Chapter 10
Bioconversion and Biorefineries: Recent Advances and Applications

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Abstract The conversion of biomass is full of challenges requiring multiples steps for attaining high efficiencies in the transformation of this material for producing valuable goods and chemicals. There exist several biological processes capable of generating different fuels and green chemicals; however, their efficiency may be too low associated with the need of biomass pre-treatments or the maturity of these technologies may be at an early stage requiring for the development of pilot-scale experiences to get an insight on their performance under different conditions and for assessing their behaviour during extended periods. Some technical aspects are still in need of deep research to consider their implications in a global economic balance when the integration into multiple phases is proposed. Technologies for the production of fuels and the valorisation of the variety of side streams are reviewed in this chapter giving an approximation of the several possibilities of integrating these biological alternatives considering the production of ethanol, butanol, biodiesel and biogas along with the production of hydrogen. A cascade approach for applying a diversity of valorisation stages has been studied taking into account the use of different side streams for coupling biological and thermal processes in an attempt to increase process yields and reduce operating costs. The integration of anaerobic digestion and fermentative hydrogen production for the valorisation of cellulosic biomass into different processes as ethanol and biodiesel production has been assessed.

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10.1 Introduction

The use of biomass and its conversion into high value-added products is of great relevance when considering the development of a sustainable economy having as main aim the reduction or substitution of non-renewable sources. However, attaining this goal makes imperative the complete valorisation of biomass, reducing waste streams and developing processes characterised by a low energy demand. This concept irremediably leads to the concatenation of different biological and thermochemical technologies integrated in a way that allow for the maximisation of yields and economic revenues, otherwise their industrial application would be compromised. Therefore, attaining a green economy requires the development of processes similar to those already taking place in petroleum refinery, which allow the production of useful chemicals at a large scale, but in this case using renewable sources as raw material, thus the name of biorefineries (Fernando et al. 2006). The different value-added products that can be obtained from biomass in a biorefinery involves chemical building blocks, raw materials for different subsequent stages, biofuels and the production of energy (heat and power) (Aresta et al. 2012; Yadav et al. 2019). This strategy must be in compliance with physical conservation laws and it is to be achieved using the principles of Green Chemistry and Clean Technologies, where only pure substances are produced without waste and using by-products from another production step or conversion into energy, which also increases profitability (Kołtuniewicz and Dąbkowska 2016).

In recent years, the research activities have extensively reported on the valorisation of different types of biomass and the evaluation of microbiological processes capable of transforming these materials into a great variety of valuable products. However, there is still a need of extrapolating these results at larger scales and what it is of most relevance, to evaluate the global performance of coupling several types of technologies intended to maximise biomass conversion. The development of sustainable biorefineries calls for the suitable integration of innovative treatments to prove the technical and economic viability of the entire value chain (Aresta et al. 2012).

The experience and knowledge gained in the operation and management of conventional petroleum refineries can serve as a starting point to aid in making biorefineries a reality. The existence of petroleum refineries for over a century has allowed for a perfect control of thermal and catalytic processes. These processes have become increasingly sophisticated with the different products moving initially from a handful of fuels and lubricants to a full suite of chemical products (Mabee and Saddler 2006). However, one of the main factors that should be considered when comparing the technological development of refineries and that expected for its renewable counterpart is the dispersion of the feeding raw materials for the latter one. Dispersion and season availability will directly affect transportation costs and therefore will negatively influence carbon emissions in any type of energy efficiency balance assessed. Therefore, the future of these technologies is highly dependent of the management activities necessary for the supply of raw materials.

The lessons learned in petroleum refinery will serve as a wide knowledge base for the development of highly efficient biorefineries. Existing pulp and paper mills may be viewed as early examples of biorefineries, thus the integration of innovative processes in already operating industrial facilities would greatly help in developing complex conversion technologies that enable the production of value-added biomaterials and energy (Mabee and Saddler 2006). The biorefinery concept although offering several benefits to society and the environment is required to evolve in a way that allows for flexibility in the treatment of different feedstocks, increasing efficiency in the conversion of lignocellulosic materials and sustain production all year round in an attempt to avoid the low capital utilisation of several agro-industrial factories which depend on seasonal availability of feeding materials (Eggeman and Verser 2006; Kour et al. 2019a; Rana et al. 2019). The European Biobased Economy (based on an intensive agriculture) allows for a large production of materials from different types of biobased chains (food as well as non-food) along with the production of by-products that act as raw materials for a great variety of conversion techniques thus resembling a cascade approach of valorisation. The aim is to generate cyclic processes within which as many by-products as possible are valorised (Fava et al. 2015).

Different conversion platforms are available for transforming any kind of biomass into chemicals and/or fuels. Sugar and starch-based platforms were the first ones to be developed due to the relative low capital investment and the facility for controlling these types of fermentations. Yeast, specially *Saccharomyces cerevisiae* presents outstanding abilities for converting sugars to ethanol and it is part of one of the oldest human technologies being essential for many biotechnological processes (Dashko et al. 2014; Yadav et al. 2020). However, this yeast cannot utilise cellulosic materials, thus the extended use of starch in this fermentation processes, requires an additional pre-treatment in the form of hydrolysis to release glucose (Apiwatanapiwat et al. 2011) as main sugar with amylases being one of the most widely used family of enzymes capable of achieving the hydrolysis of starch and facilitating the subsequent fermentation stages.

The production of ethanol as a biofuel from the fermentation of sugars/or starchy materials was initially classified as first-generation biofuels, inside this same category were included fuels derived from vegetable oils or animal fats using conventional technologies (Cherubini and Jungmeier 2010). However, the competition created with food and feed agronomic production generated the need of transforming these processes into systems capable of treating more complex materials, leading to the so-called second- and third-generation biofuels.

The second-generation biofuels can be considered as the following reasonable step in producing biofuels from feedstock of lignocellulosic biomass and non-food materials along with the use of energy crops. On the other hand, the third-generation biofuels, which is an area currently under intensive research, are based on algal biomass production. This line of work requires a huge amount of experimental work to attain a significant improvement in biofuel yields and lower further the production costs (Aro et al. 2016). In an attempt to enhance the performance of these systems, the process has evolved into the so-called fourth-generation biofuels which can be

defined as the combination of the third-generation biofuel with the enhancement of performance by means of genetic and metabolic engineering (Singh et al. 2017; Farrokh et al. 2019; Kour et al. 2019b).

