VOLUME I

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BIOFEEDSTOCKS AND THEIR PROCESSING

Edited by
Lalit Kamel Singh
Gaurav Chaudhary

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Publishers at Scrivener
Martin Scrivener (martin@scrivenerpublishing.com)
Phillip Carmical (pcarmical@scrivenerpublishing.com)

Advances in Biofeedstocks and Biofuels

Volume 1: Biofeedstocks and Their Processing

Edited by

Dr. Lalit Kumar Singh

Department of Biochemical Engineering, Harcourt Butler Technical University (Formerly Harcourt Butler Technological Institute)

Dr. Gaurav Chaudhary

Department of Biotechnology, Institute of Engineering & Technology, Mangalayatan University

WILEY

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Production of Bioenergy in the Framework of Circular Economy: A Sustainable Circular System in Ecuador

Vega-Quezada Cristhian[1,2*], Blanco María[2] and Romero Hugo[3]

[1]Academic Unit of Business Administration, Universidad Técnica de Machala, Av. Panamericana Km 5 ½, Machala, ECUADOR
[2]Department of Agricultural Economics, Universidad Politécnica de Madrid, ETSI Agrónomos, Av. Complutense 3, 28040 Madrid, SPAIN
[3]Academic Unit of Chemistry and Health, Universidad Técnica de Machala, Av. Panamericana Km 5 ½, Machala, ECUADOR

Abstract

This chapter reviews and applies the principle of the circular economy to recent advances in bioenergy production. Using Ecuador as a case study, we identify a set of production technologies for both biogas and biodiesel, that may interact in sustainable circular processes of production and by-product reuse. The main contribution of this chapter is in highlighting the synergies between different technologies of bioenergy production and waste reuse, as well as the technological requirements for implementation within a systemic approach. The example of a sustainable circular strategy in Ecuador illustrates how an integrated approach to food production, waste management and bioenergy generation can deliver multiple social, economic and environmental benefits.

***Keywords*:** Bioenergy, biofuel production, circular economsy

**Corresponding author*: cvega@utmachala.edu.ec

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1.1 Introduction

1.1.1 Energy and Bioenergy

The world's primary energy production quantified in millions of tonnes of oil equivalent (Mtoe) has more than doubled from 1973 to 2010. Figure 1.1 shows this dramatic increase from 1973 to 2010, as well as the regional share of global energy production, highlighting the increases in production across Asia, including China, as well as in the Middle East.

Global consumption of primary energy has seen an equally large increase between 1973 and 2010, rising from 4672 Mtoe in 1973 to 8677 Mtoe in 2010. During this period, natural gas has seen a slight increase in its respective proportion of total energy consumed, increasing from 14% in 1973 to 15.2% in 2010, whereas the proportion of biofuels and waste materials have dropped from 13.2% to 12.7%. In absolute values, the consumption of natural gas has increased from 654.1 to 1318.9 Mto, whereas biofuels consumptions have increased from 616.7 Mtoe in 1973 to 1101.2 Mtoe in 2010 [1]. The trend in global energy consumption growth, considered at an annual rate during from 1973–2010, was 1.68%, consumption of natural gas increasing by 1.91% and biofuels by 1.58%, suggesting that consumption of these forms of energy will continue to grow in the future.

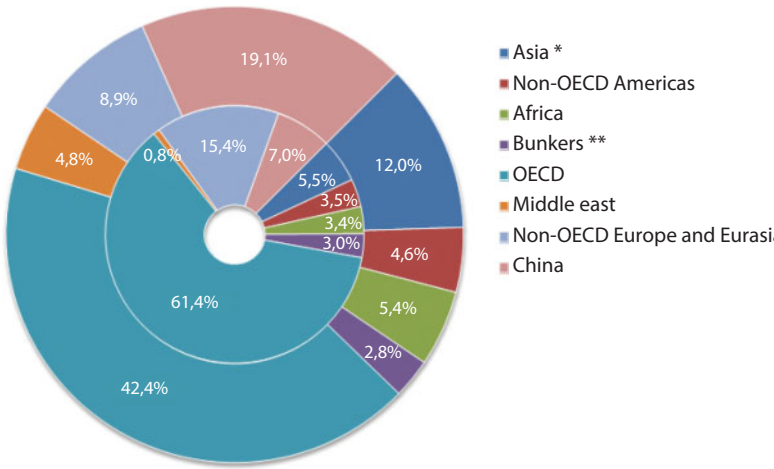

Figure 1.1 Global primary energy producing regions in the years 1973 and 2010.
*Asia doesn't include China.
**Includes international aviation and international marine bunkers.
Source: [1], Formulated by the authors.

Table 1.1 Fuels used in the generation of electricity*.

Year	1973		2010	
Fuel type	**TWh**	**Percentage**	**TWh**	**Percentage**
Total	6115,0	100%	21431,0	100%
Hydro	1284,2	21,0%	3429,0	16,0%
Other**	36,7	0,6%	792,9	3,7%
Coal/peat	2342,0	38,3%	8701,0	40,6%
Oil	1510,4	24,7%	985,8	4,6%
Natural gas	739,9	12,1%	4757,7	22,2%
Nuclear	201,8	3,3%	2764,6	12,9%

*Excludes storage pumps.
**Others includes; geothermal, solar, wind, biofuels, waste materials and heat.
Source: [1], Formulated by the authors.

Of the total global primary energy consumption used in 2010, 17.7% (equating to 1536 Mtoe) was used in the generation of electricity. The approximate percentages and amount of power consumption, in Tera watt hours (TWh) for each fuel type used in generation are presented in Table 1.1 [1].

In analysing the increase in global electrical generation between 1973 and 2010, the annual growth rate has been 3.45%, whilst generation from renewable energy sources, such as solar, wind, biofuels, geothermal amongst others has increased at an annual rate of 8.66%. This increase in renewable energy generation has been attributed in most cases to the international concern for mitigating climate change, which has generated favorable prospects for further development of activities to get the greatest potential from renewable energy technologies.

To model the global future energy supply, the International Energy Agency (IEA) has predicted two possible scenarios for the year 2035:

The first scenario, "New Policies," has been developed based upon the policies, commitments and plans announced and developed by various countries and regions across the world. The second scenario has been developed within a political-climatic framework post-2012, which seeks to stabilize the concentration of greenhouse gases to 450 ppm of CO_2 equivalent based upon policies currently under consideration [1]. The expected outcomes of both scenarios by 2035 are shown in Figure 1.2.

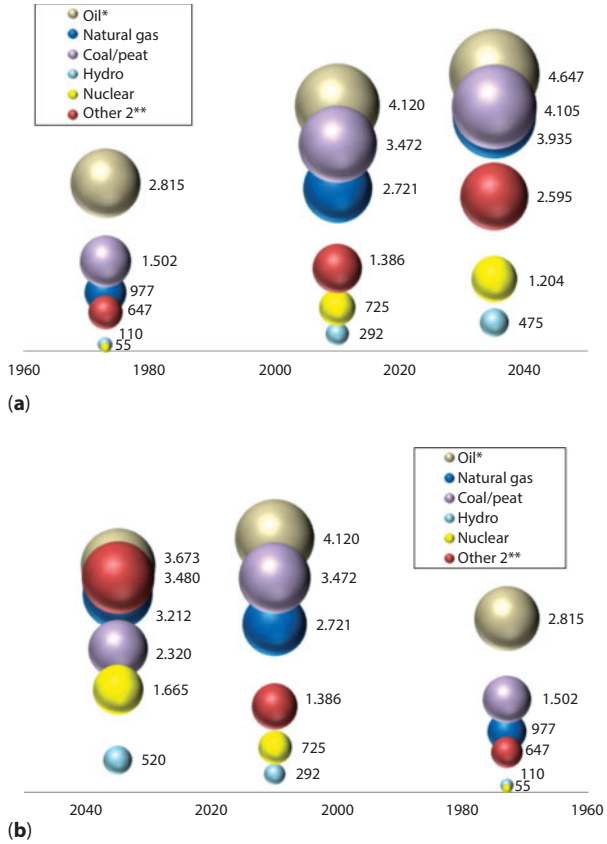

Figure 1.2 Primary energy supply in the world by 2035 under different scenarios.

NPS: New Policies 450S: Scenario 450.
*Includes international aviation and international marine bunkers.
**Other includes geothermal, solar, wind, biofuels, waste and heat.
Source: [1], Formulated by the authors.

1.1.2 Ecuadorian Case

Ecuador is the third-fastest-growing economy in Latin America, with one the lowest unemployment rates in the Americas and across the world. It is one of the most biodiverse countries in the world, with the rights of nature enshrined within its constitution. Ecuador is considered one of the richest countries in terms of mineral resources on Earth, with it being a regional leader in the production and exportation of oil. Further, Ecuador is internationally renowned for its global exportation of bananas, flowers, shrimp and cocoa.

The continuity of a long-term tendency in government policy can be seen in the National Plan for Good Living (2014–2017), within which the

importance for synergies between agriculture and bioenergy are evident. Further, the 4th Goal of the plan is particularly pertinent, to "Ensure the rights of nature and promote a healthy and sustainable environment". The Plan also references the importance of increased diversity in the energy matrix, promoting efficiency and growth in renewable energies, with a specific plan of development, which has projected scenarios of use up to 2025. Clearly demonstrating the commitment of the Ecuadorian government to sustainable development. In this context, the government and its institutions promotes the production of first-, second-, and third-generation energy crops required as raw material for biodiesel production.

This chapter will analyze the economic potential for biodiesel in Ecuador, whilst also proposing systematic initiatives that could be implemented for the formation of a circular economy strategy. This proposal is based upon current biotechnological advances, which have provided the required information used to establish the movement towards sustainable development of biofuels in Ecuador.

1.2 A Sustainable Circular System in Ecuador

Sustainable production of biodiesel is a goal for Ecuador, where currently the principal energy crop is palm. To provide an alternative for the sectoral development of bioenergy in Ecuador, we will analyze the potential for the production of microalgae within the principal of the circular economy. The proposed schemes for such production are presented in Figure 1.3, which will be explained in detail throughout this chapter. The objective is to highlight the synergies between different bioenergy technologies for production and the reuse of waste products within these systems. Further, this chapter will delve into the technological requirements for the implementation of such a cyclical approach.

A review of the scientific literature has been performed by the authors, with specific attention paid to literature addressing the elements within the proposed system (Figure 1.3). These elements include production of biogas from municipal waste and manure, assessment of the potential for biogas generation from manure and its conversion to electricity, and the production of microalgae using photobioreactor sheets, amongst others.

1.2.1 Biogas

Biogas is the result of fermentation and anaerobic digestion of organic materials; the implementation of biogas systems often leads to significant

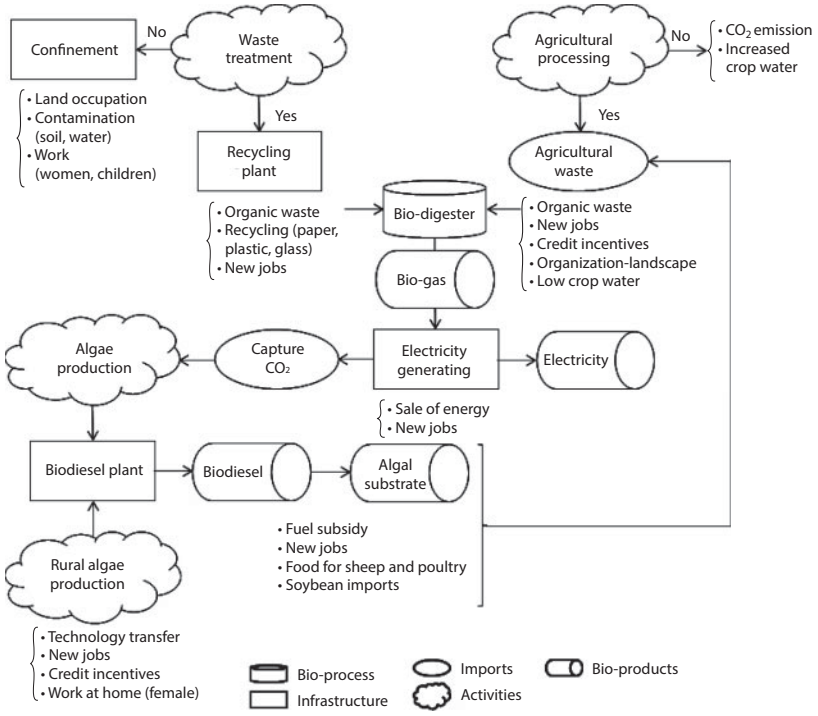

Figure 1.3 Schematic for sustainable circular system in Ecuador.
Source: [2].

improvements in resource efficiency, whilst reducing environmental impacts compared to current waste management and agricultural practices [3]. Apart from reducing greenhouse gas emissions, such biogas systems can reduce, amongst others, eutrophication and air pollution, and make better use of crop nutrients [4].

Presently, there is no established means of trading biogas on international markets; according to the IEA, as of 2009 100% of global production was consumed locally. Figure 1.4 highlights electrical generation (gigawatts hours (GWh)) and gross heat production (terajoules (TJ)) produced from global biogas combustion.

The major use of biogas is for electrical generation; however, other important uses are available for this bioenergy, including industrial consumption and residential uses, as shown in Table 1.2.

Biogas is the first biofuel proposed within Figure 1.3; the purpose of its production within our circular scheme is for electricity production, whilst using CO_2 emitted as a by-product of combustion, as an input for producing microalgae. The study by Börjesson, Pål & Berglund, Maria,

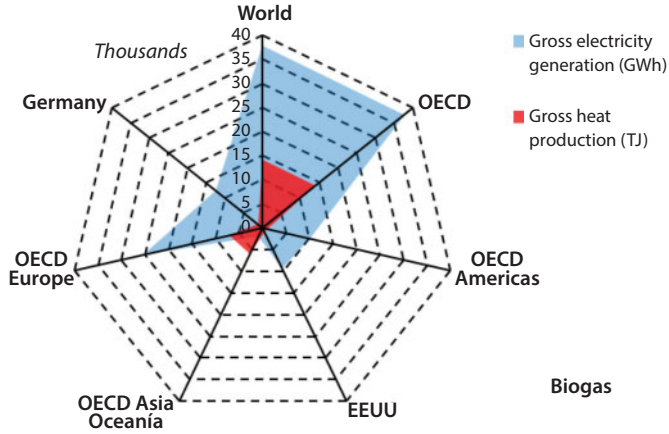

Figure 1.4 Renewable and waste energies in 2009.
Source: [5], Developed bythe authors.

Table 1.2 Uses of biogas by region.

	Rest of world	OECD Americas	OECD Asia Oceania	OECD Europe
Unit	*TJ*	*TJ*	*TJ*	*TJ*
Production	**327862**	**221994**	**32306**	**354529**
Transformation	**2351**	**115800**	**29692**	**313044**
Electricity Plant	1690	101350	21236	259121
Cogeneration Plant	629	14162	1571	50611
Thermal	32	0	6885	2780
Other transformations	0	288	0	532
Total Consumption	**325260**	**106144**	**2605**	**39747**
Industry	20	104546	328	19136
Transport	0	0	0	903
Residential	325027	0	0	2764
Commercial and Public Services	160	1586	2146	13381
Agriculture/Forestry	38	12	0	3463
Non-energy Use	15	0	131	100

Source: [5], Developed by the authors.

which compared biogas systems against fossil fuels, concluded that the introduction of biogas systems may lead to both direct and indirect benefits. Indirect benefits were found to include reduced nitrogen leaching, reduction in manure-based production of ammonia and methane, and that other organic wastes and crop residues can be utilized in the production process, rather than wasted. However, when biogas systems are introduced to replace other biofuel systems, including for heat and ethanol production or for burning organic residues, greenhouse gas emissions may increase [3]. Throughout the biogas production process, it is necessary to estimate emissions of CO_2, which may be mitigated, as well as the potential for electricity production.

1.2.1.1 CO_2 Emissions

In considering agricultural waste management, we considered the work of Macías-Corral's *et al.* [6], who demonstrated the applicability of a two-phase anaerobic digestion system. This study evaluated the co-digestion of various waste forms, including municipal solid waste (MSW) and cow manure (CM) by such a digestion system [6]. Further, the digestion of individual residues (MSW and CM) were investigated separately to evaluate the effect of co-digestion.

Amongst the principal conclusions developed after they characterized the waste type treated and the method applied to convert waste into energy were:

- The use of a reactor for the two-phase anaerobic digestion of each sample presented an average CH_4 methane content of greater than 70%.
- The mixture of 90% of MSW and 10% of CM showed the highest production of biogas with a productivity of 172 m^3 CH_4/ton in dry garbage.
- The mix between MSW (90%) and CM (10%) experienced a weight reduction of 78.3% and a reduction in volume of 98% after 141 days, proving to be the most efficient mixture of co-digestion.

To quantify GHG emissions within this work, the US Environmental Protection Agency's methodology [7] was applied. In this quantification a data series of livestock numbers and evolution (2004 to 2011) was collated from statistics sourced from the Ecuadorian Institute of Statistics and Census [8]. From this data series, we have projected livestock numbers to

2025, considering an equivalent annual rate of increase. These projections have been developed because when adjustments were made to this series using tools such as @RISK, the estimates were found to be of poor quality and differed considerably based upon the statistical criteria used (Chi -square, Anderson-Darling, Kolmogorov-Smirnov). Therefore, for each item of the dataset, annual percentage rate changes were applied based upon the period covered within the dataset to develop the projections to 2025.

Estimates of GHG emissions were calculated as follows: the Ecuadorian livestock population was firstly reclassified into five categories (following Cuéllar & Webber [9] and EPA [7]). 1) feeding cattle, 2) dairy cows, 3) other meat and dairy cattle, 4) pigs, and 5) poultry. The reclassification consisted of transforming physical units (1000kg/ livestock type) of livestock as per the original data, into animal units. This transformation profited from using the conversion factors described by Kellogg, Lander, Moffitt, & Gollehon [10] and Cuéllar & Webber [9].

Following this transformation from physical units to animal units, we proceeded to estimate the amount of manure excreted, once again following the methodology described by the EPA [7]. For this, we considered that during the processing of manure two GHGs are emitted; methane (CH_4) and nitrous oxide (N_2O). Methane excreted directly by the livestock through enteric fermentation are distinct from those emitted from the processing of manure, which are another important source of GHGs emissions. However, as part of this work, only emissions produced directly from the manure processing were considered.

The calculation of CH_4 and N_2O emissions firstly required an estimate of the volume of manure excreted by each livestock type. The volume of manure excreted by cattle was calculated using Formula 1:

$$VS excreted_{Animal,WMS} = Population_{Animal} \times VS \times WMS \qquad (1.1)$$

VS refers to the volatile solid production rate (kg VS/animal/year), whereas WMS is the distribution of manure by Waste Manure System for each animal type (percent) and Animal Population represents the number of animal units per each 1000kg. The formula estimates the amount of VS excreted within each managed WMS for each animal type (kg/yr).

To calculate the volume of manure excreted by other animals, the following formula was used:

$$VS excreted_{animal,WMS} = Population_{animal} \times VS \times WMS \times 365.25 \qquad (1.2)$$

The animal population represents the number of animal units per 1000 kg, VS refers to the volume excreted (expressed in kg per day) by animal type and WMS is expressed as a percentage which indicates the type of manure management system used based upon the livestock farming during the production process. 365.25 is a factor applied to annualize VS, with VS expressed in kg per day, with the factor correcting it to Kg per year.

Once these calculations had been made for the total annual manure excretion of manure, an estimate can be made of the amount of CH_4 emitted during the management process of the manure. The emissions emitted from the manure can be expressed in Giga grams (Gg) using the following formula:

$$CH_4 = \sum_{animal,WMS} (VS excreted_{animal,WMS} \times B_0 \times MCF \times 0.662) \quad (1.3)$$

B_0 represents the quantity (m^3) of CH_4 emitted per kg of manure excreted by animal type, with MCF representing the methane conversion factor by type of manure management system and 0.662 being a factor which corresponds to the density of methane at a temperature of 25 °C (kg CH_4/m^3 CH_4).

As mentioned, the amount of CH_4 was initially expressed in Gg, which required converting into Tera grams (Tg) of CO_2 equivalent. This could also have been expressed in millions of tons of CO_2 equivalent. To perform the conversion to Tera grams (Tg) of CO_2 equivalent Formula 1.4 was applied:

$$Tg\, equiv.\mathrm{CO_2} = \frac{(Gg\,\mathrm{CH_4} \times GWP)}{1000} \quad (1.4)$$

Following the calculation of CH_4 emissions, estimates of N_2O emissions were implemented. However, before doing so it was necessary to consider that there are two types of emissions; direct and indirect. To calculate these emissions, it was necessary to estimate the amount of nitrogen (N) excreted per animal type; therefore we applied Formula 1.5:

$$N excreted_{animal,WMS} = Population_{animal} \times WMS \times Nex \quad (1.5)$$

Animal population represents the number of animal units per 1000 kg of weight. WMS is expressed as a percentage which indicates the type of manure management system used based upon the livestock farming during

the production process. *Nex* refers to the amount of N excreted (expressed in Kg) by type of animal per year.

Formula 1.5 permitted the calculation of the volume of N emitted by cattle, however, and similar to Formula 1.1, this does not consider emissions from other livestock animals. The emissions of these other livestock types were calculated using Formula 1.6:

$$Nexcreted_{animal,WMS} = Population_{animal} \times WMS \times Nex \times 365.25 \quad (1.6)$$

Animal population represents the number of animal units per 1000 kg. WMS is expressed as a percentage which indicates the type of manure management system used based upon the livestock farming during the production process. Nex refers to the amount of N excreted (expressed in Kg) by animal type per day. 365.25 is a factor applied to annualize Nex, with Nex expressed in kg per day, with the factor correcting it to Kg per year.

Following the estimation of N excretion per animal type, we calculated direct N_2O emissions (Gg) using the following formula:

$$Direct\ N_2O = \sum_{animal,WMS} (N\,excreted_{animal,WMS} \times EF_{WMS} \times \frac{44}{28}) \quad (1.7)$$

EF_{WMS} refers to direct N_2O direct emissions per manure processing system according to the guidelines of the IPCC and is given by the ratio (kg N_2O-N/kg N). The constant 44/28 refers to the conversion factor of N_2O-N into N_2O.

In addition to direct emissions of N_2O, indirect emissions were estimated using Formula 8:

$$Indirect\ N_2O = \sum_{animal,WMS} \left(\left[N\,excreted_{animal,WMS} \times \frac{Frac_{gas,WMS}}{100} \times EF_{volatilization} \times \frac{44}{28} \right] + \left[N\,excreted_{animal,WMS} \times \frac{Frac_{runoff/\,each,WMS}}{100} \times EF_{runoff\,/leach} \times \frac{44}{28} \right] \right) \quad (1.8)$$

$Frac_{gas,WMS}$ indicates Nitrogen lost through volatilization in each WMS, $EF_{voltalization}$ indicates the emission factor for the volatilization (0.010 kg N_2O-N/kg N). $Frac_{runoff/leach,WMS}$ indicates the N lost through runoff and leaching per WMS; $EF_{runoff/leach}$ indicates the emission factor for runoff and leaching (0.0075Kg N_2O-N/kg N). The constant 44/28 refers to the conversion factor of N_2ON to N_2O.

Similar to the estimates of methane emissions, nitrous oxide emissions from manure management were converted from giga grams to tera grams (Tg) of CO_2 equivalent using Figure 1.9:

$$Tg\,equiv.\mathrm{CO}_2 = \frac{(Gg\,\mathrm{N}_2\mathrm{O} \times GWP)}{1000} \tag{1.9}$$

GWP refers to the global warming potential of N_2O.

1.2.1.2 Potential Electricity Power

The power generation potential of manure was determined by following the methodology previously applied in Cuéllar & Webber [9], whose high-level assessment aimed to consider the potential for converting manure into biogas.

To identify the amount of energy that could be generated from Ecuadorian livestock manure, the existing animal numbers (per 1000 kg) described earlier were used. The estimates of energy potential of livestock manure ($E_{biogas}[BTU]$) considered in billions of BTU per year, was calculated using Formula 10:

$$E_{biogas}\left[BTU\right] = Population_{animal} \times FEB_{animal} \times 365.25 \tag{1.10}$$

Following Chastain, Linvill, and Wolak [11], FEB_{animal} work which indicates the gross energy factor not converted into biogas per animal type, expressed in thousands of BTU/animal per day. 365.25 is used to convert FEB from a daily to an annual value.

Using the estimate of unconverted energy to gross biogas energy potential, the biogas-based electricity potential was calculated. However, to calculate this we must consider the dependence upon the efficiency of the electric generator during the conversion from biogas to electricity. Biogas can normally be converted into electric with an efficiency range of 34–40% for large turbines, and 25% for smaller generators [9, 12, 13]. We followed Cuéllar & Webber [9] and applied a range of efficiency (η) from 25% to 40% in determining the $e_{biogas}[kWh]$ biogas-based electric potential of Ecuador, calculated using Formula 1.11:

$$e_{biogas}\left[kWh\right] = E_{biogas} \times 0.000293\left[\frac{kWh}{BTU}\right] \times \eta \tag{1.11}$$

The coefficient 0.000293 allowed for the transformation from BTU units into kWh; the result of the calculation was expressed in millions of kWh per year of biogas-based electricity generation.

The potential environmental benefits of biogas use are generally higher when used as a replacement fuel for gasoline or diesel vehicles. This is due to the substantial reduction in air pollutants such as hydrocarbons, oxides nitrogen, particles, etc. However, when biogas is used to replace fuel oil or natural gas for large-scale production of heat and electric power, comparative reductions in pollutants are small or insignificant [3, 4, 14]. In consideration of this, in addition to quantifying the energy potential of manure-based biogas, the amount of CO_2 emitted during combustion of biogas, whilst generating electricity was quantified. To calculate this, emissions of CO_2 produced were determined once again following the work of Cuéllar & Webber [9], who applied the following formula:

$$kg_{CO_2total} = 1m^3_{biogas}\left(X\%CH_4 \times \rho CH_4 \times 2.75 + \rho CO_2\left(1 - X\%CH_4\right)\right) \tag{1.12}$$

kg_{CO_2total} represents the total emissions of carbon dioxide emitted from combustion of one cubic meter of biogas. X%CH_4 indicates the percentage of CH_4 in a cubic meter of biogas. ρCH_4 represents the density of methane under normal conditions. 2.75 represents the constant that indicates that the combustion of 1 kg of pure methane results in 2.75 kg of CO_2 being generated. ρCO_2 is the density of CO_2 under normal conditions; in addition, it multiplies the factor that indicates the % of CO_2 in cubic meters of biogas.

Following the estimate of the amount of CO_2 emitted from the combustion of one cubic meter of biogas, we determined the amounts of CO_2 (kg) emitted, per kWh of electric power generated during combustion. To do this, the following formula was applied:

$$Z_{CO_2} = \frac{1m^3_{biogas}\left(X\%CH_4 \times \rho CH_4 \times 2.75 + \rho CO_2\left(1 - X\%CH_4\right)\right)}{E_{\%CH4}\eta} \tag{1.13}$$

The numerator of this formula references kg_{co_2total}, which is calculated by applying formula (12). Whereas the denominator refers to the energy content according to the percentage of CH_4 in biogas, multiplied by the efficiency factor.

With the amount of CO_2 (Kg) emitted per kWh generated from the combustion of biogas calculated, it was possible to calculate potential CO_2 emissions (tonnes) from potential energy generation from manure. For this we considered a CH_4 content of 60% and η = 25%. Further, CO_2 emissions (tons) were also calculated considering a CH_4 content of 70% and η = 40% by multiplying Z_{co2} (the amount of CO_2 emitted per kWh generated) by $e_{biogas}[kWh]$ (kWh generated from livestock manure-based biogas).

1.2.2 Biodiesel

In the case of biodiesel, unlike biogas, it is found in international markets, thanks to large volumes of imports and exports. The major net exporters of biodiesel are Argentina, Brazil, Indonesia, Malaysia and the United States, with Argentina being the largest. The origins of biodiesel can be traced to Argentina and Brazil with soy, soy and rapeseed in the United States and palm oil in Malaysia and Indonesia [15].

Analysis of agricultural markets using mathematical models and partial equilibrium modeling suggests that global exports of biodiesel will increase from 626 million gallons in 2012 to 936 million by 2025 [16]. Malaysia and Indonesia are modeled to see the greatest growth as net exporters, together modeled to exceed the Argentinian soy-based exports. Figure 1.5 highlights the expected dynamics of international markets between 2010 and 2025, based upon the model FAPRI [16]. The figure demonstrates the shift in the markets and how present export leaders become increasingly less important by 2025. For example, in 2010, Argentina had a ratio of market percentage to percentage number of exporting countries of 3.29 and 3.42 in 2011. However, by 2025 this ratio reduces to 2.06, indicating a clear shift and distribution of exports caused by growth in Indonesia and Malaysia, which by 2025 will have a combined ratio of 2.59.

Figure 1.5 also presents additional information, including the profit margin of biodiesel from shop floor to the FOB price within the European Union, with an average margin of 20%. The EU is the biggest consumer of biodiesel worldwide, consuming 84% of global exports in 2012 and 99% by 2025.

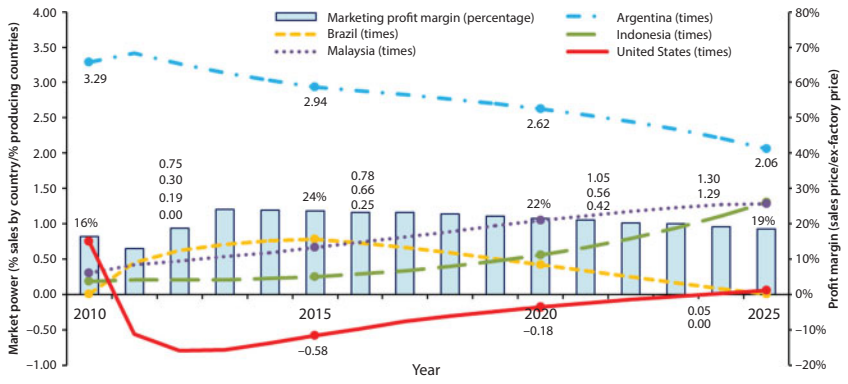

Figure 1.5 Trends of market power and biodiesel margins.
Source: [16], Formulated by the authors

1.2.2.1 Biodiesel in Ecuador

The second most important energy crop within Ecuador in terms of agricultural land occupied is the African Palm, which in 2006 was found by the Oil Palm Census to cover 207,285 hectares. From 42.43% of this area, production was found to be 709,424 (tonnes), corresponding to yields of 8/t/ha/year.

Palm crop have prepared a map of "Agro-ecological zoning of palm cultivation under natural conditions" [17]. The total area established in this map highlights an additional 408,938 hectares and represents the available locations for palm oil extraction at the national level, and disaggregated locations at the provincial level. These maps have shown the importance of two provinces (Esmeraldas and Santo Domingo), both of which contain more than 80% of the 42 national plants, with 43% and 38%, respectively.

The cultivated area of African Palm in 2011 had increased to 244,574ha, of which 98% was solely palm and another 2% mixed cropping, with the area of productive and harvested palms covering 202,650ha. Annual production totalled 2,907,356 tonnes, with yields of 10.34 tonnes per hectare, representing a 29.25% increase in productivity in 5 years.

In 2012, Ecuador consumed 10.29 billion litres of fossil fuels, of which 4.57 billion was diesel, sold at 0.26 dollars per litre [18]. Figure 1.6 shows the evolution (2008–2012) in the consumption of the three most important liquid fuels within Ecuador; the left-hand axis demonstrates the volume of fuel consumed (millions of litres). The figure shows that liquid petroleum gas (LPG) sees the lowest growth in demand, growing to only 65.3 million litres. Whereas in the cases of diesel and petrol, demand is more dynamic

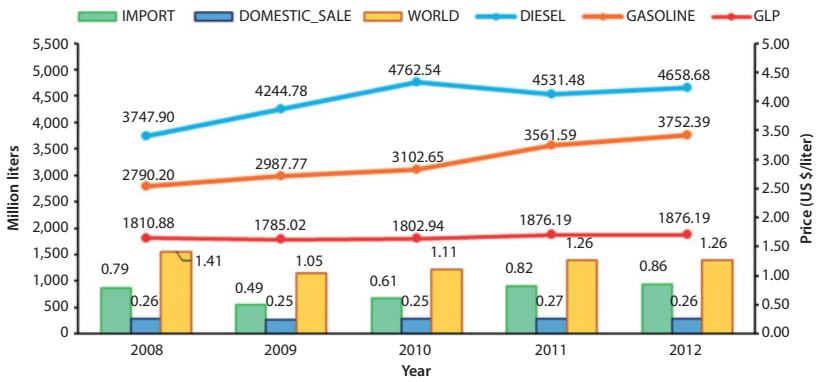

Figure 1.6 Fuel consumption, diesel price and world biodiesel price.
Source: [16, 18], Developed by the authors

with petrol consumption seeing an increase of just under 1 billion litres, up from 2.79 to 3.75 billion litres between 2008 and 2012 [18].

In this section a brief analysis will be made of the trends in diesel consumption within Ecuador, whilst projecting future consumption patterns based upon observed trends. Projections will be made of the expected volume of consumption in billions of litres by 2025; the period for this analysis is justifiable due to the timeline of the Ecuadorian government's development strategies. Although fuel demand depends on various factors such as the number of cars and income level, as well as international fuel prices, to simplify the analysis and projections we have assumed a constant growth rate in demand based upon historical information.

The right-hand vertical axis of Figure 1.6 shows fuel prices in dollars per litre, with the evolution of import prices and internal sale prices highlighted. The difference between these two prices indicates the government subsidy, which in 2012 totalled 1.6 billion dollars [18]. Figure 1.6 also demonstrates the international price changes of biodiesel, showing that the global average price up to 2012 had risen above $1.2 per litre. To project the international market biodiesel prices up to 2025, information has been taken from the model designed by the Food and Agricultural Policy Research Institute (FAPRI).

In considering data from 2012 and the implementation of the governmental Decree No. 1303 for the promotion of biofuel production, domestic Ecuadorian demand for biodiesel in the mix of premium diesel with 5% biodiesel (dieselB5) has been about 232 million litres, and if the mix requires 10% biodiesel (dieselB10) consumption would be around 465 million litres.

In order to project import prices and internal sales to 2025, the rate of annual performance was assumed to follow historical information, whilst projections of global biodiesel prices from the FAPRI model will be used [16]. Figure 1.7 shows the expected evolution in area available for energy crops across Ecuador, as well as the required amount of biodiesel to meet the characteristics of dieselB5 and dieselB10.

1.2.3 Microalgae Biodiesel

The production of biodiesel requires three steps to convert biomass to biodiesel; biomass production, extraction of lipids and the transesterification to obtain the biodiesel. There exists a range of technologies available for the production of biomass from microalgae including photoautotrophic production, heterotrophic production and mixotrophic production [19].

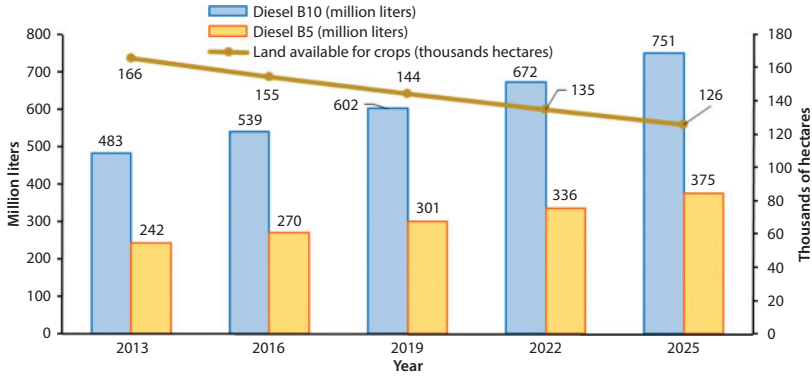

Figure 1.7 Potential demand of biodiesel and available are for energy crops.
Source: Data series 2004–2011[18][8], Projections to 2025 made by author.
Developed by the authors

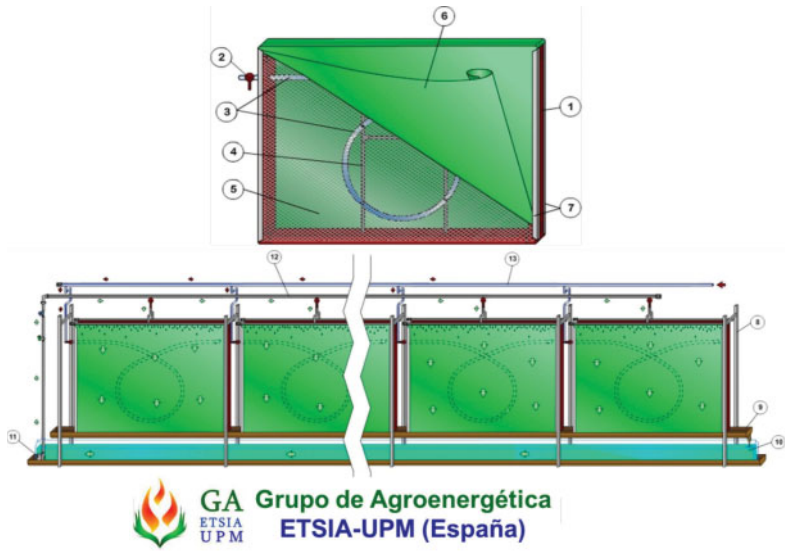

Figure 1.8 Photobioreactor sheet.
Source: [21]

The production of algal biomass aligns perfectly with the principal of the circular economy; the production of algal biomass would be fertilized with CO_2 emitted from the combustion of biogas as part of the electrical generation process. This CO_2 would then be converted to O_2 via the process of photosynthesis; as such we consider that photoautotrophic production would be the most appropriate technology within our proposed system.

1.2.3.1 Biomass Production

The recovery of microalgae biomass generally requires one or more solid–liquid separation processes, which is a particularly challenging phase of the algal biomass production process [20] accounting for 20–30% of production costs. The processes involved within production include flocculation, filtration, flotation, and centrifugal sedimentation, some of which are energy intensive [19].

Within photoautotrophic production exist a diversity of production systems including open pond, closed photobioreactor and hybrid [19]. As part of the proposed circular economy, analysis will be made of microalgae production from photobioreactor sheets [21] as well as in open ponds.

The purpose of using the photobioreactor sheets is that they can be directly supplied with the CO_2 produced from biogas combustion, allowing for harvesting of algae every 5–7 days from the vertical panels with approximate humidity values between 25–50%. The first large-scale production of microalgae using photobioreactor sheets is in Spain; however, technical results are still confidential. The next stage after production and harvesting of microalgae biomass is lipid extraction, permitting processing into biodiesel.

1.2.3.2 Lipid Extraction

A number of viable options are available for the conversion of algae biomass; such conversion considers the same processes used in the conversion of terrestrial biomass to energy.

These processes depend upon the biomass source, conservation options and the end use [19]. According to L. Brennan and P. Owende, we can separate conversion technologies of algae biomass into two basic categories, thermochemical and biochemical (Figure 1.9).

Following Figure 1.9, it appears that the most appropriate conversion technique would be thermochemical, with the processes identified for obtaining the biodiesel being thermochemical liquefaction and pyrolysis. Of these two, the process that is suggested for the circular system is thermochemical liquefaction. Thermochemical liquefaction is a low-temperature (300–350C), high-pressure (5–20 MPa) process aided by a catalyst in the presence of hydrogen [22], converting wet algal biomass material into liquid fuel [23]. However, reactors designed for thermochemical liquefaction and fuel-feed systems are extremely complex and therefore expensive [19], but are advantageous in their ability to convert wet biomass into energy [24], and one can consider that this process is a net producer of energy [25].

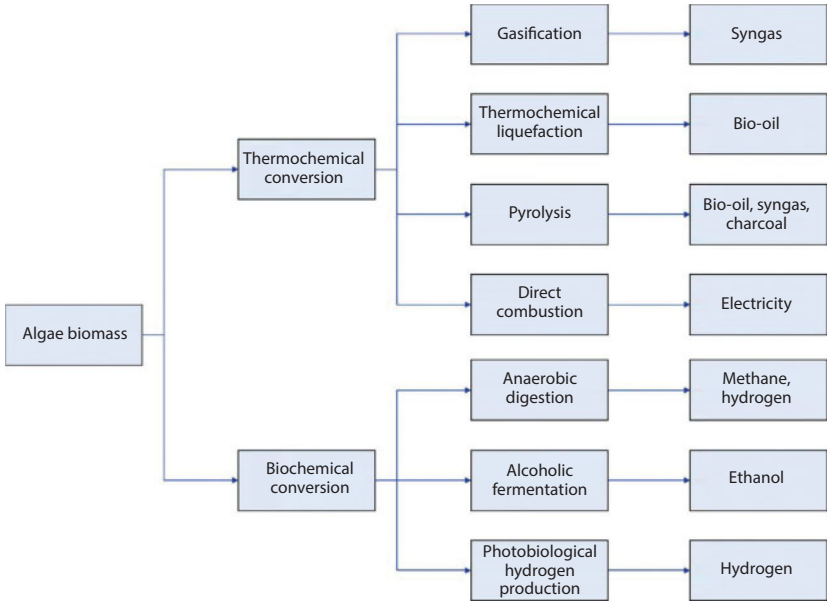

Figure 1.9 Potential algal biomass conversion processes.
Source: [19].

1.3 Microalgae versus Palm Oil in Ecuador

As part of this investigation, it is vital to compare the production of biodiesel from both microalgae and palm sources. As part of this analysis consideration will be made of environmental, technological and economic factors of both biodiesel sources. In this analysis we have considered four possible scenarios for the production of biodiesel;

S1 - access to capital and constraints on land availability for energy crops;
S2 - same as S1, but with the addition of tax incentives
S3 - constraints on land availability for energy crops, with limited access to capital, but with a government fund supporting 80% of total investment
S4 - same as S3, but with the addition of tax incentives.

In considering the environmental impacts from the production of these two sources of biodiesel, the quantity of CO_2 emitted through production of biogas, and the combustion of biogas in producing electricity were calculated. The technical factors have been highlighted previously with a

new alternative for the production of algae biomass using photobioreactor sheets. Economic factors considered relate to a cost-benefit analysis using as indicators, net present value and both private and public cost-benefits [2]. The analysis of biomass production considers various microalgae production systems, with technical data for transesterification taken from Kovacevic and Wesseler [26].

1.3.1 Palm Oil

To estimate the production of biodiesel from palm it is necessary to quantify the production and extraction processes of lipids and the transesterification for biodiesel data, which is provided by Kovacevic and Wesseler [26], who considered rapeseed oil in the European Union.

Considering the proposed scenarios for analyzing the potential of the proposed move towards a sustainable system, it is vital to consider the surface area available for energy crops in Ecuador, as well as the performance of products and by-products. These have been estimated for the period 2013 to 2025, along with the estimated costs of production at each stage; biomass production, extraction and transesterification of lipids for biodiesel. Figures 1.10 and 1.11 present revenues and estimated costs of palm biodiesel production in Ecuador.

Figure 1.10 demonstrates the yields (t/ha) generated from palm production for biomass, lipids and glycerol. The figure also highlights the potential income in USD/ha for both biomass cake and glycerol, whereas Figure 1.11 presents the expected temporal variation in capital and operational costs for each of the three phases associated with biodiesel production.

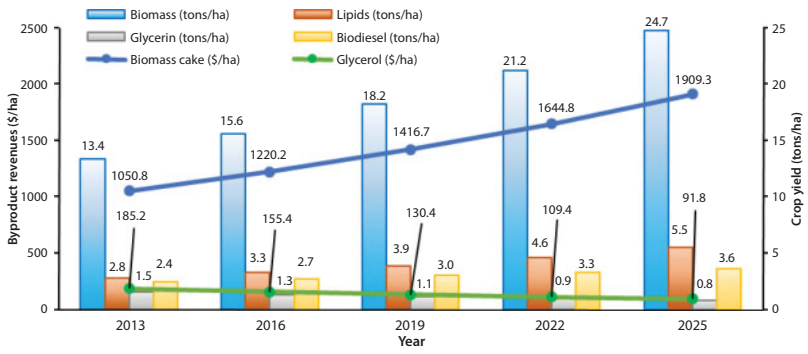

Figure 1.10 Yields and revenues from palm oil biodiesel production and by-products.
Source: [26, 27], Projections to 2025 based [8]. Developed by the authors.

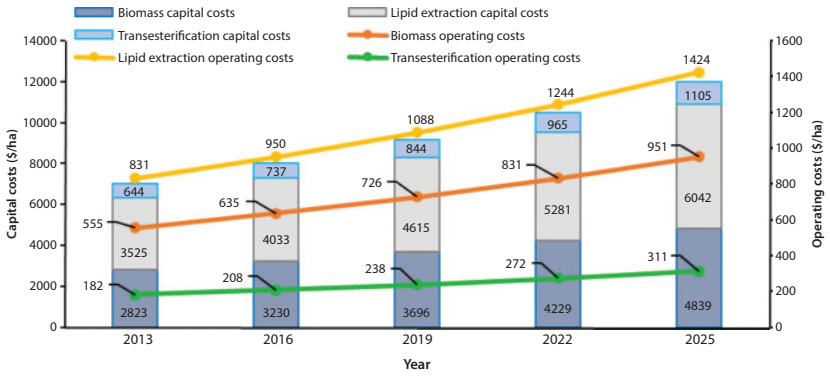

Figure 1.11 Capital and operational costs of palm based biodiesel production to 2025. *Source*: [26, 27], Projections to 2025 based [8]. Developed by the authors.

1.3.2 Microalgae Oil

In the case of biodiesel production from microalgae, it is necessary to quantify the positive effects of this crop within the proposed circular system. For this, the environmental benefits of this proposal will be analyzed, considering CO_2 emissions and the energy potential for processing of both solid urban waste and cattle manure. The following figures demonstrate total Ecuadorian cattle numbers up to 2025, estimates of potential CO_2 mitigation and the monetary equivalent from carbon credits. Further, the amounts of energy in KWh, which can be generated from biogas anaerobic digestion, along with the monetary equivalent of the electricity produced were all calculated.

Figure 1.12 shows the cattle population in Ecuador up to 2011 and projected beyond to 2025. The units within the figure correspond to the number of animals multiplied by the conversion factors, allowing for the conversion of stock weights in 1000 kg to animal numbers. Figure 1.13 highlights the estimation of CO_2 emissions from manure management and projected emissions up to 2025, based upon the change in cattle numbers as seen in Figure 1.12. In addition to CO_2 emissions, it also provides a trend of potential income, received in lieu of CO_2 emissions through the application of co-digestion systems (MSW and CM) as proposed by this circular system strategy proposal.

Figure 1.14 presents the potential energy produced from biogas production from the co-digestion of MSW+ CM; it shows the energy generated (millions of kWh) from motors with various efficiency ranges (25–40%). In addition, the figure also quantifies monetarily (millions USD) the potential electric production from biogas production.

