Lignocellulosic ethanol production plant by Biochemtex in Italy

Lignocellulosic Ethanol Process and Demonstration

A Handbook
Part I

BIO LYFE
2nd gen bioethanol

SEVENTH FRAMEWORK PROGRAMME

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Content

The BIOLYFE project

The BIOLYFE project aims at improving critical steps of the second generation bioethanol production process and at demonstrating the whole supply chain, from feedstock sourcing via fuel production to product utilization. The main result of the project is the construction of an efficient second generation industrial demonstration unit with an annual output of about 40,000 tons of lignocellulosic bioethanol.

The project is developing technologies allowing an increased and economically viable utilization of the lignocellulosic feedstock for the production of second generation bioethanol. In order to achieve this objective, BIOLYFE project focuses on hydrolysis and fermentation steps. BIOLYFE started in January 2010 and lasts for 3 years. The proejct is co-funded by the European Commission in the 7th Framework Programme (Project No. FP7-239204).

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Preface

The EU goal to reduce CO_2 emissions by 80-95% until 2050 compared to 1990 levels is a driving force for the decarbonisation of the transport sector and integration of new approaches into existing regulatory frameworks. In the Energy Roadmap 2050 (COM/2011/ 885), the European Commission identified different directions for developing a long-term European framework towards decarbonisation of the energy system and expressed the need to invest into new renewable energy technologies, including second generation biofuels. The CARS 21 High Level Group (Competitive Automotive Regulatory System for the 21 century) established by the European Commission has identified second generation biofuels as particularly promising and recommended giving a substantial support for their development.

The first European regulation on the promotion of the use of energy from renewable sources covering all areas of renewable energies is a milestone of renewable energy policy in Europe. The Renewable Energy Directive (RED, 2009/28/EC) lays down a mandatory 10% target for energy from renewable sources in transport until 2020, strengthening the role of biofuels in improving the security of transport fuels supply.

Transition towards second generation biofuels enables CO_2 savings and is promoted by the EU RD&D (Research, Development & Demonstration) activities as well as different incentives stimulating the private sector in technology development. For example, the RED provides an incentive to stimulate the second generation biofuels market by double-counting contribution made by biofuels produced from wastes, residues and lignocellulosic material.

Lignocellulosic ethanol production is one of the most promising second generation biofuel technologies. Even though different conversion technologies are available on the market, there are still challenges to overcome before launching a large scale industrial production.

This publication provides an overview of the second generation bioethanol technology in terms of available feedstock, pretreatment technologies and production processes.

Standardised units and abbreviations, which are commonly used at European level, were applied. Details on conversion units are given at the end of the handbook. The decimal sign is a point, and the thousand separator is a comma.

Part I Overview on the lignocellulosic ethanol production

1 Introduction

RITA MERGNER, RAINER JANSSEN

1.1 (Bio)ethanol as fuel and chemical

Ethanol - C_2H_6O - is a chemical and an energy product extracted from the fermentation of sugars and starches or by chemical synthesis. Ethanol (CAS 64-17-5) is a volatile, flammable, colorless liquid and can be referred to as ethyl alcohol or as pure alcohol. It can be mixed with water or gasoline at any ratio and it burns with a pale blue, non-luminous flame forming carbon dioxide (CO_2) and water (H_2O). The main chemical features of ethanol compared to diesel and gasoline are indicated in Table 1.

Table 1: Physicochemical properties of ethanol, diesel and gas (Modified from Kaltschmitt and Hartmann 2001)

Properties	Ethanol	Diesel	Gasoline
Carbon (%)	52	86	86
Hydrogen (%)	13	13	14
Oxygen (%)	35	-	0
Lower heating value (MJ/kg)	26.8	42.5	42.7
Lower heating value (MJ/l)	21.3	36	32
Density (15°C) kg/l	0.794	0.815-0.855	0.72-0.78
Kinematic viscosity (20°C) mm^2/s	1.5	4	0.6
Flash point (°C)	12.8	68	-42.8
Boiling point (°C)	78	180-360	25-215
Ignition temperature (°C)	420	250	300
Evaporization heat (kJ/kg)	904	250	380-500
Stoichiometric ratio	9	14.5	15.1
Vapour pressure 38°C (psi)	2.5	0.04	7-9
Octane number (RON)	107	93	-
Cetane number	<8	>45	-

In December 2010 the European Committee for Standardisation (CEN) approved European Standard for ethanol 'Automotive fuels - Ethanol as a blending component for petrol - Requirements and test methods' (EN 15376:2011). The standard specifies requirements and test methods for ethanol to be used as an extender for automotive fuel for petrol engine vehicles. Table 2 provides an overview on the requirements for ethanol for the use as a blending component for petrol according to the standard EN 15376:2011.

Table 2: Requirements and test methods for ethanol (Standard EN 15376:2011)

Properties	Unit	Lower limit	Upper limit
Ethanol (incl. higher saturated alcohols)	% wt.	98.7	-
Higher saturated monoalcohols (C_3-C_5)	% wt.	-	2.00
Methanol	% wt.	-	1.00
Water	% wt.	-	0.30
Inorganic chloride	mg/l	-	20.00
Copper	mg/kg	-	0.10
Total acidity (as acetic acid)	% wt.	-	0.007
Appearance	-	Clear and bright	
Phosphorous	mg/l	-	0.5
Involatile material	mg/100 ml	-	10.0
Sulphur	mg/kg	-	10.0

Ethanol is applied in a wide range of industries. In the *food sector,* ethanol is used to produce spirit drinks and added as an ingredient in a wide range of food preparations. In the *chemical sector,* ethanol is mainly used as a solvent in products such as varnishes, paints, inks, tinctures and explosives. In the *pharmaceutical industry,* ethanol is applied as a biocide or solvent for the production of different medicines, e.g. antibiotics, vaccines, syrups or vitamins. In the *cosmetic industry,* it is used to produce soap, lotions, shampoo or deodorants. Many *household products* such as screen washers, detergents, disinfectants or washing powders contain ethanol. Except for alcoholic beverages, nearly all industries use hydrous ethanol (95.5% v/v ethanol, 4.5% v/v water azeotropic solution) which is known as 95% alcohol. In the *transport sector,* ethanol is used as a vehicle fuel (E100), blended with gasoline or as a gasoline octane enhancer and oxygenate.

The EU transport sector is very much dependent on imported fossil fuels significantly contributing to Greenhouse Gas (GHG) emissions, which increased by 24% from 1990 to 2008 representing 19.5% of total GHG emissions in the EU (European Expert Group on Future Transport Fuels 2011). Therefore, alternative fuels will play a significant role in the decarbonisation of the transport sector, security of energy supply and diversification of energy sources.

One of the most common biofuel available on the market today is bioethanol derived from sugar- or starch-based crops through a natural fermentation process. It is often referred to as the 'first generation' bioethanol and is increasingly used in the transport sector as an alternative to fossil fuels all over the world. As the supply of raw materials needed to produce first generation bioethanol is limited, lignocellulosic biomass ('second generation bioethanol') is seen as an attractive feedstock for future large scale supplies of bioethanol (Gray et al. 2006).

The organic origin of bioethanol is one of the advantages over fossil fuels. Bioethanol is widely used as a blending agent with gasoline to increase the octane number and at the same time reduce toxic emissions such as carbon monoxide (CO), carbon dioxide (CO_2) or nitrogen oxides (NO_x). Since bioethanol contains roughly 2/3 of the energy content per unit of volume compared to gasoline, a larger volume of fuel is needed for the same driving range. However, its higher octane number leads to increased compression ratio and improved energy efficiency levels.

Bioethanol-blended gasoline was introduced in many countries to meet the increasing demand of fuels (Figure 1). Bioethanol blends are categorized into *low blends* and *high blends*. Low-level bioethanol blends in gasoline are E5, E10, E15 or E25. Blends such as ED diesel (10% ethanol derivative blended in diesel) or E-diesel (a mix of 7.7% bioethanol, additives and diesel) can be applied in diesel engines. Large volumes of low bioethanol blends can be introduced into road transport fuels without any additional modifications to infrastructure or vehicles.

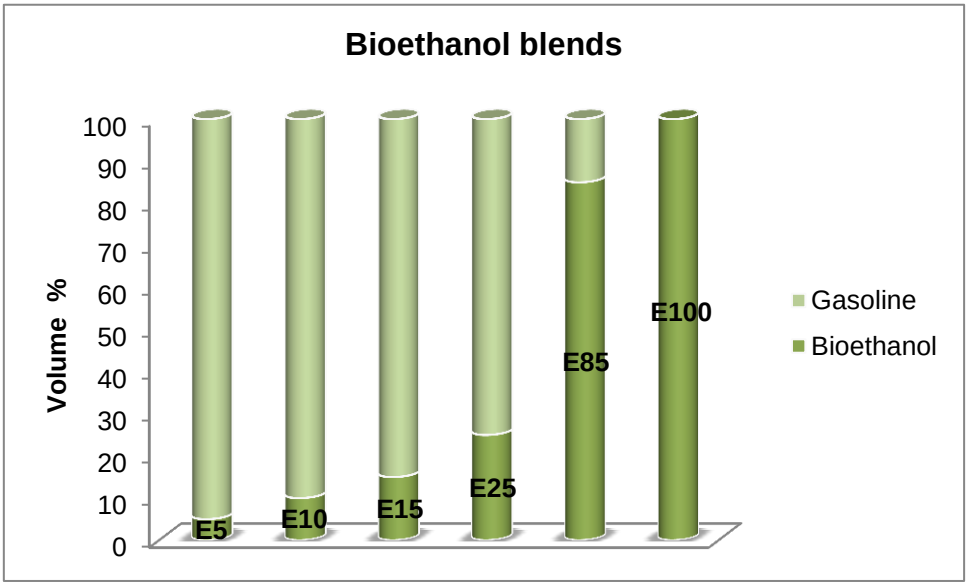

Figure 1: Bioethanol blends

High blends such as E85 and E100 contain a high proportion of bioethanol and require modified vehicles or flexi cars as well as a dedicated fuel infrastructure. Bioethanol high blends can be also used in converted diesel vehicles as well as in dedicated heavy diesel vehicles in form of ED95 (96.5% ethanol, 3.5% additives). In Sweden, UK, Germany, France and the Netherlands E85 is available on a significant scale.

Table 3 indicates the impact of different ethanol blends on gasoline vehicles and required changes depending on ethanol volume in gasoline. Up to 5% ethanol volume in gasoline does not require modifications for any vehicle with Otto Cycle Engine. The higher the blend, the more modifications are required for the vehicle.

Table 3: Impact of different ethanol/ETBE blends on gasoline vehicles (Sugarcane-based Bioethanol 2008)

Necessary modifications (Otto Cycle Engines)												
Ethanol/ETBE volume in gasoline (%)	Carburettor	Fuel injection	Fuel pump	Fuel filter	Ignition system	Fuel tank	Catalytic converter	Basic engine	Motor oil	Intake manifold	Exhaust system	Cold start system
≤ 5%	For any vehicle											
≤ 10		For vehicles manufactured since 1990										
≤ 25	Brazilian gasoline vehicles											
≤ 85	Flexible Fuel Vehicles used in USA and Canada											
≥ 85%	Flexible Fuel Vehicle used in Brazil											

No modifications are necessary

Modifications are probably necessary

Octane number is a standard measure of the performance of a motor. The higher the octane number, the more compression the fuel can withstand before detonating. There are severeal octane ratings. Research Octane Number (RON) is determined by running the fuel in a test engine with different compression ratio under controlled conditions. Motor Octane Number (MON) testing is similar to RON testing with a preheated fuel mixture, higher engine speed and variable ignition timing to further stress the knock resistance of the fuel. The impact of different ethanol blends on octane number is given in Table 4.

Bioethanol can also be used as an *additive* to replace certain chemical additives such as lead in blend with gasoline. For example, lead additive at a concentration of approximately 0.6 g/l can be replaced with a 20% blend of ethanol in gasoline (Thomas and Kwong 2001).

Table 4: Impact of different ethanol blends on octane number (Carvalho 2003)

Composition of base gasoline			Increased octane number with:							
			5% ethanol/ETBE		10% ethanol/ETBE		15% ethanol/ETBE		20% ethanol/ETBE	
Aromatics	Olefins	Saturates	MON	RON	MON	RON	MON	RON	MON	RON
50	15	35	0.1	0.7	0.3	1.4	0.5	2.2	0.6	2.9
25	25	50	0.4	1.0	0.9	2.1	1.3	3.1	1.8	4.1
15	12	73	1.8	2.3	3.5	4.4	5.1	6.6	6.6	8.6
11	7	82	2.4	2.8	4.6	5.5	6.8	8.1	8.8	10.6

There are two types of bioethanol in the market - *hydrous* and *anhydrous*. *Hydrous bioethanol* typically contains 93-95% ethanol and 5-7% water and is applied in pure ethanol engines adapted to run on 100% ethanol (E100). Hydrous bioethanol is also used in modified gasoline buses or in special fuels such as HE15 (15% hydrous bioethanol and 85% gasoline) and ED95 (95% ethanol, 5% additives). Additionally, it may be used in fuel blends after dehydration. *Anhydrous bioethanol* remains after the dehydration of hydrous bioethanol and is blended with gasoline or diesel at any ratio. Anhydrous bioethanol contains maximum 0.7% water and is used in E85 as well as in nearly all bioethanol low blends.

Ethanol-water solution makes an azeotropic mixture at 95.4% of ethanol by mass with a boiling point of 78.2°C. Azeotope is a mixture of liquids that has a constant boiling point, because the vapour has the same composition as the liquid mixture. The boiling point of an azeotropic mixture may be higher or lower than that of any of its components. The components of the solution cannot be separated by simple distillation. Figure 2 shows the vapor-liquid equilibrium of the mixture of ethanol and water.

As 95.4% ethanol cannot be separated from water by simple distillation, chemical methods should be applied to further purify ethanol. For example, molecular sieves, membranes or pressure-swing distillation can be applied to separate ethanol and water. Another solution to eliminate the azeotropic point is the addition of an entrainer, however this risks contaminating the solvent.

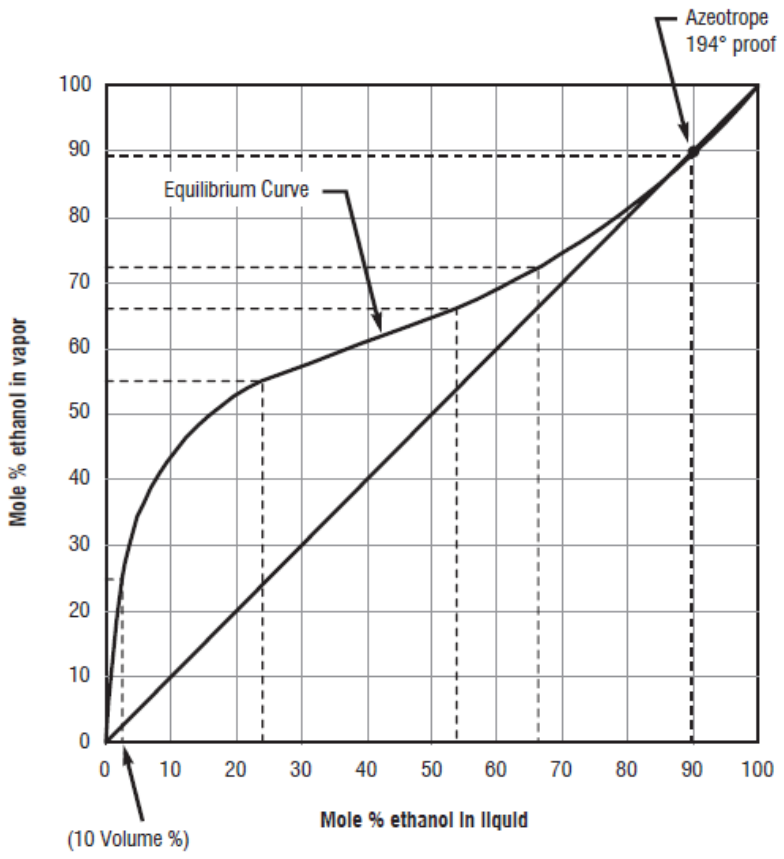

Figure 2: Vapor-liquid equilibrium for the ethanol-water mixture at atmospheric pressure (Jacques et al. 2003)

Bioethanol can be produced from any biological feedstock containing appreciable amounts of sugar or materials that can be converted into sugars such as starch or lignocellulose.

Depending on processed feedstocks, bioethanol production is classified into two different generations. Bioethanol produced through a natural fermentation of sugar-containing feedstocks (e.g. sugar cane, sugar beet or sweet sorghum) or starchy materials (e.g. wheat, corn, potatoes or barley) refers to as 'first generation' bioethanol (Figure 3).

Figure 3: First generation starch- and sugar-based feedstock for bioethanol production (top row from left: wheat, corn, rice, cassava; bottom row from left: triticale, grain sorghum, barley, potatoes)

Second generation bioethanol is produced from lignocellulosic biomass such as energy crops (corn, *Miscanthus*, *Arundo donax* etc.) or agricultural and forest residues (wood, straw, bagasse etc.) (Figure 4). In contrary to first generation biomass feedstock, a larger variety of plants and often the 'whole plant' can be used in conversion process enhancing the global resource base for bioethanol production. This leads to a potentially larger scale production and lower costs. Even though second generation technologies are already available, they are not yet commercially introduced in the market.

Figure 4: Second generation lignocellulosic feedstock for bioethanol production (top row from left: wood, straw; bottom row from left: bagasse, grass)

1.2 World ethanol markets

The contribution of alternative fuels in the transport sector is constantly growing. Currently, first generation bioethanol is the dominant biofuel worldwide. Figure 5 shows the global biofuels production in 2000-2010. Global production of fuel ethanol in 2012 was estimated around 83.1 billion litres (REN21 2013).

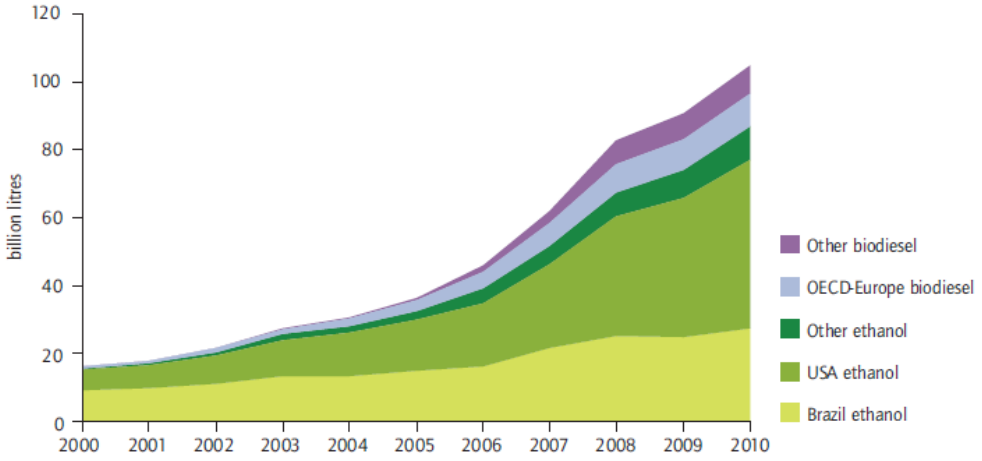

Figure 5: Global biofuels production 2000-2010 (OECD/IEA 2011)

According to OECD and FAO (2011), ethanol production shall reach around 154 billion litres until 2020 (Figure 6). However, trade in ethanol is expected to develop much slower. At the global level, growth in trade comes almost entirely by exports from Brazil and Thailand. Brazilian ethanol exports are expected to reach 9.7 billion litres by 2020. For Thailand, ethanol exports are expected to increase to about 0.5 billion litres in 2020. In Europe, due to the sustainability criteria of the RED and the expected rather slow development of cellulosic ethanol until 2020, ethanol imports are expected to amount for only 2.3 billion litres by 2020 (OECD/FAO 2011).

Figure 6: Development of the world ethanol market (OECD/FAO 2011)

In terms of feedstocks, corn and sugar cane should remain the major ethanol feedstocks over the coming decade. By 2020, 44% of global ethanol production is expected to be

produced from coarse grains and 36% from sugar cane (Figure 7). Lignocellulosic ethanol production should represent only 5% of global production by 2020.

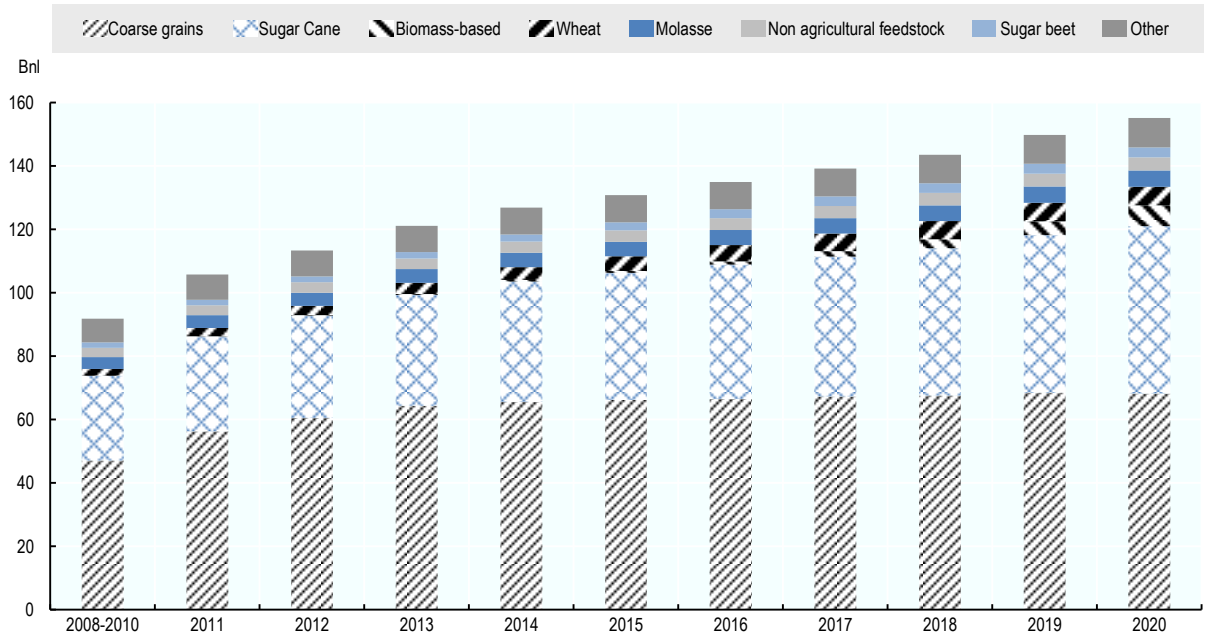

Figure 7: Evolution of global ethanol production by feedstocks used (OECD/FAO 2011)

In order to introduce bioethanol in the fuel market, most countries have set long term targets for bioethanol production. For example, Japan set a target to produce 6 billion liters of bioethanol per year by 2030 leading to 5% of transport energy. China targets to produce 13 billion liters of bioethanol per year by 2020.

In 2012, 4.8 billion liters of bioethanol were produced in the EU. The main bioethanol producers in the EU are France (1.2 billion litres), Germany (0.7 billion litres), Belgium (0.45 billion litres) and the Netherlands (0.45 billion litres) followed by Spain and Sweden (EurObserv'ER 2013). Bioethanol consumption in transport sector amongst other biofuels was 19.9% in 2012 (Figure 8). In comparison to 2011, the percentage remained stable.

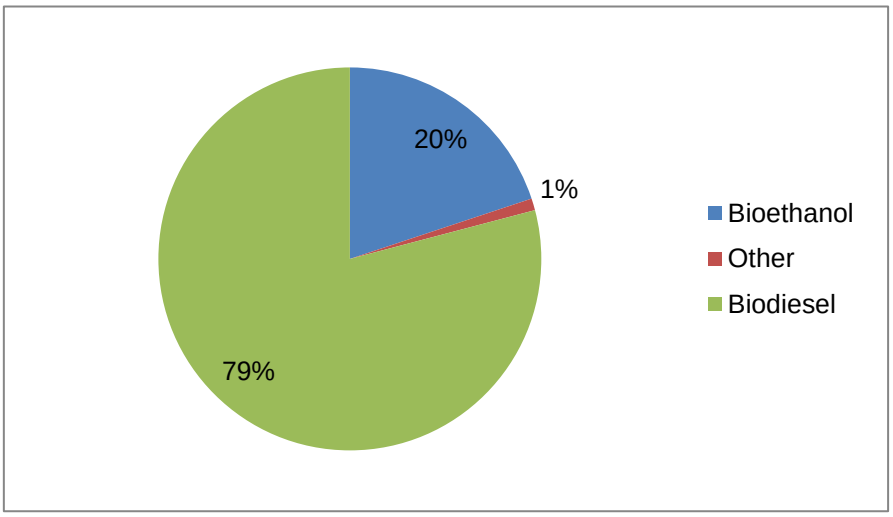

Figure 8: Share of biofuels in the EU biofuel consumption in 2012 (EurObserv'ER 2013)

The main feedstocks used in the EU for bioethanol production are sugar beet, wheat, corn and potatoes. International companies such like Abengoa, Tereos, Crop Energies, Cristanol, Agrana, Ensus, Verbio and Sekab are the main bioethanol producers in Europe.

Ethanol exports from the USA to Europe increased significantly in the past years from 13 million liters in 2009 to approximately 1.1 billion liters in 2011. This was due to bioethanol classification as a 'chemical product' eligible for reduced customs duty. However, the situation changed in March 2012 when the European Commission has published a customs regulation concerning the classification of certain goods in the Combined Nomenclature (No. 211/2012) harmonizing the classification of ethanol/gasoline blends that are imported into Europe. As of April 2012 imported ethanol/gasoline blends containing at least 70% ethanol blended with 30% gasoline are classified as denatured ethanol (CN code 2207 200 00). This new classification means that fuel blends containing at least 70% ethanol are no longer classified as a 'chemical product' and are not eligible for a lower import duty of 6.5% *ad valorem*.

1.3 Sustainability

Growing global shares of biofuels in energy sector raise serious environmental and socio-economic concerns including the protection of biodiversity, soil degradation and water pollution as well as the competition with food production. It is therefore important to ensure that feedstock for biofuels is produced in a sustainable way. In order to reach significant CO_2 reductions in the transport sector, biofuel technologies will have to make a significant contribution and be at the same time socially, environmentally and economically sustainable.

Bioethanol can be produced in different ways using various feedstocks. It is biodegradable, less toxic than gasoline and can considerably reduce GHG emissions. In addition, bioethanol does not contain olefins and sulphur which have a negative impact on air quality. For example, ethanol from sugarcane can lead to significant net savings in GHG emissions, especially when the co-product is used to provide heat and power at the processing plant (OECD/IEA 2008). However, first generation bioethanol production is increasingly questioned over the impact on climate change and the environment. In addition, it is limited due to the following reasons:

- Feedstock competition with food production
- Total production costs
- Limited land use efficiency

Nevertheless, first generation bioethanol plays an important role in establishing infrastructure and policy measures to introduce second generation bioethanol in the market. Sustainability criteria for first generation biofuels apply to second generation biofuels production (e.g. minimum GHG emission savings, definition of suitable land for biofuel cultivation, social standards etc.).

Dedicated cellulosic energy crops could produce higher bioethanol yields per hectare because the entire crop is used as a feedstock. These crops are land-using, even though some may be grown on land that would normally not be used for food production. However, in case of agricultural and non-agricultural residues that would otherwise be disposed of, there is no need for additional land to produce the feedstock, resulting in theoretical high biofuel yields per hectare and zero competition with food production (JRC 2010).

Figure 9 indicates environmental, social and economic aspects of sustainable biofuel production which should be taken into account. The main environmental sustainability criteria are GHG emissions, air quality, land use change and water quality.

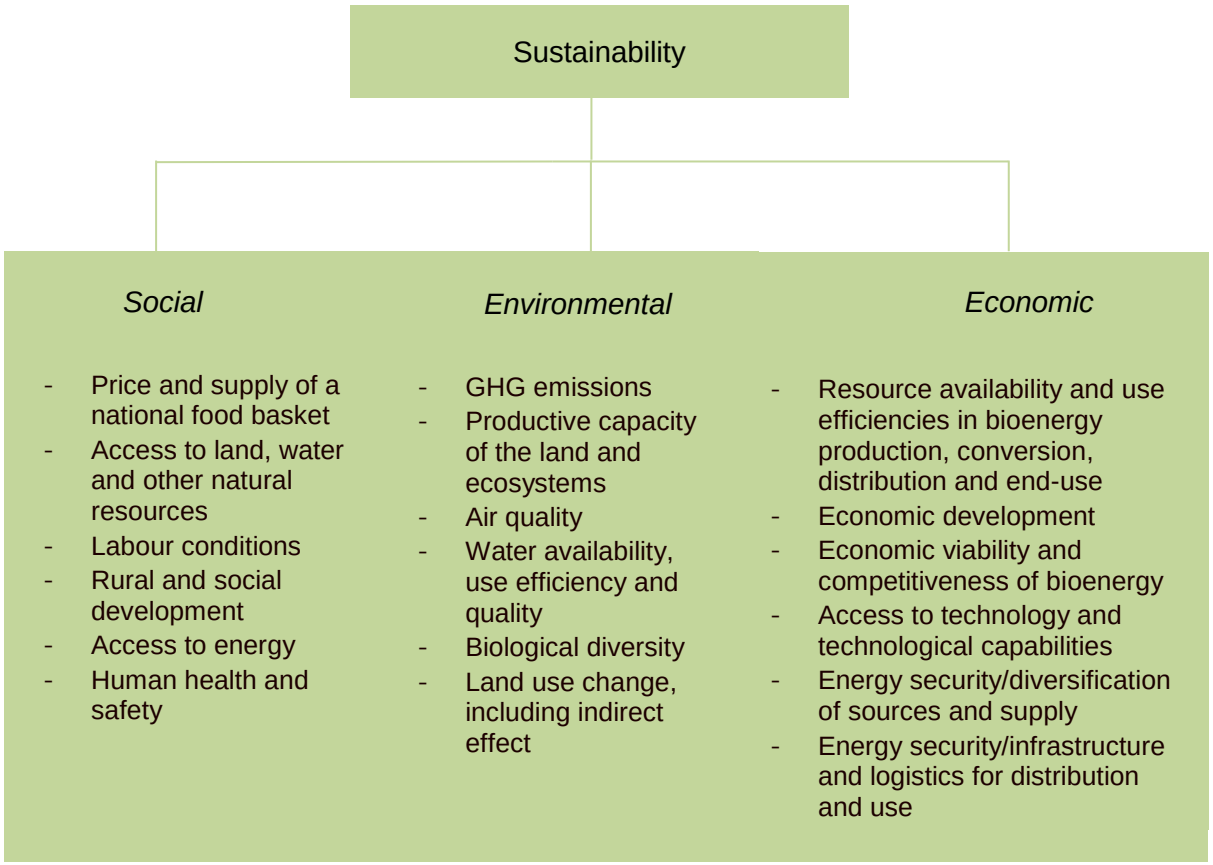

Figure 9: Pillars of sustainability (The Global Bioenergy Partnership, Chair conclusions of the 12 Task Force meeting, May 2011)

This chapter concentrates only on several widely discussed sustainability indicators for second generation bioethanol production such like GHG emissions (without land use emissions) and net energy balance. Different studies report considerable GHG emission reductions of second generation bioethanol. As mentioned, second generation bioethanol has better GHG performance than first generation bioethanol due to feedstocks which are primarily derived from residues. However, the reviewed studies do not take into account GHG emissions for enzyme production which is anticipated to have a significant impact on the GHG emission savings of second generation bioethanol.

Menichetti and Otto (2009) reviewed studies on energy balance improvements for second generation bioethanol (Table 5). In the selected number of studies GHG emission savings are typically in the 76-98% range.

Table 5: Range of results for second generation bioethanol without land use change based on selected number of studies (Menichetti and Otto 2009)

Author	Year	Scope	GHG improvement	Feedstock
Farrell et al.	2006	USA	88%	Cellulosic ethanol from switchgrass
Elsayed et al.	2003	Various	84%	Cellulosic ethanol from wheat straw
Edwards et al.	2007	Europe, Brazil	76-88%	Cellulosic ethanol from wheat straw, wood
Grood and Haywood	2007	USA	93-98%	Cellulosic ethanol from switchgrass
Veeraraghavan and Riera-Palou	2006	UK	88-98%	Cellulosic ethanol from wheat straw

In the EU, biofuels have to comply with sustainability criteria set by the European Commission in the Renewable Energy Directive (RED, 28/2009/EC) which apply since December 2010. In addition, the European Commission adopted a number of decisions to support the implementation of sustainability criteria related to GHG savings, biodiversity and land with high carbon stock such like forests or peat-lands. According to the RED directive, GHG emission savings from the use of biofuels should be at least 35%. This threshold will rise to 50% as of 2017 and to 60% as of 2018 for biofuels produced in installations which started production not earlier than 1 January 2017. Raw material obtained from land with high carbon stock (old forests, grasslands and protected areas), wetlands and continuously forested areas is not allowed to be used for biofuels production.

Fuel Quality Directive (EC 30/2009) amends a number of elements of the petrol and diesel specifications as well as introduces a requirement on fuel suppliers to reduce the GHG intensity of energy supplied for road transport (Low Carbon Fuel Standard). In addition, the Directive establishes sustainability criteria that must be met by biofuels if they are to count towards the GHG intensity reduction obligation. Accordong to Article 7b, biofuels shall not be made from raw material obtained from land with high biodiversity value (e.g. primary forest, wooded land, designated areas and highly biodiverse grassland). Also, biofuels shall not be made from raw material obtained from land with high carbon stock (e.g. wetlands, or continuously forested areas). Biofuels produced from waste and residues, other than agricultural, aquaculture, fisheries and forestry residues, need only fulfil the GHG emission saving levels which shall be at least 35%, at least 50% as of 1 January 2017 and at least 60% as of 1 January 2018 for biofuels produced in installations in which production has started on or after 1 January 2017.

The European Commission promotes the cultivation of crops on degraded land for bioenergy production. The RED attributes a bonus of 29 g CO_{2eq}/MJ in the calculation of the carbon balance if evidence is provided that the land was not in use for agriculture or any other activity in January 2008 and is severely degraded land, including such land that was formerly in agricultural use or heavily contaminated land. The bonus of 29 g CO_{2eq}/MJ applies for a period of up to 10 years from the date of conversion of the land to agricultural use, provided that a steady increase in carbon stocks as well as sizable reduction in erosion phenomena are ensured and that soil contamination for land is reduced (Annex V, 28/2009/EC).

The RED sets out special measures and requirements for wastes and residues which are different to requirements for biofuels from other feedstocks. Biofuels produced from waste, residues, non-food cellulosic material and lignocellulosic material have the following advantages:

- Double counting - Art. 21(2): Biofuels produced from wastes, residues, non-food cellulosic material and lignocellulosic material shall be considered to 'count twice' for Member States in meeting the 10% transport target and for economic operators in meeting their obligation in national schemes.
- Criteria limited to greenhouse gas (GHG) emissions - Art.17(1): Biofuels produced from waste and residues, other than agricultural, aquaculture, fisheries and forestry residues, need only to fulfill the sustainability criteria set out in Art. 17 (2) - at least 35% GHG emission savings until 2017, 50% as of 2017 and to 60% as of 2018.
- No upstream GHG emissions - RED Annex V Part C (18): Wastes, agricultural crop residues and residues from processing shall be considered to have zero life-cycle GHG emissions up to the process of collection of those materials.

1.4 Indirect land use change (ILUC)

Increased demand for biofuels is expected to produce changes in the present land use configuration. Furthermore there is a growing concern about the effect of land use change on biodiversity, food supply, soil and water quality. Biofuels production on current land and use of biomass in a given region can induce displacement of activities and land use changes elsewhere. This effect is known as indirect land use change (ILUC). Due to changes in the carbon stock of the soil and the biomass, indirect land use change has consequences in the GHG balance of a biofuel (Gnansounou et al. 2008). These indirect correlations exist at a global level and it is important to calculate and allocate different effects of biomass production, especially for energy use. However, there are problems based on the following points (IFEU 2009):

- The indirect effects are generally independent of regional correlations and have an impact via the complex mechanisms of the agricultural markets.
- The use of one hectare of land for biomass does not necessarily mean that exactly one hectare of new land will be developed for the displaced food/animal feed/fiber plant. The indirect consequences of the demand for energy plantscan also increase crop yield overall. In many regions of the world, it can be assumed that the potential yield per hectare of land is not fully exploited.
- The mix of food and animal feed sold on the market changes as a result of the connection between the production of biofuel and food/animal feed. Certain food/animal feed - already established in the market - is displaced by the newly offered products. As a result, there are complex shifts in global land use to produce the necessary food/animal feed and biofuels. If the efficiency of global land use (product yield per hectare) increases, this can curb demand for agricultural land.

ILUC impacts can be estimated using global agricultural models. There are different approaches applied to account for the risk of biofuels-induced ILUC. On general, three different approaches are applied (Croezen et al. 2010):

- Risk adder approach (WBGU/Öko, Corbey advice, in some aspects Ecometrica)
- Chain analysis, comparable with chain analysis in the RED (Ensus, E4Tech, in some aspects Ecometrica)
- Agro-economic modeling (IIASA, JRC AGLINK study, IFPRI study, FAO/OECD 2009-2018 Outlook)

The risk adder approach takes into account a standardized emission factor for the land used for biofuels feedstock production, generally a globally averaged GHG emission factor for conversion of forest to arable land. Under the RED legislation, this emission should be divided by a period of 20 years to calculate the GHG emissions per unit of biofuels. An ILUC factor for a specific biofuel is calculated by dividing the resulting annual GHG emission by the biofuels yield per hectare. This approach ignores any effects of by-products and agro-economic interactions between prices, demand, and increases in specific crop yields and trade, or does not render them explicit.

In the chain analysis, an LCA-like (Life Cycle Assessment) approach is applied. The ILUC-related GHG emissions are estimated by comparing land use in a scenario with a situation in which a certain amount of extra biofuels is produced, with the modellers estimating in which region the extra feedstock is grown. Based on anticipated market developments, they estimate how much and what kind of land use change occurs. In this calculation projected crop yield increases are taken into account, as are the effects of substitution of primary crops by biofuels by-products (e.g. substitution of coarse grains by distiller grains). In this approach, economic interactions and their effects are not taken into account.

In agro-economic model, all the parameters are interconnected. In this way feedback loops can be taken into account. However, it can be argued that agro-economic models depend extensively on critical input parameters such as land and commodity prices, and internal resolution and structure (e.g. conversion of pasture land, handling of by-products etc.) (Fritsche et al. 2010).

