Biodiesel production from lignocellulosic biomass

B.Sc. Semester 5

Long-term economic and environmental concerns have resulted in a considerable amount of work on renewable sources of liquid fuels to replace fossil fuels. The conversion of abundant lignocellulosic biomass(LCB) to biofuels for use as transportation fuels presents a viable option for improving energy security and reducing greenhouse gas emissions. Therefore, scientists have been evaluating the potential use of the inedible part of food crops to produce bioenergy and biomaterials. Currently, biodiesel is the most attractive alternative to traditional fossil-based diesel fuel, but its development and application have been hindered by the high cost of the required feedstock. The use of traditional feedstock, such as vegetable oils, could result in a shortage of edible oil and increased food prices. Therefore, in recent years, researchers have been seeking alternative sources of oil, such as microbial oils, also known as single cell oils (SCOs), which consist of lipids produced by oleaginous microorganisms, including yeasts, moulds, and micro algae. These lipids are considered to be promising candidates because their composition of fatty acids is similar to that of vegetable oil.

The most common technique for producing biodiesel is transesterification, which refers to a catalyzed chemical reaction of vegetable oil with an alcohol to produce fatty acid alkyl esters (biodiesel) and glycerol. The production of biodiesel from residual, agricultural biomass would have environmental benefits that far exceed its economic benefits. Significantly, cellulosic biomass and other waste materials that occur in abundance outside the human food chain can be obtained throughout the year and are relatively inexpensive. According to International Energy Agency (IEA, 1998), biomass currently provides approximately 14% of the total worldwide need for energy, and it represents an important contributor to the world economy. Other benefits of the use of agricultural waste biomass are that it would contribute to the stabilization of farmers' incomes and help maintain and improve ecological and social sustainability.

The major obstacle to widespread utilization of this important resource is the absence of economically feasible technologies for overcoming the impediments to converting lignocellulosic material to useful lipid products. Hence, there is considerable economic interest in the development of processes that can pretreat and convert inexpensive cellulosic wastes to useful products that can be used as fuel. Some such processes already have been developed, but, so far, they have not been found to be advantageous from production or economic perspectives. For the first time, the prospect of biodiesel production from LCB is described in this paper, including of the most recently developed biochemical processes, such as cultures of high lipid-accumulating microbes, the fermentation process, and the pretreatment of LCB.

Availability of LCB

LCB is the largest, most attractive biomass resource in the world that can be used as a raw material for the economic production of microbial oils. The total worldwide production of cellulose and hemicellulose is about 85 l09 ton/annum, with cereal straw estimated to exceed 2.9 l09 ton/annum. Although the bioconversion of lignocellulosic residues into ethanol or butanol has been performed successfully, it has been technically and economically challenging to produce biodiesel from LCB, and the technology has yet to progress from the laboratory stage to the demonstration stage. The major LCB resources come from the residues generated by agricultural, forest, and industrial sources. A detailed list of biomass sources is given in Table 1. Not all biomass residues can be utilized for energy. In some instances, their wide dispersal or their low bulk densities make recovery, transport, and storage very costly.

Food crops

Most of the arable land in the world is used for food crops. However, food scientists and world leaders agree that the whole world will face a great food crisis in the near future. The main food crops produced are rice, sugar cane, vegetables, wheat, pulses, coconuts, maize, millet, and groundnuts (Table 1). In most cases, only small percentages of the food crop residues, mainly the lignocellulosic parts, are used for heat energy in rural areas. Crop residues can be collected, mostly by bailing, either at the time the primary crop is harvested or later. But not all field residues are recoverable. In some cases, crop residues contribute to enhancing the organic matter and nutrients in the soil, improving the soil's ability to retain water, and sustaining microbial and macroinvertebrate activity. These effects typically lead to improved plant growth, soil productivity, and crop yield. But the percentage of field residues of a crop to be recycled onto the land depends upon the specific local climatic and soil conditions. Low residue crops, such as soybeans, rarely produce enough residues to maintain adequate soil cover through the winter, so they are not receiving serious consideration as biofuel feedstocks.

