006) it was observed that this plant has a low glucose yield due to a low-efficiency cellulose hydrolysis. The stems contain high concentrations of crude proteins and organic acids.

2.2 Switch grass (panicum virgatum)

This is a perennial herbaceous plant that grows mainly in the United States. Its ethanol yield per hectare is the same as for wheat. It responds to nitrogen fertilizers and can sequester the carbon in the soil. It is a highly versatile plant, capable of adapting easily to lean soils and marginal farmland (Heaton et al., 2004). Like maize, it is a type C_4 plant, i.e. it makes an alternative use of CO_2 fixation (a process forming part of photosynthesis). Most of the

genotypes of Panicum virgatum have short underground stems, or rhizomes, that enable them with time to form a grassy carpet. Single hybrids of Panicum virgatum have shown a marked potential for increasing their energy yield (Bouton, 2007), but genetic engineering methods on this plant are still in a developmental stage and for the time being only their tetraploid and octaploid forms are known; we also now know that similar cell types (isotypes) reproduce easily.

2.3 Sweet sorghum (sorghum bicolor L)

The grains obtained from this plant are rich in starch and the stems have a high saccharose content, while the leaves and bagasse have a high lignocellulose content. The plant can be grown in both temperate and tropical countries, and it tolerates drought, flooding and alkalinity. Sorghum is considered an excellent raw material because the methods for growing and transporting it are well established. Ethanol can be obtained from it by exploiting both its starch and its sugar content. Research is currently underway on the use of hybrid or genetically modified species, although those obtained so far are weaker and need to be further refined and tested as concerns energy conversion efficiency (Rooney et al., 2007).

2.4 Cassava (manihoc esculenta)

This tuber is of considerable interest not only for ethanol production but also to produce glucose syrup, and it is available in tropical countries. The ethanol yield from the whole manioc is equivalent to the ethanol produced from cereals using dry milling methods. The only known lies in that the manioc has to be processed 3-4 days after it was harvested. To avoid such lengthy processing times, the manioc is first sliced and then left to dry in the sun. The waste water produced in the process can be treated by means of anaerobic digestion to produce bio gas.

2.5 Spruce (picea abies)

This tree has attracted a great deal of attention as a raw material for ethanol production because it is a lignocellulose material mainly composed of hexose sugars, which are more readily convertible than pentose sugars.

2.6 Willow (salix)

This is a member of the Angiosperm family and is consequently characterized by a hard wood. In this species, a fraction of the xylose units is acetylated. Some of the OH groups of the xylose carbons C_2 and C_3 are replaced by O-acetyl groups. With pretreatment, these groups release acetic acid that, in high enough concentrations, inhibits the yeasts involved in the fermentation process, according to some studies (Sassner et al., 2008a). It was recently demonstrated (Sassner et al., 2008b) that, by pretreating willow with sulfuric acid before the enzymatic hydrolysis process, and then simultaneously performing saccharification and fermentation, they succeeded in obtaining a global ethanol yield of 79%.

2.7 Reed canary grass (phalaris arundinacea)

This is a type C_3 perennial herbaceous plant that grows in the cool season and has an excellent resistance to flooding. Its productivity is strongly influenced by high levels of nitrogen fertilizers, a feature that makes it very useful for the distribution of fertilizer from livestock.

2.8 Sugar cane (saccharum officinarum)

This plant only grows well in tropical and subtropical regions, which is why it is particularly common in Brazil. It has a 12-17% sugar content, 10% of which is glucose and the other 90% is saccharose. Milling can extract 95% of the total sugar content and the juice can subsequently be used to produce sugar or allowed to ferment to produce bioethanol. The bagasse (i.e. the solid residue remaining after milling) can be used as a source of energy and heat.

2.9 Sugar beet (beta vulgaris)

This plant generally grows in the cooler temperate regions, so it is abundant in Europe, North America and Asia. In the ethanol production process, the sugar beet is sliced and, while the juice is used to produce sugar or ethanol, the pulp is dried and used as animal feed or sold for pharmaceutical purposes.

2.10 Cereals

These must be ground to obtain starch, from which bioethanol is subsequently obtained. The cereals containing fewer proteins and more carbohydrates are preferable for distilling purposes because they have a higher bioethanol conversion rate. This means that the nitrogen content in the cereals can be adapted to facilitate starch accumulation instead of proteins synthesis, thereby improving both the energy yield and the quality of the fermentation process (Rosenberg et al., 2001). The principal cereals are:

2.10.1 Wheat

It grows mainly in temperate regions. The wheat treatment process is much the same as for the other cereals and it is best to use high-gravity fermentation to obtain the best performance in the fermentation process.

2.10.2 Barley

The most suitable is the so-called Winter variety, which is often underestimated as a foodstuff, despite the fact that it can tolerate drought and is highly adaptable.

2.10.3 Winter rye (secale cereale L)

This cereal relies heavily on the availability of nitrogen in the soil; it has high contents of both glucan and xylan (40.8% and 22.3% respectively) (Petersson et al., 2007).

2.10.4 Corn stover

This is what remains on the ground after maize has been harvested. This raw material is abundantly available and demands no further investment in biomass, although not all of the corn stover can be removed - 30% of it must be left on the ground to prevent erosion (by facilitating water infiltration and reducing evaporation), and as the main source of soil organic carbon (SOC) in order to preserve the soil's productivity. Corn stover contains polymeric hemicellulose and cellulose, but their biodegradability by glycosidase is strongly inhibited by a small quantity (12-15%) of lignin (Gressel, 2008; Varvel et al., 2007).

2.11 Jerusalem artichoke (helianthus tuberosus)

This plant grows in summer, reaching its maximum height in July and dying in October. The tubers are rich in inulin (a fructose polymer), which can be used to obtain a syrup for

use both in the foodstuffs industry and in the production of ethanol. It was demonstrated (Curt et al., 2006) that, towards the end of the season, the potential for bioethanol production of the stems of clones is 38% of that of the tubers.

2.12 Municipal solid waste (MSW)

The most suitable waste for converting into bioethanol is the waste from the fruit and vegetable industries, for instance, cotton fiber, milk whey from cheese-making, the waste products of coffee making, and so on. Generally speaking, such waste contains approximately 45% of cellulose (glucose polymer), which can be simultaneously hydrolyzed and fermented to produce ethanol. SSL (Spent Sulfite Liquor) is a byproduct of bisulfite "pulp" manufacturing that can also be fermented to produce ethanol. Waste varies considerably in content from one area to another, but the majority of the volume generally consists of paper (20-40%), gardening waste (10-20%), plastics, glass, metals and various other materials (Prasad et al., 2007).

2.13 Miscanthus

This is a type C_4 graminaceous perennial that forms rhizomes. Miscanthus x giganteus is generally used to obtain biofuels: this is a sterile tetraploid hybrid obtained from Miscanthus sinensis and Miscanthus sacchariflorus, characterized by a yield that in autumn reaches 30 t ha^{-1} in irrigated soils and 10-25 t ha^{-1} in those without irrigation. The contribution of Miscanthus sacchariflorus to the Miscanthus x giganteus genome lies in its adaptability to warm climates, while Miscanthus sinensis provides the genetic resources needed in the colder regions. It is often used as an ornamental grass or cover crop and it can grow as much as 4 m high. It takes three years to arrive at a stable yield (around 5 years in marginal soils) and in its first year of growth the rhizomes are particularly sensitive to low temperatures, whereas in subsequent years they can even withstand temperatures of around -40°C. The rhizomes remain inactive in winter and begin to grow when the temperatures of the soil reaches 10-12°C. As for the plant's energy value, the dry matter has a net calorific value of approximately 17 MJ/kg. The energy value of 20 t of dry Miscanthus is approximately the same as that of 8 t of coal (Heaton et al., 2004; Sánchez & Cardona, 2008; DEFRA, 2011).

When Panicum virgatum and Miscanthus (Heaton et al., 2004) - both type C_4 plants of considerable interest as energy sources - are compared, Miscanthus produces more biomass per unit than Panicum virgatum (i.e. 12 Mg ha^{-1}). Both plants are perennials and this means a saving because there is no need to replant them. In areas with an abundant rainfall but problems of nitrogen contamination of the water supply, it is better to use Miscanthus as an energy crop, whereas growing Panicum virgatum with adequate nitrogen fertilizing certainly produces a better yield in uncontaminated dry areas.