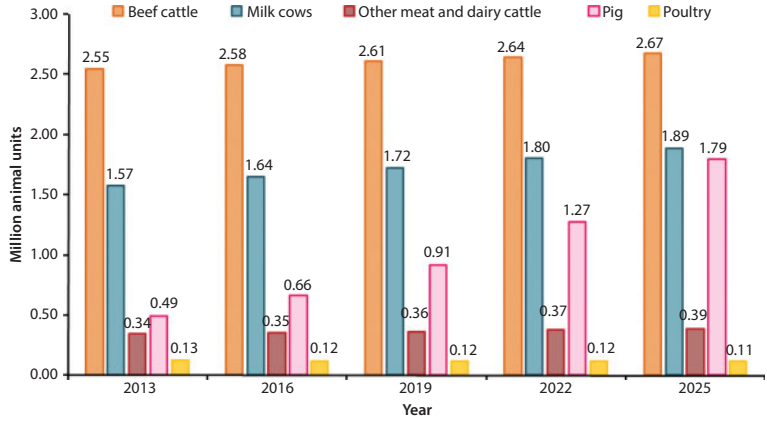

Figure 1.12 Ecuadorian cattle numbers (1000kg) to 2011 and project up to 2025.
Source: [8], Developed by the authors.

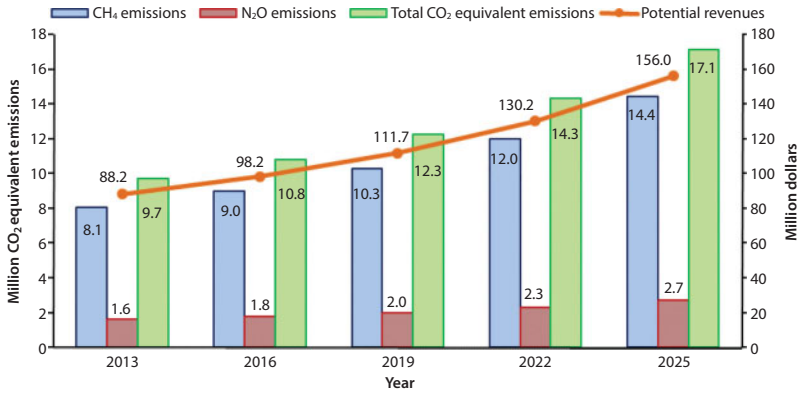

Figure 1.13 Emissions of CO_2 equivalent and potential revenues from mitigation in Ecuador up to 2025.
Source: Data 2004–2011 [8]; Projections to 2025 made by author; methodology and formulas used in calculation [7], Developed by the authors,

Figure 1.15 shows the CO_2 emissions from the combustion of biogas during the process of electric generation with regards to CO_2 emissions from cattle manure management, the values in the graph are presented in millions of tons of CO_2.

For the proposed circular strategy and the production of microalgae, we analyzed two algal biomass production systems, as well as the extraction processes for lipids and transesterification, finding that they are equivalent in both alternative systems. Capital and operating costs have been taken and adapted from the work of Kovacevic *et al.* [26].

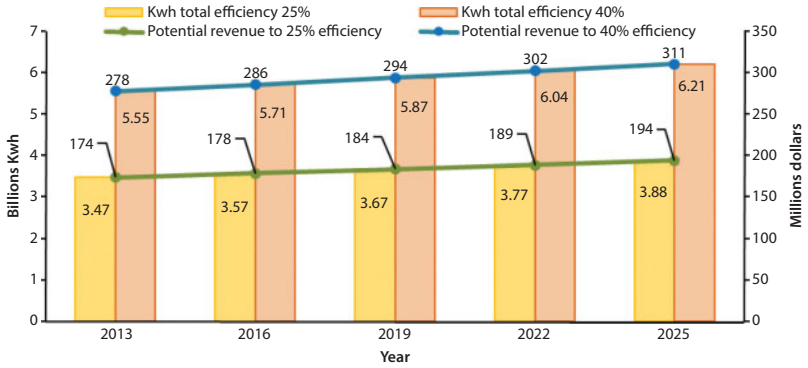

Figure 1.14 Potential revenues and potential energy production (KWh) from biogas production in Ecuador.
Source: Data 2004–2011 [8]; Projections to 2025 made by author following methodology and formulas of [9], Developed by the authors.

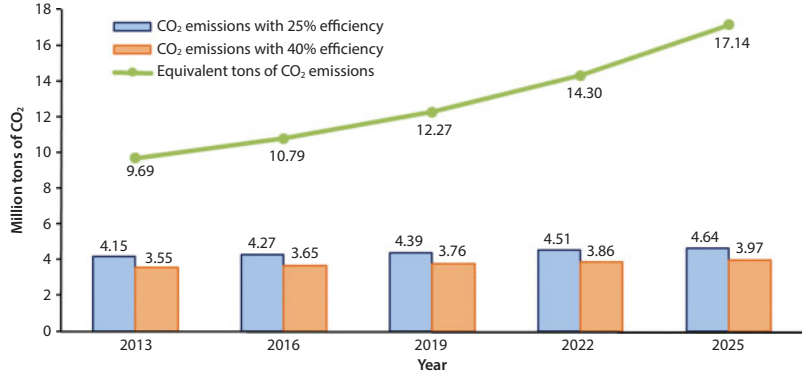

Figure 1.15 Emissions of CO_2 from biogas combustion and emissions of CO_2 equivalent.
Fuente:Methodology and formulas of [9], Developed by the authors.

1.3.2.1 Microalgae in Open Ponds

Figure 1.16 presents the projected values of the capital and operational costs (USD/ha) for the production of microalgae in open ponds. It also shows the costs for each of the three phases within the production of biodiesel, whilst highlighting the high operational and capital costs of this production system.

Figure 1.17 demonstrates the yields from microalgae in open ponds, as well as yields from other products during production of biodiesels. The yields of biomass, lipids, glycerine and biodiesel are expressed in tons per hectare. Additionally, to yields, estimates of the potential revenue made

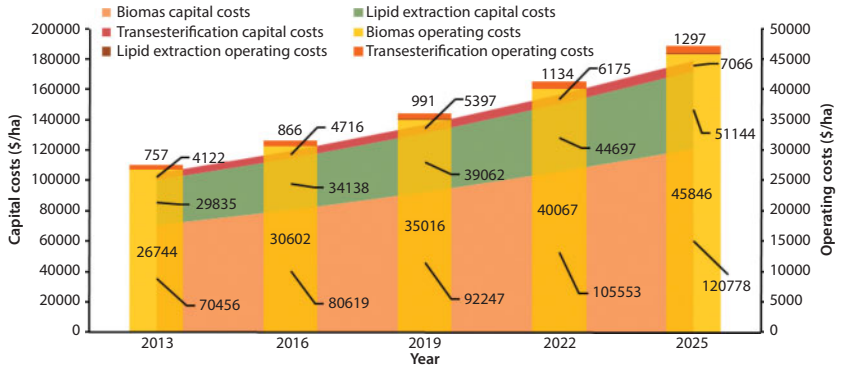

Figure 1.16 Capital and operational costs of microalgae based biodiesel production to 2025.
Source: [26], projections to 2025 based upon[8], Developed by the authors.

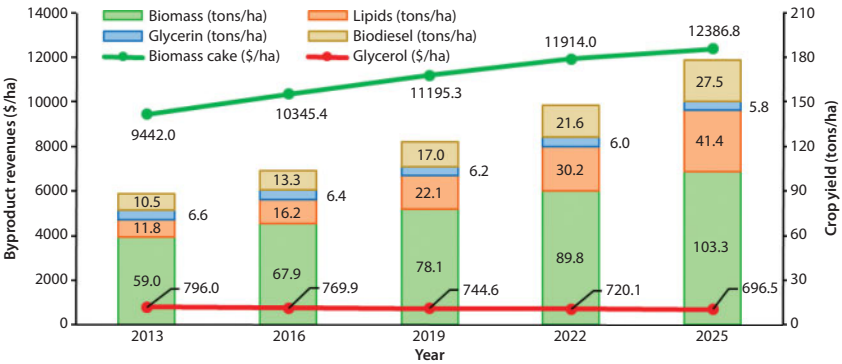

Figure 1.17 Yields and incomes from by-products of microalgae based biodiesel production.
Source: [26], projections to 2025 based upon[8], Developed by the authors.

from by-products such as biomass cake and glycerol have been included in USD/ha.

1.3.2.2 Microalgae in Laminar Photobioreactor

Figure 1.18 details the capital and operating costs for producing microalgae in photobioreactor sheets, rather than in open ponds. As with open ponds, the operating costs represent the greatest cost in the production process.

Figure 1.19 presents estimated yields and income from photobioreactors sheets. Yields of biomass, lipids, glycerine and biodiesel are all expressed

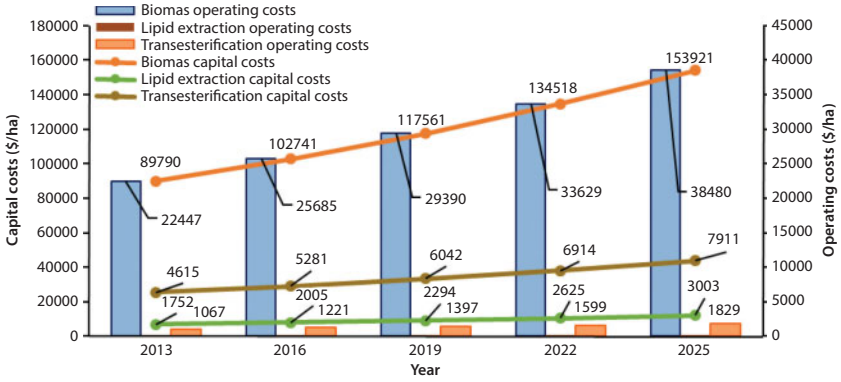

Figure 1.18 Capital and operational costs of microalgae2 up to 2025.
Source: [21, 26], projections to 2025 based upon [8], Developed by the authors.

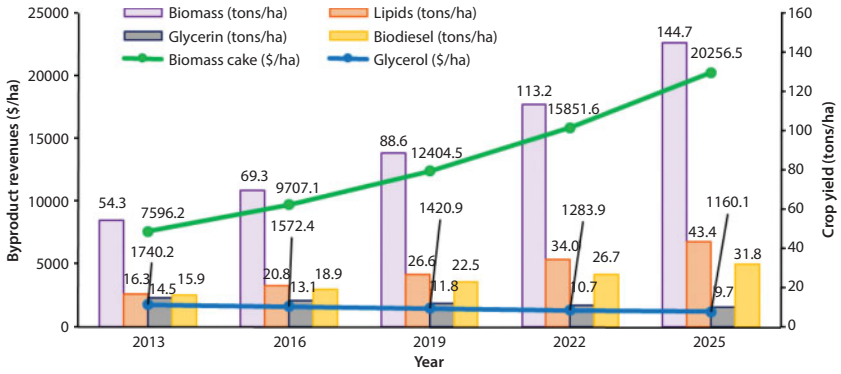

Figure 1.19 Yields and incomes from the by-products of microalgae2 based biodiesel production
Source: [21], projections to 2025 based upon [8], Developed by the authors.

in tonnes per hectare, whilst the potential income from biomass cake and glycerol are shown in USD per hectare.

One of the by-products of biodiesel production is biomass cake, which even after lipid extraction contains high levels of both protein and carbohydrates. This cake could therefore in theory provide an important input as animal feed; as such it is important to identify the potential availability of this feed, as presented in Figures 1.20 and 1.21.

Figure 1.20 shows the potential quantities of animal feed from both palm and microalgae biodiesel production in millions of tons for scenarios S1 and S2. In addition, monetary quantification of the potential revenue

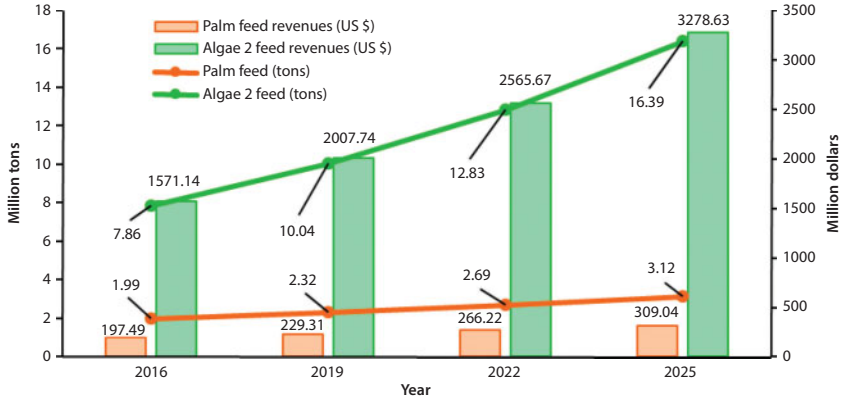

Figure 1.20 Scenarios 1 and 2- potential revenues and tons of biomass cake based animal feed.
Source: Mathematical model developed in GAMS and results from Excel solver, projections to 2025 based upon[8], Developed by the authors.

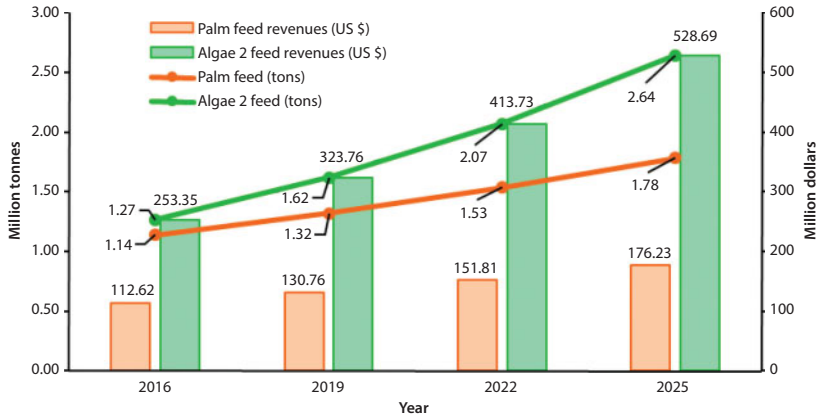

Figure 1.21 Scenarios 3 and 4 - potential revenues and tons of biomass cake based animal feed.
Source: Mathematical model developed in GAMS and results from Excel solver, projections to 2025 based upon [8], Developed by the authors.

from the sale of this feed is shown; these values are expressed in millions of dollars. Figure 1.21 shows the same elements as Figure 1.20, but represent values estimated from application of scenarios S3 and S4.

Figure 1.22 presents potential feed consumption per 1000 kg of cattle in Ecuador for the four proposed scenarios.

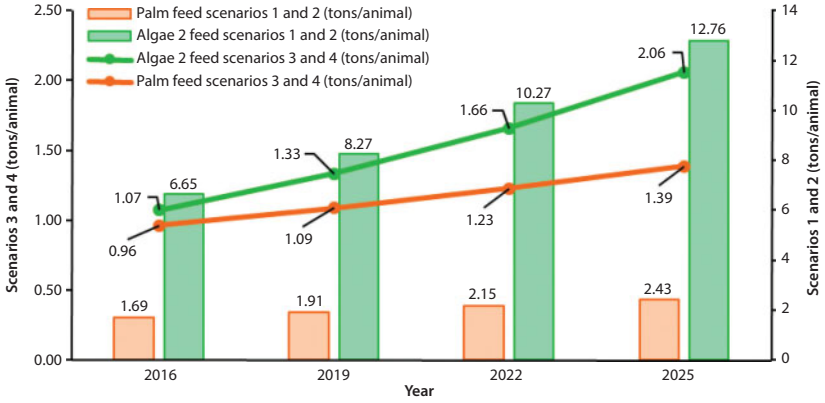

Figure 1.22 Potential animal feed availability per animal unit in Ecuador.
Source: Mathematical model developed in GAMS and results from Excel solver, projections to 2025 based upon [8], Developed by the authors.

1.4 Discussion

Detailed financial analysis of biodiesel production technologies have been made by Vega-Quezada, C. *et al.* [2], the results of which are extremely relevant to our own. Of the four scenarios implemented for the development of the bioenergy sector in Ecuador, scenario 4 should be considered the most probable based upon present trends, as the production of microalgae in open ponds was found to be relatively unprofitable both publicly and privately in scenarios 1, 2 and 3.

Table 1.3 shows income and monetary expenses for the production of biodiesel from palm; the MSW in this case has not been quantified because the data were limited on the treatment of MCW and the amounts generated per year. In the case of CM, emissions of CO_2 equivalent by 2025 were found to be 155.52 million tonnes, which in Ecuador represents an opportunity cost between $810-928 million, as the production of biodiesel from palm is not considered, or is not compatible with initiatives of a circular system and is unsustainable in terms of MSW treatment. As for the production of biodiesel, it was found that 100% of the potential surface area available for energy crops would be occupied by 2025, with the amount of biodiesel produced meeting only 90% of domestic demand for biodiesel B5. It is estimated that the Net Present Value (NPV) in terms of private investment for the production of biodiesel from palm is $2.6 billion.

Table 1.4 presents the estimated revenues and expenditures for the development of microalgae based biodiesel, as part of the proposed

Table 1.3 Individual revenues and expenditures associated with proposed initiatives.

	Activity	Quantification	Costs/Benefits
Urban organic residues	CO_2 emissions and leachates to subsoil	N/A (–)	N/A (–)
Agricultural organic residues	Livestock manure CO_2 equivalent emissions	155.52 million tonnes of CO_2 equivalent	810-928 million dollars (–)
Biodiesel production	African Palm production	Use of 161,855 ha and satisfaction of 90% of internal demand for biofuels	2.6 billion (+)

Developed by the authors.

Table 1.4 Revenues and expenditures as part of the proposed circular system.

	Activity	Quantification	Costs/Benefits
Livestock Sector	Manure storage cost	N/A	
Synergy of MSW + CM	Mitigation of emissions of CO_2 equivalents	Reduction 155.52 million tonnes of CO_2 equivalents	810–928 million dollars (+) obtainment of CER
Electricity Generation	Anaerobic digestion (ROU+ROA)	44.28–70.84 Mwh	2.39 billion dollars (+) Plant investment (–)
	Biogas combustion	52.91 million tonnes of CO_2 × biogas combustion	246–279 million dollars (–)
Biodiesel Production	Algae production in photobioreactor sheets	Use of 26,100 hectares and supply 112% of internal demand for biofuels	4.36 billion dollars
	CO_2 absorption during biogas combustion	52.91 million tonnes CO_2 × biogas combustion	246–279 million dollars (+)
	Rural algae production	N/A	N/A
	Livestock feed	8.42 billion tonnes	

Developed by the authors.

circular system. Unlike for palm production, there are potential synergies between MSW + CM for electricity generation based upon microalgae.

Unlike the production of biodiesel from palm, CO_2 emissions from manure management in the livestock sector could be mitigated to the financial benefit of $810-928 million, which could become an income through the development of carbon credits. Electricity generation from biogas has been estimated to be between 44.28 to 70.84 gross MWh, which is equivalent to revenues from electricity cogeneration of $2.39 billion, excluding investment and operational costs of the plant. The combustion of biogas produces an estimated 52.9 million tonnes, which could be deducted from revenues from carbon credits.

The production of biodiesel from microalgae in photobioreactors sheets shows promising results considering that CO_2 emissions from biogas combustion can be absorbed fully, mitigating the emissions' negative impact. Under the conditions set in scenario 4, the area required for production of biodiesel would cover only 18% of the available surface area for energy crops, whilst meeting 112% of internal demand of biodiesel B5 by 2025. In terms of monetary benefits, NPV is estimated to be $4.36 billion; also, 8.4 billion tons of microalgae-based animal feed could be made available to the livestock sector at low prices, incentivizing manure collection and transportation from the nearest processing plant.

1.5 Conclusion

This chapter is limited to a theoretical assessment of the potential to produce biodiesel from microalgae with existing technologies. It can be inferred that the analysis of the elements as a whole provides greater benefits for the development of the bioenergy sector that the sum of individual benefits. The scope of this chapter has been exemplified by a specific initiative; a circular building system for biofuels presents promising results in technological, environmental, social and economic terms, allowing the conclusion that the proposed system is feasible.

Acknowledgements

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References

1. I. E. A. IEA, "Key World Energy Statistics 2012," Paris, 2013.
2. C. Vega-Quezada, M. Blanco, and H. Romero, "Synergies between agriculture and bioenergy in Latin American countries: A circular-economy strategy for bioenergy production in Ecuador," *Posted to Rev. J. New Biotechnol.*, pp. 1–9, 2016.
3. P. Börjesson and M. Berglund, "Environmental systems analysis of biogas systems-Part II: The environmental impact of replacing various reference systems," *Biomass and Bioenergy*, vol. 31, pp. 326–344, 2007.
4. M. Lantz, M. Svensson, L. Björnsson, and P. Börjesson, "The prospects for an expansion of biogas systems in Sweden - Incentives, barriers and potentials," *Energy Policy*, vol. 35, pp. 1819–1829, 2007.
5. I. E. A. IEA, "Renewables Information 2009," Paris, 2009.
6. M. Macias-Corral, Z. Samani, A. Hanson, G. Smith, P. Funk, H. Yu, and J. Longworth, "Anaerobic digestion of municipal solid waste and agricultural waste and the effect of co-digestion with dairy cow manure," *Bioresour. Technol.*, vol. 99, pp. 8288–8293, 2008.
7. U.S. E. P. A. "Inventory of U.S. greenhouse gas emissions and sinks: 1990–2011," 2013.
8. N. I. of S. and C. of E. INEC, "Viewer Ecuador Agricultural Statistics ESPAC," *INEC*, 2008. [Online]. Available: http://www.ecuadorencifras.gob.ec/estadisticas-agropecuarias-2/.
9. A. D. Cuéllar and M. E. Webber, "Cow power: the energy and emissions benefits of converting manure to biogas," *Environ. Res. Lett.*, vol. 3, p. 034002, 2008.
10. R. L. Kellogg, C. H. Lander, D. C. Moffitt, and N. Gollehon, "Manure Nutrients Relative to The Capacity of Cropland and Pastureland to Assimilate Nutrients: Spatial and Temporal Trends for the United States," *Proc. Water Environ. Fed.*, vol. 2000, pp. 18–157, 2000.
11. J. P. Chastain, D. Ph, D. E. Linvill, and F. J. Wolak, "On-Farm Biogas Production and Utilization for South Carolina Livestock and Poultry Operations PROJECT SUMMARY Performance of Mesophilic Anaerobic Digesters Table 1. Digester volume and energy production for on-farm biogas digesters maintained at a Manure," *Construction*, no. 1991, pp. 1–8, 1999.
12. P. H. Nielsen, A. M. Nielsen, and R. H. Frederiksen, "Heat and power production from pig manure," *Inst. Prod. Dev. Denmark*, 2004.
13. S. Tafdrup, "Viable energy production and waste recycling from anaerobic digestion of manure and other biomass materials," *Biomass and Bioenergy*, vol. 9, no. 95, pp. 303–314, 1995.
14. M. Sundberg, W. Johansson, and H. Hjortsberg, "Biogas in future agriculture and sustainable societies: effects on soil, environment and economy. Report," *Swedish, Recycl. Waste (Kretslopp och avfall)*, vol. 12, 1997.

15. A. Dufey and D. Stange, "Estudio regional sobre la economía de los biocombustibles en 2010: temas clave para los países de América Latina y el Caribe," 2011.
16. F. and A. P. R. I. FAPRI, "FAPRI, Food and Agricultural Policy Research Institute," 2012. [Online]. Available: http://www.fapri.iastate.edu/outlook/2012/.
17. M. of A. Magap, "Geoportal," 2012. [Online]. Available: http://geoportal.agricultura.gob.ec/.
18. B. C. del E. BCE, "OIL SECTOR REPORT , IV Quarter 2012," 2012. [Online]. Available: http://www.bce.fin.ec/index.php/component/k2/item/756.
19. L. Brennan and P. Owende, "Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products," *Renew. Sustain. Energy Rev.*, vol. 14, no. 2, pp. 557–577, Feb. 2010.
20. B. Wang, Y. Li, N. Wu, and C. Q. Lan, "CO2 bio-mitigation using microalgae," *Applied Microbiology and Biotechnology*, vol. 79, no. 5. pp. 707–718, 2008.
21. J. Fernández González, "Laminar photobioreactor for the production of microalgae," PCT PCT/ES2011/000104-WO2011138477, 2011.
22. H. B. Goyal, D. Seal, and R. C. Saxena, "Bio-fuels from thermochemical conversion of renewable resources: A review," *Renewable and Sustainable Energy Reviews*, vol. 12, no. 2. pp. 504–517, 2008.
23. V. Patil, K.-Q. Tran, and H. R. Giselrød, "Towards sustainable production of biofuels from microalgae.," *Int. J. Mol. Sci.*, vol. 9, no. 7, pp. 1188–95, 2008.
24. J. Clark and F. Deswarte, *Introduction to Chemicals from Biomass: Second Edition*. 2015.
25. T. Minowa, S. Yokoyama, M. Kishimoto, and T. Okakura, "Oil production from algal cells of Dunaliella tertiolecta by direct thermochemical liquefaction," *Fuel*, vol. 74, no. 12, pp. 1735–1738, Dec. 1995.
26. V. Kovacevic and J. Wesseler, "Cost-effectiveness analysis of algae energy production in the EU," *Energy Policy*, vol. 38, no. 10, pp. 5749–5757, 2010.
27. I. I. de C. para la A. IICA, "Cultivo de la Palma, Guía técnica," 2006.

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The Impact of Biomass Feedstock Composition and Pre-treatments on Tar Formation during Biomass Gasification

John Corton[1]*, Paula Blanco-Sanchez P.[2], Zakir Khan[3], Jon Paul McCalmont[1], Xi Yu[2], George Fletcher[4], Steve Croxton[5], James Sharp[3], Manosh C. Paul[3], Ian A. Watson I.[3] and Iain S. Donnison[1]

[1]IBERS, Aberystwyth University, Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, UK
[2]European Bioenergy Research Institute, Aston University, Birmingham B4 7ET, UK
[3]School of Engineering, University of Glasgow, Glasgow, G12 8QQ
[4]GF Consulting, Northampton, UK
[5]EON, Westwood Way, Westwood Business Park, Coventry CV4 8LG

Abstract

Gasification is a favourable technology for distributed power generation. However, commercialisation and scale up have been hampered by problems associated with tar formation. Tars are detrimental to operational efficiency as they can condense downstream initiating corrosion and blockages, thus resulting in a reduction in an overall yield during the gasification process. So far there are two main routes to reduce tar formation, namely thermal tar cracking at higher gasification temperatures, or catalytic tar cracking by using different types of heterogeneous catalysts, depending on the reaction system's configuration. Nevertheless tar still represents a potential issue during gasification, therefore further studies have been focused on trying to find a relationship between biomass composition and tar formation and composition. In this chapter we discuss various alternatives for biomass pre-treatment as a way to reduce tar formation during gasification through compositional manipulation. Engineering solutions provide a primary route to reduce tar formation, but further integrated processing offers increased system efficiently generated using tailored feedstocks. This may be achieved by harvesting energy or products from pre-treatment stages aimed at reducing tar formation and ash composition.

**Corresponding author*: jcc@aber.ac.uk

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2.1 Introduction

Fossil fuel reserves are in decline and there are negative environmental impacts associated with its use as an energy source. As a consequence there is a necessary focus on exploiting renewable and sustainable energy resources. One source of renewable energy is biomass and gasification is a promising way of converting biomass into usable energy carriers. Biomass gasification is a technology which is appropriate across a range of scales, including off grid electricity generation. Carbon dioxide emissions from biomass are accepted as neutral because of the comparatively fast rate of carbon fixation during growth [1]. Furthermore renewable energy supplies in the form of liquid, gas and solid fuel are reliant on biomass as a feedstock [2].

Gasification is a conversion technique whereby biomass is broken down, mainly into the gaseous components CO, H_2, CO_2, CH_4, H_2O and N_2, a mixture known as synthesis gas or syngas. In addition to the primary gaseous products a tar, ash and char fraction are produced during gasification [3]. The gasification process can occur in a variety of reactor types, however it is widely accepted that the most efficient reactors currently in use are fluidized bed reactors [4]. The process conditions include a low air/oxygen environment and temperatures between 700–1500 °C. Though combustion methods and technologies have increased in efficiency, gasification potentially has promise as a conversion route with an even higher efficiency rating especially when used in gas turbines, fuel cells or catalytic reactors [1].

Gasification is also an initial process step in some liquid fuel processes. These include the production of Fischer Tropsch fuels, some of which can be blended into current transport fuel distribution systems [5]. Methanol and dimethyl ether production also depend upon gasification as an initial process step, these products are both promising clean liquid fuels for the future, able to potentially replace diesel and petrol in the transport sector [6].

However the gasification process is hampered by processing challenges including the formation, condensation and further accumulation of tar. Tar formation is thus a major issue with regards to implementing gasification technologies. Tar condensation can occur onto mechanical surfaces, blocking equipment and causing corrosion, which is detrimental to the overall operational efficiency [3, 7]. In addition the produced gases require cleaning to remove tars before they are utilised in downstream processing,

which can be expensive and time consuming [8]. Examples of the international development of gasification technology at large commercial scale include Lahti Energy, in Finland; Essent, in Netherlands, and Agnion Technologies in Germany, therefore efficiency improvements including tar reduction, would potentially have large and positive commercial impacts.

Some operational and technical influences on tar production are covered in other works and not detailed in this chapter. The primary technical and operational influences on tar formation chemistry [9] during gasification include temperature [10], pressure [11]; run parameters such as gasifying medium [12], air to fuel ratio (equivalence ratio) and residence time [13], and the inclusion of catalysts [14] such as carbonates, oxides and hydroxides of alkali metals [15].

This study examines published research regarding the influence of biomass composition upon tar formation. We review various pre-treatment and processing options that alter biomass composition and impact upon tar production during gasification. The final section of this chapter considers future processing options that may reduce tar productivity and improve gasification efficiency.

2.2 Tar Composition

Tar is a generic term used to encompass the mainly aromatic and oxygenated compounds contained in the syngas coming from the gasification of solid waste or biomass. Milne *et al.* [16] pointed out that there are many definitions of tar and provided a broad diversity of compounds that can be found in the tar fraction. Li *et al.* [3] described tar as the condensable fraction of the organic gasification products, which are further described as being primarily aromatic hydrocarbons, including benzene. Fushimi *et al.* [17] listed the primary tar components such as levoglucosan, furfural and 5-methylfurfural (5-HMF). However in general tar can be referred to as 'all the organic compounds, produced under thermal or partial-oxidation regimes (gasification) of any organic material', and it is also known to be largely aromatic [16].

Some authors [18] have grouped tar compounds into five sub categories according to their molecular weight (Table 2.1). These categories are useful for appreciating the diversity of chemical species found in tars and for implementing the correct analytical procedures when undertaking analysis of the tar fraction.

Tar formation has been widely associated with the process temperature; Elliott proposed a scheme associating the tar compounds formation with

Table 2.1 The tar subcategories as reviewed by Li *et al.*,[6] elucidated by Ponzio *et al.*,[19] Benzene is a dominant component of classes 2–5.

Tar class	Class name	Property	Representative compounds
1	GC undetectable (very heavy)	Heavy tars undetected by GC	Remaining fraction when the GC detectable tar is subtracted from the total tar.
2	Heterocyclic aromatics	Tars containing hetero atoms.	Pyridine, phenol, cresols
3	Light aromatic (1 ring)	Light single ring hydrocarbons	Toluene, ethylbenzene, xylenes
4	Light poly aromatic hydrocarbon compounds (2–3 rings)	Condense at low temperature and concentration	Indan, indene, naphthalene, methyl naphthalene, biphenyl, acenaphthylene, acenaphthene, fluorine, phenanthrene, anthracene
5	Heavy poly aromatic hydrocarbons (>3- rings)	Condense at high temperatures and low concentrations	Flouroanthene, pyrene, chrysene

GC = gas chromatography.

the increase in the gasification temperature from 400 up to 900 °C. This diagram has been modified during the years since its publication by Milne and Evans [16, 19, 20]. A more recent tar formation trend according to the process temperature, has been reported by Basu, 2006 (Figure 2.1).

The increase in the temperature of lignocellulose results in the transformation of solid cellular material into an intermediate liquid; the further increase in the temperature, leads to the non-equilibrium liquids to produce volatile organic compounds (VOC's) [21]. Oxygen content tars or primary tars are generated during feedstock decomposition at around 400 and 500 °C, and include cellulose-derived products, analogous hemicellulose derived products and lignin-derived methoxyphenols. These

Figure 2.1 Tar formation pathway according to the process temperature.

compounds are further broken down and the fragments are recombined resulting in secondary tars including double bounded compounds such as phenolics and olefins. Methyl derivatives or tertiary tars are produced between 650 and 1000 °C, and finally condensed tars are composed mainly by aromatics and are formed at temperatures above 750 °C [16, 22]. One relevant parameter for a gasifier operation is the condensation temperature of tar compounds contained in the produced syngas, this parameter is known as tar dew point [23]. In general at temperatures below 350 °C tar compounds initiate to condense, and the condensation trend increases as the syngas temperature is decreased.

2.3 Tar Formation Cell Wall Polymers and Ash Composition

Plant cell wall material is the most abundant renewable biological resource on earth with an annual production of approximately 160 x 10^9 tonnes [24]. Lignocellulosic material is primarily composed by cellulose, hemicellulose and lignin. In terrestrial plants these polymers coexist in a complex microfibril structure, contributing with 14–28% lignin, 40–51% cellulose and 28–37% hemicellulose [24]. Interspecies variation of those compositional percentages can impact upon tar production during gasification [25, 26]. The respective contributions to tar production are shown in Figure 2.2.

Rabou *et al.* [23] reported that the primary cell wall polymers differ in their contribution to tar formation. For example aromatic and phenolic compounds, that can be found in major proportions as tar compounds, are derived from lignin. Yu *et al.* [27] supported this statement by reporting that lignin contributed to the formation of more tar compounds compared to cellulose.

Conversely Worasuwannarak *et al.* [26] found that gasifying pure cellulose generated substantially more tar compared to pure lignin or a variety of biomass feedstocks. The authors theorised that the interaction of lignin and cellulose eventually produces a reduction in tar formation and an increase in char formation. Water and esters were produced at the expense of tar due to interactions between lignin and cellulose.

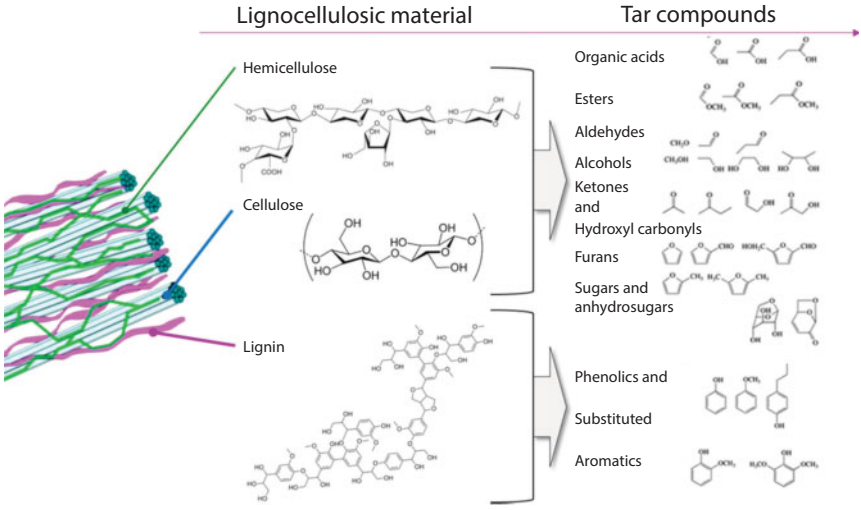

Figure 2.2 Contribution of tar compounds from lignocellulosic material.

Following pyrolysis, cellulose primarily produces water soluble tars and the production of these is decreased substantially if cellulose and lignin are pyrolysed together. Work by Fushimi *et al.* [17] illustrated this process and the work is in agreement with Worasuwannarak *et al.*'s [26] work. It was observed that water insoluble tar is increased by a cellulose-lignin interaction [17]; however the proportion of water insoluble tar is small compared to water soluble tar. The impact of hemicellulose upon tar formation is not significant according to Hosoya *et al.* [28] and Worasuwannarak *et al.* [26] linked its presence to an increase in reaction water following gasification.

2.3.1 The Impact of Plant Type and Blending Upon Tar Production

According to Rabou *et al.* [23] tar production depends largely upon the type of gasification employed rather than the composition of dry feedstock (including ash composition). However, some authors do report intra species variation in tar production and attribute it to variations in cell wall composition; this is particularly evident when lower temperature gasification was implemented.

Lv *et al.*'s [25] work examined a range of biomass feedstocks that differed in the cellulose and lignin composition. Thermogravimetric analysis (TGA) was used to discover a potential link between cellulose composition and tar formation. It was found that the species with the highest cellulose composition such as bagasse produced the higher levels of tar, whereas

rice husk with the lowest cellulose level produced the lowest tar yield. Furthermore species with intermediate cellulose levels were positioned intermediately with regards to tar formation within the tar production range. It was also observed that at higher temperatures, tar levels declined but the hierarchy of species specific cellulose levels to tar formation was resilient to this. Therefore a relationship between the cellulose content in lignocellulosic materials, can be correlated with tar formation during the gasification process.

Qin *et al.* [29] found that the comparatively lignin rich sawdust feedstock generated a higher percentage tar yield when compared to the relatively cellulose rich (lower lignin) corn stalks at temperatures up to 800 °C. At temperatures higher than 800 °C the gasification of cellulose rich corn stalks resulted in higher tar yields. Furthermore the high cellulose composition corn stalks produced a tar with a higher percentage of aliphatic compounds, whereas the sawdust produced tars with a higher percentage composition of aromatic compounds. This was attributed to the monomer structures comprising the most dominant relative cell wall polymers (lignin for saw dust and cellulose for corn stalks). It is notable that Qin *et al.* [29] reported that high molecular mass tars prevailed at higher temperatures (900 °C in this case). In some agreement with this Brage *et al.* [30] also found that temperature and feedstock were the two most important variables with regards to tar production. However, the feedstocks that they examined were coal and generalised biomass, so an analysis of intra species variation was not undertaken.

Contradictory research conclusions reflect the complexity of tar production. At lower temperature gasification the cellulose and lignin levels are key to tar production. Broadly concluding the relationship between cell wall polymer composition and tar production during gasification is multivariate (including ash) and rigorous multivariate analysis of experimental data may well lead to a better understanding. Once equipped with such knowledge the possibility of blending or selecting feedstocks with cell wall polymer configurations suited to low tar production may be more viable. Aligning optimum conditions to optimum process conditions may be a suitable methodology for optimising low tar production.

2.3.2 Blending

As previously mentioned authors report a drop in tar production using lignin and cellulose blends compared to pure components. Plant cell walls already contain a mix of these polymers (along with hemicellulose). However if there exists a ratio of lignin to cellulose that would optimise low

levels of tar formation then pre-treatments, breeding or biomass blending could be exploited to those ends. So the work by Fushimi *et al.* [17] and Worasuwannarak *et al.* [26] may have implications for biomass pre-treatments or selection prior to gasification. In a blending system cell wall polymer composition may potentially be optimised for low tar production, below that presented by either raw material when gasified in isolation and exploiting the cell wall polymer interactions described. Using blended biomass Pinto *et al.* [31] reduced the negative impacts of a high silica and alkali metal composition by blending biomass feedstocks prior to gasification. However, tar formation was also lowered (in some instances), for example blended rice husk with straw produced lower levels of tar formation compared to straw alone. So blending could perhaps have positive implications for both reducing ash (which has a negative impact upon equipment through slagging and fouling) and tar formation during gasification.

Variation in cell wall constituents occurs in purpose grown bioenergy plant genotypes as well as the highly diverse wild type flora. Hodgson *et al.* [32] found highly significant differences between Miscanthus genotypes with regards to lignin, cellulose and hemicellulose composition. Genotypes with an optimum blend of cellulose and lignin aimed at the lowest tar production potential may be worth examining as strains suitable for gasification. Alternatively blends could be made as described above.

A reduction in polymerisation of the cell wall components can be implemented by the use of enzyme [33] and chemical [34] pre-treatments. This may be a method of tuning the cell wall compositions if they are deemed to be a primary influence upon the tar production. There may be options with regards to this for feedstock blending.

2.3.3 Ash Composition

Ash composition can be sub divided into the ash that is a contaminant due to harvesting and processing, known as introduced ash [35] and inherent ash contained within the biomass.

Introduced ash is introduced from the soil during harvesting and post-harvest processing. In the case of herbaceous biomass the feedstock needs to be cut; it may be made into windrows (rows of cut biomass ready to lift for baling) and baled in some form for handling, transport and storage. There are various operational options available relating to these activities and these impact upon the introduced ash composition. Bonner [36] reports that the mean ash composition of corn stover varies between 11.5–28.2% depending upon the techniques selected for this part of the value chain. Hand cut whole plant corn stover has an ash composition of

5–7% [37]. The highest ash compositions were found to be associated with mowing and wheel raking for windrow creation. The lowest ash compositions were associated with shred flail and raking into windrows using a hydraulically driven basket rake [36].

Levels of inherent ash are influenced by plant group. In general woody biomass tends to contain lower levels of ash compared to herbaceous biomass (such as grasses) (37). The ash composition of biomass is negatively correlated with tar production [38], Skoulou *et al.* [38] found that leached (partly de-mineralised) biomass had a higher level of tar production compared to untreated biomass, the difference was highly substantial at 850 °C but the difference in tar production was reduced on increasing the temperature to 950 °C. They proposed that the metal component catalysed the destruction of the heavier tar components. The presence of carbonates, oxides and hydroxides of alkali metals in the reactor help to decompose tars [15]. However, the advantages attributed to this catalysis are countered by the advantages of a low ash feedstock such as reduced particulate matter emissions as well as the reduced slagging and fouling attributed to ash thermal behaviour [39, 40]. A balance between the substantial problem of tar formation [41] and ash related issues needs to be considered.

2.4 Thermochemical Pre-treatments for Gasification

2.4.1 Torrefaction

One of the most efficient and promising pretreatments for promoting tar reduction during gasification is torrefaction. The advantages of this pretreatment are multifaceted for gasification. Torrefaction is the heating of biomass under low oxygen conditions and at atmospheric pressure. The heating rate is less than 50 °C min^{-1} and torryfying temperatures vary from 200 °C–300 °C [42].

Torrefaction decreases the volatile composition and drives off moisture increasing the efficiency of gasification [1]. In addition torrefied biomass can be milled with energy costs that are three to seven times lower than the energy costs associated with milling the original biomass [42, 43]. With regards to tar formation Dudyński *et al.* [44] carried out biomass air gasification of ordinary wood pellets, torrefied wood pellets and sawdust in an industrial fixed bed gasifier. They reported that the torrefied pellets were associated with significantly lower tar yields compared to the other feedstocks, followed by sawdust and ordinary pellets. In order to maintain efficiency (having lost the volatiles which can act as a gasification fuel) Prins

et al. [1] advised feeding the volatiles into the out-coming syngas, to act as a chemical quench. The overall efficiency was addressed by this process step.

2.4.2 Slow Pyrolysis

Slow pyrolysis is a biomass conversion method that employs temperatures of approximately 300–500 °C with a long residence time (hours), in low/zero oxygen. Moisture and volatiles are driven off to provide the primary product which is a charcoal. The system can employ a batch or a continuous feed. Figure 2.3 is an example of the pilot scale batch slow pyrolysis unit located at the Institute for Biological Environmental and Rural Science, Aberystwyth University, UK.

In a similar system to a torrefaction to gasification dual process, the Netherlands Energy Research Foundation (ECN) developed the CASST system (clean air sustainable syngas technology [23]). In this system an alternative dual pyrolysis process set up was implemented. Instead of torrefaction ECN implemented a slightly raised pyrolysing temperature of 350 °C, providing a low temperature slow pyrolysis. The charcoal that is produced is de-volatised and when subjected to gasification generates a syngas, a ten-fold reduction in tar production was reported [23]. According to Rabou *et al.* [23] the system encountered problems and the specific details were not discussed.

Slow pyrolysis generates char. The addition of char into a gasifier can reduce tar vapours significantly according to Devi *et al.*'s's review [45]. The

Figure 2.3 A batch fed pilot scale slow pyrolysis unit, located at the Institute for Biological Environmental and Rural Sciences at Aberystwyth University (http://beaconwales.org/).

char is also mostly gasified as well so a renewable resource is required. It may be possible to exploit a fraction of the feedstock for this route. There is some consensus that too much char is required for practical application [23]. However a proportion of the gasification feedstock could take this route and if the waste heat can be rescued from char production (Figure 2.4), as proposed by Roberts *et al.* [46], and used to supplement biomass drying for both char and gasification feedstocks, the benefits might combine into a suitable system. Biomass blending and densification with biochar has been done before [47]. The blending of biomass with biochar would potentially lower the O/C ratio (which is high in biomass resulting in over oxidisation during gasification) benefitting efficiency as well as reducing tar generation. A dual conversion edition is described later in this work.

2.4.3 Intermediate Pyrolysis

Intermediate pyrolysis is conducted at temperatures of ~400–500 °C in zero/low oxygen conditions. A solids residence time of 1–30 minutes (hence intermediate) and a heating rate of 1–1000 °C s^{-1} is employed. Aston University in the UK have been instrumental in advancing this process. The chars produced by intermediate pyrolysis also have a high carbon content and low volatile composition and have been successfully gasified as reported by Sattar *et al.* [48], but no data comparing tar production following gasification of pyrolysed with none pyrolysed feedstocks is reported.

One attribute of intermediate pyrolysis is the generation of oil with a high calorific value; the oil fraction easily separates into organic and aqueous phases; the organic fraction has some beneficial characteristics compared to oil generated from fast pyrolysis including improved viscosity and heating value [48]. These qualities may be the result of a relatively prolonged residence time and contact with char [48, 49]. Following the extraction of volatiles to generate separate oil and char fractions, it is likely that the chars can be gasified with low tar production, based upon the results of gasifying slow pyrolysis derived charcoal (Figure 2.4). Further comparative studies are a research opportunity in this area.

2.4.4 Fast Pyrolysis

Fast pyrolysis produces char, gas, oil and an aqueous fraction. The process involves the implementation of a rapid heating rate (>1000 °C s^{-1}), a low/zero oxygen environment, temperatures ~500 °C and a short solid residence time in the reactor (~1 s).