Non-food/energy crops

Where crop residues are needed to maintain sustainable production, a more viable option may be crops that could be grown specifically for use as energy crops, including herbaceous energy crops such as switch grass and short-rotation, woody crops such as hybrid poplar. These crops are perennials, so they require few field passes and little soil disturbance, which results in their having low erosion rates. Paine et al. (1996) recommended growing these crops on marginal lands, such as land that is subject to significant erosion, poorly-drained soils, or areas used for wastewater reclamation. This approach would avoid competition with food crops and increase the amount of arable land.

Energy crops (Table 1) show higher productivity levels per square acre than their conventional counterparts. In addition, by comparison, such crops are more homogeneous in terms of their physical and chemical characteristics than residual resources that are often described as the biomass resource of the future. Among the most promising of the identified options so far are perennial crops. They are expected to contribute to the goal of increased bioenergy development by exhibiting low input requirements, over 10–15 years of productive life, and high yields. On the other hand, Ericsson et al. (2009) suggested a controversial result by stating that energy crops make a fairly small contribution to the biomass supply and that production is dominated totally by annual crops for the production of transportation fuels. In 2005, the cultivation area for energy crops among the European Union's 25 members (the EU25) amounted to about 2.5 M ha, 80–85% of which was rapeseed cultivation for the production of biodiesel. Traditional food and forage crops produced with current agricultural practices create negative environmental effects, such as erosion, the leaching of nutrients, and the emission of greenhouse gases. These environmental difficulties may be reduced by dedicated, perennial energy. Energy crop systems also can contribute to diminishing the toxicity of municipal waste by taking up heavy metals, thus reducing negative impacts on the community. Heavy metal may spread on soil when digested municipal waste used as fertilizer.

Energy crops are a new concept to farmers that they may view as having certain risks associated with their cultivation, securing contracts, and fluctuating market prices. Thus, it is vital for that such crops provide reliable income that is at least comparable to, or preferably higher than, their conventional counterparts.

Table 1
Recoverable LCB.

Food crops	Non-food/energy crops	Forest residues	Industrial process residues
– Rice straw – Wheat straw – Sugarcane tops – Maize stalks millet – Groundnut stalks – Corn straw – Soybean residue – Residues from vegetables – Residues from pulses	– Cardoon (Cynara cardunculus, L.), – Giant reed (Arundo donax L.) – Salix – Jute stalks – Willow – Poplar – Eucalyptus – Miscanthus – Reed canary grass – Switch grass – Hemp	– Tree residues (twigs, leaves, bark and roots) – Wood processing residues (sawmill off-cuts and sawdust) – Recycled wood (that derived from the demolition of buildings, pallets and packing crates)	– Rice husk – Rice bran – Sugarcane bagasse – Coconut shells – Coconut husks – Maize cob – Maize husks – Groundnut husks

Forest residues

Forest residues are the second largest source of lignocellulosic biomass next to the agro-residues. Much of this residue is uncontrollable, yet a large portion of it may be reduced. The irregularly round shape and varying dimensions of trees must be changed to the required shape and size by removal of extraneous material. Parts of the trees, or entire trees, are rejected for a specific use because of size, shape, quality, or species. Such residue is often increased by poor operating practice or by manufacturing issues and failures. For some wood species, faulty manufacturing is the direct cause of residue in the later stages of processing.

Forest residues typically refer to those parts of trees that are unsuitable for saw logs, i.e., treetops, branches, small-diameter wood, stumps, dead wood, and even misshapen whole trees, as well as undergrowth and low-value species. Wood processing residues (e.g., sawmill rejects and sawdust) and recycled wood (e.g., wood derived from the demolition of buildings, pallets, and packing crates) are important sources of LCB.

Industrial process residues

Advances in industrial biotechnology offer potential opportunities for economic utilization of agro-industrial residues. The residues produced in industrial crop processing (Table 1) also are abundant sources of cellulosic biomass. Such biomass is 100% collectable and reusable. It is a relatively inexpensive raw material and already has been used for the production of several industrially-important chemicals and bioethanol. Fig. 1 makes it apparent that the feedstocks that generate the largest quantities of biomass are rice bran, maize cobs, and sugar cane bagasse. These are followed by other residues from industrial processes. The physical properties, content of cellulose, and fermentable pentosans in each of these residues are different, and, accordingly, the processing technologies must differ slightly when they are used as raw material for microbial oil production for biodiesel.

