

CHAPTER II

LITERATURE REVIEW

2.1 Energy in Thailand

2.1.1 Energy Consumption in Thailand

Thailand is a net energy importer, chiefly because of oil, with imports worth 900 billion baht a year, while energy demand is rising by 4% annually (DEDE, 2012). For this reason, the government has to implement the policy and measures to promote the continuously increase of alternative energy consumption comprising of solar, wind, small hydro power, biomass, biogas, municipal solid waste (MSW) and biofuels (ethanol and biodiesel) to replace fossil energy including the policy on energy efficiency to improve energy efficiency in all economic sectors by decreasing of energy intensity.

In 2012, Thailand's final energy consumption has increased 3.9% from the previous year whereas final alternative energy consumption has grown 7.6% and energy intensity has decreased 2.4%. Final total energy consumption as shown in Table 2.1, final energy consumption by fuels and economic sectors as shown in Figure 2.1 and Figure 2.2 respectively (DEDE, 2012).

Table 2.1 Final total energy consumption in Thailand 2012

unit : ktoe

Final Energy Consumption	Agriculture	Industry**	Residential	Commercial	Transportation	Total
Coal & its Products	-	5,794	-	-	-	5,794
Petroleum products	3,765	4,070	2,161	1,195	23,996	35,187
Natural Gas	-	2,884	-	2	2,228	5,114
Electricity*	25	7,742	2,735	4,102	6	14,610
Renewable Energy	-	4,882	-	4	-	4,886
Tradition Renewable Energy	-	1,538	6,187	-	-	7,725
Total	3,790	26,910	11,083	5,303	26,230	73,316

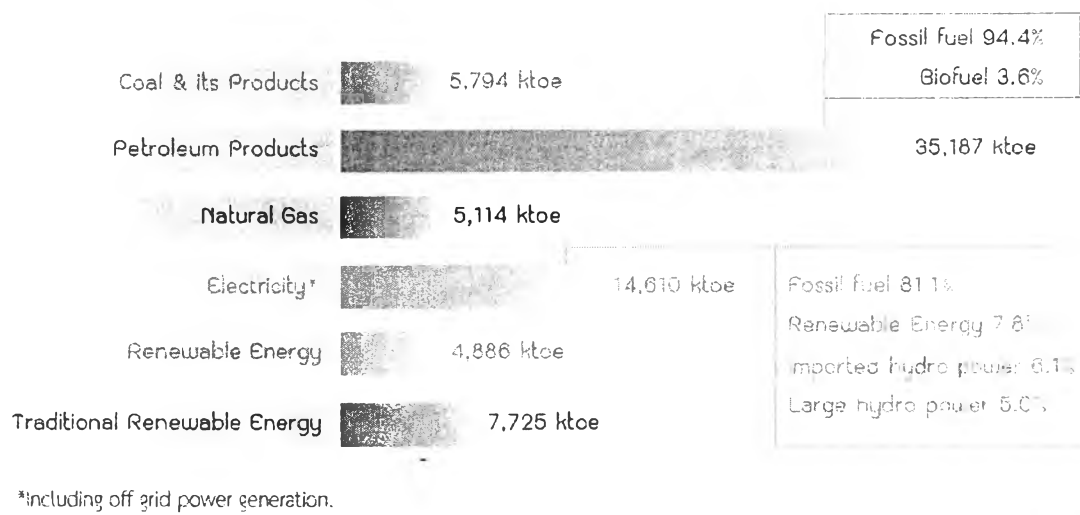


Figure 2.1 Final energy consumption by fuels in Thailand 2012.

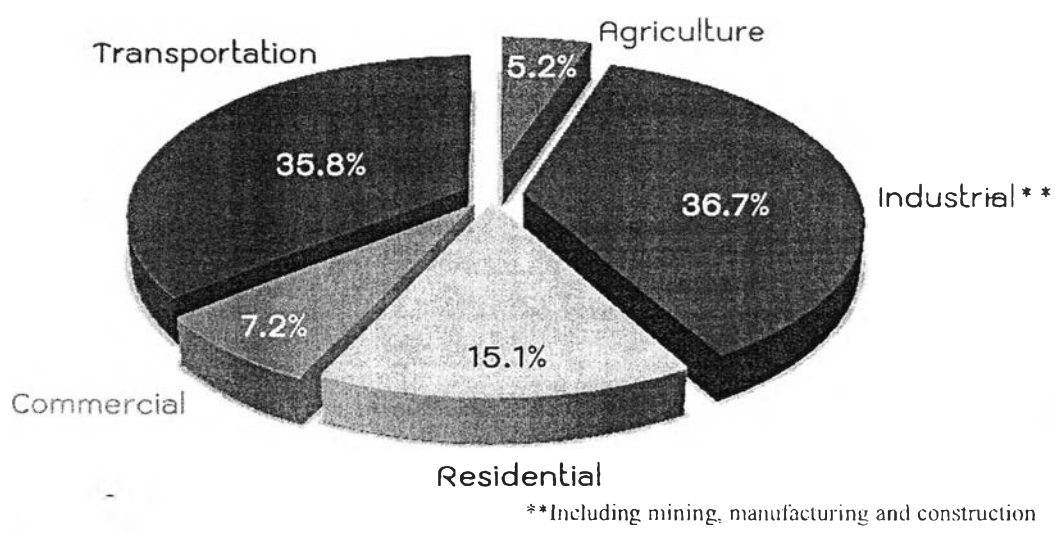


Figure 2.2 Final energy consumption by economic sectors in Thailand 2012.

2.1.2 Petroleum in Thailand

Thailand consumed an estimated 1 million bbl/day of oil in 2011, leaving total net imports of 627,000 bbl/day (EIA, 2013), and making the country the second largest net oil importer in Southeast Asia as shown in Figure 2.3. Thailand is a net importer of crude oil and a net exporter of petroleum products. The country imports over 60% of its total petroleum needs and almost 85% of its crude oil consumption, leaving Thailand highly dependent on global oil markets and volatile

prices. About 78% of its crude imports originate from the Middle East, while another 8% are from other Asian suppliers. The country's oil import dependency has spurred the government to promote the use of other fuels such as natural gas, renewable sources, and biofuels as well as to boost crude oil and product stocks and to encourage investment in marginal field production. Thailand's oil products consist primarily of diesel, liquefied petroleum gas (LPG), and naphtha as these fuels feed the transportation, petrochemical, and other industrials, and residential sectors.

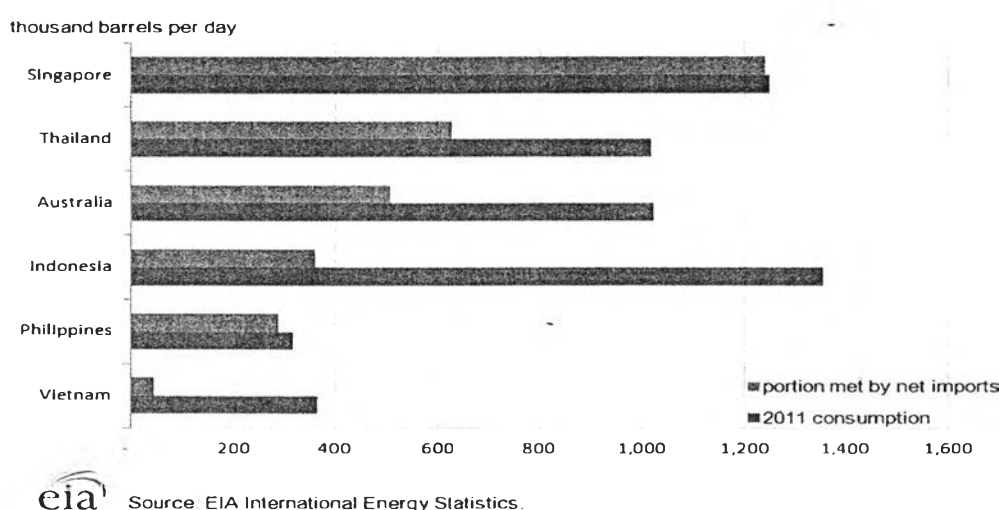


Figure 2.3 Top Southeast Asian net oil importer in 2011.

Average crude oil prices in 2012 were at historically high levels for the second year in a row. Brent crude oil averaged 111.67 \$/barrel, slightly above the 2011 average of \$111.26. West Texas Intermediate oil averaged 94.05 \$/barrel in 2012, down slightly from \$94.88 in 2011 as shown in Figure 2.4.

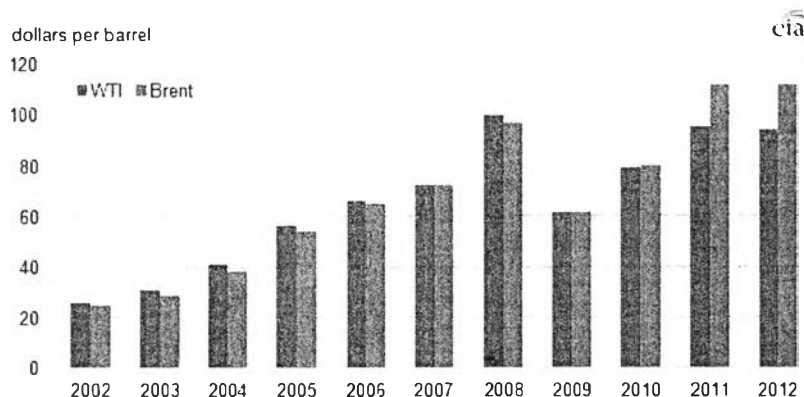


Figure 2.4 Average annual spot price for Brent and WTI crude oil in 2002-2012.

2.1.3 Alternative and Renewable Energy

Thailand final alternative energy consumption has increased continuously, due to the policy of alternative energy development is targeted to up more alternative energy consumption in all sectors, especially alternative energy which can be produced in the country comprising solar energy, wind energy, small hydro power, biomass, garbage, biogas and biofuels (bioethanol and biodiesel). To consume more alternative energy, to affect the decrease of fossil energy consumption and energy imports. Thailand consumes domestic alternative energy in the form of electricity, heat and biofuels, as well as domestic natural gas for vehicles (NGV). Alternative and renewable energy shares of Thailand final energy consumption in 2012 as shown in Figure 2.5 (DEDE, 2012).

Geographically situated in the equator, Thailand also has great potential in solar with average radiation of 18.2 MJ/m²/day; and in some areas with wind speed potential. Such abundant alternative energy sources certainly make Thailand the best potential on alternative energy development and create opportunity to strengthen energy security in the future. The government of Thailand has currently assigned the Ministry of Energy to set up the 10 year Alternative Energy Development Plan (AEDP) aiming to create the framework and direction for increasing alternative energy consumption by 25% in 2021. Performance on alternative and renewable energy policy is shown in Table 2.2 (DEDE, 2012).

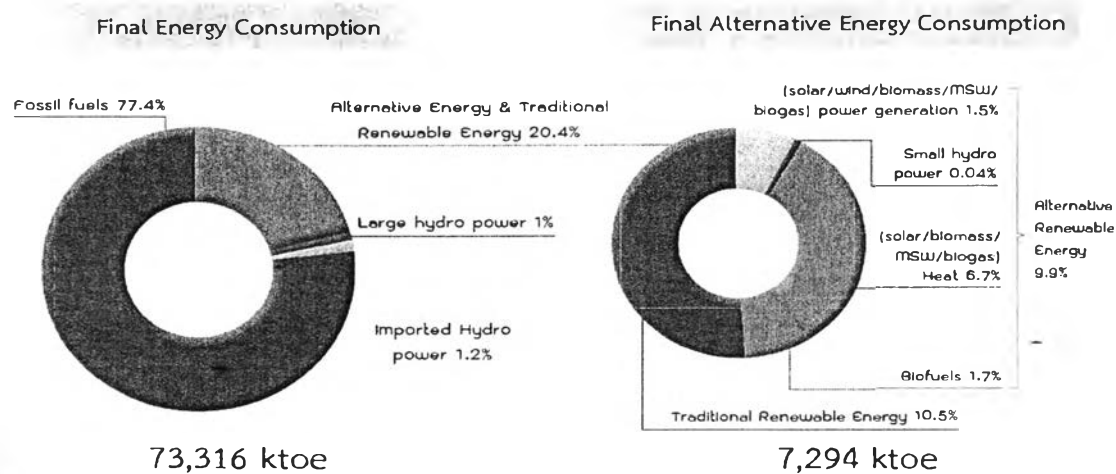


Figure 2.5 Alternative and renewable energy shares of Thailand final energy consumption in 2012.

Table 2.2 Performance on alternative and renewable energy policy in Thailand

TYPES OF ENERGY	unit	Target 2021	As of 2012
Electricity	MW	9,201	2,786
	ktoe		1,138
Solar	MW	2,000	376.72
Wind	MW	1,200	111.73
Small Hydro Power	MW	1,608	101.75
Biomass	MW	3,630	1,959.95
Biogas	MW	600	193.40
MSW	MW	160	42.72
New energy	MW	3	-
Heat	ktoe	9,335	4,886
Solar	ktoe	100	4
Biomass	ktoe	8,200	4,346
Biogas	ktoe	1,000	458
MSW	ktoe	35	78
Biofuels	million litres / day	39.97	3.5
	ktoe		1,270
Ethanol	million litres / day	9	1.4
Biodiesel	million litres / day	5.97	2.7
New Energy Replacing Diesel	million litres / day	25	-
%AE		25%	9.9%

2.2 Biofuels

2.2.1 Overview Biofuels

The biofuels produced from the renewable resources could help to minimize the fossil fuel burning and CO₂ production. Biofuels produced from biomass such as plants or organic waste could help to reduce both the world's dependence on oil. These biofuels have the potential to cut CO₂ emission because plants they are made from use CO₂ as they grow. Biofuels and bio-products produced from plant biomass would mitigate global warming. This may due to the CO₂ released in burning equals the CO₂ tied up by the plant during photosynthesis and thus does not increase the net CO₂ in the atmosphere.