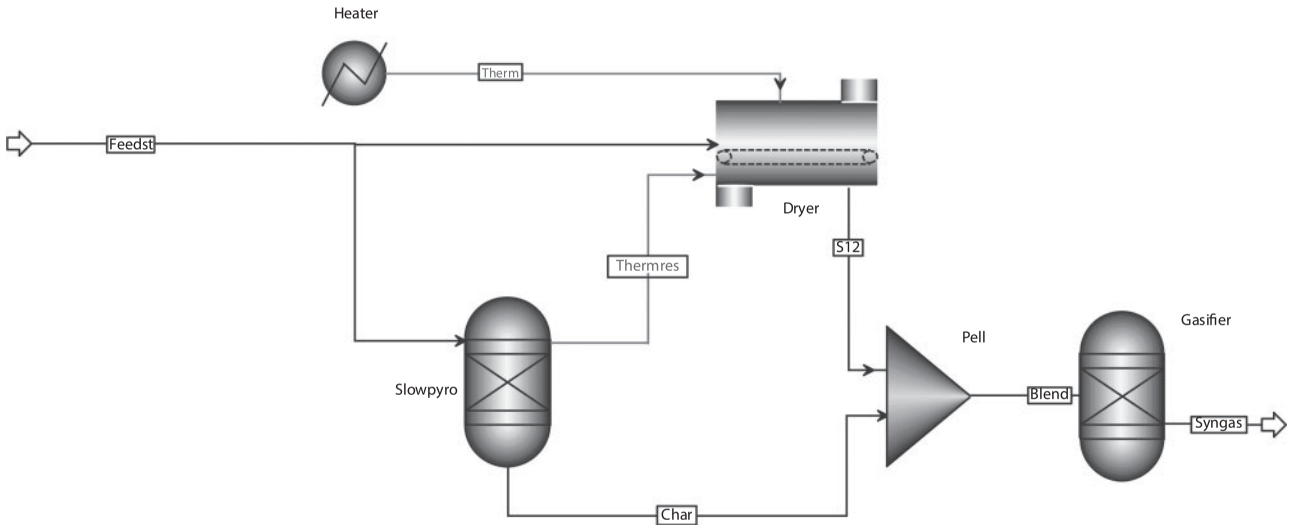

Figure 2.4 A suggested process flow chart of a slow pyrolysis and gasification unit combined process stream. The thermal waste (thermres) generated from slow pyrolysis is exploited as proposed by Roberts *et al.* [46] and used to supplement biomass drying to increase efficiency. The pyrolysing pre-gasification step is introduced to reduce tar formation during gasification (1, 20). A similar system using torrefaction instead of slow pyrolysis may be also be implemented. The torrified or charred biomass is blended and pelletized (PELL) prior to gasification. Created using Aspen Plus V 8.6, (Aspen Technology Inc, Massachusetts, U.S.A.).

The fast pyrolysis process favours oil production. Its use as a precursor process to gasification was examined by Svoboda *et al.* [50], Sakaguchi *et al.* [51] and Zhang *et al.* [52], but the impact on tar production was not reported.

Boateng *et al.* [53] gasified char generated following fast pyrolysis of switchgrass. The charcoal generated during fast pyrolysis was low in tar producing organic compounds because those organic compounds had already been volatised and collected as a an oil product. Boateng *et al.* [53] reported that there was little evidence of any tar production in the form of cendensable gases following gasification.

Fast pyrolysis yields of dry milled biomass produces ~ 20–40 wt % charcoal in the product stream. However the removal of the bio oil does mean that the volatile fraction is reduced. This would impact on the efficiency of the gasification process because the volatile fraction provides a suplementary fuel during gasification prins [1] (in addition to contributing tar). However it may be viable for the oil to be used as a supplementary burner fuel or adding into the gasifier as a feedstock. Svoboda *et al.* [50] used a spray mechanism to inject a bio-oil and charcoal slurry into a gasifier but did not report on related tar production.

A suggested process flow is presented in Figure 2.5, that combines fast pyrolysis with gasification. This is based upon research that shows that high temperature gasification (achievable with a pyrolysis oil supplementary fuel) results in lower tar production [10] and the addition of char to the feedstock lowers tar production [45].

The primary pre-processing and compositional influences upon tar formation are detailed in Table 2.2.

2.5 Processing Options that Exploit Conversion Route Integration

If there is a sufficient supply of sustainable biomass, combining process routes may be a beneficial and efficient option. These systems can be designed to potentially reduce tar formation in the gasification step by reducing the volatile composition and exploiting that volatile composition in another energy or product generating process. Kamm and Kamm [54] detailed many biorefinery options and some may potentially reduce tar production during gasification.

In what Kamm and Kamm [54] describe as a green biorefinery model (Figure 2.6), wet herbaceous biomass is subjected to screw-pressing. A press fluid is generated and that fluid can be fed into anaerobic digesters (AD) to produce biogas that can be scrubbed to methane to power

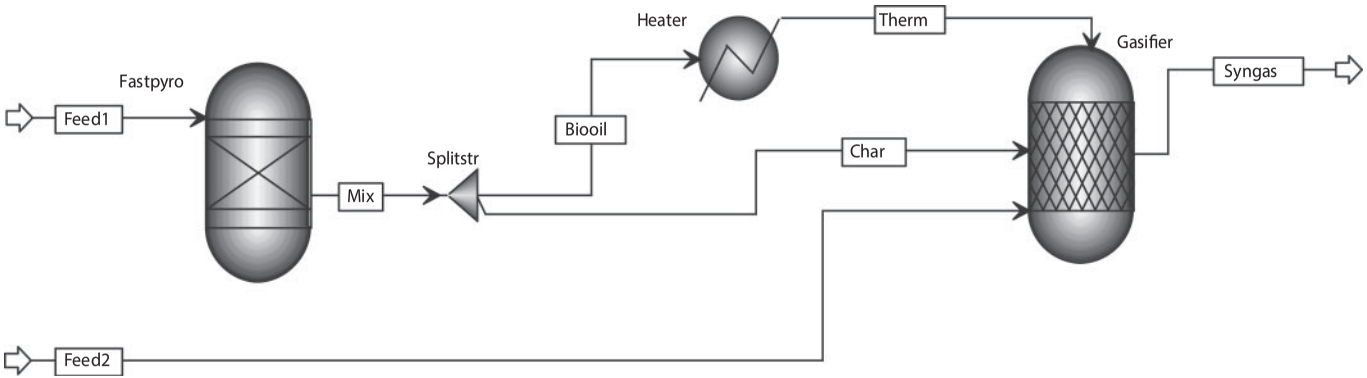

Figure 2.5 A suggested process flow diagram exploring the combination of fast pyrolysis and gasification in order to promote reduced tar production during the gasification phase. Both char (from fast pyrolysis) and higher temperature gasification (supported by bio-oil as a supplementary fuel) are associated with lower tar yields. The process may also utilise intermediate pyrolysis with potentially greater efficiency due to the ease of separation between the aqueous and oil fractions. Created using Aspen Plus V 8.6, Aspen Technology Inc, Massachusetts, U.S.A.

Table 2.2 Some components and treatments that increase and decrease tar production during gasification.

Variable		Impact upon tar formation during gasification	References
Cell wall composition	Cellulose	Increase	Worasuwannarak *et al.* [24]
	Lignin	Increase	Yu *et al.* [18, 26]
Thermochemical pre-treatment	Torrefaction (reduction of volatiles)	Decrease	Dudyński [44]
	Slow pyrolysis (addition of char)	Decrease	Devi *et al.* [45]
Ash composition		Decrease	Skoulou *et al.* [38]

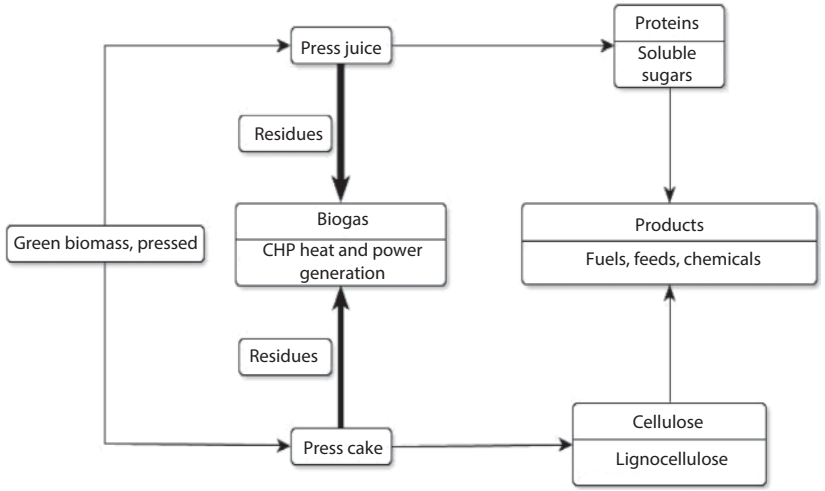

Figure 2.6 Integrated processing using multiple products may be an efficient way to manipulate biomass in order to reduce tar production during gasification. Based on Kamm and Kamm [54].

a combined heat and power (CHP) unit. The remaining fibrous fraction has essentially been partially demineralized, depolymerised and the sugar compositionhas been partially pressed out.

The Kade system was developed by the Institute of Biological Environmental and Rural Science (Aberystwyth University) in partnership

with AMW Partnership (UK). The system developed following research regarding the Integrated Generation of Solid Fuel and Biogas from Biomass system (IFBB) developed at Kassel University, Germany [55]. In this system waste biomass (*Juncus effuses* dominant biomass) generated during the conservation management of wetland landscapes was screw pressed. The fluid fraction from this procedure was digested using anaerobic digestion to generate biogas. Reed (high dry matter) was subjected to slow pyrolysis and the waste heat from the procedure was rescued and used to dry the fibrous fraction stream from the screw-pressing procedure [46]. The dry fibrous fraction (from screw-pressing) had a significantly reduced mineral composition. This fibrous fraction (65%) was blended with the slow pyrolysis char (25%) and waste wood chips (10%) to generate a solid fuel. Having been relieved of a significant amount of minerals (pressing) and following blending the emissions profile of the densified fuel was much improved (Figure 2.7) [56].

The blend that was devised for combustion had a reduced mineral composition and was blended with a char. Both these attributes are well suited for optimised gasification. The lower mineral/ash composition is favourable for reducing slagging and ash fouling. The loss of the tar cracking potential of the ash may be counteracted by the addition of char which is associated with tar reduction. Potentially this may be a well-balanced gasification fuel. As gasification runs in parallel with anaerobic digestion the energy balance of the whole system benefits substantially.

2.6 Conclusion

Cellulose and lignin appear to contribute to tar formation during gasification. Understanding the multivariate interactions between ash, cellulose, hemicellulose and lignin with regard to tar production may provide a promising potential route to understanding the impact of feedstock composition on tar formation during gasification. The overall feedstock composition may be manipulated by implementing pre-treatments and developing integrated processes.

A very efficient and promising thermochemical pre-treatment for lowering tar production during gasification appears to be torrefaction. Combining torrefaction with thermal recovery may be a route to efficiently optimise tar reduction during gasification.

Biomass composition aimed at reducing tar formation may be promising, but broad consensus from the highly experienced Energy Research Centre for the Netherlands, is that engineering solutions are primary in tar control

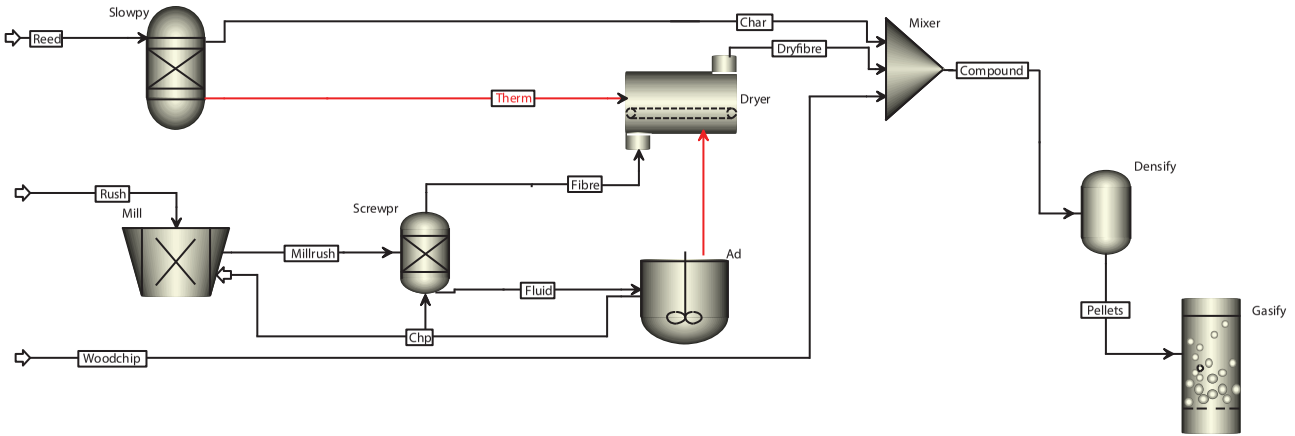

Figure 2.7 The kade system was originally designed for processing low input high diversity biomass for efficient combustion fuel generation. However, the end product is a blended pellet with reduced mineral and sugar composition. The blend contains char from slow pyrolysis of waste reed. Reduced mineral composition benefits gasification as mineral fouling of the gasifier is reduced and the addition of char reduces tar formation as described in previous research. By combining processes a suitable gasification fuel may have been generated. Created using Aspen Plus V 8.6, (Aspen Technology Inc, Massachusetts, U.S.A.).

and biomass composition is secondary. However, integrated processing that harvests energy or products from process stages aimed at reducing tar production and ash related fouling (through demineralization) is a process outlook that potentially offers increased whole system efficiently.

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References

1. Prins, M.J., K.J. Ptasinski, and F.J.J.G. Janssen, *More efficient biomass gasification via torrefaction.* Energy, 2006. 31(15): p. 3458–3470.
2. Bridgwater, A.V., *Production of high grade fuels and chemicals from catalytic pyrolysis of biomass.* Catalysis Today, 1996. 29(1–4): p. 285–295.
3. Li, C. and K. Suzuki, *Tar property, analysis, reforming mechanism and model for biomass gasification—An overview.* Renewable and Sustainable Energy Reviews, 2009. 13(3): p. 594–604.
4. Larson, E.D., H. Jin, and F.E. Celik, *Large-scale gasification-based coproduction of fuels and electricity from switchgrass.* Biofuels, Bioproducts and Biorefining, 2009. 3(2): p. 174–194.
5. Corton, J., *et al.*, *Bioenergy as a biodiversity management tool and the potential of a mixed species feedstock for bioenergy production in Wales.* Bioresource Technology, 2013. 129: p. 142–149.
6. Hanaoka, T., *et al.*, *Effect of woody biomass components on air-steam gasification.* Biomass and bioenergy, 2005. 28(1): p. 69–76.
7. Font Palma, C., *Modelling of tar formation and evolution for biomass gasification: A review.* Applied Energy, 2013. 111: p. 129–141.
8. Kumar, A., D.D. Jones, and M.A. Hanna, *Thermochemical biomass gasification: a review of the current status of the technology.* Energies, 2009. 2(3): p. 556–581.
9. Vreugdenhil, B., R. Zwart, and J.P.A. Neeft, *Tar formation in pyrolysis and gasification*. 2009: ECN.
10. Li, X., *et al.*, *Biomass gasification in a circulating fluidized bed.* Biomass and bioenergy, 2004. 26(2): p. 171–193.

11. Knight, R.A., *Experience with raw gas analysis from pressurized gasification of biomass.* Biomass and bioenergy, 2000. 18(1): p. 67–77.
12. Gil, J., *et al.*, *Biomass gasification in atmospheric and bubbling fluidized bed: effect of the type of gasifying agent on the product distribution.* Biomass and bioenergy, 1999. 17(5): p. 389–403.
13. Han, J. and H. Kim, *The reduction and control technology of tar during biomass gasification/pyrolysis: An overview.* Renewable and Sustainable Energy Reviews, 2008. 12(2): p. 397–416.
14. Sutton, D., B. Kelleher, and J.R.H. Ross, *Review of literature on catalysts for biomass gasification.* Fuel Processing Technology, 2001. 73(3): p. 155–173.
15. McKee, D.W., *Fundamentals of Catalytic Coal and Carbon Gasification Mechanisms of the alkali metal catalysed gasification of carbon.* Fuel, 1983. 62(2): p. 170–175.
16. Milne, T.A., N. Abatzoglou, and R.J. Evans, *Biomass gasifier" tars": Their nature, formation, and conversion*. Vol. 570. 1998: National Renewable Energy Laboratory Golden, CO.
17. Fushimi, C., S. Katayama, and A. Tsutsumi, *Elucidation of interaction among cellulose, lignin and xylan during tar and gas evolution in steam gasification.* Journal of Analytical and Applied Pyrolysis, 2009. 86(1): p. 82–89.
18. Ponzio, A., S. Kalisz, and W. Blasiak, *Effect of operating conditions on tar and gas composition in high temperature air/steam gasification (HTAG) of plastic containing waste.* Fuel Processing Technology, 2006. 87(3): p. 223–233.
19. Douglas, G.B., *et al.*, *Liveweight gain and wool production of sheep grazing Lotus corniculatus and lucerne (Medicago sativa)*, in *New Zealand Journal of Agricultural Research*. 1995, Taylor & Francis. p. 95–104.
20. Basu, P., *Combustion and gasification in fluidized beds*. 2006: CRC press.
21. Mettler, M.S., D.G. Vlachos, and P.J. Dauenhauer, *Top ten fundamental challenges of biomass pyrolysis for biofuels.* Energy & Environmental Science, 2012. 5(7): p. 7797–7809.
22. Fjellerup, J., *et al.*, *Formation, decomposition and cracking of biomass tars in gasification*. 2005, Technical University of Denmark. Department of Mechanical Engineering.
23. Rabou, L.P., *et al.*, *Tar in biomass producer gas, the Energy research Centre of the Netherlands (ECN) experience: an enduring challenge.* Energy & Fuels, 2009. 23(12): p. 6189–6198.
24. Pauly, M. and H. Scheller, *O-acetylation of plant cell wall polysaccharides: identification and partial characterization of a rhamnogalacturonan O-acetyltransferase from potato suspension-cultured cells.* Planta, 2000. 210: p. 659.
25. Lv, D., *et al.*, *Effect of cellulose, lignin, alkali and alkaline earth metallic species on biomass pyrolysis and gasification.* Fuel Processing Technology, 2010. 91(8): p. 903–909.
26. Worasuwannarak, N., T. Sonobe, and W. Tanthapanichakoon, *Pyrolysis behaviors of rice straw, rice husk, and corncob by TG-MS technique.* Journal of Analytical and Applied Pyrolysis, 2007. 78(2): p. 265–271.

27. Yu, H., *et al.*, *Characteristics of tar formation during cellulose, hemicellulose and lignin gasification.* Fuel, 2014. 118: p. 250–256.
28. Hosoya, T., H. Kawamoto, and S. Saka, *Cellulose–hemicellulose and cellulose–lignin interactions in wood pyrolysis at gasification temperature.* Journal of Analytical and Applied Pyrolysis, 2007. 80(1): p. 118–125.
29. Qin, Y., *et al.*, *The influence of different chemical compositions in biomass on gasification tar formation.* Biomass and bioenergy, 2015. 83: p. 77–84.
30. Brage, C., *et al.*, *Tar evolution profiles obtained from gasification of biomass and coal.* Biomass and bioenergy, 2000. 18(1): p. 87–91.
31. Pinto, F., *et al.*, *Effect of biomass type blended with rice production wastes in syngas produced by co-gasification.* Materials and Technologies for Energy Efficiency, 2015: p. 7.
32. Hodgson, E.M., *Genetic and environmentally derived variation in the cell wall composition of Miscanthus and implications for thermo-chemical conversion*, in *Bio-Energy Research Group*. 2008, Aston: Aston. p. 126.
33. Quiñones, T.S., *et al.*, *Production of xylooligosaccharides from renewable agricultural lignocellulose biomass.* Biofuels, 2015. 6(3–4): p. 147–155.
34. Li, C., *et al.*, *Comparison of dilute acid and ionic liquid pretreatment of switchgrass: Biomass recalcitrance, delignification and enzymatic saccharification.* Bioresource Technology, 2010. 101(13): p. 4900–4906.
35. Kenney, K.L., *et al.*, *Understanding biomass feedstock variability.* Biofuels, 2013. 4(1): p. 111–127.
36. Bonner, I., *et al.*, *Impact of Harvest Equipment on Ash Variability of Baled Corn Stover Biomass for Bioenergy.* BioEnergy Research, 2014. 7(3): p. 845–855.
37. Tao, G., *et al.*, *Biomass properties in association with plant species and assortments. II: A synthesis based on literature data for ash elements.* Renewable and Sustainable Energy Reviews, 2012. 16(5): p. 3507–3522.
38. Skoulou, V., *et al.*, *Effect of biomass leaching on H2 production, ash and tar behavior during high temperature steam gasification (HTSG) process.* International Journal of Hydrogen Energy, 2009. 34(14): p. 5666–5673.
39. Arvelakis, S., *et al.*, *Effect of leaching on the ash behavior of olive residue during fluidized bed gasification.* Biomass and bioenergy, 2002. 22(1): p. 55–69.
40. Fournel, S., *et al.*, *Predicting gaseous emissions from small-scale combustion of agricultural biomass fuels.* Bioresource Technology, 2015. 179: p. 165–172.
41. Neubauer, Y., *Strategies for tar reduction in fuel-gases and synthesis-gases from biomass gasification.* Journal of Sustainable Energy & Environment Special Issue, 2011. 67: p. 71.
42. Deng, J., *et al.*, *Pretreatment of agricultural residues for co-gasification via torrefaction.* Journal of Analytical and Applied Pyrolysis, 2009. 86(2): p. 331–337.
43. Arias, B., *et al.*, *Influence of torrefaction on the grindability and reactivity of woody biomass.* Fuel Processing Technology, 2008. 89(2): p. 169–175.
44. Dudyński, M., *et al.*, *Biomass gasification: Influence of torrefaction on syngas production and tar formation.* Fuel Processing Technology, 2015. 131: p. 203–212.

45. Devi, L., K.J. Ptasinski, and F.J.J.G. Janssen, *A review of the primary measures for tar elimination in biomass gasification processes.* Biomass and bioenergy, 2003. 24(2): p. 125–140.
46. Roberts, K.G., *et al.*, *Life Cycle Assessment of Biochar Systems: Estimating the Energetic, Economic, and Climate Change Potential.* Environmental Science & Technology, 2009. 44(2): p. 827–833.
47. Corton, J.L.-L., A., *The impact of pressing and co firing with biochar and woodchip on the emissions profile of Juncus effuses generated during conservation management.* Ist International Biomass Emissions Conference. p. 15 1 p. 7, 2015.
48. Sattar, A., *et al.*, *Steam gasification of rapeseed, wood, sewage sludge and miscanthus biochars for the production of a hydrogen-rich syngas.* Biomass and bioenergy, 2014. 69: p. 276–286.
49. Bridgwater, A.V., *Review of fast pyrolysis of biomass and product upgrading.* Biomass and bioenergy, 2012. 38: p. 68–94.
50. Svoboda, K., *et al.*, *Pretreatment and feeding of biomass for pressurized entrained flow gasification.* Fuel Processing Technology, 2009. 90(5): p. 629–635.
51. Sakaguchi, M., A.P. Watkinson, and N. Ellis, *Steam gasification of bio-oil and bio-oil/char slurry in a fluidized bed reactor.* Energy & Fuels, 2010. 24(9): p. 5181–5189.
52. Zhang, Y., *et al.*, *Comparative techno-economic analysis of biohydrogen production via bio-oil gasification and bio-oil reforming.* Biomass and bioenergy, 2013. 51: p. 99–108.
53. Boateng, A.A., *Characterization and Thermal Conversion of Charcoal Derived from Fluidized-Bed Fast Pyrolysis Oil Production of Switchgrass†.* Industrial & Engineering Chemistry Research, 2007. 46(26): p. 8857–8862.
54. Kamm, B. and M. Kamm, *Biorefineries – Multi Product Processes*, in *White Biotechnology*. 2007. p. 175–204.
55. Wachendorf, M., *et al.*, *Utilization of semi natural grassland through integrated generation of solid fuel and biogas from biomass. I. Effects of hydrothermal conditioning and mechanical dehydration on mass flows of organic and mineral plant compounds, and nutrient balances.* Grass and Forage Science, 2009. 64(2): p. 132–143.
56. Corton, J., *et al.*, *Press fluid pre-treatment optimisation of the integrated generation of solid fuel and biogas from biomass (IFBB) process approach.* Bioresource Technology, 2014. 169(0): p. 537–542.

3

Key Pretreatment Technologies for An Efficient Bioethanol Production from Lignocellulosics

Archana Mishra and Sanjoy Ghosh*

Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee-247667 UK, India

Abstract

Foreseeable depletion of traditional fossil fuels resulted in wide recognition of bioethanol as an efficient alternative for gasoline as transportation fuel. Ethanol production from corn is limited due to the fact that another feedstock is needed to produce it. Among all these feedstocks, lignocellulosic biomasses are most promising because of their abundance, low cost and high carbohydrate content. At commercial scale, bioconversion of lignocellulosics to ethanol needs efficient pretreatment methods for complete delignification of the biomass. A suitable pretreatment method results in an increased concentration of fermentable sugars during saccharification, which improves the efficiency of the whole production process. The main aim of the pretreatment step is to increase the digestibility of the total available fermentable sugars in the biomass. The method and condition of pretreatment should be selected appropriately according to the subsequent steps of saccharification and fermentation. This chapter will review various pretreatment technologies for maximum conversion of the holocellulosic fraction to ethanol and will highlight various key properties which need to be focused on for economy and maximum productivity.

***Keywords*:** Lignocellulosics, pretreatment, saccharification, fermentation, bioethanol

**Corresponding author*: ghoshfbs@iitr.ac.in

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3.1 Introduction

Energy security along with increase in CO_2 emissions concerns has intensified the need for non-petroleum-based alternative energy sources. Biofuels (bioethanol or biodiesel) are a renewable and suitable primary energy resource which has potential to provide alternative transportation fuels in the near future [1]. In the present scenario, bioethanol production relies on ethanol from starch but there are noteworthy debates about its sustainability. In these circumstances, bioethanol production from lignocellulosic biomasses is the most promising alternative as they do not challenge food crops in a direct way and they are also abundant and less expensive compared to conventional agricultural feedstocks. Worldwide annual production of lignocellulosic feedstocks has been estimated around 1×10^{10} metric ton [2].

Basically, lignocellulosic biomasses are composed of holocellulose (hemicellulose and cellulose), lignin and extractives. Composition of each biomass depends on its origin [3, 4]. The major component is cellulose, which is a linear crystalline β-D-glucose polymer and has a rigid structure which is very difficult to break [5]. The cellulosic fraction of the biomass is converted into glucose monomer by the chemical or enzymatic method of hydrolysis [6]. Hemicellulose is a heteropolymer which is composed of both linear and branched chain of D-glucose, D-xylose, D-mannose, D-galactose, and L-arabinose. Since its structure is not crystalline, it is easier to hydrolyse comparatively [7]. Lignin is the most rigid 3-D polymeric component of the plant cell wall which consists of three different phenyl propane precursors as its monomeric unit, which are non-biodegradable [8].

Biological conversion of lignocellulosic feedstocks to ethanol offers multiple benefits but still its development is hampered by some technical and economic obstacles [2]. Some of the important factors needed to improve ethanol production economy are: a) efficient biomass utilization to obtain high productivity, ethanol yield, and high ethanol concentration after fermentation in the distillation feed; b) to reduce energy requirement, process integration should be considered [9, 10]. Biothanol conversion from lignocellulosic feedstock mainly involves: cellulose and hemicelluloses hydrolysis to fermentable sugars, sugar fermentation and recovery and purification of alcohol to meet fuel specifications (Figure 3.1). The task of converting lignocelluloses hydrolysis into fermentable sugars is still problematic technically because of the cellulose digestibility hinderation by various compositional, physicochemical and structural factors. A pretreatment step is done to obtain potentially fermentable sugars from

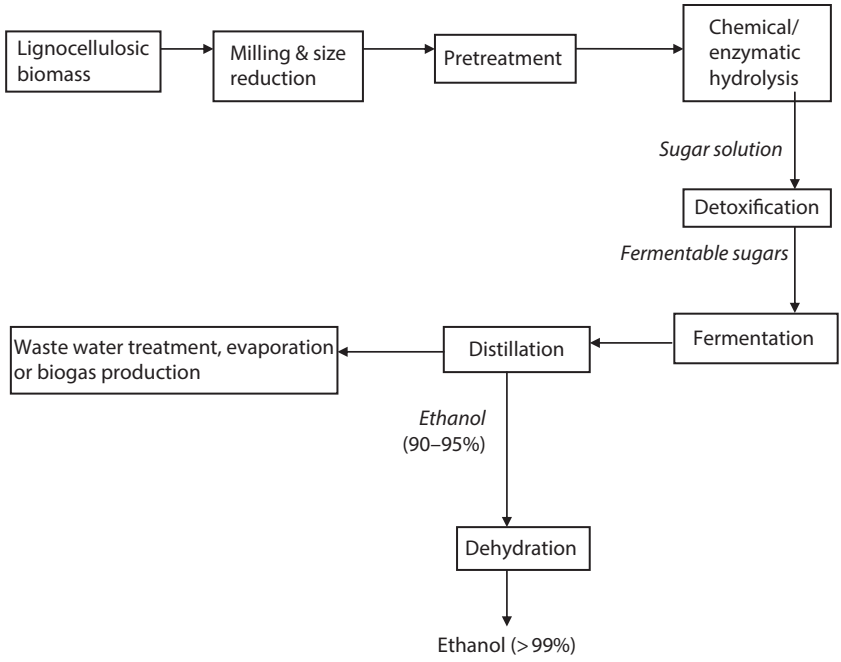

Figure 3.1 Overall process scheme for bioethanol production from lignocellulosic biomass.

lignocellulosic biomass. The main aim of pretreatment is lignin structure degradation and disruption of crystalline structure of cellulose to enhance the enzymatic accessibility during hydrolysis [6]. Currently, pretreatment research is focused mainly on identification, evaluation, demonstration and development of approaches which can show an efficient and effective subsequent hydrolysis step with reduction of the treated biomass with lower enzyme loading and reduced bioconversion times. Numerous approaches for pretreatment are being investigated on different feedstocks types. Several review articles provide a general overview in this context [11–14]. Biomass pretreatment is considered as a crucial step in bioethanol production and adds huge amounts to overall costs in the process. Mosier *et al.*, [6], have described it as the second most expensive unit cost (after feedstocks cost) in the bioethanol production process based on enzymatic hydrolysis.

As it is known that different lignocellulosic feedstocks have different physico-chemical properties, it is compulsory to select suitable pretreatment technologies based on their properties. The pretreatment method has a large influence on subsequent steps in the ethanol conversion scheme in

terms of digestibility of cellulose, toxic compounds generations which are potential inhibitors of the fermentation process, power requirements for stirring and energy requirement in the downstream process [9]. Various studies have shown that parameters like chip size requirement, pentose recovery, toxic compounds concentration and minimum energy demand are the significant factors in an effective pretreatment process [15]. This chapter will review all available pretreatment technologies, their advantages and disadvantages for lignocellulosic biomass and analyze the interrelated factors between all three steps, i.e., pretreatment, hydrolysis and fermentation.

3.2 Pretreatment Methods for Lignocellulosic Biomass

Pretreatment of lignocellulosic biomass is directed to reduce crystallinity of cellulose present in the biomass, increase the accessible surface area of biomass for hydrolysis, hemicelluloses removal and lignin barrier breakage (Figure 3.2). During the pretreatment step, cellulose becomes more accessible to hydrolytic agents which facilitate rapid conversion of polymeric carbohydrate into its monomeric fermentable sugars with more yields. There are various pretreatment methods to fractionate, solubilize, and hydrolyze the biomass and separate all three components (cellulose, hemicellulose, and lignin). On the basis of different forces or energy consumed in the whole process, these are categorized into a) physical or mechanical;

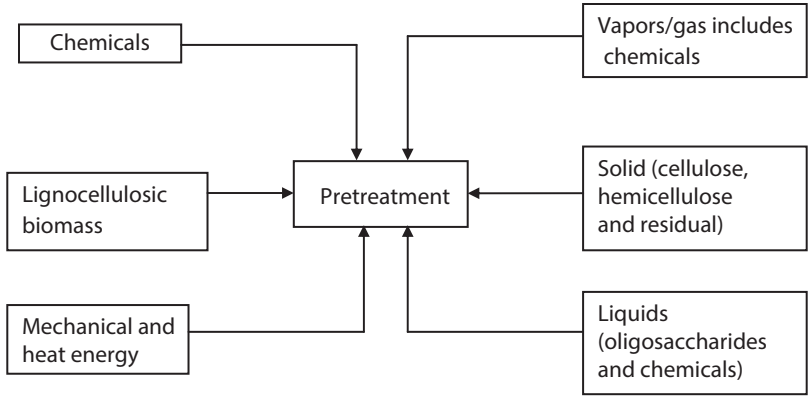

Figure 3.2 Design of pretreatment technology for bioethanol production from lignocellulosic biomass.

b) physico-chemical; c) chemical, and d) biological methods for lignocellulosic biomass pretreatment. Mechanical pretreatment mainly increases the accessible surface area for hydrolysis by biomass size reduction. Physico-chemical methods occur at high temperature and pressure; therefore high control is required of operating conditions [16]. Chemical methods loosen the network of holocellulose and lignin structure and/or dislocate lignin and hemicellulose. Biological pretreatment methods involve no chemicals and cause delignification of lignocellulosic biomass [17].

3.2.1 Parameters for Effective Pretreatment of Lignocellulosics

There are various factors which influence biodegradability of lignocellulosic biomass. The surface area which is accessible for hydrolysis is related to crystallinity of cellulose, hemicellulose and lignin content.

1. Cellulose crystallinity: Cellulose crystallinity is considered as one of the most important factors in the determination of the hydrolysis rate of cellulosic substrates which are relatively refined. Cellulosic microfibrils contain amorphous and region both. Around two-thirds of cellulose exist in crystalline form. Cellulase enzyme readily hydrolyzes amorphous portion of crystalline cellulose, which are more accessible in contrast to less accessible crystalline portion [7]. Therefore it is believed that digestibility of lignocellulosic biomass increases with reduction in cellulose crysallinity [18]. However, cellulose crystallinity is not the only factor which decides the lignocellulosic biomass digestibility; other factors also contribute to it [19].

2. Effect of surface area accessibility: Based on previous studies, it has been found that there is a good correlation between pore volume (total surface area which is accessible) and cellulose digestibility [20]. This correlation can be used to improve the hydrolysis process by lignin removal. There are two types of surface areas present in lignocellulosic biomass, i.e., external and internal. External surface area is linked with particle shape and size, while internal surface area is related to cellulosic fibres capillary structure.

3. Effect of lignin: Lignin present in lignocellulosic biomass is responsible for structural rigidity, integrity of biomass and swelling of material. In lignocellulosic biomass lignin covers cellulose and hemicelluloses [18, 19]. The presence of lignin covers the enzyme accessibility to cellulose and hemicelluloses which reduces hydrolysis efficiency. Lignin is considered as the most important factor for recalcitrance of lignocellulosic biomass.

Therefore, an efficient delignification process improves the rate and extent of the hydrolysis step [20].

4. Effect of hemicellulose: In lignocellulosic biomass, hemicellulose is present as a physical barrier which prevents the cellulose fibre from hydrolysis. In studies, it has been found that hemicellulose removal increases mean pore size of biomass and increases probability and accessibility of cellulose hydrolysis) [18, 19]. In hemicellulose, the degree of acetylation is another important factor, as acetyl groups and lignin are attached to hemicelluloses matrix and hinder breakdown of polysaccharide [7].

Other than these parameters, there are several other points which need to be taken into consideration for an economic and effective pretreatment process [13]:

1. Effective for multiple crops: Some pretreatment methods have been found suitable for specific biomasses. For instance, alkaline pretreatment methods (lime, ammonia recycling percolation and ammonia fiber expansion) have been found effective on delignification of agricultural residues but less effective for recalcitrant substrate like softwoods [19]. Although acid pretreatment methods are expensive relatively, they are very effective on a wide range of lignocellulosic biomass [6].

2. Digestiblility of pretreated solids: Cellulose obtained after pretreatment should be highly digestible, having a yield of more than 90%. In addition, residence time should be less than 3 days and enzyme loading not greater than 10 FPU/g of cellulose preferably [13].

3. No significant sugars degradation: Around 95% of fermentable holocellulosic sugars should be obtained through the pretreatment step.

4. Minimum generation of toxic compounds. Harsh conditions during the pretreatment process result in hemicellulose degradation partially and generation of toxic compounds. It affects hydrolysis and fermentation steps further [21]. The amount of toxic compounds depends on the raw material used and the pretreatment condition harshness. These compounds can be classified as follows: a) carboxylic acids; b) furan derivatives; c) phenolic compounds. Major furan derivates are 5-hydroxymethylfurfural and 2-furfuraldehyde, which derive from hexose and pentose sugar degradation, respectively [22]. Carboxylic acids include acetic acid, formic acid and levulinic acid. Phenolic compounds consist of alcohols, aldehydes and ketones [23].

5. Biomass size reduction: Prior to pretreatment, milling or grinding the biomass into smaller particle size is energy intensive and costly.

6. Pretreatment reactor: It should be less costly (by minimizing its volume, keeping reasonable operating pressure and for corrosive chemicals, appropriate material of construction should be applied).

7. Disposal challenges: Compounds and biomass formed during or after pretreatment should not face disposal or processing challenges.

8. Low moisture content: At high dry matter content, feedstock use would minimize energy consumption.

9. Fermentation compatibility: Recovery of sugar or sugar concentration should be compatible with the hemicellulose (xylose and arabinose) fermenting organism.

10. Lignin recovery: Lignin recovery after the process should lead to simplified downstream processing and it should be able to convert into other valuable products [13].

11. Minimum heat and power requirements: Energy demands for pretreatment should be minimum and/or should be thermally integrated.

3.2.2 Important Pretreatment Methods

3.2.2.1 Physical or Mechanical Methods

1. Mechanical comminution: Particle size reduction is needed to make handling of material easy and to enhance a specific surface area. This can be obtained by milling, chipping or grinding. Mechanical pretreatment is done usually as a primitive step in bioethanol production. Particle size which is desired is dependent on the subsequent steps which will be followed during the entire process. The size of raw material is kept usually between 10 to 30 mm in the case of chipping and 0.2 to 2 mm in milling or grinding [15]. Processes like vibratory milling, colloid milling, hammer milling, and two-roll milling are used to enhance lignocellulosic biomass digestibility compared to traditional ordinary ball milling [12]. Various factors like capital and operating costs, possibilities of scale-up, and equipment depreciation are crucial for this process. However, requirement of high energy input makes it economically non-feasible [24].

2. Extrusion: In this process, lignocellulosic biomass is first treated at a very high temperature (>300 °C), after that shearing and mixing is carried out to modify the physical and chemical structure of cellulose. Screw speed and barrel temperature are two factors which are believed to be responsible for disruption of lignocellulosic biomass and cause fibrillation, defibrillation

and fibre shortening [25]. This results in increased arability of polysaccharides to further attack. Bioreactor parameters must be highly efficient during this process. In one of the recent studies, application of enzyme during this process was found effective for bioethanol production [26].

3. Ultrasonic: This method is investigated only at lab scale for lignocellulosic biomass although for sludge treatment it is a well-known technique. In the experiments, the effect of this pretreatment method has been shown on a model compound (carboxy methyl cellulose), not on any lignocellulosic biomass [27]. Results show that when cellulosic suspension is treated with energy by irradiation, enzymatic hydrolysis enhances by approximately 200%, though mode of action is still unknown. Therefore it is assumed that hydrogen bond of crystalline structure of cellulose breaks upon supplying with adequate amount of energy. In a 2003 study by Bochek *et al*. [28], energy supplied was 130 KJ/g of model compound, which is significantly high compared to hydrogen bond of cellulose (0.12 KJ/g).

3.2.2.2 Physico-chemical Methods

1. Steam explosion: This is also referred to as autohydrolysis and is the most commonly used method (catalyzed or uncatalyzed) for lignocellulosic biomass pretreatment [19, 29, 30]. In this method, chipped or grilled biomass is treated for some time (30 s–20 min.) to high pressure saturated steam in a batch or continuous process. Pressure is reduced suddenly after treatment, which causes explosive decompression of biomass with hemicelluloses degradation and lignin matrix disruption. This method combines both chemical and mechanical forces due to autohydrolysis of acetyl group present in hemicellulosic portion of biomass. It is started at very high temperature (160 °C–260 °C), which results in the formation of acetic acid from acetyl group [31]. At high temperature, water acts as an acid also. Various factors which influence this process are residence time, reaction temperature, moisture content in biomass, and particle size of raw material. To improve the results, acid or alkali has been tested. Combined effect of time and temperature can be represented by $R_o = t \exp^{(T-100/14.75)}$ where R_o is severity parameter [32]. Steam explosion has many advantages over other processes (Table 3.1). It includes [33]:

- high recovery of sugars
- lower capital investment and environmental impact
- more energy-efficient process
- possibilities of using larger particle size
- addition of acid or alkali catalyst
- feasibility at industrial scale

Table 3.1 Effect of different pretreatment methods on lignocellulosic biomass structure.

Pretreatment method	Effect on accessible surface area increase	Decrystallization of cellulose	Solubilisation of hemicellulose	Removal of lignin	Generation of toxic compounds	Alteration of lignin structure
Mechanical comminution	High	High	-	-	-	-
Steam explosion	High	-	High	Medium	High	High
Liquid hot water	High	Not determined	High	Low	Low	Medium
Acid based	High	-	High	Medium	High	High
Alkaline	High	-	Low	Medium	Low	High
Oxidative delignification	High	Not determined	-	Medium	Low	High
Ammonia fibre expansion	High	High	Medium	High	Low	High
Ammonia recycle percolation	High	High	Medium	High	Medium	High
Lime	High	Not determined	Medium	High	Medium	High
CO_2 explosion	High	-	High	-	-	-

This process is less effective for softwoods due to the presence of low acetyl group content, therefore catalyst needs to be added in the hemicellulosic part of raw material [15]. SO_2 or H_2SO_4 addition is found most effective for softwood pretreatment but formation of inhibitory compound is one of the main drawbacks of this method [34, 35].

Major disadvantages of this method are formation of toxic compounds which affect hydrolysis and fermentation and energy consumption [6]. A separate detoxification step is required due to formation of by-products. Activated charcoal treatment is used to remove these, which increases the overall cost of the process.

2. Liquid hot water: This is a type of hydrothermal pretreatment and does not require any catalyst addition and rapid decompression. To maintain liquid state of water, high pressure is used at high temperature. Hydrothermal pretreatment, Hydrothermolysis, aqueous fractionation, solvolysis or aquasolv terms are also used for this process. Temperature ranges from 170 °C–230 °C and pressure (>5 MPa) is used for usually 15 minutes during the process [2].

Liquid hot water pretreatment process removes hemicelluloses from lignocellulosic biomass which increases cellulose accessibility. Slurry obtained after pretreatment contains liquid and solid fractions. Liquid fraction contains hemicelluloses derived sugars and solid fraction contains cellulose. Better pH control (4–7) minimizes non-specific degradation of sugars and avoids toxic compound formation [6].

Liquid hot water can result in high hemicellulosic sugar fraction in the form of oligomers mostly and reduces by-product formation. Residence time and reaction temperature are most significant factors in this process. To promote effective contact between liquid hot water and biomass, three different methods have been developed, i.e., cocurrent, countercurrent and flowthrough.

In the co-current method, biomass slurry and water is heated to the desired temperature and held for optimized residence time, after which it is cooled down. In the countercurrent method, slurry biomass and liquid hot water move opposite to each other. In the flowthrough method, biomass is kept stationary and liquid hot water is passed through it [36, 37].

As no catalyst or corrosion resistant material is required in this process, it is considered economical. The main advantages are high pentose sugar recovery and lower toxic compound generation, but high water demand and high energy requirement make it non-feasible at industrial scale.

3. Ammonia based pretreatment/ Ammonia fibre expansion (AFEX): This is another type of pretreatment process in which liquid ammonia is

used to treat biomass at temperature (90 °C–100 °C) for a limited time (30–60 min.) which follows rapid pressure release [38]. This results in physical disruption of lignocellulosic biomass fibres and decrystallization of cellulose to some extent. Due to swelling caused by rapid expansion of liquid ammonia, this process can either modify or reduce effective crystallinity of cellulosic fibre and lignin matrix [20]. It increases digestibility of biomass by deacetylation process [39, 40]. Toxic compounds are not produced using it; therefore washing with water is not compulsory. Woody biomass is less effective than agricultural residues and herbaceous biomass. Shao *et al.* [41] showed that ammonia fibre expansion treated biomass yielded 1.5–3-fold higher sugar recovery compared to untreated biomass. Cellulase addition if done sequentially results in 15–20% higher hydrolysis of starch compared to simultaneous addition of enzyme. After 72 h of hydrolysis, 70% glucan was converted.

4. CO_2 explosion: In this method, CO_2 is utilized as supercritical fluid, i.e., fluid exhibits gaseous mass temperature properties apart from solvating power of liquid. This process removes lignin efficiently in both hardwood and softwood [42]. Supercritical CO_2 at high pressure is used in this method. Co-solvents such as ethanol can be added to improve delignification. Supercritical CO_2 is used mostly in non-extractive purposes as an extraction solvent because of its advantages such as low-cost availability, non-inflammability, non-toxicity, easy recovery and environment friendly [43]. CO_2 from carbonic acid in aqueous form increases rate of hydrolysis. CO_2 molecule size should be compatible to NH_3 and H_2O as it can penetrate pores which are accessible to them. Disruption of cellulose and hemicelluloses and increment in surface area accessible for enzymatic attack occurs in this process. This method is cost effective compared to ammonia-based pretreatment methods and toxic compound generation is much lower compared to steam explosion [44].

5. Oxidative delignification: three types of oxidative delignification has been explained

A. Using oxidizing agents such as hydrogen peroxide (H_2O_2) or peracetic acid ($C_2H_4O_3$).
B. Ozonolysis
C. Wet oxidation

A. Using oxidizing agents such as hydrogen peroxide or peracetic acid: Most commonly used is hydrogen peroxide. At 25 °C–30°C, 1–2% H_2O_2 is effective in recovery of most of the hemicelluloses and 50% of lignin can

be dissolute using it, which is fivefold higher than NaOH [45]. During this process, several reactions occur like electrolytic substitution, breakage of alkyl or aryl ether linkage, side chain displacement or oxidative breakdown of aromatic nuclei. This method is found effective for pretreatment of rice hulls which has shown 96% sugar conversion after hydrolysis [46].

B. Ozonolysis: Ozone is a very effective oxidizing agent. In this method, lignocellulosic biomass is pretreated with ozone. Ozone does not affect holocellulose portion; it degrades only lignin by attacking its aromatic ring structure. Various types of lignocellulosic biomasses have been treated using ozone. Some of them are baggase, peanut, rye straw, cotton straw, wheat straw, poplar sawdust, pine and peanut [15, 47]. This process is performed usually at room temperature (around 30 °C) and pressure. Inhibitory compounds are not generated during this process; therefore saccharification and fermentation process does not get affected. Ozone gas is passed through the substrate vessel (fixed bed, packed bed or stirred semi-batch reactors) [47, 48].

Two major factors influence this process: type of lignocellulosic biomass and moisture content. In a recent study done by Miura *et al.* [49], the effect of wet disk milling and ozonolysis on *Cryptomeria japonica* (Japanese cedar) was shown to improve sugar recovery upon enzymatic saccharication. Ozone consumption reduced when moisture content reached greater than 40%, which resulted in reduction of lignin removal from biomass. Glucose and xylose recovery was 68.8% and 43.2%, respectively. The requirement of a large amount of ozone makes the process expensive and non-feasible at industrial scale [15].