LCB that is used at the present time to produce microbial oil Traditional feedstocks have several limitations, e.g., the use of vegetable oils would result in a shortage of edible oil and cause food prices to increase; cooking oil used in frying foods is insufficient to meet the demand; the growth time for producing feedstock for manufacturing oil from plants is an entire cultivation season (at least 6–12 months); and difficult decisions must be made that likely will spat social conflict. To overcome the limitations of traditional feedstock, the use of agro-industrial residues has attracted the attention of researchers worldwide. These residual materials can be used as nutritional source for microorganisms that accumulate intracellular lipids within short time. Table 2 shows the agro-industrial residues that have been used to produce microbial lipids in recent studies. LCB is the most abundant agricultural residues in the world, and they are mainly used as fuel, biofertilizer, and animal feed in rural areas. It is economically promising to produce microbial oil from agro-industrial residues, especially lignocellulosic materials.

Oleaginous microorganisms, which are able to produce more than 20% of their weight in the form of triacylglycerols, are receiving ever-increasing attention for several reasons such as (i) high growth rate; (ii) accumulation of large quantities of lipids; (iii)composition of fatty acids that is similar to that of vegetable oils; (iv) having potential lipids with rare fatty acid structure; and (v) minimal reutilization of stored lipids for metabolic function. Huang et al. (2009) explored the possibility of lipid production from sulfuric acid-treated rice straw hydrolysate by Trichosporon fermentans. They also reported that, among the various agricultural crop residues, rice straw, the hydrolysate of which mainly contains glucose, xylose, and arabinose, has proven that T. fermentans can grow well and accumulate lipid efficiently on glucose as well as xylose. T. fermentans could also grow well in pretreated waste molasses, and the addition of various sugars (fructose, sucrose, xylose, and lactose) to the pretreated molasses could efficiently enhance the accumulation of lipids.

When costly glucose was substituted by corn stalk and Populus euramevicana leaves hydrolytes as alternative carbon sources, encouraging results were observed for lipid production (11.78% and 28.59% respectively) on a cellular biomass basis. Poor growth of Rhodotorula glutinis was observed in rice straw hydrolytes (lipid content, 5.74%), presumably because of the presence of inhibitory compounds in these hydrolytes. A novel method was described by Peng and Chen (2008) for the production of SCO in solid-state fermentation (SSF) from steam-exploded wheat straw mixed with wheat bran, using the endophytic fungus *Microsphaeropsis sp.*, which is capable of accumulating SCO and secreting cellulase. They showed that the production of cellulase by *Microsphaeropsis sp.* was limited, leading to low SCO yield 42 mg/g dry substrate (gds), which could, however, be increased by adding cellulase to the solid-state medium, leading to a maximal SCO yield of 80 mg/gds.

Shiu et al. (2010) were the first to investigate the production of biodiesel from rice bran using an in situ process in which esterification was followed directly by transesterification without employing a separation step in between. Lin et al. (2009) studied the use of crude rice bran oil to produce biodiesel.