3. Production processes

Bioethanol production processes vary considerably depending on the raw material involved, but some of the main stages in the process remain the same, even though they take place in different conditions of temperature and pressure, and they sometimes involve different microorganisms. These stages include hydrolysis (achieved chemically and enzymatically), fermentation and distillation.

Hydrolysis is a preliminary treatment that enables sugars to be obtained from the raw materials that are then fermented. In the case of enzymatic hydrolysis, effective

pretreatments are needed, however, to increase the susceptibility of lignocellulose materials to the action of the enzymes. The following paragraphs describe the various production methods, distinguishing them according to the type of raw material involved.

3.1 Lignocellulose biomass

The biofuels obtained from wood cellulose and from organic materials in general offer considerable advantages over conventional biofuels. Burning ethanol obtained from cellulose produces 87% lower emissions than burning petrol, while for the ethanol from cereals the figure is no more than 28%. Ethanol obtained from cellulose contains 16 times the energy needed to produce it (Martinez et al., 2008), petrol only 5 times and ethanol from maize only 1.3 times. The problem is a matter of how to disrupt the bonds of this molecule in order to convert it into fermentable sugars.

In fact, this is unquestionably the type of raw material that is the most complicated to process. The starting material may be farming and forest waste, scrap woods, grassy crops grown for energy purposes or even municipal solid waste. Lignocellulose occurs in the walls of vegetable cells and consists of cellulose microfibers contained in the lignin, hemicellulose and pectin. The procedure to obtain ethanol consists first in depolymerizing the carbohydrates into their monomeric sugars, then fermenting the sugars with the aid of appropriate microorganisms. The lignocellulose biomass consists mainly of three basic polymers: cellulose, hemicellulose (such as xylane), lignin and other minor components (essential oils, acids, salts and minerals).

3.1.1 Pretreatments

These are used to modify the structure and dimensions of macroscopic and microscopic raw materials, and also their chemical composition. They have the effect of solubilizing the hemicellulose, reducing the crystallinity, and increasing the available surface area and porosity of the substrate. An effective pretreatment must meet following requirements: - it must increase sugar formation or facilitate the subsequent formation of sugars during hydrolysis, preventing any degradation or the loss of carbohydrates, and avoiding the formation of byproducts capable of inhibiting the subsequent processes of hydrolysis and fermentation, all at a competitive cost (Balat et al., 2008).

Pretreatments are particularly essential before enzymatic hydrolysis and may be of various types, i.e. physical, chemical, biological, steam explosion, and ammonia fiber explosion (AFEX).

Physical pretreatments may or may not be mechanical. The mechanical physical pretreatments include milling and grinding, that not only reduce the substrate, but also increase its surface area to volume ratio, thus making the cellulose easier to convert during hydrolysis. "Ball milling" could also be used to reduce the crystallinity of the cellulose, but this practice is not only very expensive, but also takes a long time (nearly a week) to complete, so it is hardly practicable on an industrial scale. The non-mechanical pretreatments feature a combination of high-power internal and external forces that decompose the lignocellulose.

Chemical pretreatments are used mainly to reduce the crystalline content of the cellulose. Using this type of pretreatment poses plant-related problems, however, since all the structural materials have to be capable of withstanding the severe working conditions imposed by the chemical agents.

The chemical pretreatments most often used are an alkaline treatment to delignify and solubilize the glycan, and an NaOH treatment that dissolves the lignocellulose biomass, destroying its lignin structure. Pretreatment with diluted sulfuric acid is also very important but this poses serious problems if it is associated with diluted acid hydrolysis, because the hydrolyzed end products become scarcely fermentable.
Other chemical pretreatments include: pretreatment with hydrogen peroxide (H_2O_2), which exploits oxidative delignification to separate and solubilize the lignin, and dissolve the lignocellulose matrices, thereby increasing the enzymatic digestibility of the mass; pretreatment with ozone, which degrades the lignin polymers; and pretreatment with liquid hot water (LHW), which is applied mainly to alfalfa. It was demonstrated (Laser et al., 2002) that, in ideal conditions, this method is as effective as diluted acid hydrolysis, without the need to use any acid or create any products of neutralization).
Biological pretreatments involve the use of enzymes, which are already useful in industrial processes on timber waste, in the processing of pulp and scraps. Several microorganisms studied years ago are the enzymes produced by the basidiomycetes Pleurotus ostreatus: these enzymes are homologous proteins characterized by different specifications, depending on which phenols are substituted. Another fungus in the basidiomycetes class that is effective in delignification is the Phanerochaete chrysosporium (Palmieri et al., 1997).
In the steam explosion process, saturated steam is used at very high temperatures and pressures to break up the chemical bonds in the cellulose, hemicellulose and lignin in order to break down the fibers and hydrolyze the biomass. The process consists in delivering steam under high pressure into a sealed chamber containing the lignocellulose material, then reducing the pressure and thus making the steam and matrix expand, and obtaining its explosive decompression through an orifice, which disrupts the cellular structure of the substrate, breaking up the acetyl groups of the hemicellulose. In some cases (e.g. Angiosperm), it is preferable to use acid catalysts, such as H_2SO_2 or SO_2, to make the cellulose-rich components more accessible to the enzymes. SO_2 gas is better able to attack the fibers (Shevchenko et al., 2000), but its use makes it necessary to carefully consider the working conditions in which the steam explosion takes place. In fact, it becomes necessary to find the best compromise between a strong enzymatic hydrolysis (obtainable in very severe conditions) and a good recovery of the components containing hemicellulose, that are in the form of monomeric sugars (which demand much less severe conditions) (Silverstein et al., 2007). That is why a severity indicator has been developed (Overend & Chornet, 1987), which correlates pretreatment temperatures and times, assuming that the pretreatment obeys Arrhenius's equation and has first-order kinetics. The indicator R_0 is:

$$R_o = t \cdot \exp\left[\frac{(T_r - T_b)}{14.75}\right] \tag{1}$$

where t is the duration of the pretreatment (min), T_r is the reaction temperature (°C), T_b is the baseline temperature (100°C) and the constant 14.75 is the conventional activation energy, assuming that the whole conversion is of the first order. If the version with sulfuric acid is being used, then the severity parameter, called M_0 in this case, is slightly modified:

$$M_o = t \cdot C^n \cdot \exp\left(\frac{T_r - T_b}{14.75}\right) \tag{2}$$

where *C* is the chemical concentration (wt%) and n is an arbitrary constant (Chum et al., 1990).

Ammonia fiber/freeze explosion (AFEX) pretreatment involves the use of liquid ammonia and steam explosion: in this process, the previously-humidified lignocellulose material is placed in a vessel under pressure with liquid NH_3 in proportions of 1-2 kg NH_3/kg of dried biomass. This method is very effective for non-woody materials such as bagasse and newspaper, but less so in the case of "soft" wooden materials. This system does not release any sugars directly, but it does make the polymers (hemicellulose and cellulose) easier for the enzymes to attack. The ammonia can also be replaced with carbon dioxide because the latter is relatively less costly and also because the alcohol waste product contains traces of pollutants that would thus derive only from the lignin.

The most promising pretreatments for farming waste are AFEX and LHW, while pretreatment with steam affords a high output of sugars from both farming waste and forest waste.

3.1.2 The hydrolysis process

Hydrolysis is governed by the law:

$$\left(C_6H_{10}O_5\right)_n + nH_2O \rightarrow nC_6H_{12}O_6 \tag{3}$$

and can be mainly of two types: acid (using diluted or concentrated acids) or enzymatic. A lignocellulose biomass is more complicated to hydrolyze than pure cellulose because it contains components that are not glucose-based, such as hemicellulose and lignin.

A lignocellulose biomass undergoing acid hydrolysis mainly produces xylose, while the lignin and cellulose fractions remain unchanged. This is because xylan is more susceptible to hydrolysis in moderately acid conditions because of its amorphous structure, while cellulose demands more severe conditions because of its crystalline nature.