C. Wet oxidation: This method is suitable for biomass having high lignin content. Biomass is treated with water and oxygen or air for 30 minutes at a temperature above 120 °C [50]. Variables affecting this process are reaction time, temperature and oxygen pressure [51]. When the temperature reaches more than 170 °C, process becomes exothermic, which makes it self-sufficient with respect to heat [51]. This approach for pretreatment catalyzes acid formation from oxidative reaction and hydrolytic reaction. All the portion of lignocellulosic biomass (cellulose, hemicelluloses and lignin) gets affected during the process. Hemicelluloses degrade into very low molecular weight sugars which are soluble into water. Cellulose degrades partially and lignin undergoes cleavage. Due to degradation, the surface area of cellulose becomes highly accessible for hydrolysis. Additions of alkaline agents (e.g., Na_2CO_3) help in hemicellulose solubilisation and decrease formation of toxic compounds [52].

In a study on common reed (*Phragmites australis*), around 51.7% hemicelluloses solubilization and 58% lignin solubilisation was obtained; 87%

cellulose retained in solids. At optimized condition (185 °C for 12 minutes); cellulose digestibility was improved more than three times in comparison to untreated biomass as a control. 82.4% glucose conversion was achieved, which was processed further during simultaneous saccharification and fermentation. Using it, ethanol concentration of 8.7 g/L was achieved, which was 73% of theoretical yield [53].

In another study on rice husk, alkaline peroxide was used for wet oxidation. Raw material was soaked in 1% (w/v) H_2O_2 solution at room temperature overnight. It resulted in 67% hemicellulose solubilisation and 88% lignin degradation. It gave a 13-fold increase in glucose recovery [54]. High pressure, high temperature and large reaction vessel requirements limit its use. The cost of oxygen and catalyst make it non-economical.

6. Microwave: This method is widely used because of easy operation and very high heating efficiency. Time varies from 5 minutes to 20 minutes. It modifies cellulose ultra structure and causes degradation of hemicellulose and lignin by improving the enzymatic susceptibility of biomass [55]. Alkali especially NaOH has been found the most suitable reagent for rice straw using this method [56]. In case of switchgrass low energy was required for pretreatment for an extended time where the sugar yield was found 70–90% [57]. An orthogonal design was developed to optimize this process where ethanol yield was found 148.93 g/kg of wheat straw. From untreated biomass, this yield was 26.87 g/kg of biomass [58]. In another study on *Miscanthus sinesis*, sugar recovery increased significantly using ammonium hydroxide. 75% lignin was removed and 41% total reducing sugar was obtained in case of oil palm empty fruit bunch fibre in alkaline condition [59].

Homogeneous heating and reduced reaction time are the main advantages of this process. It is a promising method because of short time, energy saving and minimum toxics generation. To modify cellulose native structure with hemicellulose and lignin degradation, it is one of the best methods to use [60]. Reagents can be used to enhance the overall yield.

3.2.2.3 Chemical Methods

1. Acid based: Acid pretreatment is one of the most widely used pretreatment methods. It causes lignin and hemicellulose solubilisation and an improvement in cellulose accessibility. Formation of inhibitory compounds like furfural, 5- hydroxymethyl furfural, phenolics, etc., are major limitations of this process. It can be categorized into two types:

A. Weak acid hydrolysis
B. Strong acid hydrolysis

A. Weak acid hydrolysis or dilute acid hydrolysis: For lignocellulosic biomass, it is one of the most effective techniques. There are two ways for approaching this process [27]:

a. High temperature, continuous flow process: used mainly for low solids loading when T > 160 °C and substrate concentration is 5–10 wt. %.
b. Low temperature, batch process: used mainly for high-solids loading when T ≤ 160 °C substrate concentration is 10–40 wt.%.

Dilute sulfuric acid is sprayed onto the lignocellulosic biomass. Mixture is held at 160 °C–220 °C for a few minutes. Hemicelluloses hydrolysis occurs, which releases soluble oligomers and monomeric sugars from cell wall matrix into hydrolysate. Removal of hemicellulose enhances enzymatic digestibility and porosity. Maximum digestibility coincides with complete removal of hemicellulose [61]. Organic acids like maleic acid, fumaric acid, etc., can be used for this pretreatment method in place of inorganic acids [62]. This method has shown good performance in recovering hemicellulosic sugars but these sugars might be further converted to furan compounds furfural and 5-hydroxymethyl furfural which are strong inhibitors of microbial fermentation. Also, acids can be corrosive in nature. This method is most suitable for lignocellulosic biomass having low lignin content, as lignin is not removed in this process.

B. Strong acid hydrolysis: Sulfuric acid and hydrochloric acid have been used widely for treatment of lignocellulosic biomass as they are very powerful reagents for cellulose hydrolysis [15]. Enzymes are not needed after concentrated acid hydrolysis for saccharification. Advantages include feedstock flexibility, high monomeric sugar yield and mild temperature requirement. Drawbacks are the corrosive nature of the acids and for economy, recycling of acid is needed. Several industries are in the process of commercialization of strong acid hydrolysis treatment of lignocellulosic biomass for bioethanol production.

Concentrated acid requires corrosion resistant equipments as they are corrosive and toxic in nature. For industrial scale, dilute acid treatment is more feasible. Various reactors such as plug flow reactor, flowthrough reactor, countercurrent reactor, shrinking bed reactor and percolation are developed for this process. Acid pretreatment can be again categorized using two different approaches. First is high temperature, short reaction time, and second is low temperature, longer reaction time (30–90 minutes).

High sugar recovery has been reported using dilute H_2SO_4. Various other acids such as HCl, HNO_3, H_3PO_4, oxalic acid, formic acid, acetic acid and maleic acid have been tested. Lee *et al.* [63] used oxalic acid for treatment of corn cobs; 13.1% of total sugar was obtained and a much lower amount of inhibitory compound was formed. Using maleic acid, 10% sugar was obtained and a high amount of inhibitory compound was formed. Kim *et al.* [38] performed two-stage hydrolysis in which aqueous ammonia was used in the first stage and dil. H_2SO_4 was used in the second in percolation mode. Total reducing sugar yield was 90.8%, which indicates that this combination resulted in better lignin removal and high sugar yield. *Eulaliopsis binate* is a perennial grass found in India and China, when treated with dilute H_2SO_4 at optimum condition, 21.02% sugar, 3.22% lignin and 3.34% acetic acid was obtained. Low amount of inhibitory compounds were formed. 78% sugar yield was obtained upon treatment of corn stover with dilute H_2SO_4. Cheapness of H_2SO_4 and HCl makes the process economical. The main drawbacks of acid hydrolysis are:

- High energy input requirement as high temperature is needed.
- Corrosion resistant specific reaction vessel required because of the corrosive nature of acids.
- Inhibitory compound generation.

2. Alkali based: Alkali removes mainly the lignin portion of lignocellulosic biomass which increases reactivity of the remaining holocellulosic part. Alkali treatment acts on hemicelluloses by removing acetyl group and various uronic acid substitutions which reduces the enzymatic accessibility to hemicellulose and cellulose [7]. Mechanism of alkaline hydrolysis is based on intermolecular ester bonds saponification which crosslink xylan hemicelluloses and lignin [15]. Various reagents used for alkali pretreatments are:

A. Calcium or sodium hydroxide: Lime ($Ca(OH)_2$) or NaOH is used widely. Salts are formed using these compounds which can get incorporated in the substrate. Hence, it needs to be recycled or removed [64]. Process conditions are kept relatively mild and reaction time can be very long. Mild conditions prevent lignin condensation, which results in high solubility of lignin, especially for biomass having low lignin content (softwood, grasses, etc.). Mild conditions also prevent sugars degradation to furan compounds and organic acids. Oxygen or air addition to the reaction mixture improves the delignification process significantly [7].

B. Aqueous ammonia based: Pretreatment of lignocellulosic biomass with aqueous ammonia reduces lignin content at elevated temperatures and also removes some hemicelluloses while doing decrystallisation of cellulose. Ammonia pretreatment techniques include

- Ammonia fibre expansion method (AFEX)
- Ammonia recycle percolation (ARP)
- Aqueous ammonia soaking or soaking in aqueous ammonia (SAA)

The AFEX process is explained briefly earlier in physico-chemical methods. In ARP, flow-through column reactor is used for biomass pretreatment with aqueous ammonia. Liquid flows through the reactor column at high temperature; the column is packed previously with lignocellulosic biomass. The reactor system should be pressurized slightly to prevent flash evaporation [65, 66]. Solid fraction which is rich in cellulose and hemicelluloses is separated after reaction from liquid. Then liquid fraction is flown through steam heated evaporator for ammonia recovery. Ammonia is then recycled back to reactor inlet and separated fraction is passed into a crystallizer. The washing step is carried out after crystallization to extract the sugars retained in solid matrix.

SAA is carried out at low temperature and removes the lignin present in biomass efficiently by minimal interaction with hemicellulose. Due to this, surface area and pore size is increased. Then retained cellulose and hemicellulose can be further hydrolyzed to fermentable sugars by commercial cellulase and xylanase mixtures. In a study by Kim *et al.* [67], 15–30% aqueous ammonia was used to treat destarched barley hull at temperature 30–75 °C for 12 h–77 days. Solid liquid ratio was 1:12 with no agitation. Solids were recovered after soaking by filtration. Then it was washed and analyzed. 66% lignin solubilisation, 83% glucan and 63% xylan was observed after biomass treatment with 15% aqueous ammonia at 75 °C for 48 h. Ammonia cost and its recovery drives the cost of the process [68, 69]. Biomass pretreatment economics are also strongly influenced by total sugar yield.

In alkali-based pretreatment, NaOH is used most widely. $Ca(OH)_2$ based pretreatment has the additional advantage of low cost of reagent and less safety requirements compared to other alkali-based pretreatments. Also, it can be easily recovered from hydrolysate obtained by reaction with CO_2 [6]. Pretreatment process combinations have also been tried for significant recovery of sugars, e.g., includes combination of lime treatment with oxidative delignification process. High downstream processing costs make it a

costly process (Table 3.2). For washing of calcium and sodium salts, a high amount of water is needed, which is difficult to remove.

3. Organosolv: Organic or aqueous organic solvent mixtures are used in combination with inorganic acid catalysts for delignification in this method. Various organic solvent mixtures have been used like triethylene glycol, ethylene glycol, ethanol, methanol, tetrahydrofurfuryl alcohol and acetone [70]. Acetylsalicylic acid, salicylic acid and oxalic acid can also be used with or without addition of organic acids at higher temperatures [71]. Wheat straw pretreatment resulted in 70% hemicelluloses recovery and 65% lignin removal by glycerol-based autocatalytic pretreatment [72]. 98% cellulose retention was also obtained using this method. In another study, modified organosolv method was performed; first ethanol was used under mild conditions which followed H_2O_2 treatment in horticultural waste. This resulted in 26.9 g/L reducing sugar recovery in hydrolyzate [73]. This hydrolysate medium was fermented using *Saccharomyces cerevisiae* which produced 11.7 g/L ethanol. Hideno *et al.* [74] studied Japanese cypress (*Chamaecyparis obtusa*) and found that the application of the ball milling process and alcohol-based organosolv pretreatment improved the enzymatic digestibility significantly and reduced the severity of process conditions of organosolv treatment. Alcohol-based organosolv pretreatment and short-time ball milling had a synergistic effect.

Organosolv pretraetment has been used extensively for high-quality lignin extraction (value-added product). Due to efficient lignin removal, around 90% sugar was recovered after enzymatic hydrolysis of treated biomass. The main drawback of the organosolv process is solvent and catalysts cost (Table 3.3). Solvent removal and recovery can reduce the operational cost considerably [15]. As organic solvents are inflammable, safety measures should be impeccable. Uncontrolled use can be the cause of fires and explosions. This additional cost makes the process non-economical. Organic solvents have shown an inhibitory effect on enzymatic hydrolysis; therefore their removal is required prior to hydrolysis, which increases operation cost [6].

4. Ionic liquids: In this method, biomass with ionic liquids in a ratio of 1:10 w/w is used. Temperature ranges from 100°C to 150 °C. The antisolvent (e.g., ethanol, methanol and water) use the soluble biomass regeneration and then subject it to enzymatic saccharification for production of fermentable sugars. Ionic liquids behave like a salt which is a combination of small inorganic anions and large organic cations. At relatively low temperature (room temperature), it exists as liquid. At high temperature, room temperature ionic liquids have the ability to form hydrogen bonds with

Table 3.2 Capital cost of some important pretreatment processes (feedstock used: corn stover) [86].

Pretreatment method	Dilute acid treatment	Hot water treatment	Ammonia fibre expansion	Ammonia recycle percolation	Lime	Untreated biomass	Ideal pretreatment
Pretreatment direct fixed capital (in million $)	25.0	4.5	25.7	28.3	22.3	0	0
Total fixed capital (in million $)	208.8	200.6	211.5	200.3	163.6	210.9	162.5
Ethanol production (million gallon/ year)	56.1	44.0	56.8	46.3	48.9	9.0	64.7
Total fixed capital ($/gallon annual capacity)	3.72	4.57	3.72	4.56	3.35	22.26	2.51

Table 3.3 Comparison of different pretreatment routes.

Pretreatment methods	Mode of action (accessible surface area included)	Potential sugar yield	Inhibitor formation	Residue formation	Need for recycling chemicals	Investment cost	Operational cost	Applicable to various biomass	Proven at pilot scale
Mechanical	–	Low	No	No	No	Moderate	High	Medium range	Successful to some extent
Liquid hot water	• Hemicellulose removal	High	Yes	No	No	Moderate	-	-	Yes
Weak acid	• Hemicellulose removal • Lignin structure alteration (minor)	High	Yes	Yes	Yes	Moderate to low	Moderate	Medium range	Yes
Strong acid	• Cellulose and hemicellulose hydrolysis	High	Yes	Yes	Yes	High	Moderate to low	Wide range	Yes
Alkaline	• Lignin removal (major) • Hemicellulose removal	High	No	Yes	Yes	Low		Medium to few range	To some extent-not proven
Organosolv	• Lignin removal (major) • Hemicellulose removal depending on solvent used	High	No	Moderate	Yes	High	High	Medium range	Yes
Wet oxidation	• Lignin removal (major) • Dissolve hemicelluloses • Cellulose decrystallization	Moderate to low	No	Moderate	No	Moderate	-	-	No
Steam explosion	• Hemicellulose removal (major) • lignin structure alteration	Moderate	Yes	Moderate	No	Moderate	Moderate	Medium to low range	Yes
Ammonia fibre expansion	• Removal of lignin (major) • Hemicellulose removal • Cellulose decrystallization	High	No	Moderate	-	-	-	Low range	-
CO_2 explosion	• Removal of hemicellulose • Decrystallization cellulose	Moderate	Moderate	No	No	High	-	-	No
Physico-chemical	• Lignin removal (major) • Hemicellulose removal	High	No	Yes	Yes	Moderate to low	Moderate to low	Medium range	To some extent

cellulose as anions like formate, acetate, chloride or alkyl phosphonate are present in it. It has enormous potential for substrate production achieving more than 90% digestibility of cellulose [75]. Ionic liquids or room temperature ionic liquids (RTIL) which remain in the biomass can show interference with activity of hydrolytic enzymes and downstream fermentation processes, which affect sugar yield and ultimately ethanol [76, 77]. Ionic liquids can be regenerated by flash distillation from antisolvents [78]. At industrial scale, recycling methods should be energy efficient. Toxicity to microorganisms and enzymes to be used during ethanol production should also be considered for using this method [13, 79]. Additionally, to recover lignin and hemicelluloses after cellulose extraction from solutions, some technology should be developed. These are some major limitations to ionic liquids use for bioethanol production from lignocellulosics. Despite these flaws, it has great potential in biorefinery industries, although no industry employs it for pretreatment as of now.

3.2.2.4 Biological Methods

Traditional methods (physical, chemical and physico-chemical) for pretreatment of lignocellulosic biomass require extensive amount of energy and are not environmental friendly. Therefore, biological pretreatment came into existence because of its efficiency, economy and environmental friendly behavior. Hemicellulolytic enzyme (mostly xylanase) is used for pretreatment of hemicellulosic part of biomass and cellulolytic (cellulase) enzymes are used for cellulosic treatment of lignocellulosic biomass. Most commonly used microorganisms for pretreatment are ubiquitous filamentous fungi which are isolated directly from soil or living plants. White rot fungi are most effective. Some of the common examples of white rot fungi are *Ceriporia lacerate, Pycnoporus cinnarbarinus Pleurotus ostreaus, Cyathus stercolerus, Ceriporiopsis subvermispora, and Phanerochaete chrysosporium*. These fungi produce lignin peroxidase which is manganese dependent. This enzyme degrades the lignin part of biomass. Brown rot fungi attack only the cellulosic part while soft rot fungi and white rot fungi attack the whole biomass except the hemicellulosic part. High delignification efficiency has been shown by white rot fungi on various lignocellulosic feedstocks [39, 80]. An effective and significant lignin removal has been reported by fungus *Ceriporiopsis subvermispora*, on various feedstocks by combined action of laccase and manganese peroxidase [81]. 24.2–56.5% glucose yield was obtained, which was 2–3 times higher.

Phanerochaete chrysosporium was used for the pretreatment of rice husks which resulted in 44.7% of total reducing sugars [82]. White rot

basidiomycetes (e.g., *Lepista nuda, Trametes versicolor, Phanerochaete chrysosporium, Fomes fomentarius, Ganoderma resinaceum, Euc-1, Irpex lacteus, and Bjerkandera adusta)* were used for wheat straw treatments by submerged and solid state fermentations. Fungal strain *Tinea versicolor* was used for enzymatic hydrolysis which proved best among all these [83]. *Streptomycin griseus* was found effective for treatment of both softwood and hardwood [84]. Hydrolysis and fermentation at high substrate concentration leads to enhance inhibitors concentration during the process. Treatment with some enzymes like laccases has been found effective in prevention of formation of inhibitory compounds [25]. Phenolic compounds recovery from the leaves of *Larrea tridentata* was found 33% more using combining biological treatment and methanol extraction methods in comparison to only methanol extraction [85]. Advantages of biological method of pretreatment include: no chemical involvement, low energy, low capital cost and environmental friendly behaviour (Table 3.4). A major limitation to use of biological pretreatment is the hydrolysis rate which is much less [15]. Hence, more research is required on various isolates like basidiomycetes fungi for quick and efficient delignification property.

3.3 Conclusion and Future Perspectives

Various pretreatment methods for lignocellulosic biomass to improve bioethanol production process have been described in this chapter. These methods should make the biomass accessible to hydrolytic reactions where cellulose crystallinity, its accessible surface area, lignin degradation and hemicelluloses removal are the main substrate-related parameters which affect the hydrolysis process. Table 3.1 summarizes the effect of different pretreatment technologies on the lignocellulosic structure. In this study, process economic impact of the different pretreatment methods has also been shown, which is related to capital and operating cost in Table 3.2**.** Among all the methods described above, chemical and physico-chemical methods are most effective and promising for industrial applications currently. Comparison of some of the important pretreatment routes is presented briefly in Table 3.3 while positive and negative aspects of all methods are described in Table 3.4. It has been concluded from this study that pretreatment reactors cost is often inversely proportional to pretreatment catalyst recovery or ethanol product recovery cost, i.e., they are often counterbalanced. However, these results serve only as a guide and should not be considered as a basis, as variation in the development state of each technology was not made. To increase sugar yields, efficient conversion of

Table 3.4 Summary of advantages and disadvantages of pretreatment technologies for lignocellulosic biomass.

S. no.	Pretreatment method	Advantages	Disadvantage
1.	**Milling**	• Reduction in crystallinity of cellulose and degree of polymerization • Partcicle size reduction improve pore size and specific surface area	• High energy • high power requirement
2.	**Steam explosion**	• Solubilisation of hemicellulose and transformation of lignin • Low cost • High glucose and hemicelluloses yield in two step process	• Toxics generation • Hemicellulose degradation is partial
3.	**Liquid hot water**	• Biomass size reduction not needed • Usually no chemicals required • Corrosion resistant material not required.	• High energy requirement • High power requirement Toxics generation
4.	**Ammonia fibre expansion**	• Less formation of inhibitors • Particle size reduction not needed	• Not suitable for biomass having high lignin content • High cost of chemicals
5.	**CO_2 explosion**	• Accessible surface area increase • Low cost availability • No formation of inhibitors • Non-inflammable • After extraction, easy recovery • ecofriendly	• High pressure requirement
6.	**Wet oxidation**	• High hemicelluloses and lignin solubilisation • Less inhibitor formation	• High cost of oxygen • High cost of alkaline catalysts

7.	**Concentrated acid**	• Ambient temperature requirement • High glucose yield	• Acid recovery needed • Requirement of corrosion resistant material • Toxic and hazardous nature of acids
8.	**Diluted acid**	• High sugar recovery • Less inhibitors	• Low reducing sugar concentration • Degradation products generation
9.	**Alkali**	• Reduction in crystallinity of cellulose and degree of polymerization • Lignin structure disruption	• High cost • Not suitable at industrial scale
10.	**Ozonolysis**	• Removes lignin effectively • No toxics • Room temperature and pressure required	• High cost of ozone
11.	**Organosolv**	• Lignin disruption • Hemicellulose hydrolysis	• Solvent reuse and recovery needed and high cost
12.	**Biological**	• Less energy required • Lignin removal • Reduction in degree of polymerization • Hemicellulose partial hydrolysis • No chemicals requirement, ecofriendly • Mild environmental conditions	• Slow process rate • Very low rate of treatment • Commercially not effective

hemicellulosic sugars is an important task which reduces overall ethanol production cost.

A major bottleneck in pretreatment technology is the presence of lignin, a major inhibitor of cellulose and hemicelluloses hydrolysis. Extensive research has been done for the development of various processes to overcome this problem. From this study, it is concluded that no treatment technology is able to convert 100% biomass into monomeric fermentable sugars. Biomass loss is always there, which affects the final yield of process and increases bioethanol cost. Hence, extensive research is needed in this area either to develop a new efficient treatment technology or to upgrade an existing process for promising results.

References

1. Hamelinck CN, van Hooijdonk and Faaij APC "Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term" *Biomass Bioenergy,* vol. 28, p. 384–410, 2005.
2. Sánchez ÓJ and Cardona CA "Trends in biotechnological production of fuel ethanol from different feedstocks" *Bioresour Technol*, vol. 99, p. 5270–5295, 2008.
3. Singla A, Paroda S, Dhamija SS, Goyal S, Shekhawat K, Amachi S and Inubushi K "Bioethanol production from xylose: problems and possibilities" *J Biofuels,* vol. 3, p. 39–49, 2012.
4. Singla A and Inubushi K "Effect of biochar on CH_4 and N_2O emission from soils vegetated with paddy" *Paddy Water Environ,* vol. 12, p. 239–243, 2014.
5. Chesson A and Forsberg CW, "Polysaccharide degradation by rumen microorganisms," In: Hobson (ed) *The Rumen Microbial Ecosystem*, pp 251–284, 1988.
6. Mosier N, Wyman CE, Dale BD, Elander RT, Lee YY, Holtzapple M and Ladisch CM, "Features of promising technologies for pretreatment of lignocellulosic biomass" *Bioresour. Technol.,* 96, p. 673–686, 2005.
7. Chang VS and Holtzapple MT "Fundamental factors affecting biomass enzymatic reactivity" *Appl Biochem Biotechnol,* vol. 84, p. 5–37, 2000.
8. Palonen H "Role of lignin in the enzymatic hydrolysis of lignocelluloses" *VTT Pub,* vol. 520, p. 1–80, 2004.
9. Galbe, M and Zacchi, G "Pretreatment of lignocellulosic materials for efficient bioethanol production" *Adv. Biochem. Eng. Biotechnol,* vol. 108, p. 41–65, 2007.
10. Tomás-Pejó, E., Oliva, J.M. and Ballesteros M "Realistic approach for full-scale bioethanol production from lignocellulose: a review" *J. Sci. Ind. Res*. Vol. 67, p. 874–884, 2008.
11. Carvalheiro F, Duarte LC and Gírio FM "Hemicellulose biorefineries: a review on biomass pretreatments" *J Sci Ind Res,* vol. 67, p. 849–864, 2008.

12. Taherzadeh MJ and Karimi K "Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review" *Int J Mol Sci,* vol. 9, p. 1621–1651, 2008.
13. Yang B and Wyman CE "Pretreatment: the key to unlocking low cost cellulosic ethanol" *Biofuels Bioprod Bioref,* vol. 2, p. 26–40, 2008.
14. Hendriks ATWM and Zeeman G "Pretreatments to enhance the digestibility of lignocellulosic biomass" *Bioresour. Technol.,* Vol. 100, 10–18, 2009.
15. Sun Y and Cheng J "Hydrolysis of lignocellulosic materials for ethanol production: a review" *Bioresour Technol,* vol. 83, p. 1–11, 2002.
16. Taherzadeh MJ and Karimi K "Enzymatic-based hydrolysis processes for ethanol from lignocellulosic materials: a review" *Bioresources,* vol. 2, p. 707–738, 2007.
17. Chandel AK, Chan EC, Rudravaram R, Narasu ML, Rao LV and Ravinda P "Economics and environmental impact of bioethanol production technologies: an appraisal" *Biotechnol Mol Biol Rev,* vol. 2, p. 14–32, 2007.
18. Jeoh T, Johnson DK, Adney WS and Himmel ME "Measuring cellulase accessibility of dilute-acid pretreated corn stover" *Prepr Symp Am Chem Soc Div Fuel Chem*, vol. 50(2), p. 673–674, 2005.
19. Chandra RP, Bura R, Mabee WE, Berlin A, Pan X and Saddler JN "Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocellulosics" *Adv Biochem Eng Biotechnol*, vol. 108, p. 67–93, 2007.
20. Laureano-Pérez L, Teymouri F, Alizadeh H and Dale BE "Understanding factors that limit enzymatic hydrolysis of biomass" *Appl Biochem Biotechnol,* vol. 121, p. 1081–1099, 2005.
21. Oliva JM, Sáez F, Ballesteros I, Gónzalez A, Negro MJ, Manzanares P and Ballesteros M "Effect of lignocellulosic degradation compounds from steam explosion pretreatment on ethanol fermentation by thermotolerant yeast Kluyveromyces marxianus" *Appl Microbiol Biotechnol,* vol. 105, p. 141–154, 2003.
22. Palmqvist E and Hahn-Hägerdal B "Fermentation of lignocellulosic hydrolysates II: inhibitors and mechanism of inhibition" *Bioresour. Technol,* vol. 74, 25–33, 2000.
23. Klinke, H.B., Ahring, B.K., Schmidt, A.S., Thomsen and A.B., 2002. "Characterization of degradation products from alkaline wet oxidation of wheat straw" *Bioresour. Technol,* Vol. 82, p. 15–26, 2002.
24. Zhu JY and Pan XJ "Woody biomass pretreatment for cellulosic ethanol production: technology and energy consumption evaluation" *Bioresour Technol,* vol. 101, p. 4992–5002, 2010.
25. Alvira P, Tomás-Pejo E, Ballesteros M and Negro MJ "Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review", *Bioresour Technol,* vol. 101, p. 4851–4861, 2010.
26. Zheng J, Choo K, Bradt C, Lehoux R and Rehmann L "Enzymatic hydrolysis of steam exploded corncob residues after pretreatment in a twin-screw extruder" *Biotechnol Rep,* vol. 3, p. 99–107, 2014.

27. Harmsen PF, Huijgen WJJ, Bermúdez López LM, Bakker RRC, *Literature Review of Physical and Chemical Pretreatment Processes for Lignocellulosic Biomass,* Biosynergy: Energy Research Centre of the Netherlands, 2010.
28. Bochek AM "Effect of hydrogen bonding on cellulose solubility in aqueous and nonaqueous solvents" *Russian Journal of Applied Chemistry,* vol. 76(11), p. 1711–1719, 2003.
29. Singh J, Suhag M and Dhaka A "Augmented digestion of lignocellulose by steam explosion, acid and alkaline pretreatment methods: a review" *Carbohyd Poly,* vol. 117, p. 624–631, 2015.
30. Maurya DP, Singla A and Sangeeta N "An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol" *3 Biotech*, vol. 5, p. 597–609, 2015.
31. Pan X, Xie D, Gilkes N, Gregg DJ and Saddler JN "Strategies to enhance the enzymatic hydrolysis of pretreated softwood with high residual lignin content" *Appl Biochem0 Biotechnol- Part A Enz Eng. Biotechnol,* vol. 124, p. 1069–1079, 2005.
32. Alfani A, Gallifuoco F, Saporosi A, Spera A and Cantarella M "Comparison of SHF and SSF process for the bioconversion of steam-exploded wheat straw" *J Ind Microbiol Biotechnol,* vol. 25, p. 184–192, 2000.
33. Avellar BK and Glasser WG "Steam-assisted biomass fractionation: Process considerations and economic evaluation" *Biomass Bioenergy*, vol. 14, p. 205–218, 1998.
34. Berlin A, Balakshin M, Gilkes N, Kadla J, Maximenko V, Kubo S and Saddler JN "Inhibition of cellulase, xylanase and β-glucosidase activities by softwood lignin preparations" *J Biotechnol*, vol. 125, p. 198–209, 2006.
35. Kumar L, Saddler JN, Arantes V and Chandra R "The lignin present in steam pretreated softwood binds enzymes and limites cellulose accessibility" *Bioresour Technol,* vol. 103, p. 201–208, 2012.
36. Liu C, Wyman CE "The effect of flow rate of compressed hot water on xylan, lignin, and total mass removal from corn stover" *Ind Eng Chem Res,* vol. 42, p. 5409–5416, 2003.
37. Yang B and Wyman CE "Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn stover cellulose" *Biotechnol Bioeng*, vol. 86, p. 88–95, 2004.
38. Kim JW, Kim KS, Lee JS, Park SM, Cho HY, Park JC and Kim JS "Two-stage pretreatment of rice straw using aqueous ammonia and dilute acid" *Bioresour Technol,* vol. 102, p. 8992–8999, 2011.
39. Kumar R and Wyman CE "Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies" *Biotechnol Prog,* vol. 25, p. 302–314, 2009a.
40. Kumar R and Wyman CE "Does change in accessibility with conversion depends on both the substrate and pretreatment technology" *Bioresour Technol,* vol. 100, p. 4193–4202, 2009b.
41. Shao Q, Chundawat SP, Krishnan C, Bals B, da Sousa LC, Thelen KD, Dale BE and Balan V "Enzymatic digestibility and ethanol fermentability of

AFEX-treated starch-rich lignocellulosics such as corn silage and whole corn plant" *Biotechnol Biofuels,* vol. 9, p. 3–12, 2010.
42. Kim KH and Hong J "Supercritical CO_2 pretreatment of lignocelluloses enhances enzymatic cellulose hydrolysis" *Bioresour Technol*, vol. 77, p. 139–144, 2001.
43. Schacht C, Zetzl C and Brunner G "From plant materials to ethanol by means of supercritical fluid technology" *J Supercrit Fluids,* vol. 46, p. 299–321, 2008.
44. Zheng Y, Lin HM and Tsao GT "Pretreatment for cellulose hydrolysis by carbon dioxide explosion" *Biotechnol Prog*, vol. 14, p. 890–896, 1998.
45. Chaturvedi V and Verma P "An overview of key pretreatment processes employed for bioconversion of lignocellulosic biomass into biofuels and value added products" *3. Biotech*, vol. 5, p. 415–431, 2013.
46. Saha BC and Cotta MA "Enzymatic saccharification and fermentation of alkaline peroxide pretreated rice hulls to ethanol" *Enzyme Microb Tech,* vol. 41, p. 528–532, 2007.
47. García-Cubero MT, González-Benito G, Indacoechea I, Coca M and Bolado S "Effect of zonolysis pretreatment on enzymatic digestibility of wheat and rye straw" *Bioresour Technol,* vol. 100, p. 1608–1613, 2006.
48. Vidal PF and Molinier J "Ozonolysis of lignin-Improvement of in vitro digestibility of poplar sawdust" *Biomass,* vol. 16, p. 1–17, 1988.
49. Miura T, Lee SH, Inoue S and Endo T "Combined pretreatment using ozonolysis and wet-disk milling to improve enzymatic saccharification of Japanese cedar" *Bioresour Technol,* vol. 126, p. 182–186, 2012.
50. Varga E, Klinke HB, Reczey K and Thomsen AB "High solid simultaneous saccharification and fermentation of wet oxidized corn stover to ethanol" *Biotechnol Bioeng,* vol. 88, p. 567–574, 2004.
51. Schmidt A and Thomsen A "Optimization of wet oxidation pretreatment of wheat straw" *Bioresour Technol,* vol. 64, p. 139–151, 1998.
52. Ahring BK, Jensen K, Nielsen P, Bjerre AB and Schmidt AS "Pretreatment of wheat straw and conversion of xylose and xylan to ethanol by thermophilic anaerobic bacteria" *Bioresources Technology,* vol. 58, p. 107–113, 1996.
53. Szijarto N, Kadar Z, Varga E, Thomsen AB, Costa-Ferreira M and Reczey K "Pretreatment of reed by wet oxidation and subsequent utilization of the pretreated fibers for ethanol production" *Appl Biochem Biotechnol,* vol. 155, p. 386–396, 2009.
54. Banerjee S, Sen R, Mudliar S, Pandey RA, Chakrabarti T and Satpute D "Alkaline peroxide assisted wet air oxidation pretreatment approach to enhance enzymatic convertibility of rice husk" *Biotechnol Prog*, vol. 27, p. 691–697, 2011.
55. Maurya DP, Vats S, Rai S and Negi S "Optimization of enzymatic saccharification of microwave pretreated sugarcane tops through response surface methodology for biofuel" *Indian J Exp Biol,* vol. 51, p. 992–996, 2013.
56. Zhu S, Wu Y, Yu Z, Wang C, Yu F, Jin S, Ding Y, Chi R, Liao J and Zhang Y "Comparison of three microwave/chemical pretreatment processes for enzymatic hydrolysis of rice straw" *Biosyst Eng,* vol. 93, p. 279–283, 2006.

57. Hu ZH and Wen ZY, Enhancing enzymatic digestibility of switchgrass by microwave-assisted alkali pretreatment" *Biochem Eng J,* vol. 38, p. 369–378, 2008.
58. Xu J, Chen H, Kádár Z, Thomsen AB, Schmidt JE, Peng H "Optimization of microwave pretreatment on wheat straw for ethanol production" *Biomass Bioenergy,* vol. 35, p. 385–386, 2011.
59. Nomanbhay SM, Hussain R and Palanisamy K "Microwave assisted enzymatic saccharification of oil palm empty fruit bunch fiber for enhanced fermentable sugar yield" *J Sustain Bioenergy Syst,* vol. 3, p. 7–17, 2013.
60. Lu X, Xi B, Zhang Y and Angelidaki I "Microwave pretreatment of rape straw for bio-ethanol production: focus on energy efficiency" *Bioresour Technol,* vol. 102, p. 7937–7940, 2011.
61. Chen Y., Sharma-Shivappa RR, *et al*. "Potential of agricultural residues and hay for bioethanol production" *Applied Biochemistry and Biotechnology*, vol. 142(3), p. 276–290, 2007.
62. Kootstra *et al*. "Optimization of dilute maleic acid pretreatment of wheat straw." *Biotechnology for biofuels*, 2: 31, 2009.
63. Lee J, Houtman CJ, Kim H, Choi I and Jeffries TW "Scale-up study of oxalic acid pretreatment of agricultural lignocellulosic biomass for the production of bioethanol" *Bioresour Technol,* vol. 102, p. 7451–7456, 2011.
64. González, G, López-Santín J *et al*. "Dilute acid hydrolysis of wheat straw hemicellulose at moderate temperature: A simplified kinetic model" *Biotechnology and Bioengineering,* vol. 28(2): 288–293, 1986.
65. Kim, TH, Kim JS, *et al*. "Pretreatment of corn stover by aqueous ammonia." *Bioresource Technology,* 90(1), 39–47, 2003.
66. Kim, TH and Lee YY "Pretreatment of corn stover by soaking in aqueous ammonia" *Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology,* vol. 124(1–3), p. 1119–1131, 2005.
67. Kim TH, Taylor F and Hicks KB "Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pretreatment" *Bioresour Technol*, vol. 99, p. 5694–5702, 2008.
68. Holtzapple, MT, Jun J-H, *et al. "Ammonia Fiber Explosion (AFEX) pretreatment of lignocelluloses"* Symposium Paper - Energy from Biomass and Wastes, 1991.
69. Holtzapple, MT, Ripley EP, *et al*. "Saccharification, fermentation, and protein recovery from low-temperature AFEX-treated coastal bermudagrass" *Biotechnology and Bioengineering,* vol. 44(9), p. 1122–1131, 1994.
70. Zhao H, Jones CL, Baker GA, Xia S, Olubajo O and Person VN "Regenerating cellulose from ionic liquids for an accelerated enzymatic hydrolysis" *J Biotechnol,* vol. 139, p. 47–54, 2009a.
71. Sarkanen KV "Acid catalyzed delignification of lignocellulosics in organic solvents" *Prog Biomass Convers,* vol. 2, p. 127–144, 1980.
72. Sun F and Chen H "Organosolv pretreatment by crude glycerol from oleochemicals industry for enzymatic hydrolysis of wheat straw" *Bioresour Technol,* vol. 99, p. 5474–5479, 2008.

73. Geng A, Xin F and Ip JY "Ethanol production from horticultural waste treated by a modified organosolv method" *Bioresour Technol,* vol. 104, p. 715–72, 2012.
74. Hideno A, Kawashima A, Endo T, Honda K and Morita M "Ethanol-based organosolv treatment with trace hydrochloric acid improves the enzymatic digestibility of Japanese cypress (Chamaecyparis obtusa) by exposing nanofibers on the surface" *Bioresour Technol,* vol. 18, p. 64–70, 2013.
75. Lee SH, Doherty TV, Linhardt RJ and Dordick JS "Ionic liquidmediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis" *Biotechnol Bioeng,* vol. 102, p. 1368–1376, 2009.
76. Sathitsuksanoh N, Zhu Z and Zhang YHP "Cellulose solvent based pretreatment for corn stover and avicel: concentrated phosphoric acid versus ionic liquid" *Cellulose*, vol. 19, p. 1161–1172, 2012.
77. Shi J, Gladden JM, Sathitsuksanoh N, Kambam P, Sandoval L, Mitra D, Zhang S, George A, Singer SW, Simmons BA and Singh S "One-pot ionic liquid pretreatment and saccharification of switchgrass" *Green Chem,* vol. 15, p. 2579–2589, 2013.
78. Joglekar HG, Rahman I and Kulkarni BD "The path ahead for ionic liquids" *Chem Eng Technol,* vol. 30, p. 819–828, 2007.
79. Zhao X, Cheng K and Liu D "Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis" *Appl Microbiol Biotechnol,* vol. 82, p. 815–827, 2009b.
80. Shi J, Chinn MS and Sharma-Shivappa RR "Microbial pretreatment of cotton stalks by solid state cultivation of Phanerochaete chrysosporium" *Bioresour Technol,* vol. 99, p. 6556–6564, 2008.
81. Wan C and Li Y "Fungal pretreatment of lignocellulosic biomass" *Biotechnol Adv* vol. 30, p. 1447–1457, 2012.
82. Potumarthi R, Baadhe RR, Nayak P and Jetty A "Simultaneous pretreatment and sacchariffication of rice husk by Phanerochete chrysosporium for improved production of reducing sugars" *Bioresour Technol,* vol. 128, p. 113–117, 2013.
83. Pinto PA, Dias AA, Fraga I, Marques G, Rodrigues MA, Colaco J, Sampaio A and Bezerra RM "Influence of ligninolytic enzymes on straw saccharification during fungal pretreatment" *Bioresour Technol,* vol. 111, p. 261–267, 2012.
84. Saritha M, Arora A and Lata "Biological pretreatment of lignocellulosic substrates for enhanced delignification and enzymatic digestibility" *Indian J Microbiol,* vol. 52, p. 122–130, 2012.
85. Martins S, Teixeira JA and Mussatto SI "Solid-State fermentation as a strategy to improve the bioactive compounds recovery from Larrea tridentata leaves" *Appl Biochem Biotechnol,* vol. 17, p. 1227–1239, 2013.
86. Eggeman, T and Elander RT "Process and economic analysis of pretreatment technologies" *Bioresour. Technol,* vol. 96, p. 2019–2025, 2005.

4

Present Status on Enzymatic Hydrolysis of Lignocellulosic Biomass for Bioethanol Production

Arindam Kuila[1], Vinay Sharma[1], Vijay Kumar Garlapati[2*], Anshu Singh[3], Lakshmishri Roy[4] and Rintu Banerjee[3]

[1]Department of Bioscience and Biotechnology, Banasthali University, Rajasthan - 304022, India
[2]Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, HP-173234, India
[3]Agricultural and Food Engineering Department, IIT Kharagpur, Kharagpur, West Bengal -721302, India
[4]Department of Food Technology, Techno India, Kolkata, West Bengal - 700091, India

Abstract

Bioethanol is becoming a better alternative to fossil fuels. Production of ethanol by using edible feedstock such as grains, sugarcane etc., became a point of concern in terms of the food supply and demand. In such a scenario lignocellulosic biomass that includes nonedible feedstocks opened up a new avenue for the second-generation bioethanol production. Lignocellulosic bioethanol production is composed of three major steps: pretreatment, enzymatic hydrolysis and fermentation. The main factor restraining the commercialization of bioethanol lies in the development of the enzymatic hydrolysis step. During the enzymatic hydrolysis step carbohydrates (cellulose and hemicelluloses) polymers get converted into free monomeric sugars. The major problems associated with enzymatic hydrolysis are cost of the enzyme, higher incubation time for complete degradation of carbohydrates, inhibition of enzyme activity in the presence of phenolic compounds and thermal inactivation of cellulase enzyme. The present article discusses recent trends and development of the enzymatic hydrolysis process for cost-effective bioethanol production. In this review the authors cover the following points: development of cellulase-producing organisms, the enzyme production process, the

**Corresponding author*: shanepati@gmail.com

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improvement or enhancement of enzymatic hydrolysis and its future prospects for commercial lignocellulosic bioethanol production.

***Keywords*:** second-generation bioethanol, lignocellulosic biomass, cellulase enzyme, enzymatic hydrolysis

4.1 Introduction

The steady increase in energy demand and the limiting of fossil fuels are creating an energy gap which poses a serious need for alternative energy sources. The best way to fill this energy gap is the use of sustainable sources of energy, i.e., renewable. Bioethanol is one such promising renewable energy source which is capable of replacing fossil fuels usage partly because of its higher energy density, greater air-fuel ratio, more specific energy and heat of vaporization [1].

Bioethanol is differentiated as first- and second-generation ethanol based on the raw material used. First-generation bioethanol is derived from food crops such as corn and sugar cane while second-generation converts lignocellulosic biomass. But due to controversy of food versus energy, ethanol production from lignocellulosic substrates has gained significant interest as a wide variety of feedstocks can be used as materials with no significant competition with the food chain. The majority of the process cost of ethanol production is dependent on the cost of raw material and in such a scenario, lignocellulosic biomass has made the process commercially feasible.

Lignocellulosic bioethanol production highly depends on two promising steps, which are pretreatment and saccharification. Pretreatment is the critical step of removing the lignin because the extent to which the biomass becomes accessible to the enzyme for saccharification highly depends on the type of pretreatment employed. Apart from the pretreatment process, another significant step is the efficient hydrolysis during saccharification of lignocellulosic substrates as it is the rate limiting step towards techno-economical feasibility of lignocellulosic bioethanol. Enzyme cellulase catalyzes the hydrolysis of cellulose by breaking the 1, 4-β-glycosidic bonds in between the cellulose chain of biomass. Complete use of carbohydrate components in lignocellulosic biomass is reliant on the improvement or development of cost-effective/cheaper technologies for cellulase production, and also on the development of enzymatic hydrolysis of carbohydrate components to monomeric sugars (hexoses and pentoses). A previous study revealed that enzyme production is the most expensive step in lignocellulosic ethanol production [2]. It covers approximately 40% of the total cost. So, for commercial lignocellulosic bioethanol production

development of cost-effective cellulase production technology is needed. Therefore, the present chapter discusses the current status of enzymatic hydrolysis to provide insight into the hydrolysis/saccharification process.

4.2 Hydrolysis/Saccharification

The saccharification process, i.e., the hydrolysis of cellulose and hemicelluloses, can be carried out mainly in two ways, i.e., biological (enzymatic) and chemical (acidic). The acidic reaction is done by using either dilute or concentrated acid. The enzymatic process has several benefits such as low toxic compound generation, high product yield, less chemical requirements, etc. (Figure 4.1).

4.2.1 Cellulase

The cellulases enzyme system is a mixture of endo-β-glucanase (EC 3.2.1.4), exo-β-glucanase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21). Cellulase acts on cellulose in the following manner: endo-β-glucanase acts randomly inside the cellulose chain, exo-β-glucanase acts on the external end of the cellulose chain and β-glucosidase degrade cellobiose into glucose or free monomeric sugar (Figure 4.2).

Individual enzymes are not capable of degrading the cellulose chain to a monomeric unit, hence synergistic action leads to a proper saccharification.

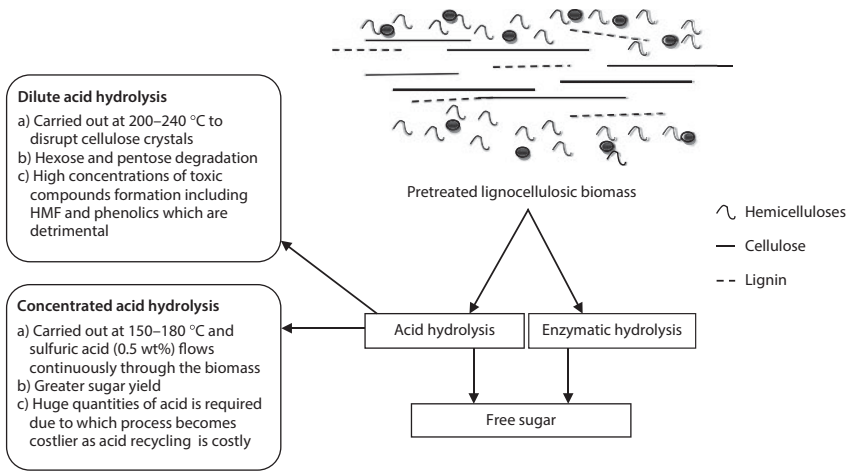

Figure 4.1 Saccharification process for lignocelluosic material.