Different bioconversion technologies have become available since the appearance of the first fermentation processes. Nowadays, bioconversion facilities are capable of integrating several technologies to attain the valorisation of lignocellulosic biomass and waste streams, in particular of agro food by-products, waste effluents and surplus materials, with the production of value-added fine chemicals, novel materials and biofuels (Fava et al. 2015). A biorefinery thus involves a multi-step valorisation approach starting with the collection of the raw material, followed by its transport to plant and selection, involving pre-treatment stages (Fig. 10.1). The development of the precursor containing biomass is a key step of the process along with the subsequent fractionation stage leading to the recovery of valuable products (FitzPatrick et al. 2010). Although there is great experience in the implementation of fermentation at large scales, there exists a great need for enhancing the yields of high-quality by-products and increasing the efficiency in energy and water use of these industrial systems, along with the optimisation of fractionation equipment given the intrinsic difficulty of operating with a great variety of components and the presence of organic compounds produced in the intermediary stages having the potential of interfering in the yields of separation and precipitation steps.

One of the main obstacles commonly reported in ethanol fermentation from lignocellulosic biomass is the insufficient separation of cellulose and lignin, the formation of by-products that inhibit ethanol fermentation, the high use of chemicals and/or energy and the considerable production of waste materials (Menon and Rao 2012; Rastegari et al. 2019). The present chapter deals with the different processes available for the conversion of biomass and integration approaches tested in an attempt to make of the biorefinery concept a reality, taking into consideration that the biorefinery concept is geared towards the production of both traditional and novel fuels and chemicals with a wider goal than simply imitating petroleum refineries but rather generating novel products, which are otherwise not obtainable from fossils (Amoah et al. 2019).

10.2 Biofuel production

The most widely used biofuels in the transport sector are bioethanol, biodiesel and biogas. Ethanol is mainly produced from sugarcane in Brazil and corn in the United States of America (USA), with these two countries being the main producers of this type of fuel worldwide (about 85% of the global production for ethanol production) (Sharma et al. 2019). Biodiesel, on the other hand, is mainly produced from plants oils and again USA and Brazil scaling the first position coping about 36% of the global production (Statista 2019). Biogas is mainly produced from wastes, energy crops and agricultural residues, although the valorisation of this gas is usually performed by means of combined heat and power units for electricity production and heat

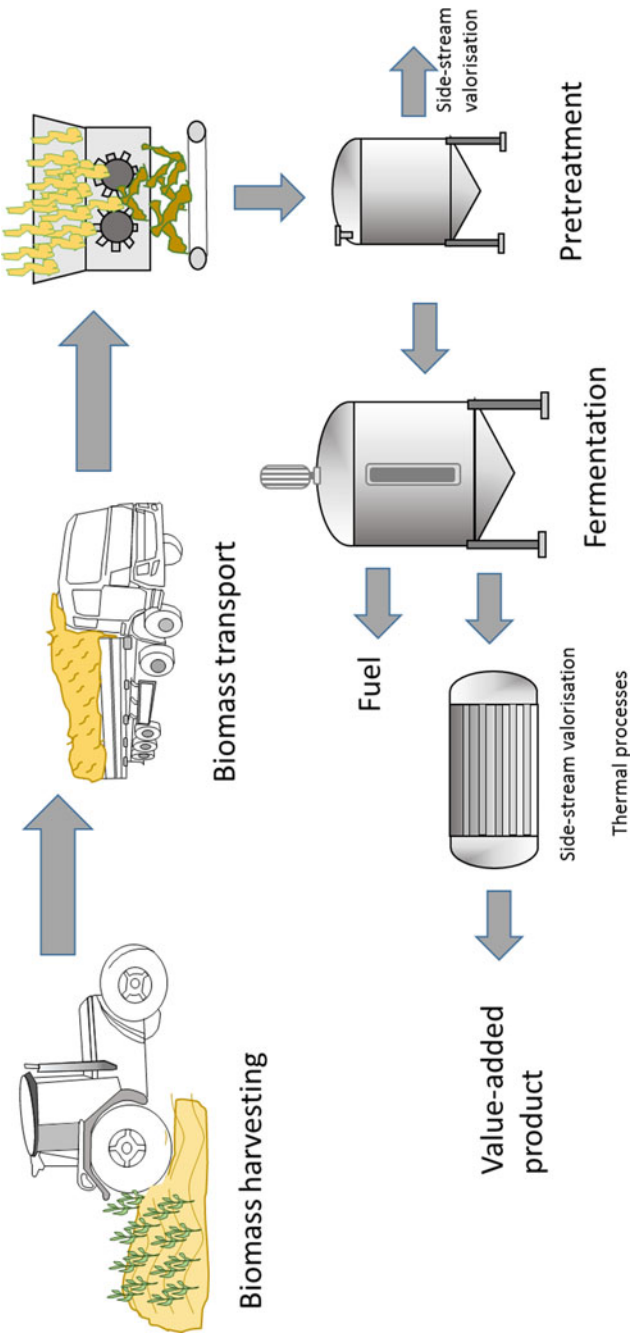

Fig. 10.1 Scheme of different stages in biorefinery concept

recovery, it also has an important share in the transport sector, with a high increase in the production of biomethane to an upgraded level compatible with injection to the natural gas grid. Germany is the leading country in the ranking of installed biogas producing plants and biomethane production in Europe (European biogas association report 2017), whereas the main producers of this gas are USA and European countries.