If hydrolysis is implemented using 1% diluted sulfuric acid, the hemicellulose is depolymerized at a lower temperature than the cellulose. This process is usually conducted in two consecutive stages.

One of the most important characteristics of this type of hydrolysis is the rate of the reactions involved, which facilitate the continuity of the process. To speed up the diffusion of the acid, the raw material is mechanically reduced to pieces a few millimeters in size.

Hydrolysis with concentrated acids (10-30%), on the other hand, rapidly and completely converts cellulose into glucose and hemicellulose into xylose, with some degree of degradation. The acids most often used are sulfuric and hydrochloric acid, and hydrogen fluoride.

This type of acid hydrolysis has the great advantage of recovering the sugars very efficiently (approximately 90% of hemicellulose and cellulose are depolymerized into monomeric sugars). From an economic standpoint, this process enables a reduction in production costs by comparison with the diluted acid solution, especially if the acids are retrieved and reconcentrated. The acids and sugars in solution are separated by ion exchange so the acid is reconcentrated by passing it through a series of multiple-effect evaporators. The remaining solid fractions, which are rich in lignin, are collected and can be made into pellets for use as fuel.

So, in short, we can divide concentrated acid hydrolysis into two stages: in the first stage, the concentrated acid (70%) destroys the crystalline structure of the cellulose, breaking up

the hydrogen links between the cellulose chains; in the second stage, hydrolysis induces a hydrolytic reaction in the single isolated cellulose chains.

The enzymatic hydrolysis of natural lignocellulose materials is a very slow process, because it is hindered by several structural parameters of the substrate, such as its of cellulose and hemicellulose content, and the surface area and crystallinity of the cellulose. Pretreatments are consequently needed to make the biomass more susceptible to attack by hydrolysis. For the same reason, a cocktail of enzymes has to be used that is capable of breaking the links in the polymeric chains. This cocktail is usually a mixture of various hydrolytic enzymes, including cellulase, xylanase, hemicellulase and mannoxidase. Enzymatic cellulose degradation is a complex process because it takes place in limit conditions between the solid and liquid phases, where the enzymes are the mobile components. Generally speaking, degradation is characterized by a rapid initial phase followed by a slower second phase that can continue until all the substrate has been used up. The reason for this behavior is usually assumed to be because the accessible fraction of cellulose is quick to hydrolyze, followed by the slow activation of the absorbed enzyme molecules.

Chopping up the biomass increases the surface area accessible to the enzymes and reduces the polymerization and crystallinity of the cellulose, thus enabling a smaller quantity of enzymes to be used and the production costs to be contained.

Both bacteria and fungi can produce the cellulase for the hydrolysis of lignocellulose materials. The bacteria may be aerobic or anaerobic, mesophylic or thermophylic. The bacteria most often used are Clostridium, Cellulomonas, Bacillus, Thermomonospora, Ruminococcus, Bacteriodes, Erwinia, Acetovibrio, Microbispora and Streptomyces. The enzymes are usually classified according to their reaction site, so they may be intracellular (or cell-associated) or extracellular. The main function of extracellular enzymes is to convert the substrate into an external medium by taking effect on the cell mass constituents. Conversely, intracellular enzymes need the substrate to spread through the cellular mass before it can be converted.

The most widely accepted mechanism for the enzymatic hydrolysis of cellulose involves the synergic action of the enzymes endoglucanase (or endo-1,4-β-glucanase, EG), exoglucanase (or cellobiohydrolase, CBH), and β-glucosidase. Both EG and CBH are extracellular enzymes, while β-glucosidase is intracellular. EG randomly disrupts the cellulose chains, consequently inducing their strong degradation. It takes effect by hydrolyzing the β-1,4-glucoside bonds, creating new ends in the chains. Exoglucanase breaks up the ends of the chains, thus enabling the release of soluble cellobiose or glucose. BGL hydrolyses the cellobiose into glucose, thus eliminating the inhibitory cellobiose; then BGL completes the process by catalyzing the hydrolysis of cellobiose into glucose. Most cellulase and hemicellulase producers are microorganisms such as the filamentous fungi, e.g. Trichoderma sp., which can be used in their natural form or genetically modified (Trichoderma viride, Trichoderma reesei, Trichoderma longibrachiatum). CBH I and CBH II are the main enzymes of Trichoderma reesei, while EG I and EG II are the dominant endoglucanases.

Enzymatic activity is influenced by various parameters, such as temperature (a 20-30°C increase in temperature leads to a 3- to 5-fold increment in the end products). The crucial issue of temperature lies in the risk of an unwanted denaturation when the temperature is too high (Balat et al., 2008). Enzymatic hydrolysis, with or without the addition of catalysts, has generally proved capable of a high yield of both glucose (>90%) and xylose (>80%).

3.1.3 Fermentation

After hydrolysis, the hydrolyzed products must be fermented by means of microorganisms such as yeasts (Hahn-Hägerdal et al., 2006). Since the hydrolyzed products are composed mainly of glucose, xylose, arabinose and cellobiose, the microorganisms used must be capable of fermenting all of them efficiently for ethanol to be produced on a large scale. The reactions that involve glucose and xylose are respectively:

$$3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2 \quad (4)$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (5)$$

The classic method used in the fermentation of the hydrolyzed biomass is separate hydrolysis and fermentation (SHF), in which the two processes are completed in different units. A commonly used alternative is simultaneous saccharification and fermentation (SSF), in which hydrolysis and fermentation are completed in the same unit. A last option is represented by consolidated bioprocessing (CBP).

When the SHF process is used, the solid fraction of the lignocellulose material undergoes hydrolysis and this process is called saccharification. The liquid fraction, on the other hand, goes first to the reactor for glucose fermentation, then it is distilled to extract bioethanol, leaving behind only the unconverted xylose, which is then fermented in a second reactor and then undergoes a second, final distilling phase.

The main advantage of this process consists in that separating the processes of hydrolysis and fermentation enables optimal working conditions to be adopted in each case. The enzymes are free to work at high temperatures, while the microorganisms can induce fermentation at more moderate temperatures.

Among the disadvantages, in addition to needing two twin reactors, there is the fact that the enzymes for hydrolyzing the cellulose are inhibited end products. The rate of hydrolysis progressively declines due to the accumulation of glucose and cellobiose.

This process has sometimes been used to produce ethanol from a mix of municipal solid waste: in this case, enzyme recycling was improved using micro- and ultra-filtering procedures, thus achieving the hydrolysis of 90% of the cellulose with a net enzyme load of 10 FPU/g of cellulose (where FPU stands for filter paper unit) (Sánchez & Cardona, 2008).

In the SSF procedure, enzymatic hydrolysis and fermentation take place simultaneously. Cellulases and microorganisms take effect in the same process, so the glucose produced by hydrolysis of the cellulose is immediately consumed by the bacterial cells that convert it into ethanol. SSF achieves the highest output of bioethanol at the lowest costs, since the lesser demand for enzymes is lower because the inhibitory effect of the cellobiose and glucose end products is alleviated by fermentation with yeast. This is a discontinuous type of process that uses natural heterogeneous materials containing complex polymers such as lignin, pectin and lignocellulose. The greatest advantages offered by SSF are a faster rate of hydrolysis thanks to the conversion of the sugars that inhibit cellulase activity, a low enzyme demand, a high product yield, the need for less sterile conditions, a shorter process time, and smaller overall reactor dimensions (Sun & Cheng, 2002).

This process also has far from negligible disadvantages, however, the most significant of which consists in the need to complete fermentation and hydrolysis in suboptimal conditions. That is why microorganism selection and preparation is so important for this process. The cocktail of enzymes for hydrolyzing the cellulose must likewise remain stable

within a wide range of temperatures and pH. As for the Saccharomyces cerevisiae cultures, the typical working conditions in SSF involve a pH of 4.5 and temperatures of around 310 K. Experiments have recently been conducted with a new variant of this process called simultaneous saccharification and cofermentation (SSCF), in which the five- and six-carbon sugars are fermented simultaneously. In SSCF, hydrolysis continuously releases hexose sugars that increase the rate of glycolysis, so that the pentose sugars can ferment more quickly and produce a higher yield.