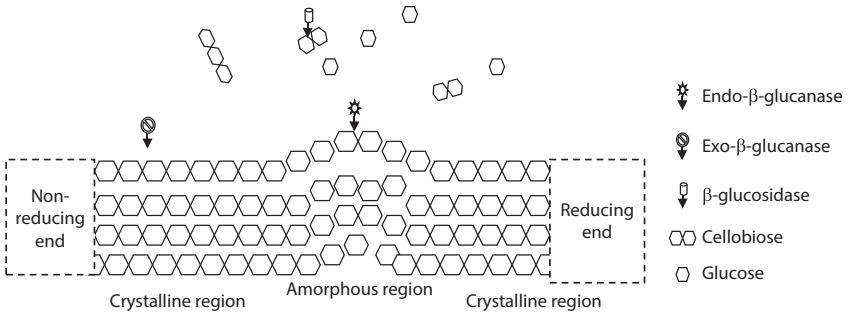

Figure 4.2 Schematic representation of cellulase mediated hydrolysis.

Major synergism has been noticed firstly between endo and exo-β-glucanase and secondly between exo-β-glucanases which act from both reducing and nonreducing end. β-glucosidase overcomes catabolic repression by preventing accumulation of cellobiose.

4.2.2 Screening of Cellulase-producing Microorganisms

There are several bacteria and fungi microorganisms capable of producing cellulase for the saccharification. Bacteria have a very low growth rate and require anaerobic growth conditions, therefore fungal cellulase have been mostly used for the given purpose (Table 4.1). The fungal cellulases production system works on the repressor/inducer phenomena where cellulose or other oligosaccharide act as inducers while glucose or other easily metabolized carbon sources act as repressors.

Trichoderma and *Aspergillus* are the most studied microorganisms for cellulase production. The crude enzyme extract of these microorganism are available for commercial use. *Trichoderma* produce endo-β-glucanase and exo-β-glucanase in higher quantity and β-glucosidase in lower quantity. In the case of *Aspergillus*, it produces endo-β-glucanase and β-glucosidase in higher quantity and exo-β-glucanase in lower quantity. It was reported that *Trichoderma reesei* QM-9414 is one of the best cellulase producers [13]. Later it was subjected to mono-colony isolation to obtain *Trichoderma reesei* KY-746. The mutated version gave higher cellulase activity [13].

Aspergillus niger is an important fungal strain for higher cellulase production. *Aspergillus niger* is a group of nine genera, and among them some possess higher potential of cellulase production. Different scientists have reported various media for cellulase production by using *Aspergillus niger* [14–16]. Abostate *et al.* [17] reported isolation of five potential *Aspergillus* sp. for cellulase production. They reported maximum

Table 4.1 Reducing sugar yield from different types of biomass.

Lignocellulosic biomass	Source of cellulase	Reducing sugar yield (mg/g dry substrate)	Incubation time (h)	Reference
Wheat straw	*Trichoderma reesei* NCIM 1186	371.44	24	[3]
Parthenium sp.	Commercial cellulase	574	48	[4]
Wheat straw	*Trichoderma reesei*	270	48	[5]
Wheat straw	*Trichoderma longibrachiatum*	294	72	[6]
Sorghum straw	*Coriolus versicolor*	440	168	[7]
Sargassum sp.	Commercial cellulase	326.89	72	[8]
Rice straw	Commercial cellulase	567	48	[9]
Rice straw	Commercial cellulase	414.16	72	[10]
Rice straw	*Aspergillus niger*	623.90	24	[11]
Pinus roxburghii	Locally isolated microorganism	334.00	24	[12]

endo-β-glucanase production in the case of *Aspergillus* MAM-F23. A previous study reported use of sorghum straw as a potential substrate for cellulase production [18]. Using *A. niger* under submerged fermentation, maximum cellulase production (0.77 IU/mL) was reported using sorghum straw as substrate, and lowest cellulose production (0.28 IU/mL) was reported using wheat straw as substrate. Maurya *et al*. 2012 [19] used *Trichoderma reesei* NCIM 992 for cellulase production under solid state fermentation. Kurup *et al.* (2005) compared cellulase production by different bacteria using water hyacinth as substrate. They found maximum cellulase production of 216 FPU/gds. Amira *et al*. 2012 [20] found higher xylanase activity (14.41 FPU/mg) using *Aspergillus niger* under solid state fermentation. Kumar *et al*. 2012 [21] reported maximum CMCase production (7.814 U/mg) from *Paenibacillus polymyxa*. Nair *et al*. 2008 [22] isolated 34 fungal strain strains for cellulase and xylanase production and they reported maximum cellulase production using *Trichoderma* sp. SBS60 and maximum xylanase production using *Aspergillus sydowii* SBS45. Ali and El-Dien 2008 [23] reported use of two different strains (*Aspergillus niger* and *Aspergillus nidulans*) for fungal cellulae production on water hyacinth.

4.2.3 Cellulase Production

Initial cellulase production was attempted on liquid culture but due to accumulation of free sugar catabolic repression took place, which hampered the cellulase synthesis during the microbial growth. Fed batch or continuous mode culturing can overcome the issue but adds to the overall cost.

Cellulase production on the agro industrial residues through solid state fermentation (SSF) is one of the promising technologies in terms of reduced processing cost. Carbohydrate moieties present in these cheap residues act as a carbon source for fungal growth. For cellulase production different substrates, such as wheat bran, rice straw, corn cob, sorghum straw, groundnut shell, cotton flower, saw dust, eater hyacinth, etc., have been reported [17, 24]. Table 4.2 shows the cellulase production under solid state fermentation by different fungal strains.

4.2.4 Factors Affecting the Cellulase Mediated Hydrolysis

Cellulase mediated hydrolysis consists of primarily three steps:

Adsorption of cellulase enzymes onto the surface of the cellulose

1. Bioconversion of cellulose to fermentable sugars
2. Desorption of cellulase

Table 4.2 Cellulase (CMCase) production by different fungal strains under SSF condition.

Microorganisms	CMCase activity (IU/gds)	Reference
Aspergillus niger	25.20	[25]
Fungal strains CG-10	29.04	[26]
Bacillus licheniformis	2.11	[27]
A. niger NRRL 567	425.3	[28]
Trichoderma atroviride	90.43	[29]
Aspergillus niger HN-1	416.3	[30]
Aspergillus awamori	19.00	[31]
Aspergillus fumigatus Z5	526.30	[32]
Trichoderma sp.	172.31	[33]
Humicola insolens TAS-13	18.98	[34]

3. The governing factors for these steps are mainly substrate concentration, enzyme dosage and reaction conditions.

At low substrate concentration the reducing sugar yield and reaction rates are increased but at high substrate concentration the reducing sugar yield and reaction rates are decreased. At high substrate concentration the decrease in the reducing sugar yield and reaction rates are due to end product inhibition of cellulase enzyme. Mojovic *et al*. 2006 [35] reported lower substrate concentrations were more suitable in order to avoid substrate inhibition. The authors found that at 16% suspension of corn flour the glucose yield was 76%, while when a 40% suspension was hydrolyzed the yield was only 50.2%.

High enzyme dosage enhances the reducing sugar yield but at the same time significantly increases the processing cost. Therefore, selection of optimum parameters such as temperature, pH, and incubation time at low enzyme dosage can be one approach to overcome the issues. Mahamud and Gomes [36] reported use of crude *Trichoderma* cellulase for enzymatic saccharification of alkali pretreated sugarcane bagasse. They reported maximum degree of hydrolysis (37.29%) at 50 °C. Ahmed *et al.* [37] reported that enzymatic saccharification of alkali treated bagasse rapidly increased up to 8 h and the rate of this increase was

Table 4.3 Effect of additive on cellulase mediated hydrolysis.

Additives	Reference
Addition of Ca(II) and Mg(II) results in lignin- metal complex formation	[42]
poly(ethylene oxide) polymer (PEO) and poly(ethylene glycol) (PEG)	[45]
Surfactants and bovine serum albumin (Tween 20, Tween 80, Triton X-100, Agrimul and SDS)	[46]
Ammoniation and various N compounds	[47]

substantially reduced at later stages. Han *et al.* [38] reported maximum reducing sugar yield (341.87 mg/g dry substrate) from alkali pretreated wheat straw at 55 °C using cellulase produced from *Penicillium waksmanii*. The variation in temperature was due to different species used for cellulase production. Moreover, the hydrolysis rate was influenced by the duration of the hydrolysis process [39]. Saha *et al.* [40] achieved maximum reducing sugar yield (554 mg/g dry substrate) after 72 h of saccharification of dilute acid pretreated wheat straw at 45 °C. In the case of alkali pretreated wheat straw maximum reducing sugar yield (343.95 mg/g dry substrate) was obtained after 30 h of enzymatic saccharification [38].

Jeya *et al*. 2009 [41] reported optimization of enzymatic saccharification of alkali-treated rice straw by using CCD based RSM. The authors found a maximum saccharification rate of 88% at an enzyme concentration of 37.5 FPU/g-substrate after optimization of the hydrolysis parameters. Liu *et al*. 2010 [42] used CCD based RSM for optimization of enzymatic hydrolysis of recycled pulp. Phuengjayaem and Teeradakorn, 2011 [43] reported that maximum yield of glucose was 0.366 g/g dry substrate at the optimal condition: 1.0–2.5% of the acid pretreated sweet sorghum straw, 30 FPU/g-substrate of cellulase, pH 3–5, at 30–50 °C in 96 h. Higher reducing sugar yield in short incubation time is required for improved process economics of bioethanol production [44].

Lignin has also an adverse effect on cellulases. It affects the whole process by nonproductive adsorption and irreversible binding of enzymes which limits the accessibility of cellulose to cellulase. Various methods have been used to eliminate lignin inhibition (Table 4.3).

4.3 Future prospects of enzymatic hydrolysis

The saccharification process, though it seems similar, faces various bottlenecks which are both technical and economical. Technical problems associated with the process are inefficient cellulase adsorption and efficacy due to limited accessible substrate surface, end product inhibition and lignin, while economic issues are related to cost of raw material, cellulase enzyme, etc. Hence, the current cellulase mediated hydrolysis problem needs to be taken care of for further advancement of lignocellulosic-bioethanol technology. Use of genetically modified cellulolytic organisms by cloning cellulase coding sequences into bacteria, fungi and plants is recommended to increase the cellulase yield and productivity under stress conditions. Even genetically engineered raw materials with higher carbohydrate content and low lignin content could reduce the cost. Simultaneous saccharification and fermentation (SSF) is also considered to be cost-effective by overcoming the end product inhibition. There is a serious need to understand the mode of action of the critical factors that control interactions between biomass, cellulase and inhibitory compounds. This knowledge will provide a new avenue to identify better pretreatment and saccharification strategizes as per industrial needs.

References

1. M.E.D. Oliveira, B.E. Vaughan, and E.J.J. Rykie, *Bioscience*, Vol. 55, pp. 593–602, 2005.
2. L. Spano, J. Medeiros, and M. Mandels, Division of Food Services Laboratories, US Army, Natick, MA, 1975.
3. A. Kuila, P.V.C. Rao, N.V. Choudary, G. Sriganesh, and H.R. Velankar, *Environmental Progress & Sustainable Energy*, Vol. 34, pp. 1243–1248, 2015.
4. K. Pandiyan, R. Tiwari, S. Surender, K.S.N. Pawan, R. Sarika, A. Anju, B.S. Shashi, and N. Lata, Enzyme Research, Vol. 2014, pp. 1–8, 2014.
5. F. Monlau, A. Barakat, E. Trably, C. Dumas, J.P. Steyer, and H. Carrere, *Critical Reviews in Environmental Science and Technology*, Vol. 43, pp. 260–322, 2013.
6. A. Barakat, and X. Rouau, *Biotechnology for Biofuels*, Vol. 7, 2014.
7. S. Phuengjayaem, A. Poonsrisawat, A. Petsom, and S. Teeradakorn, *Journal of Agricultural Science*, Vol. 6, pp. 120–133, 2014.
8. J.P. Tamayo, and E.J.D. Rosario, *Iranica Journal of Energy & Environment*, Vol. 5, pp. 202–208, 2014.
9. S.G. Wi, I.S. Choi, K.H. Kim, H.M. Kim, and H.J. Bae, *Biotechnology for Biofuels*, Vol. 6, 2013.

10. T. Srinorakutara, Y. Subkaree, N. Bamrungchue, S. Suttikul, V. Panphan, P. Pripanpong, and V. Burapatana, In: Renewable energy and global care, The 24th Annual Meeting of the Thai Society for Biotechnology, 2012.
11. M. Hashem, E.H. Ali, and R. Abdel-Basset, *Journal of Agricultural Science and Technology*, Vol. 15, pp. 709–721, 2013.
12. S. Vats, D.P. Maurya, A. Jain, V. Mall, and S. Negi, *Indian Journal of Experimental Biology*, Vol. 51, pp. 944–953, 2013.
13. S.K. Deshpande, M.G. Bhotmange, T. Chakrabarti, and P.N. Shastri, *Indian Journal of Chemical Technology*, Vol. 15, pp. 449–456, 2008.
14. A. Shweta, *Biotechnology Research Journal*, Vol. 1, pp. 108–112, 2015.
15. S.K. Sandhu, H.S. Oberoi, N. Babbar, K. Miglani, B.S. Chadha, and D.K. Nanda, *Journal of Agricultural and Food Chemistry*, Vol. 61, pp. 12653–12661, 2013.
16. M.A. Umsza-Guez, A.B. Díaz, I. Ory, A. Blandino, E. Gomes, and I. Caro, *Brazilian Journal of Microbiology*, Vol. 42, pp. 1585–1597, 2011.
17. M.A.M. Abostate, M. Swelim, A.I. Hammad, and R.B. Gannam, *World Applied Sciences Journal*, Vol. 9, pp. 1171–1179, 2010.
18. B.V. Mohite, and J.G. Magar, *Bionano Frontier*, Vol. 3, pp. 189–192, 2010.
19. D.P. Maurya, D. Singh, D. Pratap, and J.P. Maurya, *Journal of Environmental Biology*, Vol. 33, pp. 3–8, 2012.
20. D.R. Amira, A.R. Roshanida, and M.I. Rosli, *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*, Vol. 6, 2012.
21. D. Kumar, M. Ashfaque, M. Muthukumar, M. Singh, and N. Garg, *Journal of Environmental Biology*, Vol. 33, pp. 81–84, 2012.
22. S.G. Nair, R. Sindhu, and S. Shashidhar, *African Journal of Microbiology Research*, Vol. 2, pp. 82–86, 2008.
23. U.F. Ali, and H.S.S. El-Dein, *Journal of Applied Sciences Research*, Vol. 4, pp. 875–891, 2008.
24. S.W. Kang, Y.S. Park, J.S. Lee, S.I. Hong, and S.W. Kim, *Bioresource Technology*, Vol. 91, pp. 153–156, 2004.
25. G.P.K. Reddy, G. Narasimha, K.D. Kumar, G. Ramanjaneyulu, A. Ramya, B.S.S. Kumari, and B.R. Reddy, *International Journal of Current Microbiology and Applied Sciences*, Vol. 4, pp. 835–845, 2015.
26. Gupta C., Jain P., Kumar D., Dixit A.K., Jain R.K., (2015) Production of cellulase enzyme from isolated fungus and its application as efficient refining aid for production of security paper. *IJAMBR* 3: 11–19.
27. B.R. Dave, P. Parmar, A. Sudhir, K. Panchal, and R.B. Subramanian, *Journal of Bioprocessing & Biotechniques*, Vol. 5, 2015.
28. A. Shweta, *Biotechnology Research Journal*, Vol. 1, pp. 108–112, 2015.
29. P. Sangwan, V. Mor, R. Dhankhar, and S. Sukhani, *International Journal of Pharma and Bio Sciences*, Vol. 6, pp. 755–762, 2015.
30. S.K. Sandhu, H.S. Oberoi, N. Babbar, K. Miglani, B.S. Chadha, and D.K. Nanda, *Journal of Agricultural and Food Chemistry*, Vol. 61, pp. 12653–12661, 2013.

31. M.A. Umsza-Guez, A.B. Díaz, I. Ory, A. Blandino, E. Gomes, and I. Caro, *Brazilian Journal of Microbiology*, Vol. 42, pp. 1585–1597, 2011.
32. D. Liu, R. Zhang, X. Yang, H. Wu, D. Xu, Z. Tang, and Q. Shen, *International Biodeterioration and Biodegradation*, Vol. 65, pp. 717–725, 2011.
33. H. Sun, X. Ge, Z. Hao, and M. Peng, *African Journal of Biotechnology*, Vol. 9, pp. 163–166, 2010.
34. I.U. Haq, M.M. Javed, and T.S. Khan, *Biokemistri*, Vol. 18, pp. 83–88, 2006.
35. L. Mojović, S. Nikolić, M. Rakin, and M. Vukasinović, *Fuel*, Vol. 85, pp. 1750–1755, 2006.
36. M.R. Mahamud, and D.J. Gomes, *Journal of Scientific Research*, Vol. 4, pp. 227–238, 2012.
37. F.M. Ahmed, S.R. Rahman, and D.J. Gomes, *Malaysian Journal of Microbiology*, Vol. 8, pp. 97–103, 2012.
38. L. Han, J. Feng, S. Zhang, Z. Ma, Y. Wang, and X. Zhang, *Brazilian Journal of Microbiology*, Vol. 43, pp. 53–61, 2012.
39. T. Sun, H.N. Laerke, H. Jorgensen, and K.E. Bach-Knudsen, *Animal Feed Science and Technology*, Vol. 131, pp. 66–85, 2006.
40. B.C. Saha, L.B. Iten, M.A. Cotta, and Y.V. Wu, *Biotechnology Progress*, Vol. 21, pp. 816–822, 2005.
41. M. Jeya, Y.W. Zhang, I.W. Kim, and J.K. Lee, *Bioresource Technology*, Vol. 100, pp. 5155–5161, 2009.
42. Q. Liu, K.K. Cheng, J.A. Jhang, J.P. Li, and G.H. Wang, *Applied Biochemistry and Biotechnology*, Vol. 160, pp. 604–612, 2010.
43. S. Phuengjayaem, and S. Teeradakorn, In: International Conference on Asia Agriculture and Animal, Singapore, 2011.
44. M.J. Taherzadeh, and K. Karimi, *Bioresources*, Vol. 2, pp. 707–738, 2007.
45. J. Börjesson, M. Engqvist, B. Sipos, and F. Tjerneld, *Enzyme and Microbial Technology*, Vol. 41, pp. 186–195, 2007.
46. T. Erickson, J. Borjesson, and F. Tjerneld, *Enzyme and Microbial Technology*, Vo. 31, pp. 353–364, 2002.
47. V.J.H. Sewalt, W.G. Glasser, and K.A. Beauchemin, *Journal of Agricultural and Food Chemistry*, Vol. 45, pp. 1823–1828, 1997.

5

Biological Pretreatment of Lignocellulosic Biomaterials

Sandeep Kaur Saggi[1], Geetika Gupta[1]* and Pinaki Dey[2]

[1]Department of Biotechnology, Thapar University, Patiala, India
[2]Department of Biosciences and Technology,Karunya University, Coimbatore, India

Abstract
Pretreatment is the most critical and expensive step for the production of bio-ethanol in the case of lignocellulosic biomass rather than the starchy biomass. Pretreatment methods can be divided into four major types, namely physical, chemical, physico-chemical and biological. An effective pretreatment process of biomass aims for removal/breakdown of lignin to make the complex polymeric carbohydrate molecule accessible to hydrolyzing agents like acids or enzymes along with minimal loss and/or degradation of monomeric sugars, negligible production of inhibitory and toxic products, reduction in energy demands, and with reduced corrosion, time consumption and process costs. Biological pretreatment can be considered as a greener way for degradation and/or removal of hemi-cellulose and lignin seal by various microbes among all other pretreatment methods. Fungus like brown rot attacks on cellulose while white and soft rot fungi attack both on cellulose and lignin. Basidiomycetes white rot fungi were found most effective in delignification. The present chapter reviews different lignocellulolytic bacteria and fungus for their efficacy and the enzymes they produce.

***Keywords*:** Bio-ethanol, starchy biomass, lignocellulosic biomass, pretreatment, glucose

5.1 Introduction

Due to the unexpected depletion of the world's energy supply, interest in alternative sources of energy has increased globally (John *et al*., 2011).

**Corresponding author*: geetika_12_gupta@yahoo.com

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Among all other liquid biofuels used for motor vehicles, bioethanol is the most widely used (Demirbas *et al*., 2005). Bioethanol is a burning biofuel that does not contribute to global warming because the carbon dioxide produced by the combustion of ethanol is consumed by growing plants and can be considered as a zero carbon source of energy. Currently, most bioethanol is produced from food and feed crops such as sugar cane (Brazil, South Africa) and corn starch (United States), which is leading to an imbalance in food security. Hence, lignocellulosic materials such as agricultural wastes or residues, energy crops, pulp paper industry waste, kitchen waste and municipal waste rich in carbohydrates have been of great interest in the world of research into bioethanol production and found to be a promising substrate (Sarkar *et al*., 2012). The use of this lignocellulosic substrate as raw material for biofuel production requires some processing prior to fermentation by microorganism, as the microorganisms are unable to convert complex lignocellulosics into biofuels. They can only utilize the simpler fermentable sugars. The processing steps involve: firstly size reduction to increase the surface area, then pretreatment to delignification or breaking the crystalline structure of cellulose, after that hydrolysis of carbohydrates to break polymeric chains of cellulose/hemicellulose into monomeric fermentable sugars. Pretreatment and/or hydrolysis are the major cost-consuming steps involved in bioethanol production (Taherzadeh *et al*., 2007). Hence it has become important to look for the development of new processes or improvement in existing technologies for the conversion of other readily available, low-cost substrate into the simpler fermentable sugars (Zabed *et al*., 2014). Several pretreatment methods have already been developed by researchers around the world, which include physical, chemical, physico-chemical and biological pretreatments. The major goals of pretreatment methods are:

- Maximum delignification
- Maximum breakdown of crystalline structure of cellulosic polymer
- Minimum toxic and inhibitory product formation
- Minimum degradation of carbohydrate
- Economically cheaper in cost
- Reduced requirement of recovery of spent chemicals
- Ecofriendly
- Maximize renewable sources
- Production in large quantity
- Potential environment benefits

(Parveen Kumar *et al*., 2009)

For a particular lignocellulosic biomass a specific or combination of two or more pretreatment methods is required to achieve the goals of pretreatment. Each pretreatment method has different advantages and disadvantages over others. The selection of a particular pretreatment method depends on the type of lignocellulosic biomaterial and the goals of pretreatment. This chapter focuses on the biological pretreatment methods. The advantage of biological pretreatment is that it requires milder reaction conditions, i.e., pH near to neutral, room temperature, atmospheric pressure, ecofriendly, maximize renewable sources, production in large quantity, potential environment benefits, doesn't require any recovery of spent chemicals (Parveen Kumar *et al*., 2009). The only disadvantage is that it is quite slower as compared to other pretreatment methods. Bioethanol has been produced from different lignocellulosic materials (John *et al*., 2011) like crop residues (Kim and Dale 2004), municipal solid waste (Mtui and Nakamura 2005), forest product industry wastes (Kadar *et al*., 2004), and leaf and yard waste (Lissens *et al*., 2004) as well as dairy and cattle manures (Wen *et al*., 2004).

There are several challenges involved in making the process of bioethanol production from lignocellulosic materials economical and feasible. Unlike sucrose and starch-based ethanol production the lignocellulosic materials require different processing to make the carbohydrates accessible to the hydrolyzing agent. Therefore, the first challenge is the pretreatment of lignocellulosic materials to break the crystalline structure of cellulose and to remove lignin, thus improving the yield of fermentable sugars. The second challenge is to hydrolyze the lignocellulosic biomass with maximum yield of fermentable sugars at low cost. The final challenge is to ferment the pentose sugars along with hexose sugars which account for more than 20% of the total carbohydrate content of lignocellulosic biomass. The most commercial yeast *S. cerevisiae* and *Zymomonas mobilis* are unable to ferment pentose sugars. *Pachysolen tannophilus, S. stipitis* and *Candida shehate* can ferment pentose sugars, but limited due to of low ethanol tolerance and poor ethanol productivity (Saha *et al*., 2003).

5.1.1 Different Source for Bioethanol Production

In contrast to fossil fuels, bioethanol is a renewable energy source, which can be produced by the sugars fermentation. The use of corn starch, sugarcane or cereal-based ethanol production technologies may create a problem with food security as they come under the category of food crops and their production for energy will compete with the limited agricultural

land required for food and feed production. For the economical production of ethanol, lignocellulosic materials, like crop's remnants, grasses, sawdust, wood chips, solid animal waste, and industrial waste could have huge potential (Sun & Cheng, 2002). Woody crop residues, rice husk and bagasse can be considered for the production (Ravindranath *et al*., 2005). The waste water from food and agro-based industries accounts for 65–70% of the total industrial waste water in the form of organic load (Pachauri & Sridharan 1998).

5.1.2 Lignocellulosic Materials

Lignocellulosic biomass has the huge potential to extensively reduce the production cost because it is cheaper than corn and sugarcane and is also available in abundant quantity. Lignocellulosic feedstock includes agricultural residues (wheat straw, corn stover, rice straw, bagasse, grasses, etc.), forest residues, wood-based industrial waste and other low-value biomass like municipal solid waste. Lignocellulosic biomass are mainly composed of cellulose, hemicellulose and lignin; the close association of all these makes a complex crystalline structure of lignocellulose. Table 5.1 shows the amount of cellulose, hemicellulose and lignin in different lignocellulosic materials. Figure 5.1 shows the general structure of lignocellulosic material.

Table 5.1 Composition of different lignocellulosic biomass (Jørgensen *et al*., 2007).

Lignocellulosic biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Switch grass	45	31.4	12
Coastal Bermuda grass	25	35.7	6.4
News paper	40–55	25–40	18–30
Grasses	25–40	35–50	10–30
Corn cobs	45	35	15
Softwood	45–50	25–35	25–35
Hardwood	40–55	24–40	18–25
Paper	85–99	0	0–15
Wheat straw	30	50	15
Leaves	15–20	80–85	0
Cotton seed hairs	80–95	5–20	0

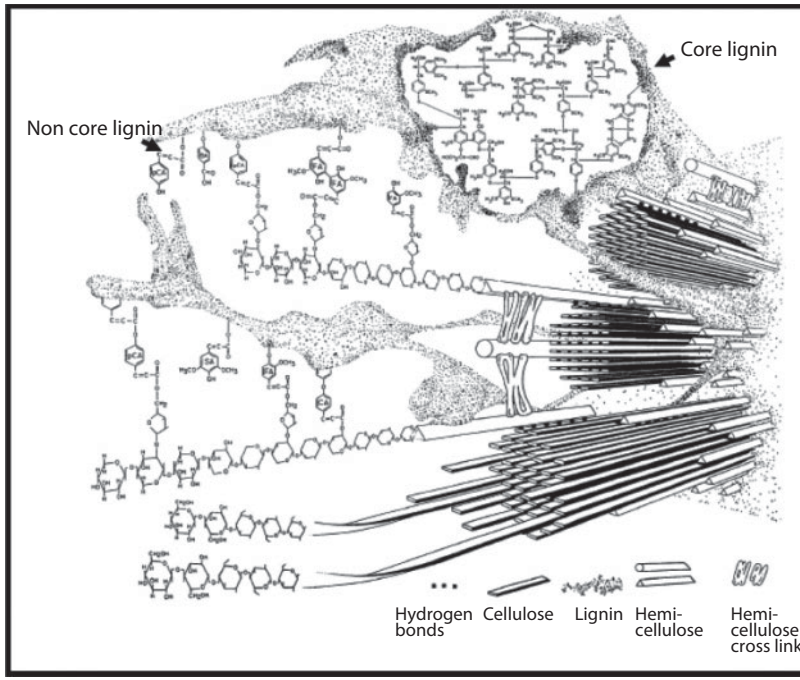

Figure 5.1 Structural of cell wall of lignocellulosic biomass (Daniela *et al*., 2011).

5.1.3 Cellulose

Cellulose is found in the cell wall of all plants and is characterized as the structural component of the plants in the form of cellulose microfibrils. It represents the major component of plants and is the most abundant biomaterial in the world. It is the linear polymer of glucose monomer linked together by $\beta(1\rightarrow4)$ glycosidic bonds (see Figure 5.2). Cellulose present in the secondary wall of higher plant ensures a range of 7,000–14,000 degree of polymerization in contrast to primary wall which has approx. 500–6,000 degree of polymerization (Richmond, 1991; Clarke *et al*., 1996). Each anhydroglucose unit constructs a chain configuration and cellobiose is the repeating unit of cellulose chain. The chemical reactivity of cellulose is determined by the main functional groups present in it, i.e., hydroxyl groups and glycosidic bonds (Fan *et al*., 1987). The free hydroxyl groups of cellulose macromolecule forms a number of intra- and intermolecular hydrogen bonds to give different ordered crystalline arrangements (Hermans *et al*., 1949). These intra- and intermolecular hydrogen bonds form a highly ordered crystalline macro-molecular structure of cellulose. Approximately 100 cellulose chains together form

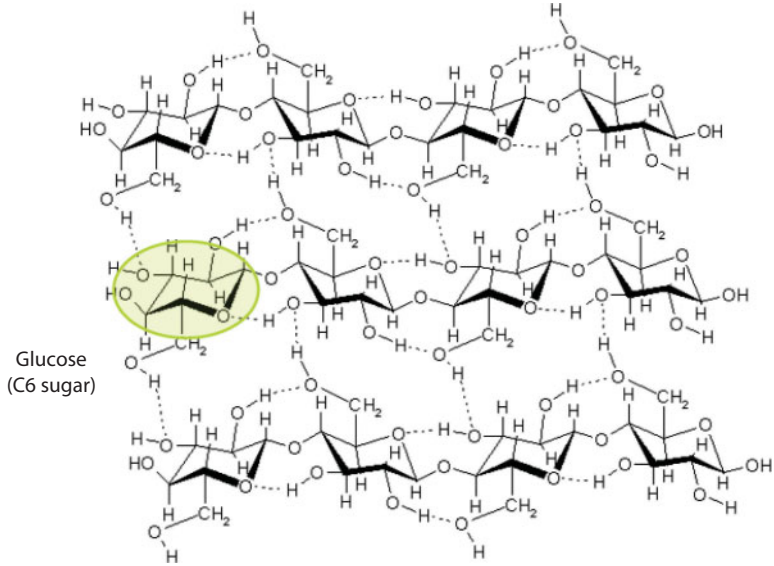

Figure 5.2 Structural of cellulose (Beguin *et al*., 1994).

the elementary fibrils of the diameter range 4–5 nm. Elementary fibril on combination forms microfibril of 10–30 nm diameter (Fengel & Wegener, 1983, Zhang & Lynd, 2004, Fan *et al*., 1987). Four principal allomorphs of cellulose have been identified: cellulose I, II, III, IV (Fengel and Wegener, 1983) and can be differentiated by its characteristic X-ray diffraction pattern. Advances in characterization of cellulose ultrastructure have shown that within all four allomorphs, subgroups exist. Cellulose I is the natural form of cellulose and apparently most abundant. It has a highly complex three-dimensional structure and is not yet fully resolved due to the coexistence of two different crystalline forms, cellulose Iα and Iβ (Atalla, & Vanderhart, 1984). Cellulose I can undergo an irreversible transition to form a stable crystalline form, cellulose II, through two different processes: regeneration and mercerization. Treatment with ammonia or certain amine like ethylene diamine converts the cellulose I or cellulose II into cellulose III. Cellulose II on treatment with glycerol at high temperature transforms into cellulose IV (Chanzy *et al*., 1978, Chanzy *et al*., 1979).

5.1.4 Hemicellulose

Hemicellulose is the second most abundant polymer of plant biomass. It is bound with cellulose and lignin component by covalent and non-covalent

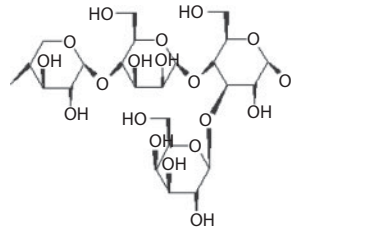

Figure 5.3 General structure of Hemicellulose [web: 4].

bonds in the cell wall (Saha *et al*., 2003). Hemicellulose is a complex carbohydrate as compared to cellulose (Figure 5.3). Hemicelluloses are easily soluble in alkaline solution while cellulose is insoluble. Based on the composition and intra-structural bonding hemicelluloses are categorized into xylans, heteroxylans, galactomannan etc. The composition of all these fractions varies in different wood species. Softwood hemicellulose is mostly composed of glucomannan while hardwood hemicellulose is made up of xylans mainly. In some softwood like larchwood, a significant amount of arabinogalactan is found while in other softwood less than 1% arabinogalactan is found (Alén *et al*., 2000). Arabinogalactan is partly and fully soluble in water. In general, grasses contain 20–40% arabinoxylan (arabino-4O-methylglucurono-xylan) with different ratio arabinan and xylan (Clarke *et al*., 1997).

5.1.5 Xylan

The general structure of xylan is shown in Figure 5.4. Pure deacetylated xylan forms crystalline hexagonal platelets with different layers of approximately 5nm thickness. Side groups of acetyl or arabinose or uronic acid inhibit the formation of strict molecular order of xylan. X-ray diffraction pattern shows a trigonal unit of xylan monohydrate, and with an increase in moisture, increase in cell size can be observed (Fengel and Wegener, 1983).

Hardwood hemicellulose is generally made up of xylans dominantly (Saha *et al*., 2003) and joined with groups of 4-O-methylglucuronic acid (Me-GluU) with an (1–2)-glycosidic linkage with xylose units. O-acetyl substitutes many –OH groups at C2 and C3 of xylose units. Hardwood xylans have very short chains with two to three branching points, linked together at C3 of backbone. The average degree of polymerization of xylan lies in the range of 100–200. Smaller amounts of rhamnose and

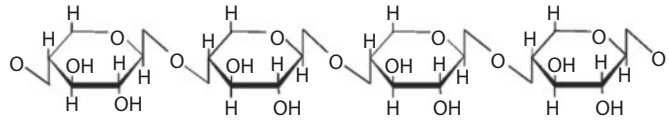

Figure 5.4 General structure of xylan [web: 5].

galactouronic acid are also found to be associated with the main chain of hardwood xylan (Clarke *et al*., 1997). In contrast to hardwood, softwood lacks acetyl group and is composed of arabinofuranose units linked by α-(1–3)-glycosidic bonds to the xylan backbone. Xylans with higher molecular weight contain increased number of arabinose units and more branching points. Arabinofuranose units are esterified with p-curamic acid and ferulic acid (Fengel and Wegener, 1983).

5.1.6 Lignin

Lignin can be characterized as a polyphenolic material growing primarily from enzymatic dehydrogenetive polymerization of three phenyl-propanoid (p-hydroxycinnamyl alcohols) units named trans-coniferyl alcohol, trans-sinapyl alcohol and trans-p-coumaryl alcohol. It is an amorphous structure due to highly cross-linked structural units (Figures 5.5 and 5.6). The degree of polymerization of lignin ranges from 450–500. Lignin serves a dual purpose in the woods. It acts as binder between cells and imparting rigidity to the cell walls. It can also be defined as thermoplastic high molecular-mass material considering its polymeric properties (Alén, 2000; Wayman & Parekh *et al*., 1990). Lignin in the plant cell wall is also responsible for the resistance towards microbes and chemicals (Himmel *et al*., 2007).

Different molecular structures of lignin have been found in softwood, hardwood and grasses. Normal softwood lignin is mainly derived from the guaiacyl units mostly originated from trans-coniferyl alcohol (90%) and the remainder consists of trans-p-coumaryl alcohol. Hardwood lignin is also known as "guaiacyl-syringyl lignin" and is composed of trans-coniferyl alcohols and trans-sinapyl alcohols in varying ratios. Grass lignin is termed as "guaiacyl-syringyl lignin", though it additionally contains significant amount of trans-p-coumaryl alcohol derived structural elements (Sakakibara & Sano, 2000). The building blocks as mentioned above are linked together by ether (C-O-C), carbon-carbon (C-C) or ester bonds (C-O-O-C). Out of all the linkages present two-thirds are of ether type linkages between phenolic ring and one of the side chain carbon at a different

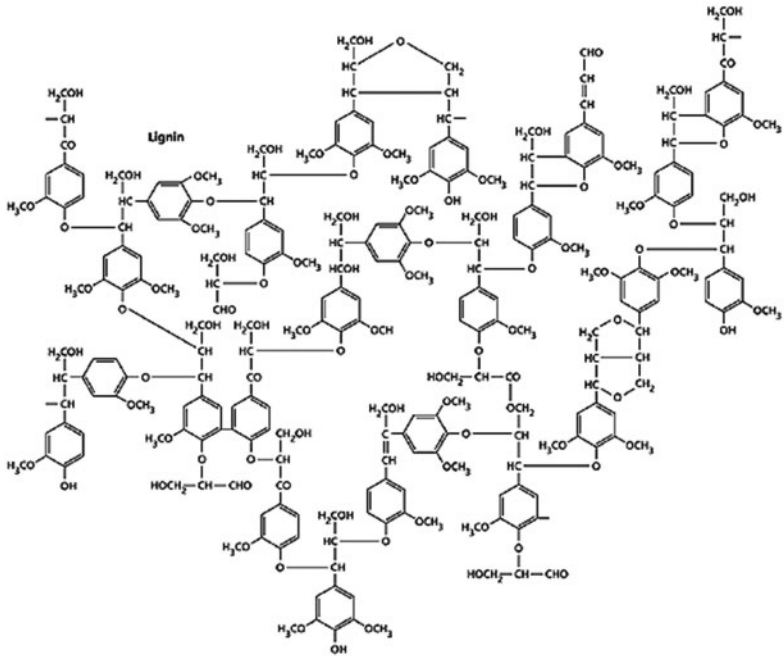

Figure 5.5 Partial structure of a hypothetical lignin molecule from European beech (*Fagus sylvatica*). The phenylpropanoid units that make up lignin are not linked in a simple, repeating way. The lignin of beech contains units derived from coniferyl alcohol, sinapyl alcohol, and *para*-coumaryl alcohol in the approximate ratio 100:70:7 and is typical of angiosperm lignin. Gymnosperm lignin contains relatively fewer sinapyl alcohol units (Nimz *et al*., 1974).

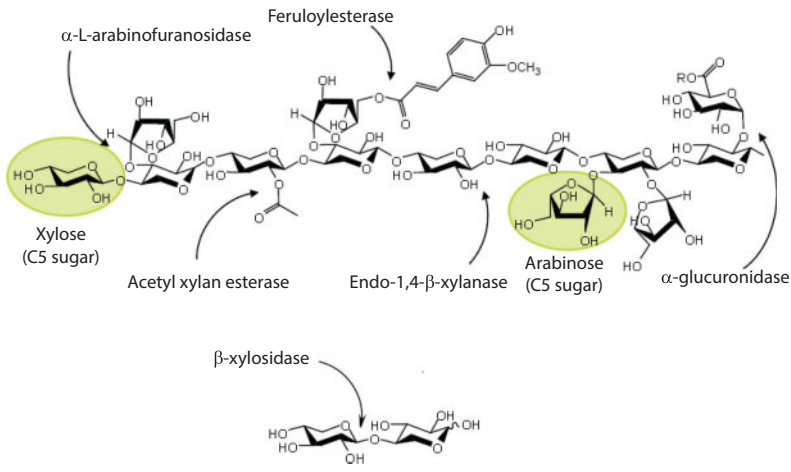

Figure 5.6 Structure of lignin (Perez *et al*., 2002).

position (β-O-4, α-O-4, γ-O-4). The second most dominating linkages are the carbon-carbon linkages between two phenolic rings or between side chain carbon and phenolic ring or between two side chain carbons. The ester linkages are found typically less than 10%. Phenolic hydroxyl, aliphatic hydroxyl, methoxyl and carbonyl are the most common functional groups found in the lignin. Isolated lignin shows maximum solubility in dioxane, acetone, methyl cellosolv, tetrahydrofuran, dimethyl formamide and dimethyl sulfoxide (Alén, 2000).

5.1.7 Lignin Carbohydrate Interactions

Different studies have shown a close and tough association of hemicellulose and lignin; this interaction could be physical or chemical. This association between hemicellulose and lignin is termed as lignin carbohydrate complex. Different covalent linkages such as benzyl ether, benzyl ester and phenyl glycoside etc. are found in this lignin carbohydrate complex. In hardwoods mainly ester and glycoside linkages are observed whereas in softwoods all kinds of linkages are found. Side groups of hemicellulose such as L-arabinose, D-galactose and 4-O-methyl-D-glucuronic acid and main chain end groups of xylan and glucomannan form linkages with functional groups. Glycosidic bonds involve the reducing end group of hemicellulose chain and the hydroxyl group of lignin. Ether and ester bonds are formed at α-carbon location in phenyl propane units. Ether linkages in the complex of lignin and carbohydrate are much more stable than all the other type of linkages. Range of the molecular weight of these complexes varies from 600–15,000 (Alén, 2000; Fengel & Wegener, 1983).

5.2 Pretreatment

5.2.1 Pretreatment

The abundance of lignocellulosic biomass with low cost and high carbohydrate content, nearly equal to the starch content of corn and other grains, makes it attractive feedstock for bioconversion to ethanol. The heterogeneous composition and complex structure of lignocellulosic biomass along with the recalcitrant nature of cellulose poses the major difficulties in the bioconversion scheme of biomass to ethanol. The highly crystalline structure of cellulosic fibers makes it very resistant to acid and enzyme-based hydrolysis (Grohman *et al.*, 1993). Thus, pretreatment becomes the important process in the bioconversion of lignocellulosic materials. The major goal of any pretreatment method is to alter or eliminate structural and

compositional obstacles to hydrolysis in order to improve the hydrolysis rate and increase the yield of fermentable sugars from carbohydrate content of lignocellulosic biomass (Mosier *et al*., 2005). The effect of pretreatment on lignocellulosic biomass for accessibility of hydrolytic enzyme. A suitable pretreatment process must have the following properties: (i) increase the formation of sugars or ability to subsequently form sugars by hydrolysis, (ii) carbohydrate degradation or loss must be avoided, (iii) toxic compound formation should be avoided which may inhibit the subsequent hydrolysis or fermentation process and (iv) it must be cost effective (Silverstein *et al*., 2005). The pretreatment facilitates the acid or enzyme catalyzed hydrolysis by promoting the physical and chemical disruption of lignocellulosic matrix. Pretreatment can have significant inferences on the assembly and productivity of the rest of the process and finally on economics (Mabee *et al*., 2006). Recently various techno-economic studies have been executed to assess the cost and performance of pretreatment technologies (Hamelinck *et al*., 2005, Eggeman & Elander *et al*., 2005, Chen *et al*., 2007). Studies show that pretreatment is an important factor involved in the success of the cellulosic bioethanol production technology, since it defines the extent to and cost of conversion of cellulose and hemicellulose to bioethanol. There is a large scope in lowering the cost of pretreatment process and it could only be possible through extensive research and development approaches (Chandel *et al*., 2007a). The major challenge in cellulosic bioethanol production is to make the pretreatment process of lignocellulosic material cost effective (Hamelinck *et al*., 2005). There are many different methods of pretreatment process such as mechanical pretreatment (Rivers & Emert, 1987), steam explosion (Brownell & Saddler, 1987, Zhang *et al*., 2008), ammonia fiber explosion (Alizadeh *et al*., 2005, Teymouri *et al*., 2004, Teymouri *et al*., 2005), supercritical CO_2 treatment (Kim & Hong, 2001), alkali or acid pretreatment (Silverstein *et al*., 2007, Martin *et al*., 2007, Champagne, 2007), biological pretreatment (Patel *et al*., 2007), etc. Different pretreatment methods and their applications are given in Table 5.2.

5.3 Microbial Pretreatment Process

5.3.1 Fungi

Fungus is the group of eukaryotic organisms which are highly important in different biological applications. Many fungal species are normally used in biological pretreatment e.g., brown, white and soft rot fungi. Brown

Table 5.2 Different types of pretreatment method.

Type of pretreatment method	Processes	Changes in biomass	References
Physical methods	**Milling**	• Surface area and pore size increases • Cellulose crystallinity decreases • Degree of polymerization decreases	(Mais *et al.*, 2002)
	• Ball milling • Two roll milling • Hammer milling • Colloid milling • Vibro energy milling		
	Irradiation		(Kumakura *et al.*, 1982)
	• Gamma ray irradiation • Electron beam irradiation • Microwave irradiation		
	Others		(Negro *et al.*, 2002, Garrote *et al.*, 1999)
	• Hydrothermal • High pressure steaming • Expansion • Extrusion • Pyrolysis		
Chemical and physicochemical	**Explosion**	• Increase in accessible surface area • Partial or complete delignification • Decrease in crystallinity • Decrease in degree of polymerization • Partial or complete hemicellulose hydrolysis	(Emmel *et al.*, 2003, Ballesteros *et al.*, 2001)
	• Steam explosion • Ammonia fibre explosion (AFEX) • CO_2 explosion • SO_2 explosion		

	Alkali • Sodium hydroxide • Ammonia • Ammonia sulphite • Calcium hydroxide or lime		(Vaccarino *et al.*, 1987, Kim & Holtzapple, 2006)
	Acid • Sulfuric acid • Hydrochloric acid • Phosphoric acid		(Taherzadeh & Karimi, 2007a)
	Gas • Chlorine dioxide • Nitrogen dioxide • Sulfur dioxide		(Fan *et al.*, 1982)
	Oxidizing agents • Hydrogen peroxide • Wet oxidation • Ozone		(Palonen *et al.*, 2004)
	Solvent extraction of lignin • Ethanol-water extraction • Benzene-water extraction • Ethylene glycol extraction • Butanol water extraction • Swelling agents		(Pasquini *et al.*, 2005)
Biological pretreatments	**Fungi and actinomycetes**	• Delignification • Reduction in degree of polymerization of cellulose • Partial hemicellulose hydrolysis	(Ahring *et al.*, 1996)

rots mainly attack cellulose, whereas white and soft rots attack both cellulose and lignin. Some enzymes are required to break the cellulose present in the lignocellulosic material. Hydrolysis of cellulose is carried out by cellulase enzyme, which is a mixture of endoglucanases which helps in the generation of free chain ends of molecule, exoglucanases enzyme which breaks the molecule by removing cellobiose units and cellobiohydrolases using β-glucosidase, which hydrolyses cellobiose to produce monomer of glucose/sugar. The endoglucanases attack the low crystallinity regions of the cellulose and create free chain ends of molecule. The exoglucanases degrade the sugar chain by removing cellobiose units from the free chain ends. The produced cellobiose unit is then cleaved and produced to monomer of glucose by enzyme β-glucosidase (Coughlan and Ljungdahl, 1988). Enzyme hemicellulase cleave the bonds of cellulose and hemicellulose respectively. Hemicellulolytic enzymes are a mixture of eight enzymes such as endo-1, 4-ß-D-xylanases, exo-1, 4-ß-D xylocuronidases, α-L-arabinofuranosidases, endo-1, 4-ß-D mannanases, ß-mannosidases, acetyl xylanesterases, α-glucuronidases and α-galactosidases (Jorgensen *et al*., 2003). Lignin breakdown by white rot fungi occurs by the action of two enzymes such as peroxidases and laccase (Lee *et al*., 2007). One of this fungi, white rot fungi, are effective microorganisms for biological pretreatment of lignocellulosic biomass (Sun & Cheng, 2002). In one study on water hyacinth, when pretreated with white rot fungi and dilute acid ethanol its yield increased by twofold over acid pretreatment (Ma *et al*., 2010). After exposure to white rot fungi *Stereum hirustum*, affect biological pretreatment on the red pine called *Pinusdensi* flora. *S. hirsutum* is considered as an effective potential fungus for biological pretreatment. When *Pinusdensi* flora Japanese pine treated with fungi *Stereum hirsutum* which enzymatically saccharified using commercial enzymes cellulase 1.5 L and Novozyme 188 for the conversion to the monomer of sugar yield was increased approximately to 21.0% compared to non-pretreated samples (Lee *et al*., 2007). When white rot fungal pretreatment was combined with the chemical pretreatment the biological pretreatment improved the performance of non-fungal pretreatment. Physical pretreatment of hot water treatment (170 °C for 3 min. at 110 psi) which altered the cell wall structure of biomass and finally facilitated the degradation of soybean straw biomass and the glucose yield, this was all carried out by the combination of liquid hot water and fungal pretreatment, which reached about 65%. As compared to chemical and thermal pretreatment, fungal pretreatment white rot fungi is an environmentally friendly and energy-efficient process (Wan and Li, 2011 and 2012). Such processes offer advantages as low cost, low energy, no chemicals requirement, and mild environmental conditions.