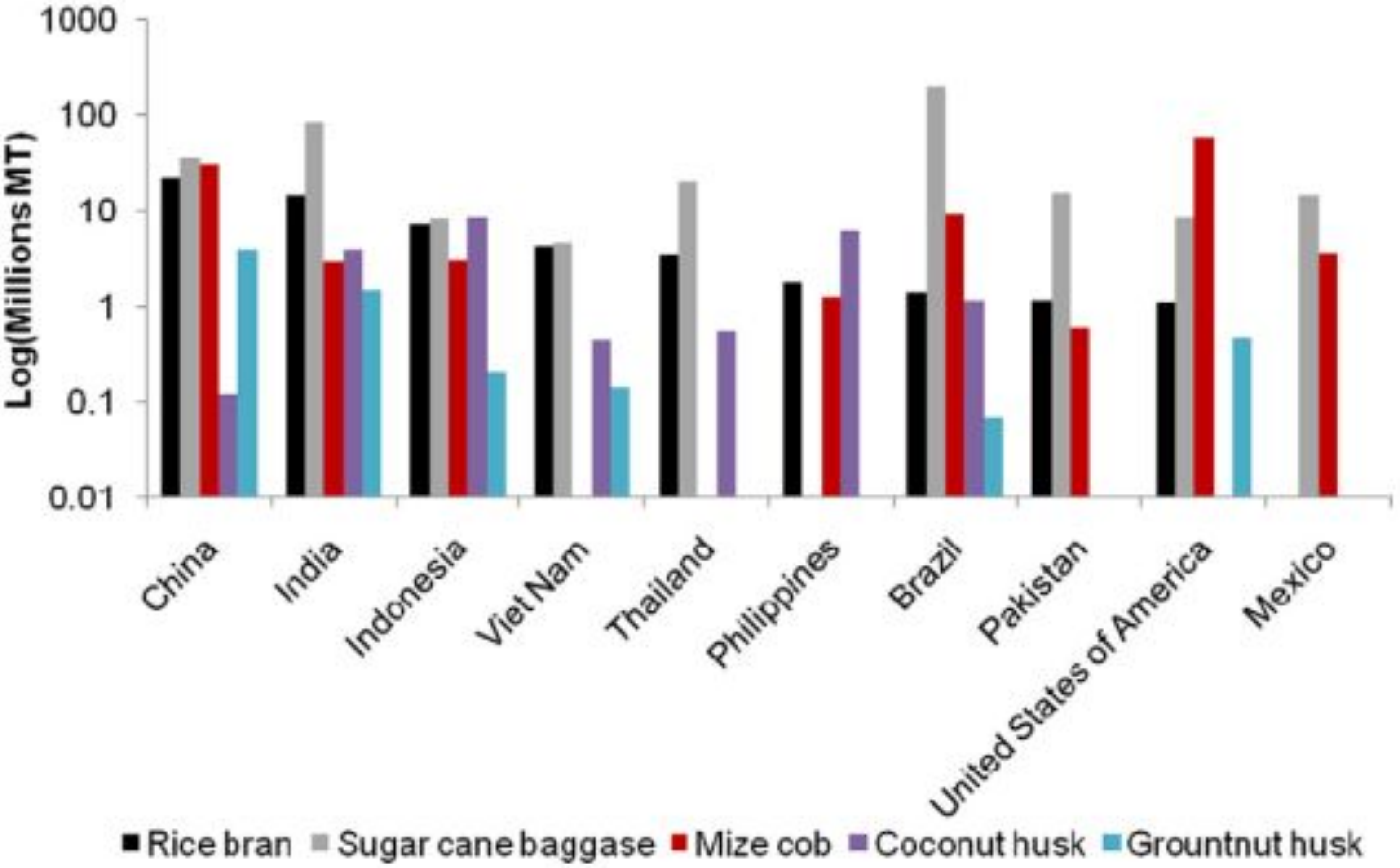

Fig. 1. LCB generated by different countries in processing industrial crops (FAOSTAT, 2009).

Table 2
The agro-industrial residues and their fermentation process to accumulate lipid by oleaginous microorganism.

Residual materials	Fermentation process	Pretreatment methods	References
Wheat straw and wheat bran	Solid-state	Steam explosion	Peng and Chen, 2008
Pitch pine	Liquid	Organosolv	Park et al., 2010
Pear pomace	Solid-state	Without treatment	Fakas et al., 2009
Rice straw	Liquid	Acid hydrolysis	Huang et al., 2009
Wheat straw	Liquid	Organosolv	Sun and Chen, 2008
Sweet sorghum	Semi-solid state	Without treatment	Economou et al., 2010
Tomato waste	Liquid	Acid hydrolysis	Fakas et al., 2008
Corn stalk	Liquid	Acid hydrolysis	Dai et al., 2007
Tree (*populus euramevicana)* leaves	Liquid	Acid hydrolysis	Dai et al., 2007
Rice straw	Liquid	Acid hydrolysis	Dai et al., 2007

Composition

Typically, LCB contains 35–55% cellulose, 20–40% hemicellulose and 10–25% lignin. The cellulose that is embedded in the lignin matrix has an insoluble, highly-crystalline structure, so it is difficult to hydrolyze into sugar or cell protein, which is easy to use. Cellulose is a long chain of glucose molecules, linked primarily by b(1–4) glycosidic bonds. In general, cellulose is very resistant to biodegradation due to its strong crystalline structure and due to interferences by other biomass constituents, such as hemicellulose and lignin. Hemicellulose consists of branched polymers of xylose, arabinose, galactose, mannose, and glucose. Hemicellulose binds bundles of cellulose fibrils to form microfibrils, which enhances the stability of the cell wall. Hemicelluloses are also cross-linked with lignin, creating a complex web of bonds that provide structural strength and prevent microbial degradation.