In CBP, four biologically-mediated conversions take place in a single process, i.e. the production of glycolytic enzymes (cellulase and hemicellulase), hydrolysis of the carbohydrate component of the pretreated biomass to obtain sugars, fermentation of the six-carbon sugars (mannose, galactose and glucose), and fermentation of the five-carbon sugars (xylose and arabinose).

The main difference between CBP and the other processes consists in that there is no single process focusing on cellulase production. CBP, also known as direct microbial conversion (DMC), requires just one microbial community for both cellulase production and fermentation. The weakness of this approach lies in the difficulty of finding an organism sturdy enough to simultaneously produce cellulase and ethanol with a high yield. Wyman (Wyman, 1994) wrote that many studies on CBP involved the use of the bacterium Clostridium thermocellum for enzyme production, cellulose hydrolysis and glucose fermentation, while Clostridium thermosaccharolyticum enabled the simultaneous conversion of the pentose sugars obtained from hemicellulose hydrolysis into ethanol. Using Clostridium thermocellum in the system also induces a 31% higher conversion of the substrate than when Trichoderma reesei or Saccharomyces cerevisiae are used. Recent studies have focused on cellulase production combined with a high ethanol yield using strains of Escherichia coli, Klebsiella oxytoca and Zymomonas mobilis as well as the yeast Saccharomyces cerevisiae. The expression of cellulase in Klebsiella oxytoca increased the yield from microcrystalline cellulose hydrolysis and enabled an anaerobic growth in the amorphous cellulose. Various cellobiohydrolases have likewise been functionally expressed in the Saccharomyces cerevisiae. Genetic engineering and metabolic studies will enable the development of new stable strains of microorganisms capable of converting the cellulose biomass into bioethanol, leading to improvements in the industrial bioethanol production process (Lynd et al., 2005).

The microorganisms used during the fermentation process must be capable of working efficiently on both monosaccharide and polysaccharide sugars, so they have to be very versatile. The survival of these bacteria is only assured in controlled pH conditions and the majority of the microorganisms cannot tolerate bioethanol concentrations in excess of 10-15% (w/v).

Saccharomyces cerevisiae is one of the microorganisms most often used because it affords a high ethanol yield from hexose sugars, and it can tolerate bioethanol and inhibitory compounds very well. It has the great disadvantage, however, of being unable to assimilate C_6 sugars.

The ethanol-generating bacteria that seem industrially most promising are Escherichia coli, Klebsiella oxytoca and Zymomonas mobilis. Zymomonas, in particular, has demonstrated an aptitude for rapidly and efficiently producing bioethanol from glucose-based raw materials and, by comparison with the other yeasts, it has demonstrated a 5-fold higher yield. The ethanol it produces in the fermentation of the glucose corresponds to a yield that is 97% of the theoretical yield and in concentrations up to 12% (w/v). This bacterium is also

capable of producing bioethanol efficiently from fructose and saccharose (C_5), but not from C_6 sugars.
There are also yeasts that naturally ferment xylose, such as Pichia stipitis, Candida Shehatae and Candida parapsilopis, and they can do so through the action first of xylose reductase (XR), which converts xylose into xylitol, and then of xylitol dehydrogenase (XDH), which converts xylitol into xylulose. Bioethanol fermentation from xylose can also be achieved by recombinant Saccharomyces cerevisiae using the heterologues XR and XDH of Pichia stipitis and xylulose kinase (XK) of Saccharomyces cerevisiae.
Table 1 summarizes the commonly used bacteria and microorganisms (Balat et al. (2008)), highlighting the principal parameters used to assess the performance of the various types of fermentation.

Species	***Characteristics***
Clostridium acetobutilicum	Useful in fermentation of xylose to acetone and Butanol.
Clostridium thermocellum	Capable of converting cellulose directly to ethanol and acetic acid.
Escherichia coli	Native strains ferment xylose to a mixture of Bioethanol.
Klebsiella oxytoca	Native strains rapidly ferment xylose and cellobiose.
Lactobacillus pentoaceticus	Consumes xylose and arabinose.
Latobacillus casei	Ferments lactose very well; particularly useful for bioconversion of whey.
Lactobacillus xylosus	Uses cellobiose if nutrients are supplied: uses nglucose, D-xylose, and L-arabinose.
Lactobacillus pentosus	Homolactic fermentation. Some strains produce lactic acid from sulfite waste liquors.
Lactobacillus plantarum	Consumes cellobiose more rapidly than glucose, xylose, or arabinose.
Zymomonas mobilis	Normally ferments glucose and fructose.

Table 1. Commonly used bacteria and microorganisms (Balat et al. (2008)).

Fermentation can occur in various ways, i.e. discontinuously, continuously, with cells immobilized, and batch-fed (Chandel et al., 2007).
A problem encountered in enzymatic hydrolysis consists in the formation of inhibitors. The activity of the enzymes is strongly influenced by certain levels of cellobiose, glucose or products such as furfural and organic acids deriving from pretreatments.
Inhibitors form in relation to the conditions in which enzymatic hydrolysis takes place. Conditions can be selected that should provide maximum solubilisation and recovery of the hemicellulose component (low severity), optimum enzymatic hydrolysis of the water

insoluble cellulosic component (high severity), and a compromise between the two conditions (medium severity). The combined severity (CS) links the severity factor (R0) to the ambient pH, and this index expresses the intensity of the previously-described factors. Its value is expressed as:

$$CS = \log R_0 - \text{pH} \tag{6}$$

When the CS increases beyond the value that generates the highest concentrations of mannose and glucose, the cellulose and hemicellulose break down and there is a drop in the concentration of fermentable sugars that coincides with the formation of furfural and hydroxy methyl furfural (HMF), which subsequently degrade into levulinic and formic acids. To achieve both the maximum fermentability and a high yield of fermentable sugars, the *CS* should be around 3 (Palmqvist & Hahn-Hägerdal, 2000)).

Inhibitors can come from various sources, e.g. equipment, carbohydrate degradation, lignin decomposition, wood extracts and their decomposition. They can be classified according to their structure as organic, acid, furanes and phenolic compounds. The fermentation inhibitors in particular include the furane derivatives, such as furfural and 5-hydroxy-methyl-furfural (5-HMF), the aliphatic acids, such as acetic acid, formic acid and levulinic acid, and the phenolic compounds. The furane derivatives can further react to form certain polymeric materials. The formation of inhibitory compounds makes it necessary to introduce changes in the production process, such as process water recirculation. It was demonstrated (Palmqvist et al., 1996), for instance, that unconcentrated hydrolyzed products have a moderately inhibitory action, while five-fold concentrations of nonvolatile components almost completely inhibit the fermentation of ethanol by Saccharomyces cerevisiae.

The formation of inhibitors and consequently of toxic compounds is a problem that has a negative fallout on the rate of both enzymatic hydrolysis and fermentation. The toxic compounds can form during steam explosion pretreatments and also during hydrolysis in the presence of low acid concentrations, and they are mainly the products of lignin degradation.

Four main groups of inhibitors have been identified in hydrolyzed lignocellulose products these are acetic acid from the hemicellulose fraction, products of lignin degradation, products of sugar degradation, and extracts that have been solubilized during the pretreatment.

The fermentation inhibitors, on the other hand, can be divided into various groups, depending on their origin:

- substances released during pre-hydrolysis and hydrolysis: acetic acid and extracts including terpenes, alcohols and aromatic compounds (e.g. tannins);
- inhibitors produced as a byproduct of pre-hydrolysis and hydrolysis, due to sugar degradation (furfural, 5-HMF);
- products of lignin degradation, including sizable groups of aromatic and polyaromatic compounds with a great variety of constituents (cinnamaldehyde, p-hydroxybenzaldehyde, syringaldehyde);
- products of the fermentation process, such as ethanol, acetic acid, glycerol and lactic acid;
- metals released by equipment and additives, e.g. nickel, iron, copper and chrome.

The compounds revealing the greatest inhibitory potential are acetic acid and the products of lignin degradation (Larsson et al., 1999).
A detoxification procedure can be used to improve the sugars' fermentability. Detox methods may be physical, chemical or biological, and they are impossible to compare directly with one another because the degree to which they can neutralize the inhibitors varies. The different microorganisms suitable for this purpose can tolerate the inhibitors to varying degrees. The choice of the most suitable method consequently depends on the raw materials involved and the composition of the hydrolyzed products. Figure 1 shows a flowchart of ethanol production from lignocellulose raw materials.