Biofuels are fuels developed from organic matter, most commonly from forestry and agricultural products. The two main types of liquid biofuels in use are bioethanol and biodiesel. Bioethanol is used in gasoline engines and is derived from grains and sugar crops, whereas biodiesel is used in diesel engines and is derived from oil producing crops, such as oil palm and rapeseed. Another distinction in types of biofuels is between first generation biofuels and advanced biofuels (sometimes referred to as second or third generation). The biofuel production pathways for various biomass feedstocks as shown in Figure 2.6.

First generation biofuels come from agricultural crops and processes such as corn, sugarcane, cassava, palm oil, etc. It can offer some CO₂ benefits and can help to improve domestic energy security. First generation biofuels are characterized either by its ability to be blended with petroleum-based fuels, combusted in existing internal combustion engines, and distributed through existing infrastructure, or by the use in existing alternative vehicle technology like flexible fuel vehicle (FFVs) or natural gas vehicles. However, the main disadvantages of first generation biofuels are biodiversity and land use and competition with food crops.

Second generation biofuels come from non-food crops such as trees and grasses or agricultural residues such as rice straw, corn stover, sugarcane bagasse, cassava rhizome, etc. Production processes for second generation biofuels vary from laboratory scale to commercial scale pilots. Although second generation biofuels are promising for future biofuel production, at present the production processes need to be

validated at commercial scale. Many advantages and disadvantages of the first generation biofuels and obvious advantages of second generation biofuels as shown in Figure 2.7, the approaches to integral utilization of biomass for sustainable development are more reasonable, where all parts of the plant such as leaves, stalk, fruits, rhizome, bagasse and seeds can be utilized to useful products.

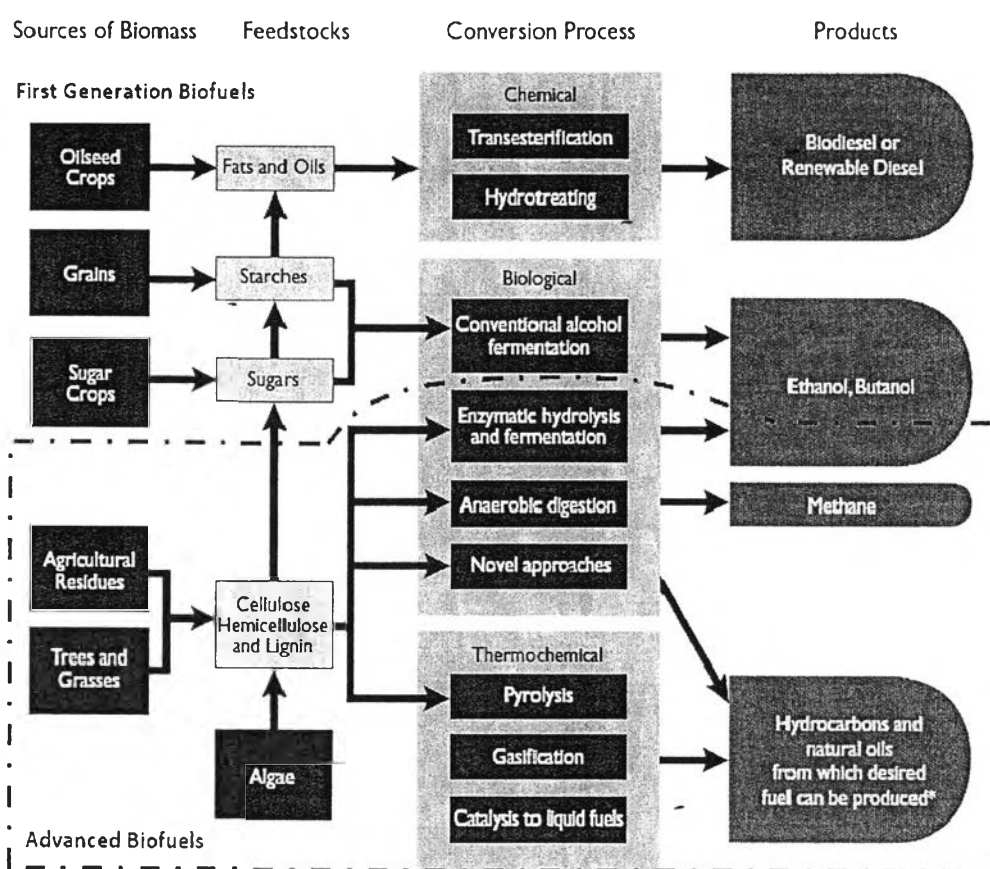


Figure 2.6 Pathways for biofuel production from various biomass feedstocks (Source: Pena and Sheehan, 2007).

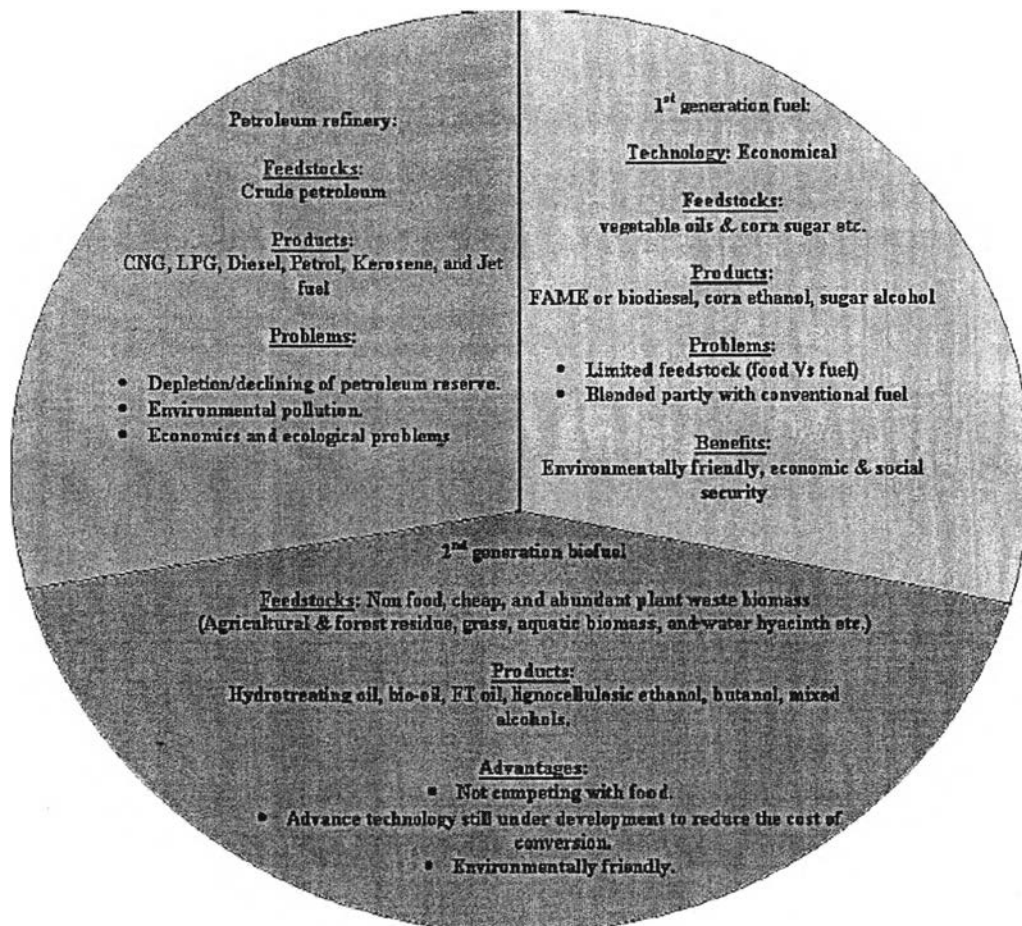


Figure 2.7 Comparison of first, second generation biofuel and petroleum fuel (Source: Naik *et al.*, 2010).

2.2.2 Biofuels Situation in Thailand

Thailand is the leading producer of biofuels in Southeast Asia and third only to China and Indonesia in Asia. The government intends to move away from crude oil dependency, particularly in the transportation sector, and the Department of Alternative Energy Development and Efficiency (DEDE) has actively promoted the use of alternative fuels such as compressed natural gas, liquefied petroleum gas, biodiesel, and bioethanol. The biofuels market in Thailand has grown substantially since 2004 when global oil prices began escalating. Thailand's key biofuels are ethanol from molasses and cassava, and biodiesel from palm oil plants. The Thai government currently subsidizes gasohol consumption through its State Oil Fund and approved the

phasing out of Octane 91 regular gasoline in favor of ethanol blends in gasoline in 2012. Likewise, on the biodiesel front, the government is introducing pilot projects for various biodiesel blends for trucks and boats and expanding production of palm oil yields.

Although the portion of biofuels is a fraction of total oil production, Thailand's biofuels output was almost 20,000 bbl/day in 2011, rising from a mere 2,400 bbl/day in 2006. In 2011, ethanol consumption rose to 8,960 bbl/day and has increased about four-fold in the past five years. The domestic consumption was 6,375 bbl/day, and as part of Thailand's new 10-Year Alternative Development Plan in 2012-2021, the country anticipates consumption climbing to 56,600 bbl/day by 2021. Thailand's ethanol exports to regional sources accounted for about 27 percent of production and jumped dramatically in 2011 as the Philippines and Singapore imported more for gas blending. Thailand anticipates exports to increase to regional markets and plans to designate some export-only-ethanol plants.

Thailand is the world's third largest palm oil producer and a leading biodiesel consumer, using nearly 11,000 bbl/day in 2011. All of the country's production feeds consumption, and the government restricts all exports of biodiesel products. Thailand's new alternative energy development plan increased the biodiesel consumption target to 37,550 bbl/day by 2021. A new mandate on B5 biodiesel blend was installed in 2012, and biodiesel intake is likely to increase in the next few years. However, the government prioritizes palm oil for food over fuel use, and biodiesel production will be subject to these demands as well as the ability to boost crops.

2.2.3 Bioethanol in Thailand

In 2012, the number of operating ethanol plants will likely increase to 21 plants with total production capacity of 3.715 million liters/day, up from 19 plants with production capacity of 3.065 million liters/day in the previous year as shown in Table 2.3. The new ethanol plants will be cassava-based plants. There are six new cassava-based ethanol plants due (Source: <http://gain.fas.usda.gov>). The government plan to terminate Octane 91 regular gasoline sales and shift it to gasohol by October 1, 2012.

For ethanol consumption, E10 Octane 91 gasohol consumption increased to 660 million liters (5.5 million liters/day), up 8.2 percent from the previous

year. Also, E20 gasohol consumption increased to 90 million liters (0.7 million liters/day), up 32.4 percent from the previous year due to the government price subsidy for E20 gasohol from the State Oil Fund, causing E20 gasohol to be cheaper than regular gasoline by 17.4 percent. Consumption of E20 gasohol accounted for approximately 7.0 percent of total gasohol consumption, up from around 5.0 percent in the previous year.

Table 2.3 Ethanol plants in Thailand (Source: <http://www.dede.go.th>)

Ethanol plants by feedstocks	Registered plants		Under-construction plants ¹		On-line plants ²	
	Number of plants	Capacity (Million liters/day)	Number of plants	Capacity (Million liters/day)	Number of plants	Capacity (Million liters/day)
Sugarcane (S)	1	0.20	-	-	1	0.20
Molasses (M)	15	2.69	-	-	5	0.78
Cassava (C)	24	8.39	6	2.22	5	0.78
(M)/(S)/(C)	8	1.23	-	-	8	1.31
Total	48	12.51	6	2.22	19	3.07

1= As of February 2012, 2= As of November 2011.

2.3 Biomass, Lignocellulosic Biomass and Agricultural Residues

2.3.1 Biomass

2.3.1.1 Biomass as Multiple Feedstocks for Biorefinery

Biomass is harvested as part of a constantly replenished crop. This maintains a closed carbon cycle with no net increase in atmospheric CO₂ levels. There are five basic categories of material, that is, virgin wood, forestry materials, materials from arboricultural activities or from wood processing; energy crops: high-yield crops grown specifically for energy applications; agricultural residues: residues from agriculture harvesting or processing; food waste, from food and drink manufacture, preparation and processing, and postconsumer waste; industrial waste and coproducts from manufacturing and industrial processes.

Feedstocks that are used directly in a manner that is given to us by nature fall under the category of natural feedstocks. The first generation biofuels use the edible biomass for producing biofuels. Some of them are sunflower seeds, jojoba oil, soy bean oil, safflower seeds for biodiesel production, and corn, sugarcane and cassava for producing bioethanol. In contrast, the second-generation biofuels are produced from nonedible feedstocks like lignocellulosic biomass which include agricultural residues (stalk, husk, stover, bagasse), forest residues (branch, twigs, bark, leaves), and several others. The important renewable feedstocks for biorefinery industry show in Figure 2.8.