1.5 Biomass potential

The availability of resources impacts the share of biomass for electricity, heat and fuel production, therefore the assessment of feedstock availability is necessary. Feedstock potential is calculated in terms of theoretical, technical, economic and realistic potential, however disagreements between definitions in different studies should be taken into account. Hoogwijk et al. (2005) distinguished five categories of biomass potential:

- Theoretical potential: the upper limit of primary biomass (the net primary productivity of biomass produced at the total earth surface by the process of photosynthesis).
- Geographical potential: the theoretical potential at land area available for the production of biomass for energy.
- Technical potential: the geographical potential reduced by losses due to the process of converting primary biomass to secondary energy carriers, defined by the conversion efficiency of the conversion.
- Economic potential: the technical potential that can be realized at profitable levels, described by a cost-supply curve of secondary biomass energy.
- Implementation potential: the maximum amount of the economic potential that can be implemented within a certain timeframe, taking institutional constraints and incentives into account.

Biomass categories most often indicated in studies are energy crops, forestry, residues from forestry, residues from agriculture and wastes. However, different studies include different types of biomass and there is no universally applied classification scheme. Accordingly, estimations provided by different authors not always can be compared directly. Therefore, global biomass potential cannot be measured, however it can be modelled.In this case the structure of the model plays an important role in determining the result. Berndes et al. (2003) provided the following distinction between estimates of potential:

- Demand-driven assessments analyze the competitiveness of biofuels or estimate the amount of biomass required to meet targets on climate-neutral energy supply (demand side).
- Resource-focused assessments focus on the total bioenergy resource base and the competition between different uses of the resources (supply side).

Different assessments were made regarding the potential bioenergy production. Several examples were chosen from literature to illustrate different biomass potential estimations. Some of examples include feedstock potentials for second generation bioethanol production, however this information is limited.

Ericsson and Nilsson (2006) assessed the potential biomass supply in EU15 (Austria, Belgium, Luxembourg, Denmark, Finland, France, Germany, Greece, Ireland, Italy, the Netherlands, Portugal, Spain, Sweden, the UK), EU10 (ten new EU member states - Bulgaria, the Czech Republic, Hungary, Poland, Romania, Slovakia, Slovenia, Estonia, Latvia and Lithuania), plus Belarus and Ukraine. Five scenarios (1, 2a, 2b, 3a and 3b) were designed to describe short-, medium- and long-term potential of biomass for energy production. Scenarios were based on assumptions concerning residues harvests, energy crop yields and surplus agricultural land. Each scenario described the potential for development of biomass production within a given time frame, dependent on a number of factors, where 1, 2 and 3 refer to periods of short-term (10-20 years), medium-term (20-40 years) and long-term (>40), respectively. The letters in different scenarios indicate (a) low and (b) high biomass harvests in terms of forest residues and energy crops. Potentials for each biomass category are shown for each scenario in Table 6. Crop residues include cereal straw and corn residues and forest biomass includes forest residues and forest industry by-products.

Table 6: Potential supply of biomass energy in Europe (Ericsson and Nilsson 2006)

Scenario (time perspective)	Countries	Forest biomass (EJ/yr)	Crop residues (EJ/yr)	Energy crops (EJ/yr)	Total (EJ/yr)
Scenario 1	EU15	1.3	0.7	1.1	3.1
	EU10	0.4	0.2	0.4	1.0
	Belarus, Ukraine	0.1	0.1	0.3	0.5
Scenario 2a	EU15	1.3	0.6	2.9	4.8
	EU10	0.4	0.3	1.4	2.1
	Belarus, Ukraine	0.1	0.2	1.3	1.6
Scenario 2b	EU15	1.7	0.6	3.7	6.0
	EU10	0.5	0.3	1.8	2.6
	Belarus, Ukraine	0.2	0.2	1.7	2.1
Scenario 3a	EU15	1.3	0.5	7.3	9.1
	EU10	0.4	0.1	3.8	4.3
	Belarus, Ukraine	0.1	0.1	4.3	4.5
Scenario 3b	EU15	1.7	0.5	9.5	11.7
	EU10	0.5	0.1	4.9	5.5
	Belarus, Ukraine	0.2	0.1	5.5	5.8

The study concludes that under certain restrictions on land availability, the potential supply of biomass energy amounts to up to 11.7 EJ/yr in the EU15 and 5.5 EJ/yr in the EU10. The study also shows that the potential biomass resources are unevenly distributed which may increase international biofuel trade within Europe and lead to biofuel imports from other continents.

Slade et al. (2011) summarized the assumptions leading to the full range of global biomass potentials found in the reviewed literature (Figure 10). Estimates up to 100 EJ (1/5 of current global primary energy supply) assume that there is limited land available for energy crops. Estimates within range 100-300 EJ (around half current global primary energy supply) assume that food crop yields keep pace with population growth and increase meat consumption. Little or no agricultural land is made available for energy crop production Estimates in excess of 300 EJ and up to 600 EJ (600 EJ is slightly more than current global primary energy supply) assume that increases in food-crop yields will outpace demand for food, with the result that an area of high yielding agricultural land (>1Gha) becomes available for energy crops. Only extreme scenarios envisage biomass potential in excess of 600 EJ. The primary purpose of such scenarios is to illustrate the sensitivity of biomass estimates to key variables such as population and diet, and to provide a theoretical maximum upper bound. According to the report, exploiting the potential in the low band of estimates could make an important contribution to future global primary energy supply through a combination of residues, wastes, and energy crops grown on different land types. Moving from the lower to the middle bands implies a dominant role for energy crops and requires increasingly ambitious assumptions about improvement in the agricultural system.

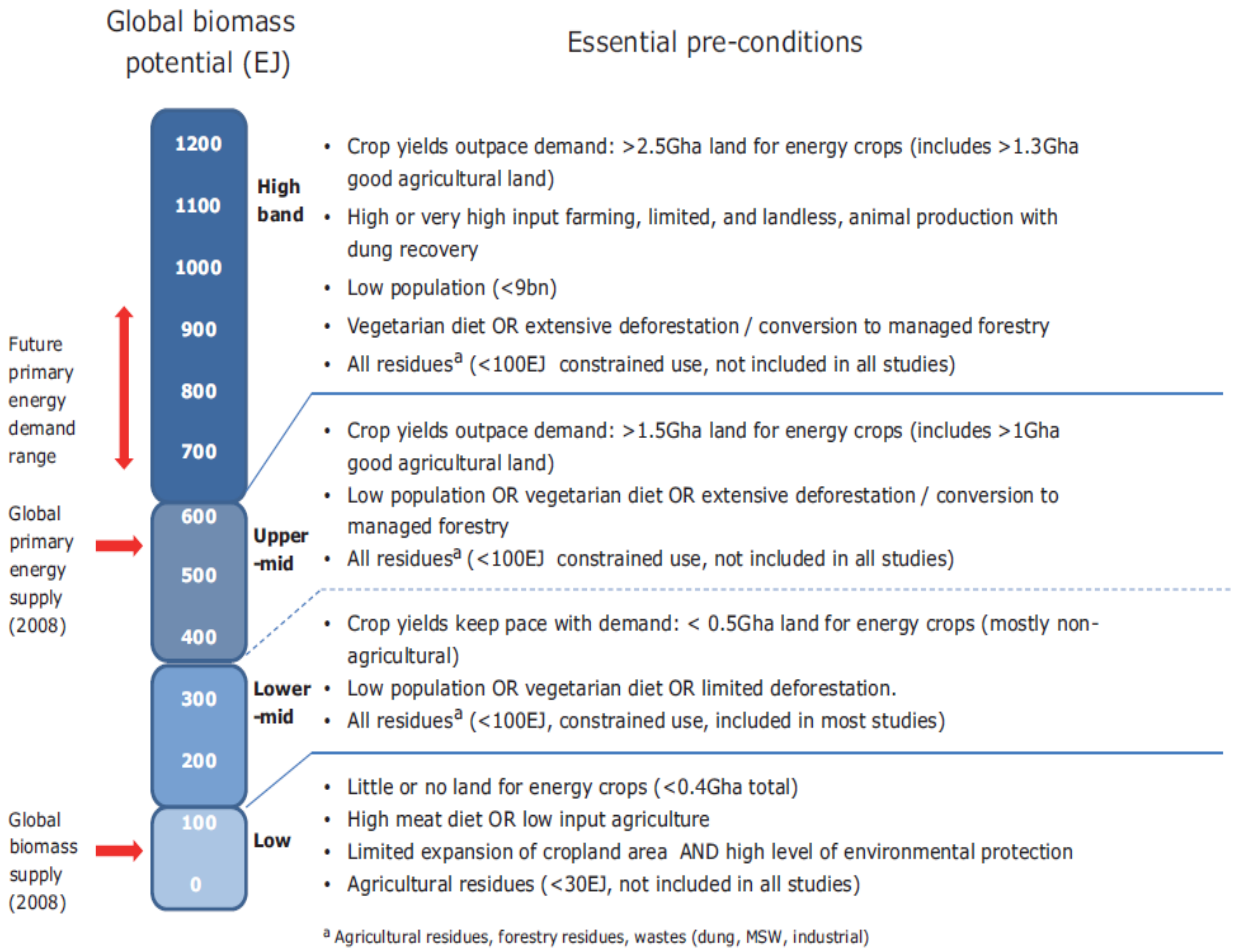

Figure 10: Assumptions for high, medium and low biomass potential estimates (Slade et al. 2011)

According to OECD/IEA (2011), biofuel demand over the next decade is expected to be highest in OECD countries. Non-OECD countries will account for 60% of global biofuel demand by 2030 and roughly 70% by 2050, with strongest demand projected in China, India and Latin America (Figure 11).

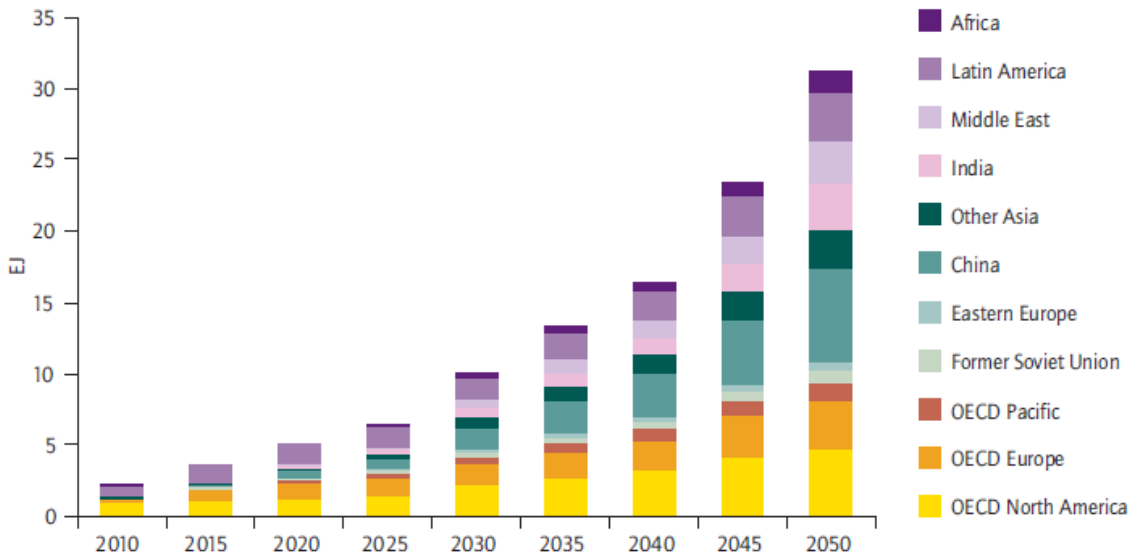

Figure 11: Biofuel demand by region 2010-2050 (OECD/IEA 2011)

A study by IEA (2010) analyzed the potential contribution of lignocellulosic residues for production of second generation biofuels. It assessed the potential of lignocellulosic residues by presenting two scenarios in which 10% and 25% of globally available residues are assumed to be available for biofuel production. The amount of residues was calculated on the basis of annual production data. The IEA assessment showed that considerable amounts of second generation biofuels can be produced from agricultural and forestry residues. Using 10% of global forestry and agricultural residues in 2007 could provide around 4.0 EJ of BTL-diesel or second generation bioethanol and up to 5.7 EJ of bio-SNG. Using 25% of global forestry and agricultural residues could produce around 10.0 EJ of BTL-diesel or second generation bioethanol, which would lead to 10.5% of current transport fuel demand. In order to asses available residues in 2030, increases in agricultural production (1.3%/yr) and roundwood consumption (1.1%/yr) were adopted from FAO (2003). It was concluded that 10% of global forestry and agricultural residues could yield around 5.2 EJ BTL-diesel or second generation bioethanol which would constitute to around 4.1% of the projected fuel demand in 2030. Up to 7.4 EJ could be provided through conversion to bio-SNG (around 5.8% of total transport fuel). 25% of global forestry and agricultural residues converted to second generation bioethanol, BTL-diesel or bio-SNG could contribute 13.0-23.3 EJ globally. That could cover 10.3-14.8% of the projected fuel demand in 2030. The study indicates that around two-thirds of the potential is located in developing countries in Asia, Latin America and Africa. Therefore it is important to include these countries in the development of new technologies.

Fischer et al. (2007) analyzed potential biofuel production in Europe by 2030 in three different scenarios. A reference scenario (‘baseline’) described likely developments under recent policy settings. It took into account effects of ongoing trends in food consumption and technological progress in food production. ‘Low’ scenario predicted a higher share of areas with organic agricultural production. ‘High’ scenario assumed an intensified agricultural production system compared to ‘baseline’. Table 7 summarizes potential biofuel feedstock production in EJ biofuel equivalent. The ‘First generation only’ scenario considers conventional biofuel feedstocks (cereals, oil crops and sugar crops) and conventional chains, whereas ‘Second generation’ scenario also considers herbaceous and woody lignocellulosic plants selecting the best yielding feedstock in energy terms for each land unit.

Table 7: Potential biofuel energy production in Europe by 2030 for different scenarios (adapted from Fischer et al. 2007)

Land availability scenario	Feedstock types scenario (in EJ)							
	First generation only				Second generation			
	EU15+	EU12	Ukraine	Total	EU15+	EU12	Ukraine	Total
Arable land								
Baseline	1.5	2.1	2.3	5.9	2.3	3.2	3.4	8.9
Low	1.3	2.1	2.3	5.6	2.0	3.2	3.4	8.6
High	1.8	2.1	2.6	6.9	2.8	3.8	3.8	10.4
Pasture land								
Baseline and low	Not used				Not used			
High	Not used				1.3	1.0	0.8	3.1

[1] EU15+ includes EU15 and Norway, Switzerland. EU12 refers to new EU members on 2004 and 2007 enlargements. Total is the sum of results for EU15+, EU12 and Ukraine.

It was concluded that the Eastern Europe plays an important role. Also, high potential contribution could come from Ukraine. In addition, significantly higher energy output per hectare implies in most areas cultivation of second generation energy feedstocks. The analysis showed that if agricultural land potentially available for biofuels would be used for cultivation of the most energy efficient biofuel feedstocks, by 2030 up to 50% of projected transport fuel consumption could be produced within Europe (EU27, Norway and Switzerland). If only first generation technologies on arable land would be applied, approximately 20% of projected transport fuel consumption could be covered with domestic resources. More than half of the potential biofuel production is located in EU12. Ukraine could contribute around a third of Europe's overall biofuel feedstock potential due to large and productive arable land area and high possible yield increases.

1.6 Overview on second generation production process

Ethanol is produced either synthetically from petrochemical feedstock (gasoline) or biomass by microbial fermentation. In comparison to first generation bioethanol from sugar and starch materials, second generation bioethanol production from lignocellulosic materials requires additional processing steps, as the crystalline structure of cellulose makes it highly insoluble and resistant to enzymatic attack. It is important to mention that efficient depolymerization of cellulose (source of hexose sugars such as glucose) and hemicellulose (mainly source of pentose sugars such as xylose) is not accessible to microorganisms producing first generation bioethanol. The combination of hemicellulose and lignin provides a protective sheath around the cellulose, which has to be modified or removed before efficient hydrolysis of cellulose can occur.

Polysaccharides - long carbohydrate molecules - are very stable and pentose sugars are difficult to ferment. First of all, polysaccharides need to be hydrolyzed or broken down into simple sugars using acid treatment or enzymes. Therefore, to economically hydrolyze cellulose, more advanced pretreatment technologies are required than for processing sugar or starch crops. After the cellulose and hemicellulose are saccharified the remainder of the ethanol production process is similar to first generation ethanol production:

Compared to sugar and starch based biomass, lignocellulosic biomass processing for bioethanol production is more complex due to recalcitrant nature of the material. Conversion of lignocellulosic biomass to fuel ethanol involves the following steps (Figure 12):

- Crushing (milling, grinding or chipping)
- Pretreatment
- Hydrolysis
- Fermentation of sugars
- Distillation
- Dehydration (only for anhydrous ethanol production)

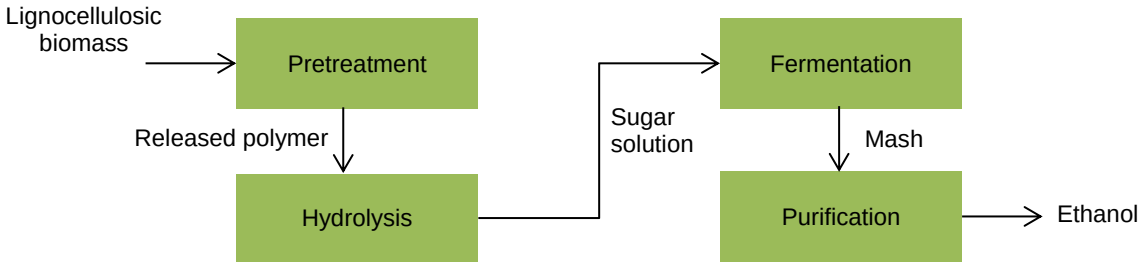

Figure 12: Production process of ethanol production from lignocellulosic materials (adapted from Taherzadeh and Karimi 2007)

First of all, the feedstock needs to be chopped into small pieces to ensure the spread of acid or enzymes in the feedstock. In the pretreatment stage the goal is to destroy the lignin shell protecting cellulose and hemicellulose. At the same time the crystallinity of the cellulose is decreased (Saratale and Oh 2012). Without breaking lignin, sugar containing materials would not be accessible for the hydrolysis process. Depending on the intensity of the pretreatment, various side-products which are inhibiting hydrolysis and/or fermentation steps may be produced such as organic acids or inorganic salts.

Pretreatment process is a crucial process step in bioethanol production process. Depending on pretreatment method the structure of lignocellulosic biomass is broken down and cellulose is made more accessible to enzymatic attack which coverts carbohydrate polymers into fermentable sugars. Pretreatment methods can be classified into three different groups, however these methods are interdependent:

- Biological pretreatment
- Physical pretreatment
- Chemical pretreatment
- Physicochemical pretreatment

Biological pretreatment is based on the use of funghi (white-rot funghi, brown-rot funghi) or special bacteria degrading the structure of lignocellulose. This method compensates disadvantages of chemical and physicochemical pretreatment, but the duration of the degradation process is long and therefore not used commercially.

The goal of *physical pretreatment* is the reduction of particle size in order to make material processing easier. Physical pretreatment can be done by grinding or milling. *Chemical*

pretreatment uses acids or lyes to access lignocellulose. The disadvantages of this method are corrosion, environmental problems as well as risk of process materials degradation.

Steam explosion methods are used in *physicochemical pretreatment.* Biomass is mixed with high pressure and temperature steam (20-75 bar and 180-280°C) and suddenly expanded. The obtained cellulose is highly porous and can be further processed in hydrolysis. The disadvantage of this method is a high energy demand. For mechanical breakdown other methods such like ammonia fiber explosion, CO_2 explosion or liquid hot water (LWH) methods can be applied.

The objective of *hydrolysis* is to split the polymeric structure of the cellulosic material and release glucose and xylose. This process might be also referred to as saccharification of lignocellulose. Using acids and enzymes cellulose and hemicellulose polymers are broken down and individual sugar molecules are formed which can be fermented into bioethanol. Enzymes are proteins that increase the rates of chemical reactions. During enzymatic process, molecules called 'substrates' are converted into different molecules called 'products'. Due to different structure of hexose dominated cellulose and pentose dominated hemicellulose hydrolysis process is complex. Hydrolysis of hemicellulose is much easier to process due to its amorphous structure and happens in the pretreatment step. In order to hydrolyze cellulose, different enzymes or fungi such as *Trichoderma Reesei* are applied.

During the *fermentation* process pentose and hexose sugars released from cellulose and hemicellulose in previous stages are converted to ethanol by a variety of microorganisms. Baker's yeast (*Saccharomyces cerevisiae*) is widely applied microorganism for bioethanol production, however it cannot ferment pentose sugars. In the fermentation stage it is important to suppress various by-products such as acetates, furfural and lignin as they can have a negative impact on pentose sugars processing.

After the fermentation process, produced ethanol has to be separated from the fermentation broth. Different procedures such like distillation or use of membranes and absorbents can be applied. *Distillation* is widely applied upgrading process of ethanol from lower concentration into pure ethanol and is carried out in different steps:

- Evaporation of ethanol from beer - crude ethanol with ~45% concentration
- Purification - ethanol concentration is increased to aezotrope, i.e ~96%
- Dehydration - removal of azeotropic water to reach ~98.7 ethanol concentration as indicated in the Standard EN 15376:2011

1.7 Support policies

The development of biofuels is strongly driven by policy mandates, commitments, regulations and fiscal incentives. Current biofuels policies are seen in a broader perspective including growing competition for productive land and increasing need for renewable energy sources, in particular in the transport sector. The European Commission defined the principal objectives of biofuels policy, namely GHG savings, security of supply and employment (Edwards et al. 2008). Biofuels, including bioethanol, are mentioned in a variety of EU strategies, roadmaps, directives and regulations. In the EU, biofuels have to meet sustainability criteria and cross compliance environmental rules as part of the Common Agricultural Policy (CAP).

The Energy 2020 strategy calls for speeding up the development and demonstration projects for second generation biofuels in order to ensure quick market uptake of sustainable second generation biofuels. The European Commission adopted a comprehensive strategy (Transport 2050 Roadmap, COM/2011/144) outlining ambitious goals to increase mobility and reduce emissions by developing sustainable fuels. Urban transport will have to fulfill 50%

shift away from conventionally fuelled cars to cleaner cars and fuels by 2030, phasing them out in cities by 2050.

The EU biofuels policy is based on several directives: Biofuels Directive (2003/30/EC), Renewable Energy Directive (RED, 2009/28/EC) and Fuel Quality Directive (FQD, 2009/30/EC) which are the key drivers for biofuels development in the EU.

The *Biofuels directive* was adopted in 2003 and sets a 2% renewable fuels target for energy consumption in transport by 2005 and 5.75% by 2010. Even though the targets were not obligatory for the Member States, it was the first attempt to promote the use of biofuels as a wider application of biomass. The *Fuel Quality Directive* was amended in 2009 and sets environmental quality standards for fuels enabling wider use of ethanol in petrol by setting a maximum limit of 10% ethanol by volume in petrol.

Renewable Energy Directive was introduced in 2009 and sets mandatory targets of 20% share of energy from renewable sources by 2020 and 10% share of renewable energy in the transport sector. National Renewable Energy Action Plans (NREAPs) are detailed roadmaps indicating how each Member State is going to meet its legally binding 2020 target. Due to the limited progress achieved by the previous directive, the target is obligatory for all Members States. In addition, RED introduces sustainability criteria for biofuels in order to ensure that only sustainable biofuels contribute towards the 2020 target. For the estimation of the 10% target, the contribution made by biofuels produced from lignocellulosic materials will be considered to be twice that made by other biofuels. Figure 13 indicates the shares of RES in the transport sector in 2020 based on the NREAPs.

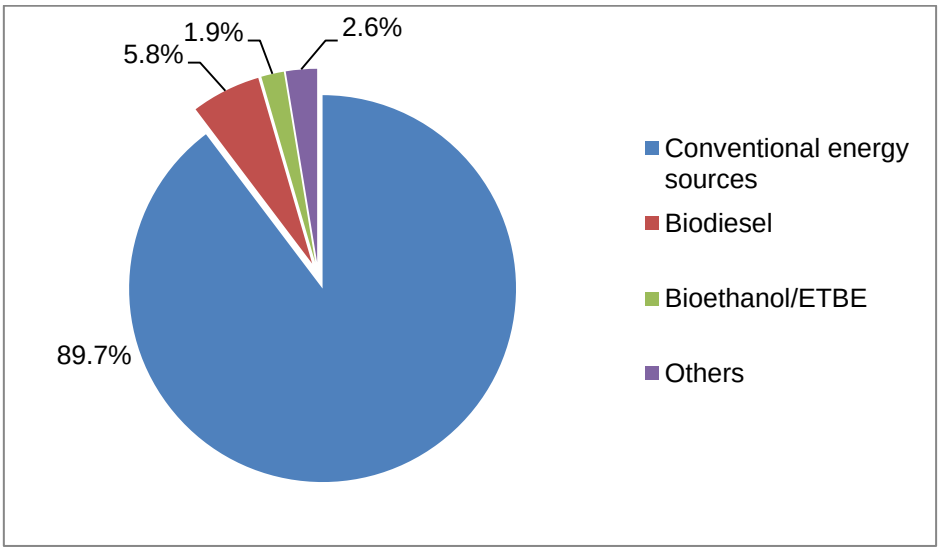

Figure 13: Renewable energy sources in the transport sector in 2020 (EREC 2011)

Tax exemptions and mandatory fuel blending in conventional fuels are the main mechanisms for introducing biofuels. However, no specific support for second generation biofuels is envisaged in the NREAPs. Only a few countries foreseen support measures for second generation biofuels.

According to the NREAPs, in 2015 and 2020 it is expected that second generation biofuels will reach around 2,039 ktoe consumption in transport. The highest consumption level is expected in Denmark, Finland, France, Germany, Sweden, Italy, Spain, the Netherlands and Poland.

Table 8 summarizes data from the NREAPs indicating total contribution from bioethanol technology in the Member States to meet the binding 2020 targets in the transport sector.

Table 8: Estimation of total contribution from bioethanol technology in ktoe (NREAPs 2010)

Country	Bioethanol/ETBE		of which second generation biofuels		of which imported		Expected consumption of second generation biofuels in transport	
	2015	2020	2015	2020	2015	2020	2015	2020
Austria	61.0	80.0	-	-	12.0	11.0	-	-
Belgium	47.4	91.2	-	-	-	-	-	63.4
Bulgaria	19.0	60.0	-	-	-	10.0	-	4.0
Cyprus	2.6	14.7	-	14.7	2.6	14.7	1.5	37.9
Czech Rep.	91.0	128.0	-	29.0	24.0	29.0	-	47.8
Denmark	95.0	94.0	8.0	47.0	95.0	94.0	21.0	131.0
Estonia	14.0	38.0	-	-	-	-	0.2	0.3
Finland	120.0	130.0	20.0	40.0	-	-	70.0	180.0
France	550.0	650.0	-	-	50.0	50.0	200.0	200.0
Germany	996.0	857.0	32.0	32.0	482.0	276.0	133.0	155.0
Greece	256.0	414.0	-	-	256.0	414.0	-	-
Hungary	106.0	304.0	-	-	-	-	21.0	27.0
Ireland	90.0	139.0	-	-	49.0	99.0	1.0	1.0
Italy	374.0	600.0	60.0	100.0	109.0	200.0	248.0	400.0
Latvia	19.0	18.0	-	-	-	-	-	44.0
Lithuania	30.0	36.0	-	-	-	-	-	-
Luxembourg	8.8	23.1	-	-	8.8	23.1	-	-
Malta	-	-	3.7	5.8	3.7	5.8	1.3	3.2
Netherlands	217.0	282.0	22.0	34.0	196.0	240.0	92.0	155.0
Poland	334.0	451.0	-	44.0	-	-	88.0	176.0
Portugal	24.0	27.0	-	-	-	-	6.0	8.0
Romania	121.0	163.0	-	35.0	-	-	-	-
Slovakia	30.0	75.0	-	25.0	-	-	-	60.0
Slovenia	7.6	18.5	-	-	-	-	-	-
Spain	301.0	400.0	-	-	-	-	161.0	252.0
Sweden	358.0	465.0	-	-	185.0	292.0	67.2	94.2
UK	692.0	1,743.0	-	-	574.4	1446.7	-	-
Total	***4,961.8***	***7,286.8***	***145.7***	***391.8***	***2,044.9***	***3,190.6***	***1,109.7***	***2,039.8***

The Danish Energy Strategy 2050 sets an ambitious target of becoming independent from fossil fuels until 2050. According to Danish NREAP, in 2007-2010 the Energy Technology Development and Demonstration Programme (ETDDP) contributed 200 million DKK for the development and demonstration of the second generation biofuels. In Denmark the companies selling fuels for land transport have an obligation to ensure that biofuels make up at least 5.75% of the company's total annual sale. This target was phased in over a three year period: 0.75% in 2010, 3.35% in 2011 and 5.7% in 2012. Second generation biofuels are planned to be introduced from 2015 onwards and will reach 50% share in biofuels by 2020 (both bioethanol/ETBE and biodiesel). Until now no specific blending requirements have been defined for second generation biofuels, however such fuels will have to comply with the CEN standards on biofuel blends in gasoline and diesel.

Finland targets to produce biofuels domestically as of 2020. Three large scale second generation biofuel plants using forest biomass are planned in Finland. In addition, there are second generation biofuel production projects planned to reach 180 ktoe production by 2020. The blending obligation of biofuels has been amended and the national obligation for 2020 is 20%. Finland was able to introduce E10 at an early stage in January 2011, because the necessary national regulations and the E10 petrol quality standards have already been adopted.

France was the first country to introduce E10 in the EU in 2009. It will introduce lignocellulosic biofuels in 2017, but biofuels will mostly remain first generation. Taking into account high uncertainty, NREAP does not include detailed figures on the production of second generation biofuels until 2020. Article 21 of the Grenelle I law states that 'The production of biofuels in France is a subject to energy performance and environmental criteria in particular including their effects on soils and water resources. France will support the establishment of a mechanism for the certification of biofuels taking into account their economic, social and environmental impact. Priority will be given to development and research of second and third generation biofuels.'

Sweden does not apply quotas to renewable energy in the transport sector, but promotes biofuels via financial instruments such as tax exemption and the obligation on service stations to offer biofuel. The Government's vision is to have a vehicle fleet independent of fossil fuels by 2030. The investment will initially relate to second generation biofuels and subsequently to the demonstration and commercialization of other energy technologies of major national importance with an extensive export potential.

In the Netherlands the greatest contribution in 2020 is expected from first generation biofuels. The share of the second generation biofuels will increase from 16% in 2015 to 19% in 2020. Bioethanol/ETBE will make a contribution of 0.28 Mtoe (12 PJ) in 2020. It is assumed that 85% of the bioethanol/ETBE will be imported into the Netherlands in 2020.

In Spain bioethanol/ETBE consumption is expected to nearly double, from 232 ktoe in 2011 to 400 ktoe in 2020. By 2020 one or more of Spain's bioethanol production projects using lignocellulosic or waste material is expected to be at the commercial stage.

Poland has a significant potential for the production of biomass and biofuels. While other EU countries start the research or even production of second generation biofuels, Poland is at the stage of necessary modification of the fiscal and legal environment created for biofuels. In accordance with the Energy Policy of Poland until 2030, the production of energy using second generation bioethanol is planned to start in the years 2020-2025. At the same time, the introduction of second generation biofuels is expected in 2017, which could allow a significant increase of renewable energy sources in transport. Consequently, it is assumed that second generation bioethanol would be used as bio-component after 2017.

Even though the share of second generation biofuels is still limited, the quantity is gradually increasing due to increased deployment of advanced technologies. Under set sustainability criteria the 2020 target is demanding as technologies for the production of second generation

biofuels have to develop strongly at a very fast pace. According to Apostolaki et al. (2012), the achievement of the 2020 target is only possible if the production development of fuels from lignocellulosic crops is strongly boosted already from 2015, as in 2020 a substantial amount of second generation biofuels is required to satisfy the demand.

2 Components of lignocellulosic feedstock

RITA MERGNER, RAINER JANSSEN

Lignocellulosic feedstock is a promising alternative feedstock for bioethanol production. In general, lignocellulosic feedstock can be divided into the following categories:

- Waste materials (municipal solid waste (MSW), paper waste)
- Straw, corn residues and other agricultural residues
- Energy crops, perennial crops and grasses (herbaceous crops)
- Woody wastes, forestry residues and short rotation coppice

The main structural materials of plants are composed mainly of three bio-based chemicals called cellulose, hemicellulose and lignin. Together they are called lignocellulose, a composite material of rigid cellulose fibers bound by lignin and hemicellulose (Figure 14). Cellulose and hemicellulose are polysaccharides that can be hydrolyzed into sugars and eventually fermented to bioethanol, whereas lignin cannot be used for bioethanol production. Lignocellulosic feedstock (second generation feedstock) is more resistant to being broken down than starch- and sugar-based biomass (first generation feedstock). Lignocellulose has a highly lignified and crystalline structure, therefore it requires additional processing steps for bioethanol production.

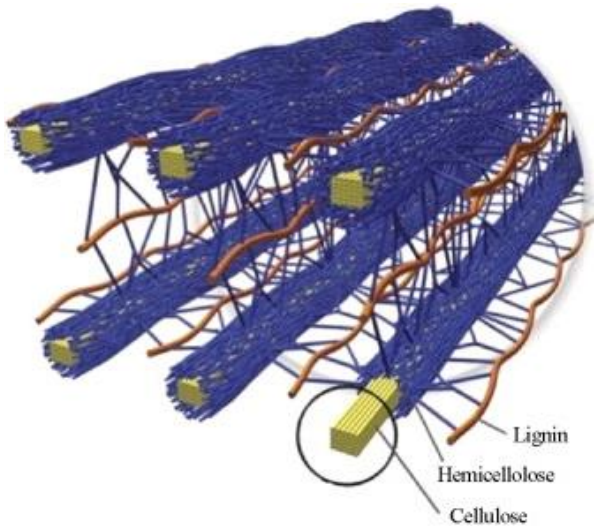

Figure 14: Cellulose strands surrounded by hemicellulose and lignin (Doherty et al. 2011)

Table 9 shows the chemical composition of common lignocellulosic biomass (hardwood and softwood) in per cent. Cellulose and hemicellulose usually comprise two thirds of the dry biomass. Remaining smaller part of lignocellusic biomass is composed of different plant specific chemicals called extractives (resins, phenolics and other chemicals), minerals (calcium, magnesium, potassium and others), salts and acids.

Table 9: Chemical composition of lignocellulosic biomass (Miller 1999)

Chemical components	Hardwood (%)	Softwood (%)
Cellulose	40-50	40-50
Hemicellulose	25-35	25-30
Lignin	20-25	25-35
Pectin	1-2	1-2
Starch	Trace	Trace

Cellulose - $C_6H_{10}O_5$ - (approximately 40-50% of the dry matter) is a sugar polymer chain of glucose - $C_6H_{12}O_6$ - which is a six carbon sugar and is often referred to as hexose sugar or C_6. Cellulose contains a long chain of α-cellulose and short-chain forms of β-cellulose and γ-cellulose. The orientation of the linkages and additional hydrogen bonding make the cellulose polymer rigid and difficult to break. In hydrolysis the polysaccharide is broken down to free sugar molecules by addition of water. This process is called saccharification and produces glucose. Figure 15 shows a cellulose polymer.

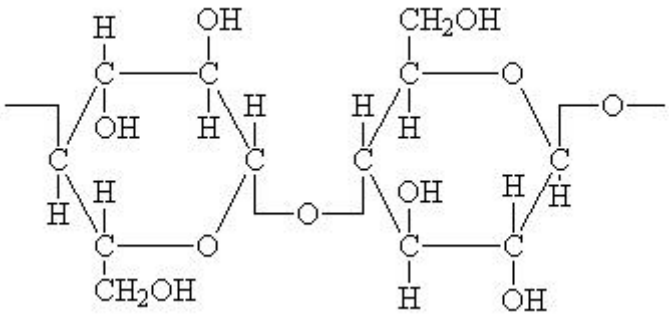

Figure 15: Cellulose polymer

Hemicellulose - $C_5H_8O_4$ - (approximately 25-35% of the dry matter) is a mixture of shortly branched chains of five and six carbon sugars (pentose C_5 and hexose C_6) with varying types of linkages. In contrast to cellulose, hemicellulose is a heteropolymer consisting of short chains of various sugars, mainly five carbon sugars such as xylose and arabinose, and six carbon sugars such as galactose, glucose and mannose. Unlike cellulose, the structure and composition of hemicellulose can vary.

Hemicellulose can be hydrolyzed to six carbon sugars (glucose, mannose and galactose) and the five carbon sugars (xylose and arabinose). It also contains non-sugar groups, such like acetyl. Hemicellulose is relatively easy to hydrolyze due to its branched and shapeless nature. In general, softwood hemicellulose mainly contains mannose as a major constituent, whereas hardwoods mainly contain xylose. Some of xylose units in hardwood species are acetylated (OH groups are replaced by O-acetyl groups) which can cause high levels of acetic acid that can inhibit subsequent yeast fermentation. Figure 16 shows a hemicellulose polymer.

Compared to hexose sugars, pentose sugars (xylose and arabinose) are not easily utilized by *Saccharomyces* yeast strains. Therefore, genetically modified strains such like *Pichia stipites*

or *Zymomonas mobilis* are used for their fermentation. *Candida shehatae* can co-ferment both pentoses and hexoses to bioethanol (Joshi et al. 2011).

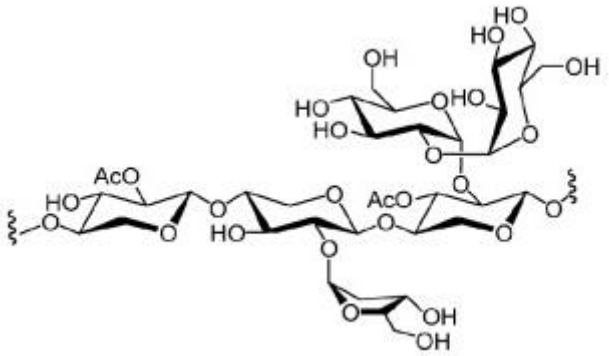

Figure 16: Hemicellulose polymer

Lignin - $C_6H_{11}O_2$ - (lat. lignum - wood) is a complex polymer containing phenyl propane and methoxy groups linked in a three-dimensional structure. Lignin is an integral part of the plant cell walls which cannot be hydrolyzed to fermentable sugars and fermented into liquid fuels as cellulose or hemicellulose. It covers the cell walls, sticks together the cellulose fibrils and is difficult to completely remove. However, it gives stiffness to the cell and may protect against microbial attack. In addition, lignin has high energy content and can be used in the ethanol production process for electricity and heat production or converted into a by-product. Depending on the feedstock, the amount of lignin present in the feedstock might be so high that additional revenue can be gained from excess electricity eliminating the need for fossil sources such as coal or natural gas. Only few organisms are capable to degrade lignin into several high value products, such as organic acids, phenols and vanillin (Hamelinck et al. 2005). Figure 17 shows lignin polymer.