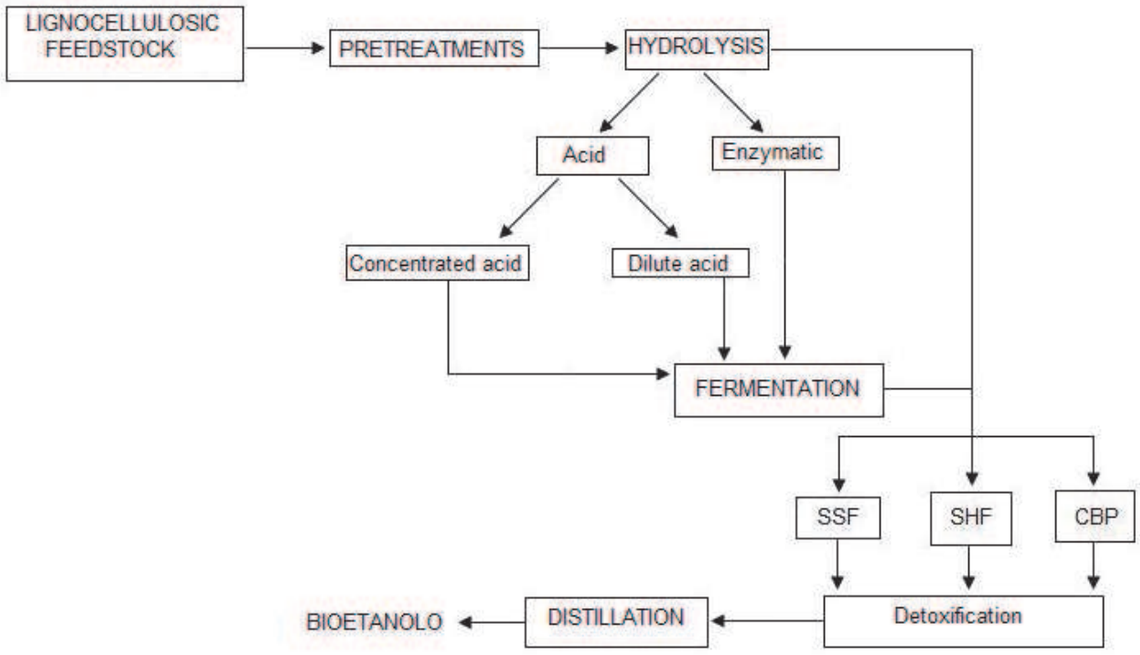

Fig. 1. Flowchart of ethanol production from lignocellulose raw materials

3.2 Raw materials containing starch

Starch is a biopolymer defined as a homopolymer. The constituent monomers are molecules of glucose held together by bonds between the oxygen atom of one molecule and the carbon atoms of adjacent molecules. These particular bonds are described as glycosidic and may be type α or type β, depending on the stereoisomery of the anomeric carbon in the molecule. Starch in plants occurs in the form of granules containing two main constituents in variable proportions, depending on the resource, i.e. amylose (16-30%) and amylopectin (65-85%). These are both type α glucose polymers. Amylose is a glucose polymer held together by α-1,4 bonds in linear chains, while amylopectin is a highly-branched glucose polymer with type α-1,6 bonds. Inside the cell, the starch is in the form of granules located in the amyloplasts. The granules contain both amorphous and crystalline regions, in proportions of approximately 30-70, respectively.
The starch for ethanol production comes mainly from cereals, wheat or corm being at the top of the list in North America and Europe, and tubers such as manioc in the tropical

regions. In order to produce bioethanol from starch, its carbohydrate chains have to be broken down to obtain glucose syrup, which can then be converted into bioethanol with the aid of yeasts.

3.2.1 Starch hydrolysis

Various microorganisms are capable of hydrolyzing starch, though a preliminary process called gelatinization is needed to ensure an efficient hydrolysis. During this preliminary process, the starch granules swell, particularly rupturing the hydrogen bonds in the crystalline regions. The long glucose chains comprising the starch must be converted into fermentable sugars by means of a process called the "hydrolysis technique", during which the starch reacts with the water normally used to break down the starch into its fermentable sugars. There are numerous microorganisms capable of hydrolyzing starch, but those involved in the starch degradation process are generally amylase, α-amylase, β-amylase and isoamylase. The most important for the purposes of the SSF process are certainly the first two. α-amylase is an endo-amylase that randomly attacks the α-1,4 bonds, rapidly reducing the starch molecule's dimensions and consequently also its viscosity, i.e. it liquefies the starch. α-amylase can be obtained by means of heat-resistant bacteria such as Bacillus licheniformis, or by means of new strains of Escherichia coli or Bacillus subtilis, used on the starch suspensions during the first hydrolysis stage. For amylase to succeed in attacking these suspensions, they must be brought up to high temperatures (90-110°C) to rupture the starch cell nuclei. The products of this preliminary hydrolysis phase, called liquefaction, is a solution containing dextrins and a small amount of glucose.

$$\text{Starch} \xrightarrow[\text{(liquefaction)}]{\alpha\text{-amylase}} \text{Dextrins} \xrightarrow[\text{(saccharification)}]{\text{glycoamylase}} \text{Glucose} \tag{7}$$

At this point, the liquefied starch undergoes saccharification at low temperatures (60-70°C), induced by the action of glycoamylase generally obtained from Aspergillus or Rhizopus species. This enzyme is an exo-amylase capable of producing units of glucose from amylose and amylopectin chains.

The factors that influence starch hydrolysis include the substrate, enzyme activity and the reaction conditions (temperature, pH and other parameters) (Prasad et al., 2007). The microorganisms take effect more easily on gelatinized starch, but this process demands large amounts of energy so on an industrial level there has been a tendency to focus on using microorganisms capable of growing on ungelatinized starch. Various studies on this issue have considered certain species of fungi for producing enzymes capable of degrading starch in its natural state (Soccol et al., 1994). Liquefaction is followed by a saccharification stage under the effect of glycoamylase.

3.2.2 Milling

The milling phase enables the starch to be extracted from the biomass and it is very important for the purposes of analyzing the bioethanol production process as a whole because it strongly influences not only the subsequent stages but also the co-products obtained at the end of intermediate stages, which also vary according to the specific raw material entering the process (wheat, barley, corn, oats). The two main options are wet milling and dry milling.

Wet milling is the standard procedure generally used in the starch-based foodstuffs industry. Though this procedure demands more energy and more economic resources, and it delivers a smaller quantity of ethanol, it is still preferred at industrial level because its capacity to separate the grain into its components enables a purer form of starch to be obtained, along with more valuable byproducts. Wet milling can be used to obtain not only ethanol, but also products such as corn oil, gluten-based foods and flour, and corn steep liquor (CSL).

Dry milling means there is no need to pre-treat the raw material, which simply has to be ground before going through the other processing stages (hydrolysis, fermentation, distillation), which are identical to those following the wet milling process. Because dry milling does not break down the cereals into their various components, the unfermentable residue leaving the process that extracts the ethanol from the fermentation broth is rich in proteins, fibers, fats and sugars.

3.2.3 From hydrolysis to bioethanol

After the preparatory stage, the glucose solution can be fermented in ethanol. The temperature of the glucose is lowered to around 35°C, and then the yeast (usually Saccharomyces cerevisiae) is added and the anaerobic fermentation process begins, which converts the glucose into ethanol and carbon dioxide.

$$\text{Glucose} \xrightarrow[\text{Alcoholic fermentation}]{\text{Saccharomyces cerevisia}} 2\,\text{EtOH} + 2\,CO_2 \tag{8}$$

As a rule, the preferred method is to conduct the saccharification and fermentation steps during the same stage of the production process. Fermentation can be completed in two stages (Verma et al., 2000) using starch treated with α-amylase and glycoamylase.

Fermentation may be continuous or discontinuous, it makes no difference. When the fermentation broth reaches an ethanol content of around 8-10% v/v (beyond which the yeast can no longer survive), the ternary mixture is distilled by adding benzene or cyclohexanone, or using molecular sieves. After distillation, the ethanol is 95% pure.