10.2.1 Bioethanol

The different bioconversion platforms for the production of biofuels usually involve a pre-treatment stage and the severity of this stage is associated with the type of substrate being treated. Bioethanol production was one of the first processes implemented at industrial scale to supplement gasolines with a renewable substitute. The fermentation for producing ethanol from sugarcane is capable of reaching extremely high yields (92–93% of the theoretical yield). The fermenter operates with high yeast cell densities (10–15% w/v) and fermentation volumes are as high as 0.5–3 billion litres (Amorim et al. 2011). Figure 10.2 shows a schematic representation of the Brazilian distillery technology for ethanol production. The fermentation from sugarcane takes advantage of the production of electricity from bagasse which favours the energy balance of the global process. Recycling of yeast cells is fundamental to achieve economic feasibility. In the Brazilian fermentation process, more than 90% of the yeast is reused from fermentation to the next one (Basso et al. 2008). It is also essential for the transformation of ethanol into ethyl tertiary butyl ether (ETBE) which is produced from a mediated catalytic reaction of isobutylene and ethanol.

The use of corn or cereals for producing ethanol involves additional steps for milling the raw material and the subsequent enzymatic hydrolysis at high temperature for liquefying starch type carbohydrates for the saccharification to take place. The fermentation in this case produces two types of stillage which are separated by centrifugation, leading to a solid fraction containing distiller wet grain and a thin stillage fraction which is further evaporated and then mixed with the wet grains to produce dried distiller grains with solubles (DDGS) (Eggeman and Verser 2006).

Due to the multiple stages in the ethanol fermentation process at large scale, the conversion of this type of plants into biorefinery centres allows for increasing the energy efficiency of the process. A remarkable case is that of the Bazancourt-Pomacle biorefinery which has developed a diversifying strategy to change its original nature of sugar factory and distillery to be transformed into a starch and glucose producing plant, and specialised research centre where start-up companies can test their demonstration and industrial pilot plants at its sites for developing lignocellulosic fractionation for ethanol and fine chemicals production (Schieb et al. 2015; Stadler and Chauvet 2018). A different story is that of Abengoa and its ethanol production plants, which due to different regulation constraints and unfavourable ethanol market prices was not able to keep industrial plants located in Spain and France, after being one of the main bioethanol producers in Europe.

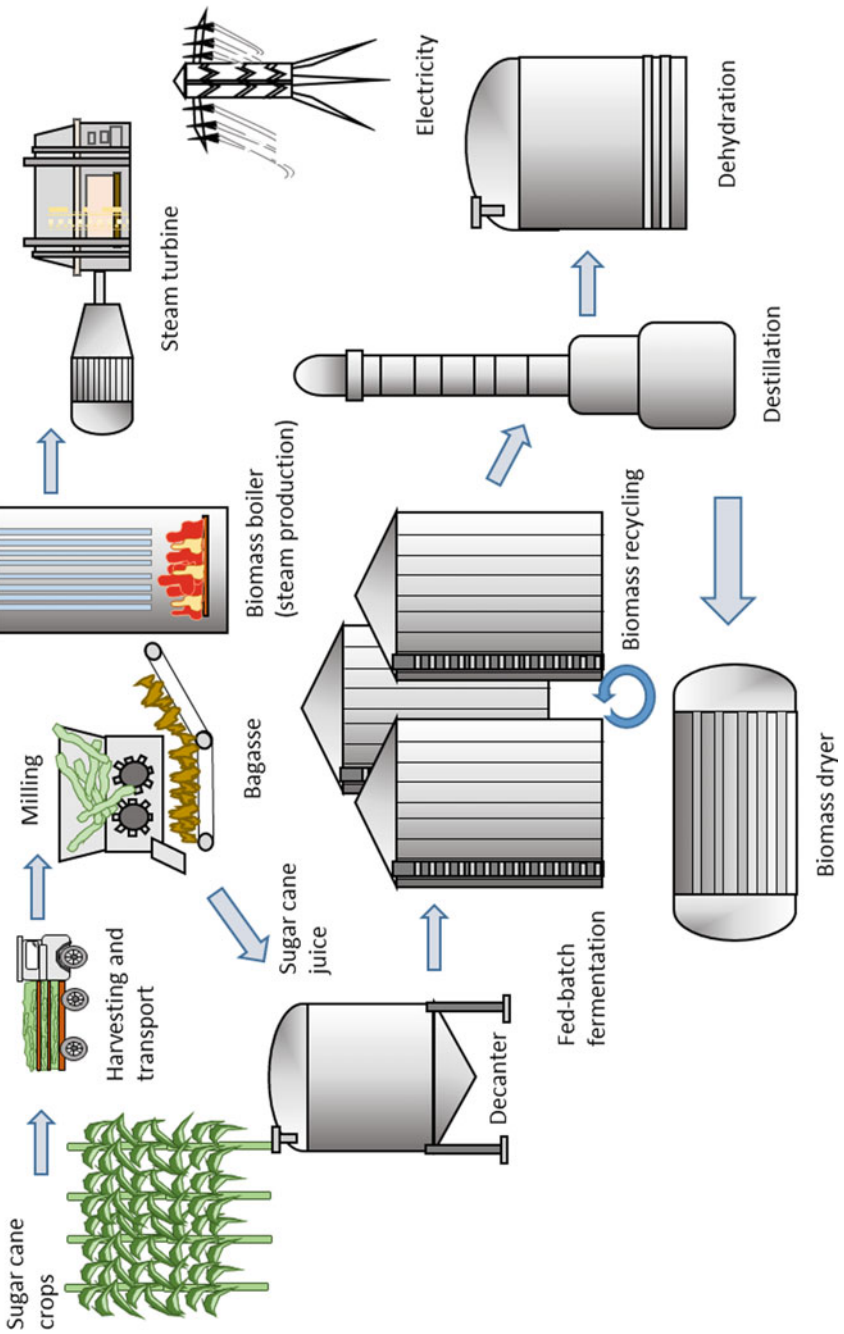

Fig. 10.2 Scheme of a typical plant for bioethanol production

The feasibility of these cereals processing plants is greatly dependent on the production of value-added by-products which can give higher revenues than the traditional sale of electricity, CO_2 and DDGS, with this later material presenting significant prices variations associated with the fluctuating price of cereals where it is produced from and thus affecting the final price of the resulting bioethanol (Pena et a1. 2012). Increasing quality (protein content) of DDGS is essential to gain profitability of ethanol industrial plants, but in any case the price reached of this by-product is limited by the market prices of animal feeding. This is the case of POET offering its DDGS improved product denominated Dakota Gold® HP™ (dakotagold.com) with higher protein content than that traditionally obtained from the standard DDGS. Increasing the protein content of this by-product has been subject of extensive research by several authors (Robinso et al. 2008; Singh et al. 2005) with Pena and co-workers (2012) proposing the use of a ligninolytic fungus selected from a screen of nine white-rot fungal strains. These authors reported a 32% increase in protein content after carrying out a secondary fermentation of DDGS. This process had the additional advantage of also producing an important ligninolytic enzyme with a variety of biotechnological applications.