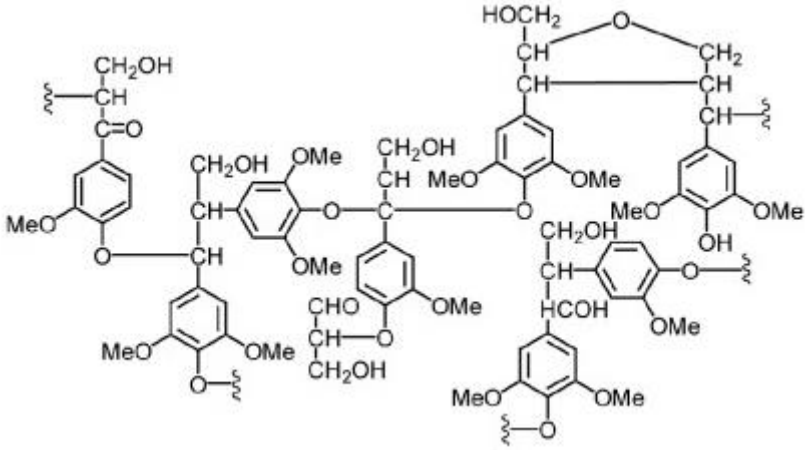

Figure 17: Lignin polymer

To sum up, cellulosic biomass contains different kinds of sugars which can be divided into *hexoses (C_6) and pentoses (C_5)*. Different sugar amounts in different lignocellulosic feedstocks are summarized in Table 10. Besides glucose, the pentose sugars (D-xylose and L-arabinose) are the most abundant hemicellulosic sugars and can make up to 30% of the sugars found in lignocellulose. However, traditional yeast cannot ferment pentose sugars

leading to lower sugar yield. Therefore, pentose sugars fermentation depends on the availability of fermenting yeast strain or hemicellulytic enzymes.

Co-fermentation of both pentose and hexose sugars can significantly improve sugar yield, however a co-fermenting yeast is needed. This creates a major constraint to the economic conversion of lignocellulosic feedstock. Many efforts have been made to construct suitable yeast strains. The greatest success was made in the engineering of pentose utilizing *S. cerevisiae*, *E. coli*, *K. oxytoca*, and *Z. mobilis*. However, it still remains a challenge to get suitable strains fulfilling the requirements of bioethanol production from lignocellulose at industrial level.

Table 10: Cell wall composition among various lignocellulosic sources considered for biofuel (% of dry matter) (Chandel et al. 2010)

Lignocellulosic source	Cellulose	Hemicellulose				Lignin
	Glucan	Xylan	Arabinan	Mannan	Galactan	
Sugarcane bagasse	40.2	22.5	2.0	0.5	1.4	25.2
Wheat straw	32.1	19.5	2.8	0.6	1.1	20
Corn stover	37.5	21.7	2.7	0.6	1.6	18.9
Switch grass	34.2	22.8	3.1	0.3	1.4	19.1
Pine wood	44.8	6.0	2.0	11.4	1.4	29.5
Aspen wood	48.6	17.0	0.5	2.1	2.0	21.4
Spruce wood	41.9	6.1	1.2	14.3	1.0	27.1
Douglas fir wood	46.1	3.9	1.1	14.0	2.7	27.3

3 Feedstock provision

FABIO SISSOT

3.1 Introduction

A number of feedstocks have been investigated with regard to their suitability for second generation bioethanol production. The choice of the most suitable feedstocks depends on many factors, such availability, high cellulose and hemicellulose content, price, contribution in reducing GHG emissions, energy efficiency, high bioethanol yields, and environmental impacts.

Biomass residues, such as agricultural and forest residues or municipal solid waste can be applied for second generation bioethanol production. However, the availability of these feedstocks can be limited due to their wide and non-homogenous distribution. Therefore, dedicated lignocellulosic energy crops are considered to have a high potential for sustainable second generation bioethanol production. In addition, dedicated lignocellulosic energy crops allow diversifying agricultural production by offering an alternative business strategy to local farmers (Francès et al. 2000). Also food processing residues (such as bagasse) can be used for second generation bioethanol production, however this option is not considered in this handbook.

Agricultural residues from the cultivation of food products such as cereal straw (wheat, barley, rice and other species such as oats or triticale and rye) and corn stalks is a valid option for second generation bioethanol production (Carriquiry 2010). However, the existing market for animal breeding has to be taken into account. Among the most promising feedstocks for second generation bioethanol production are species such as Fiber Sorghum (*Sorghum bicolor*), Miscanthus (*Miscanthus spp*), Switchgrass (*Panicum virgatum*), Giant reedgrass (*Arundo donax*), Reed canary grass (*Phalaris arundinacea*), and Cardoon (*Cynara cardunculus*). Ligneous materials from willow and poplar short rotation coppice are considered as well. In terms of availability, the fact that wood from short rotation coppice is often used in heating or power plants has to be taken into account. Picco (2010) mentions dedicated crops such as maize (*Zea mais*), hemp (*Cannabis sativa*), Kenaf (*Hibiscus cannabinus*), willow (*Salix spp*) and black locust (*Robinia pseudoacacia*) for second generation bioethanol production. A proper strategy in feedstock provision suggests selecting high-yield biomass crops, but some of the most important factors to be taken into account in the feedstock selection process are local conditions, geographical area, related pedo-climatic conditions, agricultural structure and environmental implications. This chapter considers dedicated crops and agricultural residues for bioethanol production.

3.2 Biomass sources

As mentioned, the yield in dry matter and the pedo-climate conditions have to be taken into account for the dedicated crops. In addition, the real feasibility of the crop in compliance with the agricultural rotation should be considered. On the base of the work done by Zegada-Lizarazu et al. (2011) and Alexopoulou et al. (2012), Table 11 shows an overview of different dedicated crops species. This includes general evaluation of the most suitable cultivation zones temeperature for sprouting and vegetation, water demand, as well as frost and drought resistance, harvesting period. The most suitable cultivation zones refer to the environmental stratification of Europe (Table 11). The cultivation of each crop in other areas with little difference in climate is possible, however lower yield or vegetative problem risks should be expected. Table 12 summarizes the average yields of selected dedicated crops in dry matter. The selection of dedicated crops is based on the actual level of knownledge about crop cultivation and feasibility in Europe, yields in dry matter and environmental issues.

In the future the list could be updated following the progress of on-field research and results of cultivation at industrial scale.

Table 11: Comparison between some lignocellulosic dedicated crops (modified from Zegada-Lizarazu et al. 2011; Alexopoulou et al. 2012; Cosentino et al. 2012)

Species	Vocated Zones	Temperatures, °C		Water demand	Frost resistance	Drought resistance	Harvesting period
		Seed germination	Vegetation				
Annual herbaceous							
Fiber Sorghum (*Sorghum bicolor*)	CON PAN MDN MDS LUS	12	13-40	M	L	H	Autumn
Perennial herbaceous							
Miscanthus (*Miscanthus spp*)	ATN CON PAN LUS ATC MDN	>9	11-40	H	M	L	At the phenologic drying of the epigeic part[1]
Giant reed (*Arundo donax*)	MDN MDS	>5	5-38	M	L	M/H	Winter[2]
Switchgrass (*Panicum virgatum*)	ATN ATC	>10	10-35	M	H	M/H	At the phenologic drying of the epigeic part[1]
Cardoon (*Cynara cardunculus*)	MDN MDS	>5	1-35	L	L	H	Fall/Summer
Ligneous							
Poplar (*Populus spp*)	ATN ATC NEM CON PAN	>5	3-38	M	M	M	Winter
Willow (*Salix spp*)	ATN ATC NEM CON PAN LUS	>1	1-38	H	H	L	Winter

Note: [1]Winter in Northern Italy; [2]Traditionally in Winter (for bioethanol production the crop can be harvested with a forage harvester all the year, except in summer or when weather conditions do not permit such operation); [3]The period varies with the cultivation zones: ATC, Atlantic Central; ATN, Atlantic North; CON, Continental; LUS, Lusitanian; PAN, Pannonian; MDN, Mediterranean North; MDS, Mediterranean South; NEM, Nemoral.
H - High; M - Medium; L - Low

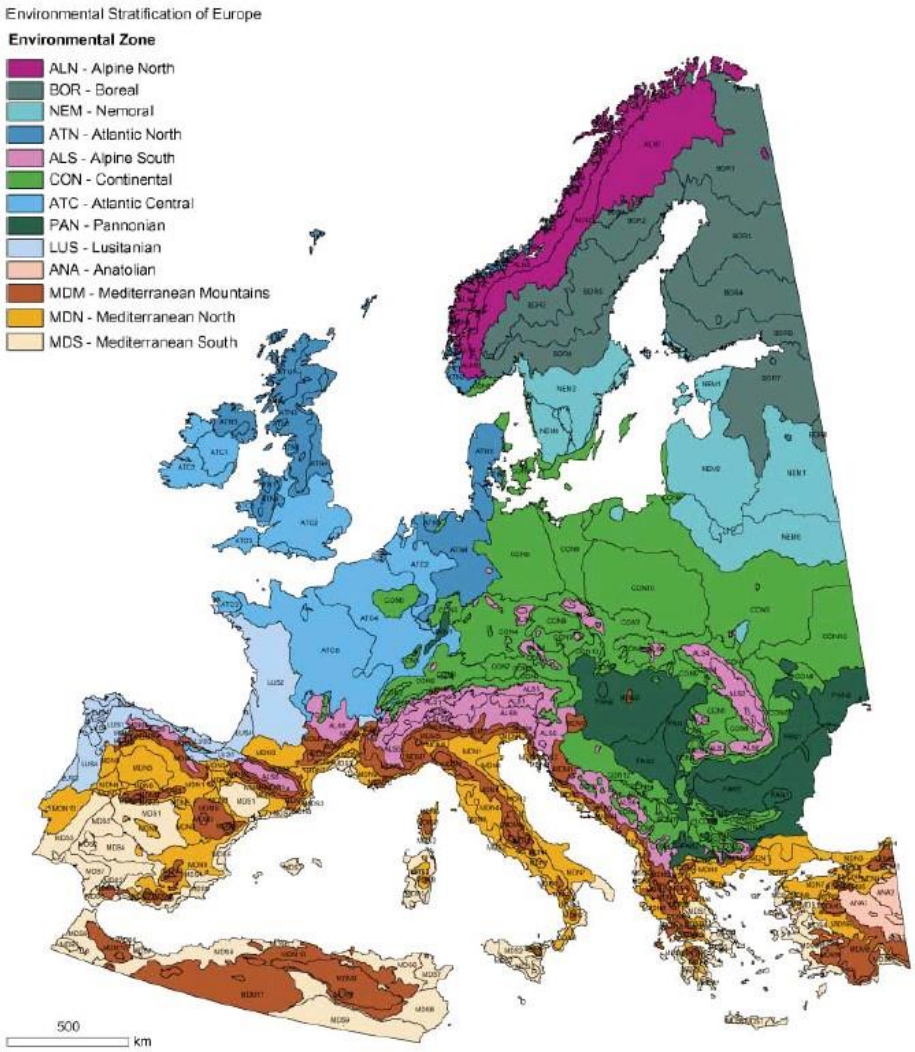

Figure 18: Environmental Zones of Europe (Metzger et al. 2005)

Table 12: Average yield in dry matter of selected dedicated crops

Species	Full production starting (year)	Yield in full production period (t dm/ha*y)	Sources
Annual herbaceous			
Fiber Sorghum (*Sorghum bicolor L. Moench*)	1	10-26	Bellocchi et al. 2000; Quaranta et al. 2010; Amaducci et al. 2000; Mahmood 2012
Perennial herbaceous[1]			
Miscanthus (*Miscanthus spp*)	>3	10-38[2]	Brosse et al. 2012; Scurlock et al. 1999; Defra 2007
Giant reed (*Arundo donax*)	3[3]	20-30	Christou et al. 2003; Angelini et al. 2004; Pari et al. 2009; Nassi et al. 2010; Cosentino et al. 2010; Fagnano et al. 2010; Pari et al. 2009; Pari et al. 2010; Angelini et al. 2004; Angelini et al. 2009
Switchgrass (*Panicum virgatum*)	>3	10-25	Canestrale et al. 2007; Picco 2007;
Cardoon (*Cynara cardunculus*)	3-4	10-20	Luger 2003; El Bassam 2010; Christou et al. 2005
Ligneous[4]			
Poplar 5 years cutting cycle (*Populus spp*)	3-10[5]	7-18	Spinelli et al. 2006; Sperandio 2007; Weger et al. 2002
Willow (*Salix spp*)	3-6	3-12	Weger et al. 2002; Karp et al. 2010; Wickham et al. 2010

Note: [1]Productive cycle ranges beween 10-15 years; [2]At industrial scale, a reducing factor of 30% should be reasonably applied; [3]After the 8th year a decrease in yield was noted; [4]Productive cycle ranges beween 10-15 years; [5]For transplanting plants of two years old are used.

Agricultural residues are by-products of the cultivation of food crops. They can be ligneous (such as fruit tree prunings) or herbaceous (straw, stalks). Fruit tree prunings are produced every year in a large amount during the production cycle of fruit trees. Yields in dry matter range between 2-3 t dm/ha (Garavaglia et al. 2001, Pari et al. 2000). The advantage of using agricultural residues is that no additional land is needed.In this way competition for land is avoided. In addition, biofuel production from agricultural residues should have a low impact on food prices since it is not widely used for animal litter. For example, for rice straw no impact on the straw market is expected.

For second generation bioethanol production the most wide source of residues is cereal straw. Straw is a by-product of cereals such wheat (*Triticum spp*), rice (*Oryza sativa*), barley

(*Hordeum vulgare*) and oat (*Avena sativa*). It consists of the dried stems and leaves of the plants after the completion of the life cycle. The availability of wheat straw is affected by the demand for animal litter and animal feeding. Considering the large amount of straw that a bioethanol industrial plant could use, the impact on the local straw market has to be taken into account.

The yield of straw varies from 2 to 6 t/ha (Larsen et al. 2011, Skøtt 2011, Kadam et al. 2000, A.P.E.V.V. 2002). It depends on the species and the areas of cultivation. Depending on the areas of cultivation, wheat and other similar cereals (e.g. barley, oat or triticale) straw is harvested in June-July, while rice straw is available in September-October.

3.3 Cultivation, supply chain and logistic

The first step in cultivation is the soil preparation, aiming at restoring a good structure of the soil to guarantee the health of the crop. For clay soil, especially in case of perennial crops, breaking the tillage pan in order to allow the capillary water movements could be necessary. The equipment vertically ridges the soil at 60-80 cm in depth.

Ploughing is the main operation, aiming at creating a suitable environment for crop growth and at increasing the water storage capacity of the soil. The common depth for ploughing is around 30 cm. After ploughing soil needs to be refined with a proper harrow. Finally, soil is levelled and ready for sowing or transplanting.

Especially in the first year weeding is required (in order to permit a fast growth of the crop and the development of a good canopy. For annual crops weeding is required before or immediately after sowing. In general, this operation is done by using a non-selective broad-spectrum systemic herbicide. If the crop height allows the tractor to enter the fields an additional mechanical weeding can be done as well by means of an inter row hoe or a rotary cultivator. Depending on the crop, sowing is done by precision or drill seeders, while for rhizomes transplanting equipment derives from machines used in potatoes cultivation. Fertilization is done with rotary fertilizer distributor. There are different methods of irrigation. Commonly a reel irrigator with a swing arm sprinkler is used.

Herbaceous crops can be harvested with forage harvesters or with balers. Forage harvesters are normally used also for maize harvesting. They harvest wet material to be utilized in a short time. Depending on the conversion technology, the material after harvesting could not be optimal for the bioethanol plant.

There are two methods for baling: large square bales (tipical size is 50-100 cm x 80-120 cm, with an adjustable length of 70-240 cm or more) and round bales (generally 90-180 cm in diameter and 100-120 cm wide). The first solution is recommended for working capacity and logistic (handling and storage), but the risk of rainfall water penetration and self-combustion is quite high if the moisture content at baling is not below 20-25%.

As reported by studies of University of Maryland (Vough 2006) and confirmed by interviews to local operators, in case the material is too wet or was not allowed to dry sufficiently in the field, it goes through a sweat process during the first months of storage. During the sweat process live plant tissue respiration and bacteria or mold activity produce heat. Such heat allows the drying of the external part of the mass. Between external dry biomass and internal wet biomass the best conditions for spontaneous combustion could occur.

Round bales are less affected by water rainfall if good weather conditions exist on site and can be left on the field for further drying. Round bales are not exempt from fire risk if the baled material is too wet. Besides hearbaceous dedicated crops, baling is always used for agricultural residues harvesting after main product recovering, such as straw, mainly in large square bales. Upon baling, mowing-conditioning, raking and windrowing has to be taken into account as needed operations. Some prototypes for mowing-conditioned of annual dedicated

crops were proposed as well (Assirelli 2009, Pari et al. 2009, Pari et al. 2010). For ligneous crops harvesting two methods are suggested:

- Self-propelled chippers: if the machines support the diameter of trees, an equipment derived from forage harvester principle cuts the trees and chips them loading in an agricultural trailer (Pari et al. 2009, Pari et al. 2010).
- Whole tree timbering and subsequent chipping: common timbering equipment, as well as specific designed machines can be used (Ibid.). The principle is to cut the whole trees in order to facilitate their drying minimizing the losses of dry matter. Plants are stored in piles and chipped on demand.

As described below, the choice between the different solutions of harvesting also depends on the strategy of supply and logistics. Table 13 shows cultivation and harvesting techniques of some dedicated crops.

Table 13: Cultivation and harvesting techniques of some dedicated crops

Species	Propagation	Operations	Harvesting
Annual herbaceous			
Fiber Sorghum (*Sorghum bicolor*)	Seeds	Soil preparation, weeding, sowing, 2nd weeding (mechanical, if necessary), fertilization, irrigation (if available)	1) Mower conditioner, wind-rake, baling 2) Forage harvester
Perennial herbaceous			
Miscanthus (*Miscanthus spp*)	Rhizomes (or micro-propagated plants)*)	Soil preparation, weeding (1st year), transplanting, fertilization (1st year), irrigation (1st-2nd year)[2]	1) Mower, wind-rake, baling 2) Forage harvester
Giant reed (*Arundo donax*)	Rhizomes (or micro-propagated plants)[1]	Soil preparation, weeding (1st year), transplanting, fertilization (1st year), irrigation (1st-2nd year)[2]	1) Mower, wind-rake, baling 2) Forage harvester
Switchgrass (*Panicum virgatum*)	Seeds	Soil preparation, sowing, weeding (1st year), fertilization (1st year)[3]	1) Mower, wind-rake, baling 2) Forage harvester
Cardoon (*Cynara cardunculus*)	Seeds	Soil preparation, weeding (1st year), sowing, fertilization (1st year), irrigation (1st-2nd year)[2]	1) Mower, wind-rake, baling 2) Forage harvester
Ligneous			
Poplar 5 years cutting *(Populus spp*)	Plants of two years old	Soil preparation, weeding (1st year), fertilization (1st year if needed), transplanting, mechanical weeding between rows (during the 1st year if needed)	1) Self propelled chipper 2) Timber (or specific equipment) harvester, fork 3) Specific equipment for timbering and loading of whole trees
Willow (*Salix spp*)	Cuttings	Soil preparation, weeding (1st year), fertilization (1st year if needed), transplanting	1) Self propelled chipper 2) Timber (or specific equipment) harvester, fork 3) Specific equipment for timbering and loading of whole trees

Note: [1]micro-propagated plants are more expensive to be trasplanted than rhizomes; [2]depending on water availability (rainfalls and soil reserve); [3] generally no more than 100 kg of N per ha are required.

The supply chain strategy is a compromise between the demand of biomass of the bioethanol plant during the year, the agricultural practices, the yield and the biomass harvesting period, the storage methods and facilities, as well as the transport logistic.

As for perennial crops, the cultivation of the propagation material has also to be taken into account. A good strategy is to plan the cultivation and supply of such material.

The crop yield in biomass affects the surface involved in the production process. This means that the supply basin could be divided into several annulus each one having its own annual production of biomass and road distance to the bioethanol plant. Moreover, each plant has a

fixed storage capacity that cannot allow to store the entire seasonal production. Thus, next to the direct delivering after harvesting, middle storages have to be suggested.

The supply strategy and logistic involves the following steps:

- Transport from the field to a bioethanol plant or to a middle storage yard
- Middle storage or storage at the plant
- Transportation from middle storage to a bioethanol plant

The choice of the transport has to take into account the distance to the bioethanol plant and the way the biomass is stored.

The material chipped during the harvesting (forage harvester or self-propelled chippers) is loaded into agricultural trailers (dumpers, more versatile; load volume ranges between 10 and 50 m^3) or road tractors (load volume from 30 to 58 m^3; size depends on the available space for manueuvre in the field) that follow the machine in the field.

Table 14 gives some examples of the biomass density. To avoid the diseconomy of scale the distance should not exceed 5 km for agricultural trailers (average speed on road 30 km/h) and 10-15 km for road tractors (average speed on road 50 km/h). Such vehicles can deliver the biomass to the bioethanol plant or to a middle storage yard.

Table 14: Density of milled biomass of some dedicated crops

Specie	Density (t/m3)	Reference moisture content (%)
Cardoon	0.15	15
Sorghum	0.60	85
Giant reed	0.16	45
Poplar	0.35	51

For the transportation of baled biomass the most common solution is an agricultural trailer. Based on the capacity, th load ranges between 18 and 26 bales for roundbales, and from 24 to 32 bales in case of large square bales.

Middle storage yards allow to optimize the supply chain following the bioethanol plant demand acting as dynamic reserves able to deliver biomass on demand. From middle storage yards the volumes of biomass and their transporting can be well planned during the year by using a high loading capacity vehicle. The choice of the location for yards depends on the availability of sites, the road network and the size of the yard.

Ligneous chipped biomass can be stored in non-covered open-air piles with an average height of 8 m. The volume of each pile and the minimum distance between piles depend on the authorization of the local fire department authority. Another solution is sheds, preferably closed on three sides. If the biomass is harvested as whole trees, they can be left to dry at the field border waiting to be chipped and transported to a middle storage yard or to a bioethanol plant. Herbaceous chipped biomass is preferably stored in sheds or in piles covered with plastic material, in order to avoid losses of material which can be caused by wind.

Table 15 show some examples of the volumes of chipped biomass. The amount of stored material varies with the density and the moisture content.

Table 15: Examples of storage of chipped dedicated crops

Specie	Moisture content (%)	Open-air pile[1] (surface[2] 400 m²; height 8 m)			Shed (surface 400 m²; height 5 m)		
		Volume (m³)	Quantity (t ar)[3]	Quantity (t dm)[4]	Volume (m³)	Quantity (t ar)[3]	Quantity (t dm)[4]
Cardoon	15	-	-	-	1.600	240	205
Sorghum	85	-	-	-	1.600	960	145
Giant reed	45	-	-	-	1.600	256	140
Poplar	51	1.070	375	184	1.600	560	275

Note: [1]For an angle of repose of 45°C; [2]Base of pile 20 m x 20 m; ar: as received; dm: dry matter

Herbaceous biomass bales can be stored in open-air piles or in shed. The latter solution implies higher investment costs when existing sheds are already used for other purposes. However, it ensures better material drying and the protection of harvest from rainfalls and snow.

Open-air piles of bales are generally covered with plastic material (Figure 19). Using this method particular care has to be taken about the on-field drying before baling, expecially for material with a moisture content at harvesting higher than 30%. Other covering materials, such as waterproof geotextile material, were tested with good results on biomass drying (Pari et al. 2009). However, the cost of investment has to be seriously considered. Due to the fact that the soil is not perfectly plain, putting bales on a plain surface (concrete or stabilizated soil) is suggested. This also aims at protecting biomass from soil moisture. In order to avoid infiltrations of water and/or breaking of the covering, the better solution is to built a pile. The amount of biomass stored in each pile has generally to be approved by the local fire department authority. Pile height depends on the available equipment. A height of around 6 m is generally adopted. Bales handling is done with tractor forks.

Table 16 gives some indications about 400 m² of storage surface and the number of stored bales. As for chipped material, the amount of stored material depends on the weight of a single bale, density, species and moisture content.

Figure 19: Biomass bales storage in open-air piles

Table 16: Examples of bales storage on a surface of 400 m^2

	Plastic covered pile Number of bales	Sheld Number of bales
Round bales		
Round bales on the width	6	14
Round bales on the length	15	14
Round bales on the height	5	6
Total of round bales	***300***	***1.176***
Large square bales		
Straw large square bales on the width	5	8
Straw large square bales on the length	28	16
Straw large square bales on the height	8	9
Total of large square bales	***924***	***1.152***

If the distance is longer than 10-15 km from the middle storage yard to the bioethanol plant, the transportation can be done by vehicle with a higher load capacity, such as road tractor (plus trailer) or semitrailers. The transportation volumes range between 80 and 115 m^3, while the recommended length for load is from 7 to 14 m. This helps to optimize longer distances of delivering and decreases the cost of transport.

4 Pretreatment technologies

ISABELLA DE BARI

4.1 Introduction

Pretreatment processes are necessary to reduce the crystallinity and polymerization degree of cellulose, increase biomass surface area, remove hemicellulose and break the lignin seal (Figure 20). It makes hydrolysis of lignocellulose more efficient by breaking the high stability of the materials and by making it accessible to enzymatic or microbial attacks.

The pretreatment of lignocellulosic materials for the production of bioethanol plays an important role in the commercialization of lignocellulosic ethanol. Pretreatment is one of the most expensive processing steps, therefore an effective and economical pretreatment should meet the following requirements: (a) producing reactive cellulosic fiber for enzymatic attack, (b) avoiding destruction of hemicelluloses and cellulose, (c) avoiding formation of possible inhibitors for hydrolytic enzymes and fermenting microorganisms, (d) minimizing the energy demand, (e) reducing the cost of size reduction for feedstocks, (f) entailing low capital costs, (g) producing few wastes, (h) consuming the minimum amount of chemical reagents (Taherzadeh and Karimi 2008). The pretreatment process represents roughly 33% of the total cost of the process, therefore it is essential to develop efficient technologies to reduce its incidence (Mosier et al. 2005).

The selection of pretreatment method determines the process configuration for the further processing steps such as hydrolysis and fermentation. Harsh conditions during pretreatment lead to a partial hemicellulose and lignin degradation and generation of toxic compounds (Tomas-Pejo et al. 2011). Recently, pretreatment research focused on developing promising approaches supporting the enzymatic hydrolysis with lower enzyme dosage and shorter conversion time.

Pretreatment methods are classified into four main categories: *biological, physical, chemical and physicochemical.* However, this classification is rather relative, as different configurations of pretreatment methods can be applied depending on feedstock characteristics. Each pretreatment method has its own advantages and disadvantages and no single pretreatment approach is suitable for all biomass species.

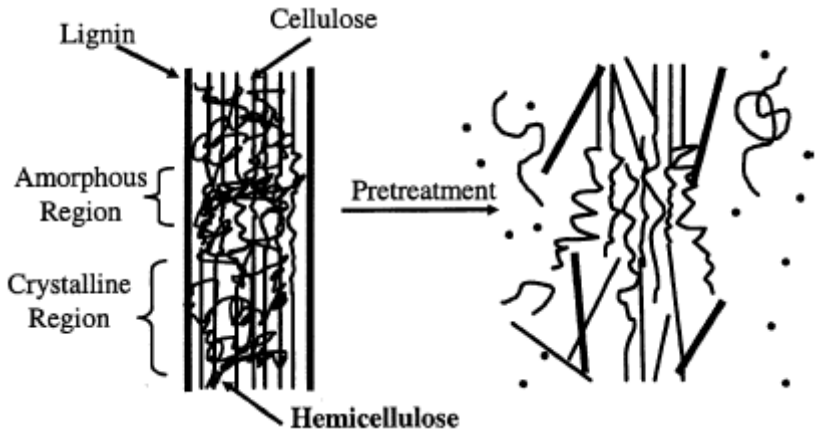

Figure 20: Pretreatment of lignocellulosic material (Mosier et al. 2005)

4.2 Biological pretreatment

Biological pretreatment methods use microorganisms, mainly brown, white and soft-rot fungi to digest lignin and hemicellulose at relatively mild environmental conditions. Biological pretreatment attracted much interest as it offers potential advantages over physical or chemical pretreatments, such as lower energy requirements, lower pollution and high yields of desired products. However, biological methods have been less investigated, compared to physical and chemical methods. The drawbacks of biological pretreatment are time requirements as well as potential carbohydrate loss due to cellulose and hemicellulose degradation.

Some of the most promising microorganisms for biological pretreatment are white-rot fungi that can mineralise lignin to CO_2 and water. Several white-rot fungi such as *Phanerochaete chrysosporium, Ceriporiopsis subvermispora, Phlebia subserialis* and *Pleutrotus ostreatus* have been examined on different lignocellulosic biomass showing high delignification efficiency. The fungi have been studied in connection with several ligninolytic enzymes, such as lignin peroxidases (LiP), manganese peroxidases (MnP), laccase (Lac) and versatile peroxidases (VP). With these enzymes white-rot fungi can be applied in biopulping, biobleaching, ruminant feed, xylose, ethanol, biogas and enzymes production.

Some recent findings demonstrate that delignification cannot be considered as the only parameter to assess the effectiveness of microorganism in biological pretreatment.

The main drawback of biological processes is the low hydrolysis rate compared to other technologies. Furthermore, some species consume part of carbohydrates for the fungal growth. In order to overcome these disadvantages, usually the biological pretreatment is combined with other pretreatment such as a mild chemical pretreatment to enhance the saccharification yields. Consequently, biological pretreatment requires longer process time increasing the operating costs. Overcoming these challenges will play a crucial role in the future development of this pretreatment method. Table 17 shows some examples of biologically pretreated materials.

Table 17: Biological pretreatment of rice straw and wheat straw using different fungi

Biomass	Microorganism	Biological pretreatment conditions	Hydrolysis	Yields %	Ref
Rice straw	Wood-rot fungus (*Dichomitus squalens*)	Inoculum size 1.5×10^4 spores/mL 100 mL colture medium+2 g rice straw at 29°C for 15 days	0.1 g equivalent of glucan in 5.0 mL of buffer (pH 4.8), 60 FPU/g glucan of Celluclast 1.5 L and 30 CBU Novozym 188, 50°C	58.1	Bak et al. 2010
Rice straw	Wood-rot fungus *(Phanerochaete chrysosporium*)	Inoculum size 2.7×10^6 spores/mL 100 mL colture medium+ 2 g rice straw at 29°C for 15 days	0.1 g equivalent of glucan in 5.0 mL of buffer (pH 4.7), 60 FPU/g glucan Celluclast 1.5 L and 30 CBU Novozym 188, 50°C	64.9	Bak et al. 2009
Wheat straw	*Bjerkandera*	2 g of wheat straw in 6 mL + 2 mL of preinoculated mycelia (5 days), 28°C for 21 days. Biological pretreatment was followed by mild alkali treatment	5% (w/v), 15 FPU/ g of Celluclast and NS50010 and 30 U/g (NS50013 and NS50030), pH 4.8, 50 °C	15*	Salvachua et al. 2011
	C.rigida			5*	
	F.fomentarius			17*	
	G.australe			33*	
	I.lacteus			81*	
	P.tigrinus			80*	
	P.radiata			66*	
	P.ostreatus			53*	
	P.alveolaris			76*	
	P.subvermispora			78*	
	S.hirsutum			29*	
	T.versicolor			54*	

* Cellulose digestibility was estimated from the histograms

4.3 Physical pretreatment

4.3.1 Mechanical comminution

Chipping, grinding or milling mainly reduce the biomass size and the crystallinity degree (Figure 21). Several milling procedures were developed depending on the biomass composition. In particular extruder, roller mill, cryogenic mill and hammer mill are used for dry material. Colloid mill, fibrillator and dissolver are used for wet material such as paper from domestic wastes. Ball milling can be used for both dry and wet materials (Taherzadeh and Karimi 2008).

Mais et al. (2002) reported the effect of ball milling used as pretreatment method or prior to the enzymatic hydrolysis of SO_2-impregnated steam exploded *Douglas fir* wood chips. They found the optimal number of ball beads for improving the enzymatic hydrolysis. More recently Jin and Chen (2006) investigated the combination of low severity steam explosion (180°C for 5 min) and superfine grinding (60 µm) with respect to side products generation and enzymatic hydrolysis efficiency. The use of superfine grinding improved the enzymatic hydrolysis of suspensions consisting of 10% steam exploded rice straw by 25-27%.

The energy used for this pretreatment method depends on the final particles size. Vidal et al. (2011) reported that the required energy to mill herbaceous biomass to size smaller than 2 mm is ≤50 kWh/t (corresponding to 2% of the energy that can be recovered from the ethanol produced) and at least twice this value to achieve a similar size reduction for woody biomass. The particle sizes below which the pretreatment does not show significant improvement are dependent on the specific technology: steam explosion (8-50 mm), liquid hot water (1.4-15 mm), dilute acid and base pretreatments (0.8-3 mm). In addition to the energy consumption another disadvantage of this process is that it does not remove lignin.

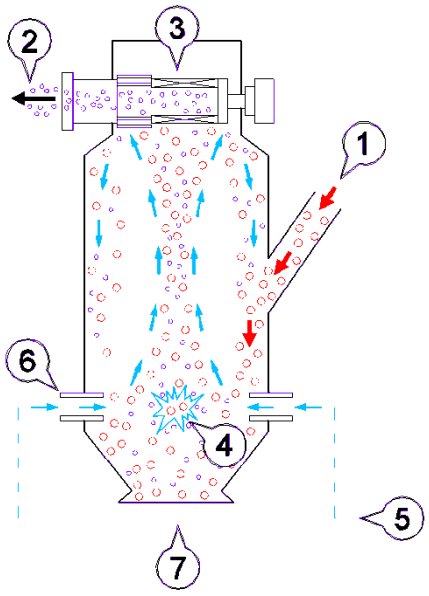

Figure 21: Superfine grinding 1. Infeed 2. Output fine material 3. Classification section 4. Grinding section 5. Compressed air 6. Nozzles 7. Discharge opening (Isabella De Bari)

4.3.2 Irradiation

Irradiation by gamma rays, electron beam and microwaves increases the surface area, reduces the cristallinity of cellulose and can improve enzymatic hydrolysis. It is possible to combine this method with other methods such as acid pretreatment to further accelerate enzymatic hydrolysis. This method selectively acts on the glicosidic bonds and high irradiation above 100MR produces the cleavage of ß-1-4 glicosidic bonds. This pretreatment is particularly useful for material containing significant amount of lignin because, but it is less effective on materials containing small amount of lignin such as newspaper (Kumakura and Kaetsu 1983). On the whole, this method is expensive and leads to difficulties in commercial application.

4.3.3 Extrusion

Extrusion is an option to pretreat lignocellulose due to its adaptability to process modifications such as the addition of chemicals or removal of materials and the application of high pressure. This method includes heating, mixing and shearing resulting in modifications during the passage through an extruder. Extrusion can be a viable pretreatment method due to its ability to simultaneously expose biomass to a range of disruptive conditions in a continuous flow process. Extruder screw speed, barrel temperature, and feedstock moisture content are important factors that can influence sugar recovery from biomass (Karunanithy et al. 2012).

4.4 Chemical pretreatment

A broad range of chemical pretreatment methods has been studied. This chapter provides an overview of the main methods such as organoslov, ozonolysis, acid and alkali pretreatment in which biomass is destructured and fractionated through the use of chemicals.

4.4.1 Ozonolysis

Ozone is an oxidizing agent showing high delignification efficiency (Shatalov and Pereira 2008). Ozone pretreatment mainly acts as lignin oxidant. It is usually performed at room temperature and normal pressure and has a minimum effect on biomass degradation. Ozonolysis has been applied on several agricultural residues such as wheat straw and rye straw increasing the enzymatic hydrolysis yield in both cases (Cubero et al. 2009).

An important drawback to consider is the large amount of ozone needed, which can affect the economic viability of the process (Sun and Cheng 2002). The chemical pretreatment with ozone of rye and wheat straw was investigated by Cubero et al. (2012) by using a fixed bed reactor. Insoluble lignin reduction was about 50%. The hydrolysis tests were carried out at 3% (w/v) initial dry solid loading, supplemented with enzymatic cocktails from Novozymes, namely NS50013 (0.11 g/g cellulose), NS NS50010 (0.05 g/g cellulose). Pricess conditions were pH 4.8 and 50°C for 96 h. The glucose yield after 120 min ozonation ranged from 40% to 50% for rye straw and from 34% to 39% for wheat straw, whereas xylose yields were about 30%. Independently of the cereal straw, longer ozonation time sharply reduced the production of monosaccharides, probably due to the formation of side products. Figure 22 shows a scheme of the ozonolysis process.

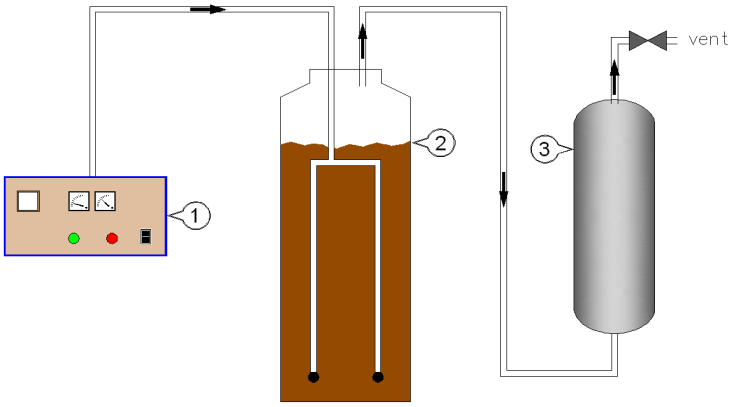

Figure 22: Ozonolysis process 1. Ozone generator, flow meter and concentration monitor 2. Ozonation reactor 3. Ozone destruction column (Isabella De Bari)

4.4.2 Acid pretreatment

Concentrated acids can hydorlyze holocellulose to sugars with high yields. However, this process requires the use of special materials for the reactor construction and typically generates high concentrations of inhibiting compounds. Furthermore, it entails the acid recovery. On the contrary, dilute acid pretreatment could be used to enhance the hemicellulose solubility and increase the cellulose enzymatic digestibility. Direct saccharification of cellulosic biomass by dilute acid was in operation in Germany since 1940s. In recent years, this process has been mainly used as a mean of pretreatment for enzymatic hydrolysis of cellulose.

The most commonly used acid is diluted sulphuric acid (H_2SO_4). Acid pretreatment followed by alkali pretreatment results in relatively pure cellulose. This chemical pretreatment consist of the addition of acids (usually between 0.2% to 2.5% w/w) to the biomass, followed by mixing at temperatures between 130ºC and 210ºC (Brodeur et al. 2011).