However, the main drawback is the low hydrolysis rate and it requires careful control of growth conditions with a large amount of space (Alvira *et al*., 2010). It is done with the help of an enzyme like cellulase after pretreatment of biomass to convert cellulose into fermentable monomers of sugars. It is done under mild conditions because of the optimum range of enzyme activity. Because of these reasons such as low corrosion problems, low utility consumption, and low toxicity of the hydrolysates there are advantages to this process.

The fungal pretreatment method, which affects in enzymatic hydrolysis process, applied for the fermentation to produce biofuel. After pretreatment of maize silage with enzyme cellulase, the yield of sugar was increased to 90.1% which is a much higher conversion than hydrolysis of untreated maize silage which showed 62.3% (Popiel *et al*., 2008). It shows that glucose yield from enzyme hydrolysis changes according to the pretreatment process (Ingram *et al*., 2011; El-Zawawy *et al*., 2011). Saccharification step is one of the critical steps for bioethanol production where complex molecules are converted into simple monomers of sugar. These enzymes used for hydrolysis can be produced by fungi such as *Trichoderma reesei* and *Aspergillus niger* and/or bacteria such as *Clostridium cellulovorans* (Arai *et al*., 2006). These enzymes are highly substrate specific for enzyme activity to hydrolysis for the biomass (Banerjee *et al*., 2010; Taherzadeh & Karimi, 2007b). Cellulose is hydrolysed to monomer of glucose whereas hemicellulose gives rise to various pentoses and hexoses sugars. Among the various cellulolytic microbial species, Trichoderma is one of the most studied as cellulase and hemicellulase producing fungal strains (Xu *et al*., 1998). One of the fungi species, *Trichoderma* is able to produce two cellobiohydrolases and five endoglucanases and three endoxylanases enzymes (Xu *et al*., 1998; Sandgren *et al*., 2001). On the other hand, *Aspergillus* is very efficient in ß glucosidase production (Taherzadeh & Karimi, 2007b). *Trichoderma* cellulase supplemented with extra ß-glucosidase has been studied several times (Krishna *et al*., 2001; Itoh *et al*., 2003 and Ortega *et al*., 2001). Many fungi such as *Phanerochaete, Humicola, Schizophillum, Trichoderma, Penicillium, Fusarium*, species also have been reported for cellulase production (Sun & Cheng, 2002; Rabinovich *et al*., 2002). Various factors influence monomer of sugars from lignocellulose biomass. These various temperatures, pH and mixing rate are the main factors for enzymatic hydrolysis of lignocellulosic biomass material (Taherzadeh & Karimi, 2007a; Olsson *et al*., 1996). Belkacemi and Hamoudi (2003) have studied enzymatic hydrolysis of corn stalk hemicelluloses at 30 °C and pH 5.0. Saccharification was 90% and sugar was released after 10 h.

5.3.2 Bacteria

Different species of bacteria such as *Clostridium, Cellumonas, Bacillus, Microbispora* and *Streptomyces* etc., are able to produce cellulase and amylase (Rabinovich *et al*., 2002; Sun and Cheng, 2002). Hydrolysis of starchy materials break down the starch into fermentable sugars called saccharification. Hydrolysis of biomass is carried out at temperatures of 90 to 110 °C. At low temperatures, hydrolysis of starchy waste is also possible and can contribute further to energy savings (Sanchez *et al*., 2008). For the conversion of starch into fermentable sugars, two types of pretreatment can be used such as acid hydrolysis and biological hydrolysis which needs to be performed. Each of these pretreatments has their own set of advantages and disadvantages for use. Biological hydrolysis is chosen though there is a high cost of enzymes and initial investment needed because of the high conversion yield of glucose (Tasic *et al*., 2009). Amylase enzyme (α-amylase, β-amylase and glucoamylase) are employed for hydrolysis of starchy biomass materials. These amylolytic enzymes are derived from various sources such as plants, animals and microorganisms. Microbial amylases are in use commonly for hydrolysis of biomass (Kunamneni *et al*., 2005). Enzyme α-Amylase randomly cleaves the 1,4 α-D-glucosidic linkages of amylose in the linear amylase chain. Another enzyme of amylase, amyloglucosidase cleaves the 1, 6-α-D-glucosidic linkages at the branching points of amylopectin as well as 1, 4-α-linkages of amylose also (Pandey *et al*., 2000). Production of enzyme α-amylase showed under solid state fermentation by *Bacillus cereus* MTCC 1305 has been used with wheat bran and rice flake manufacturing waste as substrates. Wheat bran as carbon source used as highest enzyme production expressed as units per mass of dry substrate approx. 94 U/g was observed. Production parameters of wheat bran biomass were optimized an inoculum size of 10% volume per mass. Substrate and moisture ratio showed as 1:1. Carbon sources, supplemented such as glucose 0.04 g/g showed enhanced enzyme production up to 122 ± 5 U/g. It was the same when supplementation of nitrogen sources was added as 0.02 g/g showed decline in enzyme production. For this starch hydrolysis optimum amylase enzyme activity was observed at 55 °C, pH = 5. Some potato tubers were used and ground into a mash, which was highly viscous. Mash viscosity was reduced by the pretreatment of biomass using with mixed enzyme such as pectinase, cellulase and hemicellulase. Starch in the pretreated mash was hydrolysate to maltodextrins by the action of enzyme α-amylase from 30 °C to 85 °C. SSF of this starchy material mash was performed at 30 °C with the addition of enzyme and supplements as glucoamylase and ammonium sulphate as nitrogen source, respectively. In a specific case study, the addition of glucoamylase,

supplement ammonium sulphate concentration and fermentation time were optimized as at 1.65 AGU g/1, 30.2 mM and 61.5 h, respectively using the response surface methodology (RSM) Using the optimized condition, ethanol yield obtained 16.61% (v/v), which was equivalent to 89.7% of the theoretical yield value according to RSM. Production of ethanol from waste potato needs more attention because limited research conducted about potato waste for ethanol production. The waste potato industry can be an enriched carbon source for yeast during alcohol fermentation (Fadel *et al*., 2000 & Liimatainen *et al.,* 2004).

5.4 Conclusion

Selection of suitable raw material for bio-ethanol production is the major concern regarding its worldwide commercial sustainability. Variations in the price of raw materials and availability of feedstock for bio-ethanol production are totally dependent on geographic locations. In such a context, waste lignocellulose-based materials have been proved to be potential, easily available and economically viable material over the existing feed stock for bio-ethanol production. As recovery of simple sugars from the lignocellulosic biomass is the major challenge, design of a suitable pretreatment method to facilitate the process plays a central role in determining the market price of bio-ethanol. Physical, chemical and biological pretreatments are the vital methods for the conversions of simple sugars from lignocellulosic biomass. A judicious combination of a chemical pretreatment method with biological pretreatment improved the performance of the process in achieving maximum yield of simple sugar. Being an eco-friendly process, biological (fungal, bacterial) pretreatment is considered a highly promising pretreatment regime for lignocellulosic biomass.

References

Ahring, B. K., Jensen, K., Nielsen, P., Bjerre, A. B., & Schmidt, A. S. (1996). Pretreatment of wheat straw and conversion of xylose and xylan to ethanol by thermophilic anaerobic bacteria. *Bioresource Technology*, *58*(2), 107–113.

Alén, R. (2000). Structure and chemical composition of wood. *Forest products chemistry. Helsinki: Fapet Oy*, 35.

Alizadeh, H., Teymouri, F., Gilbert, T. I., & Dale, B. E. (2005). Pretreatment of switchgrass by ammonia fiber explosion (AFEX). *Applied Biochemistry and Biotechnology*, *124*(1–3), 1133–1141.

Alvira, P.; Tomás-Pejó, M.; Ballesteros, M. & Negro, M.J. (2010). Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresource Technology*, Vol. 101, 4851–4861.

Arai T, Kosugi A, Chan H, Koukiekolo R, Yukawa H, Inui M (2006). Properties of cellulosomal family 9 cellulases from *Clostridium cellulovorans*. *Applied Microbiology and Biotechnology* 71, 654–660.

Atalla, R. H., & Vanderhart, D. L. (1984). Native cellulose: a composite of two distinct crystalline forms. *Science*, *223*(4633), 283–285.

Ballesteros, I., Oliva, J. M., Navarro, A. A., González, A., Carrasco, J., & Ballesteros, M. (2001). Effect of chip size on steam explosion pretreatment of softwood. *Applied Biochemistry and Biotechnology*, *84*(1–9), 97–110.

Banerjee, Goutami, John S. Scott-Craig, and Jonathan D. Walton. Improving enzymes for biomass conversion: a basic research perspective. *Bioenergy Research* 3, no. 1 (2010): 82–92.

Beguin P, Aubert J P (1994). The biological degradation of cellulose. FEMS Microbiology Reviews 13, 25–58.

Belkacemi K, Hamoudi S (2003). Enzymatic hydrolysis of dissolved corn stalk hemicelluloses: Reaction kinetics and modeling. *Journal of Chemical Technology and Biotechnology* 78, 802–808.

Brownell, H. H., & Saddler, J. N. (1987). Steam pretreatment of lignocellulosic material for enhanced enzymatic hydrolysis. *Biotechnology and Bioengineering*, *29*(2), 228–235.

Champagne, P. (2007). Feasibility of producing bio-ethanol from waste residues: a Canadian perspective: feasibility of producing bio-ethanol from waste residues in Canada. *Resources, Conservation and Recycling*, *50*(3), 211–230.

Chandel AK, Chan EC, Rudravaram R, Narasu ML, Rao LV, Ravindra P (2007a). Economics and Environmental Impact of Bioethanol Production Technologies: An Appraisal. *Biotechnol. Mole. Biol. Rev*., 2: 14–32.

Chanzy, H., Imada, K., & Vuong, R. (1978). Electron diffraction from the primary wall of cotton fibers. *Protoplasma*, *94*(3–4), 299–306.

Chanzy, H., Imada, K., Mollard, A., Vuong, R., & Barnoud, F. (1979). Crystallographic aspects of sub-elementary cellulose fibrils occurring in the wall of rose cells cultured in vitro. *Protoplasma*, *100*(3–4), 303–316.

Chen, Y., Sharma-Shivappa, R. R., & Chen, C. (2007). Ensiling agricultural residues for bioethanol production. *Applied Biochemistry and Biotechnology*, *143*(1), 80–92.

Clarke, A. J. (1996). Chemistry and structure of cellulose and heteroxylan. *Biodegradation of cellulose: Enzymology and Biotechnology.* Lancaster, Pa.: Technomic Press, 9.

Clarke, A. J., (1997). Enzymology of biodegradation of cellulose and hemicellulose, Chapter 2 In: *Biodegradation of cellulose: Enzymology and Biotechnology.* Technomic Publishing Co., Inc., Lancaster, Basel.

Coughlan, M.P., Ljungdahl, L.G., 1988. Comparative biochemistry of fungal and bacterial cellulolytic enzyme system. In: Aubert, J.-P., Beguin, P., Millet, J. (Eds.), *Biochemistry and Genetics of Cellulose Degradation*, pp. 11–30.

Daniela, Bocchini M, Heloiza F, Alves do Prado, Rodrigo S, Henrique F, Márcia M de Souza, Roberto da and Eleni G. (2011). Agroindustrial Wastes as Substrates for Microbial Enzymes Production and Source of Sugar for Bioethanol Production, Integrated Waste Management - Volume II, *Environmental Engineering. DOI: 10.5772/23377*.

Demirbas A (2005). Bioethanol from cellulosic materials: a renewable motor fuel from biomass. *Energy Sources* 27, 327–33.

Eggeman, T., & Elander, R. T. (2005). Process and economic analysis of pretreatment technologies. *Bioresource Technology*, *96*(18), 2019–2025.

El-Zawawy, W. K., Ibrahim, M. M., Abdel-Fattah, Y. R., Soliman, N. A., & Mahmoud, M. M. (2011). Acid and enzyme hydrolysis to convert pretreated lignocellulosic materials into glucose for ethanol production. *Carbohydrate polymers*, 84(3), 865–871.

Emmel, A., Mathias, A. L., Wypych, F., & Ramos, L. P. (2003). Fractionation of *Eucalyptus grandis* chips by dilute acid-catalysed steam explosion. *Bioresource Technology*, *86*(2), 105–115.

Fadel, M. (2000) Alcohol production from potato industry starchy waste. *Egypt J. Microbiol.* (35)273–287.

Fan, L. T., Gharpuray, M. M., & Lee, Y. H. (1987). Nature of cellulosic material. In *Cellulose Hydrolysis* (pp. 5–20). Springer Berlin Heidelberg.

Fan, L., Lee, Y. H., & Gharpuray, M. (1982). The nature of lignocellulosics and their pretreatments for enzymatic hydrolysis. *Microbial Reactions*, 157–187.

Fengel, D., & Wegener, G. (Eds.). (1983). Cellulose in *Wood: chemistry, ultrastructure, reactions*. Walter de Gruyter.

Garrote, G., Dominguez, H., & Parajo, J. C. (1999). Hydrothermal processing of lignocellulosic materials. *HolzalsRoh-und Werkstoff*, *57*.

Grohmann, K., (1993). Bioconversion of Forest and Agricultural Plant Residues. *Biotechnology in Agriculture* No. 9. C.A.B. International, Wallingford, UK.,

Hamelinck, C.N., G van Hooijdonk and A.P.C. Faaij, 2005. Future prospects for the production of ethanol from ligno-cellulosic biomass. Biomass & Bioenergy 28: 384–410.

Hermans, P. H., and Weidinger, A. (1949). X -ray study of the crystallinity of cellulose, *Journal of Polymer Science, 4*(2), 135–144.

Himmel, M. E., Ding, S. Y., Johnson, D. K., Adney, W. S., Nimlos, M. R., Brady, J. W., & Foust, T. D. (2007). Biomass recalcitrance: engineering plants and enzymes for biofuels production. *Science*, *315*(5813), 804–807.

Ingram, T., Wörmeyer, K., Lima, J. C. I., Bockemühl, V., Antranikian, G., Brunner, G., &Smirnova, I. (2011). Comparison of different pretreatment methods for lignocellulosic materials. Part I: conversion of rye straw to valuable products. *Bioresource technology*, 102(8), 5221–5228.

Itoh H, Wada M, Honda Y, Kuwahara M, Watanabe T (2003). Bioorganosolv pretreatments for simultaneous saccharification and fermentation of beech wood by ethanolysis and white rot fungi. *Journal of Biotechnology* 103, 273–80.

John, R.P., Anisha, G.S., Nampoothiri, K.M. and Pandey, A. (2011). Micro and macroalgal biomass: A renewable source for bioehanol, Bioresour, Technol, 102: 186–193.

Jorgensen H, Kutter J P, Olsson L. (2003). Separation and quantification of cellulases and hemicellulases by capillary electrophoresis. *Analytical Biochemistry* 317(1), 85–93.

Jørgensen, H., Kristensen, J. B., & Felby, C. (2007). Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities. *Biofuels, Bioproducts and Biorefining, 1*(2), 119–134.

Kadar, Z., Szengyel, Z. and Reczey, K. (2004). Simultaneous saccharification and fermentation (SSF) of industrial wastes for the production of ethanol, Ind. Crops Prod. 20:103–110.

Kim, K. H., & Hong, J. (2001). Supercritical CO_2 pretreatment of lignocellulose enhances enzymatic cellulose hydrolysis. *Bioresource Technology*, *77*(2), 139–144.

Kim, S. and Dale, B.E. (2004). Global potential Bioethanol production from wasted crops and crop residues, *Biomass Bioenerg*. 26:361–375.

Kim, S., & Holtzapple, M. T. (2006). Delignification kinetics of corn stover in lime pretreatment. *Bioresource Technology*, *97*(5), 778–785.

Krishna S. H., Reddy T. J., Chowdary G. V. (2001). Simultaneous saccharification and fermentation of lignocellulosic wastes to ethanol using a thermo tolerant yeast. *Bioresource Technology* 77(2), 193–6.

Kumakura, M., &Kaetsu, I. (1982). Radiation degradation and the subsequent enzymatic hydrolysis of waste papers. *Biotechnology and Bioengineering*, *24*(4), 991–997.

Kumar, P., Barrett, D. M., Delwiche, M. J., & Stroeve, P. (2009). Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Industrial & *Engineering Chemistry Research*, 48(8), 3713–3729.

Kunamneni A., Permaul K., Singh S. (2005). Amylase production in solid state fermentation by the thermophilic fungus Thermomyces lanuginosus. *J Biosci Bioeng.*(100)168–171.

Lee, D. H., & Lee, D. J. (2007). Biofuel Economy and Hydrogen Competition†. *Energy & Fuels*, 22(1), 177–181.

Liimatainen, H.; Kuokkanen, T.; Kaariainen, J. (2004). Development of Bio-Ethanol Production from Waste Potatoes. In *Proceedings of the Waste Minimization and Resources Use Optimization Conference*, 123–129.

Lissens, G., Klinke, H., Verstraete, W., Ahring, B. and Thomsen, A.B. (2004). Wet oxidation pretreatment of woody yard waste: parameter optimization and enzymatic digestibility of ethanol production, *J. Chem. Technol. Biotechnol.* 79:889–885.

Ma, F., Yang, N., Xu, C., Yu, H., Wu, J., & Zhang, X. (2010). Combination of biological pretreatment with mild acid pretreatment for enzymatic hydrolysis and ethanol production from water hyacinth. *Bioresource technology*,*101*(24), 9600–9604.

Mabee, W. E., Saddler, J. N., Nielsen, C., Nielsen, L. H., & Jensen, E. S. (2006). Renewable-based fuels for transport. Rise Energy report

Mais, U., Esteghlalian, A. R., Saddler, J. N., & Mansfield, S. D. (2002).*Enhancing the enzymatic hydrolysis of cellulosic materials using simultaneous ball milling* (pp. 815–832). Humana Press.

Martin, C., Alriksson, B., Sjöde, A., Nilvebrant, N. O., &Jönsson, L. J. (2007a). Dilute sulfuric acid pretreatment of agricultural and agro-industrial residues for ethanol production. *Applied Biochemistry and Biotechnology*, *137*(1–12), 339–352.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., & Ladisch, M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, *96*(6), 673–686.

Mtui, G. and Nakamaura, Y. (2005). Bioconversion of lignocellulosic waste from selected dumping sites in Dares Salaam, Tanzania, Biodegradation 16:493–499.

Negro, M. J., Manzanares, P., Ballesteros, I., Oliva, J. M., Cabañas, A., & Ballesteros, M. (2002). Hydrothermal pretreatment conditions to enhance ethanol production from poplar biomass. *Applied Biochemistry and Biotechnology*, *105*, 87–100.

Nimz, H. (1974). Beech lignin—proposal of a constitutional scheme. *AngewandteChemie International Edition in English*, *13*(5), 313–321.

Oleskowicz-Popiel, P., Lisiecki, P., Holm-Nielsen, J. B., Thomsen, A. B., & Thomsen, M. H. (2008). Ethanol production from maize silage as lignocellulosic biomass in anaerobically digested and wet-oxidized manure. *Bioresource technology*, *99*(13), 5327–5334.

Olsson L, Hahn-Hagerdal B (1996). Fermentation of lignocellulosic hydrolysates for ethanol production. *Enzyme and Microbial Technology*, 18(5), 312–31.

Ortega N, Busto M D, Perez-Mateos M (2001). Kinetics of cellulose saccharification by *Trichoderma reesei* cellulases. *International Biodeterioration Biodegradation* 47(1), 7–14.

Pachauri, R. K., & Sridharan, P. V. (Eds.). (1998). *Looking back to think ahead, GREEN India 2047: Growth with resource enhancement of environment and nature*. TERI Press.

Palonen, H., Thomsen, A. B., Tenkanen, M., Schmidt, A. S., &Viikari, L. (2004). Evaluation of wet oxidation pretreatment for enzymatic hydrolysis of softwood. *Applied Biochemistry and Biotechnology*, *117*(1), 1–17.

Pandey, A., Nigam, P., Soccol, C.R., Soccol, V.Y., Singh, D.,Mohan, R., (2000). Advances in microbial amylases. *Biotechnol.Appl. Biochem.* (31)135–152.

Pasquini, D., Pimenta, M. T. B., Ferreira, L. H., &Curvelo, A. A. D. S. (2005). Extraction of lignin from sugar cane bagasse and *Pinus taeda* wood chips

using ethanol–water mixtures and carbon dioxide at high pressures. *The Journal of Supercritical Fluids*, *36*(1), 31–39.

Patel, S. J., Onkarappa, R., & Shobha, K. S. (2007). Fungal pretreatment studies on rice husk and bagasse for ethanol production. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *6*(4), 1921–1926.

Pérez, J., Muñoz-Dorado J., de la Rubia, T., Martinez, J. (2002). Biodegradation and biological treatments of cellulose, hemicellulose, and lignin: an overview. *International Microbiology*, 5: 53–63.

Rabinovich M. L., Melnik M. S., Boloboba A. V. (2002). Microbial cellulases (review). *Applied Biochemistry and Microbiology* 38(4), 305–21.

Ravindranath, N. H., Somashekar, H. I., Nagaraja, M. S., Sudha, P., Sangeetha, G., Bhattacharya, S. C., & Abdul Salam, P. (2005). Assessment of sustainable non-plantation biomass resources potential for energy in India. *Biomass and Bioenergy*, *29*(3), 178–190.

Richmond, P. A. (1991). Occurrence and functions of native cellulose. *Biosynthesis and biodegradation of cellulose.* Dekker, New York, 5–23.

Rivers, D. B., & Emert, G. H. (1987). Lignocellulose pretreatment: a comparison of wet and dry ball attrition. *Biotechnology Letters*, *9*(5), 365–368.

Saha, B. C. (2003). Hemicellulose bioconversion. *Journal of Industrial Microbiology and Biotechnology, 30*(5), 279–291.

Sakakibara, A., & Sano, Y. (2000). Chemistry of lignin. *Wood and Cellulosic Chemistry*, 109–174.

Sanchez, O. J., & Cardona, C. A. (2008). Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresource technology*, 99(13), 5270–5295.

Sandgren M., Shaw A., Ropp T. H., Wu S., Bott R., Cameron A. D. (2001). The X-ray crystal structure of the *Trichoderma reesei* family 12 endoglucanase 3, Cel12A, at 1.9 Å resolution. *Journal of Molecular Biology* 308(2), 295–310.

Sarkar N, Ghosh S K, Bannerjee S, Aikat K (2012). Bioethanol production from agricultural wastes: An overview. *Renewable Energy* 37, 19–27.

Silverstein, R. A. (2005). A comparision of chemical pretreatment methods for converting cotton stalks to ethanol. Master's thesis (adv: R. Sharma).

Silverstein, R. A., Chen, Y., Sharma-Shivappa, R. R., Boyette, M. D., & Osborne, J. (2007). A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. *Bioresource Technology*, *98*(16), 3000–3011.

Sun, Y., & Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology,* 83(1), 1–11.

Taherzadeh, M. J., & Karimi, K. (2007). Enzymatic-based hydrolysis processes for ethanol from lignocellulosic materials: A review. *BioResources*, 2(4), 707–738.

Taherzadeh, M. J., & Karimi, K. (2007a). Acid-based hydrolysis processes for ethanol from lignocellulosic materials: a review. *BioResources*, *2*(3), 472–499.

Taherzadeh, M. J., & Karimi, K. (2007b). Enzymatic-based hydrolysis processes for ethanol from lignocellulosic materials: A review. *Bio Resources*, 2(4), 707–738.

Tasić, M. B., Konstantinović, B. V., Lazić, M. L., &Veljković, V. B. (2009). The acid hydrolysis of potato tuber mash in bioethanol production. *Biochemical engineering journal,* 43(2), 208–211.

Teymouri, F., Laureano-Perez, L., Alizadeh, H., & Dale, B. E. (2005). Optimization of the ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover. *Bioresource Technology*, *96*(18), 2014–2018.

Teymouri, F., Laureano-Pérez, L., Alizadeh, H., & Dale, B. E. (2004). Ammonia fiber explosion treatment of corn stover. *Applied Biochemistry and Biotechnology*, *113*, 951.

Vaccarino, C., Lo Curto, R. B., Tripodo, M. M., Bellocco, E., Laganá, G., & Patané, R. (1987). Effect of SO_2, NaOH and Na_2CO_3 pretreatments on the degradability and cellulase digestibility of grape marc. *Biological Wastes*, *20*(2), 79–88.

Wan, C., & Li, Y. (2011). Fungal pretreatment of lignocellulosic biomass. *Biotechnology advances*, *30*(6), 1447–1457.

Wan, C., & Li, Y. (2012). Fungal pretreatment of lignocellulosic biomass. *Biotechnology Advances*, *30*(6), 1447–1457.

Wayman M, Parekh SR (1990) Biotechnology of biomass conversion; Fuels and chemicals from renewable resources. Open University Press Milton Keynes.

web4:http://www.emeraldbiology.com/2013/07/fuel-for-biofuels-part-2-cellulosic.html access date 25th November, 2013.

web 5: http://www.biotek.com/resources/articles/enzymatic-digestion-of-polysaccharides-2.html access date 4th December, 2013.

Wen, Z., Liao, W. and Chen, S. (2004). Hydrolysis of animal manure lignocellulosics for reducing sugar production, *Bioresour, Technol*, 91: 31–39.

Xu J, Takakuwa N, Nogawa M, Okada H, Morikawa Y (1998). A third xylanase from *Trichoderma reesei PC-3–7*. *Applied Microbiology and Biotechnology* 49, 18–724.

Zabed, H., Faruq, G., Sahu, J. N., Azirun, M. S., Hashim, R., & Nasrulhaq Boyce, A. (2014). Bioethanol production from fermentable sugar juice. *The Scientific World Journal*, 2014.

Zhang, L. H., Li, D., Wang, L. J., Wang, T. P., Zhang, L., Chen, X. D., & Mao, Z. H., (2008). Effect of steam explosion on biodegradation of lignin in wheat straw. *Bioresource Technology*, *99*(17), 8512–8515.

Zhang, Y. H. P., & Lynd, L. R., (2004). Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems. *Biotechnology and Bioengineering*, *88*(7), 797–824.

6

Anaerobic Digestion and the Use of Pre-treatments on Lignocellulosic Feedstocks to Improve Biogas Production and Process Economics

Laura Williams, Joe Gallagher, David Bryant and Sreenivas Rao Ravella*

IBERS, Aberystwyth University, Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, UK

Abstract

Biogas from anaerobic digestion (AD) has been identified as an important source of clean energy that can make a contribution towards renewable energy provision in order to mitigate climate change. Many of the crops and agricultural residues used in today's AD systems consist primarily of lignocellulosic materials. The efficiency of conversion of these feedstocks to biogas have been shown to be improved through the use of physical, chemical or biological pre-treatments. The benchmarking of commercial biogas plants throughout Europe demonstrated several challenges that need to be addressed to further optimize biogas yield and to improve its overall economics. Central to this is the need for more efficient release of sugars from recalcitrant lignocellulose feedstocks. In this review we discuss AD and assess the use of pre-treatments for improved production of biogas from agricultural residues and crops.

***Keywords*:** Biogas, methane, anaerobic digestion (AD), lignocellulosic biomass, pre-treatment

6.1 Introduction

Much research has been undertaken regarding the possibilities of anaerobic digestion (AD) providing an alternative source of renewable energy to

**Corresponding author*: rsr@aber.ac.uk

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reduce CO_2 emissions from fossil fuels and partly replace fossil fuels as they become increasingly unavailable [1–3]. AD is a four-stage process; hydrolysis, acidogenesis, acetogenesis, and methanogenesis [4] (Figure 6.1) whereby organic material (termed "substrate") such as cattle effluent, food waste and plant biomass, is broken down by microorganisms in the absence of oxygen, to produce biogas and digestate [5]. The creation of such useful products from organic waste has generated global interest in AD as a means of waste valorisation. Additionally, research suggests that AD could be more economical than bioethanol production, as AD uses less energy in the unit processes to produce biogas than is used in the production of alcohol-based transport fuels such as ethanol, and the output energy ratio for methane is higher than that of alcohol-based biofuels [6]. AD performance is measured by weighing the viability of the system inputs against the product output, i.e., the biogas yield. Soluble chemical oxygen demand (sCOD), total solids (TS) and volatile solids (VS) are all methane yield indicators [2, 7, 8]. An elevated sCOD indicates that microorganism activity is occurring, and thus dictates the rate of digestion depending on how much the sCOD is increased. TS and VS decrease with microorganism activity as solid material is broken down and indicates increases in biodegradability.

The use of pre-treatments has been researched to investigate the possibility of increasing the digestibility of the substrate, and reducing the residence time taken to produce these products, i.e., improving the efficiency of AD and making it more cost effective.

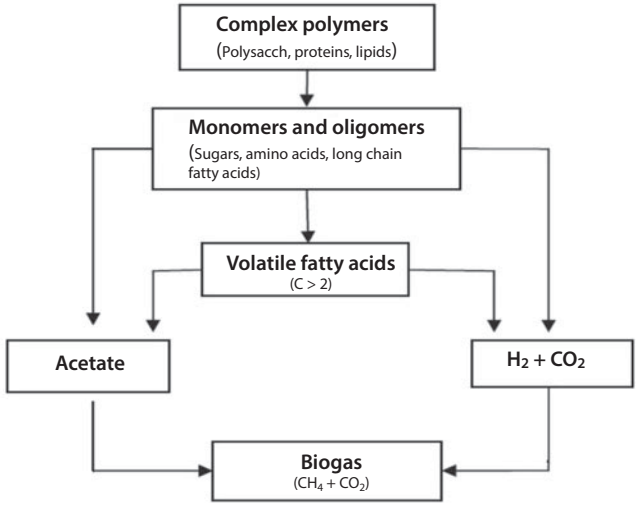

Figure 6.1 The stages of the methane fermentation process [9].

Although cattle effluent is one of the most well-researched AD substrates on account of it being a problematic waste product, more recently there has been a focus on plant biomass in the way of first-generation (1G) feedstock, which includes food crops such as vegetable oils and grains [10], and second-generation (2G) feedstocks, which are primarily lignocellulosic and consist of agricultural residues such as wheat straw [11], Maize stover [12–14], oat straw [15], rye [16], barley straw [17, 18], sorghum forage, wheat straw [3], oilseed rape straw [19], grass silage [20–23], non-herbaceous and herbaceous phytomass [7], as well as energy crops such as miscanthus [24, 25], reed canary grass [26, 27], switch grass [28], willow [29, 30], and Salix [31, 32] (Figure 6.2). Other feedstocks include fodder maize and forage grasses [33–35]. The AD process can be categorized as either liquid AD (L-AD) or solid-state AD (SS-AD), depending on the total solids (TS) content of the feedstock that is being digested [36]. L-AD is typically defined as containing 0.5–14.0% TS and is usually used for liquid waste including cattle effluent and other animal manures; whereas SS-AD contains 15–40% TS and is used for the treatment of lignocellulosic biomass in addition to solid fraction municipal solid waste [4, 36]. The fact that 1G feedstocks are primarily food crops has raised concerns about its sustainability; the use of food crops for fuel is contestable when the global

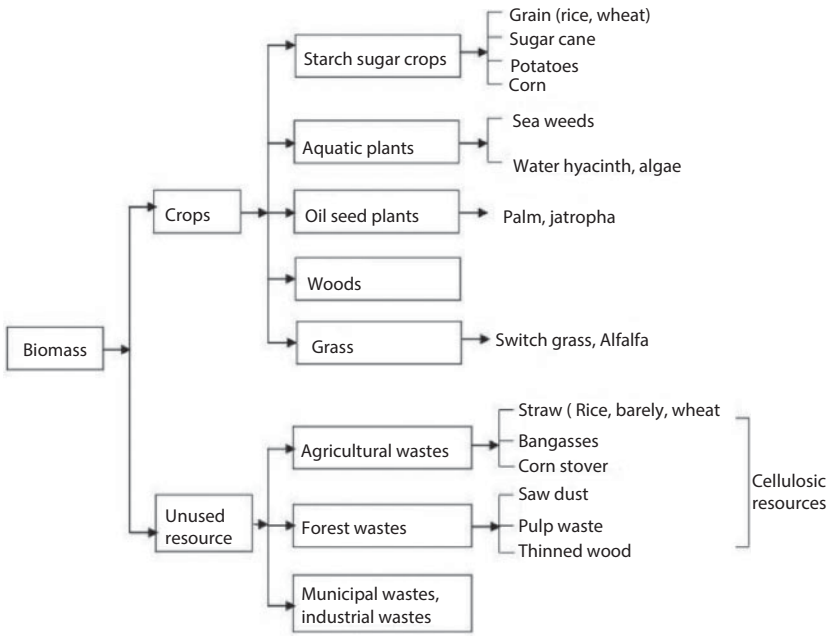

Figure 6.2 Biomass as a renewable feedstock [42].

population continues to increase and demand for food and agricultural land is rising [3, 37, 38]. In an attempt to overcome the problem of using food crops, the possibilities of using 2G feedstocks are being explored. 2G feedstocks are considered by some to be more sustainable than 1G feedstocks as they are derived from agricultural residues or energy crops, which do not compete with food crops for land or fertilisers [3, 37]. In addition, the abundance of low-value, high-yielding lignocellulosic biomass coupled with their environmentally friendly features has strengthened the popularity of 2G feedstocks as substrates for AD [39–41].

6.2 Feedstocks Available for AD

It is important to recognize that one of the key benefits of AD as an energy production system is that it provides a closed energy cycle whilst disposing of organic waste products, many of which would be destined for landfill. Substrates include wastes from the agricultural, processing, food and drink and domestic sectors. Agricultural waste ranges from livestock effluent to crop residues, and it is these feedstocks which have been most widely investigated in AD. However, the abundance of food waste at the post-production stage of food production is well documented in the UK, and must also be considered as a substrate for co-digestion in AD. It has been calculated that 7 million tonnes of food are wasted by UK households each year [43] and it has been suggested and demonstrated in German biogas plants that the digestion of energy crops and plant residues combined with food waste would be beneficial [44]. Co-digestion of carefully selected feedstocks to produce an optimal substrate would further enhance an already promising energy generation process. More recently, there have been studies investigating the potential of algal derived biomass, on account of the abundance of this organic matter. However, this chapter focuses on the pre-treatment of lignocellulosic biomass and energy crops.

6.2.1 Lignocellulosic Feedstock Analysis and Substrate Suitability

While ensiling is a common agricultural practice to preserve organic biomass for long periods of time, research regarding the use of ensiled feedstocks for AD is currently in its infancy. The principle of ensiling is to promote an anaerobic lactic acid fermentation to stabilize plant material at low pH in anaerobic conditions. To achieve this, plant material with sufficient water soluble carbohydrate (WSC) content is harvested, wilted

to a dry matter (DM) content of 25–30% DM and compacted and sealed with plastic film which is impermeable to air. Residual oxygen is rapidly depleted by aerobic micro-organisms and this creates conditions which are favourable to lactic acid bacteria (LAB), which ferment WSC to lactic acid resulting in a reduction in pH. For stable preservation it is necessary to achieve a pH < 4.5. This can be facilitated by application of an LAB inoculant or direct application of mineral or organic acids which prevents deterioration of the organic matter by detrimental microorganisms such as yeasts [45]. More recently, alkali treatments have been assessed for their suitability for the ensiling process. The storage of feedstocks by ensiling has been examined to determine whether or not ensiling can begin the process of breaking down some of the recalcitrant cell wall bonds present in the lignocellulosic material [45]. Ensiling is considered an economic storage solution for wet feedstocks rather than drying [46]. Zheng *et al.* [46] investigated the ensilage of sugar beet pulp (SBP), and found that together with providing a suitable storage solution for this wet material, it also acted as a biological pre-treatment which enhanced biofuel yield. Zheng *et al.* [46] examined SBP for the production of ethanol biofuel as opposed to biogas, although there has been some research in this area regarding the use of ensiling as a biological pre-treatment for biogas production. A problem associated with ensiling is feedstock losses during the aerobic digestion period, which then reduces the amount of available biomass for AD [47]; however, Herrmann *et al.* [47] found that cutting the biomass smaller resulted in a reduced biomass loss through aerobic digestion at the beginning of the ensilage process. This study also found that cutting the plant matter to shorter lengths resulted in an increase in harvesting and fuel costs. There is a trade-off between gaining a higher methane yield and the costs incurred in order to achieve such a yield; in order for AD to be viable, the benefits must outweigh the costs.

Lignocellulosic biomass is increasing in popularity as a feedstock for AD owing to its abundance in the form of forestry residues and organic fractions of municipal waste [48]. Crops grown for energy and agricultural residues including rice straw, wheat straw and corn stover have been researched as a feedstock for AD. A key benefit of agricultural residues is the multiple opportunities to eradicate waste and generate energy; these include residues such as stalks and leaves but also residues from the processing stage when the crop is milled and sieved [49]. Rice straw and wheat straw are among the most abundant sources of agricultural residue biomass on account of their status as a global food staple [49, 50].

In a study conducted by Amon *et al.* [51], 14 crop varieties and 6 permanent grassland varieties were compared for their methane-yielding

abilities. Variables included optimum harvest time and methane yield per hectare, and it was found that the maize varieties yielded the highest quantity of methane; however, the yield potential was dependent on the time of harvesting; other crops included sunflower varieties, winter wheat and rye varieties. Although the subject of growing crops for energy is controversial, the data from this study has led to the development of the methane energy value model (MEVM), which can estimate the specific methane yield of organic substrates in addition to optimum harvest time of various species and crop varieties. These models have the potential to improve the cost-effectiveness of AD in facilitating the selection of the best harvest period to the maximum methane yield. The difference between optimum harvest times for maximum yield across different energy crop varieties could also be monitored by means of careful crop rotation. This would ensure an equal distribution of plant residue available for AD throughout the year to avoid fluctuations in availability and also assure that residues become available for use only when they are at their peak for maximum methane yield in the digester [52].

A similar study using energy crops was conducted by Godin *et al*. [53], who found that autumn harvested energy crops had the most promising composition for AD as it had a high dry biomass yield and digestibility, as opposed to winter crops which had begun to store away sugars in the rhizomes and thus were less digestible, although additional fertilizer would be needed as nutrients would not be recycled into the rhizome and the autumn crop would have a higher moisture content. Similar findings have been observed by Kandel *et al*. [54], who investigated the biogas potential of reed canary grass (*Phalarisarundinacea L.*), a perennial crop which can grow in poor, waterlogged soils in colder climates. The study found that time of harvest significantly impacted the methane potential of the crop, with there being a trade-off between a lower lignin content in younger plants to facilitate digestibility, or, a higher lignin content in a more mature plant which would be more recalcitrant to break down but that would produce higher yields. Younger plants with more leaves have a higher protein content which improves the quality of the methane, but the final yield is lower than if a more mature plant were selected [54]. However, although a more mature plant produces a larger amount of methane, the increase in process time required to obtain the methane would need to be taken into account to ensure that the system is cost effective. Kandel *et al*. [54] suggests that, since reed canary grass has optimum harvest times, it would be advantageous to grow multiple crops throughout the year in an attempt to obtain biogas of higher quality methane from younger plants, and in quantity from those that are more mature.

However, in order for this to be a viable option it is essential that the biogas production be greater than the costs incurred through additional fertiliser and labour for its generation [54].

The biochemical methane potential (BMP) yield of each feedstock is different on account of variable organic composition. BMP and anaerobic biodegradability is dependent on the lignin content of the matter and also the cellulose crystallinity [7]. In a study investigating BMP yield for a range of herbaceous (plants without a persistent woody stem) and non-herbaceous (plants with a persistent woody stem) feedstocks, including green waste such as hedge trimmings, Triolo *et al.* [7], found that the non-herbaceous feedstocks produced less methane, which is unsurprising owing to non-herbaceous matter having a higher lignin content and thus being more difficult to decompose. Lawn cuttings were among the 57 herbaceous and non-herbaceous samples studied for BMP yield, and it was observed that lawn waste, although lignocellulosic, had a lower lignin content and a higher BMP yield than hedge cuttings; the lignin content of lawn cuttings was 8.2% compared to 29.4% in hedge cuttings [7]. One reason for these differences lies in the physiological vegetation age; as grass is mown it reduces the physiological age of the vegetation which in turn reduces the lignin content of the plant, whereas woodier plants such as hedges are cut less frequently, resulting in a higher lignin content [7]. It should also be noted that there will be variations in lignin content when comparing different plant species. Another finding of this paper was that BMP fluctuated in accordance with time of harvest; summer samples were found to have a higher BMP than autumn ones for grass cuttings. Triolo *et al.* [7], suggests that the reason for this is likely due to the vegetation advancing with time; in the summer the environment is milder and the vegetation is producing more sugar, whereas in autumn the environment becomes more unpredictable and sugar production is lower. This observation is in agreement with Amon *et al.* [51], who investigated optimum harvest time of energy crops, and confirms that in order to generate the greatest methane yield, feedstock must be carefully considered before being utilised as a substrate.

In order to assess feedstock suitability in terms of BMP yield, it is possible to use a technique that has been used in animal feed analysis to distinguish the structural features of plant cells; the neutral detergent fibre assay (NDF), to enable the composition of a feedstock to be calculated. The NDF assay calculates how much cellulose, hemicellulose and lignin a feedstock contains [55], and was originally developed by Van Soest [56]. The NDF analysis is frequently used in studies attempting to enhance the AD process in addition to similar BMP "yield prediction" methods; Raju *et al.* [55], used *in-vitro* organic matter digestibility

assay (IVOMD) and near infrared spectroscopy (NIRS) as well as NDF to assess methane potential of meadow grasses. IVOMD differs from NDF in that instead of calculating the amount of each component that is present in the material, it demonstrates the percentage of material that is available for digestion by rumen microbes [55]. NIRS is a quicker method that relies on correlations between observed NIR peaks and wet chemistry based determinations. Correlation models are set up for each feedstock. During the study, Raju *et al*. [55], found that NIRS prediction statistics of BMP were the best, and that the NDF and IVOMD prediction statistics were very poor in comparison. It is suggested in the study that a possible reason for this is that the data set was quite homogenous; but even when NDF and IVOMD analyses were combined to offer a prediction statistic, the result was not very accurate [55]. Jacobi *et al*. [57], conducted a study into the use of NIRS to predict biogas production of maize silage. The samples turned out to be very similar in composition and quality, and though the NIRS proved to be accurate in predicting biogas production, the lack of variability in the samples led the authors to suggest that a more heterogenous data set would better demonstrate the benefits of NIRS.

It would seem that NIRS could be extremely useful in identifying BMP yields of feedstocks owing to the rapidity and accuracy of this analysis; therefore further research of this technology would be advantageous in optimizing AD. Although NDF is one of the first technologies that was developed to determine composition of plant matter and is perhaps less accurate than the newer NIRS, the NDF assay is still a useful tool used in much AD research for analysis of lignocellulosic biomass to calculate methane potential [53, 54].

As AD is comprised of four stages with each of them dependent on a specific microbial population, it is important that feedstock composition is suitable for optimal microorganism activity at each stage. In order for the AD process to be balanced, and for the four stages to complement each other, it is essential that each stage be completed within a similar time scale [44]. If either stage is completed too quickly, it has a knock-on effect to the subsequent stage which can lead to inhibition at the methanogenesis (methane production) stage [44]. Feedstock composition is important because some feedstocks have a higher level of solubility than others and thus are broken down faster; i.e., lignocellulosic biomass, which is composed of many insoluble compounds, would take more time to decompose than cattle effluent, for example. Consequently, feedstock suitability in terms of its composition and how it relates to breakdown by the AD microorganisms is another factor that can strongly affect AD efficiency. Co-digestion is a popular solution

as it involves balancing the substrate to have an optimized combination of highly soluble and less soluble compounds.

6.2.2 Substrate Parameters and Co-digestion

There have been a range of studies investigating the efficiency of different feedstocks used in AD, either as a stand-alone substrate or by means of co-digestion with other feedstocks [58–61]. Extensive literature suggests that the optimum methane yield is achieved when several feedstocks with complementary qualities are homogenized to enhance digestibility of the substrate. Energy crops yield the best results when combined with other feedstocks; for example, in conjunction with feedstocks such as cattle effluent which contains macro and micronutrients resulting in improved microorganism performance in the digester [52, 62]. Additionally, cattle effluent is an ideal co-feedstock on account of its neutral pH and its high buffering capacity [63] which enables the production of methane (pH 6.5 and 8.5) [44]. However, the methane yield potential of cattle effluent is low, which is why it is frequently co-digested with energy crops, which have the greatest methane yield potential. The pH of the feedstock being used for an AD substrate is therefore an important parameter to consider, as a substrate which is too acidic will restrict methane production and reduce the efficiency of the AD process. Ensiling renders the feedstock more acidic, and requires the addition of a neutral or alkaline feedstock as a buffering agent. pH can also be influenced by the volume of volatile fatty acids (VFAs) formed during the first three stages of the AD process; hydrolysis, acidogenesis and acetogenesis [64]. The most common VFAs are acetic acid, propanoic acid and butyric acid, and their presence in a high volume results in the digester becoming too acidic, and inhibits or even halts microorganism activity and methanogenesis [65].

Another important consideration during substrate selection is the carbon-to-nitrogen ratio (C:N) in order to reduce ammonia inhibition. Ammonia is a nutrient which is necessary for microbial growth, and produced as a result of the digestion of nitrogenous material [66]. Ammonia inhibition occurs when ammonia levels exceed those which are tolerable by digester microorganisms, and reactor failure has been observed when ammonia levels reach 1700–1800 mg l^{-1} [66]. C:N ratio varies across feedstocks, for example; the C:N ratio of lignocellulosic biomass can be between 40 and 130:1, resulting in a C:N imbalance which in turn can lead to inhibition as it limits the amount of available nitrogen for microbial growth and thus inhibits biogas production [4, 50, 67]. An ideal C:N ratio is 20-30:1 [68], therefore lignocellulose can be a difficult feedstock to

utilize with regard to its high C:N ratio. A solution is to co-digest the lignocellulosic biomass with a feedstock which has a low C:N ratio, such as cattle effluent, to balance the substrate in the digester by providing the nitrogen that is required by the microorganisms [67, 68].