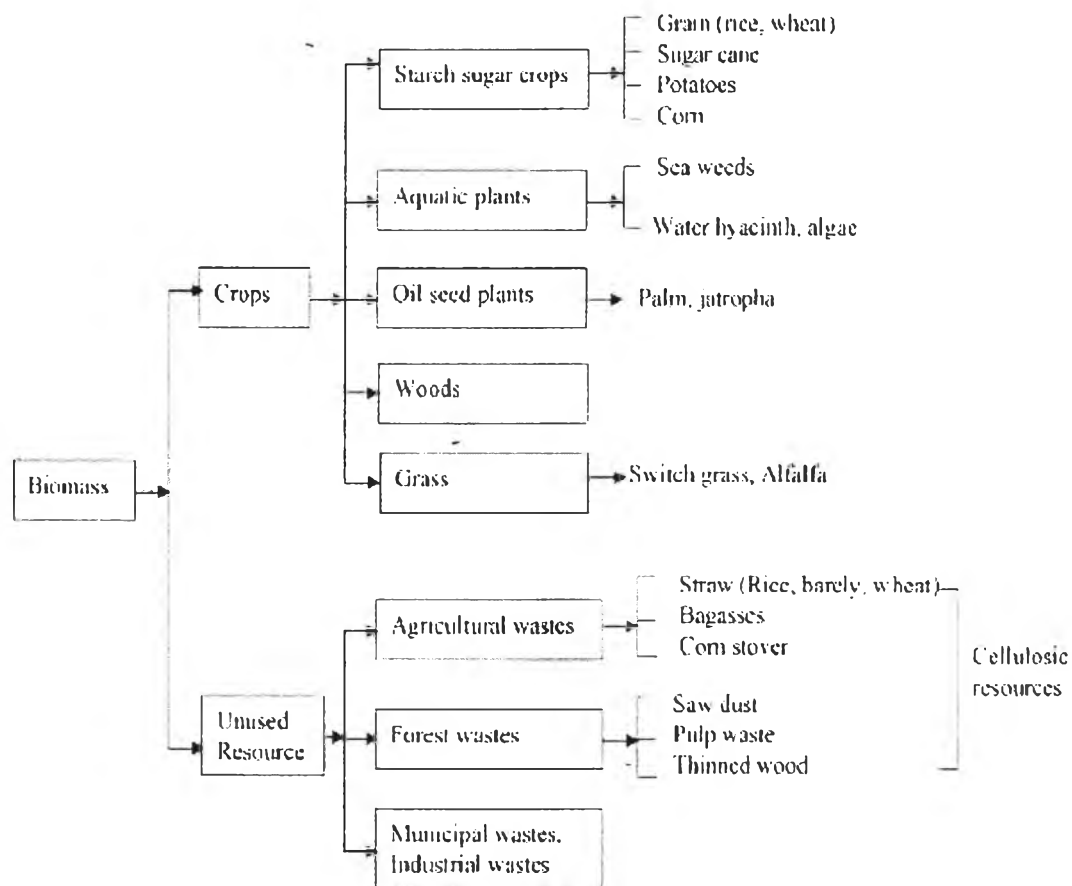


Figure 2.8 Biomass as renewable feedstock for biorefineries (Naik *et al.*, 2010).

2.3.1.2 Biorefinery System

The sustainable use of bio-based carbon suggests integrated manufacturing in biorefineries to selectively transform the variety of molecular structures available in biomass into a range of products including transportation biofuels, chemicals, polymers, pharmaceuticals, pulp and paper, food, or cattle feed.

The biorefinery concept embraces a wide range of technologies able to separate biomass resources (wood, sugarcane, corn, etc.) into their building blocks (carbohydrates, proteins, fats, etc.) which can be converted to value-added products, biofuels, and chemicals. A biorefinery is a facility (or network of facilities) that integrates biomass conversion processes and equipment to produce transportation biofuels, power, and chemicals from biomass. Figure 2.9 gives an overview of the possible conversion pathways to produce the desired energy and material products from different biomass feedstocks, through jointly applied technological processes (Cherubini *et al.*, 2009). The biorefinery concept is analogous to today's petroleum refinery, which produces multiple fuels and products from petroleum.

Biomass is constituted of an enormous variety of plant species with varying morphology and chemical composition. However, regardless of the phenotype, five main biomass components can be identified worldwide: lipids, starch, cellulose, hemicelluloses, lignin, and proteins. The average biomass available in the world is reported in Figure 2.10 (Cherubini and Strømman 2011). It clearly appears that lignocellulosic biomass components such as cellulose, hemicelluloses, and lignin are by far the most abundant. Since they can be even gathered from waste streams (e.g., crop residues, paper and wood industries), or directly harvested from forests or biomass stands through sustainable management, their price tend to be lower than other biomass sources which need a dedicated agricultural plot. For this reason, this chapter has a special focus on the possibility to produce commodity chemicals from lignocellulosic sources, which have the largest chances for a massive market penetration in the near future.

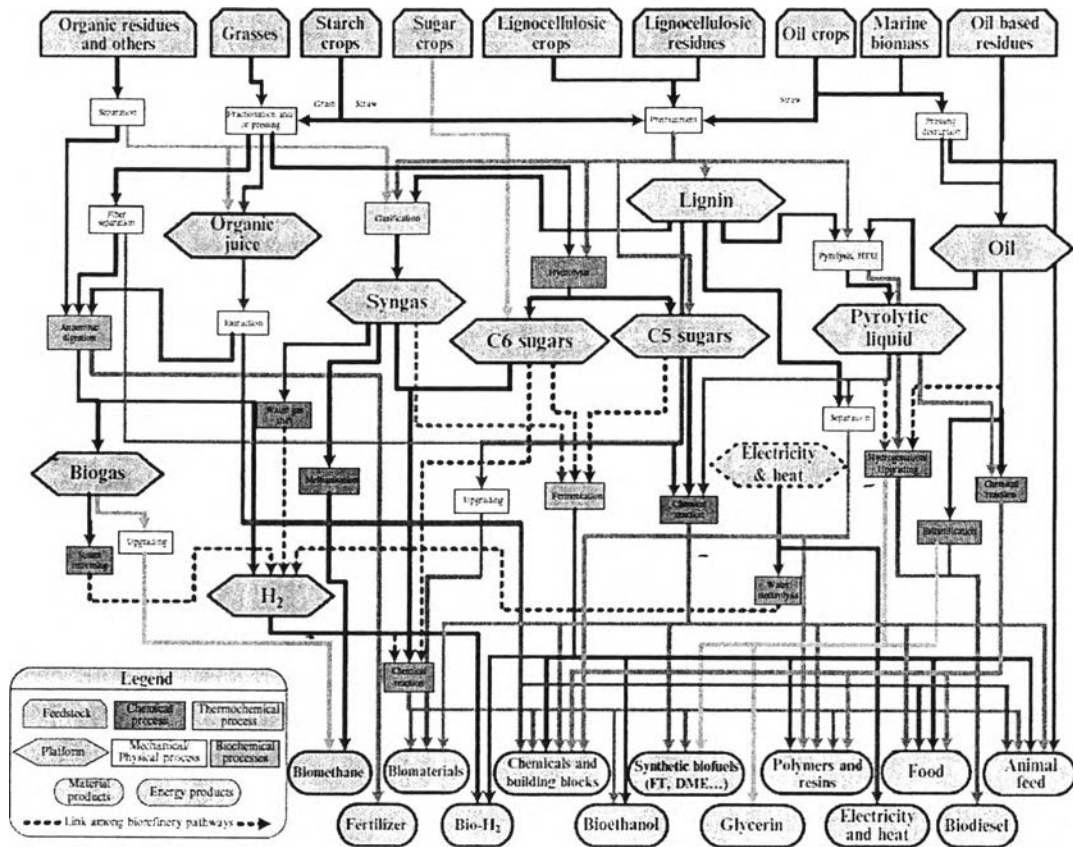


Figure 2.9 Main conversion routes for production of biofuels, energy, and chemical from different biomass sources (Cherubini *et al.*, 2009).

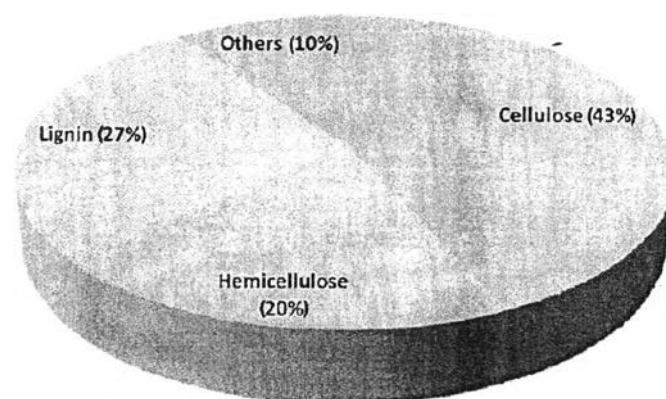


Figure 2.10 World average composition of the above ground standing biomass (Cherubini and Strømman 2011).

2.3.2 Lignocellulosic Biomass

2.3.2.1 *Composition of Lignocellulosic Biomass*

It consists of three major components, that is, cellulose, hemicellulose, and lignin:

i) Cellulose: It contains linear polysaccharides in the cell walls of wood fibers, consisting of D-glucose molecules bound together by β -1,4-glycoside linkages. Biomass comprises 40-50% cellulose.

ii) Hemicellulose: It is an amorphous and heterogeneous group of branched polysaccharides (copolymer of any of the monomers of glucose, galactose, mannose, xylose, arabinose, and glucuronic acid). Hemicellulose surrounds the cellulose fibers and is a linkage between cellulose and lignin (15-30%). Hemicelluloses are heterogeneous polymers of pentoses (e.g., xylose, and arabinose), hexoses (e.g., mannose, glucose and galactose), and sugar acids.

iii) Lignin: It is a highly complex three-dimensional polymer of different phenylpropane units bound together by ether ($C_{22}O$) and carbon-carbon ($C_{22}C$) bonds. Lignin is concentrated between the outer layers of the fibers, leading to structural rigidity and holding the fibers of polysaccharides together (15-30%). Generally, softwoods contain more lignin than hardwoods. Lignins are divided into two classes, namely, guaiacyl lignins and guaiacyl-syringyl lignins. Although the principal structural elements in lignin have been largely clarified, many aspects of their chemistry remain unclear. The contents of cellulose, hemicelluloses, and lignin in common lignocellulosic materials are shown in Table 2.4.

Table 2.4 Contents of cellulose, hemicellulose, and lignin based lignocellulosic materials

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Rice straw ^[Yoswathana <i>et al.</i>, 2010]	39.50	23.00	12.90
Corn stover ^[Chaiklangmuang <i>et al.</i>, 2011]	48.93	20.46	30.61
Cassava rhizome ^[Pattiya <i>et al.</i>, 2010]	29.93	42.73	23.36
Sugarcane bagasse ^[Buaban <i>et al.</i>, 2010]	45.86	28.84	23.35
Corn Cobs ^[Wongkasemjit <i>et al.</i>, 2012]	41.27	46.00	7.40
Oil palm fronds ^[Wanrosli <i>et al.</i>, 2007]	47	35	15
Oil palm EFB ^[Alriols <i>et al.</i>, 2009]	37	24	24

2.3.2.2 Lignocellulose Biomass Structure

Lignocellulose is by far the major component of biomass, comprising around half of the plant matter produced by photosynthesis and representing the most abundant renewable organic resource in soil. It consists of three types of polymers, cellulose, hemicellulose, and lignin, that are strongly intermeshed and chemically bonded by non-covalent forces and by covalent cross-linkages (Pe´rez *et al.*, 2002; Sanchez, 2009). Compared with starchy biomass, it is considered as a quite recalcitrant material due to its highly lignified and crystalline structure.

Cellulose and hemicellulose are macromolecules constructed from different sugars, whereas lignin is an aromatic polymer synthesized from phenylpropanoid precursors (Sanchez, 2009).

Cellulose is a linear polymer that is composed of D-glucose subunits linked by b-1,4 glycosidic bonds forming the dimer cellobiose. These form long chains (or elemental fibrils) linked together by hydrogen bonds and intra- and intermolecular van der Waals forces. This polymer usually is present as a crystalline form and a small amount of nonorganized cellulose chains forms amorphous cellulose. In the latter conformation, cellulose is more susceptible to enzymatic degradation (Pe´rez *et al.*, 2002). Cellulose appears in nature to be associated with other plant compounds, mainly lignin, and this association may affect its biodegradation.

Hemicellulose is a polysaccharide with a lower molecular weight than cellulose. It is formed from D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, 4-O-methyl-glucuronic, D-galacturonic, and D-glucuronic acids, depending on the hemicellulose source. Sugars are linked together by β -1,4- and sometimes by β -1,3-glycosidic bonds.

Lignin is present in the cellular wall to give structural support, impermeability, and resistance against microbial attack and oxidative stress. It is an amorphous heteropolymer, non water-soluble, and optically inactive that is formed from phenylpropane units joined together by nonhydrolyzable linkages. This polymer is synthesized by the generation of free radicals, which are released in the peroxidase-mediated dehydrogenation of three phenyl propionic alcohols: coniferyl alcohol (guaiacyl propanol), coumaryl alcohol (p-hydroxyphenyl propanol), and sinapyl alcohol (syringyl propanol). This heterogeneous structure is linked by C₂₂C and aryl ether linkages, with aryl-glycerol β -aryl ether being the predominant structure (Sanchez, 2009). Diagrammatic illustration of the framework of lignocellulose is shown in Figure 2.11.

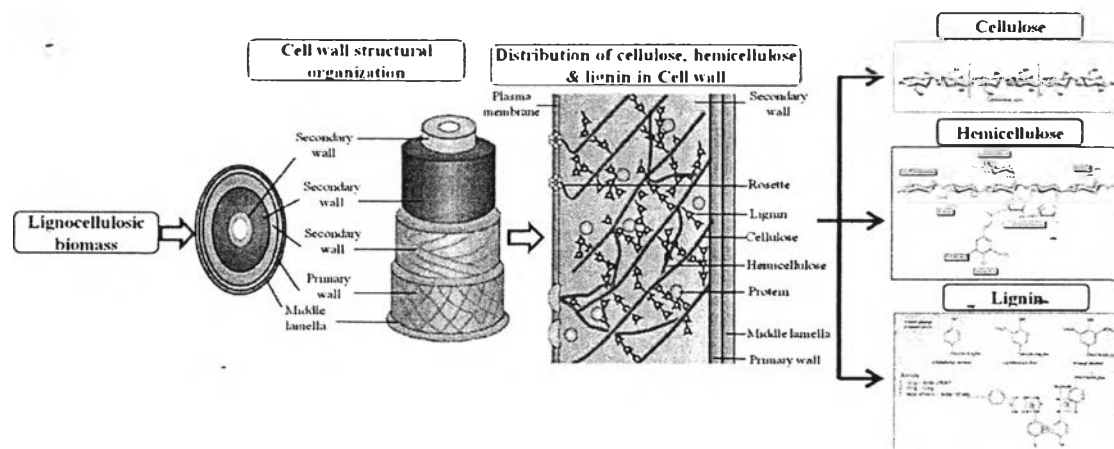


Figure 2.11 Diagrammatic illustration of the framework of lignocelluloses (Menon and Rao, 2012).