Processing/pretreatment of LCB

A major problem in the processing of lignocellulosic materials is the natural resistance of the hemicellulose and cellulose to the conversion process required to generate zermentable sugars. Pretreatment is a primary or prerequisite condition if agro-industrial residues are to be converted to fuels and chemicals via biochemical proceses. The main purposes of pretreatment are to separate the components of the LCB, reduce the crystallinity of the LCB, make the cellulose accessible (Fig. 2), and remove lignin. Lignocellulosic materials are very complicated, so a simple pretreatment process is not feasible. The best pretreatment method and the conditions of that method depend significantly on the type of lignocelluloses that make up the LCB. Different types of pretreatment methods are used, depending on the properties of the substrate. Table 2 shows the most frequently-used pretreatment methods.

Enzymes produced by a variety of microorganisms are capable of breaking down lignocellulosic materials to sugars, but they require longer retention times. The digestibility of the cellulose that is present in LCB is hindered by many physicochemical, structural, and compositional factors.

Peng and Chen (2008) studied the use of steam explosion treatment with wheat straw, and they found that nitrogen content was markedly lower (0.56%) than that of untreated wheat straw (2.62%). This pretreatment method removes most of the hemicellulose, thus improving enzymatic digestion. However, in order to achieve lipid accumulation in a microorganism, the medium should be composed of an excess of carbon and a limiting amount of nitrogen. Ruiz et al. (2008) studied steam explosion for pretreatment of sunflower stalks before enzymatic hydrolysis at a temperature in the range of 180–230 C. The highest glucose yield was obtained in steam pretreated sunflower stalks at 220 C, while the highest hemicellulose recovery was obtained at 210 C. Xin and Geng (2010) pretreated horticultural waste by steam alone and by dilute NaOH solution. The steam pretreatment was conducted at 121 C for 2 h, and the pretreatment with dilute NaOH was conducted at various concentrations (1–3%) at 105 C overnight for enzyme production by Trichoderma reesei. They observed that the highest enzyme activities were obtained when steam-pretreated horticultural waste powder was used. Pretreatment with both steam and NaOH did not improve the enzyme (microbial) activities significantly compared to pretreatment with steam alone. It was also observed that pretreatment with increased concentrations of NaOH, e.g. 1–3%, did not show a significant increase in the yield of any the enzymes.

A wide range of organic or aqueous-organic solvents as well as catalysts, such as oxalic, salicylic, and acetylsalicylic acid, can be used in the organosol pretreatment of lignocellulosic materials at temperatures of 150–200 C. In addition, the solvent may along with acetic acid released from acetyl groups developed by hydrolysis of hemicelluloses. A variety of organic solvents, such as alcohols, esters, ketones, glycols, organic acids, phenols, and ethers, have been used. However, the price of the solvent and the simplicity of the recovery of the solvent also should be considered. The operational cost could be reduced by recovering the solvents by evaporation and condensation and reusing them. Removal of solvents from the pretreated cellulose is usually necessary because the solvents might inhibit the enzymatic hydrolysis and fermentation or digestion of hydrolysate. For economic reasons, the use of low-molecular weight alcohols, such as ethanol and methanol, has been favored over alcohols with higher boiling points, e.g., ethylene glycol and tetrahydrofurfuryl alcohol . Ethanol is a common solvent, but it inhibits hydrolytic enzymes. Therefore, it should be removed from the solid fraction before proceeding with enzymatic hydrolysis. The main advantage of the use of solvents over other chemical pretreatments is that relatively pure, low-molecular weight lignin is recovered as a byproduct.

Acid hydrolysis is the most commonly used pretreatment method for lignocellulosic materials, and sulfuric acid is the most frequently used acid, while other acids, such as hydrochloric acid,phosphoric acid, and nitric acid, were also reported. Table 3 shows that acid pretreatment can be conducted at high temperature and low acid concentration (dilute-acid pretreatment) or at low temperature and high acid concentration (concentrated-acid pretreatment). Lenihan et al. (2010) hydrolyzed hemicellulosic biomass in the form of potato peels using dilute phosphoric acid and obtained the optimum yield at 135 C and 10% (w/w) acid concentration. They produced 55.2 g sugar/100 g of dry potato peel after a time of 8 min.