The transformation of starch ethanol producing plants to those using lignocellulosic biomass is close to become a reality but yet, there is room for many intermediate stages needing optimisation. Despite the abundance of lignocellulosic biomass, the arrangement of its components presents a recalcitrant structure, requiring severe pre-treatments to allow the access of C-6 and C-5 sugars to the fermenting biomass, resulting also in a lignin fraction needing further valorisation (Amoah et al. 2019). The process and pre-treatment technologies for valorising this type of biomass are subject of a previous chapter. However, here it addressed the relevance in accomplishing high production yields on different by-products which would allow for attaining economic feasibility of industrial plants. One way of reaching this goal is by the integration of different fermentations capable of producing a variety of fuels from the valorisation of secondary streams from the multi-stage ethanol production process. This is the idea proposed by Ahring and Westermann (2007) for enhancing biofuel yields from biomass. The major fuels considered in this novel type of refinery are ethanol, hydrogen and methane from the use of corn- or grain-based bioethanol plants by the coupling of photofermentation and anaerobic digestion of volatile fatty acids, and the use of fuel cell systems along with catalytic reformation of methane for hydrogen production. This idea will be developed in the subsequent sections.

The different pre-treatment methods involved in the processing of lignocellulosic biomass are extrusion, steam explosion, liquid hot water, ammonia fibre explosion, supercritical CO_2 explosion and organosolv pre-treatment, other novel methods are ozonolysis pre-treatment, ionic liquids pre-treatment and biological pre-treatments along with enzymatic hydrolysis (Capolupo and Faraco 2016). Thus, the feasibility of refining lignocellulosic biomass to obtain either ethanol or any other class of fine chemicals is still in need of extensive research to make this process economically attractive.

Demonstration plants for the production of ethanol from cellulosic biomass are running at a pilot scale (some of them as large-scale pre-commercial prototypes) in an

attempt to evaluate the technical feasibility of these technologies. It should be borne in mind that the process should confront several burdens associated with the collection and transport of a diffuse source of lignocellulosic material in addition to the set of high energy-intensive steps necessary for the complete turnover of this component into ethanol and by-products. Table 10.1 shows a source of different demonstration plants constructed for the production of cellulosic ethanol, the common feature of most of them is that after promising a prosperous production, many suffered from adverse financing and the lack of a favourable regulation leading to either the shut-down of the production line or selling the industrial plant in an attempt of refinancing. The DuPont cellulosic ethanol plant (Nevada, Iowa) which was inaugurated under the promise of becoming one of the largest commercial ethanol-producing plant using non-feed feedstock had to find a new investor to be reconverted into a different line of business to produce renewable natural gas.

The cellulosic production of ethanol at industrial scale is based on the PROESA® technology, developed by Chemtex. The process requires physical pre-treatment of the feedstock by steam explosion to release the cellulosic material. By means of enzymatic hydrolysis, (either Novozyme technology or DMS technology) the cellulose is transformed into simple sugars which can be fermented into ethanol and other types of fine chemicals. When comparing this process with the traditional fermentation from soluble sugars, it is obvious that several difficulties arise in this technology. Figure 10.3 represents a scheme of the basic approach of the patented PROESA® process for producing ethanol listing also some of the particular points needing optimisation at large-scale implementation, having special relevance the effect of inhibitory compounds and the need of adapting harvesting and pre-treatment stages of biomass to the specific lignocellulosic material (Green Car Congress 2019). What is considered an efficient and economical pre-treatment for one type of feedstock may not necessarily translate into an efficient process for another type of biomass (Menon and Rao 2012).

Understanding enzyme pre-treatment and the main characteristics of the solubilisation of biomass polysaccharides is the central core of the biomass-to-bioethanol process. Xyloglucan-active hydrolases are enzymes which carry out hydrolysis and transglucosylation. Xyloglucans cover and cross-link the cellulosic microfibrils in plant cell walls making cellulose inaccessible to saccharification by cellulases. This compound is the major hemicellulosic polysaccharide in plant biomass. Xyloglucan hydrolases which are known to act synergistically with cellulases and xylanases are vital enzymes to release the plant cell wall and attain a successful bioconversion process (Saritha et al. 2016). The further conversion of polymeric cellulose or hemicellulose into simple saccharides (sugars) is highly dependent on the use of another type of enzymes such as endo-1, 4-β-glucanases, cellobiohydrolases and β-glucosidases which act randomly breaking down cellulose by attacking the amorphous regions to produce more accessible new free chain ends for the action of cellobiohydrolases (Annamalai et al. 2016).

Based on the currently available ethanol production process, a classification of biorefineries was proposed by Kam and Kam (2004) considering ‘phase I’ biorefinery as those current dry-milling ethanol plants. These types of plants use grain

Table 10.1 Some examples of industrial plants built for the production of second-generation ethanol

Project	Characteristic	Location
COMETHA Project	Industrial-scale pre-commercial plant. Finalising construction	Porto Marghera, (Italy)
Beta Renewables	Industrial scale: The refinery was built and operated by Grupo M&G. It was the world's first commercial-scale refinery, but economic crisis and the need of restructuring effort forced the cease of operation	Crscentino (Italy)
Abengoa	Industrial scale using cereals as substrate and demonstration plant for valorisation of cellulosic biomass. Shut-down and sold to an investment group for refinancing the company along with other similar plants of Abengoa	Babilafuente, Spain
Granbio	The first commercial-scale cellulosic ethanol plant in the Southern Hemisphere. The biorefinery, named Bioflex 1, transforms sugarcane residue, straw and bagasse into 'second generation' ethanol	Alagoas, Brazil
Canergy	Primary feedstock will be energy cane which is an approved EPA cellulosic feedstock. Energy cane is a perennial highly fibrous form of sugarcane with high content of cellulose. The company is experiencing delays in getting the plant operating and running	Imperial county, California, USA
POET/DSM's Project Liberty	Corn stover is used as cellulosic substrate for the fermentation process. It took additional efforts to integrate the different multi-steps of the large-scale process to get the whole plant running. Optimisation of corn stover pre-treatment stage proved to be challenging	Emmetsburg, Iowa, USA