2.3.3 Potential of Agricultural Residues in Thailand

Thailand is agricultural country and has a lot potential of agricultural goods and also the country is one of the leading producers and exporters in the world market. Each year the country has produces a lot of agricultural products such as rice, cassava, sugar cane and corn. In the same time a lot of agricultural residues also produce. The country total agricultural residues has more than 60 million ton per year. Focusing on agricultural residues, it is a big challenge for change that residues to ethanol especially from rice, sugarcane, and cassava. All of them is the major crops and release a lot of residues. The quantities of agricultural residues are calculated from agricultural product multiply by crops to residue ratio (CRR), this result is call total agricultural residues. However, some of agricultural residues are used in other activity for example fodder for animal. Therefore, the potential of agricultural residues that use for energy (Available unused residues) are calculated from total agricultural residues multiply by surplus available factor (SAF). These two ratios are conversion factor. The potential of agricultural residues in Thailand is shown in Table 2.5.

Table 2.5 Energy potential of agricultural residues in Thailand 2011

(Source: <http://www.dede.go.th>)

Products	Productions (Tons)	Residues	Available unused residues for energy (Tons)	Price of residues (\$/kg)
Cassava	21,912,400	Rhizome	1,335,999.02	0.0167 ^a
Corn	4,816,650	Stover	2,878,622.71	0.0300 ^b
Sugarcane	95,950,400	Bagasse	6,018,105.04	0.0100 ^c

a: Jongpluenmpiti and Tangchaichit, 2012; b: http://www.dld.go.th/nspk_pkk/ac01.html; c: Pattaratierasakul 2010

1 dollar = 30 baht.

2.4 Bioethanol Production from Lignocellulosic Biomass

For large-scale biological production of fuel ethanol, it is desirable to use cheaper and more abundant substrates. Lignocellulosic biomass is considered as an attractive feedstock fuel ethanol production because of its availability in large quantities at low cost and its reduced competition with food but not necessarily with feed. To introduce ethanol as a large-scale transportation fuel, the production cost must be lowered to about the same level as oil and diesel. Generic block diagram of bioethanol production from lignocellulose materials is shown in Figure 2.12 .

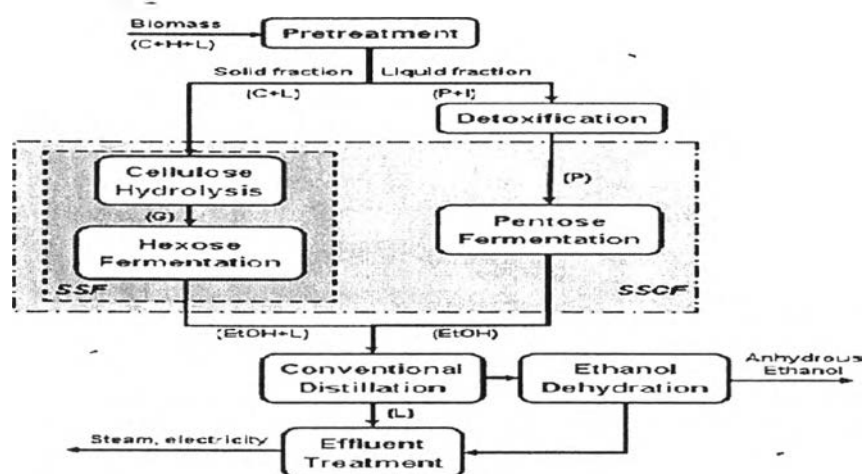


Figure 2.12 Generic block diagram of bioethanol production from lignocellulose biomass (Cardona Alzate and Sánchez Toro 2006).

2.4.1 Pretreatment Technologies for Lignocellulosic Biomass

Lignocellulosic biomass, consisting mainly of cellulose, hemicellulose, and lignin, need to be hydrolyzed to monomeric sugars before being utilized by fermenting microorganisms. The preceding hydrolysis step can be performed through acid or enzymatic catalysts. In general, bioethanol production processes from lignocellulose based on enzymatic hydrolysis offer many more advantages than processes employing acids. While acid hydrolysis requires relatively high temperatures and implies corrosive operating conditions and generation of toxic compounds, enzymatic hydrolysis is advantageous due to its higher conversion

efficiency and lower process energy requirements. However, many physicochemical, structural, and compositional factors make the native lignocellulosic biomass recalcitrant and difficult to hydrolyze by enzymes because the enzymatic digestibility of native cellulose in biomass is usually less than 20% unless extremely excessive enzyme dose is used. Thus, a previous pretreatment step is necessary to overcome these drawbacks and perform an efficient enzymatic hydrolysis. The aim of the pretreatment is to break down the lignin structure and disrupt the crystalline structure of cellulose to increase enzyme accessibility (Mosier *et al.*, 2005). The mechanism for making the cellulose more accessible to enzymes depends on the pretreatment employed and nature of the raw material.

Pretreatment is an important tool for practical cellulose conversion processes, and is the subject of this article. Pretreatment is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars as represented in the schematic diagram of Figure 2.13.

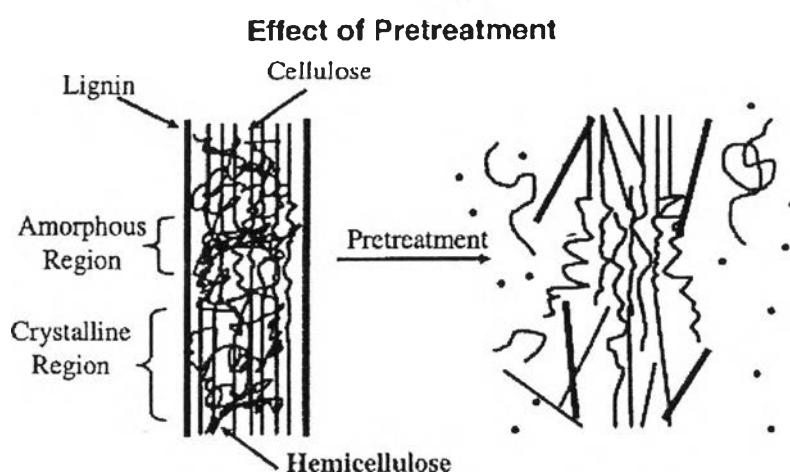


Figure 2.13 Schematic of goals of pretreatment on lignocellulosic biomass (adapted from Hsu *et al.*, 1980).

In principle, an effective pretreatment is characterized by several criteria: high yields from multiple crops, site ages and harvesting times, solid fraction

highly digestible, no requirement of size reduction, preserving hemicellulose fractions, limiting formation of inhibitors due to degradation products, minimizing heat and power requirements, and being cost-effective. Except for these criteria, several other factors are also needed to be considered, including recovery of high value-added co-products (e.g., lignin and protein), pretreatment catalyst, catalyst recycling, and waste treatment. When comparing various pretreatment options, all the mentioned criteria should be comprehensively considered as a basis.

During the last decades, a large number of diverse pretreatment technologies have been suggested. Those methods are usually classified into physical, physicochemical, chemical, and biological pretreatments.

2.4.1.1 Physical Pretreatments

- *Mechanical Comminution*

Milling (cutting the lignocellulosic biomass into smaller pieces) is a mechanical pretreatment of the lignocellulosic biomass. The objective of a mechanical pretreatment is a reduction of particle size and crystallinity. The reduction in particle size leads to an increase of available specific surface and a reduction of the degree of polymerization (DP). The increase in specific surface area, reduction of DP, and the shearing are all factors that increase the total hydrolysis yield of the lignocellulose in most cases by 5-25% (depends on kind of biomass, kind of milling, and duration of the milling), but also reduces the technical digestion time by 23-59% (thus an increase in hydrolysis rate) (Chang and Holtzapfel, 2000) such as ball milling have been integrated in SSF processes for ethanol production from sugarcane bagasse with *Pichia stipitis* (Buaban, Inoue *et al.*, 2010). As no inhibitors (like furfural and HMF (hydroxymethylfurfural)) are produced, milling is suited for ethanol production. It has, however, a high-energy requirement and was found therefore not economically feasible as pretreatment. Taking into account the high-energy requirements of milling and the continuous rise of the energy prices, it is likely that milling is still not economically feasible.

- *Extrusion*

Extrusion process is a novel and promising physical pretreatment method for biomass conversion to ethanol production. In extrusion, the materials are subjected to heating, mixing and shearing, resulting in physical and

chemical modifications during the passage through the extruder. Screw speed and barrel temperature are believed to disrupt the lignocellulose structure causing defibrillation, fibrillation and shortening of the fibers, and, in the end, increasing accessibility of carbohydrates to enzymatic attack. In recent studies application of enzymes during extrusion process is being considered as a promising technology for ethanol production. It has been recently employed for increasing the enzymatic hydrolysis yields of corn stover (Karunanithy and Muthukumarappan, 2010b).

2.4.1.2 Physicochemical Pretreatments

- *Steam Pretreatment/Steam Explosion*

During steam pretreatment, the biomass is put in a large vessel and steamed at a high temperature, (temperatures up to 240 °C) and pressure is applied for a few minutes. After a set time, the steam is released and the biomass is quickly cooled. The objective of a steam pretreatment/steam explosion is to solubilize the hemicellulose and then to make the cellulose better accessible for enzymatic hydrolysis while formation of inhibitors is avoided. The difference between “steam” pretreatment and “steam explosion” pretreatment is the quick depressurization and cooling down of the biomass at the end of the steam explosion pretreatment, which causes the water in the biomass to “explode.” During steam pretreatment, parts of the hemicellulose hydrolyze and form acids, which could catalyze the further hydrolysis of the hemicellulose. However, the role of the acids is probably not to catalyze the solubilization of the hemicellulose, but to catalyze the hydrolysis of the soluble hemicellulose oligomers (Mok and Antal, 1992). During steam pretreatment, the moisture content of the biomass influences the needed pretreatment time. The higher the moisture content, the longer the optimum steam pretreatment times (Brownell *et al.*, 1986). The positive effect of steam pretreatment is mostly due to removal of a large part of the hemicellulose, causing an increase in cellulose fiber reactivity.

Steam explosion is the most commonly used method for the pretreatment of lignocellulosic materials. Since cost reduction and low-energy consumption are required for an effective pretreatment, high particle sizes as well as nonacid addition would be desirable to optimize the effectiveness of the process (Ballesteros *et al.*, 2002; Hamelinck *et al.*, 2005). This pretreatment has been scaled-up and operated at the pilot-plant scale at the Iogen demonstration plant in Canada.

- *Liquid Hot Water*

Liquid hot water (LHW) is used instead of steam. The objective of the liquid hot water is to solubilize mainly the hemicellulose to make the cellulose better accessible and to avoid the formation of inhibitors. To avoid the formation of inhibitors, the pH should be kept between 4 and 7 during the pretreatment (Mosier *et al.*, 2005b) If catalytic degradation of sugars occurs, it results in a series of reactions that are difficult to control and result in undesirable side products. A difference between the LHW and steam pretreatment is the amount and concentration of solubilized products. In a LHW pretreatment, the amount of solubilized products is higher, while the concentration of these products is lower compared to steam pretreatment (Bobleter, 1994). This is probably caused by the higher water input in LHW pretreatment compared to steam pretreatment. The yield of solubilized (monomeric) xylan is generally also higher for LHW pretreatment; though this result diminishes when the solid concentration increases, because (monomeric) xylan is then further degraded by hydrolytic reactions to xylose and furfural (Laser *et al.*, 2002). At lower concentrations, the risk on degradation products like furfural and the condensation and precipitation of lignin compounds is reduced. It has been shown to remove up to 80% of the hemicellulose and to enhance the enzymatic digestibility of pretreated biomass materials such as corn stover (Mosier *et al.*, 2001) and sugarcane bagasse (Laser *et al.*, 2002). LHW reduces the need for neutralization of liquid streams and conditioning chemicals since acid is not added (Mosier *et al.*, 2005).

In general, LHW pretreatment is attractive from a cost-savings potential: no catalyst requirement and low-cost reactor construction due to low-corrosion potential. It has also the major advantage that the solubilized hemicellulose and lignin products are present in lower concentration, due to higher water input, and subsequently concentration of degradation products is reduced. In comparison to steam explosion, higher pentose recovery and lower formation of inhibitors are obtained, however, water demanding in the process and energetic requirement are higher and it is not developed at commercial scale.

- *Ammonia Fiber Explosion*

In the Ammonia fiber explosion (AFEX) pretreatment, biomass is treated with liquid anhydrous ammonia at temperatures between 60 and 100

°C and high pressure for a variable period of time. After the residence time the pressure is released, vaporizing the ammonia and allowing its recovery and recycling. The ammonia has a marked effect on lignocellulose causing swelling and physical disruption of biomass fibers, partial decrystallization of cellulose, and breakdown of lignin-carbohydrates linkages.

AFEX produces a solid pretreated material because during the pretreatment only a small amount of the material is solubilized and most of the biomass components remain in the solid fraction. Thus, since considerable hemicellulose is retained in the pretreated material, both cellulases and hemicellulases will be required in enzymatic hydrolysis process. The AFEX process demonstrates attractive economics compared to several leading pretreatment technologies based on a recent economic model for bioethanol from corn stover (Teymouri *et al.*, 2005).

An advantage of AFEX is that the ammonia used during the process can be recovered and reused. Also, the downstream processing is less complex compared to other pretreatment processes. Over 90% hydrolysis of cellulose and hemicellulose was obtained after AFEX pretreatment of bagasse (15% lignin). However, the AFEX works only moderately and is not attractive for the biomass with high lignin content such as hardwood, softwood and newspaper (Taherzadeh *et al.*, 2008). Furthermore, ammonia must be recycled after the pretreatment to reduce the cost and protect the environment. Both the ammonia cost and the cost of recovery processes drive up the cost of the AFEX pretreatment.