High enzymatic hydrolysis yields (74%) have been reported when straw was subjected to 0.75% v/v of H_2SO_4 at 121ºC for 1 h (Saha et al. 2005). Olive tree biomass was pretreated with 1.4% H_2SO_4 at 201ºC resulting in 76.5% of hydrolysis yields (Cara et al. 2008). Other acids, such as hydrochloric acid (HCI), phosphoric acid (H_3PO_4) or nitric acid (HNO_3) have also been studied.

Despite the mentioned advantages, it should be taken into account that washing and/or detoxification steps are typically required to remove acid before proceeding with the fermentation process. One more drawback is the production of fermentation inhibitors which reduce the effectiveness of further processes. In fact, one more drawback is the production of fermentation inhibitors, even if to a minor extent with respet to concentrated hydrolysis, which reduce the effectiveness of further processes.

The optimum conditions for acid pretreatment depend on the purpose of the pretreatment. It was found that the optimal conditions for obtaining maximum sugar yield depend on whether the goal is to maximize the yield after the pretreatment or after the enzymatic hydrolysis of the pretreated solids or if the goal is to obtain maximum yield after both steps. In addition, finding the optimal conditions is extremely important to reduce the formation of inhibitory products that will reduce the efficiency of the fermentation step (Brodeur et al. 2011). Different types of reactors such as percolation, plug flow, shrinking-bed, batch and countercurrent reactors are applied for pretreatment of lignocellulosic materials (Lee et al. 1999).

Batch reactor ensures high yields of hemicellulose hydrolysis in 80-85% range. The continuous version of the batch reactor is a plug-flow reactor (PFR). This is a flow-through reactor in which the liquid and the solid travel through the reactor at the same velocity. One available design consists in a twin screw extruder used as a biomass moving-bed mechanism. Solid-liquid separation is required to recover the sugar in liquid form. In order to retain high sugar concentration in the products, it is necessary to use a high solid/liquid ratio in the reactor.

A *percolation reactor* is a packed-bed flow-through reactor. The main advantages of this reactor are the following:

- The sugar product is removed as it is formed thus reducing the sugar decomposition
- High solid/liquid ratios can be used thus producing relatively high concentration of sugars
- Easy operation

Improvements of this process include the use of two-stages processing of biomass, low-temperature and high-temperature (typical difference is 30°C), two-stage reverse-flow and shrinking-bed reactor (Figure 23).

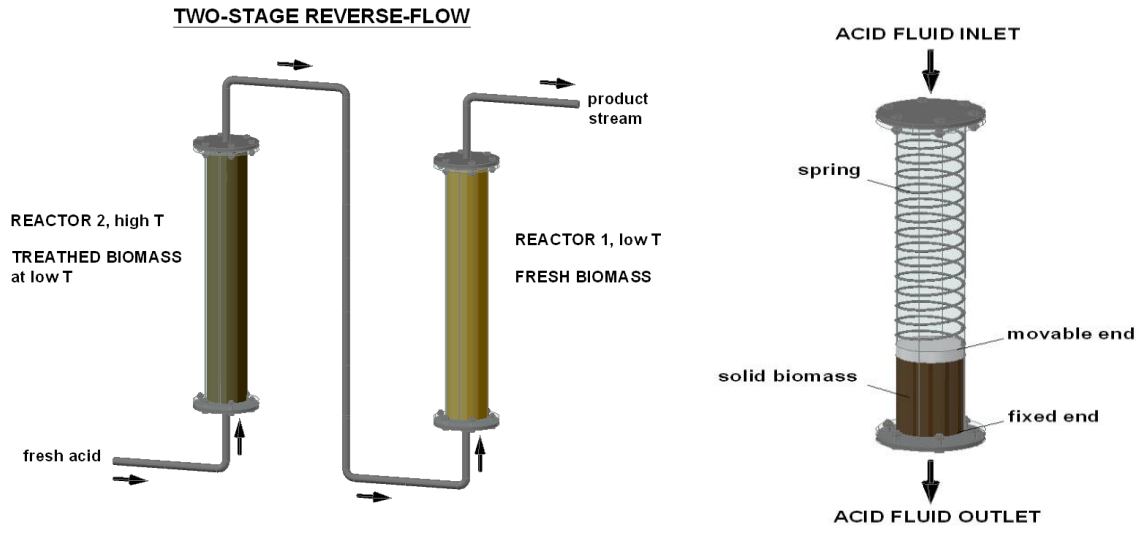

Figure 23: Two stage reverse-flow and shrinking-bed reactor (Isabella De Bari)

Wang et al. (2011) investigated the aqueous dilute acid pretreatment of corncob in cylindrical pressure vessels put in an oil bath. The pretreated material was enzymatically hydrolyzed at 2% (w/v), with 7.5 FPU/g substrate of Cellulast and 11.25 CBU/g substrate of Bovozyme, pH 4.8 at 50°C using a shaking incubator. It was found that at 170°C and acid charge of 2.2% on cob, total glucose yield and xylose recovery were 97% and 75%, respectively, which resulted in an overall monomeric sugar recovery of about 88%.

Weiss et al. (2009) demonstrated high xylose yields at high solids loadings in two different batch pretreatments (4-l Steam Digester and a 4-l stirred ZipperClave® reactor). Corn stover was loaded at 45% wt dry matter after sulfuric acid catalyst impregnation 1.5-1.6% wt/wt acid/water. Pretreatment was carried out at temperatures between 180 and 200°C at a

residence time of either 90 or 105 s. Results demonstrated an ability to achieve high xylose yields (>80%) over a range of pretreatment conditions.

Vancova et al. (2011) investigated prehydrolysis of wheat stubble using moderate temperatures and dilute acid. Conditions for the enzymatic hydrolysis were 5% wt, 5.2 pH, 50°C, and a cellulase dosage of 10 FPU/g. A maximum sugar yield of 541.2 mg/g wheat stubble was obtained by pretreatment at 2% H_2SO_4/90 min/121°C followed by enzymatic saccharification. This represents a conversion efficiency of 87% based on monomeric sugar recovery.

Organic acids such as fumaric or maleic acids are appearing as alternatives to enhance cellulose hydrolysis for ethanol production. Both acids were compared with sulfuric acid in terms of hydrolysis yields from wheat straw and formation of sugar degradation compounds. Some investigations showed that fumaric acid was less effective than maleic acid and that less amount of furfural was formed in the maleic and fumaric acid pretreatments than with sulfuric acid (Kootstra et al. 2009).

4.4.3 Alkali pretreatment

Different bases can be used to pretreat lignocellulosic materials. The effect of alkali pretreatment depends on the lignin content of the materials. Compared to other pretreatment technologies, alkali pretreatment processes utilize relatively low temperatures and pressures. The limitation of this method is that some alkali is converted to irrecoverable salts or incorporated as salts into the biomass during the pretreatment process.

Alkali pretreatment mainly acts on lignin solubilization along with acetyl groups and various uronic acid substitutes (Carvalheiro et al. 2008, Mosier et al. 2005). Lignin removal reduces the non-productive adsorption of enzymes and increases the enzyme effectiveness. It was reported that more than 95% lignin and about 88% hemicellulose in corn stover were removed after alkaline pretreatment with 10% NaOH at 120°C (Varga et al. 2002).

Different feedstocks such as wheat straw, poplar wood, switchgrass and corn stover were studied. Agricultural residues and herbaceous crops have been shown to be more suitable to alkaline pretreatment than woody biomass (Galbe and Zacchi 2007). NaOH, KOH, $Ca(OH)_2$ or NH_4OH are suitable bases for alkaline pretreatments. NaOH increases hardwood digestibility from 14% to 55% by reducing the lignin content (Kumar at el. 2009). Besides NaOH, $Ca(OH)_2$, has been widely investigated thanks to its lower cost and possibility to be recovered after use by reaction with CO_2. $Ca(OH)_2$, known as lime, also enhances cellulose digestibility. This effect was observed for enzymatic hydrolysis with corn stover and poplar wood where lime pretreatment was proven successful.

Alkaline soaking at room temperature instead of elevated temperatures was also found to improve the cellulose digestibility of corn stover (Li et al. 2004). The major problem is washing and/or neutralization of the pretreated slurry. Wan et al. (2011) compared two pretreatment processes for the enzymatic conversion of soybean straw: liquid hot water (170-210°C for 310 min) and alkaline soaking (4-40 wt%) at room temperature to increase the cellulose digestibility. Alkaline pretreatment solubilized xylan up to 46% and the subsequent glucose yield of pretreated solids reached up to 64.55%. It was concluded that liquid hot water yielded better performances. Some authors also tested alkaline pretreatment at cold temperatures. In particular Zhao et al. (2008) investigated the effect of this pretreatment on softwood that is considered as the most recalcitrant material for enzymatic hydrolysis. The results indicated that after biomass soaking with 7% NaOH/12% urea solution for 24 hours, up to 70% glucose yield from diluted suspension of biomass (2% of wood component (w/v), 20 FPU/g dry biomass, 25 CBU/g, pH 4.8 and 50°C) could be obtained from spruce while lower yields were obtained at 23°C and 60°C.

4.4.4 Oxidative delignification

Oxidative pretreatments include the use of oxygen, air and/or H_2O_2. It has been proven to be an efficient method to increase digestibility of cellulose and solubilize hemicellulose and lignin (Table 18).

Wet oxidation (WO) is an oxidative pretreatment method which uses oxygen or air as a catalyst. During the process, phenolic compounds are degraded to carboxylic acids. Oxidation is performed for 10-15 min at temperatures from 170 to 200°C and at pressures from 10 to 12 bar. The addition of Na_2CO_3 has been shown to help the pH stabilization thus decreasing the formation of inhibitory compounds.

The advantage of wet oxidation, especially when combined with alkali, is very limited formation of fermentation inhibitors and efficient removal of lignin. Wet oxidation was investigated for wood, wheat straw, corn stover and sugarcane bagasse. In addition, wet oxidation and steam explosion were compared for the enzymatic digestibility of pretreated sugarcane bagasse (Martin et al. 2007).

Major differences in biomass fractionation and by-product formation have been found. Wet oxidation leads to a major solubilization of hemicellulose and lignin and to almost complete recovery of cellulose, whereas steam explosion (described later) leads to a minor solubilisation of hemicelluloses and lignin. During wet oxidation, aliphatic acids were produced in high amounts whereas the production of furan aldehydes was quite low. In addition, solid material obtained after wet oxidation displayed higher enzymatic convertability than the solid material obtained by steam explosion. It is a strong evidence of the potential of wet oxidation to pretreat sugarcane bagasse prior to enzymatic hydrolysis. Wet oxidation was also recognised as a promising process to remove around 90% of the hemicellulose present in pulp fiber waste. However, cost of oxygen and catalyst are considered one of the major disadvantages for wet oxidation technologies.

Another oxidant agent is peracetic acid having numerous effects on the biomass structure (Duncan et al. 2010). It oxidizes the hydroxyl groups to carbonyl groups, cleaves the β-aryl bonds converting them to hydrophilic groups, oxidizes the hydroquinones to quinones and subsequently to water-soluble muconic, maleic, and fumaric acid derivatives. In addition, it reduces lignin hydrophobicity thereby reducing the non-productive binding of cellulases.

Table 18: Pretreatment by Wet Oxidation (WO)

Biomass	Pretreatment conditions			Hydrolysis		Ethanol yields		Ref
	T [°C]	*Time [min]*	*P*	*Conditions*	*Yields*			
Common reed (Phragmites australis)	195	12 min	Oxygen 12 bar+2 g/L Na_2CO_3	2% (w/w),pH 4.8, 50 °C for 48h, 25 FPU/g solids DM of Celluclast 1.5 L, + 25 IU/g solids DM of Novozym 188	90.5% glucose	SSF, 5% (w/v) DM, *Saccharomyces cerevisiae* (2 g/L DM), 30°C	73.2%	Szijártó 2009
Clover (Trifolium repens) + ryegrass (Lolium perenne)	195	10 min	Oxygen 1.2 MPa	2% (w/w),pH 4.8, 50 °C for 24h, 25 FPU/g solids DM Celluclast 1.5 L, + 0.46 CBU/mL Novozym 188	93.6%, glucose	Presaccharification +SSF, 10% (w/v) with *Saccharomyces cerevisiae* at 32°C for163 h	20 g/L (87.5% cellulose conversion yield)	Martin 2008
Grape stalks	170	60 min	Oxygen 5 bar + NaOH 20%,	Standard conditions, 20 FPU cellulase and 40 IU beta-glucosidase per g cellulose	45% glucose			Ping et al. 2011
Rapre straw 6%DM	205	3 min	Oxygen 12 bar			Prehydrolysis +SSF of the whole slurry. 1. liquefaction: 12.5% DM, 15 FPU/g, DM of Cellubrix , pH 4.8, 50°C , 120 rpm for 24 h. 2.SSF: 20 FPU/g DM of Cellubrix L, and 2.5 g/l dry baker's yeast, at 32°C for 333h	67% ethanol	Arvaniti 2012
Sugarcane bagasse	195	15 min	Oxygen 12 bar + Na_2CO_3 3.4% wt (2/g L),	2% DM, 25 FPU/g DM and 0.46CBU/mL, pH 4.8, 50°C	74.9%			Martin 2007

4.4.5 Organosolv

Organosolv pretreatment processes use organic or aqueous solvents to extract lignin from lignocellulose. Among the pretreatment technologies, organosolv has been considered as one of the most promising for second generation ethanol (Mesa et al. 2011). In this process, lignocellulose is mixed with organic liquid and water and heated. A large number of organic or aqueous organic solvents at temperature ranging from 150 to 200°C can be used with or without addition of catalysts such as oxalic, salicylic, and acetylsalicylic acid that typically produce higher solubilization of hemicellulose.

Organic solvents including alcohols with low boiling point (methanol and ethanol), alcohols with higher boiling point (ethylene glycol, glycerol, tetrahydrofurfuryl alcohol), esters, ketones, organic acids, phenols and ethers have been tested. The main advantage of using solvents is that they produce relatively pure, low molecular-weight lignin. However, the price and recovery of solvent is often a drawback. In addition, organosolv pretreatment has to be performed under extremely tight and efficient control due to the volatility of organic solvents that raise risks of fire and explosion (Zhao 2009). Furthermore, the removal of solvents from the pretreated product is usually necessary because they could have and inhibitory effect on enzymes and microorganisms. This implies the addition of a washing step.

Organosolv pretreatment can be used together with acid hydrolysis to enhance the glucose content in the solid for enzymatic hydrolysis and or to separate hemicellulose and lignin in a two stage process. Mesa et al. (2011) suggested a two-step process including a dilute acid pretreatment followed by the organosolv pretreatment with NaOH under optimized conditions (60 min, 195°C using 30% (v/v) ethanol) for sugarcane bagasse fractionation. This yielded a residual solid material containing 67.3% (w/w) glucose whose subsequent enzymatic hydrolysis gave 29.1 g glucose/100 g sugarcane bagasse. Del Rio et al. (2010) compared several pretreatment conditions including solvent and catalyst for the pretreatment of mountain pine beetle-killed lodgepole pine (*Pinus contorta*). The results are shown in Table 19.

Table 19: Summary of pretreatment conditions used for the generation of pretreated material and relevant hydrolysis yields at 2% solids and 10 FPU/g cellulose (Estimated from the histograms reported by Del Rio et al. 2010)

Chemical/solvent	Conditions	Hydrolysis yields %
NAEM60/EtOH	200°C, 60 min, 0.025 M $MgCl_2$, 78% EtOH	47
NAEM30/EtOH	205°C, 30 min, 0.025 M $MgCl_2$, 78% EtOH	65
H_2SO_4/EtOH	170°C, 60 min, 1.1% H_2SO_4, 65% EtOH	100
SO_2/EtOH	170°C, 60 min, 1.1% SO_2, 65% EtOH	70
NaOH/EtOH	170°C, 60 min, 20% NaOH, 65% EtOH	32
NAEM60/BuOH	200°C, 60 min, 0.025 M $MgCl_2$, 78% BuOH	95
NAEM30/BuOH	205°C, 30 min, 0.025 M $MgCl_2$, 78% BuOH	97
H_2SO_4/BuOH	170°C, 60 min, 1.1% H_2SO_4, 65% BuOH	93
SO_2/BuOH	170°C, 60 min, 1.1% SO_2, 65% BuOH	100
NaOH/BuOH	170°C, 60 min, 20% NaOH, 65% BuOH	85

*EtOH ethanol, BuOH butanol, NAEM neutral alkaline earth metal

Some of the Organoslv processes include phenol-pulping (Batelle Geneva), alcohol-water-pulping (Alcell, Organocell, Lignocell-Bioraff), acetic acid/formic acid pulping (Acetosolv, Formacell, Milox) and monoethanolamine-pulping (MEA). Among these, the only processes that reached the industrial scale were the Alcell process in Newcastle (Canada) which was in operation for about 10 years since 1991 (fed with aspen wood and with a capacity of 1,500 t lignin per year) and the Organocell process in Kelheim (Germany) which started operation in 1992 based on spruce (capacity 430 t pulp per day).

4.4.6 Ionic Liquids (ILs)

As already underlined in the previous paragraphs, the main challenge in the biomass pretreatment is the removal of lignin and hemicellulose increasing the cellulose biodigestibility. However, since the use of bioresources has become significant in several countries another important topic emerged concerning the highest recovery of all biomass components. In particular, besides its energetic utilization, lignin can be used for the production of several chemicals and materials according to the biorefinery approach (Labbé 2012). In order to do that, the pretreatment process should have a reduced effect toward the degradation of its structure. Ionic liquids (ILs) mediated pretreatment seem promising in ensuring this objective. They are organic salts with a melting temperature below 100°C (Figure 24). Certain ionic liquids have been shown to dissolve cellulose, other biopolymers, and even raw biomass under relatively mild conditions. The notable features of ILs are their thermal and chemical stability, nonflammability, wide liquid temperature range and good solvating properties for various types of materials. As no toxic or explosive gases are formed, ILs are called 'green solvents'.

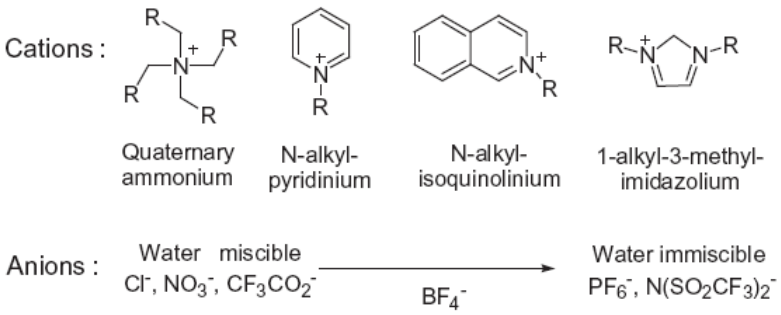

Figure 24: ILs (Liu et al 2012)

The most used salts are 1-ethyl-methylimidazolium l'1- 1-Ethyl-3-methylimidazolium chloride or [EMIM]Cl or 1-Butyl-3-Methyl-Imidazolium chloride [BMIM][Cl].

The process is typically operated in the temperature range of 80-130°C. Methods utilizing ILs require low equipment and energy costs, and research demonstrates that ILs are recyclable. Figure 25 shows a typical biomass fractionation process that makes use of ILs while Figure 26 shows the scheme of an apparatus for ILs recycle.

Recently, several groups have reported the dissolution of several lignocellulosic materials in ILs followed by cellulose hydrolysis with acid or enzymes (Fort et al. 2007; Li et al. 2008).

In order to reduce the accumulation of lignin in the ionic liquid, pretreatment of biomass has been investigated in 1-ethyl-3-methylimidazolium acetate at 140°C with ammonia and/or oxygen to enhance the delignification of miscanthus (Rodríguez 2011). After a treatment at 9

bar for 3 hours, water was added as antisolvent to regenerate the dissolved biomass. The content of lignin in the final product was halved with respect to the raw material.

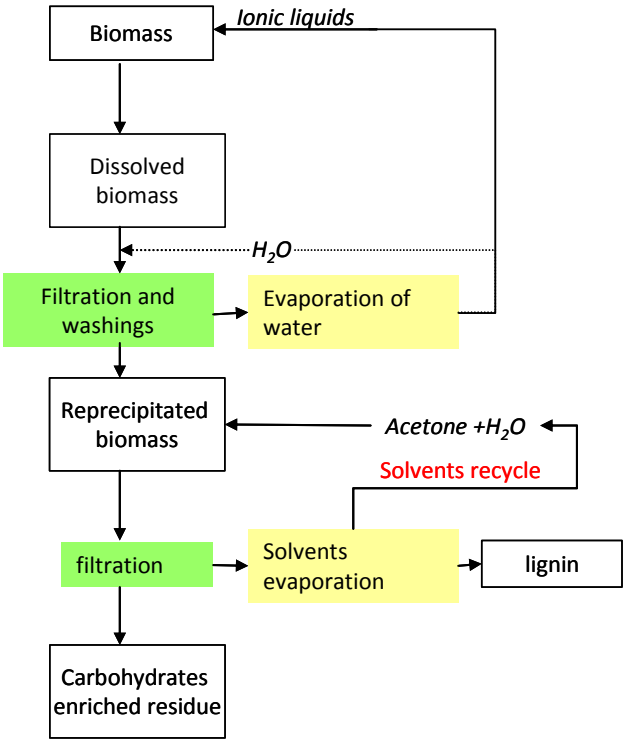

Figure 25: Schematic process for the application of ILs (adapted from Rodrigues et al 2011)

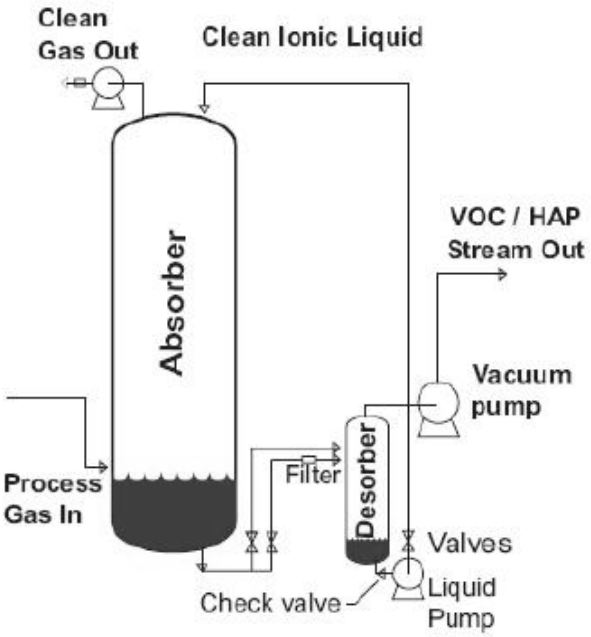

Figure 26: Schematic process for application of ILs in treatment of wood processing exhaust gases (Adapted from Han et al. 2009)

Despite this pretreatment seems to be promising for the development of the biorefinery approach, reducing the cost of ionic liquids still remains a challenge (Han et al. 2009).

4.5 Physicochemical pretreatment

4.5.1 Ammonia Fiber Explosion (AFEX)

Ammonia fiber pretreatment uses anhydrous liquid ammonia to treat the material in a pressurized reactor at temperature between 60 and 140°C for a variable period of time. The pressure is then suddenly reduced producing a rapid expansion of the ammonia gas that disrupts the lignin carbohydrates linkages and produces a partial decrystallization of cellulose, a pre-hydrolysis of hemicellulose, and modifications of lignin (Chundawat et al. 2010) (Figure 27).

Normally, AFEX processes require 1-2 kg ammonia/kg dry biomass, 90°C temperature and 30 min processing time. Compared to acid pretreatment or acid catalyzed steam explosion, the AFEX processes dissolve hemicellulose at a lower extent. Firthermore, small amounts of ammonia left can serve as a nitrogen source in subsequent fermentations (Li et. Al 2010). AFEX pretreatment can improve the saccharification rates of various herbaceous crops, grasses and agricultural residues, but has limited effectiveness on woody biomass and other high lignin feedstocks (Wyman et al. 2005). One possible reason for this is the low effect of this technology on the lignin sheet present in recalcitrant woody biomass that prevents the effective diffusion and reaction of ammonia (Balan et al. 2008).

At optimal conditions, AFEX pretreatment yielded up to 90% conversion of cellulose and hemicellulose to fermentable sugars for a wide range of lignocellulosic materials such as alfalfa, barley straw, corn residue, wheat straw, rice straw, corn fiber, sugarcane bagasse, switch grass and rye grass straw. AFEX processes solubilize only a small amount of material and most of the biomass components remain in the solid fraction. Low formation of inhibitors for the downstream biological processes is one of the main advantages of the AFEX pretreatment. Table 20 summarizes performance of the AFEX pretreatment with several type of biomass.

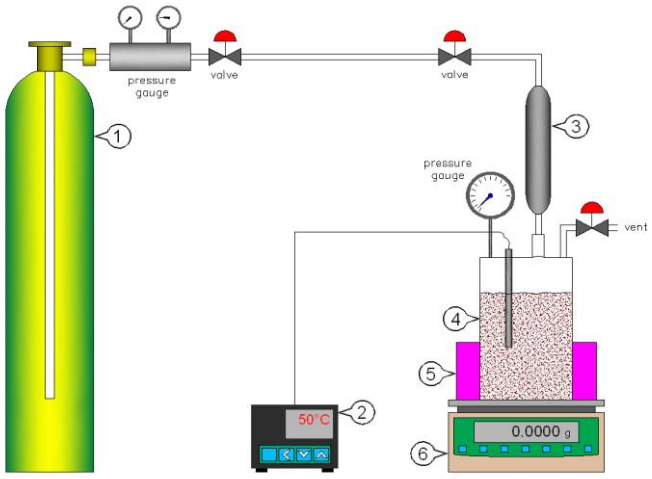

Figure 27: AFEX process (adapted from Teymouri et al. 2004) 1. Ammonia tank 2. Temperature controller 3. Sample cylinder 4. Biomass 5. Heating mantle 6. Balance

Table 20: AXEX pretreatment results on different types of lignocellulosic biomass

Biomass	Pretreatment conditions				Hydrolysis		Ethanol	
	T [°C]	*Time [min]*	*NH_3/biom DM (w/w)*	*Biomass moisture* (g water/g dry biomass)	*Conditions*	*Yields %*	*Conditions %*	*Yields*
Switchgrass	90	30	1:1	1:5.6	5% (w/v), 5IU/g biomass, 48°C	94% reducing sugars at 24 hr		
Corn fiber	90	30	1:1	1:2.3	5% (w/v), 5IU/g biomass, 48°C	Near theoretical reducing sugars at 24 hr		
Rye straw	90	30	2:1	1:2.3	5% (w/v), 5IU/g biomass, 48°C	90 % reducing sugars at 24 hr		
Corn stover	90	5	1:1	1.5:1	3% (w/v), 60 FPU/g glucan, 50 °C	98% glucose		
Sugarcane bagasse	140	30	2:1	1.5:1	6%, w/w, glucan, Spezyme (33 mg/g glucan) + Novozym 188 (31 mg/g glucan), 50°C	85 % (glucan) at 168hr	SHF , recombinant S. cerevisiae 424A LNH-ST, at OD600 of 2, 30°C, pH 4.8, 150 rpm	92% (34-36 g/L)
Forage sorghum	140	30	2:1	1.2: 1	6% glucan, mixtures of enzymes (89.1 g/kg glucan), 50°C	74% glucose; 72.8% xylose at 72 hr	SHF , recombinant S. cerevisiae 424A LNH-ST, at 0.275 g dry cell/L30°C, pH 4.8, 150 rpm	77% (at 96 hr 30-32 g/L) with respect to the initial glucose and xylose in the hydrolyzates
Empty palm fruit bunch fiber	135	45	1:1	1:1	1% glucan, Accelerase (84 μL/g biomass) multieffect xylanase (31 μL/g biomass), multieffect pectinase (24 μL/g biomass), 50°C	90% glucose and xylose at 72hr		

Recently, the AFEX pretreatment was evaluated by numerous researchers in a comparison with other leading technologies to pretreat switchgrass that is considered a promising herbaceous crop for North America (Garlock 2012; Bals 2010; Kim 2011). Garlock et al. (2012) found that under optimized AFEX conditions (2.49 g NH_3:gDM, 2 g H_2O:g DM, 152°C for 12.3 min) the monomeric glucose yield obtained from the variety Alamo at 1% glucan loading and 27 mg enzymatic proteins per g glucan proteins was about 85%, whereas the xylose yield was close to 80%.

The main advantages of the AFEX pretreatment are:

- Low degradation of biomass in molecules with toxic effect for the fermentation microorganisms
- No liquid fraction in the treated product thereby enabling the use of high solid to liquid ratio during the enzymatic hydrolysis
- Recovery of ammonia after the treatment

Lau et al. (2010) investigated the feasibility of fermenting lignocellulosic hydrolyzates from AFEX pretreated corn stover at high biomass loading (18% by weight) without any preliminary detoxification. The pretreatment conditions were 90°C,1.5 H_2O:1 g DM; 1 kg NH_3:1 kg DM. After 6 days pretreatment, the monomeric sugars yields were 65% glucose (52 g/L) and 42% xylose (22 g/L). The enzymatic hydrolyzates were fermented with metabolic yield almost theoretical and volumetric productivity of ethanol of 1.2 g/h/L. Among the potential inhibitors generated by the AFEX pretreatment, the most likely are pyrazine, pyrazine derivatives and its alkyl derivatives. According to Chundawat et al. (2010), these compounds should not have significant effects at concentrations expected even at high solids loading (20% wt) during fermentation (<0.5 g/L total pyrazine and derivatives).

Concerning the ammonia recovery and reuse, some economic evaluations were done to optimize the ammonia loading, recycling concentration and recovery (Sendich et al. 2008). In fact, ammonia loading and residence time have the greatest impact on the economics of ethanol production (Bals et al. 2011). The results of these simulations indicated that the predicted minimum ethanol selling price in an AFEX based biorefinery can be reduced from $1.41/gallon to $0.8/gallon (1 gallon - 3.7 L).

As alternative to the use of liquid ammonia, aqueous solutions containing variable concentrations of ammonia in the range of 5-15 wt% can be used. The corresponding process, known as ammonia recycle percolation (ARP), consists in the passage of aqueous ammonia through a reactor packed with biomass with a percolation rate of about 5 mL/min (Alvira et al. 2010). Typical temperatures are in the range of 140-210°C. It was proved that ARP can solubilize hemicellulose. Another process option is represented by Soaking Aqueous Ammonia (SAA) that can be performed at lower temperatures (30-75°C) than the ARP (Kang 2012; Isci 2008; Kim 2009). This alkaline pretreatment leaves both glucan and xylan in the solids and could be very interesting for being used with pentose fermenting microorganisms.

4.5.2 Steam explosion

Steam explosion (SE) has been recognized as one of the most effective pretreatments for biomass fractionation and bioethanol production (Avellar and Glasser 1998). It uses saturated water steam at a high temperature (180-220°C) for a period of time (from seconds to few minutes) followed by a sudden pressure release. Figure 28 shows a typical apparatus for steam explosion.

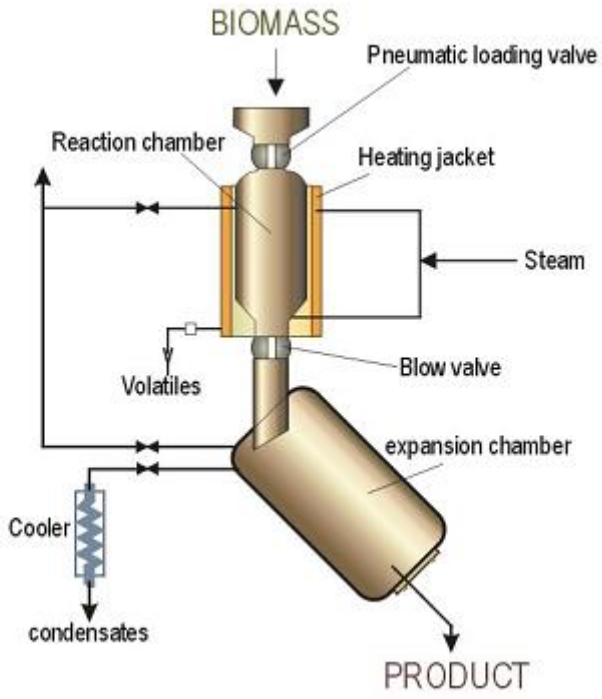

Figure 28: Steam explosion process (Isabella De Bari)

Steam explosion fractionates the biomass in two streams: a liquid fraction rich in monomeric and oligomeric sugars mainly from hemicelluloses solubilisation and a solid fraction of digestable cellulose and lignin (Tomas-Pejo et al. 2011).

The important variables in steam explosion pretreatment are residence time, particle size, and temperature. Steaming at high temperatures causes autohydrolysis conditions due to cleavage of the hemicellulose acetyl groups. The sudden pressure release provokes an explosive decompression that opens the densely packed cell wall in the biomass structure. As effect of these conditions, hemicellulose is partially solubilized and hydrolyzed and the inner surface area is increased, thus improving the accessibility to hydrolytic enzymes. An increase in temperature up to a certain level can effectively release hemicellulosic sugars. However, the loss of sugars steadily increases by further increasing the temperature, resulting in a decrease in total sugar recovery. Milder pretreatment conditions can minimize sugar degradation and generation of inhibitors. Steam explosion process of poplar (Populus nigra) biomass at 210°C and 4 min resulted in cellulose recovery above 95% and enzymatic hydrolysis yield of about 60% as well as 41% xylose recovery in the liquid fraction (Negro et al. 2003).

Some lignocellulosic biomass especially that with high lignin content, are often recalcitrant to steam explosion pretreatment, thus requiring severe process conditions that may help to overcome this drawback but, at the same time, reduce the sugars recovery. Already in the mid 80's some publications proved that biomass impregnation with sulfuric acid combined with mild SE pretreatments improves the enzymatic hydrolizability of water-insoluble fibres and yields higher sugars recovery from water soluble hemicellulose (Schultz 1984, Brownell 1986).

In some cases, especially in case of hardwood varieties, the impregnation with gaseous SO_2 ensured higher recovery of glucose than impregnation with H_2SO_4. More recent results were published by De Bari et al. (2007) concerning the effect of SO_2 catalyzed steam explosion pretreatment of poplar. Remarkable advantages in using SO_2 impregnation have also been proved for several varieties of softwoods (Wu 1999, Shevchenko 2000, Soderstrom 2002),

corn fibre (Bura 2002) and sugar cane bagasse (Martin 2002). Table 21 lists some recent results of acid catalyzed steam explosion pretreatment.

The steam explosion process offers several attractive features such as the potential for significantly lower environmental impact, lower capital investment, increased energy efficiency, less hazardous process chemicals and conditions and high sugar recovery (Avellar and Glasser 1998). Despite its versatility towards different biomass feedstocks, the main drawback of this method is the generation of toxic compounds derived from sugar degradation during pretreatment.

Table 21: Steam explosion pretreatment of different lignocellulosic biomass

Biomass	Pretreatment conditions				Hydrolysis		Ethanol	
	T [°C]	*Time [min]*	*SO_2 wt%*	*H_2SO_4 wt %*	*Conditions*	*Yields %*	*Conditions*	*Yields %*
Aspen chips	205	3	0.9				SSF, 13%, w/w DM, Cellulast (12.6 FPU/g DM) + Novozym 188 (64 IU/g DM), S.cerevisiae Sigma II 5 g/L, pH 4.8, 35°C	75% (3.6% w/v).)
Corn stover	190	5		3	2%, w/w DM, Celluclast (15 FPU/g DM) + Novozym 188 (23 IU/g DM), pH 4.8, 40°C	93		
Salix	200	4		0.5	2%, w/w DM, Celluclast (15 FPU/g DM) + Novozym 188 (23 IU/g DM), pH 4.8 40°C	82	5%, w/w DM, Celluclast (15 FPU/g DM) + Novozym 188 (23 IU/g DM), pH 5, 50°C, S. cerevisiae baker yeast	79 % (16 g/L)
Corn stover	190	1.5		1.1			10% wt, 15 FPU/g of cellulose, pH 5.2, S. cerevisiae D_5A, 32°C	85%
Lodgepole Pine	200	5	4				**Prehydrolysis** for 6 hr 5% (w/v) of Washed solids+water soluble fraction, pH 5, 40 FPU/g cellulose (Spezyme) and 20 CBU/g cellulose (Novozym 188) ,at 50°C. **SSF**+ 5 g/L of *S. crevisaie* at 37°C	77% (244 g ethanol /kg raw material)

Some papers investigated various additives which increase the efficiency of the sugar production. In particular, Zabihi et al. (2010) investigated the pretreatment of wheat straw by steam explosion soaked with acetic acid or ethanol in the temperature range 180-225°C and retention time 3-60 min. The results showed that the pretreatment of wheat straw by steam explosion soaked with acetic acid or ethanol was more effective than that by steam explosion alone. The optimal conditions maximizing the sugar recovery were steam/acetic acid 50% v/v in 150 mL pretreatment vessel at 220°C for 8 min and steam/ethanol 75% v/v in 150 mL

pretreatment vessel at 220°C for 5 min. However the effect of additives on the products fermentability should also be taken into account. The choice of additives in the steam explosion process depends on the final fractionation. Besides acids, steam pretreatment can be performed in alkaline environment. Sodium hydroxide has been used as a catalyst for the extraction of hemicelluloses from softwood (Palm and Zacchi 2004) and barley straw (Persson et al. 2009). The mechanism of hydrolysis consists in the cleavage of the ester linkages between plant polysaccharides and lignin, which increases the solubility of the hemicelluloses, without reducing their molecular mass. These hemicelluloses can be used in several high-value-added applications In particular, Persson et al. (2009) demonstrated that by using NaOH concentrations of 1 wt% in a steam explosion process at 180°C for 50 min, 30% of the arabinoxylan in barley straw could be extracted with high-molecular-mass, without dissolving the cellulose. Even higher percentages, namely 44%, were recovered from wheat straw by using NaOH concentrations of 5 wt% in a steam explosion process at 190°C for 5 min.

Most of the steam explosion pretreatments were carried out in small-size batch reactors. However, the continuous processing is of major interest for the industrial application. In particular the disruption and hydrolysis of cellulose fibers is more effective in the continuous reactor. On the other hand, the mechanical compression causes reduction of fiber accessibility to the enzyme at high pretreatment severity and lignin could recondense on the biomass pores. Figure 29 shows conceptual design of a continuous steam explosion device.