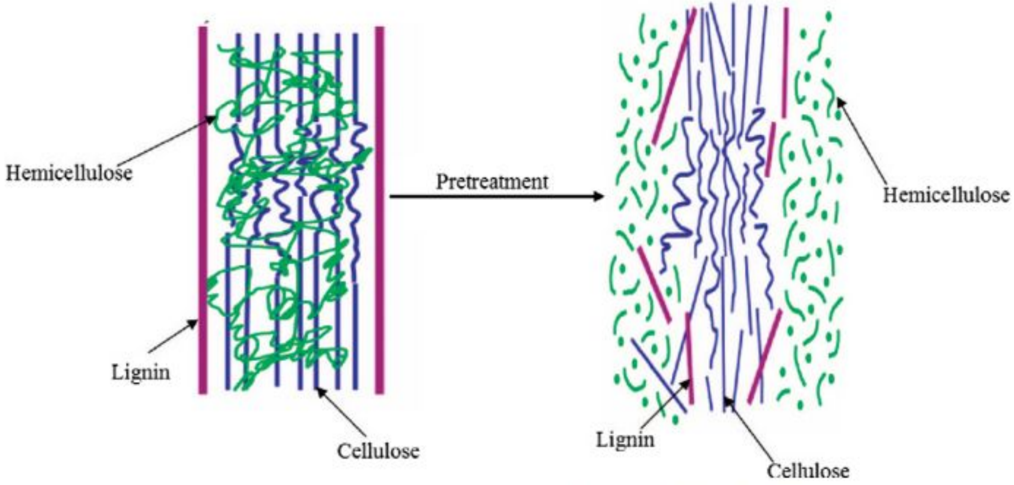

Fig. 2. Illustration of pretreatment phenomena (adapted from Hsu et al., 1980).

Table 3
Variation of hydrolysis parameters implemented on agro-industrial residues.

Residual materials	Hydrolysis with		Solid: liquid	% (w/v) of solution	Temperature (°C)	Treatment time (minute or hour)	References
	Acid	Alkali					
Rice straw	H_2SO_4		1:10	1.5	121	90 min	Huang et al., 2009
Tomato waste	H_2SO_4		1:3	2	121	2 h	Fakas et al., 2008
Corn stalk, *populus euramevicana* leaves and rice straw	H_2SO_4		1:10			8 h	Dai et al., 2007
Cane molasses	H_2SO_4		NA[a]	49	60	2 h	Liu et al., 2008
Horticulture waste		NaOH	NA	1–3	105	Overnight	Xin and Geng, 2010
Cassava bagasse, sugarcane bagasse, wheat bran, rice straw		NaOH	NA	0.4	NA	12 h	Singhania et al.,2006

[a] NA = Not available.

Microbial strains and their lipid storage capacity

An important advantage offered by the application of the oleaginous microorganisms is that they can produce lipids from residual organic matter aerobically. Consequently, in order to optimize the cost of the process, as well as to increase its environmental benefit, residual materials have been tested as possible nutrients for the oleaginous microorganisms, such as nutritional residues from agricultural and industrial operations, sewage sludge, and olive-mill wastewater, thus lowering the cost of the oils. It is remarkable that, in comparison to the production of vegetable oils, the culture of oleaginous microorganisms is not affected by seasons or climates. Microbial lipids, which also are known as SCO, were not manufactured commercially until 1995, and after six years of operation, the facilities were closed because they were no longer cost effective. By this time, several oleagenous yeasts and microalgae had been reported to grow and accumulate significant amounts of lipids that were similar to vegetable oil. Table 4 shows some of the most efficient, lipid-accumulating, oleaginous microorganisms that can be used.