In 2006, a research group (Robertson et al., 2006) experimented with the so-called "cold hydrolysis" of starch, concluding that the potential use of this method relies on the discovery and characterization of more efficient enzymes and the development of processes with a high level of integration, such as simultaneous liquefaction, saccharification and fermentation, along with other factors. Figure 2 shows the flow chart for bioethanol production from materials containing starch.

3.3 Raw materials containing saccharose

For the purposes of bioethanol production, the most important raw materials containing saccharose are unquestionably sugar cane and sugar beet. Two thirds of the world's sugar production derives from cane, the other third from beet.

3.3.1 Sugar cane (saccharum officinarum)

Sugar cane contains approximately 12-17% of total sugars, 90% of which are saccharose and 10% are glucose. Milling can extract approximately 95% of the sugar, leaving behind the solid residue. This cane residue goes by the name of bagasse. Sugar cane is washed in order

to undergo a primary “crushing" process before milling. The cane juice obtained undergoes a clarification process in which the pH is balanced and cachaça is obtained, which can be sold as animal feed or as a component in mixtures. Fermentation is usually done with the aid of a yeast, Saccharomyces cerevisiae, which is separated in a continuous phase by centrifugation and reused in the fermenter. The fermentation process differs slightly, depending on whether all the juice is used to obtain bioethanol or whether part of it is drawn off to obtain sugar: in the former case, the juice is heated up to 110°C (to reduce the risk of bacterial contamination), then decanted and fermented; in the latter, the crystals formed by concentration are centrifuged, leaving a viscous syrup called molasses.

The extract leaving the fermenter must then be distilled to extract the hydrated ethanol (an azeotropic solution containing 95.5% v/v of ethanol and 4.5% v/v of water), which is dehydrated using molecular sieves or azeotropic distillation (i.e. with cyclohexanone or benzene) to obtain a higher-grade, anhydrous ethanol. In addition to ethanol, there is also an aqueous solution leaving the distillation process, that is called residue.

Molasses obtained from sugar cane are the most important raw material for the purposes of bioethanol production. In recent years, however, there have been rising prices and restrictions on the availability of molasses, which have strongly influenced the production of bioethanol (Quintero et al., 2008). Figure 3 shows the flow chart for bioethanol, energy and sugar production from sugar cane.

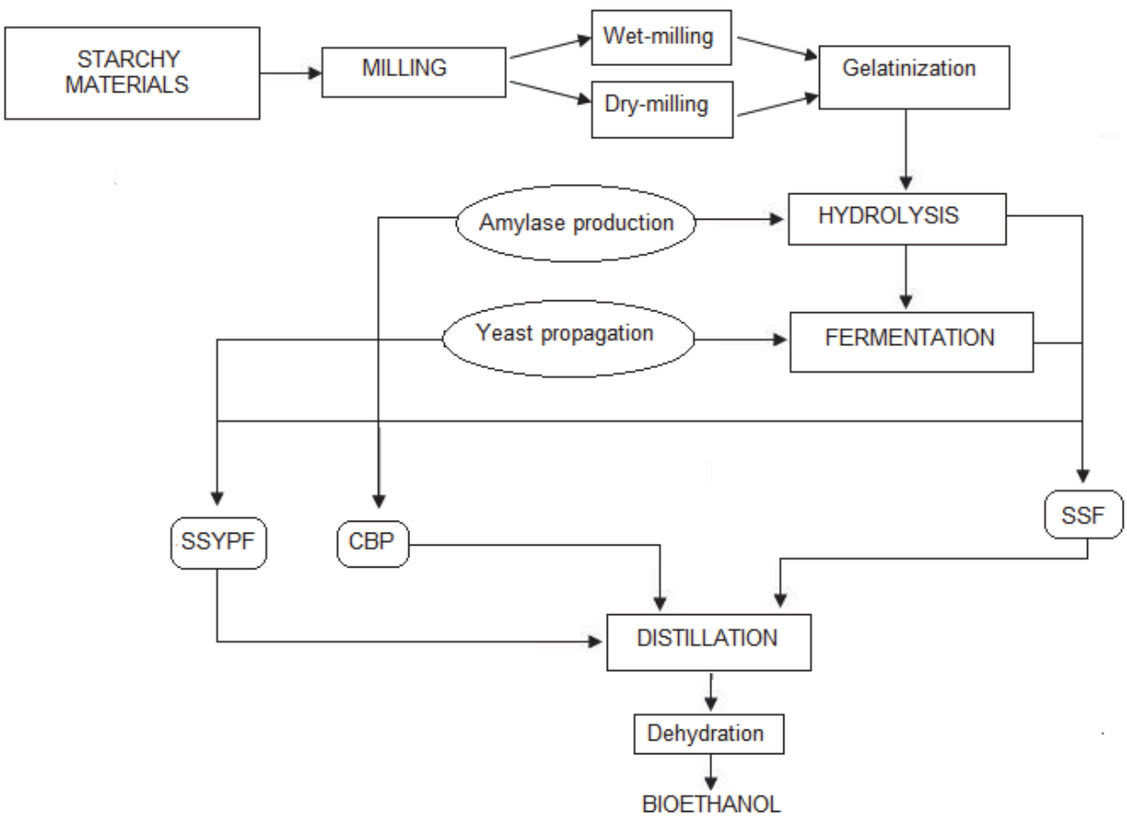

Fig. 2. Flow chart for bioethanol production from materials containing starch

3.3.2 Sugar beet (beta vulgaris)

Like sugar cane, sugar beet can also be used to obtain bioethanol by fermenting and distilling its juice. The beet is first cut into thin slices, then placed in contact with a medium (water or juice extracted from a previous process) and brought up to a temperature of about 70-80°C. In the case of sugar beet, temperature is a fundamental extraction parameter because it must be high enough to rupture the proteins in the cell walls containing the sugars, which has the effect of allowing the sugars to spread through the medium. Once this process has been completed, the sugar beet pulp is dried and sold as animal feed or to the pharmaceutical industry for use in the production of citric acid and its esters. The beet juice proceeds instead through the stages that convert it into bioethanol. At plants where sugar and bioethanol are both produced together, the juice can either be used directly or it can be concentrated in evaporators and stored for several months. Both the fresh and the concentrated sugar juice can be used in production processes involving cold crystallization and fermentation. The fermentation process relies on the use of yeasts (preferably Saccharomyces cerevisiae) or bacteria such as Zymomonas mobilis (Linde et al., 1998), which is only used in the case of a discontinuous fermentation. The great interest focusing on the bacteria is due to their capacity to convert the glucose into ethanol more efficiently than yeasts succeed in doing. Figure 4 shows the flow chart for the production of bioethanol and byproducts from sugar beet.