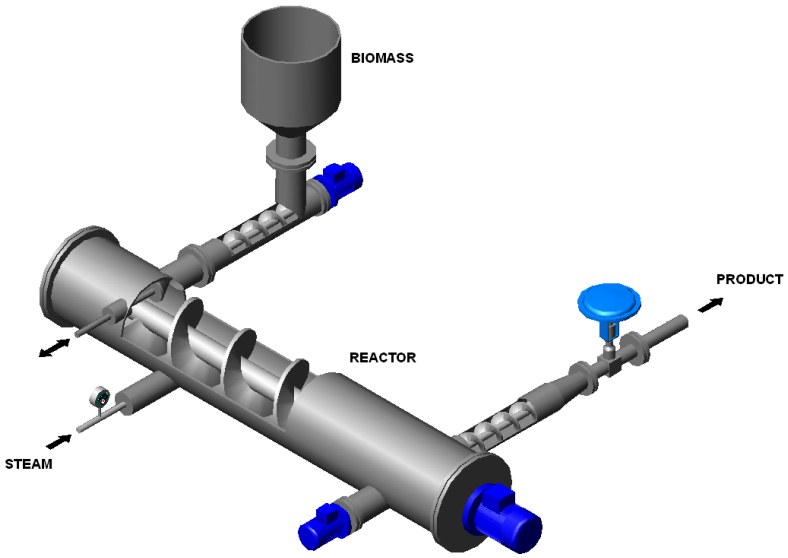

Figure 29: Conceptual design of continuous steam explosion device

Some authors investigated post-steam explosion refining for the conversion of Douglas fir. They used a mechanical pulping refiner as a mean to decrease fiber size and increased the pore volume. More recently Fang et al. (2011) used the Andritz pressurized refiner. The system consists in a pilot scale continuous pressurized reactor (50-200 kg/h of dry biomass) combining the steam explosion and mechanical refining (Figure 30).

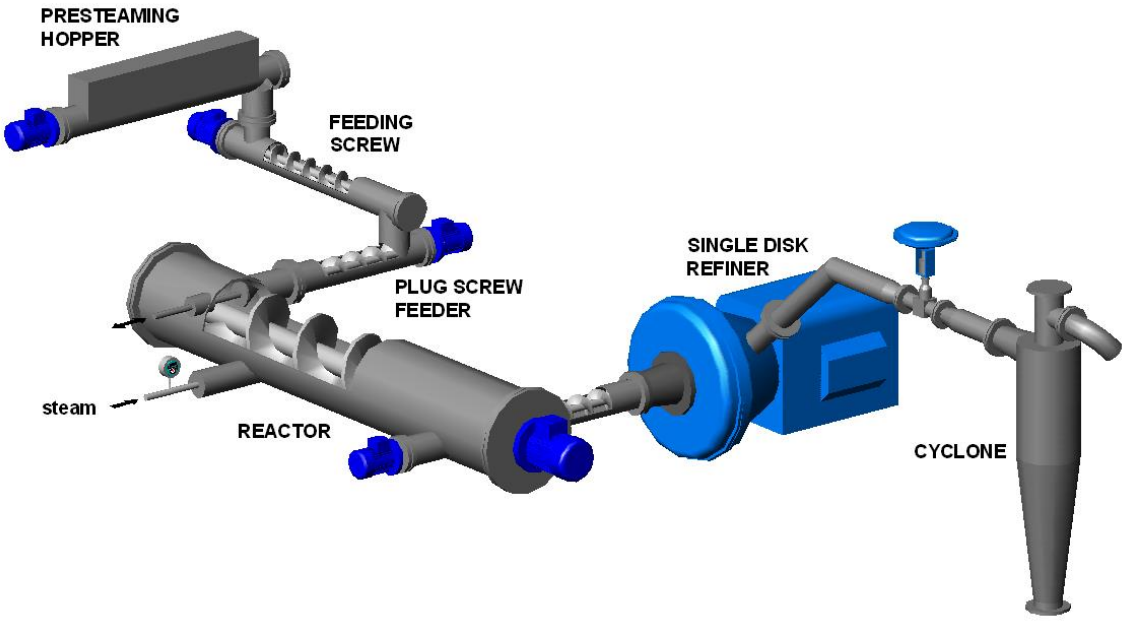

Figure 30: Conceptual design of pressurized refiner (adapted from Fang et al. 2011)

4.5.3 Liquid hot water pretreatment (LHW)

The Liquid hot water process is a hydrothermal treatment in which the pressure is applied to maintain water in the liquid state at elevated temperatures (120-260°C), thus producing alterations in the structure of the lignocellulose. This method does not require any chemical or catalyst and its advantage is low generation of inhibiting by-products and high yields of sugars recovery.

The thermal conditions produce the hydrolysis of the acetyl groups within hemicelluloses that create the acidic medium producing a partial solubilization of hemicellulose. The effectiveness of LHW pretreatment on cellulose digestibility is dependent on the pretreatment severity. This technology enables high degrees of biomass solubilization. However, the yield of undesired degradation products increases with treatment severity as well (Rogalinski 2008). The use of a pH-controlled process could reduce the biomass degradation (Mosier et al. 2005). Complete delignification is not possible at this stage, however lignin is partially depolymerized and solubilized.

Many studies have been carried out on the pretreatment of lignocellulosic biomass with LHW, especially for herbaceous feedstocks. LHW pretreatment can remove up to 80% hemicellulose from these biomasses and enhance the enzymatic hydrolysis in herbal feedstocks, such as corn stover, sugar bagasse or wheat straw.

Wan et al. (2011) investigated the use of LHW for soybean pretreatment. They found that xylan dissolution from the raw material increased with the pretreatment temperature and time. At the optimized conditions (210°C for 10 min), the maximum glucose yield from tests at 2.5 % (by weight) consistency and 20 FPU/g solid was 70.76%. Under these conditions 80% of xylan was removed. Cara et al. (2007) compared the effectiveness of LHW with steam explosion pretreatment of olive tree pruning biomass at 210°C for 10 min. The enzymatic hydrolysis of pretreated residues was performed at high solid loadings up to 30% (w/v) with 15 FPU/g substrate. The hydrolyzates from LHW-pretreated solids at 20%consistency contained 52 g/L (yield 64%).

This method uses high amounts of water and the biomass-to-liquid ratio ranges from 5 to 10% (w/v). This is considered one of the main disadvantage. There are three types of LHW

configurations: co-current, countercurrent and flow through (Mosier et al. 2005). In co-current reactor, liquid hot water is used to pretreat biomass and the biomass liquid slurry containing approximately 16% of un-dissolved solids passes through heat exchangers, is heated to 140-180°C and is held for 15-20 min. The slurry is then cooled and heat is recovered by countercurrent heat exchange with the incoming slurry. Countercurrent technology is designed to move water and lignocellulose in opposite directions through the pretreatment reactor. Flow-through technology passes hot water at 180-220°C and 350-400 psig to achieve overall sugar yields up to 96% from hemicellulose even if low concentrations of hemicellulose sugars in the range 0.6–5.8 g/L are achieved. More recently some authors investigated different reactors in order to reduce the amount of water required. Two different types of reactors, a batch autoclave and a continuous-flow apparatus, were compared by Rogalinski et al. (2008). The batch autoclave has the advantage that no substrate size reduction is necessary and that high solid-to-water-ratios can be loaded. The continuous-flow reactor allows a continuous removal of the solubilized products with the effect of reducing the degradation. However, high energy demand for the comminution and low substrate concentrations to obtain the slurry to be pumped into the reactor are required. In a subsequent study, the authors developed semi-continuous fixed-bed reactors that could help a more efficient hydrolysis (Ingrama et al. 2009). The substrate can be packed into the reactor and flown through by smaller amounts of water compared with the continuous flow device.

4.5.4 Supercritical Fluid Pretreatment (SCF)

Supercritical fluids are compounds in a gaseous form compressed at temperatures above their critical point to a liquid-like density. A number of different supercritical fluids, mainly including water and CO_2 have been studied.

Explosive release of CO_2 pressure is known as CO_2 explosion technology. CO_2 acts as an acid catalyst and at atmospheric conditions it is immiscible in water and can be easily separated and recycled. Moreover, the low temperature prevents any appreciable decomposition of monosaccharides by the acid. Upon an explosive release of the carbon dioxide pressure, the disruption of the cellulosic structure increases the accessible surface area of the substrate to hydrolysis. Supercritical CO_2 with a critical temperature (T_c) of 31°C and a critical pressure (P_c) of 7.4 MPa has an excellent potential for biomass pretreatment. Typically used as an extraction solvent, it has gained importance as a solvent for pretreatment of biomass.

This pretreatment was used by Zheng et al. (1998) on recycled paper mix. The obtained hydrolysis yield of biomass CO2-exploded (at 20.7 MPa and 35°C) and subsequent hydrolysis (0.1% w/v of enzyme liquor, for 24 h) was 72.6%. The authors also tested the effectiveness of other gases like nitrogen and helium but carbon dioxide appeared to be the most effective. The method has been recently applied to other substrates (Table 22).

The advantage of this technique is that the supercritical fluid possesses both the properties of a gas (easy mass transfer) and a liquid (solvating power). Moreover, it does not leave any residual material on the treated substrate, which could affect subsequent steps.

Even though SCF is being investigated in a number of studies for pretreatment, the whole process has not been proven economically viable with high pressures involved being an obstacle. Also further improvements need to be achieved to implement the process on a large scale.

Table 22: CO_2 explosion pretreatment on different types of lignocellulosic biomass

Biomass	Pretreatment conditions			Hydrolysis	
	T [°C]	*Time [min]*	*P*	*Conditions*	*Yields*
Wheat straw ball milled and impregnated with water (0.833 mm)	185	30	CO_2-12 MPa	3% wt, 25 FPU g^{-1} Cellulose, for 72 h	208.4 g sugars kg^{-1} (total reducing /mass of wet straw)~26%
Corn stover	150	60	CO_2-3500 psi	100 mg biomass/30 mL buffer, 50 U of cellulase and 20 U of b-glucosidase, at 47 °C for 24 h	30% glucose yield
Switchgrass	160	60	CO_2-H_2O 200 bar	1 (wt.%) cellulose ,pH 4.8, 15 FPU/g cellulose of spezyme + xylanase +β-glucosidase, 50°C	81% glucose yield
Corn stover	160	60	CO_2-H_2O 200 bar	1 (wt.%) cellulose ,pH 4.8, 15 FPU/g cellulose of spezyme + xylanase +ß-glucosidase, 50°C	85% glucose
Aspen (hardwood), 27% DM	165	30	CO_2 -3100 psi	0.5 g of dry biomass/ 30 ml buffer,100 mg cellulase pH 5.0, at 50°C for 72 h	85% reducing sugars

4.6 Comparison of pretreatment methods

Pretreatment methods aim at making lignocellulose accessible to enzymes and have been studied for improving ethanol production processes. The most appropriate pretreatment method depends on feedstock characteristics.

Table 23 shows a comparison of various pretreatments in terms of their efficiency on reducing the cellulose DP. For instance it appears that lime and controlled pH pretreatments have similar effect on the DP reduction of poplar, but showed wider differences for corn stover.

Table 23: Cellulose DP after the pretreatment (Hallac et al. 2011)

Biomass	Pre-treatment	DPa
Corn Stover	AFEX	6800
	ARP	4500
	Controlled pH	5700
	Dilute Acid	2700
	Lime	3100
Poplar	SO_2	3000
	AFEX	2600
	ARP	3100
	Controlled pH	1800
	Lime	1500
Bagasse	SO_2	500
	O_3	800
	CO_2-explosion	572
	Alkaline explosion	550
Wheat straw	O_3	908
	CO_2-explosion	698
	Alkaline explosion	662
	Organosolv (acetic acid)	1594
	Organosolv (formic and acetic acid)	2182
	Organosolv (methanol)	1519
Eucalyptus regnans	Organosolv (ethanol)	1356
	O_3	1065
	CO_2-explosion	815
Pinus radiata	O_3	2900
Spruce:Pine	Dilute Acid	200

Different sources of literature compare various pretreatment methods and introduce results of demonstrating experiments. However, it is not possible to identify an ideal pretreatment method as technologies not only combine different methods applied on the theoretical or experimental basis, but also because the pretreatment phase must be seen all together with the overall process, as for instance happens in the case of the tar removal (conversion step in the thermochemical gasification plants). Results depend very much on pretreatment conditions, feedstock characteristics, and downstream process, thus the indication of advantages and disadvantages is theoretical and must be seen as a general guideline. Table 24 summarizes the advantages and disadvantages of main pretreatment methods. In general methods such as AFEX, wet oxidation and LHW treatment seem to be more successful for agricultural residues whereas steam pretreatment has resulted in high sugar yields for both forestry and agricultural residues (Hahn-Hägerdal et al. 2006).

Table 24: Advantages and disadvantages of different pretreatment methods

Pretreatment method	Advantages	Disadvantages
Biological	Degrades lignin and hemicellulose Low energy consumption	Low hydrolysis rate Requires long incubation time Requires careful control of growth conditions
Milling	Reduces cellulose crystallinity	High power and energy consumption
Steam explosion	Causes hemicellulose auto hydrolysis and lignin transformation Cost-effective especially for hardwoods and agricultural residues	Destruction of a portion of the xylan fraction Incomplete disruption of the lignin-carbohydrate matrix Generation of inhibitory compounds Less effective for softwoods
AFEX	Increases accessible surface area, Low formation of inhibitors No liquid fraction in the treated product (high solid to liquid ratio during the enzymatic hydrolysis)	Does not modify lignin neither hydrolyzes hemicellulose High cost of large amount of ammonia
LHW	High degree of biomass solubilization, absence of chemicals Low cost reactor	High water demand, high energy requirements, low solids processing during pretreatment
CO_2 explosion	Does not produce inhibitors for downstream processes Increases accessible surface area Cost effective	Very high pressure requirements Does not affect lignin and cellulose
Wet oxidation	Efficient removal of lignin Low formation of inhibitors Minimizes the energy demand (exothermic)	High cost of oxygen and alkaline catalyst
Ozonolysis	Reduces lignin content Does not imply generation of toxic compounds	High cost of large amount of ozone needed
Organosolv	Causes lignin and hemicellulose hydrolysis	High cost Solvents need to be drained and recycled
Concentrated acid	High glucose yield Ambient temperatures	High cost of acid and need to be recovered Reactor corrosion problems Formation of inhibitors
Alkaline	Effective lignin and hemicellulose solubilization	Requires alkali removal
Diluted acid	Less corrosion problems than concentrated acid Less formation of inhibitors	Generation of degradation products Low sugar concentration in exit stream

PROESA™ is a technology requiring no chemicals in the pretreatment stage to generate high yields from lignocellulosic biomass. The technology is based entirely on physical process resulting in increased yields of recovered sugars when compared to alternative technologies.

For sure, some criteria for the choose of the pretreatment technology at industrial scale could be an highly versatile technology with respect to the feed or a technology based on the locally available biomass. Furthermore, other key elements are process scalability, capital and running costs. Tao et all (2011) carried out an economic evaluations of six pretreatment processes to convert switchgrass to cellulosic ethanol: (AFEX), dilute acid (DA), lime, liquid hot water (LHW), soaking in aqueous ammonia (SAA), and sulfur dioxide-impregnated steam explosion (SO_2). Table 25 summarizes the pretreatment costs and the plant steam and power usages for plant size of 2,000 metric tons per day and 8,406 operating hours per year. The data indicate that the investigated technologies vary greatly in terms of their process design and projected capital costs.

Table 25: Pretreatment costs

	Pretreatment capital($MM)	Total installed cost ($MM)	Total capital	Plant electricity use (kWh/gal)	Excess electricity	Plant steam use (kgsteam/gal)	MESR ($/gal)*
AFEX	$31	$191	$348	3.3	9.6	16.5	2.7
DA	$45	$192	$349	2.4	9.0	21.0	2.8
Lime + O2	$57	$212	$385	3.8	9.9	20.8	3.2
LHW	$20	$179	$325	3.0	8.6	61.7	3.3
SAA	$45	$200	$364	4.4	15.1	41.4	4.1
SO2 steam explosion	$35	$187	$340	2.4	8.9	19.6	2.9

*Minimum ethanol selling price. These data have been estimated from histograms in the paper of Tao et al. (2011).

5 Hydrolysis processes

RITA MERGNER, RAINER JANSSEN

5.1 Introduction

The objective of hydrolysis is to split the carbohydrate polymers of cellulose and hemicellulose into monomeric sugars. This process might also be referred to as saccharification. During hydrolysis, cellulose and hemicellulose polymers are broken down into individual sugar molecules which can be subsequently fermented into bioethanol. Due to the different structure of the hexose dominated cellulose and the pentose dominated hemicellulose, the hydrolysis process is very complex. Hydrolysis of hemicellulose is usually easier due to its more amorphous structure and in order to hydrolyze cellulose, severe acid conditions or different cellulose enzymes are applied.

There are two main hydrolysis methods producing sugar monomers from cellulose for the subsequent fermentation. These include acid hydrolysis (with dilute or concentrated acids) and enzymatic hydrolysis (Saha et al. 2005). For the time being, the prevailing hydrolysis technology for bioethanol production is based on enzymes due to environmental reasons and better hydrolysis yields compared to acid hydrolysis. In addition, cellulose can be hydrolyzed by ionic liquids (ILs), gamma-rays, electron-beam irridation or microwave irridation. However, these processes are not widely applied and therefore are not further discussed in this chapter.

5.2 Acid hydrolysis

5.2.1 Diluted acid hydrolysis

Diluted acid hydrolysis is the most commonly applied method among the chemical hydrolysis methods and it can be carried out in either a one-stage or a two-stage process. Dilute acid hydrolysis can be used as a pretreatment process followed by enzymatic hydrolysis or as a method to hydrolyze lignocellulose to sugars. One of the first established dilute acid hydrolysis processes was the Scholler process. It was a batch process in which the wood material was kept in 0.5% sulphuric acid at 11-12 bar for approximately 45 min. Today, most of dilute acid hydrolysis processes are performed in a batch mode with a retention time of a few minutes (Taherzadeh and Karimi 2007).

Degradation of sugars and formation on undesirable by-products are the main drawbacks of one-stage dilute acid hydrolysis. This leads to lower sugar yields and inhibition of ethanol formation processes during the fermentation (Palmqvist 2000). Possible inhibitors are furfural, 5-hydroxymethylfurfural (HMF), acetic acid, formic acid, uronic acid, 4-hydroxybenzoic acid, vanillic acic, phenol, formaldyhyde, etc.. Some inhibitors are initially present in the biomass (e.g. acetic acid), however most of the inhibitors are formed during the hydrolysis process due to sugar degradation (Figure 31). Although several detoxification methods, such as activated charcoal adsorption and lime treatment process, have been devised, an appropriate strategy for efficient hydrolysis of lignocellulose to fermentable sugars is still lacking (Kaya et al. 2000; Aden et al. 2002).

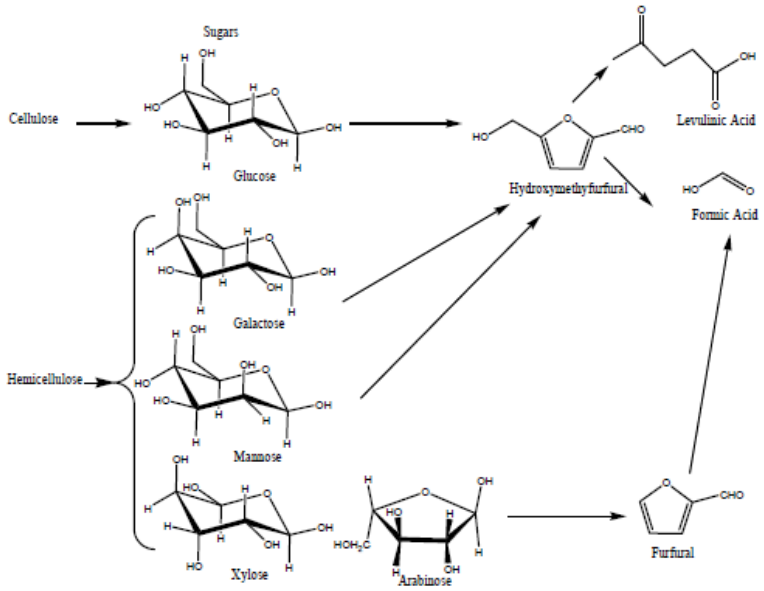

Figure 31: Reactions occurring to carbohydrates during hydrolysis of lignocellulosic materials (Hu et al. 2008).

In order to avoid formation of inhibitors and degradation of sugars at high temperatures, dilute acid hydrolysis can be carried out in two or more stages (Figure 32). In the first stage, hemicellulos is converted to sugar monomers. As hemicellulose has an amorphous structure, hydrolysis occurs under relatively mild conditions. This step is equivalent to a dilute-acid pretreatment step. In general, the maximum yield of pentoses and hexoses recovered from hemicellulose in the first-stage hydrolysis is high, i.e. 80-95% of the hemicellulose sugars available. However, the yield of cellulose hydrolysis to glucose is usually low (40-60%). In the second stage, the residual solid is hydrolysed under harsher conditions, which allow cellulose to be hydrolysed. The separate stages for hydrolysis of the hemicellulose and cellulose result in higher total sugar yields and less by-product formation since the solid cellulose fraction is separated from the liquid (the hydrolysed hemicelluloses fraction) before the second hydrolysis step. In the second-stage hydrolysis step a product with high glucose content can be obtained. This stream can then easily be fermented to ethanol or combined with the pentose rich hemicellulose fraction and then fermented, mixtures of pentoses and hexoses are usually more difficult to ferment though (see subsequent chaper on fermentation).

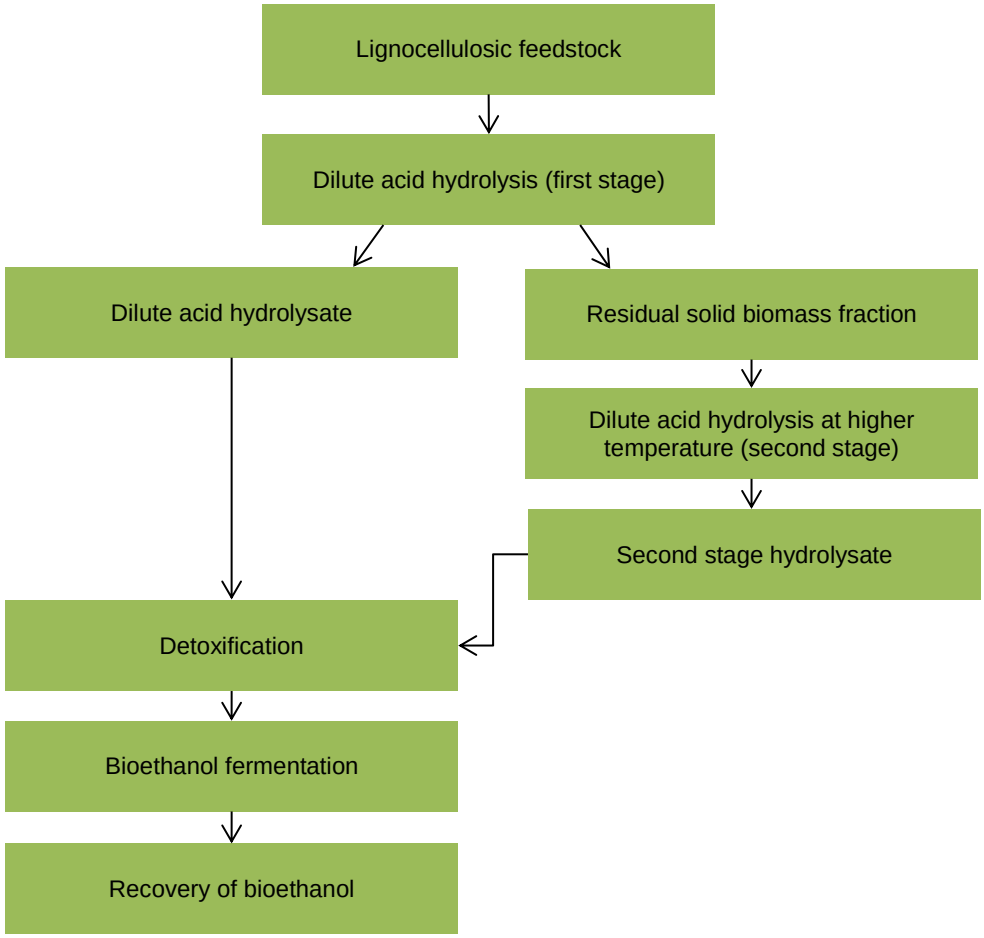

Figure 32: Two-stage dilute acid hydrolysis process

Usually batch reactors are used for acid hydrolysis processes. However, there have been experiments carried out in other types of reactors, such as plug flow, percolation, counter current and shrinking-bed reactors. Thompson and Grethlein (1979) developed an isothermal plug flow reactor analyse acid hydrolysis of cellulosic substrates. It was found that at least 50% of the potential glucose can be obtained at 240°C, 1% acid and 0.22 min of residence time. Compared to the batch reactor where the maximum glucose yield from cellulose was 40% that was a significant improvement. Further improvement of plug flow reactors has been reported by McParland et al. (1982) where 55-58% glucose yield was achieved at 240°C and 6 seconds residence time. However, the disadvantages of the plug flow reactor are heat transfer limitation within the biomass particles and control of the retention time in the range of a few seconds (Lee at al. 1999).

Kim et al. (2011) analysed cellulose hydrolysis using batch and bed-shrinking flow-through (BSFT) reactors. The experiments were conducted at 205, 220, and 235°C using 0.07 wt% H_2SO_4. The feedstock was yellow poplar and alpha-cellulose.The maximum yield of glucose obtained from batch reactor experiments was about 60% for alpha-cellulose, which occurred at 205 and 220°C. In experiments using BSFT reactors, the glucose yields of 87.5, 90.3, and 90.8% were obtained for untreated yellow poplar feedstocks at 205, 220, and 235°C, respectively (Table 26). The hydrolysis rate for glucan was about three times higher with the BSFT than with the batch reactors.

Table 26: Maximum glucose yields in batch and BSFT reactors (Kim et al. 2011)

Bioreactor/feedstock	Maximum yield (%)/reaction time (min)		
	205°C	220°C	235°C
Batch (alpha-cellulose)	61.77/30	59.23/25	40.17/16
Batch (pretreated yellow poplar)	26.62/16	35.45/13	20.43/10
Batch (untreated yellow poplar)	49.82/16	50.98/16	35.22/13
Bed shrinking (untreated yellow poplar)	87.54/25	90.32/20	90.78/20

5.2.2 Concentrated acid hydrolysis

Hydrolysis of lignocellulosic biomass by concentrated sulphuric or hydrochloric acids has a long history. In 1819 Braconnot discovered that cellulose can be converted to fermentable sugars by concentrated acids (Sherrard and Kressman 1945). The ability to dissolve and hydrolyze cellulose using sulphuric acid followed by dilution with water was reported in the literature already in 1883. In 1948, a concentrated sulphuric acid hydrolysis process was commercialized in Japan where membranes were used to separate sugars and acid.

The concentrated acid can disrupt the hydrogen bonding between cellulose chains and convert it into a completely amorphous state. Once the cellulose has been decrystallized, it forms a homogenous gelatin with the acid, therefore at this point cellulose it is susceptible to hydrolysis. To avoid high degradation, dilution with water at modest temperatures provides complete and rapid hydrolysis to glucose. In general, concentrated acid processes provide higher sugar yield compared to dilute acid processes (Table 27). In addition, the concentrated acid processes can operated at lower temperatures. However, the concentration of acid is high (30-70%) and dilution and heating of the concentrated acid during the hydrolysis process make it corrosive. The process requires special non-metallic constructions, such as ceramic or carbon-brick lining. In addition, environmental impacts limit the application of hydrochloric acid. The concentrated acid has to be recovered after hydrolysis to make the process economically feasible. High investment and maintenance costs have reduced the potential commercial interest and research in this process. However, the invention of new acid recovery technologies has revived interest in this process.

Table 27: Comparison of concentrated and dilute acid methods (Taherzadeh and Karimi 2007)

Hydrolysis method	Advantages	Disadvantages
Concentrated acid	High sugar yield	High acid consumption
	Low temperatures	Equipment corrosion
		High energy consumption for acid recovery
		Longer reaction time
Dilute acid	Low acid consumption	Operated at high temperature
	Short residence time	Low sugar yield
		Formation of undesirable by- products

Ismail et al. (2012) carried out two-stage acid hydrolysis with concentrates of perchloric acid using wheat straw. The effects of perchloric acid concentration on the hydrolysis were investigated. The perchloric acid concentration ranged from 17.5 to 40% (w/w). The glucose concentration from hydrolysis increased as the perchloric concentration increased, until a level of 35% was reached, after which it stabilized (Table 28). Based on the results, the optimum concentration of perchloric acid for hydrolysis of cellulose was when 35% HClO4 was used. This guaranteed to produce a high yield of glucose. In order to prevent degradation of xylose and arabinose and to minimize the amount of by-products, the hydrolysis of hemicellulose and cellulose was split into two separate stages. Radillo at al. (2011) analysed a two-stage hydrolysis process using only concentrated hydrochloric acid to generate fermentable carbohydrates from *L. rotundiflorus* biomass. First and second stage acid concentrations were 32% and 42.6% respectively. Total monosaccharide yields with respect to dry matter after the first stage, second stage and the overall process were 27.5%, 21.0% and 48.4% respectively. Xylose was the main first stage carbohydrate in the hydrolysate, followed by glucose, arabinose and galactose. After the second stage only glucose and a small amount of xylose were detected.

Table 28: Effects of HClO4 concentrations on hydrolysis at 100°C for 60 min (Ismail et al. 2012)

HClO4 %	Glucose g/L	Xylose g/L	Arabinose g/L	Acetic acid g/L	HMF g/L	Furfural g/L	Ethyl vanillin g/L
17.5	4.226	4.926	0.958	0.749	0.345	0.195	0.26
20	5.097	4.906	0.946	1.099	0.364	0.308	0.29
25	6.123	4.876	0.923	1.783	0.407	0.396	0.29
30	6.502	4.812	0.902	2.117	0.446	0.442	0.28
35	6.765	4.656	0.889	2.378	0.479	0.422	0.27
40	6.769	4.520	0.867	2.524	0.490	0.382	0.25

5.3 Enzymatic hydrolysis

Enzymatic hydrolysis of cellulose and hemicellulose, carried out by fungal or bacterial enzymes (cellulases and hemicellulases), is an alternative to acid hydrolysis in order to produce sugar monomers. Unlike acid hydrolysis, enzymatic hydrolysis is conducted at milder conditions, with optimums usually around pH 5.0 and 45-55°C. Due to this, enzymatic hydrolysis does not create corrosion problems or generate any inhibitors. However, this process has the disadvantage of being a much slower process and coupled withthe relatively high cost of enzymes this process has previously been regarded as a bottleneck for bioethanol production.

Cellulases are classified in glycosyl hydrolase families based on their sequence homology and hydrophobic cluster analysis and traditionally the degradation of cellulose to glucose has been regarded as a synergistic action of mainly three classes of enzymes (Pandey 2011) (Figure 33):

- Endogluconases (EGs) (EC 3.2.1.4), which randomly cleaves internal ß-1,4-glucosidic linkages in the cellulosic chain, forming two new chain ends

- Cellobiohydrolases (CBHs, also known as exogluconases) (EC 3.2.1.91), which are the most abundant component in most naturally occurring cellulose systems, progress along the cellulose and cleave off cellbiose units from the ends of the cellulosic chain in a processive manner
- ß-glucosidases (BG, also known as ß-glucoside glucohydrolases) (EC 3.2.1.21), which hydrolyze cellbiose to glucose and cleave off glucose units from cello-oligosaccharides

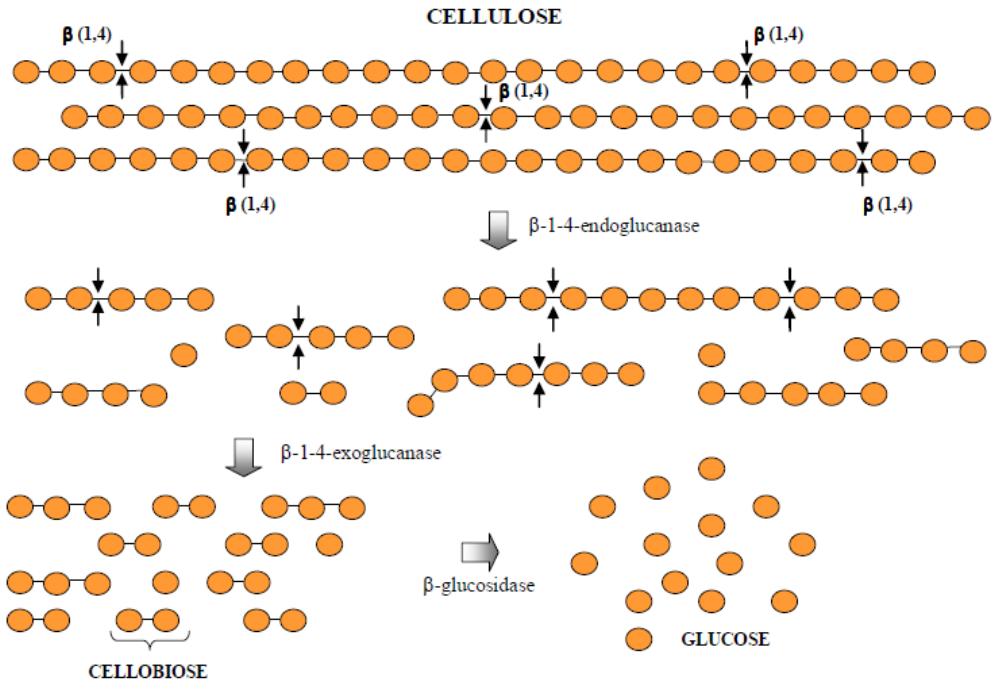

Figure 33: Enzymatic hydrolysis (Mussatto and Teixeira 2010)

Recently though, a new type of enzyme was identified as an important component for efficient enzymatic hydrolysis (Horn et. 2012). The enzyme works, as opposed to the other hydrolytic enzymes, by oxidating carbon in the glucan chain and in such a way create two new free chain ends for the CBHs to work on. Supplementation of these new enzymes gives a potential to break crystalline cellulose structures and hence enhance the enzymatic hydrolysis rate.

Before saccharification can take place, the cellulase have to adsorb onto the surface of the cellulose. Enzymes can bind both to reactive and non-reactive substances such as lignin. However, lignin may irreversibly adsorb enzymes and decrease their activity towards a reactive substrate. As the saccharification reaction takes place, a fraction of adsorbed cellulase is gradually released the supernatant because of the solubilisation of substrate while a substantial amount of the enzymes still remains in the residue (Converse et al.1990).

5.3.1 Production of cellulolytic enzymes

The commercial cellulases are relatively costly, therefore cellulase production from a wide range of microorganisms has been studied extensively to make the enzyme production process feasible.

Although many cellulolytic bacteria, particularly the cellulolytic anaerobes such as Clostridium thermocellum and Bacteroides cellulosolvens, produce cellulases with high specific activity, they do not produce high enzyme titres. As anaerobes have a very low growth rate and require anaerobic growth conditions, most research for commercial cellulase production has focused on fungi. Fungi are the main cellulase producing organisms and have been investigated over the years. Fungi that have been reported to produce cellulases include *Sclerotium rolfsii, P. chrysosporium* and species of *Trichoderma, Aspergillus, Schizophyllum and Penicillium* (Table 29). However, the microbes commercially exploited for cellulase production are mostly limited to *T. reesei, H. insolens, A.niger, Bacillus sp* and a few other organisms. *Trichoderma reesei* has been most extensively studied for cellulase production as they contain high activities of exo-glucanase and endo-gluconase and low activity of ß-glucosidases.

Table 29: Major microorganisms employed in cellulase production (Sukumaran et al. 2005)

Major group	Genus	Representative species
Fungi	Aspergillus	*A.niger, A. nidulans, A. oryzae (recombinant)*
	Fusarium	*F. solani, F. oxysporum*
	Humicola	*H. insolens, H. grisea*
	Melanocarpus	*M. albomyces*
	Penicillium	*P. brasilianum, P. occitanis, P. decumbans*
	Trichoderma	*T. reesei, T. longibrachiatum, T. harzianum*
Bacteria	Acidothermus	*A. cellulolyticus*
	Bacillus	*Bacillus sp, Bacillus subtilis*
	Clostridium	*C. acetobutylicum, C. thremocellum*
	Pseudomonas	*P. cellulosa*
	Rhodothermus	*R. marinus*
Actinomycetes	Cellulomonas	*C. fimi, C. bioazotea, C. uda*
	Streptomyces	*S. sp, S. lividans, S. drozdowiczii*
	Thermononospora	*T. fusca, T. curvata*

Xia et al. (1998) conducted a study on saccharification of corn stover by immobilized *Trichoderma reesei*. Under repeated batch processes with 60 g/L corn stover pretreated by 2% NaOH at 85°C, the average cellulase activity was 0.70 IU/ml, the concentration and yield of the reducing sugar were 26.41 g/L and 89.11% respectively after shaking culture at 150 r/min, 30 degrees C, pH 4.8 for 108 h. The fed-batch process was also studied with the same

immobilized cells. Total of 120 g/L corn stover was added in different feeding manners. The reducing sugar of 52.8 g/L was produced after 7 days and the saccharification efficiency (89.2%) was almost the same as the batch process. The results indicated that the cellulase production and cellulosic material saccharification in situs by the immobilized *Trichoderma reesei cells* is an effective process for bioethanol production.

Feng et al. (2011) evaluated the potential of *Trichoderma viride, Trichoderma pseudokoningii, Trichoderma koningii* and *Trichoderma reesei* with addition of exogenous ß-glucosidase with steam pretreated *Lespedeza*. The *T. viride* enzyme achieved the highes glucose conversion (90%), while *T. pseudokoningii* cellulase achieved the highest ratio of cellbiose to glucose (4.94%) at the end of hydrolysis.

5.3.2 Enzyme recycling

Hydrolysis processes may be further improved by strategies to recycle enzymes after hydrolysis as it to make the overall conversion process more economically feasible. Three rounds of recycling can increase the efficiency of enzymatic hydrolysis by 25% (Tu et al. 2009). Different methods are used to recycle enzymes such as sedimentation followed by ultra-filtration or micro centrifugation, cation exchange chromatography, re-adsorption and immobilization (Figure 34).