Lipids serve as storage materials in some lipid-accumulating yeasts, e.g. Rhodotorula graminis. Guerzoni et al. (1985) reported that yeasts can store up to 70% of lipids in dry matter. The first data related to lipid accumulation and the conditions for fermentation
were reported more than 50 years ago. They also monitored that the presence of a carbon-source in excess and under nitrogen limiting conditions organisms started to store lipids. Therefore, a high carbon-to-nitrogen (C/N) ratio of around 100 is a basic requirement for the accumulation of lipids. A similar result was found by Zhu et al. (2008) when they studied T. fermentans. The lipid content was quite low at a C/N molar ratio of 108, but it showed a sharp increase when the C/N molar ratio increased to 140, and it reached the maximum of 63.1% at a molar ratio of 140. Additional increases in the molar ratio above 140 resulted in a slight decrease in the lipid content, but the biomass increased continuously up to 163, where the highest lipid yield of 14.8 g/l was achieved.

Lypomyces starkeyi seemed to store the largest quantities of lipids compared to other lipid-accumulating yeasts, such as Candida curvata D, Trichosporon cutaneum, and Rhodosporidium toruloides, and it also showed only a minimal reutilization of the stored lipids. Physical factors such as the concentration of some ions, such as Zn2+ and Mn2+, affected lipid accumulation. The same was true for Fe3+, Ca2+, K+, and NH4+ , although to a lesser extent. The natural habitat of L. starkeyi is soil and ensilage in which the organism degrades carbohydrates using extracellular carbohydrolases. Both a-amylase and dextranase from L. starkeyi and the biodegradation of triazine herbicides have been the subjects of recent studies.

Fermentation process

Submerged/liquid fermentation and solid-state fermentation were studied mainly for the biotechnological production of single cell oil from lignocellulosic residues. Submerged fermentation requires prior sugar extraction from agro-biomass to the bulk liquid, which is a process that consumes both time and money. On the other hand, by solid-state fermentation (SSF) in which the microorganisms grow on moist, solid materials in the absence of free-flowing water, limited lipid accumulation can be obtained. However, solid-state fermentation has many advantages over liquid fermentation, e.g., simpler technique, smaller bioreactor or volume, reduced downstream processing cost, reduced energy requirement, and low wastewater output.

Economou et al. (2010) modified the SSF system by increasing the water content. They called it a semi-solid fermentation system that ensured easier growth of fungus and higher efficiency of SCO production. The SSF process is feasible for utilizing renewable and low-cost natural resources, e.g., agricultural and wood residues, energy crops, and byproducts of the food industry. But this process has some limitations, one of which is of significant concern, i.e., when plants or crop residues with high sugar content are utilized, sugar availability may be low or inhibition of the microbial growth may occur, resulting in low system productivity. In that case, semi-solid state fermentation is effective for increasing the availability of sugar.

Conclusions

The demand for liquid transportation fuels is constantly increasing, and biodiesel might be one of the most important solutions for this problem. Although biomass may be cheap, the costs of processing it can be high. Technologies for converting biomass to biodiesel also are at various stages of development. The numerous bottlenecks encountered in the development of such technologies, which include the pretreatment of biomass, enzymatic saccharification of the pretreated biomass, and fermentation of the hexose and pentose sugars released by hydrolysis and saccharification, have been discussed in this review. Each requires substantial research and development to improve efficiency and process economics. Thus, before this process can be successfully applied in large-scale operations, attention must be concentrated on the following issues:

(i) Development of methods for the hydrolysis of LCB that allow higher sugar yields. Enzymatic hydrolysis appears to be very promising for this purpose, but improvements are required for various aspects of the process, especially enzyme separation and reutilization after biomass treatment.

(ii) A more complete characterization of the waste materials that potentially could be useful for the production of microbial oils. Currently, second-generation biofuels are being sought that can be produced without using fertile soils. Consequently, lignocellulosic materials should be obtained from plants that can grow in semi-fertile soils that usually are not used for agriculture.

(iii) Optimization of the fermentation of oleaginous microorganisms to obtain higher concentration levels of lipids.

(iv) Optimization of the methanolysis of the microbial oils to increase the yields of biodiesel. Since the microbial oils contain high levels of free fatty acids (FFA), suitable catalysts are required, because the traditional alkaline catalyst (NaOH) may interact with FFA, leading to soap formation, reducing the yield of biodiesel and reducing the quality of the co-produced glycerol. The enzymatic synthesis of the biodiesel, based on the use of microbial lipases, offers significant improvements in process efficiency.