Recently, various research groups have done a significant amount of research to determine the optimum conditions for ammonia pretreatment of lignocellulosics biomass. (Teymouro *et al.*, 2004) evaluated the optimum process conditions and parameters, namely, ammonia loading, moisture content of biomass, temperature, and residence time, necessary for maximum effectiveness of the ammonia fiber explosion process on corn stover.

- *Microwave Pretreatment*

Microwave-based pretreatment combines both thermal and nonthermal effects generated in aqueous environment. The movement of ions and the vibration of polar molecules give rise to heat and extensive intermolecular collisions which accelerate chemical, physical, and biological processes. Microwave uses the

ability of direct interaction between a heated object and an applied electromagnetic field to increase heat. Some of the advantages of employing microwave heating over conventional heating include reduction of process energy requirements, uniform and selective processing and capacity of starting and stopping the process instantaneously. Furthermore, since the heat is generated internally via direct interaction between the electromagnetic field and components of the heated material, the heating is a faster process. When microwave is used to pretreat lignocellulose, it selectively heats the more polar part and this unique heating feature results in an improved disruption of the recalcitrant structures of lignocellulose. Regarding nonthermal effects, the electromagnetic field helps to accelerate the destruction of crystalline structures and changes the super molecular structure of lignocellulosic biomass improving its reactivity.

The short length of the process as well as the low inhibitor production is reflected in high cost effectiveness. However, the feasibility of using a pretreatment method that involves microwave irradiation and chemicals in commercial scale is unknown, and it would be necessary to study the possibilities for performing the method in the future.

- *Wet Oxidation*

Wet oxidation is an oxidative pretreatment method which employs oxygen or air as catalyst. When oxygen is not added, the process is similar to a hydrothermal pretreatment and comparable to the well-known steam explosion pretreatment. It allows reactor operation at relatively low temperatures and short reactor times. The oxidation is performed for 10-15 min at temperatures from 170 to 200 °C and at pressures from 10 to 12 bar O₂ (Olsson *et al.*, 2005). The addition of oxygen at temperatures above 170 °C makes the process exothermic reducing the total energy demand. It has been proven to be an efficient method for solubilization of hemicelluloses and lignin. However, wet oxidation does not catalyze the hydrolysis of solubilized hemicellulose. In steam explosion and dilute acid pretreatments sugar monomers are produced, while in wet oxidation soluble sugars from hemicellulose are oligomers (Klinke *et al.*, 2003). Regarding toxic products generated during pretreatment, phenolic compounds are not end products during wet oxidation because they are further degraded to carboxylic acids, formic and acetic being the major

degradation products. Furthermore, furfural and HMF production is lower during wet oxidation when compared to steam explosion or LHW methods.

Na_2CO_3 addition has been shown to decrease formation of toxic products by maintaining pH in the neutral to alkaline range. High enzymatic hydrolysis yields have been also obtained after wet oxidation pretreatment of corn stover (Palonen *et al.*, 2004). This technology has been widely used for ethanol production followed by simultaneous saccharification and fermentation (SSF) from corn stover (Varga *et al.*, 2004); however, cost of oxygen and catalysts are considered one of the main disadvantages for wet oxidation development technologies.

- *CO₂ Explosion*

The supercritical fluids are compounds that are in a gaseous form but are compressed at temperatures above their critical point to a liquid like density and it has been considered as an extraction solvent for non-extractive purposes, due to several advantages such as availability at relatively low cost, non-toxicity, non-flammability, easy recovery after extraction, and environmental acceptability. Supercritical pretreatment conditions can effectively remove lignin increasing substrate digestibility; thus, delignification has been studied by using supercritical fluids to pretreat lignocellulosic biomass. Furthermore, the addition of co-solvents such ethanol enhances delignification.

The compared CO_2 explosion with steam and ammonia explosion for pretreatment of recycled paper mix, sugarcane bagasse, and found that CO_2 explosion was more cost-effective than ammonia explosion. Further, it did not cause the formation of inhibitory compounds that could occur in steam explosion. An increase in pressure facilitated faster penetration of CO_2 molecules into the crystalline structures, producing more glucose after the explosion. Because CO_2 explosion is operated at low temperatures, it does not cause degradation of sugars such as the degradation of sugars observed with steam explosion due to the high temperature involved. However, current efforts to develop these methods do not guarantee economic viability yet. A very high pressure requirement is specially a concerning issue. On the other hand, CO_2 utilization could be an attractive alternative to reduce costs because of its coproduction during ethanol fermentation.

2.4.1.3 Chemical Pretreatments

- *Acid Pretreatment*

Acid pretreatments employ acids as catalysts which have stronger effect on hemicellulose and lignin than on crystalline cellulose. The main objective of the acid pretreatments is to solubilize the hemicellulosic fraction of the biomass and to make the cellulose more accessible to enzymes. This type of pretreatments can be performed with concentrated or diluted acid but utilization of concentrated acid is less attractive for ethanol production due to the formation of inhibiting compounds. Furthermore, equipment corrosion problems and acid recovery are important drawbacks when using concentrated acid pretreatments. The high operational and maintenance costs reduce the interest of applying the concentrated acid pretreatment at commercial scale (Wyman, 1996).

Diluted acid pretreatment appears as more favorable method for industrial applications and have been studied for pretreating wide range of lignocellulosic biomass. High enzymatic hydrolysis yields have been reported when pretreating lignocellulosic materials with diluted H_2SO_4 which is the most studied acid although HCl , H_3PO_4 and HNO_3 have also been tested (Mosier *et al.*, 2005). It can be performed at high temperature (e.g. 180 °C) during a short period of time; or at lower temperature (e.g. 120 °C) for longer retention time (30-90 min) (Taherzadeh and Karimi, 2008). It presents the advantage of solubilizing hemicellulose, mainly xylan, but also converting solubilized hemicellulose to fermentable sugars. Hemicellulose is removed when H_2SO_4 is added and this enhances digestibility of cellulose in the residual solids. Nevertheless, depending on the process temperature, some sugar degradation compounds such as furfural and HMF and aromatic lignin degradation compounds are detected, and affect the microorganism metabolism in the fermentation step (Saha *et al.*, 2005). Anyhow, this pretreatment generates lower degradation products than concentrated acid pretreatments.

The major advantage of dilute acid pretreatment over steam-explosion is significantly higher xylose yield. Using batch dilute sulfuric acid pretreatment process, xylose yield was showed to approach 80%-90% of theoretical value (Grohmann *et al.*, 1986 ; Grohmann *et al.*, 1992 ; Torget *et al.*, 1994). Dilute sulfuric acid has some important disadvantages such as corrosion that mandates

expensive materials of construction, acidic prehydrolyzates must be neutralized before the sugars proceed to fermentation, gypsum has problematic reverse solubility characteristics when neutralized with inexpensive calcium hydroxide, formation of degradation products and release of natural biomass fermentation inhibitors are other characteristics of acid pretreatment, disposal of neutralization salts is needed and biomass particle size reduction is necessary. Plus, the current sulfuric acid price has increased quickly so that the economic feasibility of dilute acid pretreatment might need to be reconsidered.

- *Alkali Pretreatment*

Alkali pretreatment refers to the application of alkaline solutions such as NaOH, Ca(OH)₂ (lime) or ammonia to increase cellulose digestibility and they are more effective for lignin solubilization, exhibiting minor cellulose and hemicellulose solubilization than acid or hydrothermal processes (Carvalho *et al.*, 2008). It is described to cause less sugar degradation than acid pretreatment, and it was shown to be more effective on agricultural residues than on wood materials. Nevertheless, possible loss of fermentable sugars and some production of inhibitory compounds must be taken into consideration to optimize the pretreatment conditions.

Sodium, potassium, calcium and ammonium hydroxides are suitable alkaline pretreatments. NaOH causes swelling, increasing the internal surface of cellulose and decreasing the degree of polymerization and crystallinity, which provokes lignin structure disruption (Taherzadeh and Karimi, 2008). NaOH has been reported to increase hardwood digestibility from 14% to 55% by reducing lignin content from 24–55% to 20% (Kumar *et al.*, 2009).

Ca(OH)₂, known as lime, also removes acetyl groups from hemicellulose reducing steric hindrance of enzymes and enhancing cellulose digestibility (Mosier *et al.*, 2005). This effect has been observed for enzymatic hydrolysis with corn stover (Kim and Holtzapfel, 2006) in which lime has been proven successfully at temperatures from 85 to 150 °C and for 3–13 h. To produce bioethanol with lime pretreatment, it is necessary to reduce pH as well as to separate the solid fraction to remove the alkali. However, solid fraction separation is not interesting owing to the significant amounts of fermentable sugars present in the liquid fraction.

In comparison with other pretreatment technologies, alkali pretreatment usually uses lower temperatures and pressures, even ambient conditions. Pretreatment time, however, is recorded in terms of hours or days which are much longer than other pretreatment processes. A significant disadvantage of alkaline pretreatment is the conversion of alkali into irrecoverable salts and/or the incorporation of salts into the biomass during the pretreatment reactions so that the treatment of a large amount of salts becomes a challenging issue for alkaline pretreatment.

- *Ionic Liquid (ILs) Pretreatment*

The use of ILs as solvents for pretreatment of cellulosic biomass has received much attention during the last decade. They are capable to break down the extensive hydrogen-bonding network in the polysaccharides and promote its solubilization. ILs are salts, typically composed of large organic cations and small inorganic anions, which exist as liquids at relatively low temperatures, often at room temperature. The notable characteristics of ILs are their thermal and chemical stability, non-flammability, wide liquid temperature range, and good solvating properties for various types of materials (Hayes, 2009). Their solvent properties can be varied by adjusting the anion and the alkyl constituents of the cation. Since no toxic or explosive gases are formed, ILs are called “green” solvents.

As cellulose solvents, ILs possesses several advantages over regular volatile organic solvents of biodegradability, low toxicity, broad selection of anion and cation combinations, low hydrophobicity, low viscosity, enhanced electrochemical stability, thermal stability, high reaction rates, low volatility with potentially minimal environmental impact, and non-flammable property. The dissolution mechanism of cellulose in ILs involves the oxygen and hydrogen atoms of cellulose hydroxyl groups in the formation of electron donor-electron acceptor (EDA) complexes which interact with the ILs. Upon interaction of the cellulose-OH and ILs, the hydrogen bonds are broken, resulting in opening of the hydrogen bonds between molecular chains of the cellulose. The interaction finally results in the dissolution of cellulose. Solubilized cellulose can be recovered by rapid precipitation with some anti-solvents such as water, ethanol, methanol, or acetone. The recovery cellulose was found to have the same DP and polydispersity as the initial cellulose, but significantly

different macro- and micro-structure, especially the decreased degree of crystallinity (Zhu, 2008).

However, there are still many challenges in putting these potential applications into practical use, for example, the high cost of ILs, regeneration requirement, lack of toxicological data and knowledge about basic physico-chemical characteristics, action mode on hemicellulose and/or lignin contents of lignocellulosic biomass and inhibitor generation issues.

- *Organosolv*

The organosolv pretreatment uses organic or aqueous solvents (ethanol, methanol, ethylene glycol, acetone, glycerol, tetrahydrofurfuryl alcohol, etc.) to extract lignin and provide more accessible cellulose. The organic solvents used in the organosolv process include methanol, ethanol, acetone, ethylene glycol, triethylene glycol, tetrahydrofurfuryl alcohol, glycerol, aqueous phenol, aqueous n- butanol. The organic solvent is mixed with water in various portions, added to the biomass and then heated to temperatures ranging 100-250 °C. Typically, acids (HCl, H₂SO₄, oxalic, or salicylic) can also be added as catalysts if the process is conducted at temperatures below 185-210 °C (Nahyun *et al.*, 2010).

For economic reasons, the use of low-molecular-weight alcohols such as ethanol and methanol has been favored (Sidiras and Kouios, 2004). The usual operation temperature of organosolv is in the range of 150-200°C. (Aziz and Sarkanen, 1989) found that the addition of catalyst was unnecessary for satisfactory delignification when temperature is higher than 185°C. Organic solvents are costly and their use requires high-pressure equipment due to their high volatility. The used solvents should be recovered and recycled to reduce the operation costs. Removal of solvents from the pretreated biomass is necessary because the residual solvents may be inhibitors to enzymatic hydrolysis and fermentation. The main advantage of organosolv over other chemical pretreatments is that relatively pure, low molecular weight lignin is recovered as a by-product (Katzen R *et al.*, 1995). The organosolv process is still too complex and expensive to attract industrial interests.

- *Ozonolysis*

Ozone is a powerful oxidant that shows high delignification efficiency. Ozonolysis is usually performed at atmospheric conditions, room temperature, and normal pressure. Its effect is mainly limited to lignin, hemicellulose is slightly affected, and cellulose is not. Thus, the amount of degradation compounds derived from hemicellulose and cellulose is very low. Notwithstanding, ozone could react with lignin-based aromatic compounds generating some lignin-derived degradation products. Ozone has been used to pretreat numerous lignocellulosic raw materials such as bagasse, and poplar among others (Kumar *et al.*, 2009). Despite some interesting results, further research has to be performed regarding ethanol production from lignocellulosic materials pretreated with ozone. An important drawback to consider is the large amounts of ozone needed, which can make the process economically unviable.

2.4.1.4 *Biological Pretreatments*

Fungal pretreatment has been previously explored to upgrade lignocellulosic materials for feed and paper applications. Recently, this environmentally friendly approach has received renewed attention as a pretreatment method for enhancing enzymatic saccharification of lignocellulosic biomass in ethanol production processes. Biological pretreatment employs wood degrading microorganisms, including white-, brown-, soft-rot fungi, and bacteria to modify the chemical composition and/or structure of the lignocellulosic biomass so that the modified biomass is more amenable to enzyme digestion.