6.3 Feedstock Pre-treatment to Improve AD

The purpose of a pre-treatment is to optimize the release of available substrate for the maximal production of the biogas. Pre-treatment is a necessary additional step in the AD of plant residues, particularly for 2G feedstocks due to the recalcitrant cell wall structure, composed of extensive cross-linked cellulose, hemicellulose and lignin bonds, designed to be insoluble in water and recalcitrant to enzymatic and microbial decomposition [41, 69, 70]. Figure 6.3 represents a schematic of the cells of a lignocellulosic plant showing primary and secondary walls, which vary in the distribution of cellulose, hemicellulose and lignin [71]. The composition of the middle lamella is predominantly lignin, a hydrophobic cross-linked polymer, and the many secondary walls encased within contain cellulose which is attached to hemicellulose and covered in lignin [71]. It is evident that such tightly knit recalcitrant bonds pose a challenge for AD and biogas production (Figure 6.3).

Pre-treatments are used to reduce the strong bonds between the cellulose, hemicellulose and lignin of recalcitrant lignocellulosic material; this facilitates digestion in the reactor and increases the biogas potential that can be produced from the biomass. Woody biomass in particular has

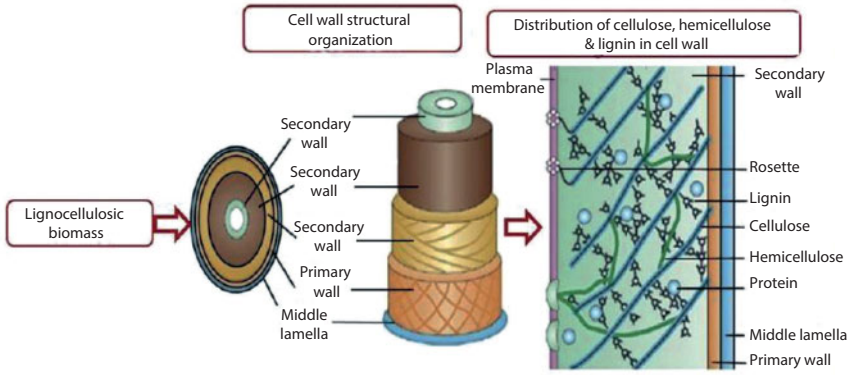

Figure 6.3 Diagrammatic illustration of the framework of lignocellulose [71].

a high lignin content which makes it more resistant to deconstruction than other 2G feedstocks; therefore pre-treatment is particularly important for the processing of these feedstocks if the maximum output is to be achieved [72].

6.3.1 Available Pre-treatment Processes

As there are many factors that can affect the efficiency of biogas production from AD, pre-treatment of the substrate is particularly important in beginning the AD process by improving the substrate BMP. There is a variety of pre-treatments available to begin the process of decomposing the cross-linked bonds in the recalcitrant plant biomass; their classification varies across the literature, but in this article they are classified as mechanical, chemical and thermo-chemical.

Mechanical pre-treatments involve reducing the particle size of the substrate to facilitate AD by creating a larger surface area for decomposition [73, 74]. These include extrusion, centrifugation, grinding and ultrasonic treatment and have been explored particularly in studies using lignocellulosic and woody biomass, to expose more of the substrate to enzyme attack improving scarification and overall process efficiency [75].

Dilute-acid and alkaline are both examples of a chemical pre-treatment. The purpose of this treatment type is to separate cellulose and hemicellulose to isolate the lignin, and enable the enzymes in the hydrolysis treatment to react with the cellulose [37, 40, 70]. Other solvents can also be used in chemical pre-treatment; the organosolv method uses solvents such as methanol and ethanol. These solvents are often used owing to their low boiling point, low cost and ease of recovery at the end of the process [76]. During the organosolv process, organic liquid and water are homogenized and added to the lignocellulose for heating in order to dissolve the lignin and hemicellulose, leaving the cellulose behind for hydrolysis reaction [77].

The thermo-chemical pre-treatment procedure involves the substrate being exposed to a moderate or high temperature whilst also being subjected to rapid changes in pressure in the presence of a chemical catalyst. Steam explosion (SE), ammonia fibre explosion (AFEX) and wet oxidation (WO) can all be classified as thermo-chemical processes. SE delignifies the hemicellulose of the biomass to facilitate enzyme access to the cellulose [78]. This is achieved by subjecting the biomass to high-pressure saturated steam for a pre-determined time period in the presence of acid or alkali, before rapidly reducing the pressure, causing the biomass to explode thus increasing cellulose availability [79]. SE pre-treatment enhance biogas production has been demonstrated in a number of laboratories [11, 19, 31,

32, 80–82]. AFEX is not dissimilar to SE in that the substrate is exposed to high-pressure liquid ammonia steam at a moderate temperature of 90 °C for several minutes; the pressure is then rapidly reduced which causes the ammonia to expand and deconstruct the biomass fibres, increasing enzyme digestibility [71, 83]. WO is a high-temperature, high-pressure method which uses oxygen or air as a catalyst to retrieve cellulose for hydrolysis and fermentation [84]. During this treatment there is a low-temperature hydrolytic reaction and a high-temperature oxidative reaction [85]. In addition to mechanical pre-treatments, SE and WO are among the most used processes to prepare lignocellulosic biomass for AD [8]. Our recent study on evaluation of different pre-treatment methods for increasing hydrolyzing of the energy crops and crop residuals and its influence on biogas production demonstrated that SE pre-treatment (Figure 6.4) yielded more biogas than untreated biomass. The methane potentials of High Sugar Rye Grass (HSRG) and Miscanthus varied from 0.23 to 0.42 m^3 CH_4 kg1 VS added (www.beaconwales.org).

Microwave heating is another thermal process which is being researched in the pre-treatment of lignocellulosic biomass. During this process, heat is transferred directly to the substrate through molecular interaction with

Figure 6.4 Steam explosion rig at BEACON biorefining facility (Aberystwyth University, UK). (www.beaconwales.org)

an electromagnetic field, and the electromagnetic energy is converted from radiation energy to thermal energy [86]. Among the advantages of microwave heating is a more controlled heating process and a reduction in processing time on account of this treatment's ability to heat a large amount of material very rapidly, thus increasing energy efficiency [86, 87]. Jackowiak *et al.* [87] investigated the efficiency of microwave heating as a pre-treatment of wheat grass at different temperatures, using pig manure as an inoculum. It was found that solubility and soluble chemical oxygen demand (sCOD) of the substrate increased with an increase in temperature. An increased sCOD indicates a larger methane production, and is linked to a decrease in total solids (TS) and volatile solids (VS) which are also indicative of biomass being broken down to produce methane [8]. A drawback of microwave heating as a pre-treatment is the production of inhibitory compounds such as VFAs [87].

6.3.2 Pre-treatment Effects on Substrate

Following pre-treatment, a number of modifications are made to the substrate. These can be identified as biodegradability enhancement [88], solubilisation [89], particle size reduction [90, 91] and formation of inhibitory products [88, 92]. It is expected that one or all of these effects take place on the substrate as a result of pre-treatment. Biodegradability enhancement, solubilisation and particle size reduction are desired effects as they break down the bonds in the biomass to reduce its recalcitrance, enable access to cellulose by solubilisation of the lignin and hemicellulose, and increase surface area available for decomposition. The combination of these effects should increase the potential methane yield produced during AD, thus improving the efficiency of the AD process. The formation of inhibitory products such as furfural and hydroxymethylfurfural (HMF) is undesirable as these weak acids can be detrimental to microorganism performance; biomass microorganisms have been known to be stressed by furfural when it occurs at concentrations exceeding 0.5 g L^{-1}, and HMF reduces microorganism action at concentrations of 0.15 g L^{-1} or more [93].

Of all of the pre-treatment effects, solubilisation is the one which is most frequently observed in lignocellulosic biomass studies as it occurs as a result of all pre-treatment methods available; whereas particle size reduction and biodegradability enhancement are restricted to either mechanical pre-treatments or occur to a much lesser degree than solubilisation [8]. Solubilisation is highly important in enhancing methane yield as it degrades the hemicellulose and lignin which are the recalcitrant components of the cell wall [89]. Particle size reduction has been observed as an

effect of extrusion pre-treatment [94] and there is some increase in temperature during extrusion as a result of friction between the extruder screw and the dry biomass being pretreated, which further aids the pre-treatment process.

It is not possible to give a detailed account of the pre-treatment effects on lignocellulosic biomass as there are a number of variables to consider. Biomass composition affects the way(s) in which a pre-treatment behaves; for example, a 1G feedstock has a lower lignin content than a 2G or woody feedstock, and so is less recalcitrant from the offset which may mean that mechanical treatment can be averted [72]. Pre-treatment type must be carefully considered in accordance with the feedstock composition that is being pre-treated, in order to produce the least possible amount of inhibitory products and achieve the optimum level of digestibility to maximize the methane yield in AD later. Optimum digestibility can be achieved by selecting the pre-treatment which results in solubilisation, biodegradability enhancement and particle size reduction of the substrate, or as many of these effects as possible. Lastly, intensity of the treatment must be considered. Factors including temperature, duration of treatment and chemical loading can all either optimize or degrade the effects intended by the pre-treatment and have major implications on process economics.

6.3.3 Effects of Pre-treatment on Methane Yields

Different pre-treatments used on different plant residues result in a different methane yield. For example, Mendez *et al.* [89] studied the effect of temperature and treatment duration during thermal hydrolysis pre-treatment of microalgae biomass and found that following the thermal treatment, the methane yield increased by 60% compared to untreated microalgae. The study found that solubilisation, brought about by the thermal treatment, was responsible for this increase, as well as the higher treatment temperatures (the temperatures tested were 140 °C, 160 °C and 180 °C). Duration of exposure to the pre-treatment had no bearing on the ultimate methane yield, as it was the same regardless of whether the substrate was subject to thermal treatment lasting 10 minutes or 20 minutes [89]. Temperature therefore plays a key role in the solubilisation of microalgae biomass and consequently a crucial role in increasing methane production, as demonstrated by Frigon *et al.* [95] and Tedesco *et al.* [96], both of whom used temperature in addition to a mechanical and chemical pre-treatment.

In a study examining two different plant substrates using alkaline and thermo-alkaline pre-treatments, Sambusitiet *et al.* [4] found that for both substrates, temperatures of 40 °C and 100 °C increased the level

of solubilisation when combined with 10% sodium hydroxide (NaOH). Both pre-treatments had a solubilisation of 40%, which suggests that energy could be saved by opting for the lower temperature treatment combined with the alkaline catalyst, without compromising on solubilisation. This pre-treatment increased the methane yield by 32% for the sorghum forage substrate and by 43% for the wheat straw substrate [4]. This latter substrate produced a methane yield that was higher still (67%) when pre-treated at 100 °C with 10% NaOH. Similar findings were made by Chandra *et al*. [6] and Monlau *et al*. [97], who found that methane yield increased with an increase in temperature and an alkaline reagent to facilitate solubilisation.

With regard to inhibitory product formation, Sambusitiet *et al*. [4] found that low traces of furfural were present at 100 °C but there was no HMF detected. This aligns with other studies examining inhibitory product formation during a thermal pre-treatment [98, 99]. Zhang, C. *et al*. [100] investigated the co-digestion of banana stem and pig manure using a NaOH pre-treatmentat 2%, 6% and 10% concentrations. The study found that there was a significant increase in the amount of biogas produced when a 6% NaOH pre-treatment was applied, but that the 2% concentration had little effect. The 10% concentration decreased the level of biogas production and therefore behaved as an inhibitor rather than a stimulant [100].

For all of the studies mentioned there was a cut-off point with regard to the temperature required to enhance methane yield. At more intense temperatures of 170 °C or more, there was a loss of organic biomass which reduced the amount of methane that could be produced. This effect was also observed by González-Fernández *et al*. [101], who examined the effects of thermal pre-treatment on the methane yield of *Scenedesmus* (microalgae) biomass. González-Fernández *et al*. [101] also found that the rate at which organic matter was solubilised was not constant, but fluctuated, and suggested that this fluctuation is due to the chemical reactions that were taking place at several stages, thus affecting the rate of solubilisation. It was also observed that this fluctuation took place more markedly at lower temperatures (90 °C) than at the higher temperatures of 175–200 °C. Overall, it was concluded that thermal pre-treatment enhanced methane production when conducted at a temperature of 90 °C, but not at a temperature of 70 °C. This suggests that the higher temperature is essential in facilitating the breakdown of the bonds of the plant cell wall and increasing accessibility for anaerobic deconstruction, but as microalgae is a relatively new substrate to be studied in AD, further research of this substrate is necessary [101].

6.4 Pre-treatment and Optimizing AD

6.4.1 Advances in Pre-treatment Methods and AD Conditions

It is clear from the literature that there is no one particular pre-treatment or substrate that will guarantee the maximum possible methane yield during AD. Methane production is dependent upon several factors, including substrate composition, and the solubility, biodegradability and particle size of said substrate, in addition to environmental conditions such as temperature and intensity of treatment. The literature in this paper suggests that thermal pre-treatments are a highly popular choice on account of the enhancement in methane yield that can be achieved. Temperature is a key operational factor which affects biogas production in AD [102]. AD can be either mesophilic or thermophilic; mesophilic AD is usually carried out at a temperature of 30–40 °C, and thermophilic AD within the range of 45–65 °C [66]. Mesophilic AD has been more widely used than thermophilic on account of the lower temperature requiring a lower energy input, and the reduced possibility of ammonia inhibition which can be brought about by fluctuations in temperature and higher temperatures, resulting in a reduced methane yield [44, 66]. However, thermophilic AD is gaining ground on account of having a reduced hydraulic retention time (HRT) which allows for a swifter substrate turnover and biogas production [103], but it is important to note that VFA accumulation is greater in thermophilic AD which increases the risk of reactor failure [66]. It would seem that in order for thermal pre-treatment to be most effective in terms of increased methane and process economics, the pre-treatment temperature would ideally need to be consistent with the temperature intended for AD, either mesophilic or thermophilic, to prevent ammonia accumulation and methanogenesis. It is important to observe that there is a point during the pre-treatment process at which the temperature and reagent intensity become damaging to the organic biomass and enhancement in methane yield is greatly reduced [2, 99]. Therefore, pre-treatment conditions must be carefully monitored, in addition to pre-treatment type and substrate composition, to avoid wasting valuable energy resources and reducing the final output.

The literature supports that solubility is very important in determining the possible methane yield for a substrate, as demonstrated by Sambusitiet *et al.* [4] and Mendez *et al.* [89]. There has been some research into the viability of ionic liquid pre-treatment, a relatively new technology, which increases surface area of the biomass available for hydrolysis via cellulose dissolution [104, 105]. A large range of more than 20 ionic liquids (ILs) are

ideal solvents for cellulose and lignin on account of their ability to completely dissolve these polymers at a range of concentrations, dependent on factors including the operational temperature, duration of treatment, IL characteristics and particle size of the substrate [106]. Imidazonium salts are commonly used for this pre-treatment during which, the lignocellulosic substrate is treated with ILs at concentrations ranging from 5.0 wt% up to 40.0 wt% and heated to <100 °C, before being centrifuged on cooling, to remove the supernatant from the precipitate which is then washed and dried for use in AD [107, 108]. During pre-treatment, the structure of the biomass has been observed to swell as the ILs disrupt the bonds of the organic material and loosen the smaller molecules such as lignin. Gao *et al.* [109] examined the effect of ILs pre-treatment on water hyacinth (*Eichhorniacrassipes*) in terms of composition, structure and biogas production, and observed a lignin removal of 27.1–60.4% in the pre-treated sample and the biogas yield was increased by 97.6% compared to the untreated sample. The low melting points of <100 °C of ILs is advantageous of this treatment in addition to high thermal stabilities and low volatility, as it requires a smaller energy input to generate the temperature suitable for pre-treatment [107]. At present, the use of ILs for pre-treatment is expensive and therefore it is necessary to recover and recycle the IL solution in an attempt to make the process more cost-effective [106]. ILs have also been combined with dimethyl sulfoxide (DMSO) to lessen the cost of the process by reducing the viscosity of the ILs and prolonging the length of time for which they can be used [100]. However, Gao *et al.* [109] found that the ability of the ILs to dissolve and delignify the water hyacinth was hampered by the use of the Dimethylacetamide (DMAC) compound, which was applied to reduce IL viscosity. Therefore it would appear that there is a trade-off between reducing the cost of the IL pre-treatment by reducing viscosity, and the inhibitory effects which can occur as a result of the use of such compounds.

The use of ruminal liquid in AD is currently being explored to enhance biogas production following pre-treatment. This process involves the application of rumen microorganisms to mimic the environment in which lignocellulosic material is most successfully digested. Ruminants such as cattle and sheep are able to digest the recalcitrant lignocellulosic material as a result of microorganism activity that occurs in the rumen; rendering animal manure to be an ideal co-feedstock for AD as many of the strong-bonds of the plant biomass have already been broken down by the animal prior to the AD process. Therefore, research regarding the possibility of adding rumen microorganisms to anaerobic digesters to improve digestion rates is being undertaken. However, a large obstacle of this idea is our lack

of knowledge of the rumen microorganisms themselves. It is estimated that only 10–20% of rumen microorganisms have been identified [110, 111]. Nonetheless, there is technology available to assist with this; fluoresence *in situ* hybridisation (FISH) is used to detect and identify microbes with which we are familiar and also those which require our attention to better understand their complex functions [111]. A study by O'Sullivan *et al.* [112] found that the use of a rumen inoculum resulted in a greater digestion rate (almost twice) than a digester leachate comparator. A clear advantage of this treatment is that in addition to having the potential to be a cost-effective pre-treatment for AD, it also utilizes another waste product which reduces the size of the load requiring disposal; waste rumen contents is a large contributor to slaughterhouse waste [113]. Several studies [113–115] have already been made into the potential of using rumen microorganisms in AD and found that using a ruminal liquid inoculum resulted in an increased rate and volume of methane production compared to a digester leachate inoculum. Quintero *et al.* [115] used a combination of ruminal liquid and pig waste sludge as an inoculum on fique's bagasse, a lignocellulosic material, and found that methane production was significantly higher than when using either inoculum on the bagasse alone. It would seem that using ruminal liquid in conjunction with animal waste, which improves the C:N ratio and balances the digester, further increases the potential methane yield. Further research into the efficiency and economic viability of this technique could enable real advances in AD over the coming years.

6.4.2 Value-added Products and AD

In recent years there has been a focus on the possibility of obtaining "value-added products" from the plant biomass substrate to extract items that would not be available if the biomass were to be pre-treated using more traditional methods. The process is known as fractionation, during which the plant biomass is pressed to separate the liquid fraction from the solid fibrousfraction [116]. Fractionation is not a new concept; between 1970 and 1980, fractionation was investigated as a means to provide protein-free liquid for ruminant feed, and a protein-concentrated leafy solid for human consumption [116]. However, the return to fractionation in recent years has been primarily to establish its viability with regard to the benefits and utilization of value-added products that can be obtained from the liquid fraction, with the fibre-rich press-cake going to biogas production. In order to achieve a combination of waste management, energy generation and obtain value-added products, the concept of "green biorefinery"

has been introduced for phase III biorefinery. Biorefinery typically involves the particle size reduction of biomass prior to pressing by means of chopping the plant matter to ease passage through the press and facilitate the release of the press-juice, which is nutrient-rich and contains proteins and amino acids [117]. The purpose of retrieving the liquid fraction is twofold; in some refineries the liquid fraction is used as a substrate in AD, and more recently, the liquid fraction is retrieved so that the valuable nutrients, of higher value than their value in biogas production, can be utilized in a range of other industries.

To date, there are three types of biorefineries, the first two of which are more restricted with regard to the substrates that they process for anaerobic digestion. Biorefineries of type I and II deal exclusively with grain feedstocks, and although type I produces a fixed end product, type II is more flexible in that it can produce multi-products including starch, glucose and corn oil from the corn or wheat feedstock [118]. However, it is type III biorefineries which are the most promising in terms of combining waste management, energy generation and production of value-added products, as these refineries involve much more elaborate processes. Green, whole-crop and lignocellulosic feedstock (LCF) biorefineries are all classed as type III biorefineries; green biorefineries handle various wet feedstocks from untreated products, such as grass, while whole-crop biorefineries utilize the whole crop e.g., miscanthus to acquire a multitude of products [118]. LCF biorefineries offer huge potential as they combine the features of both green and whole-crop biorefineries, to utilize lignocellulosic biomass and then separate the cellulose, hemicellulose and lignin fractions to then further degrade the cellulose and hemicellulose to create useful products including fuel and chemicals [118]. Owing to their adaptability, there is a greater spectrum of products that can be derived from cellulose and hemicellulose. Lignin is more restricted in its uses and is therefore primarily used as a fuel source via combustion; however there has been some research into the possibility of lignin being used for high-value-added products, such as a dispersant in cement gypsum blends [119].

Value-added products derived from lignocellulosic biomass offer a renewable solution to providing biochemicals for the chemical industry when the use of fossil fuels is becoming less viable. Plant acids, steroids, rubber, gums and waxes are some of the high-value biochemicals that are available in low volume in plants [42]. These biochemicals are then used for pharmaceuticals, cosmetics, food flavourings and other products [42, 120]. In terms of biogas yield, however, the extraction of value-added products is detrimental to the total methane yield that can be obtained [121]. At present, the primary obstacle faced by value-added product

extraction is the high costs as a result of the high temperatures required to separate chemicals before any chemistry is carried out to generate the desired products [120]. Although the extraction of value-added products in addition to biogas via biorefinery is not currently commercially viable, it is likely that with development, biorefinery including AD will become one of the key areas for dealing with a multitude of industrial pressures in the years to come.

6.5 Conclusion

Pre-treatment of biomass is of the utmost importance if the full methane potential of any substrate is to be achieved in AD, but the pre-treatment must be suitable for the substrate and conducted under the optimum conditions to prevent the loss of organic biomass and the formation of inhibitory products, to make the process as efficient and cost-effective as possible. Equally important in AD optimization is the substrate selected for biogas production; feedstock suitability with regard to BMP, C:N ratio and inhibitory product formation must be carefully considered when selecting a substrate. Thermal and thermo-chemical pre-treatments appear to be leading the way in current AD research on account of their applicability to a variety of substrates, and the ability to easily control the temperature at which treatment is conducted; this enables the pre-treatment to be tailored to complement the mesophilic or thermophilic AD process that follows, reducing the accumulation of inhibitory products and methanogenesis inhibition. With the improved economic viability of ILs and further research into reducing the formation of inhibitory products in microwave heating, pre-treatment will vastly improve the AD process for biogas generation. It is difficult to diagnose the optimum conditions for each and every available substrate, especially when lignocellulosic biomass is so abundant in quantity and variable in composition, which is why feedstock composition is essential in the substrate selection process. The use of NIRS in new research will enable a more accurate prediction of the methane potential of a feedstock to be made, by calculating its organic composition. Additionally, as biorefinery looks set to become a fundamental method of waste valorisation over the coming years, it will be essential for this process to be developed to the extent that it does not significantly compromise biogas yield as a result of the extraction of value-added products. If AD is to be a truly renewable and cost-effective energy source, it is imperative that pre-treatments and their effects are examined in more detail across a broad range of substrates, to tailor the process according to

the substrate features and AD conditions, such as those of the biorefineries of future energy generation.

There are many different crops grown for AD, including maize, grasses, beet and rye as a lignocellulosic feedstock [29, 35, 44, 51]. Accumulation of biogas from biomass sources that include manure and energy crops have been shown to be improved through pre-treatment that may include physical, chemical or biological options. In the EU most agricultural biogas plants operate based on co-digestion. In Germany alone energy crops occupy 60% of substrate load in agricultural biogas plants [44]. Approximately 80% of biogas systems in Germany use corn silage as a co-substrate for biogas production [122]. Some large-scale biogas plants produce 1.8 to 2.0 million m^3 of biogas per annum, with a feed stock handling capacity of 20,000 tonnes per annum [122]. Most agricultural biogas plants operate below their optimum performance levels, and it has been demonstrated that the potential for optimisation in existing biogas plants is up to 40% [33]. Benchmarking of commercial biogas plants to improve performance has shown several challenges to optimise biogas yield [123]. Pre-treatment technologies of lignocellulosic biomass are capable of solving some of these problems and improving its economics [8, 11, 18, 80]. Current research on pre-treatment is focused on lab scale or pilot scale, there is a need for it to be tested in commercial agricultural biogas plants to optimize the whole economy of biogas production.

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References

1. T. Prade, S. E. Svensson, and J. E. Mattsson, *Biomass Bioenergy*, Vol. 40, p. 36, 2012.
2. C. Zhang, J. Jihong Li, C. Liu, X. Liu, J. Wang, S. Li, G. Fan, and L. Zhang, *Bioresource Technology*, Vol. 149, p. 353, 2013.
3. C. Sambusiti, F. Monlau, E. Ficara, H. Carrère, and F. Malpei, *Appled Energy*, Vol. 104, p. 62, 2013.
4. F. Xu, J. Shi, W. Lv, Z. Yu, and Y. Li, *Waste Management*, Vol. 33, p. 26, 2013.

5. L. Levén, K. Nyberg, and A. Schnüre, *Journal of Environmental Management*, Vol. 95, p S99, 2012
6. R. Chandra, H. Takeuchi, and T. Hasegawa, *Renewable Sustainable Energy Reviews*, Vol. 16, p. 1462, 2012.
7. J. M. Triolo, L. Pedersen, H. Qu, and S. G. Sommer, *Bioresource Technology*, Vol. 125, p. 226, 2012.
8. M. Carlsson, A. Lagerkvist, and F. Morgan-Sagastume, *Waste Management*, Vol. 32, p. 1634, 2012.
9. P. Gupta, R. J. Singh, A. Sachan, A. S. Vidyarthi, and A. Gupta, *Renewable Sustainable Energy Reviews*, Vol. 16, p. 4908, 2012.
10. A. Mohr, and S. Raman, (2013). *Energy Policy*, Vol. 63, p. 114, 2013.
11. L. C. Ferreira, P. J. Nilsen, F. Fdz-Polanco, and S. I. Pérez-Elvira, *Chemical Engineering Journal*, Vol. 242, p. 254, 2014.
12. S. Menardo, G. Airoldi, and P. Balsari, *Bioresource Technology*, Vol. 104, p. 708, 2012.
13. J. Bacenetti, M. Negri, M. Fiala, and S. González-García, *Science of the Total Environment,* Vol. 463, p. 541, 2013.
14. G. Santi, S. Proietti, S. Moscatello, W. Stefanoni, and A. Battistelli, *Biomass and Bioenergy*, Vol. 83, p. 17, 2015.
15. X. Wu, W. Yao, J. Zhu, and C. Miller, *Bioresource Technology*, Vol. 101, p. 4042, 2010.
16. C. Herrmann, M. Heiermann, and C. Idler, *Bioresource Technology*, Vol. 102, p. 5153, 2011.
17. L. Neves, R. Ribeiro, R. Oliveira, and M. M. Alves, *Biomass & Bioenergy*, Vol. 30, p. 599, 2006.
18. G. D. Girolamo, L. Bertin, L. Capecchi, C. Ciavatta, and L. Barbanti, *Biomass & Bioenergy*, Vol. 71, p. 318, 2014.
19. V. Vivekanand, P. Ryden, S. J. Horn, H. S. Tapp, N. Wellner, V. G. H. Eijsink, and K. W. Waldron, *Bioresource Technology*, Vol. 123, p. 608, 2012.
20. S. Xie, J. P. Frost, P. G. Lawlor, G. Wud, and X. Zhan, *Bioresource Technology*, Vol. 102, p. 8748, 2011.
21. A. Prochnow, M. Heiermann, M. Plöchl, B. Linke, C. Idler, T. Amon, and P. J. Hobbs, *Bioresource Technology*, Vol. 100, p. 4931, 2009.
22. A. S. Nizami, A. Orozco, E. Groom, B. Dieterich, and J. D. Murphy, *Applied Energy*, Vol. 92, p. 783, 2012.
23. J. McEniry, and P. O'Kiely, *Bioresource Technology*, Vol. 127, p. 143, 2013.
24. R.Wahid, S. F. Nielsen, V. M. Hernandez, A. J. Ward, R. Gislum, U. Jørgensen, and H. B. Møller, *Biosystems Engineering*, Vol. 133, p. 71, 2015.
25. Kiesel, A. and Lewandowski, I. (2016), Miscanthus as biogas substrate – cutting tolerance and potential for anaerobic digestion. GCB Bioenergy. doi:10.1111/gcbb.12330
26. T. P. Kandel, R. Gislum, U. Jørgensen, and P. E. Lærke, *Bioresource Technology*, Vol. 146, p. 282, 2013.

27. M. Oleszek, A. Król, J. Tys, M. Matyka, and M Kulik, *Bioresource Technology*, Vol. 156, p. 303, 2014.
28. H. K. Ahn, M. C. Smith, S. L. Kondrad and J. W. White, *Applied Biochemistry Biotechnology*, Vol. 160, p. 965, 2010.
29. N. Martınez-Perez, S. J. Cherryman, G. C. Premier, R. M. Dinsdale, D. L. Hawkes, F. R. Hawkes, G. Kyazze, and A. J. Guwy, *Biomass and Bioenergy*, Vol. 31, p. 95, 2007.
30. D. Brown, J. Shi, and Y. Li, *Bioresource Technology*, Vol. 124, p. 379, 2012.
31. M. M. Estevez, R. Linjordet, and J. Morken, *Bioresource Technology*, Vol. 104, p. 749, 2012.
32. S. J. Horn, M. M. Estevez, H. K. Nielsen, R. Linjordet, and V. G.H. Eijsink, *Bioresource Technology*, Vol. 102, p. 7932, 2011.
33. http://www.rtd-services.com/euagrobiogas
34. T. Amon, B. Amon, V. Kryvoruchko, W. Zollitsch, K. Mayer, L. Gruber, *Agriculture, Ecosystems and Environment*, Vol. 118, p. 173, 2007.
35. D. M. Wall, P. O'Kiely, and J. D. Murphy, *Bioresource Technology*, Vol. 149, p. 425, 2013.
36. Y. Li, S. Y. Park, and J. Zhu, *Renewable Sustainable Energy Reviews*, Vol. 15, p. 821, 2011.
37. A. K. Mathew, K. Chaney, M. Crook, and A. C. Humphries, *Renewable Energy*, Vol. 36, p. 2424, 2011.
38. A. Giuliano, D. Bolzonella, P. Pavan, C. Cavinato, and F. Cecchi, *Bioresource Technology*, Vol. 128, p. 612, 2013.
39. D. J. Hayes, *Catalysis Today*, Vol. 145, p. 138, 2009.
40. V. S. H. Suhardi, B. Prasai, D. Samaha, and R. Boopathy, *International Biodeterioration and Biodegradation*, Vol. 85, p. 683, 2013.
41. L. Yang, J. Cao, J. Mao, and Y. Jin, *Industrial Crop Production*, Vol. 43, p. 711, 2013.
42. S. N. Naik, V. V. Goud, P. K. Rout, and A. K. Dalai, *Renewable Sustainable Energy Reviews*, Vol. 14, p. 578, 2010.
43. DEFRA. Food statistics pocketbook 2012.
44. P. Weiland, *Applied Microbiology and Biotechnology*, Vol. 85, p. 849, 2009.
45. C. Herrmann, A. Prochnow, and M. Heiermann, *Biosystems Engineering*, Vol. 110, p. 310, 2011.
46. Y. Zheng, C. Yu, Y-S. Cheng, C. Lee, C. W. Simmons, T. M. Dooley, R. Zhang, B. M. Jenkins, and J. S. VanderGheynst, *Applied Energy*, Vol. 93, p. 168, 2012.
47. C. Herrmann, M. Heiermann, and C. Idler, *Bioresource Technology*, Vol. 102, p. 5153, 2011.
48. L. N. Liew, J. Shi, and Y. Li, *Biomass Bioenergy*, Vol. 46, p. 125, 2012.
49. J. S. Lim, Z. A. Manan, S. R. W. Alwi, and H. Hashim, *Renewable Sustainable Energy Reviews*, Vol. 16, p. 3084, 2012.
50. K. Risberg, L. Sun, L. Levén, S. Jarle, S. J. Horn, and A. Schnürer, *Bioresource Technology*, Vol. 149, p. 232, 2013.

51. T. Amon, B. Amon, V. Kryvoruchko, A. Machmüller, K. Hopfner-Sixt, V. Bodiroza, R. Hrbek, J. Friedel, E. Pötsch, H. Wagentristl, M. Schreiner, and W. Zollitsch, *Bioresource Technology*, Vol. 98, p. 3204, 2007.
52. I. A. Nges, F. Escobar, X. Fu, and L. Björnsson, *Waste Management*, Vol. 32, p. 53, 2012.
53. B. Godin, S. StéphaneLamaudière, R. Agneessens, T. Schmit, J-P. Goffart, D. Stilmant, P. A. Gerin, and J. Delcarte, *Industrial Crop Production*, Vol. 48, p. 1, 2013.
54. T. P. Kandel, S. Sutaryo, H. B. Møller, U. Jørgensen, and P. E. Lærke, *Bioresource Technology*, Vol. 130, p. 659, 2013.
55. C. S. Raju, A. J. Ward, L. Nielsen, and H. B. Møller, *Bioresource Technology*, Vol, 102, p. 7835, 2011.
56. P. J. Van Soest, *Association Of Agricultural Chemistry*, Vol. 56, p. 825, 1963.
57. H. F. Jacobi, S. Ohl, E. Thiessen, and E. Hartung, *Bioresource Technology*, Vol. 103, p. 162, 2012.
58. M. Macias-Corral, Z. Samani, A. Hanson, G. Smith, P. Funk, H. Yua, and J. Longworth, *Bioresource Technology*, Vol. 99, p. 8288, 2008.
59. S. Tedesco, K. Y. Benyounis, and A. G. Olabi, *Energy*, Vol. 61, p. 27, 2013.
60. D. Brown, and Y. Li, *Bioresource Technology*, Vol. 127, p. 275, 2013.
61. Z. Yue, R. Chen, F. Yang, J. MacLellan, T. Marsh, Y. Liua, and W. Liao, *Bioresource Technology*, Vol. 128, p. 65, 2013.
62. H. Pobeheim, B. Munk, J. Johansson, and G. M. Guebitz, *Bioresource Technology*, Vol. 101, p. 836, 2010.
63. M. Seppälä, V. Pyykkönen, A. Väisänen, and J. Rintala, *Fuel*, Vol. 107, p. 209, 2013.
64. J-F. Peng, Y-H. Song, Y-L. Wang, P. Yuan, and R. Liu, *International Biodeterioration and Biodegradation*, Vol. 80, p. 60, 2013.
65. I. Siegert, and C. Banks, *Process Biochemistry*, Vol. 40, p. 3412, 2005.
66. O. Yenigün, and B. Demirel, *Process Biochemistry*, Vol. 48, p. 901, 2013.
67. X. Wu, W. Yao, J. Zhu, and C. Miller, *Bioresource Technology*, Vol. 101, p. 4042, 2010.
68. A. H. Igoni, M. J. Ayotamuno, C. L. Eze, S. O. T. Ogaji, and S. D. Probert, *Applied Energy*, Vol. 85, p. 430, 2008.
69. S. Zhang, D. R. Keshwani, Y. Xu, and M. A. Hanna, *Industrial Crop Production*, Vol. 37, p. 352, 2012.
70. Z. Zhang, A. A. Donaldson, and X. Ma, *Biotechnology Advances*, Vol. 30, p. 913, 2012.
71. V. Menon, and M. Rao, *Progress in Energy Combustion*, Vol. 38, p. 522, 2012.
72. Z. Wang, M. Qin, Y. Fu, M. Yuan, Y. Chen, and M. Tian, *Industrial Crop Production*, Vol. 50, p. 510, 2013.
73. D. B. Rivers, and G. H. Emert, *Biotechnology Letters*, Vol, 9, p. 365, 1987.
74. H. J. Kim, S. Lee, J. Kim, R. J. Mitchell, and J. H. Lee, *Bioresource Technology*, Vol. 144, p. 50, 2013.

75. E. Khullar, B. S. Dien, K. D. Rausch, M. E. Tumbleson, and V. Singh, *Industrial Crops and Production*, Vol. 44, p. 11, 2013.
76. X. Zhao, K. Cheng, and D. Liu, *Applied Microbiology and Biotechnology*, Vol. 82, p. 815, 2009.
77. M. J. Taherzadeh, and K. Karimi, *BioResources*, Vol. 2, p. 707, 2007.
78. G. J. M. Rocha, A. R. Gonçalves, B. R. Oliveira, E. G. Olivares, and C. E. V. Rossell, *Industrial Crop Production*, Vol. 35, p. 274, 2012.
79. P. Kumar, D. M. Barrett, M. J. Delwiche, and P. Stroeve, *Industrial Engineering Chemical Research*, Vol. 48, p. 3713, 2009.
80. E. Bruni, A. P. Jensen, and I. Angelidaki, *Bioresource Technology*, Vol. 101, p. 7668, 2010.
81. J.Wanga, Z. Yue, T. Chen, S. Peng, H Yu, H. Chen, *Bioresource Technology,* Vol. 101, p. 6610, 2010.
82. Z. Yu, B. Zhang, F. Yu, G. Xu, and A. Song, *Bioresource Technology*, Vol. 121, p. 335, 2012.
83. S. H. Mood, A. H. Golfeshan, M. Tabatabaei, G. S. Jouzani, G. H. Najafi, M. Gholami, and M. Ardjmand, *Renewable Sustainable Energy Reviews*, Vol. 27, p. 77, 2013.
84. E. Arvaniti, A. B. Bjerre, and J. E. Schmidt, *Biomass Bioenergy*, Vol. 39, p. 94, 2012.
85. C. Martín, H. B. Klinke, and A. B.Thomsen, *Enzyme Microbial Technology*, Vol. 40, p. 426, 2007.
86. Z. Sapci, *Bioresource Technology*, Vol. 128, p. 487, 2013.
87. D. Jackowiak, D. Bassard, A. Paus, and T. Ribeiro, *Bioresource Technology*, Vol. 102, p. 6750, 2011.
88. J. Ma, T. H. Duong, M. Smits, V. Verstraete, and M. Carballa, *Bioresource Technology*, Vol. 102, p. 592, 2011.
89. L. Mendez, A. Mahdya, M. Demuez, M. Ballesteros, and C. González-Fernández, *Fuel*, Vol. 117, p. 674, 2014.
90. G. G. D. Silva, M. Couturier, J-G. Berrin, A. Buléon, X. and Rouau, *Bioresource Technology*, Vol. 103, p. 192, 2012.
91. A. Banerji, M. Balakrishnan, and V. V. N. Kishore, *Applied Energy*, Vol. 104, p. 197, 2013.
92. S. Baroutian, M. Robinson, A-M. Smit, S. Wijeyekoon, and D. Gapes, *Bioresource Technology*, Vol. 146, p. 294, 2013.
93. I. Cybulska, G. Brudecki, K. Rosentrator, H. Lei, and J. Julson, *Biomass Bioenergy*, Vol. 46, p. 389, 2012.
94. M. Hjorth, K. Gränitz, A. P. S. Adamsen, H. B. Møller, *Bioresource Technology*, Vol. 102, p. 4989, 2011.
95. J-C. Frigon, P. Mehta, and S. R. Guiot, *Biomass Bioenergy*, Vol. 36, p. 1, 2012.
96. S. Tedesco, T. M. Barroso, and A. G. Olabi, *Renewable Energy*, Vol. 62, p. 527, 2014.

97. F. Monlau, E. Latrille, A. C. Da Costa, J-P. Steyer, and H. Carrère, *Applied Energy*, Vol. 102, p. 1105, 2013.
98. A. Barakat, F. Monlau, J-S. Steyer, and H. Carrere, *Bioresource Technology*, Vol. 104, p. 90, 2012.
99. S. Schwede, Z-U. Rehman, M. Gerber, C. Theiss, and R. Span, *Bioresource Technology*, Vol. 143, p. 505, 2013.
100. J. Zhang, Y. Wang, L. Zhang, R. Zhang, G. Liu, and G. Cheng, *Bioresource Technology*, Vol. 151, p. 402, 2014.
101. C. González-Fernández, B. Sialve, N. Bernet, and J. P. Steyer, *Biomass Bioenergy*, Vol. 40, p. 105, 2012.
102. J. Shi, Z. Wang, J. A. Stiverson, Z. Yu, and Y. Li, *Bioresource Technology*, Vol. 136, p. 574, 2013.
103. B. K. Ahring, *Advances in Biochemical Engineering and Biotechnology*, Vol. 81, p. 1, 2003.
104. K. Shill, S. Padmanabhan, Q. Xin, J. M. Prausnitz, D. S. Clark, and H. W. Blanch, *Biotechnology Bioengineering*, Vol. 108, p. 511, 2011.
105. N. I. Haykir, E. Bahcegul, N. Bicak, and U. Bakir, *Industrial Crop Production*, Vol. 41, p. 430, 2013.
106. D. A. Fort, R. C. Remsing, R. P. Swatloski, P. Moyna, G. Moyna, and R. D. Rogers, *Green Chemistry*, Vol. 9, p. 63, 2007.
107. V. B. Agbor, N. Cicek, R. Sparling, A. Berlin, and D. B. Levin, *Biotechnology Advances*, Vol. 26, p. 675, 2011.
108. J. Gao, L. Chen, Z. Yan, and L. Wang, *Bioresource Technology*, Vol. 132, p. 361, 2013.
109. J. Gao, L. Chen, K. Yuan, H. Huang, and Z. Yan, *Bioresource Technology*, Vol. 150, p. 352, 2013.
110. J. T. Sylvester, S. K. R. Karnati, Z. Yu, M. Morrison, and J. L. Firkins, *Journal of Nutrition*, Vol. 134, p. 3378, 2004.
111. Z-B. Yue, W-W.,Li, and H-Q. Yu, *Bioresource Technology*, Vol. 128, p. 738, 2013.
112. C. A. O'Sullivan, P. C. Burrell, W. P. Clarke, and L. L. Blackall, *Bioresource Technology*, Vol. 97, p. 2356, 2006.
113. Y. Baba, C. Tada, Y. Fukuda, and Y. Nakai, *Bioresource Technology*, Vol, 128, p. 94, 2013.
114. C. A. O'Sullivan, and P. C. Burrell, *Waste Management*, Vol. 27, p. 1808, 2007.
115. M. Quintero, L. Castro, C. Ortiz, C. Guzmán, and H. Escalante, *Bioresource Technology*, Vol. 108, p. 8, 2012.
116. P. A. Fowler, A. R. McLauchlin, and L. M. Hall, Biocomposites Centre, University of Wales, Bangor, U.K.: p. 16, 2003.
117. C. King, J. McEniry, P. O'Kiely, and M. Richardson, *Biomass Bioenergy*, Vol. 42, p. 179, 2012.
118. L. Luo, E. van der Voet, and G. Huppes, *Bioresource Technology*, Vol. 101, p. 5023, 2010.

119. Y. Matsushita, M. Imai, A. Iwatsuki, and K. Fukushima, *Bioresource Technology*, Vol. 99, p. 3024, 2008.
120. J. H. Clark, *Journal of Chemical Technology and Biotechnology*, Vol. 82, p. 603, 2007.
121. G. Luo, F. Talebnia, D. Karakashev, L. Xie, Q. Zhou, and I. Angelidaki, *Bioresource Technology*, Vol. 102, p. 1433, 2011.
122. M. Pöschl, S. Ward, and P. Owende. *Applied Energy* Vol. 87, p. 3305, 2010.
123. P. J. Hobbs, S. R. Ravella. A. J. Ward, A. Schattauer, A. Retter, J. Williams, M. Eder, T. Amon, *Proceedings of the 14th RAMIRAN International Conference,* Lisbon, 2010.

7

Algae: The Future of Bioenergy

Nivas Manohar Desai

Department of Botany, Shri Pancham Khemraj Mahavidyalaya, Sawantwadi, MS (INDIA)

Abstract

Algae have an ability to grow in non-arable land without the use of potable water sources as well as having the capacity of production of high-yield feedstock. Nowadays algae is being explored in huge measure because of its significant lipid and carbohydrate production as well as production of other potential bimolecular substrates. Algae can produce 40 times more oil for biodiesel production compared to other plant resources such as oilseed crops per unit land area. The technical and economic aspects of high-yield production of bioenergy from algae are currently in focus. It's a clean, economical and sustainable source of energy. But more research in this area is needed in order to minimize technical hurdles as well as create cost-efficient feedstock production. Algal lipids comprising saturated and polar lipids are most suitable for exploitation as fuel feedstock. The present chapter reflects current knowledge on technological innovation towards cultivation, harvesting and drying of algal biomass, as a sustainable source for the production of biomethane, biodiesel, bioethanol and biohydrogen as well as the approaches towards the biorefinery.

Keywords: Alage, cultivation, processing, sustainable bioenergy

7.1 Introduction

Energy is the most essential requirement for human survival. Energy crisis and global warming are the two major problems we are facing today. Increasing energy costs and a decrease in reserves of fossil fuels shifted the mind-set in the conversion of biomass to biofuels production. The complete

Corresponding author: nivasdesai88@gmail.com

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dependence on fossil fuels for the fulfillment of fuel demand is unsustainable. To overcome such problems, algae are the chief source for renewable energy production. Because of the huge diversity in algae, huge options are available for the production of algae-based energy. Using algae as a source of bioenergy is not a new idea, but it is now being taken seriously due to the increasing cost of petroleum and, more significantly, the emerging concern about global warming caused by burning fossil fuels. Algal bioenergy would lower impacts on environmental degradation in comparison to biofuel feedstock and will help in the improvement of developing and developed communities. Algal biomass is ideal for bioenergy production concepts which are significantly more sustainable than other alternatives due to its characteristics. Algae have high biomass productivity. The fusion of algae and its cultivation sources such as land, saline water, waste streams for nutrient supply and combustion gas in the form of CO_2 source will definitely help to generate a wide range of fuel and non-fuel products. Algae helps in CO_2 capturing. Algae are a rich source of lipids and carbohydrates. Optimizing the strains and culture conditions, the obtained biomass can be easily converted into a whole range of bioenergy products including biodiesel or bioethanol. After the first- and second-generation biofuels, the focus now being concentrated on third-generation biofuels from algal biomass is due to the significant amount of biomolecules such as lipids and carbohydrates, from which biodiesel and bioethanol may be obtained. The thermochemical and biochemical conversion of algal biomass helps in the production of biofuel oil and gases or even production of bioethanol, biodiesel, biobutanol and biohydrogens. Cultivation and processing of algal biomass has significant benefits like year-round production, higher productivity (40–50%), their offshore production, and the fact that there is no need of arable land and recycling of nutrients.