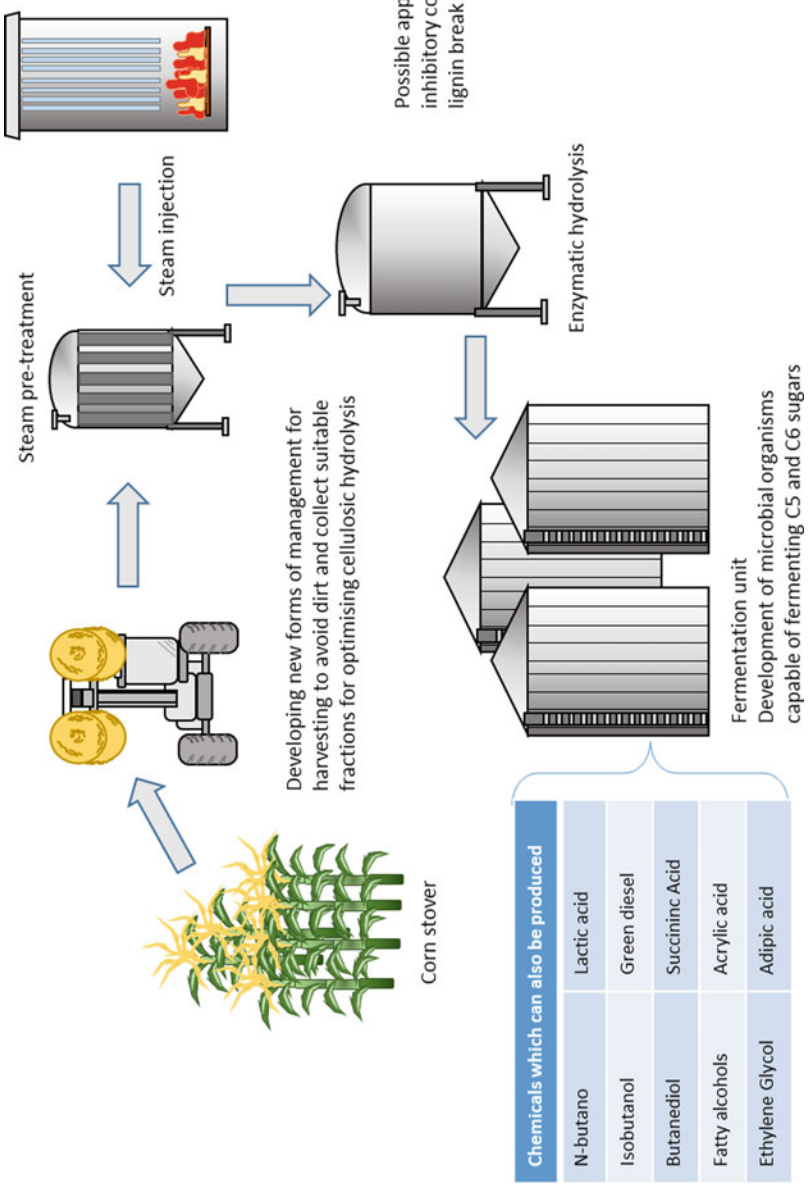

Fig. 10.3 Schematic representation of the production of ethanol from cellulosic biomass highlighting difficulties overcome at industrial scale to make the process a reality at running large-scale operating plants

as feedstock, have fixed processing capability producing a fixed amount of ethanol, feed co-products and carbon dioxide with no flexibility in processing. Phase II were considered by these same authors as those having current wet-milling technology. This technology uses grain feedstocks but it has the capability of producing various end products, depending on demand. Such products include starch, high fructose corn syrup, ethanol, corn oil and corn gluten feed and meal. Thus different fermentations can be connected to these biorefineries using the resulting stream of one previous stage as raw materials for different fermentation products, such as succinic acid, butanol and poly 3-hydroxybutyric acid among others (Du et al. 2007; Zverlov et al. 2006; Nonato et al. 2001; Rastegari et al. 2020).

On this same line, Kam and Kam (2004) classified a phase III biorefinery as the installation capable of producing not only a variety of chemicals, fuels and intermediates or end products, but also use a variety of feedstocks and processing methods to produce several types of goods for the industrial market. The flexibility on the use of different feedstock is the factor of first priority for adaptability towards changes in demand and supply feed, food and industrial commodities. The competition for the use of land for producing fuels and chemicals and that for producing food and feeds has led to an attempt for a new classification of biorefineries based on the use of feedstocks that would avoid interfering with traditional markets. This is why a great emphasis has been set on the valorisation of lignocellulosic biomass.

The products derived from the forest sector, and in particular the pulp and paper industry is currently undergoing a transitioning process where their traditional market may confront new opportunities for the development of novel products and market streams. Therefore, great opportunities exist for the cost-effective utilisation of wood components—hemicellulose, lignin and extractives (Kumar and Christopher 2017). Based on the different available ways for valorising lignocellulosic biomass, a classification of biorefineries has been developed by Dong et al. (2019). These authors denoted Type I biorefinery as those that attain a complete dissociation of lignin but keep it in the spent liquor. In this process, the majority of hemicelluloses remain in the fibre bundles so the structural sugars can be recovered after enzymatic hydrolysis. Solid–liquid separation operations are necessary for recovering lignin and the remaining solvents/catalysts are removed to avoid affecting bioconversion.

Type II biorefinery was classified by these authors as the complete removal of hemicelluloses intended to reduce the recalcitrance of lignocellulosic biomass but avoiding the generation of inhibitors to the subsequent saccharification and fermentation steps. The third classification (Type III) considers the decomposition of lignin and hemicelluloses along with the reduction of the crystallinity of cellulose. The decrystallised substrate can be regenerated after washing and become easily accessible by cellulose.