White rot fungi with selectivity to lignin degradation over cellulose can be successfully applied in microbial pretreatments. However, the patterns of cell wall deconstruction by white rot fungi vary among species and strains. Several white rot fungi such as *Phanerochaete chrysosporium*, *Ceriporia lacerata*, *Cyathus stercolerus*, *Ceriporiopsis subvermispora*, *Pycnoporus cinnabarinus*, and *Pleurotus ostreus* have been examined on different lignocellulosic biomass showing high delignification efficiency (Keller *et al.*, 2003; Kumar *et al.*, 2009; Shi *et al.*, 2009).

(Wan and Li, 2010) found that *C. subvermispora* can effectively reduce recalcitrance of corn stover with high selectivity of lignin, high degradation rate, and minimal cellulose loss. In this case, when 5 mm corn stover was

pretreated at 28 °C with 75% moisture content, overall glucose yields of 57.7%, 62.2%, and 66.6% were obtained after 18, 28, and 35 days of microbial pretreatment, respectively.

The biological pretreatment appears to be a promising technique and has very evident advantages, including no chemical requirement, low energy input, mild environmental conditions, and environmentally friendly working manner (Sun and Cheng, 2002). However, its disadvantages are as apparent as its advantages since biological pretreatment is very slow and requires careful control of growth conditions and large amount of space to perform treatment. In addition, most lignolytic microorganisms solubilize/consume not only lignin but also hemicellulose and cellulose. Therefore, the biological pretreatment faces techno-economic challenges and is less attractive commercially.

2.4.1.5 Concluding Remarks of Pretreatments

Different pretreatment methods to make the lignocellulose accessible to enzymes have been described and widely studied for improving ethanol production processes. The effects that some of the most studied pretreatments have on structure of lignocellulose are summarized in Table 2.6 and the most promising pretreatment categories that can be commercialized for biorefinery shown in Table 2.7; however, none of those can be declared outstanding as each pretreatment has its intrinsic advantages and disadvantages. An effective pretreatment is characterized by several criteria: avoiding size reduction, preserving hemicellulose fractions, limiting formation of inhibitors due to degradation products, minimizing energy input, and being cost-effective. Except for these criteria, several other factors are also needed to be considered, including recovery of high value added co-products (e.g., lignin and protein), pretreatment catalyst, catalyst recycling, and waste treatment. When comparing various pretreatment options, all the above mentioned criteria should be comprehensively considered as a basis.

Table 2.6 Effect of various pretreatment methods on the chemical composition and chemical/physical structure of lignocellulosic biomass (Menon and Rao, 2012; Tomás-Pejó, Alvira *et al.*, 2011)

	Increases accessible surface area	Cellulose Decrystallization	Hemicellulose Solubilization	Lignin removal	Lignin structure alteration	Generation of toxic compounds	Applicability of different feedstock's
Mechanical	H	H	0	0	0	0	Yes
Extrusion	H	H	0	-	-	-	Yes
Steam explosion	H	-	H	M	H	H	Yes
LHW	H	-	H	L	M	L	-
AFEX	H	M	M	L	-H	L	-
Microwave	H	H	L	H	H	L	-
Wet oxidation	H	-	H	M	H	L	-
Co: explosion	MH	-	M	H	M	M	-
Acid	H	-	H	M	H	H	Yes
Alkali	H	H	MH	H	H	L	Yes
Ionic liquids	M	H	H	MH	H	ML	Yes
Organosolv	M	-	H	MH	M	ML	Yes
Ozonolysis	M	M	MH	H	M	L	-
Biological	M	0	0	H	-	0L	-

H, high effect; M, moderate effect; L, low effect; 0, no effect.

Table 2.7 Most promising pretreatment technologies (Adapted from Menon and Roa 2012 ; Tomás-Pejó, Alvira *et al.*, 2011)

	Sugar yield	Inhibitor formation	Success at pilot scale	Advantages	Limitation & disadvantages
Mechanical	L	Nil	Yes	Reduce cellulose crystallinity and decrease particle size	High power and energy consumption
Steam explosion	H	H	Yes	Hemicellulose solubilization and lignin transformation; cost-effective	Generation of toxic compounds and partial hemicellulose degradation
LHW	H	H	Yes	Partial hydrolysis of hemicelluloses and requires no catalyst and low-cost reactor	Long residence time, less lignin removal, high water demanding and high energy requirements
AFEX	H	L	Yes	Increases accessible surface area, remove lignin and hemicellulose and low formation of inhibitors	No efficient for biomass with high lignin content and high cost of large amount of ammonia
Wet oxidation	H/L	Nil	-	Removal of lignin, dissolves hemicellulose, causes cellulose decrystallization and minimizes the energy demand (exothermic)	High cost of oxygen and alkaline catalyst and only for low lignin
CO ₂ explosion	H	L	-	Hemicellulose removal, cellulose decrystallization and cost-effective	Does not affect lignin and very high pressure requirements
Acid	H	H	Yes	Hydrolysis of cellulose and hemicellulose and alters lignin structure	Generation of degradation products and equipment corrosion
Alkali	H	L	Yes	Removal of lignin and hemicellulose, increases accessible surface area	Long residence time and irrecoverable salts formed
Ionic liquids	H/L	L	-	Dissolution of cellulose and increased amenability to cellulose	Still in initial stages
Organosolv	H	H	Yes	Lignin and hemicellulose hydrolysis	Solvents need to be drained, evaporated, condensed and reused
Ozonolysis	H	L	No	Reduce lignin content and no toxic residues	Large amount of ozone required
Biological	-	-	-	Degrades lignin and hemicellulose, less formation of inhibitors and low energy consumption	Very slow rate of hydrolysis and requires careful control of growth condition

2.4.2 Saccharification

2.4.2.1 *Enzymatic*

Microbial degradation of lignocellulosic waste is accomplished by the action of several enzymes, the most important of which are the cellulases. Three major types of cellulase activities are recognized: Endoglucanases (1,4-b-D-glucanohydrolases), Exoglucanases, and b-Glucosidases (b-glucoside glucohydrolases). Endoglucanases cut at random the internal amorphous sites in the cellulose polysaccharide chain generating oligosaccharides of various lengths, and consequently shorter chains appear. Exoglucanases act, in a progressive manner, on the reducing and non-reducing ends of the cellulose chains liberating either glucose (glucanohydrolases) or cellobiose (cellobiohydrolase) as major products. Exoglucanases can also act on microcrystalline cellulose peeling the chains from the microcrystalline structure. b-Glucosidases hydrolyze soluble cellodextrins and cellobiose to glucose. The cellulase system of *Trichoderma reesei* consists of at least two exoglucanases, five endoglucanases, and two b-glucosidases.

In addition to three major groups of cellulase enzymes, there are also a number of ancillary enzymes that attack hemicellulose, such as glucuronidase, acetylerase, xylanase, b-xylosidase, galactomannanase, and glucomannanase. The enzymatic hydrolysis of lignocellulose is limited by several factors: crystallinity of cellulose, degree of polymerization (DP), moisture, available surface area, and lignin content (Chang and Holtzapple, 2000; Koullas *et al.*, 1992; Laureano-Perez *et al.*, 2005; Puri, 1984). (Caulfield and Moore, 1974) mentioned that decreasing particle size and increasing available surface rather than crystallinity affect the rate and extent of the hydrolysis. Other researchers (Grethlein, 1985; Grous *et al.*, 1986; Thompson *et al.*, 1992) concluded that the pore size of the substrate in relation to the size of the enzymes is the main limiting factor in the enzymatic hydrolysis of lignocellulosic biomass. Removal of hemicellulose increases the mean pore size of the substrate and therefore increases the probability of the cellulose to get hydrolyzed. On the other hand, drying of pretreated lignocellulose can cause a collapse in pore structure, resulting in a decreased enzymatic hydrolyzability (Grous *et al.*, 1986). (Zhang and Lynd, 2004) mention that cellulases can get trapped in the pores if the internal area is much larger than the external area, which is the case for many lignocellulosic

biomasses. Lignin limits the rate and extent of enzymatic hydrolysis by acting as a shield, preventing the digestible parts of the substrate to be hydrolyzed.

2.4.2.2 Dilute Acid

The dilute acid hydrolysis process is one of the oldest, simplest, and most efficient methods of producing ethanol from biomass. Dilute acid is used to hydrolyze the biomass to sugars. The first stage uses 0.7% sulfuric acid at 190 °C to hydrolyze the hemicelluloses present in the biomass. The second stage is optimized to yield the more resistant cellulose fraction. This is achieved by using 0.4% sulfuric acid at 215 °C. The liquid hydrolyzates are then neutralized and toxic compounds are removed before fermentation of sugar solution (Brennan *et al.*, 1986).

2.4.3 Fermentation

2.4.3.1 Microorganisms

Fungi, bacteria, and yeast microorganisms can be used for fermentation, specific yeast (*S. cerevisiae* also known as Baker's yeast) is frequently used to ferment glucose to ethanol. Theoretically, 100 g of glucose will produce 51.4 g of ethanol and 48.8 g of carbon dioxide. However, in practice, the microorganisms use some of the glucose for growth and the actual yield is less than 100%.

2.4.3.2 Bacteria

Ethanol-producing bacteria have attracted much attention in recent years because their growth rate is substantially higher than that of the *Saccharomyces* which is currently used for fuel ethanol production. With the recent advances in biotechnology, they have the potential to play a key role in making production of ethanol more economical (Dien *et al.*, 2003). Among such ethanol-producing bacteria, *Z. mobilis* is a well-known organism used historically in tropical areas to make alcoholic beverages from plant sap. The advantages of *Z. mobilis* are its high growth rate and specific ethanol production; unfortunately, its fermentable carbohydrates are limited to glucose, fructose, and sucrose. On the other hand, the Gram-negative strain *Zymobacter palmae*, which was isolated by (Okamoto *et al.*, 1993) using a broad range of carbohydrate substrates, is a facultative anaerobe that ferments hexoses, α -linked di- and tri-saccharides, and sugar alcohols (fructose, galactose, glucose, mannose, maltose, melibiose, sucrose, raffinose, mannitol, and sorbitol). This strain produces approximately 2 mol of ethanol per mole of glucose

without accumulation of byproducts and shows productivity similar to that of *Z. mobilis* (Okamoto *et al.*, 1993).

2.4.3.3 *Yeasts*

Metabolic pathway engineering is constrained by the thermodynamic and stoichiometric feasibility of enzymatic activities of introduced genes. Engineering of xylose metabolism in *S. cerevisiae* has focused on introducing genes for the initial xylose assimilation steps from *P. stipitis*, a xylose-fermenting yeast, into *S. cerevisiae*, a yeast traditionally used in ethanol production from hexose. However, recombinant *S. cerevisiae* created in several laboratories have used xylose oxidatively rather than in the fermentative manner that this yeast metabolizes glucose (Jin and Jeffries, 2004). D-Xylose is a major component of the hydrolyzate of hemicellulose from biomass. Therefore, ethanol production from xylose is essential for successful utilization of lignocellulose. Many bacteria, yeast, and fungi assimilate xylose, but only a few metabolize it to ethanol (Skoog and Hahn-Hagerdal, 1988).

2.4.3.4 *Fungi*

The filamentous fungus *Fusarium oxysporum* is known for its ability to produce ethanol by simultaneous saccharification and fermentation (SSF) of cellulose. However, the conversion rate is low and significant amounts of acetic acid are produced as a byproduct (Panagiotou *et al.*, 2005). A few microbial species such as *Neurospora*, *Monilia*, *Paecilomyces*, and *Fusarium* have been reported to hold the ability to ferment cellulose directly to ethanol (Singh *et al.*, 1992). *F. oxysporum* produces a broad range of cellulases and xylanases, which has been characterized earlier (Christakopoulos *et al.*, 1996). Acetic acid was the major fermentation product of *Neocallimastix* sp., another ethanol-producing fungus (Dijkerman *et al.*, 1997).

2.4.4 Technological Configurations

The classic configuration employed for fermenting biomass hydrolyzates involves a sequential process where the hydrolysis of cellulose and the fermentation are carried out in different units. This configuration is known as separate hydrolysis and fermentation (SHF). In the alternative variant, the simultaneous saccharification and fermentation (SSF), the hydrolysis and fermentation are performed in a single unit. The most employed microorganism for fermenting

lignocellulosic hydrolyzates is *S. cerevisiae*, which ferments the hexoses contained in the hydrolyzate but not the pentoses.

2.4.4.1 *Separate Hydrolysis and Fermentation (SHF)*

When sequential process is utilized, solid fraction of pretreated lignocellulosic material undergoes hydrolysis (saccharification). This fraction contains the cellulose in an accessible form to acids or enzymes. Once hydrolysis is completed, the resulting cellulose hydrolyzate is fermented and converted into ethanol. One of the main features of the SHF process is that each step can be performed at its optimal operating conditions. The most important factors to be taken into account for saccharification step are reaction time, temperature, pH, enzyme dosage, and substrate load (Sa'nchez and Cardona, 2008).

2.4.4.2 *Simultaneous Saccharification and Fermentation (SSF)*

The SSF process has been extensively studied to reduce the inhibition of end products hydrolysis (Zheng et al., 1998; Saxena *et al.*, 1992). In the process, reducing sugars produced in cellulose hydrolysis or saccharification are simultaneously fermented to ethanol, which greatly reduces the product inhibition to the hydrolysis. However, the need of employing more dilute media to reach suitable rheological properties makes the final product concentration to be low. In addition, this process operates at non-optimal conditions for hydrolysis and requires higher enzyme dosage, which influences substrate conversion positively, but process costs negatively. Considering that enzymes account for an important part of production costs, it is necessary to find methods reducing the cellulases doses to be utilized (Sa'nchez and Cardona, 2008).

SSF has some advantages such as increase of hydrolysis rate by conversion of sugars that inhibit the cellulase activity, lower enzyme requirement, higher product yield, lower requirements for sterile conditions since glucose is removed immediately and ethanol is produced, shorter process time and less reactor volume because a single reactor is used. However, ethanol may also exhibit inhibition to the cellulase activity in the SSF process. Some disadvantages of this configuration include incompatible temperature of hydrolysis and fermentation, ethanol tolerance of microbes, and inhibition of enzymes by ethanol.