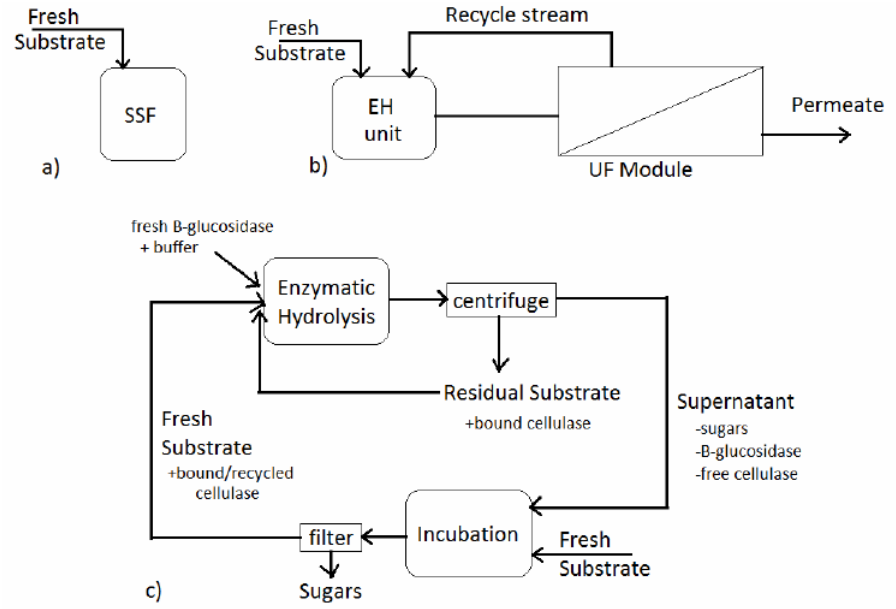

Figure 34: Strategies for recycling enzymes a) fed-batch simultaneous saccharification and fermentation (SSF), b) continuous ultra-filtration (UF), c) recycling of free cellulose from hydrolysis supernatant (Volynets and Dahman 2011)

Moniruzzaman et al. (1997) made a research on enzymatic hydrolysis of high-moisture corn fiber pretreated by AFEX and recovery and recycling of the enzyme complex. It was concluded that during initial stages of enzyme recycling, most of the initial enzyme activity could be recovered. However, a gradual decrease in enzyme activity at later stages was proved.

Mores at al. (2011) investigate a combined sedimentation and membrane filtration process for recycling cellulase enzymes in the biomass-to-ethanol process. In the first stage, the larger lignocellulose particles were removed by means of sedimentation in an inclined settler. Microfiltration was then utilized to remove the remaining suspended solids. Finally, the

soluble cellulase enzymes were recovered by ultrafiltration. The result indicates that 75% of the cellulase enzymes can be recovered in active form.

5.3.3 Factors affecting hydrolysis

Chemical and structural modifications occurring in the feedstock during the pretreatment stage significantly affect sugar release and enzymes used in the hydrolysis process and hence the choise of pretreatment process and conditions highly influence enzymatic hydrolysis. As the severity factor of pretreatment process decreases, the sugar yield after enzymatic hydrolysis typically also decreases. In addition, feedstock composition plays an important role in the effectiveness of the hydrolysis processand therefore optimization of different enzyme types and dosages is required to achieve optimal sugar yields. The main factors influencing enzymatic hydrolysis can be categorized into two major groups: substrate-related factors and enzyme-related factors (Table 30). The substrate specific factors that have been identified to affect the hydrolysis of cellulose include porosity (accessible surface area), cellulose fiber crystallinity and lignin and hemicellulose content (McMillan 1994). The presence of lignin and hemicellulose makes the access of cellulase enzymes to cellulose difficult, thus reducing the efficiency of the hydrolysis. For example, softwoods are more lignified than hardwoods and agricultural residues and therefore they are generally regarded as articularly recalcitrant for enzymatic hydrolysis.

Table 30: Factors affecting enzymatic hydrolysis

Substrate-related factors	Enzyme-related factors
Crystallinity degree of cellulose	Enzyme concentration
Accessible surface area of cellulose fibers	Incubation temperature
Degree of water swelling of cellulose fiber	Effect of surfactants
Molecular structure of cellulose	Inhibitors in enzymatic hydrolysis
Content of associated material such a lignin	Enzyme adsorption
Degree of polymerization	

Different authors have studies the relationship between substrate-related and enzyme-related factors. Crystallinity degree of cellulose and accessible surface area of cellulose fibers are the most important features with significant impact on the hydrolysis process. The surface area is important because direct physical contact between the enzyme molecules and the surface of cellulose is a prerequisite for the initial adsorption of the hydrolysis process. Any structural features limiting accessibility of cellulase enzymes to the cellulose will reduce the receptivity to hydrolysis. The crystallinity of cellulose is important due to the fact that the cellulolytic enzymes degrade the more accessible amorphous part of cellulose and not easily attack the less accessible crystalline region. As the crystallinity level of cellulose increases, cellulose becomes more resistant to further hydrolysis.

Since the hemicellulose polymer is composed of a mixture of pentose and hexose sugars several different enzymes (i.e. hemicellulases) are required for efficient decomposition. The hemicellulases could for example be comprised of endoxylanase, ß-xylosidase, α-arabinofuranosidase, α-glucouronidase, acetylxylan esterases, α-galactosidases and ß-mannosidases depending on the used substrate. This requirement makes enzymatic hydrolysis of hemicellulose rather complicated and since the general trend is moving towards milder pretreatments, where a substantial amount of hemicelluloses is retained in polymeric form, research have started to focus more and more on hemicellulases.

Maintaining high solids concentrations throughout the conversion process is important from an energy and economic viability viewpoint (Pandey 2011). High substrate concentration allows the production of a concentrated sugar solution, which in turn is beneficial for subsequent fermentation. However, high substrate concentration can also cause substrate inhibition which negatively affects the yield. The extent of substrate inhibition depends on the ratio of total enzyme to total substrate load. The maximum solid loading in hydrolysis processes depends on feedstock characteristics. 12-20% total solids is considered to be the upper limit at which pretreated biomass can be mixed and hydrolyzed in conventional stirred tank reactors.

Temperature influences enzymatic conversion of lignocellulosic biomass. The optimum temperatures of different cellulases typically range from 40 to 55°C. Tengborg et al. (2001) investigated the influence of temperature, residence time, and pH for washed pretreated spruce. The optimal temperature was found to be dependent on both residence time and pH and the maximum degree of cellulose conversion (69.2%) was obtained at 38°C and 4.9 pH for a residence time of 144 h. However, when the substrate concentration was changed from 5% to 2% DM, the cellulose conversion increased to 79.7%.

End-product inhibition of cellulase activity is another important factor affecting enzymatic hydrolysis process. Several methods have been developed to reduce the inhibition, including the use of high concentrations of enzymes, the supplementation of ß-glucosidases during hydrolysis and the removal of sugars during hydrolysis by ultrafiltration or simultaneous saccharification and fermentation (SSF).

Xiao et al. (2004) studied the inhibition effects of glucose and other sugar monomers during cellulase and ß-glucosidase hydrolysis of acetic acid pretreated softwood. The increased glucose content in the hydrolysate resulted in a dramatic increase in the degrees of inhibition on both ß-glucosidase and cellulase activities. Supplementation of mannose, xylose and galactose during cellobiose hydrolysis did not show any inhibitory effects on ß-glucosidase activity. However, these sugars were shown to have significant inhibitory effects on cellulase activity during cellulose hydrolysis.

A number of surfactants have been studied to improve enzymatic hydrolysis. Surfactants are amphiphilic compounds that are capable of self-assembling into micelles and adsorb onto surfaces depending on the surfactant structure and the polarity of the surface. Addition of surfactants to enzymatic hydrolysis of cellulose can cause the following positive effects:

- Cause surface structure modification or disruption of the lignocellulose that increase enzyme accessibility to cellulose
- Affect enzyme substrate interaction by preventing non-productive adsorption of enzymes
- Act as enzyme stabilizers preventing enzyme denaturation

Surfactants used in enzymatic hydrolysis include Tween 20, Tween 80, Emulgen 147, Tween 81 and others. Yao et al. (2007) investigated that the presence of nonionic surfactants (Tween 80) during enzymatic hydrolysis increased the conversion of cellulose into fermentable sugars. Cui et al. (2011) investigated the effect of surfactant on the hydrolysis. 0.06 g/g dry solids (DS) Tween 80 in hydrolysate lead to higher glucose yields (from 418 to 486 g/kg DS). Figure 35 shows the effect of different kinds of surfactants on the release of glucose at 2% w/v solids loading. The enzymatic hydrolysis time was 60 min. It was reported that different kinds of surfactants have distinct effects on the glucose yield during enzymatic hydrolysis. Nonionic surfactant Tween 80 showed positive effects, whereas cationic and anionic surfactants showed negative effects on enzymatic hydrolysis. This might be due to the fact that the cationic and anionic surfactants had a higher toxicity to enzymatic hydrolysis than the nonionic surfactants. Denaturation of enzymes was the probable cause for the decreased sugar conversion when cationic and anionic surfactants were used.

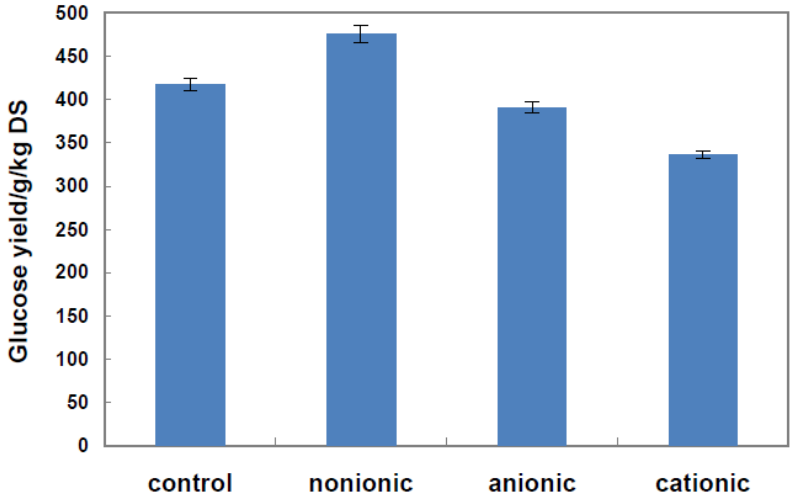

Figure 35: Effect of surfactants on enzymatic hydrolysis (Cui et al. 2011)

6 Fermentation and process configurations

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6.1 Introduction

Fermentation is the biological process in which sugars are converted to ethanol by a wide range of microorganisms. The most common fermentation process – both industrially and naturally - is the conversion of one mole of glucose into two moles of ethanol and two moles of CO_2. Also other hexoses, such as fructose and galactose, may be converted in a similar manner. The fermentative conversion of hexoses is the process behind leavening of bread, and making of beer and liquor. The methods of (although not the reason behind) fermentation were known at least 6,000 years ago, when Sumerians, Babylonians and Egyptians described the process of making beer from grain.

A large number of yeasts, bacteria and fungi are reported to produce ethanol as the main fermentation product. Of the fermenting microorganisms, yeasts – and in particular Baker's yeast, *Saccharomyces cerevisiae*- are most common in today's ethanol industry. Ethanol formation comes about primarily under anaerobic conditions in which a fermentative end-product is needed to regenerate the co-factor NAD+. However, also aerobic formation of ethanol can take place in so-called Crabtree positive yeasts. Some yeast species producing ethanol as the main fermentation product are shown in Table 31. Another often-mentioned fermenting organism is the bacterium *Zymomonas mobilis.* This well-known ethanol producing bacterium was allegedly first isolated from a glass of Capirinhas in the city of Recife, Brazil. It produces ethanol at high yields, which is a consequence of its use of an alternative pathway in the catabolic production of pyruvate from glucose (the so-called Entner-Duodoroff pathway instead of the more common Embden-Meyerhof Parnas pathway).

Table 31: Yeast species producing bioethanol as the main fermentation product (Lin and Tanaka 2006)

Strain species	T [°C]	pH value	Carbon source and concentration (g/L)	Nitrogen source and concentration (g/L)	Incubation time (h)	Concentration of bioethanol produced (g/L)
27817-Saccharomyces cerevisiae	30	5.5	Glucose (50-200)	Peptone (2), ammonium sulfate (4)	18-94	5.1-91.8
L-041-S. cerevisiae	30 or 35	-	Succrose (100)	Urea (1) or ammonium sulfate (1-2)	24	25-50
181- S. cerevisiae (aerobic)	27	6.0	Glucose (10)	Peptone (5.0)	40-160	-
UO-1-S. cerevisiae	30	5.0	Sucrose (20)	Ammonium sulfate (1)	60-96	-
V5- S. cerevisiae	24	-	Glucose (250)	-	36	-
ATCC 24860- S. cerevisiae	30	4.5	Molasses (1.6 - 5.0)	Ammonium sulphate (0.72-2.0)	24	5-18.4
Baker's yeast - S. cerevisiae	30	4.5	Sugar (150-330)	-	192	53 (max)
Baker's yeast - S. cerevisiae	28	5.0	Sucrose (220)	Peptone (5) and ammonium duhydrogen phosphate (1.5)	96	96.71
Fisio - S. cerevisiae	30	5.0	Galactose (20 - 150)	Peptone, ammonium sulphate and casamino acid (10)	60	4.8-40
A3 - S. cerevisiae	30	5.0	Galactose (20 - 150)	Peptone, ammonium sulphate and casamino acid (10)	60	4.8-36.8
L52 - S. cerevisiae	30	5.0	Galactose (20-150)	Peptone, ammonium sulphate and casamino acid (10)	60	2.4-32.0
GCB-K5-S. Cerevisiae	30	6.0	Sucrose (30)	Peptone (5)	72	27
GCA-II-S. Cerevisiae	30	6.0	Sucrose (30)	Peptone (5)	72	42
CMI237-S. Cerevisiae	30	4.5	Sugar 160	Ammonium sulphate (0.5)	30	70 (max)
2.399-S. cerevisiae	30	5.5	Glucose (31.6)	Urea (6.4)	30	13.7 (max)
27774-Kluyveromyces Fragilis	30	5.5	Glucose (20-120)	Peptone (2) and ammonium sulphate (4)	18-94	48.96 (max)
30017-K.fragilis	30	5.5	Glucose (20-120)	Peptone (2) and ammonium sulphate (4)	18-94	48.96 (max)

6.2 Mixed sugar fermentation

Contrary to sucrose- and starch-based bioethanol production – so-called first generation ethanol - lignocellulose-based production is a mixed-sugar fermentation, which furthermore takes place in the presence of several inhibiting compounds, such as low molecular weight organic acids, furan derivatives, phenolics and inorganic compounds. These compounds are primarily formed during pretreatment of the raw material. The reason behind the need to convert several sugars is that lignocellulosic materials do not only contain cellulose but also hemicellulose. The latter are heteropolymers made up of many different monosaccharides – both hexoses and pentoses. Especially hardwood and agricultural raw materials are rich in pentoses and can contain up to more than 20% of the pentose sugars – primarily xylose and arabinose (Table 32). The pentoses cannot be fermented to ethanol by the most commonly used fermentation microorganism, i.e. *Saccharomyces cerevisiae*. Since pentose sugars represents a high percentage of the available sugars in some feedstocks, their recovery and fermentation into bioethanol become an important factor for the efficiency and economics of the conversion process using these feedstocks.

There are three principal options to handle the pentoses in the process: a) Use a naturally occurring microorganism for pentose fermentation; b) Genetically engineer a suitable host organism for conversion of pentoses; or c) Ferment only the hexoses and use the remaining pentoses for other purposes. The dominating pentose sugar is xylose – in almost all feedstocks – and for this reason the main issue has been xylose fermentation.

A compilation of some natural xylose fermenting organisms are shown in Table 30. In the 1980s considerable efforts were made to identify wild type *yeast species* able to convert xylose to ethanol. A number of yeast species were indeed found, for example *Pachysolen tannophilus, Candida shehatae, Candida tropicalis, Kluyveromyces marxianus* and *Pichia stipitis* (Slininger et al., 1982, Hagerdal et al. 1985, Bruinenberg et al. 1984, Flores et al. 2000). The last of these yeasts – recently renamed *Scheffersomyces stipitis* - has often been regarded the most promising natural xylose fermenting yeast. The hemicellulosic hydrolysates of the plant *Prosopis juliflora* (18.24 g sugar/L broth) fermented with *P. stipitis* produced 7.13 g/L ethanol (Gupta et al. 2009). Detoxified xylose rich hydrolysate of L. camara when fermented with *P. stipitis* 3498 at pH 5 and 30°C for 36 h resulted 0.33 g alcohol/g lignocellulose used (Kuhad et al. 2010). The detoxified water hyacinth hemicellulose acid hydrolysate rich in pentose sugars fermented with *P. stipitis* NCIM-3497 at pH 6.0 and 30°C resulted 0.425 g ethanol/g lignocellulose. The ability of a recently isolated *Scheffersomyces stipitis* strain (UFMG-IMH 43.2) to produce ethanol from xylose was evaluated. A hemicellulosic hydrolysate produced by dilute acid hydrolysis of sugarcane bagasse was used as the fermentation medium. The best results (ethanol yield and productivity of 0.19 g/g and 0.13 g/l/h, respectively) were obtained using the hydrolysate containing an initial xylose concentration of 30 g/l, supplemented with 5.0 g/l yeast extract and inoculated with an initial cell concentration of 2.0 g/l. *S. stipitis* UFMG-IMH 43.2 was demonstrated to be a yeast strain with potential for use in xylose conversion to ethanol (Ferreira et al. 2011). A drawback of the natural xylose fermenting yeasts is that a little bit of oxygen is needed for efficient fermentation. This needs to be carefully controlled to avoid oxidative loss of the sugar.

A few *fungal species* belonging to genera *Fusarium, Rhizopus, Monilia, Neurospora* and *Paecilomytes* were found to have potential for fermenting both glucose and xylose. *Fusarium oxysporum* has shown better performance than *Neurospora crassa* (Singh et al. 1992). Xiros and Christakopoulos (2009) explored *F. oxysporum* for ethanol production from brewer's spent grain (BG) under consolidated system. An ethanol yield of 109 g ethanol per kg of dry BG was obtained with alkali-pretreated BG under microaerobic conditions (0.01 vvm), corresponding to 60% of the theoretical yield based on total glucose and xylose content of BG. However, fungal strains have disadvantages such as their long generation and

fermentation time, low tolerance to substrate and products and secretion of organic acids, which make them less attractive for ethanol production (Chandel et al. 2011).

Unlike yeast and filamentous fungi, some *bacteria* can convert xylose to bioethanol under strictly anaerobic conditions. These bacteria include *Bacillus macerans, Bacillus polymyxa, Kiebsiella pneumoniae, Clostridium acetobutylicum, Aeromonas hydrophila, Aerobacter sp., Erwinia sp., Leuconostoc sp and Lactobacillus sp.* Some bacteria, such as *Escherichia coli*, *Klebsiella, Erwinia, Lactobacillus, Bacillus* and *Clostridia*, can utilize mixed sugars, but produce no or only a limited quantity of ethanol.Instead of using a natural xylose fermenting microorganisms, one may aim for genetically engineer to obtain a good pentose fermenter. The starting point may be either a good hexose-fermenting organism, which will have to be engineered to accept also pentose as a substrate – or a good xylose-utilizing microorganism, which will have to equipped with the ethanol fermentative pathway (and possibly deleted for other fermentative pathways). The main development focus has been on the former path, in which the work-horse of the first generation ethanol production – *S. cerevisiae* – has been engineered for xylose utilization. There are two main options to introduce the enzymatic pathway which will enable conversion of the pentose xylose: Either the two-step conversion via the enzymes xylose reductase (XR) and xylitol dehydrogenase (XDH), or the one-step isomerization catalyzed by xylose isomerase (XI), is used (reviewed by e.g. Almeida et al., 2011; Van Vleet and Jeffries, 2009, Matsushika et al., 2009).

As mentioned earlier, the bacterium *Z. mobilis* is a good hexose fermenter. The pathways for xylose utilization have been introduced also into *Z. mobilis* resulting in a glucose and pentose fermenting recombinant strain (US Pat 6566107). A weakness is that most strains of Z. mobilis are highly sensitive to inhibitors, especially acetic acid but a new *Z. mobilis* strain 8b (a integrant of Zymomonas mobilis 31821(pZB5)) achieved a bioethanol yield of 83%, w/w and showed a remarkable tolerance to acetic acid (8-16 g/L) at pH 6 and 37°C (Mohagheghi et al. 2004).

Many bacteria have a wide range of substrate utilization and often a high substrate uptake rate. The drawbacks are also here that the ethanol tolerance is often limited, and sometimes a mixed fermentation occurs, e.g. for the common bacterium *Escherichia coli.* Nevertheless, several bacteria have been considered potential production. The engineering of *E.coli* strains to produce ethanol from both hexose and xylose was a relatively early successful application of metabolic engineering and was awarded patent US Pat 5000000.

Table 32: Growth characteristics of natural pentose-fermenting microorganisms (Abbi 1996)

Microorganisms	*Glucose*	*Xylose*	*Arabinose*	*Mannose*	*Cellulose*	*Temperature range [°C]*	*pH range*
Filamentous fungi							
Fusarium oxysporum	+	+	+	+	+	28-32	5-6
Neurospora crassa	+	+	-	-	+	28-37	5-6
Monilia sp.	+	+	-	-	-	26	5
Mucor sp.	+	+	-	-	-	30	5.4
Yeast							
Saccharomyces cerevisiae	+	-	-	+	-	30-35	3-7
Klyuvermyces marxians	+	+	+	+	-	30-35	3-7
Pachysolen tannophilus	+	+	+	-	-	28-32	2.5-7
Candida shehatae	+	+	+	+	-	28-32	3-7
Pichia stiptis	+	+	+	+	-	28-32	3-7
Mesophilic bacteria							
Bacillus polymyxa	+	+	+	+	-	35-37	5.5-8
Aerobacter hydrophila	+	+	+	+	-	35-37	5.5-8
Klebsiella pneumonia	+	+	+	+	-	35-37	5-6
Clostridium acetobutylicum	+	+	+	+	+	35-37	4-8
Thermophilic bacteria							
Clostridium thermocellum	+	+	+	-	+	65	4-8
C. thermohydrosulfuricum	+	+	+	-	-	65	4.7-8
C. thermosaccharolyticum	+	+	+	+	-	60	5-8
C. thermosulfurogenes	+	+	+	+	-	60	4.5-7.5
Thermoanerobacter ethanolicus	+	+	+	+	-	69	4.4-9.5

6.3 Overview on integrated fermentation technologies

The following process configurations are applied in the fermentation process (Figure 36):

- Separate Hydrolysis and Fermentation (SHF)
- Simultaneous Saccharification and Fermentation (SSF)
- Simultaneous Saccharification and Co-current Fermentation (SSCF)
- Consolidated Bioprocessing (CBP)

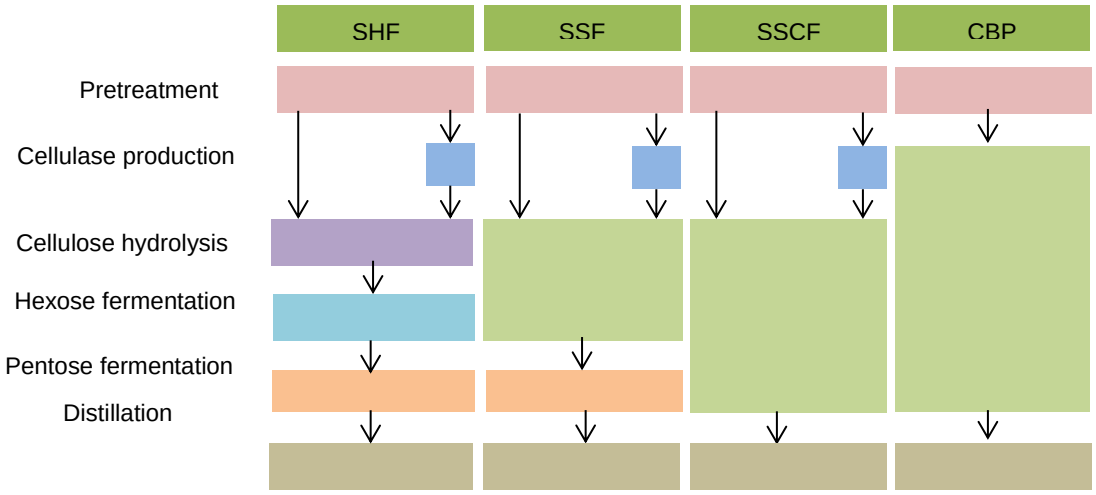

Figure 36: Integrated fermentation technologies

Separate Hydrolysis and Fermentation (SHF) technology performs hydrolysis separately from fermentation. As hydrolysis and fermentation processes are carried out in separate vessels, each step can be performed under optimal conditions. This process configuration therefore should enable more efficient performance of the enzymes. However, a drawback of is that the rate of hydrolysis is typically reduced as the activities of some of the cellulases may be inhibited by hydrolysis products – e.g. cellobiose or glucose (Table 33).

Simultaneous *Saccharification and Fermentation (SSF)* technology performs hydrolysis and fermentation in one reactor and sugars produced in hydrolysis are simultaneously fermented to ethanol. Hydrolysis and hexose fermentation are performed in one step. Pentose fermentation is performed independently from hexose processing. As the sugars are consumed when formed in the hydrolysis, the product inhibition of the enzymatic hydrolysis can be significantly reduced. However, in comparison to SHF, the process is carried out under non-optimal conditions, in particular for the enzymatic hydrolysis which takes place at a lower temperature than the optimal.

When pentose fermentation and hexose fermentation coincide with the enzymatic hydrolysis, the process is called **Simultaneous Saccharification and Co-Fermentation (SSCF)**. For the co-fermentation of pentose and hexoses, an organism capable of both conversions is required.

Separate Hydrolysis and Co-Fermentation (SHCF) In SHCF, the hydrolysis and fermentation processes occur in different reactors - to enable optimal conditions for each process separately – but the fermentation of both pentose and hexoses take place simultaneously.

Consolidated Bioprocessing (CBP) accomplishes cellulase production, hydrolysis and fermentation in one reactor by using only one microbial consortium.

Table 33: Comparison of different fermentation process configurations (Harun et al. 2011)

Process	Advantages	Disadvantages
SHF	Hydrolysis and fermentation take place at optimum conditions	Inhibitory effects Increased contamination
SSF	Low quantity of enzyme input High ethanol yield Reduced foreign contamination Less inhibitory effects Lower cost	Either hydrolysis or fermentation can be performed under optimal conditions Difficulty in process control
SHCF	High ethanol yield Hydrolysis and fermentation take place at optimum conditions	High enzyme load Increased contamination risk Inhibitory effects
SSCF	Shorter process time High ethanol yield Less contamination risk	High enzyme load Either hydrolysis or fermentation can be performed under optimal conditions
CBP	Cost effective Energy efficient	Lack of suitable organisms Difficulty in process control

6.4 Separate Hydrolysis and Fermentation (SHF)

Separate hydrolysis and fermentation processes can be designed in two ways:

- Enzymatic hydrolysis followed by hexose fermentation, while pentose fermentation occurs in a consecutive process (Figure 37)
- Fermentation of soluble pentose sugars in parallel with hexose sugars

The potential need for separate fermentation is due to the fact that pentose utilizing microorganisms ferment pentose sugars slower than hexose sugars, and the hexose sugars are preferentially utilized. In addition, some microorganisms utilizing pentose sugars may be more sensitive to the inhibitors and to the produced ethanol, and the hydrolyzate may therefore have to be more extensively detoxified.

The main advantage of SHF is that both enzymatic hydrolysis and fermentation can be run at their respective optimal temperatures, which are different. The optimum temperature for cellulase enzymes is between 45 and 50°C, whereas the optimum temperature for most ethanol-producing microorganisms is between 30 and 37°C. Furthermore, in SHF it is possible to run the fermentation process in a continuous mode with cell recycling – provided that the solid remaining fraction after enzymatic hydrolysis is separated before the fermentation. A major drawback of SHF is that the released sugars may inhibit cellulase and ß-glucosidase activity during hydrolysis, which requires the use of lower solids concentrations at higher enzyme loadings. Low solids concentrations, however, result in low ethanol concentrations, hence increasing the cost of fermentation and ethanol recovery.

However, considerable efforts have been made in developing cellulases that are less sensitive to end-product inhibition and improvements have resulted in this respect. Contamination is a further drawback of the SHF. As hydrolysis processes might take 1-4 days, there is a risk of microbial contaminations even though the temperature ranges between 45 and 50°C.

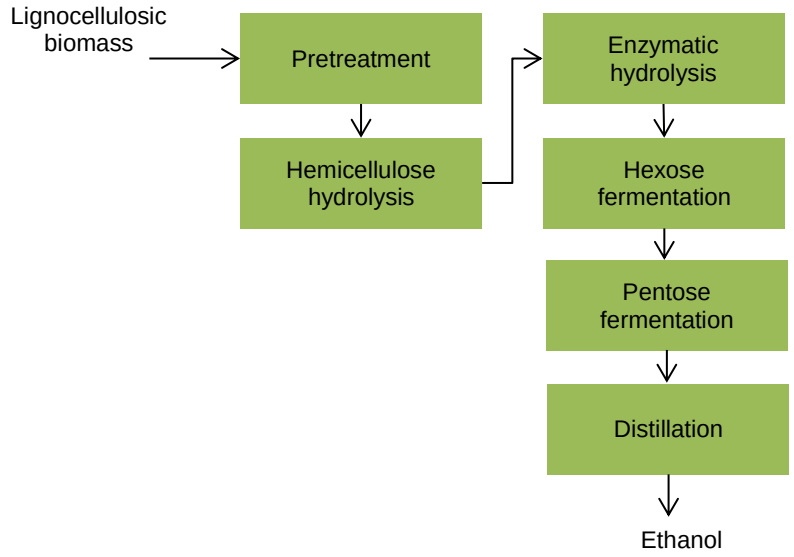

Figure 37: SHF process strategy with sequential fermentation of hexoses and pentoses

6.5 Simultaneous Saccharification and Fermentation (SSF)

The main advantages of SSF in comparison to SHF is that end-product inhibition of the cellulases can be minimized since the sugars formed in the hydrolysis are continuously removed due to the fermentation. Another advantage is that there are no sugar losses in the separation of the solid fraction and the liquid fraction after the enzymatic hydrolysis. Furthermore, SSF involves lower capital costs as the number of required vessels is reduced. On the other hand, a disadvantage of SSF is the previously mentioned different optimum temperatures for hydrolysing enzymes and fermenting microorganisms. Running the SSF at sub-optimal temperatures for the hydrolysis results in a higher enzyme dosage requirement than SHF or a longer process time. Ethanol may also exhibit inhibition of cellulase activity, but this is normally less significant than the inhibition caused by the mono- and di-saccharides. Contamination risks in SSF are reduced by the presence of ethanol (if high enough), but more important is the fact that the sugar concentration is maintained low. There is a number of options for production of yeasts and enzymes, which need to be considered in the process outline (Figure 38).

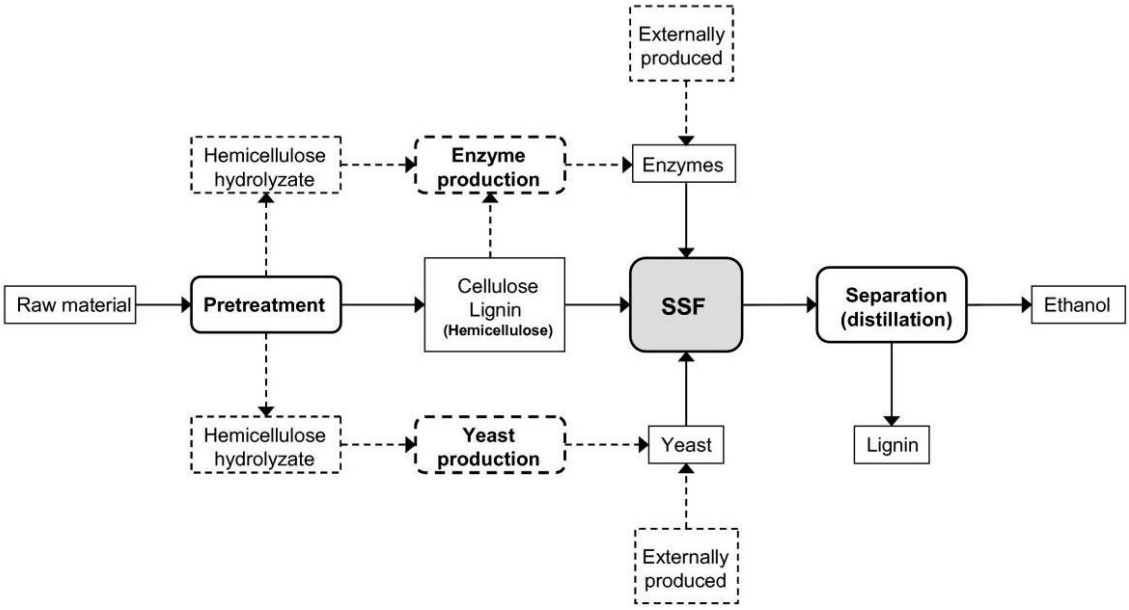

Figure 38: SSF process (Olofsson et al. 2008)

A large number of SSF studies have been conducted. The development of thermotolerant yeast strain that perform above 40°C with high ethanol tolerance can improve SSF processes. Several thermotolerant microorganisms and yeasts were studied to address this problem. Different organisms such like Saccharomyces, Schizosaccharomyces, *Candida brassica* were investigated at 30 and 40°C. Saccharomyces carlsbergensis IAM 4787 and Candida brassicae IFO 1664 showed optimal performance at 40 with ethanol yields of 62% on pure cellulose and 48% on sulphate pulp (Wyman 1996). In addition, *Candida lusitaniae, Kluyveromyces marxianus* and *Zymomonas mobilis* or mixed culture of microorganisms such like *Brettanomyces clausenii* can be applied in the process (Karimi et al. 2006).

Tu et al. (2009) investigated the effects of surfactants on SHF and SSF of steam exploded lodgepole pine (SELP) and ethanol pretreated lodgepole pine (EPLP). Supplementing Tween 80 during cellulase hydrolysis of SELP resulted in a 32% increase in glucose yield. The effect of surfactant addition on final ethanol yield of SSF was investigated by using SELP in the presence of additional furfural and hydroxymethylfurfural (HMF). The results showed that the surfactants slightly increased the conversion rates of furfural and HMF during SSF process by *Saccharomyces cer*evisiae. The presence of furfural and HMF at the experimental concentrations did not affect the final ethanol concentration either.

Ballesteros et al. (1991) screened 27 yeast strains belonging to the groups *Candida*, *Saccharomyces* and *Kluyveromyces* for their ability to grow and ferment glucose at temperatures ranging 32-45°C *K. marxianus* and *K. fragilis* were found to be the best ethanol producing organisms at the higher temperature tested and were selected for subsequent simultaneous saccharification and fermentation (SSF) studies. SSF experiments were performed at 42 and 45°C, utilizing Solkafloc (10%) as cellulose substrate and a cellulase loading of 15 FPU/g substrate. Best results were achieved at 42°C with *K. marxianus* and *K. fragilis* both of which produced close to 38 g/L ethanol in 78 h.

Watanabe et al. (2012) investigated fed-batch simultaneous saccharification and fermentation (SSF) of alkali-treated rice straw using immobilized yeast. *Saccharomyces cerevisiae* cells were immobilized by entrapping in photocrosslinkable resin beads. In batch SSF of 20% (w/w) rice straw, the ethanol yields based on the glucan content of the immobilized cells were slightly low (76.9% of the theoretical yield) compared to free cells (85.2% of the theoretical yield). In repeated-batch SSF of 20% (w/w) rice straw, stable

ethanol production of approx. 38 g/L and an ethanol yield of 84.7% were obtained. The immobilizing carrier could be reused without disintegration or any negative effect on ethanol production ability.

Chadha et al. (1995) investigated physicochemical pretreatment of ball milled rice straw with different oxidizing agents, peracetic acid, alkali-peroxide, and manganese-peroxide compounds under steaming pressure. The hydrolysate was fermented using coculture of a temperature resistant strain of *Saccharomyces cerevisiae* and *Pachysolen tannophilus* resulting in 1.5% (w/v) ethanol. The SSF of 10.0% (w/v) H_2O_2-$MnSO_4$ treated straw yielded maximum ethanol (2.9%, w/v) after 72 h at 40°C. As a consequence of the well-balanced cellulase production by mixed fungal culture, the supplementation of cellobiase or xylanase was not necessary in the SSF.

Instead of a batch SSF process, one may instead use a *fed-batch SSF process*. In this way the following advantages are gained (Olofsson et al. 2010):

- The viscosity of the medium can be maintained low due to a gradual feeding of new material to the reactor, in which the viscosity decreases due to enzymatic degradation.
- The effect of toxicity of the hydrolyzate can be decreased as a result of both adaptation of the yeast and gradual biological detoxification.
- There may be a beneficial effect on the xylose uptake from a changed concentration ration of xylose to glucose in the medium.

Whether or not the overall ethanol yield will be higher in SSF or in SHF depends on both feedstock used and process conditions.

When SHF and SSF of SO_2-impregnated and steam-pretreated (215°C, 5 min) spruce were compared using the same dry matter content (5%) and enzyme dosage (21 FPU/g cellulose), the overall ethanol yield was 60% of the theoretical for SSF and 40% for SHF. When the enzyme concentration was increased to 32 FPU/g cellulose, the overall ethanol yield in the SSF was 280 l/metric ton raw material, corresponding to 68% of the theoretical based on the content of hexose sugars in the raw material (Stenberg et al. 2000). However, in two-stage pretreatment of spruce with SHF and SSF no difference was found in overall ethanol yield The assay with SHF was performed using washed pretreated material at 2% dry matter while SSF was performed with the whole slurry from the pretreatment at 5% dry matter (Soderstrom et al. 2002).

Tomas-Pejo et al. (2010) compared SHF and SSF process from steam-exploded wheat straw by xylose-fermenting and robust glucose-fermenting *Saccharomyces cerevisiae* strains (F12 and Red Star). The strain F12 has been modified to allow xylose consumption as cereal straw contains considerable amounts of pentose sugars. Red Star is a robust hexose-fermenting strain used for industrial fuel ethanol fermentations. The highest ethanol concentration, 23.7 g/L, was reached using the whole slurry (10%, w/v) and the recombinant strain (F12) in an SSF process. It showed an ethanol yield on consumed sugars of 0.43 g/g and a volumetric ethanol productivity of 0.7 g/L h for the first 3 h. Ethanol concentrations obtained in SSF processes were in all cases higher than those from SHF at the same conditions. However, it was found that the inhibitors furfural and HMF were completely metabolized by the yeast during SSF by metabolic redox reactions.

Olsson et al. (2006) compared SHF and SSF of wheat hemicellulose with three different xylose-utilizing recombinant *Saccharomyces cerevisiae* strains (F12, CR4 and CB4). With CR4 and F12, the maximum ethanol concentrations obtained were 4.3 and 4 g/L, respectively, but F12 converted xylose 15% faster than CR4 during the first 24 h. The comparison of SHF and SSF with F12 showed that the highest, maximum ethanol concentrations were obtained with SSF. In general, the volumetric ethanol productivity was initially, highest in the SHF, but the overall volumetric ethanol productivity ended up being

maximal in the SSF, at 0.013 and 0.010 g/Lh, with starch free fibers and vinasse, respectively.