The previous classification is important since the pre-treatment applied of lignocellulosic material selected as feedstock needs to be specifically designed based on their intrinsic characteristics. Wood hemicelluloses contain mainly five types of sugars (mannose, galactose, glucose, xylose, arabinose), which are partially acetylated and have some lateral groups like 4-O-methyl glucuronic acid. The major hemicelluloses found in softwoods are galactoglucomannans, whereas in hardwoods

the dominant components are arabinoxylans (Bajpai 2018). Although one of the major features of a biorefinery is flexibility of treating different biomass materials, the complexity of each feedstock sets relevant technical impediments for optimising biomass fractionation and subsequent conversion.

In addition to lignocellulosic biomass, wastes are also important raw materials for obtaining biofuels and green chemicals. The initial concept of waste to energy (WtE) was defined by Villar and co-workers (2012) and refers to all technologies that convert, transport, manage and recover or reuse energy from any type of waste (solid, liquid, gas and heat) in a continuous industrial process. This concept associated with the transformation of any kind of waste stream is easily integrated into the biorefinery one, either by setting the conversion of biowastes into goods and energy or by valorising by-products derived from biomass fractionation technologies into the production of energy. Thus, the conversion of biomass (or wastes) needs to consider an integral approach of valorisation where all types of streams find an industrial use leading to zero emissions.

Table 10.2 presents different conversion alternatives for obtaining chemicals and energy. In addition to ethanol, butanol is also a short chain organic fuel compatible with gasoline which presents several advantages associated with the behaviour of butanol and gasoline mixtures. However, the production of this type of alcohol although being a well-known process presents several limitations associated with the low concentration levels tolerated by the fermentation broth and the multiple production of several solvents needing costly final refining stages.

10.2.2 Butanol

Butanol is produced in the so-called acetone-butanol-ethanol (ABE) fermentation. This fermentation was one of the main biological processes for producing chemicals having a scale of production similar to that of ethanol fermentation by yeast but its decline started after 1950 due to the increasing costs of substrate and the lower production price of chemical solvent synthesis by the petrochemical industry (Dürre 1998). However, the advances in the development of microbial processes for increasing product yield and new configurations of fermentation reactors have led to reviving the interest in solvent production in an attempt to decrease the high cost of butanol recovery stages associated with the low concentration attained in fermentation broth and the diversity of solvent product obtained (Qureshi and Blaschek 2001). Fed-batch reactor operation along with gas-stripping product recovery has led to increasing fermentation yields from 0.29 g/L h of total solvent productivity to 1.16 g/L h (Ezeji et al. 2004) which is a considerable success. The use of packed bed reactors under a continuous operation was evaluated by Wang et al. (2016) using immobilised *Clostridium acetobutylicum.* The continuous process was performed in the presence of oleyl alcohol used as extractant for in situ butanol recovery achieving high productivity (11 g/L h) while this value is significantly much lower when basic batch operating configuration was performed (0.2–0.4) (Formanek et al. 1997).

Table 10.2 Different non-conventional raw materials for producing ethanol and butanol

Fermentation product	Substrate	Characteristics	References
Ethanol	Municipal solid wastes	*Saccharomyces cerevisiae*	Li et al. (2007)
	Cotton gin waste pre-treated with organic acids	*Saccharomyces cerevisiae* and *Pichia stipitis* yeast strains	Sahu and Pramanik (2018)
	Lignocellulosic (agricultural wastes)	*Zymomonas mobilis*, *Candida tropicalis*	Patle and Lal (2007)
	newspaper waste	*Saccharomyces cerevisiae*	Bilal et al. (2017)
	Waste wheat straw	*Saccharomyces cerevisiae*	Han et al. (2015)
	Glycerol from biodiesel production	*Enterobacter aerogenes* HU-101, producing hydrogen and ethanol	Ito et al. (2005)
	Solka Floc, waste cardboard and paper sludge	*Kluyveromyces marxianus* (simultaneous saccharification and fermentation)	Kádár et al. (2004)
Butanol	Starch (cassava)	*B. subtilis* WD 161 and *C. butylicum* TISTR 1032	Tran et al. (2010)
	Cellulosic biomass	*C. thermocellum* and *C. saccharoperbutylacetonicum* N1–4	Nakayama et al. (2011)
	Alkali pre-treated rice straw	*C. thermocellum* NBRC 103,400 and *C. saccharoperbutylacetonicum* strain N1–4	Kiyoshi et al. (2015)
	Cheese whey	*K. marxianus* DSM 5422 and *S. cerevisiae* Ethanol Red	Díez-Antolínez et al. (2018)
	Food-industry wastes	*C. beijerinckii, C. acetobutylicum, C. saccharobutylicum* and *C. saccaroperbutylacetonicum*	Hijosa-Valsero et al. (2018)
	Orange peels	*Saccharomyces cerevisiae* NCIM 3495 and *C. acetobutylicum* NCIM 2877	Joshi et al. (2015)
	Paper mill sludge	*C. sporogenes* NCIM 2337	Gogoi et al. (2018)
	Grape pomace	*C. beijerinckii*	Jin et al. (2018)

Another interesting approach for by-passing the energy-intensive butanol recovery process is the use of biodiesel as the extractant. Fermentations of *Clostridium acetobutylicum* were evaluated using biodiesel as the in situ extractant by Li et al. (2010). Biodiesel added to the fermentation preferentially extracted butanol, minimising product inhibition, and increasing butanol production from 11.6 to 16.5 g/L. The fuel properties of the ABE-enriched biodiesel were also evaluated indicating that the key quality indicators of diesel fuel, such as the cetane number increased from 48

to 54 and the cold filter plugging point decreased significantly from 5.8 to 0.2 ºC, resulting in an outstanding improvement of biodiesel characteristics.