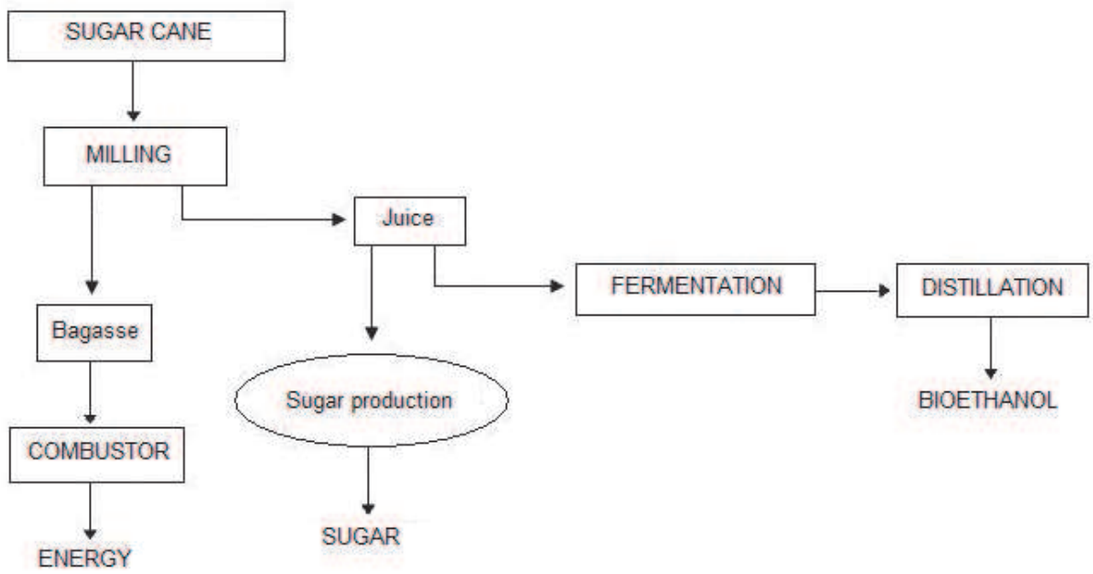

Fig. 3. Flow chart for bioethanol, energy and sugar production from sugar cane

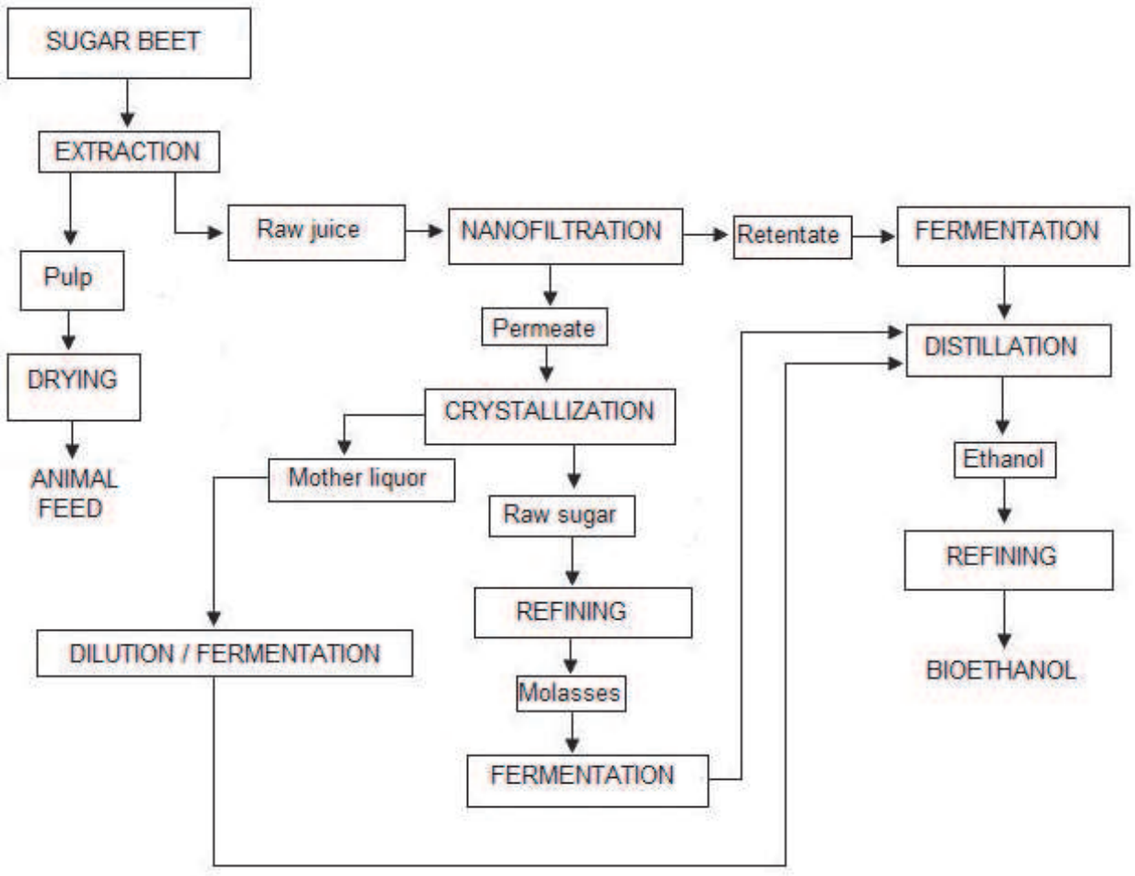

Fig. 4. Flow chart for the production of bioethanol and byproducts from sugar beet

3.4 Comparing the various raw materials

The choice of the raw materials to use to produce ethanol depends largely on local climatic conditions. North America and Europe, for instance, have based their ethanol production on materials containing starch, because of their particular farming and ecological conditions, which make it unfeasible to sugar cane adequately, although this plant offers a higher ethanol yield. In these areas, the most often grown energy crops are cereals. Using these raw materials poses some energetic sustainability limits (Patzek et al., 2005; Pimentel, 2003). The yield per ton of raw material is higher for sugar beet molasses than for cereals, so although growing sugar beet is less productive in quantitative terms than growing cereals, the annual ethanol yield from beet is higher than from cereals. The importance of analyzing the geographical position of crops helps us to see that growing the same type of cereal in tropical regions would produce a distinctly lower yield than could be achieved from the same plant grown in more suitable areas (Espinal et al., 2005). The lignocellulose materials represent the future as concerns raw materials for ethanol, because of their excellent energy value, great availability, low cost and high bioethanol yield.

These materials cannot be used to produce food, but they provide important secondary products such as methanol, syngas, hydrogen and electricity. The choice of which lignocellulose material also depends on the nature of the waste products in a given country (Kim & Dale, 2004). Cereals that are discarded during the distribution process can be

destined to ethanol production, together with farming waste and sugar cane bagasse. The drawback of these raw materials consists in the complexity of the phenomena involved in converting the biomass into ethanol. Various studies have been conducted on the process of bioethanol production starting from various raw materials, including lignocellulose materials, cereals (McAloon et al., 2000; Cardona et al., 2005), and sugar cane (Quintero et al., 2008).

3.5 Converting syngas into ethanol

Bioethanol can also be obtained by means of chemical processes (Sánchez & Cardona, 2008; Demirbas, 2005), which may or may not demand the presence of microorganisms in the fermentation stage. Gasification of a biomass to obtain syngas (CO + H_2), followed by the catalytic conversion of the syngas, has the potential for producing ethanol in large quantities. The catalysts most often used and studies are those based on rhodium (Rh) (Holy & Carey, 1985; Yu-Hua et al., 1987; Gronchi et al.; 1994).
The geometrical structure of the active site seems to be:

$$\left(Rh_x^{\ 0} Rh_y^{\ +}\right) - O - M^{n+} \tag{9}$$

where part of the *Rh* occurs as Rh^+ and the promoter ion (M^{n+}) is in close contact with these *Rh* species. The carbon monoxide is then hydrogenated to form an absorbed species -CH_x- that is then inserted in the absorbed CO. Hydrogenation of these absorbed species leads to the formation of ethanol (Subramani & Gangwal, 2008).
Another mechanism considered valid for ethanol formation involves the use of acetate (acetaldehyde formation followed by reduction) and is known, in the cases of Rh-based catalysts, to be promoted by manganese (Luo et al., 2001).
In this case, ethanol is formed by direct hydrogenation of tilt-absorbed CO molecules, followed by CH_2 insertion on the surface of the CH_2-O species to form an absorbed intermediate species. Ethanol is produced by hydrogenation of the intermediate species of CH_2-O. Acetaldehyde is formed by the insertion of CO on the surface of the CH_3-*Rh* species, followed by hydrogenation. The catalyst's performance can be improved by modifying its composition and preparing the ideal conditions for the reaction (Subramani & Gangwal, 2008). Manganese (Lin et al., 1995), Samarium and Vanadium (Luo et al., 2001) can also be used as promoter ions in processes involving Rh.

4. Environmental issues

The greenhouse gases (GHGs) are gases occurring in the Earth's atmosphere that absorb in the infrared field (carbon dioxide, ozone, methane, nitrogen oxides, carbon monoxide and so on). This feature enables them to trap the heat of the sun reflected back from the Earth's surface.
The GHG that occurs in the largest quantities is carbon dioxide, and that is why it attracts so much attention. In fact, the carbon cycle is a delicate balance between carbon accumulation, release and recycling that enables vegetable and animal species to survive. Problems linked to CO_2 began to emerge at the start of the industrial era: the ever-increasing use of fossil fuels as a source of energy meant that the carbon dioxide trapped for centuries in the fossils was being put back into the atmosphere, with no correspondingly reinforced recycling mechanism, which relies on chlorophyllic photosynthesis).