2.4.4.3 Pentoses Fermentation

One of the main problems in bioethanol production from lignocellulosics is that *S. cerevisiae* can ferment only certain mono- and disaccharides like glucose, fructose, maltose, and sucrose. This microorganism is not able to assimilate cellulose and hemicellulose directly. In addition, pentoses obtained during hemicellulose hydrolysis (mainly xylose) cannot be assimilated by this yeast. A way to overcome this obstacle is through recombinant DNA technology (genetic engineering). Other approach to this problem is the use of pentose-fermenting microorganisms such as some species of yeasts and bacteria. In this case, configurations involving the separate fermentation of pentoses and hexoses have been proposed. Yeasts such as *Pichia stipitis*, *Candida shehatae*, and *Pachysolen tannophilus* can digest pentoses, but their ethanol production rate from glucose is at least five times less than that observed for *S. cerevisiae*. Moreover, their culture requires oxygen and ethanol tolerance is 2-4 times lower (Claassen *et al.*, 1999). Pentose-fermenting yeasts require a careful control for maintaining low oxygen levels in the culture medium needed for their oxidative metabolism. Additionally, these yeasts successfully ferment pure xylose but not the aqueous hemicellulose streams generated during the biomass pretreatment, due to the presence of different inhibitors (Chandrakant and Bisaria, 1998).

2.4.4.4 Simultaneous Saccharification and Cofermentation (SSCF)

Other promising integration alternative is the inclusion of the pentose fermentation in the SSF, process called simultaneous saccharification and cofermentation (SSCF). In this configuration, it is necessary that both fermenting microorganisms be compatible in terms of operating pH and temperature. (Chandrakant and Bisaria, 1998) suggest that a combination of *C. shehatae* and *S. cerevisiae* is suitable for this kind of process. Similarly, a system including the isomerization of xylose and the fermentation with *S. cerevisiae* in a simultaneous way can be utilized. Some drawbacks of this configuration are the high byproduct formation in the form of CO₂ and xylitol, poor enzyme stability, incompatible pH and temperature (pH of 7.0 and 70 °C for the isomerization process), and the reversibility of the enzyme transformation (Chandrakant and Bisaria, 1998). Technological Configurations of each process are shown in Figure 2.14.

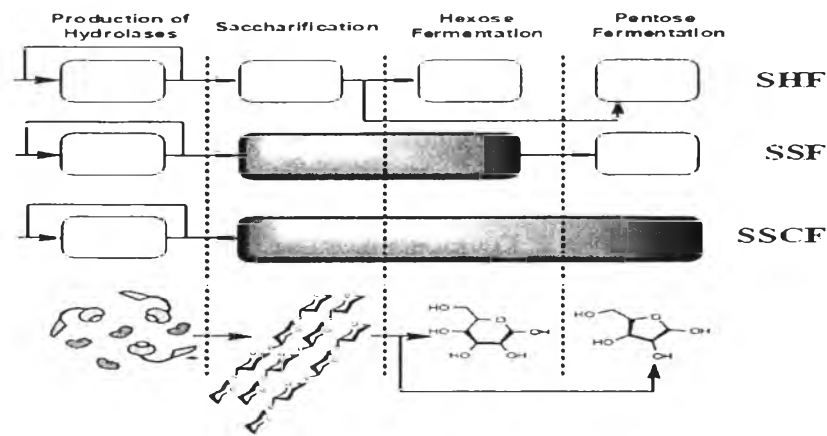


Figure 2.14 Technological Configurations of each process

(Source: <http://dx.doi.org/10.5772/55309>).

2.5 Sustainable Development

2.5.1 Definition of Sustainable Development

Sustainable development (SD) is development that meets the needs of the present without compromising the ability of future generations to meet their own needs. In other words, development that meets the needs of current generation without compromising the needs of future generations is termed as sustainable development. Sustainable development has three components: environment, society, and economy. If you consider the three to be overlapping circles of the same size, the area of overlap in the center is human well-being - as shown in Figure 2.15. (<http://www.det.wa.edu.au>).

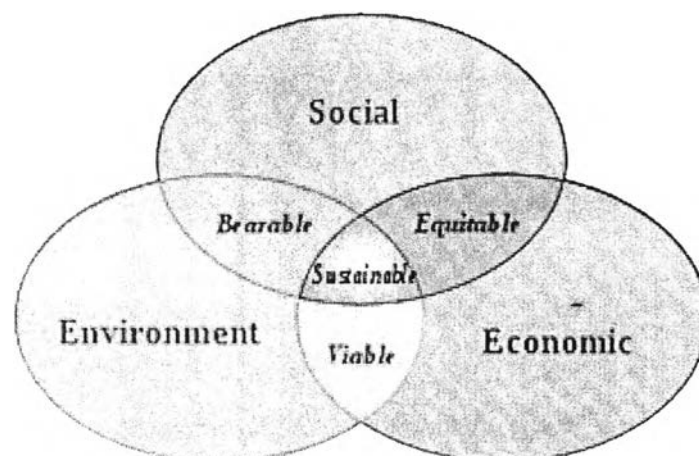


Figure 2.15 Sustainable development concept (Source: <http://en.wikipedia.org>).

2.5.2 Sustainable Energy for the Future

At present, the environmental problem is the main issue for the entire world to be interested. The major energy source is still the fossil fuels which are non-renewable and also impact to environmental. The energy that sustainable, renewable and environmental friendly is now replacing fossil fuel.

Sustainable energy is the provision of energy that meets the needs of the present without compromising the ability of future generations to meet their needs. Sustainable energy sources are most often regarded as including all renewable energy sources, such as hydroelectricity, solar energy, wind energy, wave power, geothermal energy, bioenergy, and tidal power. It usually also includes technologies that improve energy efficiency.

Apart from the energy sources that have to sustain, the processes which producing these energy are also importance because the main part that influence environment problem are released from the processes. If the processes are designed to minimize waste and optimize the utility consumption, the environmental impact will decrease. The next topics will mention about the energy situation and the way to improve the energy usage for more sustainable in Thailand.

2.6 Life Cycle Assessment (LCA)

As mention in the sustainable development topic, environmental issue is one of the importance parts that have to be analyzed. The efficiency of biofuel in terms of energy and environmental aspect can evaluate by the method call “Life Cycle Assessment (LCA)”.

2.6.1 Definition of LCA

Life cycle assessment (LCA) is a technique to assess environmental impacts associated with all the stages during its entire life cycle of a product, process or activity, encompassing, extracting and processing raw materials; manufacturing, transportation and distribution; use, re-use, maintenance; recycling, and final disposal by identifying and quantifying energy and materials used and wastes released to the environment; to assess the impact of those energy and materials used and releases to the environment; and to identify and evaluate opportunities to affect environmental improvements as shown in Figure 2.16 (SETAC, 1993).

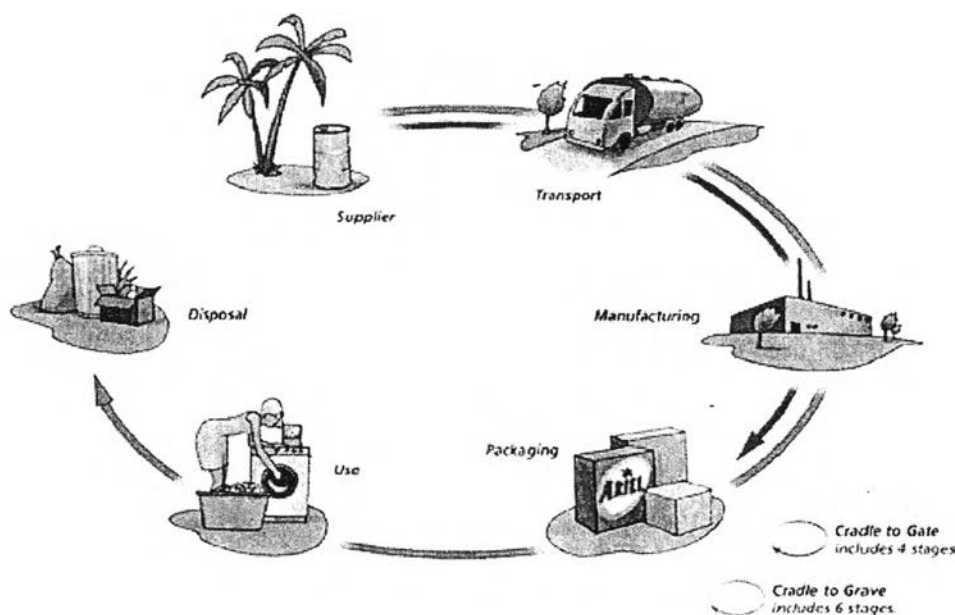


Figure 2.16 Structure of the life cycle assessment

(Source: <http://www.scienceinthebox.com>).

In the case of petroleum-derived fuels, this means LCA includes everything from the time the oil is extracted from the ground, transported to the refinery, made into fuel and distributed to your local gas station. This is also known as a Well-to-Wheels Study because it starts at the oil well and ends at the wheels or more specifically the tailpipe of your car or truck.

For a crop like corn ethanol, the LCA is much more complex. Tracking of the energy and emissions it takes to plant the corn, and make the fuels, fertilizers, and pesticides to grow the corn. Estimating whether growing the corn increases or decreases carbon in the soil. Appraising how much fuel it takes to get the corn to the ethanol refinery and how much energy is consumed and the amount of emissions that are generated in the bioethanol plant. Corn ethanol refineries typically make a co-product called distillers grain, which is a high-protein feed for cattle. This production is counted as a credit in our accounting spreadsheet. It also includes the impact of getting ethanol to the service station by rail and truck.

2.6.2 Overview of LCA

In LCA substantially broader environmental aspects can be covered, ranging from GHG emissions and fossil resource depletion to acidification and toxicity aspects, hence it is a good tool for quantifying environmental impacts of a defined product system. However, LCA as it stands has its limitations such as the difficulties in data acquisition and validation, and the misleading results due to the choice of methodology especially on allocation issues. Figure 2.17 illustrates the life cycle of biofuels involving CO₂ emission.

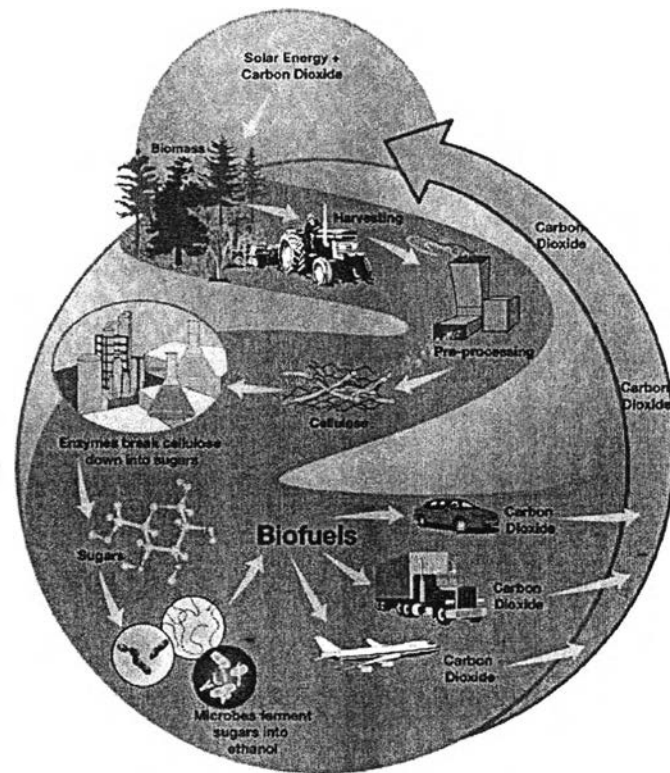


Figure 2.17 Life cycle of biofuels (Source: <http://peda.gov.in>).

The objectives of LCA are to compare the full range of environmental effects assignable to products and services in order to improve processes, support policy, provide a sound basis for informed decisions and also increase environmental awareness on the part of the general public, industry and governments. The precursors of life cycle analysis and assessment (LCAs) were the global modeling studies and energy audits of the late 1960s and early 1970s. These attempted to assess the resource cost and environmental implications of different patterns of human behavior.

LCAs were an obvious extension, and became vital to support the development of eco-labeling schemes which are operating or planned in a number of countries around the world. In order for eco-labels to be granted to chosen products, the awarding authority needs to be able to evaluate the manufacturing processes involved, the energy consumption in manufacture and use, and the amount and type of waste generated. To accurately assess the burdens placed on the environment by the manufacture of an item, the following of a procedure or the use of a certain process,

two main stages are involved. The first stage is the collection of data, and the second is the interpretation of that data.

2.6.3 Methodology of LCA

The LCA framework was standardized by the International Organization for Standardization (ISO). According to the ISO 14040 and 14044 standards, a life cycle assessment is derived in four distinct phases consisting of:

2.6.3.1 *Goal and Scope Definition*

The first step is where the intention of the use of LCA is defined, and where the setting of the boundaries (what is and is not included in the study) for the product system takes place and assumptions based upon the goal. In this phase, formulate and specify the goal and scope of study in relation to the intended application are required. For the example of a packaging study might choose to define the functional unit as - packaging of 1,000 liters of milk in containers of 1 liter. Taking this, the relevant significant comparison can be between 1,000 carton boxes and 40 returnable polycarbonate bottles, which can be used in average 25 times.

Usually what LCA does is compare different ways of obtaining the same function. Therefore in order to guarantee fairness and relevance it is crucial to be comparing between product systems that actually provide the same function, being this assured through carefully defining the functional unit. Finally, the goal and scope including a description of the method applied for assessing potential environmental impacts and which impact categories those are included.