The first-generation biofuels (FGBs) are obtained from cereal crops and sugarcanes and production is primarily limited by environmental and social concerns such as competition for land and water used for food and fiber production causing an increase in world commodity prices for food and animal feeds [1]. Owing to these important limitations the next-generation, or second- and third-generation biofuels are being developed from non-edible lignocellulosic biomasses such as woody biomass and wood wastes, crop residues, dedicated energy crops such as switch grass, municipal wastes, and algae through advanced technologies. Researchers today are engaged in the utilization of algal biomass for various bioenergy products. Algae are able to fix approximately 1.8 kg of CO_2 for every 1 kg of algae biomass produced [2]. Approximately 40 ha of algae ponds are required to fix the carbon emitted from one MW of power generated from a coal plant [3]. These recent researches not only indicated the great

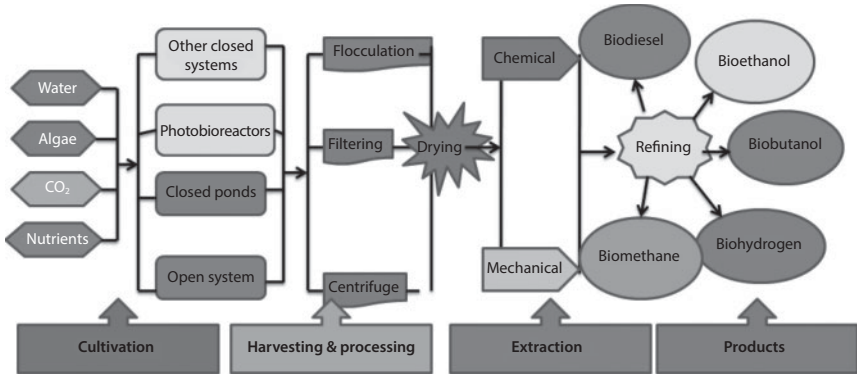

Figure 7.1 Schematic representation of algal biomass, their processing and end products.

potential of mass production of algal biomass on waste streams for simultaneous wastewater bioremediation and biofuel and other applications. Algae cultivated in such water, which is rich in nitrogen and phosphorus; these nutrients utilized by algae provide the co-benefit of producing biofuels and removing nitrogen and phosphorus [4]. Through the different cultivation, harvesting and processing methods algal biomass can yield different energy options (Figure 7.1.)

7.2 Technological Innovations for Algae Cultivation, Harvesting and Drying

Development of efficient technology for algae-based fuel is still to be furnished. Prior to the bioenergy production from algae, it is necessary to understand the basics of algae cultivation systems. A production system is geared towards a high yield per hectare because it reduces the relative costs for land and some operation costs. Realistic estimates for productivity are in the order of magnitude of 40–80 tons of dry matter per year per hectare, depending on the technology used and the location of production [5]. This is still substantially higher than almost all agricultural crops. Surpassing yields of 80 tons per year per hectare will likely require genetically improved strains or other technologies able to counteract photosaturation and photo-inibition [6]. The high capital cost associated with producing microalgae in closed culture systems is the main challenge for commercialization of such systems [7]. For the cultivation of macroalgae (seaweed) and microalgae different culture systems are used. Microalgae are smaller in size (μm), thus they have to be cultivated in a system designed for specific purpose, while seaweed can be grown directly in the open sea.

Seaweed is mainly utilized for food product, or used in many processed foods as stabilizers or emulsifiers. Besides culturing seaweed, part of the current seaweed production comes from harvesting natural populations or collecting beach-cast seaweed. Besides the disturbance of the ecosystem by these practices, they are clearly unsustainable for application on a very large scale. Therefore cultivation of microalgae in a cultivation system is worth considering. The cultivation practices for microalgae is discussed below.

7.2.1 Cultivation Practices

7.2.1.1 Open Cultivation Systems

The raceway pond is the main large-scale microalgae cultivation technique. It includes simple closed-loop channels, and by the use of paddle wheels, the water is kept moving through channels (Figure 7.2). These channels are usually made of concrete or compacted earth, often lined with white plastic, and are 20–30 cm deep. They are specially designed for optimal light capture and low construction costs. The land should be

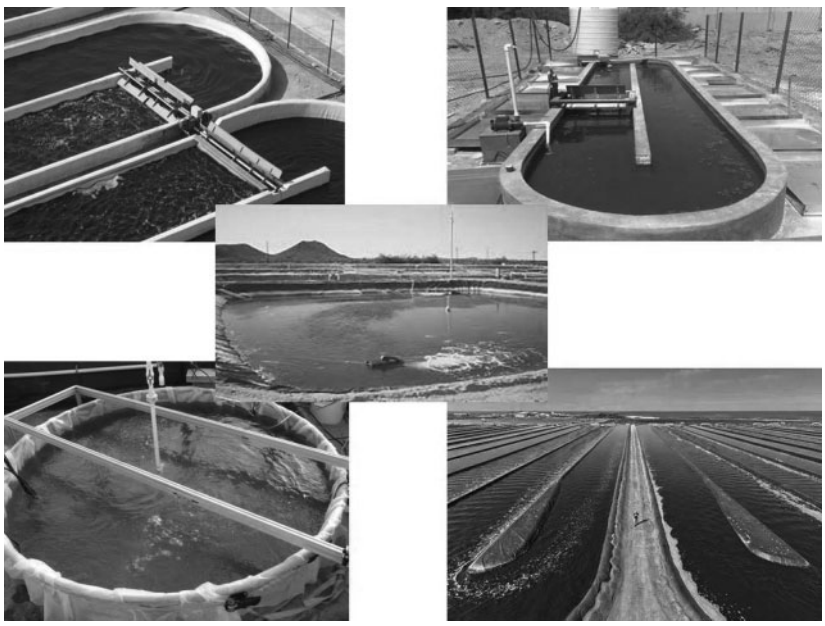

Figure 7.2 Different types of open cultivation system.

of flat land. But these systems have certain demerits regarding process controlling, such as

- Unstable ecosystem.
- Complete dependence on weather for temperature and other climatic conditions. The water is either lost by evaporation or added by rainfall.
- Challenges of naturally growing algae or algae predators that compete with the algae species intended to be cultivated.
- Challenges regarding maintenance of monoculture under an extreme environment like high salinity (e.g., *Dunaliella)*, high pH (e.g., *Spirulina*) or high nitrogen (e.g., *Chlorella*) in water.
- The slow diffusion of nutrients and flotation and sedimentation of dead and living algae, limiting the usage of available sunlight.

These conditions generally limit optimal growth and operate at a low algae concentration, making production of high biomass and harvesting more difficult. In conclusion, there is an important trade-off between a low price for the cultivation system and its production potential.

7.2.1.2 Closed Cultivation Systems (Photobioreactors)

Taking into consideration the demerits of an open cultivation system, the problems can be mitigated by building a closed system which is less affected by the environment. The effects of temperature, gas exchange and competition problems can be eased by using closed systems. The closing is done by covering it with transparent material or a greenhouse, but this is an expensive practice for large surfaces. Use of large polythin bags for batch culture is one of the simple, but inexpensive examples. Several more advanced innovations have been made based on more durable transparent materials: glass, polyethylene and polycarbonate (Figure 7.2). These reactors offer continuous operation, a high level of controllability and elevated biomass concentrations, which results in a requirement of minimum space and lower harvesting costs per ton of algae. One example is the use of a bubble column, a vertical tubular reactor.

The long horizontal tube has its own scaling problem: algae will consume nutrients and CO_2 while producing O_2; which could hamper algal growth at higher concentrations, so growth conditions deteriorate further along the tube. A further innovation has been made by installing individual modules with optimized size vs. tube length ratios. The flat

Figure 7.3 Different models of closed cultivation systems (photobioreactors).

photobioreactors have been used recently to make optimal use of surface area and solar irradiation (Figure 7.3). This system helps in the higher biomass yield, but it still requires certain improvement. Difficulties in the closed system are the complicated flow regime inside the reactor and scalability, although the latter has been greatly improved by a design called the green wall panel [8]. The closed systems are more expensive and suffer from higher energy expenditures for mixing and cooling than open ponds and are also technically more difficult to build and maintain. The comparison of open cultivation systems versus closed land-based systems are summarized in Table 7.1.

7.2.1.3 Algal Turf Scrubber (ATS)

In addition to the open ponds or photo bioreactors, an attached algal culture system such as an algal turf scrubber (ATS) can be used, in which benthic algae grow on the surface of solid support for removing nutrients from animal wastewater [10] (Figure 7.4). It is an eco-technology specifically designed to decant the nutrients from water reservoir and to produce biomass feedstock for various purposes including bioenergy, fertilizers and health products (Figure 7.3). This is a low-cost and eco-friendly technology. ATS was developed focusing on natural algal communities growing on the crests of coral reefs. Coral reef algal turfs have among the highest

Table 7.1 Comparison of open cultivation systems versus closed land-based systems.

Parameter or issue	Open ponds and raceways	Photobioreactors (PBR)
Required space	High	For PBR itself low
Water loss	Very high, may also cause salt precipitation	Low
CO_2-loss	High, depending on pond depth	Low
Oxygen concentration	Usually low enough because of continuous spontaneous outgassing	Build-up in closed system requires gas exchange devices (O_2 must be removed to prevent inhibition of photosynthesis and photo oxidative damage)
Temperature	Highly variable, some control possible by pond depth	Cooling often required (by spraying water on PBR or immersing tubes in cooling baths)
Shear	Usually low (gentle mixing)	Usually high (fast and turbulent flows required for good mixing, pumping through gas exchange devices)
Cleaning	No issue	Required (wall-growth and dirt reduce light intensity), but causes abrasion, limiting PBR lifetime
Contamination risk	High (limiting the number of species that can be grown)	Low (Medium to Low)
Biomass quality	Variable	Reproducible
Biomass concentration	Low, between 0.1 and 0.5 g/l	High, generally between 0.5 and 8 g/l
Production flexibility	Only few species possible, difficult to switch	High, switching possible
Process control and reproducibility	Limited (flow speed, mixing, temperature only by pond depth)	Possible within certain tolerances
Weather dependence	High (light intensity, temperature, rainfall)	Medium (light intensity, cooling required)
Start-up	6–8 weeks	2–4 weeks
Capital costs	High ~ US$100,000 per hectare	Very high ~ US$250,000 to $1,000,000 per hectare (PBR plus supporting systems)

(*Continued*)

Table 7.1 Cont.

Parameter or issue	Open ponds and raceways	Photobioreactors (PBR)
Operating costs	Low (paddle wheel, CO_2 addition)	Higher (CO_2 addition, oxygen removal, cooling, cleaning, maintenance)
Harvesting cost	High, species dependent	Lower due to high biomass concentration and better control over species and conditions
Current commercial applications	5,000 (8 to 10,000) t of algal biomass per year	Limited to processes for high added value compounds or algae used in food and cosmetics

Source: (Pulz, 2001 [9]).

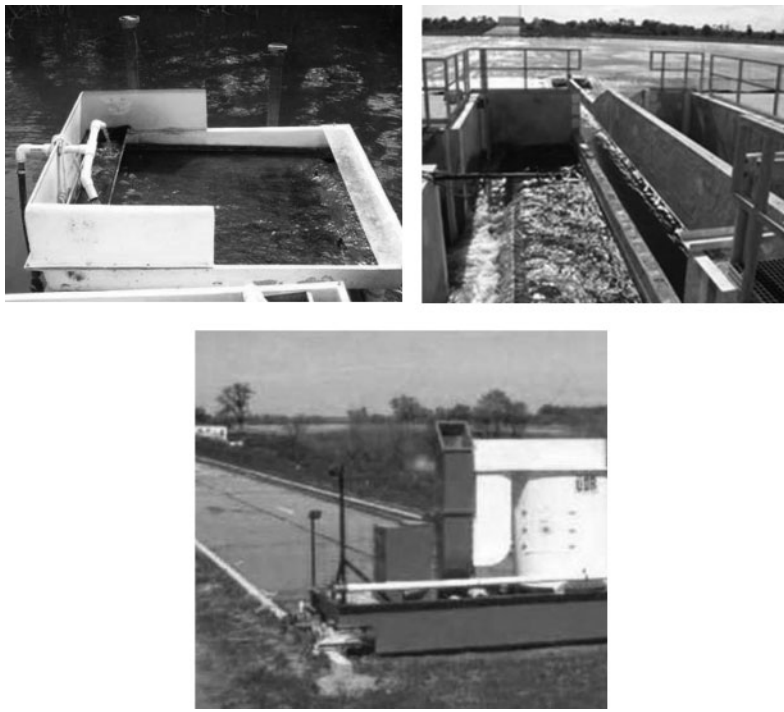

Figure 7.4 Algal turf scrubber (ATS).

productivities of the biosphere. The ATS simulates the conditions of the reef crest with surges of water from pumping that flow across shallow beds of attached algae. The ATS system consists of an attached algal community, which takes the form of a "turf," growing on screens in a shallow trough or basin (referred to as a *raceway*) through which water is pumped. The

algal community provides water treatment by the uptake of inorganic compounds and release of dissolved oxygen through photosynthesis. Water is pumped from a body of water onto the raceway, and algae remove the nutrients through biological uptake and produce oxygen as the water flows down the raceway. At the end of the raceway, water is released back into the water body, with a lower nutrient concentration and a higher dissolved oxygen concentration than when it was pumped onto the raceway. The nutrients that have been removed, or "scrubbed," from the water body are stored in the biomass of the algae growing on the screen. The algae are harvested approximately once per week during the growing season, thus removing nutrients from the waterway in the algal biomass. Harvesting is important because it rejuvenates the community and leads to higher growth rates; harvesting also prevents or reduces the potential effects of invertebrate micrograzers. In fact, biomass production rates of ATS are among the highest of any recorded values for natural or managed ecosystems [11]. Because of the fast growth rate of algae on ATS, this technology can remove nutrients and produce oxygen at a high rate. Design features of ATS include the flow rate of water, the slope of the raceway, the loading rate of nutrients in the water, and the type of screen used to grow algae.

7.2.1.4 Sea-based Cultivation Systems

The technical approaches have been developed for the cultivation of macroalgae (seaweeds), which is done by using shallow water and coastal areas that are safe, easily accessible and allow for easy control of the culture system to the seabed (Figure 7.5). This practice required a large amount of labor, hence it is expensive, so it is restricted to lowest income. To make an impact as renewable energy production, harvesting of only natural populations is not the way, thus seaweed cultivation is done with growing of seaweed using underwater ropes or similar kind of supports. Seaweed should be produced in floating cultivation systems on both sides of hundreds of hectares. Some species necessitate a substrate to hook to, which requires a network of ropes. The best cultivation conditions are the use of vertical ropes, which allow the cultivated seaweed to catch all available light until the maximum light penetration depth; these also help in minimizing the quantity and cost of rope material required per unit of area, or hybrid systems combining horizontal and vertical lines. In all cases the systems can be floating, anchored to the sea, or both. Problems of damage to rope structures and washed off biomass have been reported [12], so a cultivation system that prevents these problems needs to be designed. During experiments at sea [13], using rings (diameter of 5m, surface of 19.6 m^2 and 80–100 m substrate rope) with ropes as a base for seaweed to attach

Figure 7.5 Seaweed cultivation practices.

to, gave the best results, especially under high flow or heavy weather conditions. These rings can be attached to each other and/or the seabed and can include a slow-release fertilizer. The only problem of this system is the individual harvesting of rings, making cost-price reduction through economy of scale more difficult. Sea-based systems are less well developed than land-based systems, although some R&D initiatives have been undertaken and are still ongoing. When selecting a site for seaweed cultivation, several considerations have to be made, such as temperature, nutrient and light consideration, and distance from shore. The water content of seaweed should be reduced at the harvesting site using pressure filtration, which helps to remove around 20% of the water. Alternative options need to be investigated. Also technological innovations required for the minimizing energy spent on dewatering as well as transportation.

7.2.2 Harvesting of Biomass

When cultivating seaweed, every system requires a specific method of harvesting the biomass, but most commonly a specially developed harvesting vessel is used, which cuts the seaweed and hauls it inside [14]. The traditional methods used to harvest microalgae include concentration through

centrifugation [15], foam fractionation [16], flocculation [17], membrane filtration [18] and ultrasonic separation [19].

7.2.2.1 Settling Ponds

The simplest way to harvest the biomass is the use of settling ponds. Once a day the settling ponds are filled with a fully grown algae culture and drained at the end of that day, leaving a concentrated biomass volume at the bottom, which is stored for further processing [20]. But the settling pond requires significant additional space.

7.2.2.2 Filtration

Filtration is another way to separate the algae from the water they grown in. There are several other options available, which include different materials, vacuum, pressured and rotating filtering. Some acceptable results have been obtained for colonial microalgae, but not for unicellular species [21]. As the filtration method is a slow process [22], its use on a large scale is quite difficult. For the small cells of *Dunaliella*, filtration through sand filters, cellulose fibers and other filter materials has not proved practical [23]. One exception was filtration through diatomaceous earth. *Dunaliella* grown in salt ponds in Australia could be recovered by passing diluted culture broth through diatomaceous earth. The filtered alga was then directly extracted with organic solvent to recover b-carotene [24]. Large-scale recovery of microalgae using this technique is not possible because of continuous fouling and the subsequent need to replace membranes. Although this method appears as an attractive dewatering method, the significant operating cost requirements cannot be overlooked.

7.2.2.3 Centrifugation

Most microalgae can be harvested from suspension by centrifugation. Centrifugal recovery methods are commonly treated in textbooks [25], but practical guidelines are rarely given. Centrifugation is similar to sedimentation, wherein gravitational force is replaced by centrifugal acceleration to enhance the concentration of solids. Particle size and density difference are the key factors in centrifugal separation. Once separated, the algae concentrate can be obtained by simply draining the supernatant. Many researchers have advocated this method for reliable recovery of microalgae [21]. Different types of centrifuges have been used, and their respective reliability and efficiency have been documented by several researchers. Heasman *et al*. [26] reported that 90% to 100% harvesting efficiency can be achieved

via centrifugation. Sim *et al.* [27] compared centrifugation, chemical flocculation followed by dissolved air flotation (DAF), and membrane filtration processes for harvesting algae from pilot-scale ponds treating piggery wastewater, and they found that none of these processes were completely satisfactory. Centrifugation was reported to be very effective but too costly and energy intensive to be applied on a commercial scale. This kind of harvesting is usually recommended in the production of high-value metabolites or as a second-stage dewatering technique for concentrating algal slurries from 1% to 5% solids to >15% solids, as it does have some limitations. Undoubtedly, it is an efficient and reliable technique for microalgal recovery but one should also keep in mind its high operational cost. The recovery of the biomass in a sedimenting centrifuge depends on the settling characteristics of the cells, the residence time of the cell slurry in the centrifuge, and the settling depth. Settling depth can be kept small through the design of the centrifuge. The residence time of the slurry in the centrifuge can be controlled by controlling the flow rate. Heasman *et al.* [26] evaluated the extent of cell recovery and the effects of cell viability at different conditions of centrifugation for nine different strains of microalgae.

7.2.2.4 Flotation

Flotation is a kind of separation technique based on the attachment of air bubbles to solid particles. The resulting flocs float to the liquid surface and are then harvested by skimming and filtration. The success of flotation depends on the nature of microalgal cells in the harvesting process. Air bubbles drift up the smaller particles (<500 μm) more easily [28]. Also, the lower instability of suspended particles results in relatively higher air–particle contact. The attachment of air bubbles also depends on the air, solid, and aqueous phase contact angle. The larger the contact angle, the greater the tendency of air to adhere [29]. Dissolved air flotation (DAF), electrolytic flotation, and dispersed air flotation are some commonly used flotation techniques according to the method of bubble production. DAF is the most extensively used method for the treatment of industrial effluent. The DAF procedure by chemical flocculation is reported to recover up to 6% (w/v) algal biomass slurries from algae culture [30]. Although flotation has been used by several researchers as a potential harvesting method, there is only limited evidence of its technical and economic viability.

7.2.2.5 Flocculation

Flocculation is more convenient method of harvesting of algal cells than centrifugation or gravity filtration. Large number of chemicals has been

tested as flocculants, but the most effective one is use of aluminum sulfate followed by certain cationic polyelectrolyte [31]. The flocculating reactions of an algal biomass are particularly sensitive to the pH, properties of the cellular surface, concentrations of the flocculants and divalent cations, ionic strength of the culture solution and other factors [32]. These flocculants are of two types, namely (Group A) inorganic agents, which includes polyvalent metal ions such as Al^{+3} and Fe that form polyhydroxy complexes at suitable pH; and (Group B) polymeric flocculants, that includes ionic, nonionic, natural, and synthetic polymers. Among the group A, aluminum sulfate, ferric chloride, and ferrous sulfate are commonly used multivalent flocculants whose efficiency is directly proportional to the ionic charge. The mechanism of polymer flocculation involves ionic interaction between polyelectrolyte and algal cells, resulting in the bridging of algae and formation of flocs and charge on the polymer. Algal properties such as the pH of broth, concentration of biomass, and its charge are equally important to consider when selecting a polymer. Organic flocculants are reported to be beneficial in terms of their lower sensitivity to media pH, low dosage requirements, and wider range of applications.

7.2.2.6 Electrolytic Coagulation

The electrolytic coagulation (EC) process is a recently adapted method by wastewater treatment plants for final polishing and removal of algae from partly treated wastewater. Active polyvalent metal anodes (usually iron Or aluminum) are used to generate ionic flocculants such as Al^{+3} and Fe ions. The latter agglomerate algae to form flocs due to the net negative charge and colloidal behavior of algal cells [33]. The entire coagulation process involves the formation of coagulants through dissolving the reactive anodes which results in the formation of algal flocs.

7.2.3 Energy Efficiencies of Harvesting Processes

In terms of energy inputs, harvesting of algal biomass is the most energy-consuming process in biomass production. A specific commercial-scale algal harvesting technique yet not been developed, and the approach has been to adapt separation technologies already in use in wastewater treatment and food processing industries. Therefore, the energy consumption and energy efficiency information available is compared with respect to the energy efficiency of different algal harvesting techniques. The highest possible solids recovery (as % (w/v) total suspended solids (TSS)) and energy requirements for each of the harvesting processes are given in Table 7.2.

Table 7.2 Summary of energy usage and highest possible solids (%w/v) yields of different algae harvesting techniques.

Harvesting process	(% solids)	(kW-hm)
Centrifugation	22.0	8.00
Gravity sedimentation	1.5	0.1
Filtration (natural)	6.0	0.4
Filtration (pressurized)	27.0	0.88
Tangential flow filtration	8.9	2.06
Vacuum filtration	18	5.9
Polymer flocculation	15.0	14.81
Electro-coagulation	NA	1.5
Electro-flotation	5.0	5.0
Electro-flocculation NA		0.331

Source: Singh *et al*., [34].

7.2.4 Algal Drying

The dewatering or drying process helps to reduce the water content of the algae preceding extraction of oil. The algae mass obtained after harvesting contains as much as *ca*. 90% water content. But for the production of solid materials for easy handling, algae must be dried to *ca*. 50% water content. Amongst the various drying methods solar drying is a popular and inexpensive method and is used commercially in grains and timber drying. It also requires a considerable area of land. A more efficient method would make use of the low-grade waste heat from the power plant to dry the algae contained in a vessel. The biomass harvested from the attached culture system is paste-like pulpy slurry having a water content to that of the cell pellet centrifuged from the suspension culture system. This implies a great advantage of the attached algal culture system in terms of ease of biomass harvesting [35].

7.3 Algae-based Bioenergy Products

The extremely large arable land utilization for crop plant biomass production to be used as a raw material for production of bioethanol as well as biofuels could result in shortages in basic foods, such as corn, cereals, soy, mustard, barley, etc. Thus, they have brought much controversy and debate on their sustainability [36]. In this respect, cultivation of algae at sea

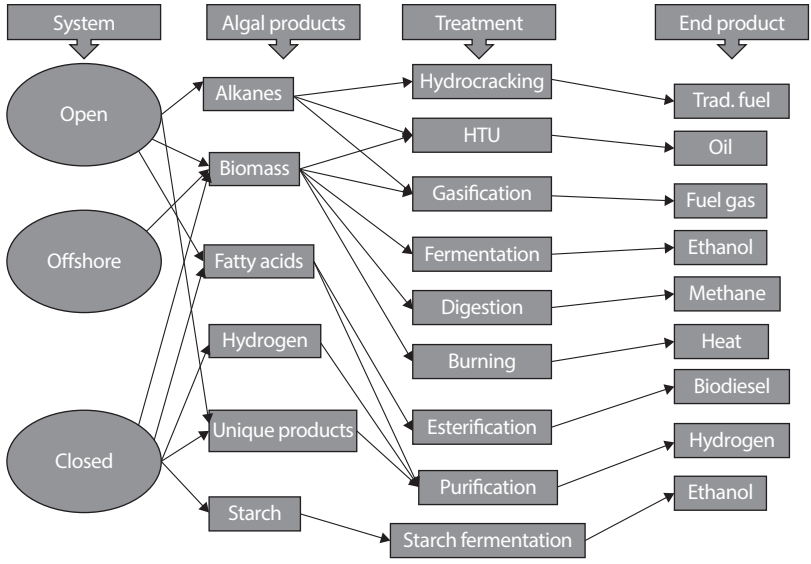

Figure 7.6 Different algae to energy options.

water or industrial or other wastewater provides a possible solution for this energy issue. Algal bioenergy products have some unique features that can greatly reduce some of the sustainability problems faced by many terrestrial sources, for example little or no competition for agricultural land, or even positive effects, such as fertilizer production instead of consumption. In the last few years the focus has shifted to a renewed interest and a great increase in activity in algae as a sustainable source of energy. Potentially algae has a high productivity and biomass production which shun competition with other productive land uses. But still there are some loopholes regarding the potential for the technologies, and there is no consensus about the optimum role for algae, with many algal strains and routes to energy under consideration. Different processing methods of algae and its harvesting and processing technologies give different options for bioenergy products (Figure 7.6).

7.3.1 Biofuel and Biodiesel

Algae are oil-rich and give a higher yield of oil per unit of land in a year compared to terrestrial crops (Table 7.3). Lipids are one of the main components of microalgae; depending on the species and growth conditions 2–60% of total cell dry matter [38]. Microalgae contain lipids and fatty acids as membrane components, storage products, metabolites and sources

Table 7.3 Oil output of different biofuel feed stocks.

Crop	Oil yield (gal/acre-yr)
Corn	18
Cotton	35
Soybean	48
Canola	127
Jatropha	202
Oil palm	635
Microalgae (15% oil)	1,200

Source: Khan *et al.* (37)

of energy. Such microalgal strains with high lipid value are of great interest in the search for a sustainable feedstock for biodiesel. These lipids can be used as a liquid fuel in adapted engines as Straight Vegetable Oil (SVO). Tri-glycerides and free fatty acids, a fraction of the total lipid content, can be converted into biodiesel. A few microalgal species have been reported to have the capacity of accumulating large quantities of lipids in cells under favorable conditions. A selction of algal strains with high efficiency of biodiesel production is prime. Use of a photobioreactor can prevent the competition from other algae, and optimum growth conditions can be easily maintained. Certain environmental and nutritional conditions during culture help in the accumulation of lipids. The viscosity of raw microalgal oil is high, thus requiring conversion to lower molecular weight constituents in the form of fatty acid alkyl esters. Transesterfication helps in the conversion of raw microalgal lipid into renewable, non-toxic and biodegradable biodiesel. During the transestrefication, glycerides in fats and oils react with alcohol in presence of catalyst. The end products of the reaction are fatty acid methyl esters (FAME) and glycerol. The use of acid catalyst has been found to be useful but the reaction rates for converting triglycerides to methyl esters are too slow [39]. Acid catalysis is suitable for transesterification of oils containing high levels of free fatty acids [40]. Alkali-catalyzed transesterification is about 4,000 times faster than the acid catalyzed reaction and hence most frequently used commercially [41].

7.3.2 Biogas (Biomethane Production)

Nowadays the production of biogas from algal biomass is gaining increasing demand worldwide. An anaerobic digester contains

synergistic microbial populations to convert a variety of organic substrates to methane and carbon dioxide. Thus algal organic compounds such as lipid, protein, or carbohydrate can be converted to methane. Methane is widely used as both a fuel and a chemical feedstock; however, under normal conditions it is a gas and therefore bulky to handle; its use as a transportation fuel is limited [7]. The growth rates of marine macroalgae exceed those of land plants; however, growth is often limited by the availability of nutrients. Conversion of methane to methanol through photochemical conversion is possible. An anaerobic biodegradability is quite inadequate because of its complex cell wall structure. The pretreatment techniques have been thus investigated so as to improve algal methane yield. Different pretreatment techniques aiming for faster anaerobic digestion, increase in biomass yield, and minimum processing hurdles are developed now. Pretreatment methods are thermal, mechanical, chemical and biological processes used to disintegrate microalgae cells, solubilize the organic content, and increase the anaerobic digestion rate and extent. Chemical pretreatments have been proved successful, particularly when combined with heat [42]. Enzymatic pretreatment seem to improve microalgae hydrolysis [43]. Biogas can be derived via anaerobic fermentation of any organic matter, including the cellulose and hemicelluloses within macroalgae, although the biomass must be subjected to pretreatment processes in order to liberate the sugars needed for fermentation [44].

The complex cell wall structure of microalgae is resistant to biological attack. Microalgal species without cell wall like *Dunaliella* sp. and *Pavlova* or the species containing glycoproteinous cell wall (Chlamydomonas sp., *Euglena*) showed higher methane yield than those with a more complex cell wall, containing recalcitrant compounds (e.g., *Scenedesmus* sp. and *Chlorella* sp.) [44]. Rates and yields of CH_4 formation from microalgal biomass often increase with digestion temperature. The incorporation of algae in photobioreactors to purify biogas has several advantages over conventional chemical methods of CO_2 removal. Obtaining algae is relatively inexpensive because culturing algae requires minimal nutrients for their growth. Growth of the algae requires a light source as well, which does not necessarily have to be expensive if illumination is provided by natural sunlight, which is not limited in supply [44].

7.3.3 Bioethanol

Bioethanol can be used as a biofuel which can replace part of the fossil-derived petrol. Research on improving bioethanol production has been

hastening for both ecological and economical purposes. Bioethanol is as an alternative to petroleum-based fuels [46]. Algae are considered as the only alternative to current bioethanol crops such as corn and soybean as they do not require arable land [47]. The unicellular marine microalgae were considered to be an abundant resource for carotenoids, lipids, and polysaccharides, and were widely investigated in the fields of food supplements and bio-fuel production [48]. Microalgae can convert up to 5% of the solar energy into chemical energy [49]. Few microalgae species have the ability of producing high levels of carbohydrates than that of lipids as reserve polymers, which are an ideal source for the production of bioethanol as carbohydrates from microalgae can be extracted to produce fermentable sugars. With new technologies, cellulose and hemicelluloses can be hydrolyzed to sugars [50], creating the possibility of converting an even larger part of algal dry matter to ethanol. It has been estimated that approximately 5,000–15,000 gal of ethanol/acre/year (46,760–140,290 L/ha) can be produced from microalgae [51]. Algal cell walls are largely made up of polysaccharides, which can be hydrolyzed to sugar. Blue-green algae including *Spirogyra* species and *Chlorococum* sp. have been shown to accumulate high levels of polysaccharides both in their complex cell walls and as starch. This starch accumulation can be used in the production of bioethanol [52, 53]. Harun *et al*. [52] have shown that the blue-green algae *Chlorococum* sp. produces 60% higher ethanol concentrations for samples that are pre-extracted for lipids versus those that remain as dried intact cells. Another algae-specific technology for ethanol production is being developed, in which green algae are genetically modified to produce ethanol from sunlight and CO_2 [54]. Bioethanol production from microalgae begins with the collection and drying of algae that have been cultivated in a suitable water environment. In the next step of the process, the algae mass is ground and hydrolyzed and then the hydrolyzed mass is fermented and finally distilled [55]. Bioethanol from algae holds significant potential due to their low percentage of lignin and hemicellulose as compared to other lignocellulosic plants [56]. While having low lignin content, macroalgae contain a significant amount of sugars (at least 50%) that could be used in fermentation for bioethanol production [57]. However, in certain marine algae such as red algae the carbohydrate content is influenced by the presence of agar, a polymer of galactose and galactopyranose. Current research seeks to develop methods of saccharification to unlock galactose from the agar and further release glucose from cellulose leading to higher ethanol yields during fermentation [57, 58]. Ethanol production from algae has very interesting prospects but it needs more development to analyze a full-scale production system.

7.3.4 Biohydrogen

Use of microalgae for photo-biological hydrogen production from water are being developed into a potentially emission-free fuel stream for the future, which will also help in atmospheric CO_2 sequestration. Algae biofuel projects were focused on obtaining biodiesel fuel, but at present, owing to innovative technologies, producers are becoming interested in the possibility of obtaining other kinds of fuel from algae that are close in composition to fuel products obtained by petroleum distillation, e.g., aviation fuel, which is obtained by subjecting algae oil to hydroprocessing. Bio-hydrogen production from microalgae was first observed in the green alga *Scenedesmus obliquus* and in many other photosynthetic species 65 years ago [59] (Boichenko and Hoffmann, 1994). Biohydrogen production from algae is of two types, which are as follows:

7.3.4.1 Direct Biophotolysis

This method involves the dissociation of water under sunlight in the presence of microalgae. Microalgae have perfect genetic, enzymatic, metabolic and electron transport machinery to produce hydrogen gas under the influence of light. In the biophotolysis, solar energy is used to convert a readily available substrate, water to oxygen and hydrogen (Figure 7.7).

The overall general reaction is

$$2H_2O \ \ \text{Light} \ \ 2H_2 + O_2$$

The well-known hydrogen-producing green algae under anaerobic conditions is *Chlamydomonas reinhardtii*, which may generate H_2 or use H_2 as

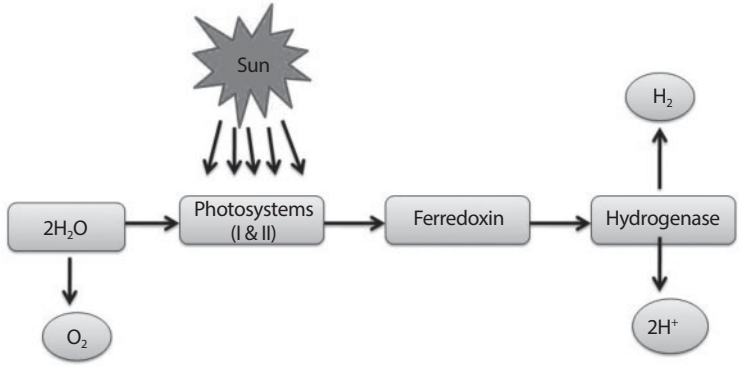

Figure 7.7 Mechanism of photolysis.

an electron donor [60]. These generated hydrogen ions are converted into hydrogen gas in the presence of enzyme hydrogenase and electrons which are donated by reduced ferredoxin.

7.3.4.2 Indirect Biophotolysis

In this method, Cyanobacteria (blue green algae) can evolve hydrogen by indirect biophotolysis. The general reaction for hydrogen formation from water by cyanobacteria is

$$12H_2O + 6CO_2 + \text{light energy} = C_6H_{12}O_6 + 6CO$$

$$C_6H_{12}O_6 + 12H_2O + \text{light energy} = 12H + 6O$$

7.3.4.3 Photo Fermentation

The general reaction for photo fermentation can be written as:

$$CH_3COOH + 2H_2O + \text{light} = 4H_2 + 2CO_2$$

A large number of microalgal screenings have been done for photo-fermentative hydrogen production. The most promising species are *Rhodopseudomonas capsulate, Rhodobacter spheroids* and *Rhodospirillum rubrum* [61]. Application of photobiological technology is a promising technology but the production of oxygen along with hydrogen causes a major setback for the technology. Large-scale electrolysis of water is also possible, but this costs more electricity than can be generated from the hydrogen it yields. For the future, more knowledge of the organisms that can produce hydrogen and their optimum conditions for growth and development is necessary, as well as optimization of the biological route of solar energy to hydrogen, through genetic modification. If these improvements prove to be possible, this would constitute a profitable and renewable hydrogen production [62].

7.4 Concluding Remarks

For sustainable biofuel, there are three principal considerations: *technical feasibility*; *economic viability*; and *resource sustainability*. Algal-based biofuel is technically feasible. However, to date, economic viability has not been achieved. Furthermore, resource sustainability, in terms of land, water, nutrient and energy utilization, must be meticulously quantified for each type of production system in order for the feedstock to be considered truly "sustainable". With large-scale biofuel production processes, this

water-energy-nutrient nexus is the subject of significant consideration and debate. Considering "algae-to-energy", hydrogen production by algae is mostly far from commercial implementation, although yield improvement options are being investigated. Algal biodiesel is generally the most favored and focused algae-for-energy option and has been researched the most. Both open and closed land-based cultivation systems appear suitable for this option. The conversion of the extracted lipids to biodiesel is relatively easy, and the product price can easily be compared with fossil fuel prices. Since nutrient-limitation is often used as an increasing lipid production strategy, this technology requires strict nutrient input control; therefore using manure or wastewater as a nutrient source may be relatively convoluted. Hydrogen has great potential to be a major contributor of clean and renewable energy. Biohydrogen production from algae on commercial scale can be useful as it fulfills most of the criteria of a clean and renewable source of energy. The process through which hydrogen is produced by algae has its pros and cons both in terms of technology and productivity. These processes are yet to be evaluated and modified for productivity and costing of commercialization. Bioethanol production from marine algae has also great potential for sustainable development. Several algae species have been studied; however, the main difficulties are its widely commercialization of bioethanol as an alternative to petroleum-based fuels, and more studies are needed in the future to dissolve these troubles.

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References

1. R. Sims, W. Mabee, J. Saddler and M. Taylor, *Bioresource Technology*, Vol. 101, p. 1570, 2010.
2. D. Antoni, V.V. Zverlov, and H. Schwarz, *Appl Microbiol Biotechnol*, Vol. 77 p. 23, 2007.

3. E.W. Becker, *Microalgae: Biotechnology and Microbiology*, Cambridge University Press, 1994.
4. C.Bigogno, I.Khozin-Goldberg, S.Boussiba, A.Vonshak and Z.Cohen, *Phytochemistry,* Vol. 605, p. 497, 2002.
5. R. Wijffels, and J.Barbosa, *Biofuels, Bioproducts and Biorefining*, Vol. 4(3), p. 287, 2010.
6. M. R. Tredici, *Biofuels*, vol. 1(1), pp. 143, 2010.
7. M. Awasthi, and R. K. Singh, *Int J Curr Sci*, Vol. 1, p. 14.
8. L. Rodolfi, G. ChiniZittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini, and M. R. Tredici, *Biotechnology and Bioengineering*, Vol. 102(1), p. 100, 2009.
9. O. Pulz, *Appl. Microbiol. Biotechnol*. Vol. 57(3), p. 287, 2001.
10. R. Bosma, W.A. van Spronsen, J. Tramper, and R.H. Wijffels, *Journal of Applied Phycology,* Vol. 15, p. 143, 2003.
11. W.H. Adey, and K. Loveland, *Dynamic aquaria: building living ecosystems*. 3rd Edn, Academic Press, New York, 2007.
12. D. P. Chynoweth, Review of Biomethane from Marine Biomass, Department of Agricultural and Biological Engineering, University of Florida, 2002.
13. B. H. Buck, and C. M. Buchholz, *Journal of Applied Phycology* Vol. 16(5), p. 355, 2004.
14. J. H. Reith, E. P. Deurwaarder, K. Hemmes, A. Curvers, P. Kamermans, W. Brandenburg, and G. Zeeman, Bio-offshore: grootschalige teelt van zeewieren in combinatie met offshore windparken in de Noordzee. Petten [etc.], Energieonderzoek Centrum Nederland [etc.]., 2005.
15. C. Briens, J. Piskorz, and F. Berruti, *Int J Chem*, *React Eng,* Vol. 6, p. 1–49, 2008.
16. M.R. Brown, S.W. Jeffrey, J.K.Volkman, and G.A. Dunstan, *Aquaculture*, Vol. 151, p. 315, 1997.
17. M. Canakci, and J. Van Gerpen, *Trans ASAE*, Vol. 44, p. 1429, 2001.
18. Y. Chisti, *Biodiesel from microalgae Biotechnology Advances*. Vol. 25, p. 294, 2007.
19. D.P. Chynoweth, C.E. Turick, J.M. Owens, D.E. Jerger, and M.W. Peck, *Biomass and Bioenergy*, Vol 5, p. 95. 1993.
20. J.R. Benemann, and W.J. Oswald, 1996. Systems and Economic Analysis of Microalgae Ponds for Conversion of CO to Biomass. Pittsburgh Energy Technology Center, pp. 260.
21. E. Grima, E.H. Belarbi, G.A. Fernandez, A.R. Medina, and Y. Chisti, *Biotechnology Advances*, Vol. 20, p. 491, 2003.
22. N.Sazdanoff, Modeling and Simulation of the Algae to Biodiesel Fuel Cycle, College of Engineering, Department of Mechanical Engineering, Ohio State University, 2006.
23. Ben-Amotz, and M. Avron. The biotechnology of mass culturing of Dunaliella for products of commercial interest. In: Cresswell RC, Rees TAV, Shah N, editors. *Algal and cyanobacterial technology*. London: Longman, p. 90–114, 1987.
24. M. Ruane, Extraction of caroteniferous materials from algae. Australian patent No. 7,239,574, 1977.

25. P.A. Belter, E.L. Cussler, and W.S. Hu, *Bioseparations: Downstream Processing for Biotechnology*. New York: Wiley, 1988.
26. M. Haesman, J. Diemar, W.O. Connor, T. Soushames, and L. Foulkes, *Aquaculture Research*, Vol. 31, p. 637, 2000.
27. T.S. Sim, A. Goh, and E.W. Becker, *Biomass*, Vol. 16, p. 51, 1988.
28. K.A. Matis, G.P. Gallios, and K.A. Kydros, *Separations Technology*, Vol. 3, p. 76, 1993.
29. G. Shelef, A. Sukenik, and M. Green, Microalgae Harvesting and Processing: A Literature Review. Report, Solar Energy Research Institute, Golden, CO, SERI Report No. 231–2396, 1984.
30. W.F. Bare, N.B. Jones, and E.J. Middlebrooks, *Journal of the Water Pollution Control Federation*, Vol. 47, p. 153, 1975.
31. S. Conover, *Mar Biol*, Vol. 32, p. 231, 1975.
32. A. Csordas, and J.K. Wang, *Aquacultural Engineering*, Vol. 30, p. 15, 2004.
33. S. Gao, J. Yang, J. Tian, F. Ma, G. Tu, and M. Du, *Journal of Hazardous Materials*, Vol. 177, p. 336, 2010.
34. M. Singh, R. Shukla, and K. Das, "Harvesting of Microalgal Biomass" in Faizal Bux, ed., *Biotechnological Applications of Microalgae*, CRC Press, London, pp. 77–87, 2013.
35. D. Das, and T.N. Veziroglu, *Intern J Hydrogen Energy*, Vol. 26, p. 13, 2001.
36. C. S. Goh, and K. T. Lee, *Sustain.Energy Rev.*, Vol. 14, p. 842, 2010.
37. S. Khan, A. Rashmi, M.Z. Hussain, S. Prasad, and U.C. Banerjee (2009). *Renewable and Sustainable Energy Reviews*, Vol. 13, p. 2361–2372, 2009.
38. Wijffels, R. (2006). *Energie via microbiologie: Status en toekomst perspectief voor Nederland*. Utrecht, Senter Novem.
39. X.Meng, J. M.Yang, X. Xu, L.Zhang, Q. J. Nie, M.and Xian, *Renew. Energy,* Vol. 34(1), p. 1, 2009.
40. P. Metzger, and C. Largeau, *Appl Microbiol Biotechnol*, Vol. 66, p. 486, 2005.
41. X.L. Miao, and Q.Y. Wu, *Bioresour Technol*, Vol. 97, p. 841, 2006.
42. F. Passos, M. Solé, J. Garcia, and I. Ferrer, *Applied Energy*, Vol. 108, p. 168, 2003.
43. A. Mahdy, L. Mendez, S. Blanco, M. Ballesteros, and C. González-Fernández, *Bioresource Technology*, Vol. 171, p. 421, 2014.
44. R. Ramaraj, and N. Dussadee, *International Journal of Sustainable and Green Energy* Vol. 4(1–1), p. 20–32, 2014.
45. P. Bohutskyi, M. J. Betenbaugh, and E. J. Bouwer, *Bioresource Technology*,Vol. 155, p. 366, 2014.
46. S. Prasad, A. Singh, N. Jain, and H. C. Joshi, *Energy Fuels*, Vol. 21, p. 2415, 2007.
47. A. Singh, D. Pant, N. E. Korres, A. S. Nizami, S. Prasad, and J. D. Murphy, *Bioresour. Technol*, Vol. 101, p. 5003, 2010.
48. B. C. Liau, C. T. Shen, F. P. Liang, S. E. Hong, S. L. Hsu, T. T.Jong, and C. M. Chang, *J. Supercrit. Fluids,* Vol. 55, p. 169, 2010.
49. C. Rösch, J. Skarka, and N. Wegerer, *Bioresour. Technol*, Vol. 107, p. 191, 2012.
50. C. N. Hamelinck, G. van Hooijdonk, and A. P. C. Faaij, *Biomass Bioenerg*, Vol. 28(4), p. 384, 2005.

51. L. Chaudhary, P. Pradhan, N. Soni, P. Singh, and A. Tiwari, *International Journal of ChemTech Research*, Vol.6(2), p. 1381, June 2014.
52. R. Harun, M. Singh, G.M. Forde, and M.K. Danquah, *Energy Reviews*, Vol. 14, p. 1037, 2010.
53. F. S.Eshaq, M. N. Ali, M. K. Mohd, *Int J Eng Sci Technol*, Vol. 3, p. 1749, 2011.
54. M. D. Deng, and J. R. Coleman, *Appl. Environ. Microbiol*, Vol. 65(2), p. 523, 1999.
55. A. Demirbas, and M.F. Demirbas, *Algae energy: algae as a new source of biodiesel.* London: Springer-Verlag, 2010.
56. R. Harun, M. K. Danquah, and G.M. Forde, *J Chem Technol Biotechnol,* Vol. 85, p. 199, 2010.
57. S. G. Wi, H. J. Kim, S. A. Mahadevan, D. J. Yang, and H. J. Bae, *Bioresour. Technol*, Vol. 100, p. 6658, 2009.
58. J. J. Yoon, Y. J. Kim, S. H. Kim, H. J. Ryu, J. Y. Choi, G. S. Kim, and M. K. Shin, *Adv Mater Res*, Vol. 93, p. 463, 2010.
59. V.A. Boichenko, and P. Hoffmann, *Photosynthetica*, Vol. 30, p. 527, 1994.
60. T. Happe, B. Mosler and J.D. Naber, *Eur J Biochem*, Vol. 222, p. 769, 1994.
61. A. Tsygankov, A. Federov, I. Talipova, T. Laurinavichene, J. Miyake, and I. Gogotov, *Appl Biochem Microbio*l, Vol. 34, p. 362, 1998.
62. A.Melis, and T. Happe, *Plant Physiol*, Vol. 127(3), p. 740, 2001.

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