Vintila et al. (2011) compared the productivity and efficiency of SHF and SSF. The ethanol production from lignocellulosic biomass was higher in SSF than in SHF. The results indicate that SSF is more efficient that SHF in terms of total production time, energy consumption and total production costs. This can be explained by inhibition of cellulase activity in the hydrolysis step of SHF process due to glucose accumulation. This inhibition can be avoided in SSF.

6.6 Simultaneous Saccharification and Co-Fermentation (SSCF)

When pentose fermentation is included in an SSF process it is called Simultaneous Saccharification and Co-fermentation (SSCF) (Figure 39). SSCF has been recognized as a feasible option for ethanol production from xylose-rich lignocellulosic materials. In SSCF the hydrolysed hemicellulose during pretreatment and the solid cellulose are not separated after pretreatment, which is otherwise a possibility if pentoses are not to be fermented.

The preferred microorganism for pentose fermentation also in SSCF is genetically engineered *Saccharomyces cerevisiae* strains, which are able to ferment xylose present in lignocellulosic biomass. However, better xylose fermenting strains are required to reach a complete xylose uptake in SSCF, and a continuous development is taking place both on XI and XR-XDH based strains. Haploid *Saccharomyces cerevisiae* strains expressing a heterologous xylose pathway including either the native xylose reductase (XR) from P. stipitis, a mutated variant of XR (mXR) with altered co-factor preference, a glucose/xylose facilitator (Gxf1) from Candida intermedia or both mXR and Gxf1 were assessed in SSCF of acid-pretreated non-detoxified wheat straw. The xylose conversion in SSCF was doubled with the *S. cerevisiae* strain expressing mXR compared to the isogenic strain expressing the native XR, converting 76% and 38%, respectively. The xylitol yield was less than half using mXR in comparison with the native variant. As a result of this, the ethanol yield increased from 0.33 to 0.39 g g-1 when the native XR was replaced by mXR. In contrast, the expression of Gxf1 only slightly increased the xylose uptake, and did not increase the ethanol production. The results suggest that ethanolic xylose fermentation under SSCF conditions is controlled primarily by the XR activity and to a much lesser extent by xylose transport (Olofsson et al. 2011) in XR-XDH strains.

Jin et al. (2012) investigated SSCF of AFEX pretreated corn stover for ethanol production using commercial enzymes and *Saccharomyces cerevisiae* 424A(LNH-ST). However, the xylose consumption was still unsatisfactory during 6h SSCF. By extending the pre-hydrolysis time to 24h or longer, the xylose consumption was improved significantly. In order to better understand the reasons for such improvement, the hydrolysate slurries after 6h pre-hydrolysis and 24h pre-hydrolysis were studied and compared. It was found that the glucose concentration after pre-hydrolysis was the critical factor that determined cell viability and hence xylose consumption during SSCF. Low temperature (30°C) and ethanol inhibition were shown to be the factors limiting hydrolysis rate and hence productivity during SSCF.

Temperature and pH are important factors in SSCF process configurations. The co-culture of *P. stipitis* and *Brettanomyces clausennii* was used in the SSCF of aspen at 38°C and 4.8 pH yielding 369 L EtOH per ton of aspen during 48 h batch process (Olsson and Hahn-Hägerdal 1996). *C. shehate* and *S. cerevisiae* are also suitable for the SSCF process. The main drawbacks of this process are high by-product formation in the form of xylitol, poor enzyme stability and incompatible pH and temperature.

As mentioned above, the development of microbial strains able to grow at elevated temperatures may improve techno-economic indicators of also SSCF processes significantly. It was reported, that the development of ethanologenic microorganisms capable to ferment at temperatures higher than 50°C can potentially reduce the cost of cellulase enzymes by 50%

considering that an increment of 20°C during saccharification may imply a doubling of the cellulose hydrolysis rate (Cardona and Sanchez 2007).

One main advantage of SSCF in comparison to Separate Hydrolysis and Co-fermentation (SHCF) is that the glucose released into the medium by enzymatic hydrolysis is simultaneously fermented, resulting in a low glucose concentration in the medium. This is beneficial for the enzymatic hydrolysis in terms of minimizing end-product inhibition, but it also gives a high xylose-to-glucose concentration ratio, which favours xylose uptake. Olofsson et al. (2010) investigated SSCF of pretreated wheat straw using both enzyme loading and substrate feeding. The results showed that by using enzyme and substrate feeding, the xylose conversion in SSCF could be increased from 40% to 50% in comparison to substrate feeding only. In addition, by this design of the feeding strategy, it was possible to process a WIS content corresponding to 11% in SSCF and obtain an ethanol yield on fermentable sugars of 0.35 g g-1. A combination of enzyme and substrate feeding was shown to enhance xylose uptake by yeast and increase overall ethanol yield in SSCF. Thus, SSCF is a feasible process option for co-fermentation of glucose and xylose, because it allows a slow, constant release of glucose throughout the process that is beneficial for xylose uptake by xylose-fermenting strains of *S. cerevisiae.* In line with the above, it has also been reported that a two-step SSCF process can significantly increase ethanol yield by improving xylose consumption. In the two-step SSCF, 4% of total cellulases were applied in the first half of the fermentation process and the rest of the cellulases were used in the second half of the fermentation process (Jin et al. 2010).

There is an upper level of WIS content due to the increasing viscosity with an increasing WIS content. This may result in severe mixing problems. A fed-batch SSCF also serves the purpose of making a higher total WIS loading in the reactor is possible, since the maximum viscosity can be kept lower than in a batch process.

To conclude, SSCF is a variant of SSF, which provides a number of principal advantages. The hydrolysis and fermentation steps are combined in one vessel for SSCF, thus it has the same characteristics as SSF, such as lower investment cost, a potentially shorter overall process time, and most likely a reduced contamination risks. Whether or not the overall ethanol yield will be higher in SSCF compared to SHCF will depend on both substrate, enzymes and microorganisms used.

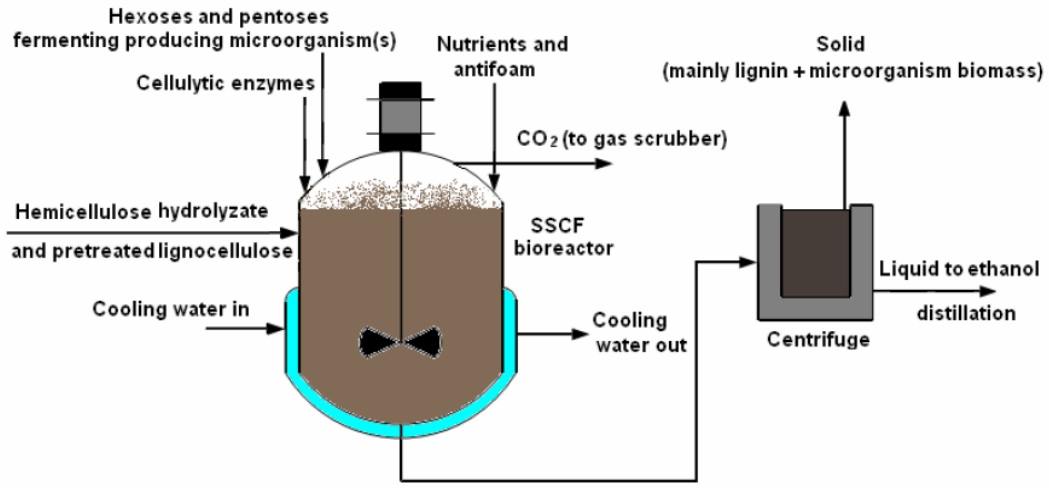

Figure 39: Process flow diagram for simultaneous saccharification and co-fermentation (Taherzadeh and Karimi 2007)

6.7 Separate Hydrolysis and Co-Fermentation (SHCF)

In SHCF, the hydrolysis and fermentation processes in SHCF take place in separate vessels so that each step can be performed at its optimal conditions, and subsequently the hexoses and pentoses are co-fermented.

There are relatively few published investigations on SHCF. Erdei et al. (2012) performed SHCF in an integrated first and second generation ethanol process using an engineered xylose fermenting strain of *S. cerevisiae*. Steam-pretreated wheat straw (SPWS) hydrolysates was fermented with wheat-starch hydrolysate in two different fed-batch configurations. In one configuration, wheat starch hydrolysate was fed to the SPWS hydrolysate (7.0% WIS) initially present in the reactor. This resulted in an average ethanol yield of 93% based on glucose and xylose and complete xylose consumption. In an alternative configuration, part of the enzymatically hydrolyzed (18.5% WIS) unwashed SPWS was initially present in the reactor and part was co-fed with the wheat starch hydrolysate. The average yield of ethanol and the xylose consumption reached 86% and 69%, respectively, in this case. The integration of first and second generation ethanol may thus offer not only investment savings but also process performance advantages.

6.8 Consolidated Bioprocessing (CBP)

An alternative route to production of bioethanol is the utilization of microorganisms that can both hydrolyze the biomass carbohydrates (through the action of excreted enzymes) to fermentable sugars and also ferment the resultant sugars to ethanol in a process known as consolidated bioprocessing (CBP). Consolidated bioprocessing (CBP) combines the following processes in a single step and in one reactor: production of lignocellulose-degrading enzymes, hydrolysis of polysaccharides present in pretreated biomass and fermentation of hexose and pentose sugars (Figure 40). The process is also known as direct microbial conversion (DMC). Usually, only one microbial consortium is applied for cellulase production and fermentation. This method offers lower production costs due to its simple feedstock processing, lower energy demand and higher conversion rates than SSF or SSCF process technologies. The potential of CBP is currently limited as there are no readily available natural microorganisms fulfilling all process requirements. However, CBP is gaining increasing recognition as a potential breakthrough for low-cost biomass processing. Although no natural microorganism possesses all properties of lignocellulose utilization and ethanol production desired for CBP, some bacteria and fungi exhibit some of the needed traits (Table 34).

Attention is also devoted to the challenges ahead to integrate all required enzymatic activities in an industrial *S. cerevisiae* strains and the need for molecular and selection strategies pursuant to developing a yeast capable of CBP. Different bacteria and yeasts have been in the focus of research and some progress has been made.

There are different strategies to apply CBP on industrial scale (Lynd et al. 2005):

- Engineering naturally occurring cellulolytic microorganisms such as *Clostridium thermocellum* to improve product-related properties such as yield and titer
- Engineering non-cellulolytic microorganisms such as *Saccharomyces cerevisiae* or *Zymomonas mobilis* that exhibit high product yields and titers to express a heterologous cellulase system enabling cellulose utilization

However, both strategies have advantages and disadvantages. For example, cellulase producers lack ethanol tolerance. In addition, it is difficult to have multiple saccharification enzyme genes efficiently expressed in ethanol producing microorganisms. Among all the CBP potential microbes, thermophilic bacteria, such as *Clostridium thermocellum*, are believed feasible as they possess cellulolytic and ethanologenic characteristics under high

temperature conditions (Georgieva et al. 2008). Complexes of cellulolytic enzymes contained in *C. thermocellum* known as cellulosome are responsible for cellulose degradation and sugar release. According to the finding from Xu et al. (2010), the temperature of 65°C was used with pH ranging from 6.5-7.4 to compromise between the optimal conditions of the growth of *C. thermocellum* and cellulosome activity.

Table 34: Comparison of potential microorganisms in CBP (Xu et al. 2009)

Microorganism	**Yeast *(S.cerevisiae)***	**Bacteria *(Z. mobilis)***	**Bacteria *(C. thermocellum)***	**Fungi *(T. reesei)***
Cellulase genes	Some attempts to express heterologous genes for key cellulases have failed	Unknown	Naturally express cellulases in cellulosomes	Naturally produce several cellulases
Cellulase production	Barely detectible activity for some enzymes from cloned genes	Unknown	Produce a few grams per liter	Produce more than 100 g/L
Ethanol production	Up to 160 g/L of ethanol	Up to 130 g/L ethanol	Very slow rate and low yield	Very slow rate and low yield
Ethanol tolerance	Very high	High	Very low	Low
Multi-sugar usage in native strains	No	No	Do not utilize xylose	Yes
Resistance to inhibitors in biomass hydrolysates	High	High	Low	Very high
Amenability to genetic manipulation	Excellent	Good	Very poor	Good
Commercial acceptance	Very high	Acceptable	Unknown	Very high

South et al. (1993) compared the conversion efficiencies of pretreated hardwood in SSF by *T.reesei* and *S.cerevisiae* and in a CBP process by *C. thermocellum* through continuous experiments. The SSF system achieved substrate conversions varying from 31% in 9 h retention time to 86% at 48 h retention time. At compatible substrate concentrations (4-5 g/l) and residence times (12-14 h), substrate conversion in the CBP system was significantly higher (77%) than in the SSF system (31%).

Jin et al. (2011) evaluated the performance of the CBP organism *Clostridium phytofermentans* (ATCC 700394) on AFEX-treated corn stover (AFEX-CS). Fermentation conditions including temperature, inoculation size, nutrients, and initial pH were investigated. At optimal conditions with 0.5% (w/w) glucan loading of AFEX-CS, *C. phytofermentans* hydrolyzed 76% of glucan and 88.6% of xylan in 10 days. These values reached 87% and 102% of those obtained by SSCF using commercial enzymes and *S. cerevisiae* 424A. Ethanol titer for CBP was found to be 2.8 g/L which was 71.8% of that yielded by SSCF (3.9 g/L). Decomposition products from AFEX-CS helped to increase ethanol yield somewhat during CBP. Particle size played a crucial role in the enhancement of sugar conversion by CBP.

Xu et al. (2010) investigated factors influencing cellulosome activity in CBP. The cellulosome, a multi-subunit protein complex catalyzing cellulose degradation in cellulolytic *Clostridium*

thermocellum, plays a crucial role in CBP. Activity of cellulosome was tested under varying concentrations of chemical compounds derived from lignocellulose pretreatment and fermentation. It was found that, firstly, the cellulolytic activity of cellulosome was actually promoted by formate, acetate and lactate; secondly, cellulosome was tolerant up to 5mM furfural, 50mM p-hydroxybenzoic acid and 1mM catechol. Furthermore, the cellulosome exhibited higher ethanol tolerance and thermostability than commercialized fungal (*Trichoderma reesei*) cellulase. To probe the implication of these unique enzyme-features, *C. thermocellum* JYT01 was cultured under conditions optimal for cellulosome activity. This CBP system yielded 491 mM ethanol, the highest level reported thus far for *C. thermocellum monocultures.* These findings demonstrate the potential advantages of bacterial cellulosome, and provide a novel strategy for design, selection and optimization of the cellulosome-ethanologen application.

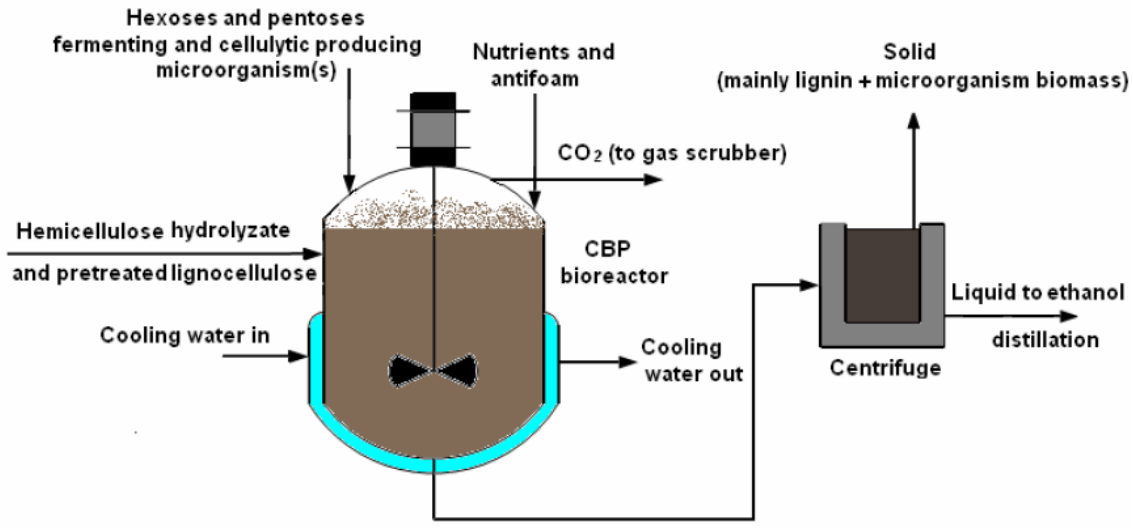

Figure 40: Process flow diagram for consolidated bioprocessing (Taherzadeh and Karimi 2007)

CBP has the potential to provide the lowest cost route for biological conversion of cellulosic biomass to fuels and other products in processes featuring hydrolysis by enzymes and/or microorganisms. To realize this potential, microorganisms must be developed that utilize cellulose and other fermentable compounds available from pretreated biomass with high rate and high conversion, and which produce ethanol at high yield. Both of these capabilities are possessed by known microorganisms, but to date have not been combined in a single microorganism or microbial system (Xu et al. 2009).

7 Downstream processing (DSP)

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7.1 Ethanol recovery

Downstream processing can be split into the following steps:

- Liquid-solid separation and distillation
- Dehydration
- Co-product (lignin) recovery and utilization

When the fermentation process is completed, ethanol concentration in the fermentation broth is usually 10-15% (w/w), thus it needs to be purified. The fermentation broth contains a mixture of ethanol, water, cell mass and other components such as residual sugars, non-fermentable sugars and hydrolysis by-products. A stripping column known as 'beer column' separates the solids and some water from the ethanol-water mixture. It separates most of the bioethanol from water producing a top stream rich in ethanol and a bottom stream rich in water (Figure 41). The beer column overhead stream contains 60-70% ethanol and is further rectified in a distillation tower to obtain an azeotropic mixture of ethanol and water. The maximum concentration of ethanol tolerated by the microorganisms is around 10 wt% at 30°C and decreases with increasing temperature (Hamelinck et al. 2005). This azeotropic mixture then passes over a molecular sieve made of zeolite to produce anhydrous or fuel grade ethanol at the outlet. As water content is normally over 80%, distillation is an energy intensive operation requiring large amounts of steam to concentrate ethanol to 95.6%. However it has to be further dehydrated to >98.7% (ahydrous ethanol) to enable its blending with gasoline (Taherzadeh and Karimi 2007).

The upgrading of ethanol from the lower concentrations towards 99.6% v/v concentration is performed using the known and widely applied technological steps:

- Evaporation of ethanol from beer: in this step the first evaporation of ethanol is performed in order to obtain 'crude' ethanol with concentration ~45% v/v
- Rectification: in rectification the ethanol concentration is increased to 96% v/v
- Dehydration: in the dehydration the remaining azeotropic water is removed in order to obtain the fuel bio-ethanol with concentration 98.7% v/v

Different separation techniques are applied to recover ethanol produced during fermentation. The first group of techniques contains non-membrane processes, whereas the second group contains the membrane processes. The non-membrane processes include product removal into a gas phase (gas stripping or vacuum fermentation), liquid phase and solid phase. The membrane separation techniques include product removal into a gas phase (pervaporation) and into a liquid phase by using membranes.

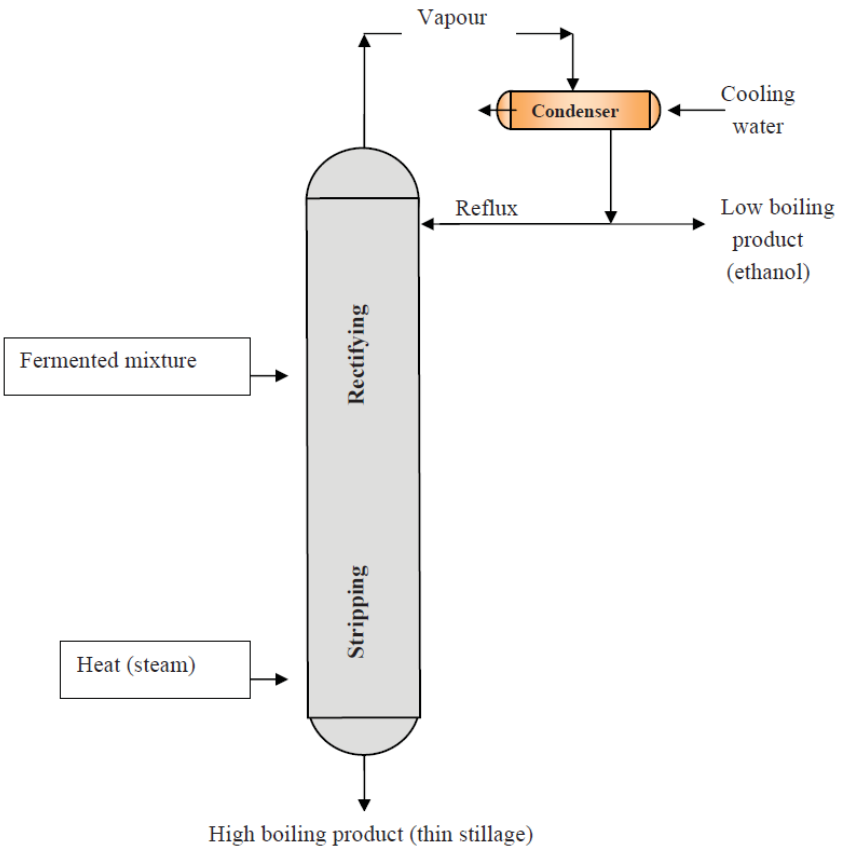

Figure 41: Distillation column (Walker 2010)

In the vacuum fermentation (Figure 42), separation is taking place under a vapour pressure which is higher than that of water. The coupling of fermenter vessel with a vacuum chamber extracting the more volatile ethanol from fermentation broth allows the partial product removal and the increase of overall process productivity (Cardona and Sanchez 2007). However, the process has disadvantages such as large amount of non-condensable carbon dioxide produced and the unused oxygen present in the vapour stream. Nguyen et al. (2011) investigated continuous fermentation integrated with separation process at atmospheric and vacuum pressures. An initial glucose concentration of 200 g/L was used to produce yeast cells under batch operating mode. After a period of 18-20 h, a medium of 350 g/L glucose concentration was fed continuously to the fermentation-separation column. During the continuous experiments, fermented broth was recirculated back to the fermentation-separation column. At atmospheric pressure, the dry mass concentration decreased rapidly and remained at 2.4 g/L after long period, while glucose concentration still remained at 240 g/L. At vacuum pressure, a quasi-steady state condition was reached after 96 h, corresponding to 4.2 g/L of cell dry mass, 165 g/L of glucose and 44.2 g/L of ethanol levels in the fermentation broth and 33.2 wt% of ethanol concentration at the outlet.

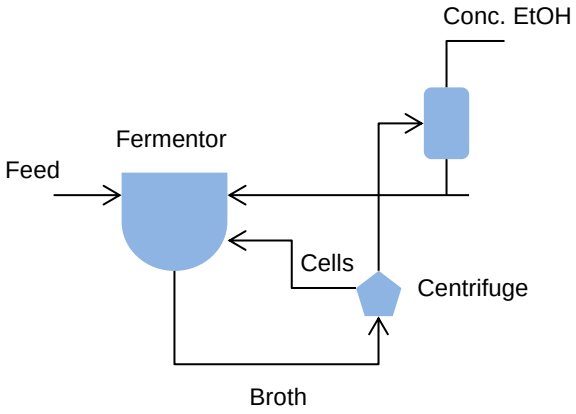

Figure 42: Ethanol removal from culture broth (Vacuum fermentation with cell recycling)

Ethanol can be removed from the culture broth through gas stripping that makes possible the increase in the concentration of sugars in the stream feeding the fermentor. Taylor et al. (1998) studied this integrated process in the case of dry-milling ethanol process in a pilot plant coupling a 30 L fermentor with a 10 cm packed column for ethanol removal by the CO_2 (stripping gas) released during the fermentation. The proposed model showed that ethanol inhibition influences especially the cell yield obtaining a value of 60 g/L of ethanol in the broth above which the inhibition is very strong.

Dominquez et al. (2000) studied ethanol production rom xylose with the yeast *Pichia stipitis* and simultaneous product recovery by gas stripping using a gas-lift loop fermentor with attached side-arm (GLSA). The bioconversion of xylose into ethanol with the yeast Pichia stipitis CBS 5773 is inhibited when 20 g/L of ethanol are present in the fermentation broth. In order to avoid this limitation, the fermentation was carried out with simultaneous recovery of product by CO_2 stripping. The fermentation was also improved by attaching a side-arm to the main body of a classical gas-lift loop fermentor. This side-arm increases the liquid circulation, mass transfer, and gas distribution, reducing the amount of oxygen in the inlet gas necessary to perform the fermentation of xylose under microaerobic conditions. The continuous stripping of ethanol from the fermentation broth in this new bioreactor system allowed the consumption of higher xylose concentrations than using Erlenmeyer shaker flasks, improved significantly the process productivity and provided a clean ethanol solution by using an ice-cooled condenser system.

The use of membrane processes for the recovery of fermentation products has been gaining increased acceptance in recent years. Pervaporation refers to separation using a membrane with liquid feed on one side and a low pressure, gaseous permeate output on the other side. Components in the liquid feed preferentially permeate through the membrane and then evaporate into the gaseous phase. Because pervaporation does not involve a large heat input, the process could save on costs associated with the heat and steam needed for the reboiler of a conventional distillation column (Kazi et al. 2010).

During pervaporation processes separation is carried out with the combined process of evaporation and permeation through a membrane. Coupling the fermentation process with pervaporation allows the removal of the product thus reducing the inhibition caused by high concentrations of ethanol. Sanchez et al. (2005) carried out the modelling of a simultaneous saccharification and fermentation process coupled with pervaporation for ethanol fuel production. Their results showed that increased concentrations of ethanol leads to a reduction in energy costs during distillation of permeate in comparison with direct distillation of fermentation broth where the concentration of ethanol is very low.

7.2 Residual solids and wastewater treatment

The main solid by-product from the conversion process is lignin which is a high energy value product. Its amount depends on the feedstock as well as on the process application. Lignin and remaining solid materials can be burned in order to produce steam for different processes such as hydrolysis, distillation or evaporation. In addition, it can be used for electricity production or heat supply. Lignin can also be processed through gasification and Fischer-Tropsch process to produce synthesis gas and hydrocarbon fuel additives.

Solids are separated by centrifuge and are dried in a rotary drier. Around 25% of the centrifuge effluent is recycled to fermentation and the rest is sent to the second and third evaporator effects. Most of the evaporator condensate is returned to the process as fairly clean condensate (a small portion, 10%, is split off to waste water treatment to prevent build-up of low-boiling compounds) and the concentrated syrup contains 15%-20% by weight total solids (McAloon et al. 2000).

The stillage or wastewater remaining after distillation has to be treated as the residual water contains significant amounts of organic compounds such as acetic acid, furfural, HMF, residual sugars and other compounds. Ethanol stillage from lignocellulosic materials is comparable to first generation feedstocks (sugar cane or corn). Therefore, methods of stillage treatment und utilization applied to first generation feedstocks might also be applicable to lignocellulosic feedstocks (Wilkie et al. 2000). There are different solutions for the treatment, utilization and disposal of stillage. The main methods are physical/mechanical separation, single cell protein production, calcium magnesium acetate, anaerobic digestion and algae production.

Physical/mechanical separation is applied to the stillage to recover and remove suspended solids containing yeast and other materials. The separated solids can be dried and used as a high-value product for animal feed. However, this method is rather limited to whole grains (corn). For sugar crops and lignocellulosic crops the separation of suspended solids is more difficult.

By using evaporation, the stillage is concentrated to syrup in multi-effect evaporators with co-production of evaporator condensate. Stillage evaporation is an energy demanding process requiring around 10% of the energy content of ethanol and can affect the energy balance of ethanol production (Figure 43). Even though evaporator condensate has lower organic content than stillage, it still contains volatile organics such as ethanol, acetic acid and formaldehyde. The evaporator condensate can undergo aerobic or anaerobic treatment. Aerobic and anaerobic digestion can be a solution for removing COD from stillage and converting it to biogas. The presence of inhibitors in the stillage should be taken into account.

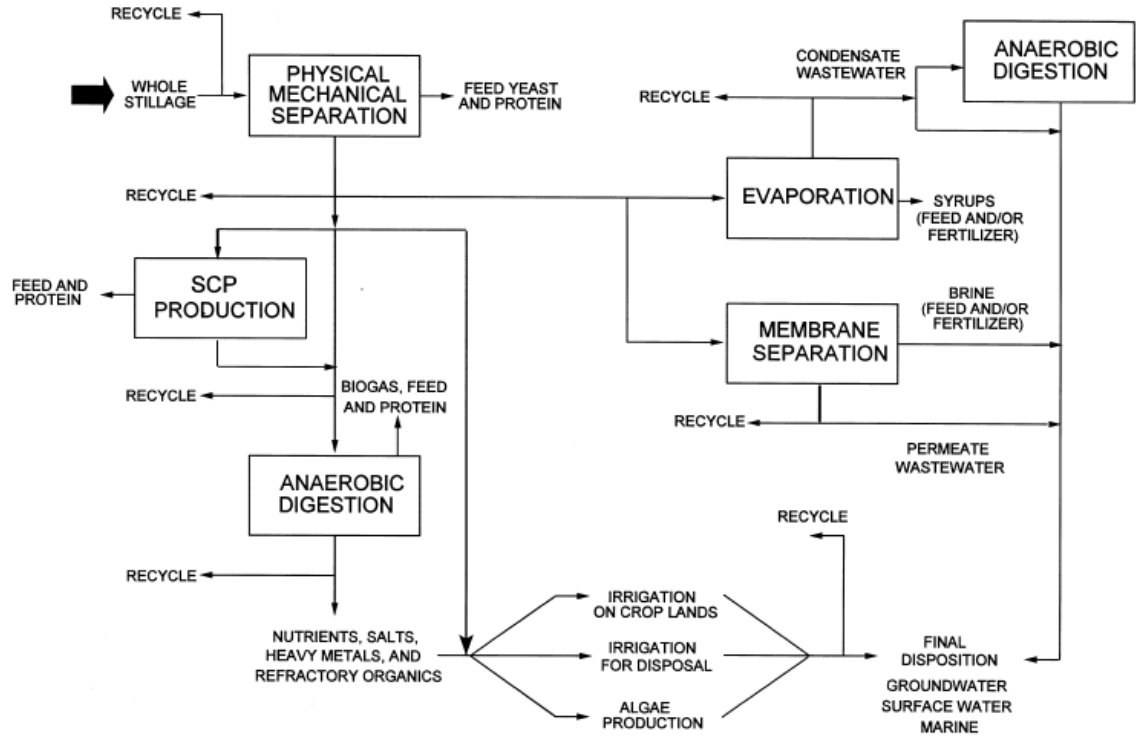

Figure 43: Stillage treatment technology and utilization options (Wilkie et al. 2000)

8 Demonstration projects

RITA MERGNER, RAINER JANSSEN

8.1 Introduction

Large efforts are dedicated to the production of biofuels from lignocellulosic raw materials. Demonstration projects for lignocellulosic ethanol production are under development in several countries worldwide. Facilities demonstrate the capability of second generation technology for continuous production and cover entire production processes. While only few production facilities are operational yet, many projects are under construction or planned.

IEA Bioenergy Task 39 has collected data on pilot and demonstration projects and displays the results in an interactive map (Figure 44). Lignocellulosic ethanol demonstration plants are already running, being constructed or planned in Spain, Denmark, Canada, USA, Sweden, Germany, Italy, Austria, France, Finland, Norway and Poland. This chapter presents main demonstration projects by providing short information on existing demonstration plants.

One of the challenges second generation facilities confront are high capital costs, higher than corn-to-ethanol facilities. Financing has been a challenge, and a number of sources, both private and government of capital are being utilized. Private sources include venture capital funds and internal funds from large corporations (Janssen et al. 2013).

There are a number of biomass feedstocks proposed for these facilities, including grain residues (e.g. corn stover, corn cobs, wheat straw), energy crops (e.g. switchgrass, sorghum, energy cane, Miscanthus, poplar), wood, wood waste, and municipal solid wastes (MSW). Both biological (e.g. enzymatic hydrolysis and fermentation) and thermal (e.g.

gasification) processes, as well as hybrid (with both biological and thermal components) systems are being developed.

Figure 44: Demonstration and pilot projects for advanced biofuels production (IEA Task 39 interactive map http://demoplants.bioenergy2020.eu/projects/mapindex)

8.2 Mossi&Ghisolfi/Biochemtex demo plant, Crescentino, Italy

Biochemtex is currently completing the world's first commercial-scale cellulosic ethanol plant in Crescentino, Italy, was opened in October 2013. The project is supported by the European Commission under the FP7 framework programme. The Crescentino plant will be the first industrial facility in the world producing second generation bioethanol. The plant will produce 15 MW of green power from lignin for own consumption and injection into the grid. The plant will apply Biochemtex's PROESA® technology which allows delivering superior economics to convert lignocellulosic biomass to sugars for the production of bioethanol or biochemicals. The technology will be licensed in the global marketplace by newly founded Beta Renewables, a joint venture with TPG Capital and TPG Biotech. Extensive description is available in Part II of this Handbook.

8.3 Biocombustibles de Castilla y León (BCyL) plant, Spain

Biocarburantes de Castilla y León, S.A. (BCyL), the company that is 50% owned by Ebro Puleva, S.A. and 50% by Abengoa Bioenergía, S.A. constructed the second generation bioethanol plant in Babilafuente (Salamanca) (Figure 43). The plant began operating in 2009 and is designed to produce bioethanol to be directly mixed with petrol in the Spanish market and promote the use of biofuels.

The project, which received financing for its construction and start-up through the FP5 Program of the European Commission, has an annual production capacity of 5 million litres, and uses wheat and corn straw as its raw material. It is the world's first plant to utilize

enzymatic hydrolysis technology at this level of output. A steam explosion pretreatment stage from SunOpta was installed. 25,000 t of the substrate per year is used as an input material. Produced ethanol is distilled to 42% and then concentrated and dehydrated. The plant's target is to achieve a yield higher than 300 L/ton of feedstock, as well as to ferment pentose sugars, opening the door to the next stage in the technological evolution of the process.

The biocatalysts and enzymes used are so important to the production of ethanol from biomass that the company has established a specific research line (the Enzyme Development Technological Program) to develop more effective and optimized enzymes to reduce consumption and therefore to reduce their financial impact on the process. In fact, some of the enzymes used in Babilafuente are produced by Abengoa, making it the only company with the capacity to prove their functional capabilities under industrial conditions.

These results demonstrate the feasibility of the enzymatic hydrolysis technology, which the company has been working on for a long time and has made major investments in. Abengoa intends to put this technology into practice on a commercial scale at the plant that it will construct in Hugoton, Kansas (USA), which will have an annual capacity of 100 million litres. This project, which is being jointly developed with the US Department of Energy (DOE), will become the largest commercial bioethanol production plant using biomass to date, enabling approximately 135,000 tonnes of CO_2 to be saved every year, equivalent to the annual emissions from 35,000 cars.

The production process involves the following steps (Figure 45):

- Preparation of biomass
- Thermochemical pretreatment (steam explosion, no fractionation)
- Enzymatic hydrolysis and fermentation with enzymes and yeast)
- Distillation to recover ethanol and solid co-product

The facility is being used to improve the design of the commercial plants of tomorrow, reduce operational costs, identify bottlenecks and streamline operations (Abengoa Bioenergy Annual Report 2011).

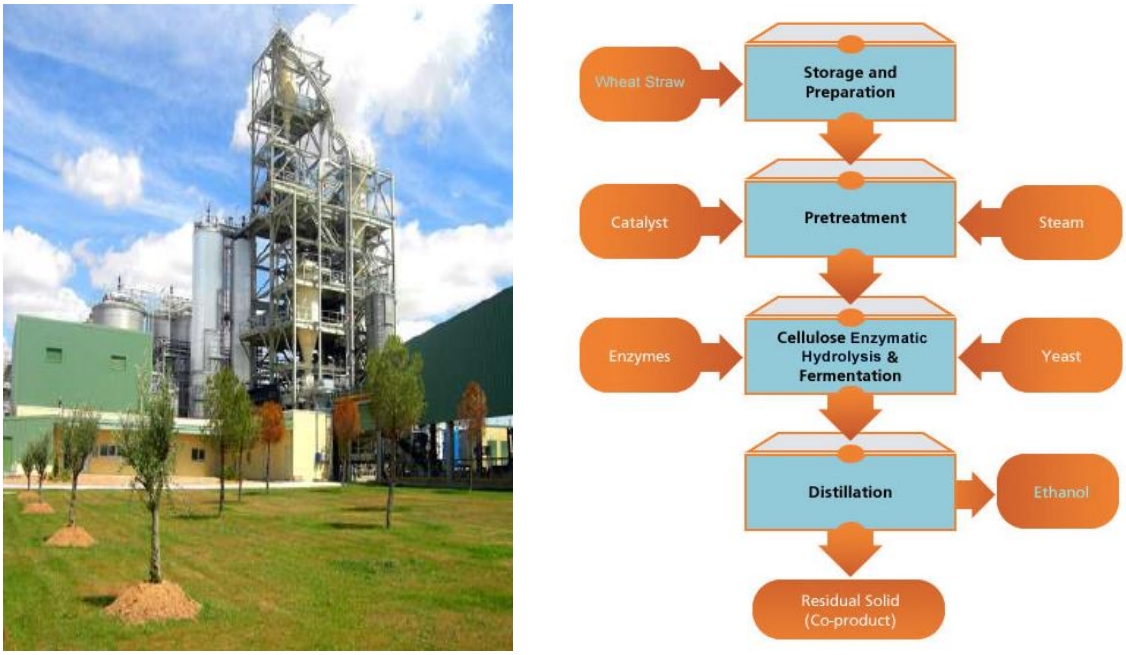

Figure 45: Abengoa demo plant in Salamanca (Abengoa Bioenergy Annual Report 2011)

8.4 Demonstration plant in Lacq, France

Abengoa Bioenergy France owns Abengoa Bioenergy's fourth ethanol production plant in Europe. It is 69% owned by Abengoa Bioenergy and 31% owned by Oceol, an association of the region's main agricultural cooperatives and industries.

This plant employs corn and low-quality vegetable alcohols as raw materials and is located at the Petrochemical Platform at Lacq, Pyrénées-Atlantiques (France). The demonstration project will apply biochemical conversion process to convert agricultural and forest residues into lignocellulosic ethanol. The total lignocellulosic biomass input to the process is 522 dry tons/day, resulting in an ethanol production of 40,000 t/a (50,000 m^3/a). In addition it will produce lignin and distiller biomass. Total investment is around 10.5 million EUR, 8.6 million thereof are funded by the EU under FP7 program. The start-up of demonstration plant is scheduled in June 2013.

Projected total annual bioethanol production capacity amounts to 250 ML, broken down into 200 ML using corn as the raw material, and 50 ML produced from the distillation of lower-quality vegetable alcohols. Annual DGS production is approximately 145,000 tons. Estimated annual corn consumption is roughly 500,000 t. The plant employs currently 73 professionals. Bioethanol produced at the plant will be used in public fleets. In addition, the project aims to improve enzymes involved in cellulose hydrolysis.