However, the use of low-cost material for making feasible the industrial production of butanol is necessary and thus involves the use of starchy materials or lignocellulosic biomass. Solventogenic *Clostridium* sp. utilise starch ineffectively due to its inexpression of amylases. Therefore, hydrolysis of starch is required to obtain sugars for the ABE fermentation (Jiang et al. 2018a, b). In this line, a novel butanol fermentation process was developed by Qureshi et al. (2016) using as lignocellulosic biomass sweet sorghum bagasse pre-treated with liquid hot water (190 ºC) followed by enzymatic hydrolysis. The hydrolysate was successfully fermented without inhibition, and an ABE productivity of 0.51 g/L h was achieved which was comparable to the 0.49 g/L h observed in the control fermentation using glucose as a feedstock. In this same line of research, the use of pre-treated corn stover as substrate was evaluated by Xue and co-workers (2016), in this case accompanying the fermentation process of butanol recovery by vapor stripping–vapor permeation (VSVP). The condensate produced from this separation technique contained butanol in a range from 212.0 to 232.0 g/L (306.6–356.1 g/L ABE) from a fermentation broth containing ~10 g/L butanol.

The high cost associated with pre-treatments of lignocellulosic and starchy materials along with the energy demand of these processes supposes an important obstacle to circumvent. Co-culturing systems are an ideal and simple way to achieve direct butanol production from starchy-based feedstocks, in which starch is firstly hydrolysed by amylolytic strains, and then released sugars are converted to butanol by mesophilic solventogenic organisms, such as *Clostridium beijerinckii* and *C. Acetobutylicum* (Jiang et al. 2018b). An increase in the efficiency of the whole process may be attained by coupling hydrolytic enzyme production, lignocellulose degradation and microbial fermentation in one single step. This microbial co-cultivation system was studied by Jiang and co-workers (2018b) consisting of *Thermoanaerobacterium* sp. M5 and *C. acetobutylicum* NJ4 achieving a butanol titer of 8.34 g/L from xylan.

The technical and economic feasibility of revitalising butanol production lies not only in the use of inexpensive lignocellulosic hydrolysates and high productivity bacteria, but also in the optimisation of techniques capable of detoxification and efficient continuous fermentation technologies along with in situ product recovery to avoid inhibitory conditions which are typical of this fermentation (Maiti et al. 2016). Life cycle assessment was performed by Pereira et al. (2015) to integrate the biobutanol production process in a sugarcane biorefinery in Brazil. This evaluation indicated that butanol derived from bagasse and straw pentoses using genetically modified microorganism presented the best environmental performance. The introduction of butanol and acetone to the product portfolio of biorefineries leads to an increase of revenues that should not be overestimated.

10.2.3 Biodiesel

Biodiesel, along with ethanol, is also a widely used biofuel compatible with diesel fuels consisting of a mixture of fatty acid methyl esters. Legal mandates for commercialising blends of petrol and diesel fuels with their compatible homologous sets the demand of these biofuels to be directly linked to the consumption of conventional transport fuels. Blends at 5 and 10% of biofuels are commercialised worldwide without the need of making changes in engines. These features have allowed the great expansion of bioethanol and biodiesel industry. The Brazilian transport sector has adapted to include flex-fuel motors capable of running on E0 to E100 (from zero to a hundred percent content in ethanol); thanks to the presence of sensors in the fuel system that automatically recognises the ethanol level in the fuel (Goldemberg 2008).

The production of biodiesel is also linked to the use of land, just as in the case of ethanol production, but regarding the harvesting of oil accumulating plant species. The fabrication of biodiesel is based on chemical reactions involving the transformation (transesterification) of lipids with alcohols (usually methanol or ethanol) in the presence of a catalyst for producing the methyl (or ethyl) esters. In this process, the transesterification reaction involves the separation of glycerine from the fatty acid by means of sodium or potassium hydroxide as catalysts (Refaat 2011). Glycerine is obtained as valuable by-product requiring neutralisation and further refining to be used in pharmaceutical and cosmetic industry. The alcohol used in excess is recovered in the final stage by distillation and returned to the fabrication process. A general scheme is presented in Fig. 10.4 where the main crops for obtaining lipids are also represented.

The great demand for the production of biodiesel worldwide has not been exempted of polemic. The substitution in the use of land traditionally dedicated to human and animal feeding is a risk that should be avoided. Another important burden for the further promotion of biodiesel and in general of any other type of biofuel is the price. High production costs make biofuels unprofitable without subsidies. Biodiesel in principle provides sufficient environmental advantages to merit subsidy in an attempt to lower the price of transportation biofuels, including also in this characteristic synfuel hydrocarbons and cellulosic ethanol (Hill et al. 2006). However, the environmental benefits may not be clear in all available production schemes, since a conscious emission study may result in negative outputs when all resources involved in the production of biofuels are considered. When compared to petroleum-derived fuels, it is usually assumed that biofuels derived from biomass feedstock provide substantial emission savings, due to the simple reasoning that emissions released from biofuel combustion are absorbed from the atmosphere throughout plant growth, thus resulting in a zero emissions footprint. The evaluation of the whole biofuel production process which should involve also the cultivation of the biomass feedstock, the effect on the increase in feedstock prices and economic incentives to acquire additional land to site plantations substituting the original use of land may result in a disappointing outcome where the released CO_2 with the use of biofuel