In addition to reducing carbon dioxide emissions, bioethanol can be seen as a no-impact fuel because the amount of CO_2 released into the atmosphere is compensated by the amount of CO_2 converted into oxygen by the plants grown to produce the bioethanol (Ferrel & Glassner, 1997).

4.1 Carbon sequestering

In the analysis of the environmental impact of bioethanol (and other biofuels too), some of the key factors concern the impact of the increasing quantities of dedicated crops on soil carbon levels and subsequent photosynthesis: these changes will also influence the atmospheric concentrations of GHG such as CO_2 and CH_4.

The main problem concerns the fact that, when a system in equilibrium experiences persistent changes, it can take decades before a new equilibrium with a constant carbon level is reached. Taking the current situation in Europe as concerns wheat and sugar beet crops, there is an estimated depletion of approximately 0.84 t of C or 3.1 t CO_2 equivalent ha^{-1} $years^{-1}$ from the ground. If no crops were grown on the soil, this depletion would be even greater, i.e. 6.5 t of C each year for sugar beet and 4.9 t of C for wheat. Apart from the effects on ground carbon levels, there are also signs of other adverse effects indirectly linked to crops grown for energy purposes, such as the increase in the amount of C in the atmospheric levels of GHG. Irrigation with good-quality water also exacerbates carbon sequestering: the water used for irrigation contains dissolved calcium and carbon dioxide (in the form of HCO_3^-); Ca and HCO_3^- react together, giving rise to the precipitation of $CaCO_3$ and the consequent release of CO_2 into the atmosphere. In the typical dry conditions of the USA, further reactions take place and irrigation is responsible for the transfer of CO_2 from the ground into the atmosphere (Rees et al., 2005). An important type of crop that can be used to reduce soil carbon sequestering is defined as "zero tillage", which means that it can be grown year after year without disturbing the soil. Seed crops (such as wheat) may be zero tillage, but not root crops (such as Panicum virgatum). Zero tillage has variable effects, and in some cases carbon sequestering in the soil may even increase, but this phenomenon can be completely overturned by a one-off application of conventional tillage. If only the carbon in the soil is considered, zero tillage leads in the long term to less global warming than growing conventional crops in damp climates, but in areas with dry climates, there is no certainty of any such beneficial effect (Six et al., 2004). Using straw from cereals can increase the carbon levels in the soil. Such residue is useful in maintaining soil carbon levels (Blair et al., 1998; Blair and Crocker, 2000) because it has a low rate of breakdown, so it is important for the residue to go back into the ground in order to keep the farming system sustainable. Since removing the residue from the ground has other negative effects too, such as an increased soil erosion and a lesser availability of macro- and micronutrients, some have suggested in the United States (Lal, 2005) that it would be advisable to remove only 20-40% of the residue for the purposes of bioethanol production, whereas it was claimed (Sheehan et al., 2004) that if up to 70% of the residue were removed to produce bioethanol, the carbon levels would initially decline and then remain stable for about 90 years. Increasing the land used to grow energy crops would have a substantial impact on the concentrations of carbon-containing gases in the atmosphere. If areas covered with forest were converted into arable land, the carbon sequestering would go from values of around 50-145 $t \cdot ha^{-1}$ to approximately 50-200 $t \cdot ha^{-1}$, assuming a 60-year rotation (Reijinders & Huijbregts, 2007).

4.2 Emissions

Mixing bioethanol with petrol, even in modest proportions, increases the octane number of the fuel and reduces the percentage of aromatic and carcinogenic compounds, and emissions of NO_x, smoke, CO, SO_x and volatile organic compounds (VOC). But there is also an increase in the emissions of formaldehyde and acetaldehyde. On the other hand, modern bioethanol production systems have an energy ratio (or net usable energy) of around 2 to 7, depending on the crops and processes used. The composition of petrols can influence the emissions of organic compounds: those containing aromatic hydrocarbons such as benzene, toluene, xylene and olefins produce relatively high concentrations of reactive hydrocarbons, while petrols formulated using oxygenated compounds (such as those mixed with bioethanol) may contain lower quantities of aromatic compounds.

The problem of petrols with high concentrations of aromatic compounds lies in their marked tendency to emit uncombusted hydrocarbons, which are difficult for catalytic converters to oxidize as well as being precursors of photochemical contamination. All oxygenated fuels have the potential for reducing the emissions of carbon monoxide (CO) and uncombusted hydrocarbons, which are also "photochemically" less reactive than the hydrocarbons of normal petrols. Because ethanol acts as an oxygenating agent on the exhaust gases of an internal combustion engine fitted with a three-way catalytic converter, adding ethanol to petrol (Poulopoulos et al., 2001) leads to an effective 10% reduction in the emission of CO, as well as a general reduction in aromatic hydrocarbon emissions. Using four-stroke engines, with four cylinders and electronic injection, fueled with various ethanol and petrol mixtures (Al-Hasan, 2003) reduced the CO emissions by about 46.5%. The anti-detonating features of petrols are very important and depending essentially on their chemical composition.

Life cycle analysis taking the "well to wheel" approach showed that the GHG emissions from bioethanol obtained from sugar beet are around 40-60% lower than the emissions from petrols obtained from fossil fuels (Reijinders & Huijbregts, 2007). Mixing bioethanol with diesel oil improves the fuel's combustion (Lapuerta et al., 2008) and reduces the size of the particles in the exhaust without increasing their quantity. Using an E10 mixture reduces the total hydrocarbon emissions because of ethanol's greater heat of vaporization.

CO emissions increase if moderate amounts of ethanol are added to diesel oil, while they diminish as the proportion of ethanol increases (Li et al., 2005). Conversely, NO_x emissions decrease with a low or moderate quantity of ethanol, but increase if more ethanol is added. The total hydrocarbons (THC) also increase with different proportions of ethanol and different speeds.

5. Conclusions

Although bioethanol is a valid alternative to fossil fuels and has a low environmental impact, its use is nonetheless posing problems relating to the use of raw materials such as cereals, which are fundamental to the food industry.

Increasing the farmland used to grow energy crops for the production of biofuels means competing with food crops. Many studies have attempted to assess the need for farmland for crops for producing ethanol. The yield in bioethanol per hectare naturally depends on the crops used, but reference can be made to the mean productivity in Europe (weighted according to the type of crop), which is currently estimated at around 2790 liters/hectare (based on a mean yield in seeds of 7 tons/hectare and 400 liters/ton).

Although bioethanol can be produced successfully in temperate climates too, the tropical climates are better able to ensure a high productivity. In Brazil, sugar cane is used to produce approximately 6200 liters/hectare (an estimate based on a crop yield of 69 tons/hectare and 90 liters/ton). The productivity of bioethanol from sugar cane is high in India too, with a yield of approximately 5300 liters/hectare. If bioethanol from sugar cane becomes a commodity used worldwide, then South America, India, Southeast Asia and Africa could become major exporters.

Research is focusing on alternatives, concentrating on innovative raw materials such as Miscanthus Giganteus, an inedible plant with a very high calorific value (approximately 4200 Kcal/kg of dry matter), or filamentous fungi such as Trichoderma reesei, which can break down the bonds of complex lignocellulose molecules.

This article summarizes the main raw materials that can be used to produce bioethanol, from the traditional to the more innovative, and the principal production processes involved. It also analyses the issues relating to emissions and carbon sequestering.

6. References

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This book aspires to be a comprehensive summary of current biofuels issues and thereby contribute to the understanding of this important topic. Readers will find themes including biofuels development efforts, their implications for the food industry, current and future biofuels crops, the successful Brazilian ethanol program, insights of the first, second, third and fourth biofuel generations, advanced biofuel production techniques, related waste treatment, emissions and environmental impacts, water consumption, produced allergens and toxins. Additionally, the biofuel policy discussion is expected to be continuing in the foreseeable future and the reading of the biofuels features dealt with in this book, are recommended for anyone interested in understanding this diverse and developing theme.

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