This project is coordinated by Abengoa Bioenergía Nuevas Tecnologías and has the participation of another four companies from different countries: Green Value (Switzerland), TNO (The Netherlands), Communauté d'Agglomération of the Pau-Pyrenées (CDAPP) and Communauté de Communes of Lacq (CCL) (France).

Figure 46: Abengoa Arance EC demonstration plant, France (Abengoa Bioenergy Annual Report 2010)

8.5 BioGasol plant, Denmark

Since 2011 BioGasol has been working on phase II of the 'BornBioFuel' demonstration plant project, after developing cost-effective solutions within pretreatment and pentose fermentation during phase I (2008-2010). The integrated plant will demonstrate BioGasol core technologies as well as in a coherent process for second generation bioethanol production. The core technologies are developed at BioGasol in Ballerup, Denmark, however other unit operations such as distillation, separation, drying and anaerobic digestion will be delivered by third party vendors. The plant will be located in Aakirkeby on the island of Bornholm.

The integrated BornBioFuel plant is designed to demonstrate feedstock flexibility, which means the plant will be demonstrating conversion of agricultural residues such as straw, garden waste, energy crops and grass from road sides. In addition, it will be possible to run campaigns on other feedstock for testing purposes. The heat and power in the plant can be integrated with an external energy system. Furthermore all process water is to be re-used in the plant, thus minimizing the waste as well as water consumption. The process uses thermochemical pretreatment and a unique fermentation process based on proprietary microbes converting both hexose and pentose sugars to ethanol. The total lignocellulosic biomass input will be 2.5 tons/hour (100% DM), resulting in an ethanol production of 5 million l/yr. During elaboration of this Handbook the planned start-up of the plant was announced in 2013. However, there is no official information available on the progress.

The project has been granted 27.5 million DKK (3.7 million EUR) for phase I, where the core technologies based on BioGasol proprietary developments have been developed to scale. This has been followed up by a second grant for the technology integration of 78.2 million DKK (10.5 million EUR) in phase II. Furthermore, BioGasol together with scientific partner Aalborg University received a grant of 12.4 million DKK (1.7 million EUR) for supporting pilot plant activities. All grants have been awarded by the Danish Ministry of Energy and Climate under the Energy Research (EFP) and Energy Technology Development and Demonstration (EUDP) programs.

In April 2012 BioGasol announced a partnerhsip with Sweetwater Energy Inc., who will integrate the BioGasol pretreatment technology (Carbofrac™) into its systems to maximise sugar production from lignocellulosic biomass. BioGasol's 'Carbofrac' pretreatment technology is a pretreatment system specifically designed for the biochemical conversion of lignocellulosic feedstock. The technology is based on a method called wet explosion and is a combination of steam explosion and wet oxidation, applying both the addition of oxygen and a pressure release at high temperature (170-200°C). Figure 47 shows the process diagram on BioGasol demonstration plant.

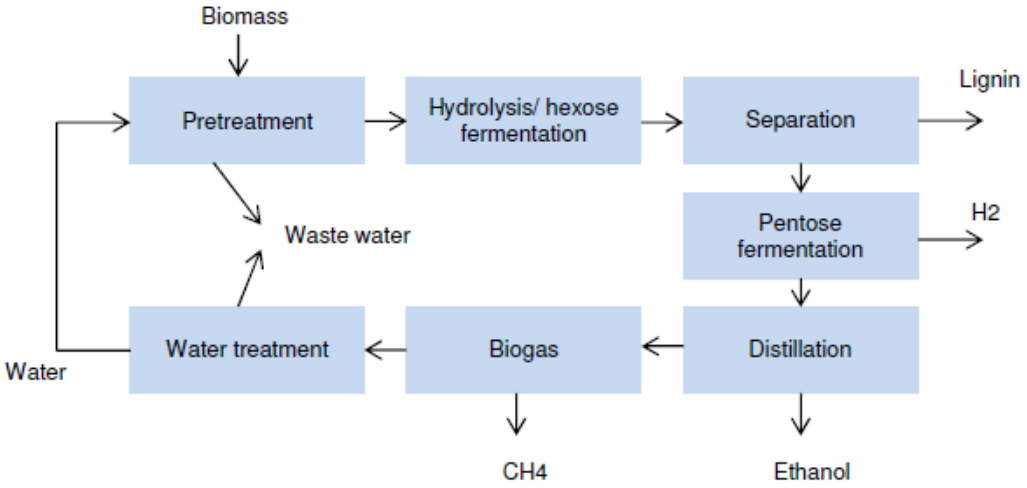

Figure 47: Process diagram of BioGasol demonstration plant in Denmark (Bacovsky et al. 2010)

8.6 Kalundborg plant, Denmark

DONG Energy constructed a demonstration plant in Denmark. The principal purpose of the demonstration plant is to show that second generation technology can be applied on a large scale production of ethanol from straw is possible.

The Kalundborg plant (Figure 48) demonstrates energy integration with a power station. Steam from the power plant cooks the straw, and residual biofuel from the ethanol plant is burned by the power plant. Since the cellulosic ethanol plant produces more energy than it consumes to convert the biomass, the end result is an energy surplus that brings down the cost for both plants and demonstrates the efficiency and financial viability of the Inbicon process. The demonstration plant started operation in 2009 has four outputs: ethanol, a powdered biofuel, molasses syrup for animal feed and a natural bacteria inhibitor.

Figure 48: Plant in Kalundborg (www.inbicon.com)

The power station sends waste steam to the biomass refinery, where it breaks down the straw fibers in order to convert them into sugars, ethanol, and lignin, the woody part of the straw. The plant is fully integrated, designed for commercial production with automatic operation 24/7. When integrated with a grain ethanol plant, the Inbicon Biomass Refinery can produce enough thermal and electrical energy to offset up to 50% of the grain plant's process

utility costs. In addition, it can supply 100% of its own steam and electricity. When integrated with a coal-fired plant's CHP system and waste-heat capture, the power station's efficiency is generally doubled. Table 35 provides an overview on the Kalundborg plant.

Table 35: Main facts about the Kalundborg plant (www.inbicon.com)

Raw materials	4 MT/h equivalent to about 30.000 metric tons of straw a year Enzymes supplied by by Danisco Genencor, Novozymes and Royal DSM
Annual production	5.4 million liter of cellulosic ethanol 11,400 metric tons of lignin pellets 13,900 metric tons of C5 molasses
Employees	30 employees
Costs	Total construction costs: about 400 million DKK (54 million EUR)
Grants	Design and construction is supported by the Danish EUDP program with 76.7 million DKK (10.3 million EUR) Demonstration is supported by the European Seventh Framework Programme with 67.7 million DKK (9.1 million EUR) Support from the European Commission under FP5 program for development of the technology in an earlier stage was granted

8.7 Iogen demonstration plant, Canada

Iogen operates the world's first demonstration facility, opened in 2004 where lignocellulosic ethanol is made from agricultural residues. The demonstration plant located in Ottawa is designed to prove the feasibility of Iogen's cellulosic ethanol process by validating equipment performance and identifying and overcoming production problems prior to the construction of larger plants. The plant can handle all functions involved in the production of lignocellulosic ethanol, including: receipt and pretreatment of up to 30 t/d of feedstock, conversion of cellulose fibre into glucose, fermentation and distillation. Wheat, oat and barley straw are used as raw materials. At full capacity Iogen's demonstration plant is designed to process about 20-30 tons per day of feedstock, and to produce approximately 5,000-6,000 litres of second generation ethanol per day. The fuel, produced from the Ottawa demonstration facility is being purchased by Royal Dutch Shell for use in fuel applications. The basic process steps of Iogen's Ottawa plant are shown in Figure 49.

Different potential feedstocks have been researched at Iogen's demonstration plant such like corn stover, switch grass, miscanthus, oat straw, sugarcane bagasse and hard wood chips. Soft wood was found to be not compatible with Iogen's technology. To be used with Iogen's second generation ethanol process, a feedstock must have at least 60% carbohydrate content, and to remain cost effective, must be available in large quantities.

Iogen applies pretreatment method to increase the surface area and 'accessibility' of the plant fibre to enzymes. This is achieved through a modified steam explosion process which improves ethanol yields, increases pretreatment efficiency, and reduces overall cost. The enzyme plant employs innovative automated production systems and operates seven days a week. Separate hydrolysis and fermentation using a multi-stage hydrolysis process is applied. Iogen uses advanced microorganisms and fermentation systems that convert both hexose and pentose sugars into ethanol. The 'beer' produced by fermentation is then distilled using conventional technology to produce cellulosic ethanol for fuel grade applications.

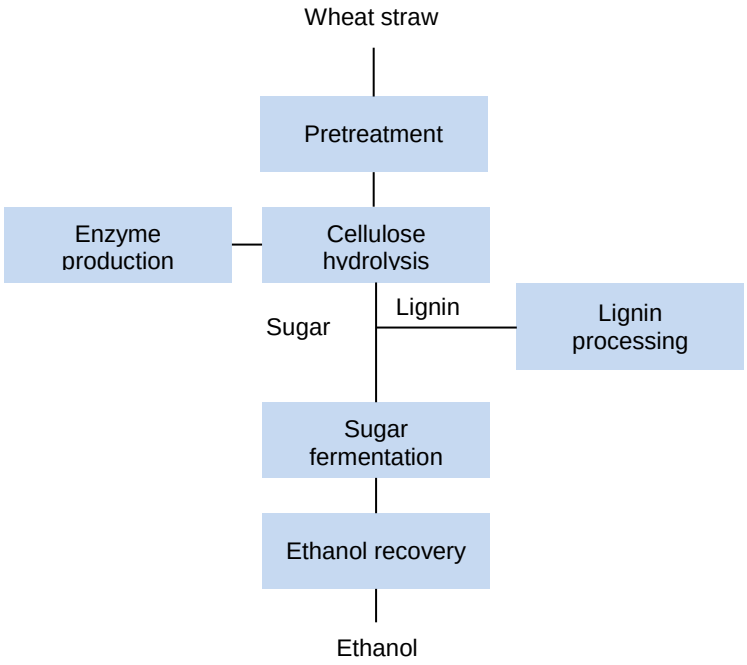

Figure 49: Iogen process for converting wheat straw to ethanol (www.iogen.ca/)

Table 36: Lignocellulosic ethanol production in Iogen's demonstration plant (www.iogen.ca/)

Year	Ethanol (L)	Cumulative (L)
2005	129,547	219,418
2006	16,811	236,229
2007	2,598	238,827
2008	206,525	445,352
2009	581,042	1026,394
2010	208,781	1535,175
2011	371,606	1906,781
2012	219,090	2125,871

8.8 Demonstration plant in Straubing, Germany

Süd-Chemie constructed Germany's largest demonstration scale lignocellulosic ethanol production plant in Straubing which started operation in July 2012. The plant processes agricultural residue feedstock to produce cellulosic ethanol. In July 2012 Süd-Chemie joined Clariant Corporation.

Construction of the plant started in 2011 and estimated cost of the project is around 28 million EUR. 16 million EUR thereof are allocated to the construction and 12 million EUR are

used for research and development. The Bavarian State Government and German Federal Ministry of Education and Research (BMBF) provided subsidies to the project of about 10 million EUR.

The main feedstocks include wheat and cereal straw, corn stover and sugar cane bagasse; but one feedstock is processed at a time. The plant will utilize around 4,500 tons of wheat straw to produce 1.000 tons of lignocellulosic ethanol per annum. The commercial scale plants will have production capacity of about 50,000-150,000 t/yr. Integrated bioengineered enzyme production units on site will produce the necessary feedstock-specific biocatalysts and optimise them as demanded by the processes.

The Straubing demonstration plant will use Süd-Chemie proprietary process technology called Sunliquid. The process is expected to increase the final yields of ethanol by about 50% due to proprietary yeast which converts pentose and hexose sugars simultaneously. Chopped feedstock undergoes hydrothermal pretreatment in a vapour pressure container. Highly optimized enzymes breaking hemicellulose and cellulose into sugar monomers in a short time are used in the hydrolysis step. Integration of the enzyme production facility on site will eliminate the dependence on enzyme suppliers, the need for transport, storage, formulation, purification and stabilisation of the enzymes. These enzymes break the hemicellulose and cellulose into sugar monomers in a short reaction time. The C5 and C6 sugars undergo fermentation simultaneously in a one pot reaction using the proprietary strain and process technology of the Sunliquid process.

Unlike the traditional distilled spirits plant routes, the cellulosic ethanol recovery process uses a proprietary downstream processing technology. The patented purification Süd-Chemie ethanol recovery process consumes 50% less energy than conventional distillation, based on the process conditions. The energy required for the entire process is sourced from the non-fermentable lignin fraction, making the production climate neutral. Produced ethanol emits 95% less CO_2 compared to fossil gasoline (www.chemicals-technology.com).

Figure 50: Süd-Chemie bioethanol plant (http://www.sud-chemie.com)

8.9 Jennings demonstration plant, USA

This project involves the operation of a biorefinery producing ethanol from sources such as dedicated energy crops and lignocellulosic agricultural residuals that could serve as major feedstocks for biorefineries of the future. The Jennings facility is located in Jennings, Louisiana that is comprised of a pilot plant and a demonstration facility. The facility started to run in 2009 and is operated by Verenium Corporation.

This project is operating the demonstration facility to validate the findings from the pilot plant operation in the production of cellulosic ethanol from dedicated energy crops and agricultural residuals. This is an operating demonstration facility that is fully integrated from feedstock pretreatment to recovery and distillation of the biofuel product. Feedstock is pretreated and subjected to proprietary enzymatic hydrolysis systems and the subsequent hexose and pentose sugars are fermented separately with proprietary strains of ethanologens to produce ethanol. The capacity of the plant is 5.3 Ml /year (40 t/d feedstock).

Biomass is delivered to the facility, and is prepared for processing by milling and washing. The biomass is hydrolyzed using steam and mildly acidic conditions. This portion of the process creates pentose syrup from the hemicellulose found in the biomass, and prepares the remaining cellulose fiber for further enzymatic conversion into glucose. The cellulose fiber and pentose syrup slurry is then sent to a liquid/solid separation step where the pentose syrup is separated from the fiber solids.

Figure 51: Jennings demonstration facility (http://demoplants.bioenergy2020.eu)

In one tank, the pentose syrup is fermented directly through the action of a proprietary industrial fermentation microorganism to make a pentose beer. In another tank, the cellulose fibers are mixed with specialized enzymes and additional proprietary industrial fermentation microorganism. The enzymes and the microorganism then work in concert with each other to simultaneously breakdown the cellulose into glucose, and ferment the glucose into a hexose beer. The pentose and hexose beers are combined and sent to distillation for recovery of the ethanol from the beer. Distillation residues are collected, dewatered, and sent to the biomass boiler as fuel to create steam and power used for the entire facility.

One of important lessons learned from the project was the data gathered from compositional data of the feedstocks. In addition, during this project was learned that sometimes research organisms are not capable of scaling up for industrial use. Research organisms are typically used in highly controlled environments, and it is not practical to maintain a highly controlled

environment at commercial scale. Industrial organisms need to be robust enough to survive and thrive in environments that may be subject to a high degree of variation.

8.10 INEOS Bio pilot plant, USA

INEOS Bio pilot plant in Fayetteville (Arkansas) represents an important step on the road to commercialisation. It enables the most promising results from laboratory, bench-scale work to be tested and optimised on a much larger integrated process. Pilot plant is providing the company with all the necessary data needed to progress with speed to full commercial scale. As an outcome of the available new data, several important innovations have been made over the last 5 years.

The pilot plant was built by Bioengineering Resources Inc (BRI) in 1994 and expanded in 2003 with the addition of a gasifier. It applies thermochemical and biochemical technology for ethanol and power production (350 t/y dry ethanol) from wood waste chips (200kg/ton) in the following steps:

- Gasification - the prepared organic carbon material is gasified using a controlled amount of oxygen to produce synthesis gas, a mixture of principally carbon monoxide and hydrogen. The gasifier design and operating conditions have been carefully chosen to inhibit the formation of dioxins and furans and to suppress the carry-over of volatile metals. The hot synthesis gas is quenched and cleaned. Heat is recovered to generate renewable power for use in the process.
- Fermentation - the cleaned, cooled synthesis gas is passed into a patented fermentation process, where it is converted selectively into ethanol by naturally occurring anaerobic bacteria (the biocatalyst). The fermentation environment, containing the right quantity and type of nutrients, is maintained at carefully controlled conditions. The bacteria, in this healthy state, achieve an extremely high selectivity to ethanol and high yield of ethanol. The high selectivity and yield translate to outstanding process efficiencies. The off-gas from the fermenter is used to generate additional power and heat.
- Purification - the ethanol solution is purified and refined to make anhydrous ethanol (>99.7% ethanol). This can be blended into gasoline as required for the local, renewable road transport fuel market.

Part II 2° Generation Bioethanol

The world's largest demo plant ready to be transferred all over the world

ARIANNA GIOVANNINI, DAVID CHIARAMONTI, STEFANIA PESCAROLO, RENANO NISTRI

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Glossary

The Glossary and Abbreviations list describes and defines various specific or common expressions, terms and words, which are used in this handbook. Several expressions are adapted from Wikipedia.

Acidity: The presence of acid-type constituents whose concentration is usually defined in terms of neutralization number. The constituents vary in nature and may or may not markedly influence the behavior of e.g. oils.

Additives: Chemicals added to fuel in very small quantities to improve and maintain fuel quality. Detergents and corrosion inhibitors are examples of gasoline additives.

Alcohols: Organic compounds that are distinguished from hydrocarbons by the inclusion of a hydroxyl group. The two simplest alcohols are methanol and ethanol.

Aldehydes: A class of organic compounds derived by removing the hydrogen atoms from an alcohol. Aldehydes can be produced from the oxidation of an alcohol.

Aliphatic: A class of saturated or unsaturated carbon compounds, in which the carbon atoms are joined in open chains.

Alternative Fuel: Fuel which is not broadly applied today and which is a niche product in the fuel market.

Anhydrous: Describes a compound that does not contain any water.

Anhydrous alcohol: Alcohol that is free of water and at least 99% pure. This ethanol may be used in fuel blends. Hydrous alcohol on the other hand contains some water and usually has a purity of 96%. In Brazil, this ethanol is being used as a 100% gasoline substitute in cars with dedicated engines. The distinction between anhydrous and hydrous alcohol is of relevance not only in the fuel sector but may be regarded as the basic quality distinction in the ethanol market.

Aromatics: Hydrocarbons based on the ringed six-carbon benzene series or related organic groups. Benzene, toluene and xylene are the principal aromatics, commonly referred to as the BTX group. They represent one of the heaviest fractions in gasoline.

Bagasse: By-product of sugarcane production. It is the biomass remaining after sugarcane stalks are crushed to extract their juice. Bagasse is often used as a primary fuel source for sugar mills; when burned in quantity, it produces sufficient heat energy to supply all the needs of a typical sugar mill, with energy to spare.

Barrel: A unit of volume measurement used for petroleum and its products. 1 barrel (bbl) = 42 U.S. gallons or 35 British gallons.

Biochemical Conversion: The use of enzymes and catalysts to change biological substances chemically to produce energy products.

Biodiesel: Biodiesel is composed of monoalkyl esters (methyl/ethyl esters), a long chain of fatty acids derived from renewable lipid sources. It is an ester based, renewable fuel made from vegetable oils, recycled fryer oils, tallow and other biological products which have had their viscosity reduced using a process called tranestrification. The by-product of this process is glycerin, the thick component of vegetable oil. Biodiesel is biodegradable, non-toxic, and essentially free of sulfur and aromatics. Originally biodiesel was considered a by-product of glycerin soap production.

Bio-ETBE: Ethyl-Tertio-Butyl-Ether produced from bioethanol. ETBE is used as a fuel additive to increase the octane rating and reduce knocking.

Bioethanol: Ethanol produced from biomass and/or the biodegradable fraction of waste, for use as biofuel E5 contains 5% ethanol and 95% petrol E85 contains 85% ethanol and 15% petrol

Biofuel: Liquid or gaseous fuel for transport produced from biomass: Alcohols, esters, ethers, and other chemicals (biodiesel, ethanol, and methane) made from biomass sources (herbaceous and woody plants, animal fats, agricultural and forest waste, or municipal solid and industrial waste) within an active carbon cycle. Production and combustion of biofuels take and replenish the CO_2 in a circular, sustainable fashion. These fuels are used for stationary and mobile applications, i.e., electricity and transportation. Two commonly used biofuels are ethanol and biodiesel.

Biogas: A fuel gas produced from biomass and/or the biodegradable fraction of waste, which can be purified to natural gas quality (biomethane) for use as biofuel or woodgas.

Biomass: Renewable organic matter such as agricultural crops, crop waste residues, wood, animal waste, animal fat, municipal waste, aquatic plants; fungal growth; etc., used for the production of energy.

Biomass-to-Liquid (BTL): This second-generation fuel belongs to the group of synthetic fuels. Its components are designed for the requirements of modern motor concepts. For the production of BTL-fuels many types of feedstock can be used.

Blending: Mixing of two compatible fuels having different properties in order to produce an intermediate fuel.

BTL: Biomass-to-Liquid

CAP: Common Agricultural Policy in the EU

Carbon Dioxide (CO_2): A product of combustion that has become an environmental concern in recent years. CO_2 does not directly impair human health, but is a greenhouse gas that traps the Earth's heat and contributes to the potential for global warming.

Carbon Monoxide (CO): A colorless, odorless gas produced by the incomplete combustion of fuels with a limited oxygen supply, as in automobile engines. CO contributes to the formation of smog ground-level ozone, which can trigger serious respiratory problems.

Catalyst: A substance whose presence changes the rate of chemical reaction without itself undergoing permanent change in its composition. Catalysts may be accelerators or retarders. Most inorganic catalysts are powdered metals and metal oxides, chiefly used in the petroleum, vehicle, and heavy chemical industries.

CBP: combined bioprocessing

Centrifuge: A machine using centrifugal force produced by high-speed rotation for separating materials of different densities. Applied to Diesel engine fuels and lubricating oils to remove moisture and other extraneous materials.

Cetane Number (Cetane Rating, Cetane Index): A measure of ignition quality of diesel fuel. The higher the cetane number, the easier the fuel ignites when it is injected into the engine. Biodiesel has a higher cetane number than petrol diesel because of its higher oxygen content. This means that engines run smoother and create less noise when running on Biodiesel. It is the equivalent to the octane number of gasoline.

CH_4: Methane

CO: Carbon monoxide

CO_2: Carbon dioxide

Combined bioprocessing (CBP): technology for cellulosic ethanol production

Common Agricultural Policy (**CAP**): The CAP is a system of European Union agricultural subsidies. These subsidies work by guaranteeing a minimum price to producers and by direct payment of a subsidy for crops planted. This provides some economic certainty for EU farmers and production of a certain quantity of agricultural goods.

Corrosion: Detrimental change in the size or characteristics of material under conditions of exposure or use. It usually results from chemical action either regularly and slowly, as in rusting (oxidation), or rapidly, as in metal pickling.

Cryogenic Storage: Extreme low-temperature storage.

Density: Density is the term meaning the mass of a unit of volume. Its numerical expression varies with the units selected.

Detergent: Additives used to inhibit deposit formation in the fuel and intake systems in automobiles.

Distillation: Distillation is a method of separating substances based on differences in their volatilities. In the distillation process a liquid is heated up to its boiling point and the vapors are collected after condensing. This process is used for ethanol production.

E10 (Gasohol): Ethanol mixture that contains 10% ethanol, 90% unleaded gasoline.

E85, E93, E95: Ethanol/gasoline mixture that contains 85% (93%, 95%) denatured ethanol and 15% (5%, 2%) gasoline, by volume.

EC: European Commission

ETBE: Ethyl tertiary butyl ester

Ethanol: (also known as Ethyl Alcohol, Grain Alcohol, CH_3CH_2OH) Can be produced chemically from ethylene or biologically from the fermentation of various sugars from carbohydrates found in agricultural crops and cellulosic residues from crops or wood.

Ethyl Tertiary Butyl Ether (ETBE): A fuel oxygenate used as a gasoline additive to increase octane and reduce engine knock.

EU: European Union

Fahrenheit: Temperature scale based on 32F for the temperature at which water freezes and 212F for the temperature at which water boils (180 difference). Conversion to Fahrenheit from Celsius (centigrade) temperature scale is by the following formula: F = 9/5C + 32, where C is the temperature in Celsius degrees.

Feedstock: Any material converted to another form of fuel or energy product. For example, cornstarch can be used as a feedstock for ethanol production.

Fermentation: The enzymatic transformation by microorganisms of organic compounds such as sugar. It is usually accompanied by the evolution of gas as the fermentation of glucose into ethanol and CO_2.

FFV: Flexible fuel vehicle

First-generation feedstock: Feedstock which is used to produce first generation biofuels. Usually, this feedstock was originally cultivated for food production. It comprises only parts of the plants, such as stalks, kernels and tubes. Examples are rape-seed, cereals, potatoes, sugar-cane etc.

First generation biofuels: Biofuels which are available on todays fuel markets, such as PPO, biodiesel and bioethanol.

Flash point: The lowest temperature in °C at which a liquid will produce enough vapor to ignite, if the vapor is flammable and when exposed to a source of ignition. The lower the

flashpoint is the higher is the risk of fire. Biodiesel has an abnormally high flashpoint (for a fuel), making it very safe to handle and store.

Flexible-Fuel Vehicle (FFV): A Vehicle with a common fuel tank designed to run on varying blends of unleaded gasoline with either ethanol or methanol.

Fossil Fuel: A hydrocarbon deposit, such as petroleum, coal, or natural gas, derived from living matter of a previous geologic time and used for fuel. Combustion of fossil fuels emits large amounts of CO_2 into the atmosphere.

GHG: Greenhouse gas

HC: Hydrocarbons

HHV: Higher heating value

High Compression Ignition Engine: Also know as Diesel engine. Unlike gasoline engines which use a spark plug to ignite the fuel, there is no external ignition spark in a high compression engine. Air is compressed, driving its temperature up to a point that it ignites fuel which has been injected into the chamber.

Hydrocarbons: Compounds containing various combinations of hydrogen and carbon atoms. Hydrocarbons contribute heavily to smog.

Hydrogen (H_2): A colorless, highly flammable gaseous fuel.

Hydrous alcohol: Alcohol that contains some water and usually has a purity of 96%. In Brazil, this ethanol is being used as a 100% gasoline substitute in cars with dedicated engines. The distinction between anhydrous and hydrous alcohol is of relevance not only in the fuel sector but may be regarded as the basic quality distinction in the ethanol market.

Indirect Land Use Change (ILUC): When biofuels are produced on existing agricultural land, the demand for food and feed crops remains, and may lead to someone producing more food and feed somewhere else. This can imply land use change (by changing e.g. forest into agricultural land), which implies that a substantial amount of CO_2 emissions are released into the atmosphere.

Infrastructure: In transportation, this term generally refers to the charging and fueling network necessary to successful development, production, commercialization, and operation of alternative fuel vehicles. It includes fuel supply, public and private charging and fueling facilities, standard specifications for fueling outlets, customer service, education and training, and building code regulations.

Kinematic Viscosity: The ratio of the absolute viscosity of a liquid to its specific gravity at the temperature at which the viscosity is measured. Expressed in Stokes or Centistokes.

LHV: Lower Heating Value

Methane (CH_4): The simplest of the hydrocarbons and the principal constituent of natural gas. Pure methane has a heating value of 1,012 Btu per standard cubic foot.

Methanol (Methyl Alcohol, Wood Alcohol, CH_3OH): A liquid fuel formed by catalytically combining CO with hydrogen in a 1 to 2 ratio under high temperature and pressure. Commercially, it is typically manufactured by steam reforming natural gas. Also formed in the destructive distillation of wood. It is commonly used in biodiesel for its reactivity.

Methyl Alcohol: See Methanol.

MJ: Megajoule

MON: Motor Octane Number

Motor Octane: The octane as tested in a single-cylinder octane test engine at more severe operating conditions. Motor octane number (MON) affects high-speed and part-throttle knock and performance under load, passing, climbing, and other operating conditions. Motor octane is represented by the designation M in the (R+M)/2 equation and is the lower of the two numbers.

NO_x: Oxides of nitrogen

OECD: Organization of Economic Co-operation and Development

Octane Enhancer: Any substance such as MTBE, ETBE, toluene, or xylene that is added to gasoline to increase octane and reduce engine knock.

Octane Rating (Octane Number): A measure of a fuel's resistance to self-ignition, hence a measure as well of the antiknock properties of the fuel.

Olefins: Class of unsaturated paraffin hydrocarbons recovered from petroleum. Typical examples include: butene, ethylene and propylene.

Oxidation: Combining elemental compounds with oxygen to form a new compound. A part of the metabolic reaction.

Oxides of Nitrogen (NOx): Regulated air pollutants, primarily NO and NO_2 but including other substances in minute concentrations. Under the high pressure and temperature conditions in an engine, nitrogen and oxygen atoms in the air react to form various NOx. Like hydrocarbons, NOx are precursors to the formation of smog. They also contribute to the formation of acid rain.

Oxidizing agent: Any substance (oxygen, chlorine) that can accept electrons. When oxygen or chlorine is added to wastewater, organic substances are oxidized. These oxidized organic substances are more stable and less likely to give off odors or to contain disease bacteria.

Oxygenate: A term used in the petroleum industry to denote fuel additives containing hydrogen, carbon, and oxygen in their molecular structure. It includes ethers such as MTBE and ETBE and alcohols such as ethanol and methanol.

Ozone (O_3): Tropospheric ozone (smog) is formed when volatile organic compounds (VOCs), oxygen, and NOx react in the presence of sunlight (not to be confused with stratospheric ozone, which is found in the upper atmosphere and protects the earth from the sun's ultraviolet rays). Though beneficial in the upper atmosphere, ground-level ozone is a respiratory irritant and considered a pollutant.

Ozonation: The application of ozone to water, wastewater, or air, generally for the purposes of disinfection or odor control.

Petrochemical: An intermediate chemical derived from petroleum, hydrocarbon liquids or natural gas, such as: ethylene, propylene, benzene, toluene and xylene.

pH: pH is an expression of the intensity of the basic or acidic condition of a liquid. Mathematically, pH is the logarithm (base 10) of the reciprocal of the hydrogen ion concentration. The pH may range from 0 to 14, where 0 is most acidic, 14 most basic, and 7 is neutral. Natural waters usually have a pH between 6.5 and 8.5.

Phenol: An organic compound that is an alcohol derivative of benzene.

Polymer: A chemical formed by the union of many monomers (a molecule of low molecular weight). Polymers are used with other chemical coagulants to aid in binding small suspended particles to form larger chemical flocs for easier removal from water. All polyelectrolytes are polymers, but not all polymers are polyelectrolytes.

Polymerization: Process of combining two or more simple molecules of the same type, called monomers, to form a single molecule having the same elements in the same

proportion as in the original molecules, but having increased molecular weight. The product of the combination is a polymer.

Propane (C_3H_8): A gas whose molecules are composed of three carbon and eight hydrogen atoms. Propane is present in most natural gas in the United States, and is refined from crude petroleum. Propane contains about 2,500 Btu per standard cubic foot. Propane is the principal constituent in liquefied petroleum gas (LPG).

R&D: Research and development

Refinery: A plan used to separate the various components present in crude oil and convert them into usable products or feedstock for other processes.

Renewable Energy: Designated commodity or resource, such as solar energy, biodiesel fuel, or firewood, that is inexhaustible or replaceable by new growth.

Research Octane Number (RON): The octane as tested in a single-cylinder octane test engine operated under less severe operating conditions. RON affects low-to medium-speed knock and engine run-on.

Second generation biofuel: Biofuel which is not yet competitive and available on the fuel market. It is made from second generation feedstock, such as cellulosic materials and plants. An example is BtL fuel.

SHF: Separate hydrolysis and fermentation (technology for cellulosic ethanol production)

Sodium Hydroxide (Caustic Soda, Lye, NaOH): It is a metallic alkaline salt that is extremely corrosive and is used in Biodiesel production to make methoxide.

Soluble: Matter or compounds capable of dissolving into a solution.

Solvent: A substance, normally a liquid, which is capable of absorbing another liquid, gas, or solid to form a homogeneous mixture.

SO_x: Oxides of sulfur

SSF: Simultaneous saccarification and fermentation (technology for cellulosic ethanol production)

Sulfur (S): An element that is present in crude oil and natural gas as an impurity in the form of its various compounds.

Surfactant: Surface-active agent. It is the active agent in detergents that possesses a high cleaning ability.

Tax Incentives: In general, a means of employing the tax code to stimulate investment in or development of a socially desirable economic objective without direct expenditure from the budget of a given unit of government. Such incentives can take the form of tax exemptions or credits.

Toxic: A substance which is poisonous to a living organism.

Vapor Pressure or Volatility: The tendency of a liquid to pass into the vapor state at a given temperature. With automotive fuels, volatility is determined by measuring RVP.

Viscosity: Measure of the internal friction or resistance of an oil to flow. As the temperature of an oil is increased, its viscosity decreases and it is therefore able to flow more readily. Biodiesel is much less viscous than the oil from which it is made. Viscosity is measured on several different scales, including Redwood No. 1 at 100F, Engler Degrees, Saybolt Seconds, etc. The most common method for designation of viscosity is kinematic viscosity, measured in centistokes.

Volatile: A volatile substance is one that is capable of being evaporated or changed to a vapor at a relatively low temperature. Volatile substances also can be partially removed by air stripping.

Volatile Organic Compound (VOC): Reactive gas released during combustion or evaporation of fuel. VOCs are a major component of air pollution and react with NOx in the presence of sunlight and form ozone. A wide range of carbon-based molecules, such as aldehydes, ketones, and hydrocarbons are VOC's.

Volatility: Ability of a substance (gasoline) to turn from a liquid to a vapor. Low volatility refers to low RVP, indicating less light hydrocarbons in the gasoline front end.

General conversion units

Table 37: Conversion of temperature units

	Unit	Celsius	Kelvin	Fahrenheit
Celsius	°C		°C = K − 273.15	°C = (°F − 32) × 1.8
Kelvin	K	K = °C + 273.15		K = (°F + 459.67) × 1.8
Fahrenheit	°F	°F = °C × 1.8 + 32	°F = K × 1.8 – 459.67	

Table 38: Conversion of pressure units (pascal, bar, technical atmosphere, standard atmosphere, torr, pound per square inch)

	Pa	bar	at	atm	Torr	psi
1 Pa		0.00001	0.000010197	9.8692×10^{-6}	0.0075006	0.0001450377
1 bar	100,000		1.0197	0.98692	750.06	14.50377
1 at	98,066.5	0.980665		0.9678411	735.5592	14.22334
1 atm	101,325	1.01325	1.0332		760	14.69595
1 Torr	133.3224	0.001333224	0.001359551	0.001315789		0.01933678
1 psi	6894.8	0.068948	0.0703069	0.068046	51.71493	

Table 39: Metric prefixes

Prefix	Symbol	10^n
pico	p	10^{-12}
nano	n	10^{-9}
micro	µ	10^{-6}
deca	da	10^{1}
kilo	k	10^{3}
mega	M	10^{6}
giga	G	10^{9}
tera	P	10^{12}

Table 40: Conversion of energy units

	kJ	kcal	Wh	Btu
1 kJ		0.2388459	0.27777778	0.94781708
1 kcal	4.1868		1,163	3.96832054
1 Wh	3.6	0.85984523		3.41214148
1Btu	1.0550559	0.25199577	2.29307108	

Table 41: Conversion of volume units

	L	m^3	US gallons	in^3
1 L		0.001	0.26417205	61.02374409
1 m^3	1,000		264.17205	61.023,74409
1 US gallon	3.78541	0.00378541		231
1 in^3	0.01639	0.00001639	0.004329	

Table 42: Conversion factors for ethanol

Ethanol	kg	toe	GJ
1 L	0.789		0.02106
1 m^3		0.51	

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Annex I

Biofuels Digest, one of the leading online news titles in the bioenergy field, released the top rankings of the companies in bioenergy sector. The magazine's ranking was based on votes cast by a 100-person international panel (60%) and over 130,000 votes cast by readers of the online editions of Biofuels Digest and Renewable Chemicals Digest (40%). Innovations and achievements in developing bioenergy production played a major role in deciding how people ranked the over 1,000 companies involved.

Below is the list of top 100 companies in the bioenergy field. The BIOLYFE project partners Novozymes and Biochemtex were ranked at place four and fourteen respectively. Most of the companies on the Biofuels Digest list come from the US.

1. Solazyme
2. KiOR
3. LanzaTech
4. Novozymes
5. POET
6. DuPont Industrial Biosciences (Genencor)
7. Gevo
8. Sapphire Energy
9. Joule Unlimited
10. ZeaChem
11. Honeywell's UOP
12. BP Biofuels
13. LS9
14. Biochemtex / Beta Renewables
15. Amyris
16. VIrent
17. INEOS Bio
18. Enerkem
19. Abengoa Bioenergy
20. Ceres
21. Neste Oil
22. Renmatix
23. Coskata
24. DSM
25. Mascoma
26. Virdia
27. Codexis
28. Butamax
29. Waste Management
30. Petrobras
31. Elevance Renewable Sciences
32. Cobalt Technologies
33. Inbicon
34. Dynamic Fuels
35. Algenol
36. Fulcrum BioEnergy
37. Valero
38. EdeniQ
39. Rentech
40. Boeing
41. OriginOil
42. SG Biofuels
43. Cosan
44. Ensyn
45. Renewable Energy Group
46. Dyadic

47. OPX Biotechnologies
48. Bluefire Renewables
49. Catchlight Energy
50. Fiberight
51. Phycal
52. BioProcess Algae
53. Raizen
54. Cool Planet
55. Aurora Algae
56. Iogen
57. Sweetwater Energy
58. Blue Sugars
59. Propel Fuels
60. Mendel Biotechnologies
61. Syngenta
62. Chromatin
63. Green Plains Renewable Energy
64. Genomatica
65. NexSteppe
66. Solix
67. Green Biologics
68. Sud-Chemie
69. Graal Bio
70. Glycos Biotechnologies
71. BioArchitecture Lab (BAL)
72. Aemetis
73. Praj Industries
74. Primus Green Energy
75. TMO Renewables
76. Sundrop Fuels
77. American Process
78. Algae.Tec
79. Albemarle
80. Borregaard Industries
81. Imperium Renewables
82. Comet Biorefining
83. Proterro
84. Chemrec
85. Lignol
86. Cargill
87. Clariant Produckte
88. ThermoChem Recovery International (TRI)
89. Genera Energy
90. Biogasol
91. Heliae
92. Parabel
93. Great Plains – the Camelina Company
94. Roundtable on Sustainable Biofuels Services Foundation
95. Agrisoma
96. Direvo Industrial Biotechnology
97. Mintz Levin
98. Bunge
99. DOD
100. Algix