2.6.3.2 *Inventory Analysis (LCI)*

This step is where all the necessary input and output data for the processes regarding the product system is gathered. The energy and raw material inputs and environmental releases associated with each stage of production are quantified. Other types of exchanges or interventions such as radiation or land use can also be included. These gathered data are related with the reference flow given by the functional unit. Typically the data for the different processes is combined over the life cycle and presented as the total emissions of a substance or total use of resource.

Finally, the results of the inventory which provides information about all inputs and outputs in the form of elementary flow to and from the environment from all the unit processes involved in the study are obtained.

2.6.3.3 Impact Assessment (LCIA)

Analyze and compare the impacts on human health and the environment burdens associated with raw material and energy inputs and environmental releases quantified by the inventory. In this phase, “Life Cycle Impact Assessment” (LCIA) is aimed at evaluating the contribution to impact categories such as global warming, acidification, ozone depletion and etc.

The first step of LCIA is termed “characterization”. Here, impact potentials are calculated based on the LCI results. The next steps are “normalization” and “weighting”, but these are both voluntary according the ISO standard. Normalization provides a basis for comparing different types of environmental impact categories (all impacts get the same unit). Weighting implies assigning a weighting factor to each impact category depending on the relative importance.

2.6.3.4 Interpretation

Evaluate opportunities to reduce energy, material inputs, or environmental impacts at each stage of the product life-cycle. In this phase, “Interpretation” is the most important one. An analysis of major contributions, sensitivity analysis and uncertainty analysis leads to the conclusion whether the ambitions from the goal and scope can be met.

All conclusions are drafted during this phase. Sometimes an independent critical review is necessary, especially when comparisons are made that are used in the public domain. Finally, an improvement, in which recommendations are made based on the results of the inventory and impact stages, is also analyzed. These may include modifying a production process, using different raw materials, or choosing one product over another (ISO 14040 and 14044, 2006).

To understand easily, the framework within which life cycle assessment is carried out is shown in Figure 2.18. Two main activities—inventory analysis and impact assessment—are preceded by a vitally important planning phase and followed by extended interpretation, which will normally involve checking the results both against the initial goals and for self-consistency.

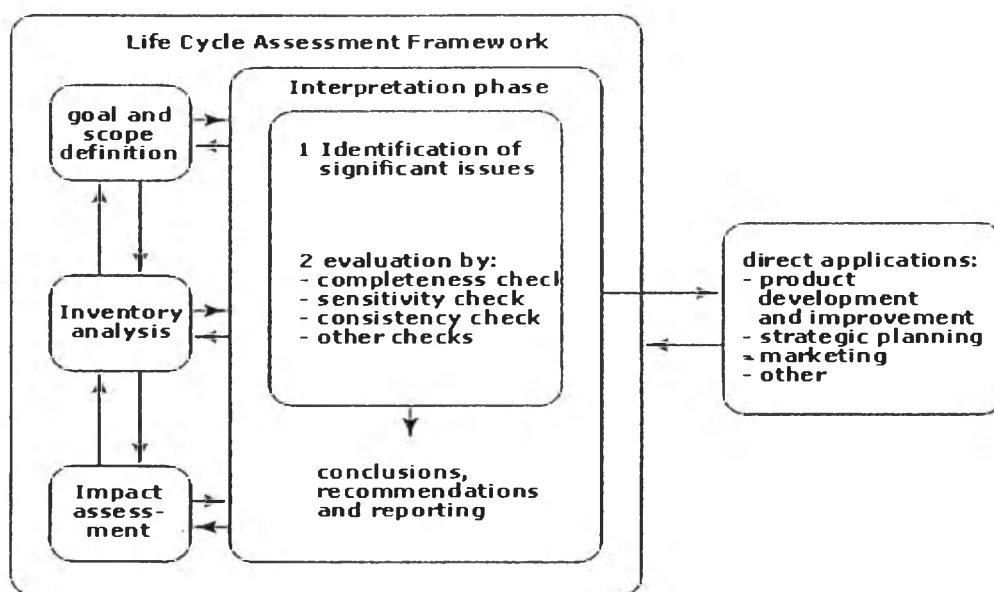


Figure 2.18 Life cycle assessment framework (Source: <http://www.ami.ac.uk>).

2.6.4 LCA studies on bioethanol

Bioethanol has become the new challenge on the reduction of fossil resource use and global warming concern. After that, many research teams have conducted the LCA on bioethanol in various materials including sugar, starchy, and lignocellulosic biomass.

(Searcy *et al.*, 2008) compared the LCA emission renewable energy routes that convert straw/corn stover into usable energy were examined. The conversion options studied were ethanol by fermentation, syndiesel by oxygen gasification followed by Fischer Tropsch synthesis, and electricity by either direct combustion or biomass integrated gasification and combined cycle (BIGCC). The greenhouse gas (GHG) emissions were 830 g CO₂ e/kWh for direct combustion, 839 g CO₂ e/kWh for BIGCC, 2,060 g CO₂ e/L for ethanol production, and 2,440 g CO₂ e/L for FT synthesis of syndiesel. The comparison in unit per mega joules is shown in Table 2.8.

Table 2.8 Comparison of GHG emission from difference sources(Source: Searcy *et al.*, 2008)

Method	Emission (g CO₂/MJ)
Direct Combustion	230.56
BIGCC	233.06
Fermented Ethanol	97.31
FT_Syndiesel	67.40

The result showed that bioethanol choice gave more attractive than those from electricity choices. However, syndiesel emitted the lowest emission with 67.40 g CO₂ per mega joules. By this, it means that the use of lignocellulosic materials in conversion process to be ethanol is better than use it to generate electricity.

(Luo *et al.*, 2009) worked on lifecycle assessment and life cycle costing of bioethanol from sugarcane two cases in Brazil. The two cases engaged were: base case—bioethanol production from sucrose, and heat and electricity generation from bagasses using the current technology (1); future case—bioethanol production from both sucrose and bagasses (2), and heat and electricity generation from wastes. His study performed LCA and compared gasoline with E10, E85 and Ethanol as well. The result is shown in Figure 2.19.

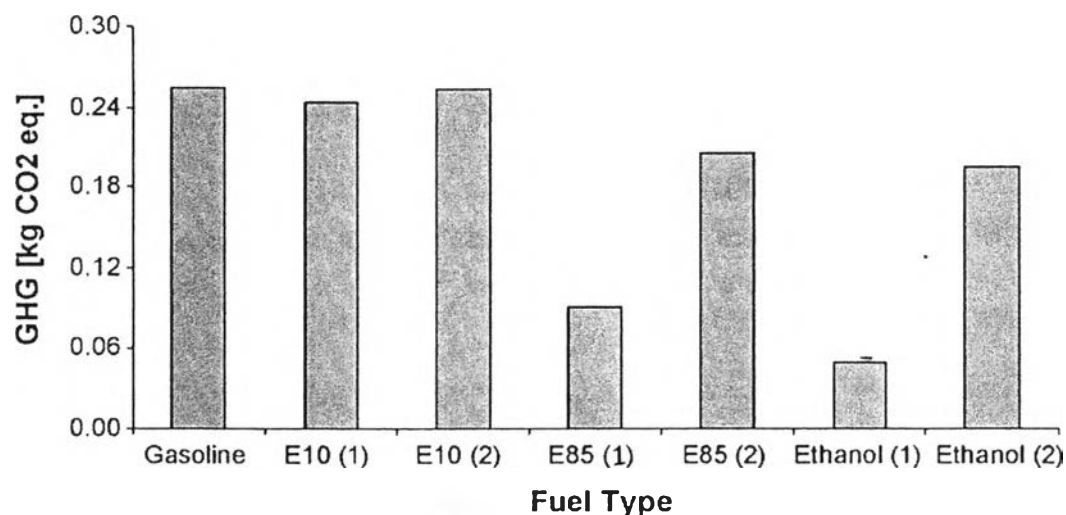


Figure 2.19 Greenhouse gas emission of ethanol from sugarcane
(Source: Luo *et al.*, 2009).

When GHG emissions were concerned, burning bagasse for electricity generation (base case) was a much better option than converting bagasse to ethanol (future case). They also performed life cycle costing, the result indicated that driving with ethanol fuels was more economical than gasoline, and the future case was economically more attractive than the base case, which have been the driving force for the promotion of advanced technologies converting bagasse to ethanol.

(González-García *et al.*, 2009) studied on the life cycle assessment of flax shives in Spain. They compare the emission in difference allocation method, economic and mass. Three scenarios (EA1, EA2 and EA3) based on economic allocation were evaluated according to the large difference in the market prices (from 15 to 36 €/ton regardless of their final destination). Mass allocation (scenario MA) was also assumed in order to estimate the effect of allocation. The comparison of global warming potential in difference allocation methods are shown in Figure 2.20.

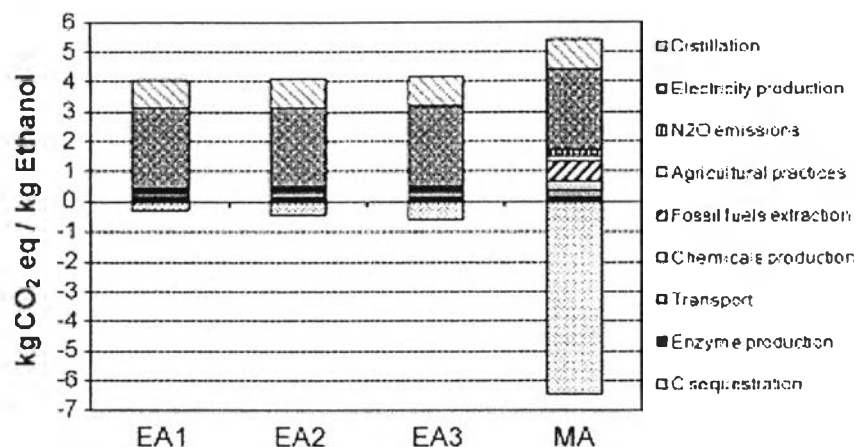


Figure 2.20 The comparison (between allocation factors) of CO₂ equivalent emission for ethanol production and main process involved (Source: González-García *et al.*, 2009).

Activities related to the ethanol conversion plant, such as distillation and electricity production, are the main hot spots in this impact category. In addition, when mass allocation is assumed, there is a remarkable contribution from fossil fuel extraction due to a higher amount of diesel from agricultural machineries being allocated to the flax shives. Moreover, it is important to remark the positive effect of the carbon sequestered during crop growth (9.9 ton CO₂/ha), which contributes to offset the GHG emissions. This effect is more outstanding in the mass allocation (highest allocation factor) since more CO₂ taken up during the crop growing is allocated to flax shives.

(Neupane *et al.*, 2011) worked on the attributional life cycle assessment of woodchips for bioethanol production. An in-depth LCA of woodchips shows that harvesting and woodchips processing stage and transportation to the facility stage emit large amount of environmental pollutants compared to other life cycle stages of ethanol production as shown in Figure 2.21. Their analysis also found that fossil fuel consumption and respiratory inorganic effects are the two most critical environmental impact categories in woodchips production. They have used Eco-indicator 99 based cradle-to-gate LCA method with a functional unit of 4 m³ of dry hardwood chips production.

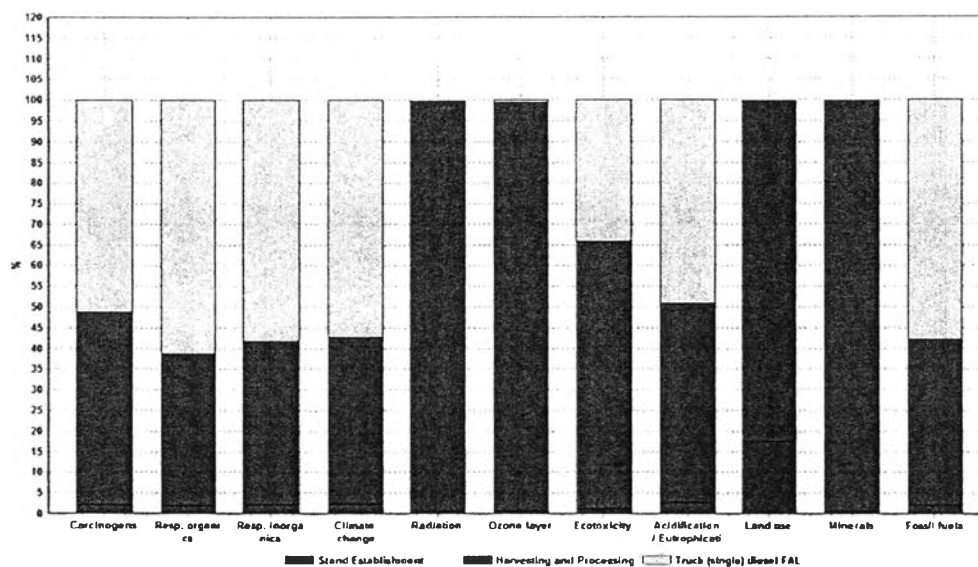


Figure 2.21 The characterized impact factor for natural regeneration scenario (Source: Neupane *et al.*, 2011).

In their work, they applied an LCA approach to analyze the environmental impacts of each process steps for woodchips production in view of the impending large scale bioethanol production in the U.S. Using an appropriate LCA method and following the ISO 14040 standards have allowed them to assess the different environmental impacts in each process step of woodchips production. The dominant environmental contributors are fossil consumptions and respiratory inorganics in the natural regeneration and the artificial regeneration scenarios. Transportation of woods from forest site to a facility has significant impact factor, followed by harvesting and processing of woodchips. Since most of the impacts are due to the combustion of fossil fuels (diesel and gasoline) used for operating machineries, one option to reduce the life cycle impacts of woodchips production on the environment is to increase the fuel efficiency of equipment used in harvesting and processing and transportation. This could be done by balancing the size and power capacity of equipment with the tree size to be harvested. They also recommend establishing a prospective biorefinery at proximity to biomass area in order to minimize transportation distance and consequently cost. This however, might increase the transportation distance and costs for producing biomass-based end products.