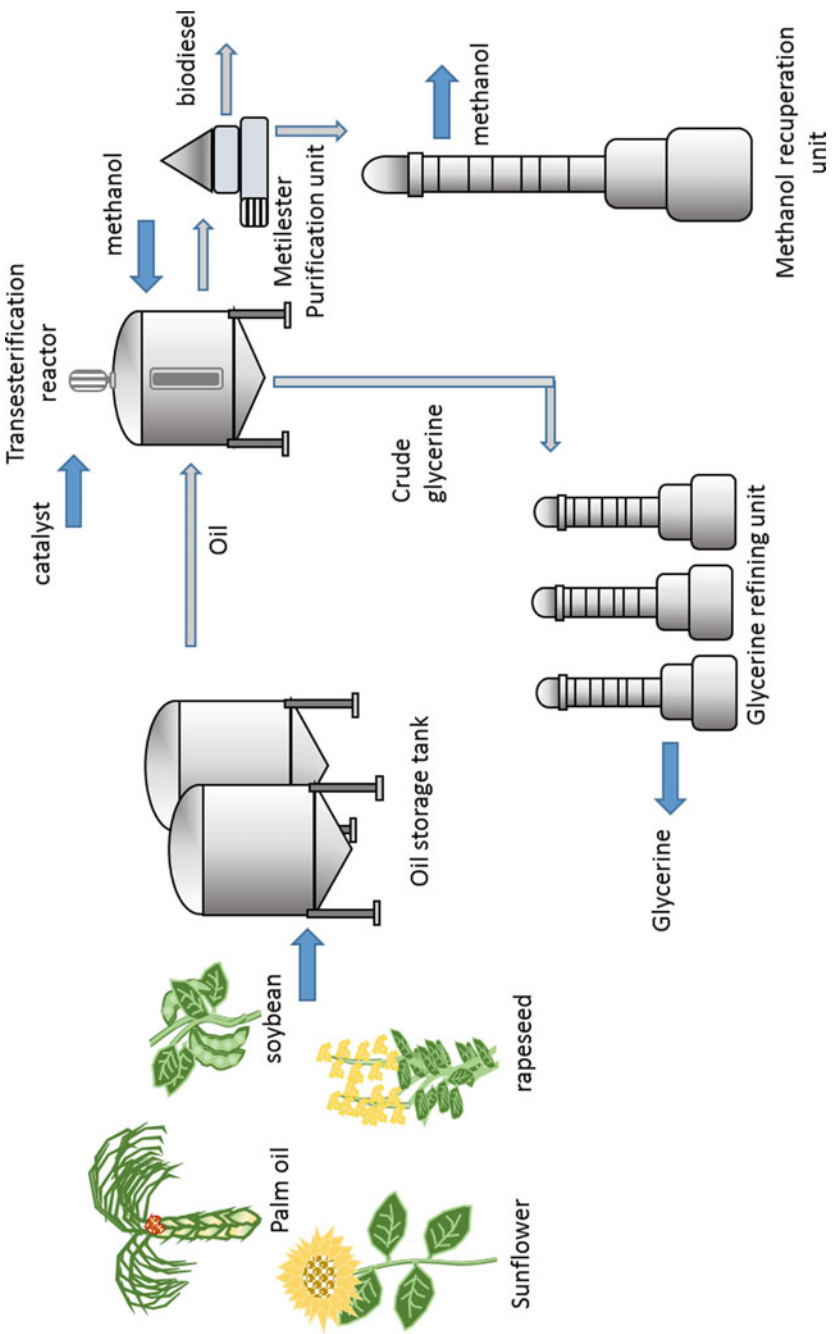

Fig. 10.4 General scheme of biodiesel fabrication process based on transesterification of lipid molecules

would be higher than if a traditional fossil fuel was combusted (Blakey et al. 2011). To all these previous facts, another point that should be taken into consideration is that the financing of governments is obtained to a great extent from taxes associated with conventional fuels sales. If the biofuel market becomes an important part of the transport sector, then the financing of governments should have to be derived from additional taxes associated with other industrial and social sectors.

There are tremendous opportunities for exploring alternative fuels especially with the growing importance of biodiesel and jet fuel in the trucking and aviation industries (Li and Mupondwa 2014). The need for these alternative fuels to be derived from biomass materials to keep a low greenhouse gas (GHG) emission balance causes an extra increase in the costs of production when low-input biomasses grown on agriculturally marginal lands or waste biomass are used as raw materials. However, this increase in production cost should counterbalance against the environmental benefits and the market distortions avoided against the use of food-based biofuels (Hill et al. 2006).

In this regard, crops such as jatropha and camelina are gaining attention as new feedstocks for biodiesel and jet fuel production based on the fact that nutrient needs of these crops are much lower than that of the traditional lipid crops. The study of Li and Mupondwa (2014) reported on GHG emissions from camelina derived biodiesel indicating that 1 MJ of energy contained in biodiesel derived from this source required a consumption ranging from 0.40 to 0.67 MJ/MJ non-renewable energy and for producing HRJ fuel ranged from −0.13 to 0.52 MJ/MJ. Camelina oil as a feedstock for fuel production accounted for the highest contribution to overall environmental performance, demonstrating the importance of reducing environmental burdens during the agricultural production process.

The interest in producing biofuels that are completely compatible with existing engines in all transportation sectors has set the focus on the development of processes for producing the so-called drop-in biofuels. The name is derived from the advantage these fuels offer for completely behaving in an equivalent manner to petroleum fuels. Currently, conventional/oleochemical feedstocks (lipids) can be easily upgraded and integrated into oil-refinery processes but the future interest is in developing thermochemical processes capable of using directly lingnocellulosic biomass to be transformed into drop-in biofuels (van Dyk et al. 2019). Thermal processes as pyrolysis and gasification allow for the conversion of lignocellulosic biomass for producing biocrude in the first case and mainly hydrogen and carbon monoxide (main constituents of syngas) in the later. Therefore, the two common alternative technologies for producing biodiesel fuel is the Fischer–Tropsch (FT) fuels to replace conventional kerosene and hydroprocessed renewable jet (HRJ) fuels made from hydroprocessed oils (Li and Mupondwa 2014).

Another source of lipid raw materials which is currently under intensive research is the culturing of microalgae for producing lipid feedstocks and also in a completely different but parallel novel line of research is the fermentation systems for producing single cell oils. Microalgae species can accumulate substantial contents of lipids based on the culturing conditions to which they are submitted. The oil content in microalgae may reach values as high as 75% (w/w of dry biomass) (Metzger and

Largeau 2005; Gonçalves and Silva 2018) but a relevant factor to take into account is the rate of lipid accumulation, since this parameter is crucial to set the volumetric productivity of the culturing pond. Most of the microalgae accumulate oils in a range between 20 and 50% (e.g. *Chlorella, Dunaliella, Isochrysis, Nannochloris, Nannochloropsis, Neochloris, Nitzschia, Phaeodactylum and Porphyridium* spp.) and the fact that many of these species can be grown on seawater makes of this option an interesting harvesting platform. However, some other factors besides high productivity should be carefully examined as it is the lipid profile of the microalgae cell since it will dictate the resulting characteristics of biodiesel (Amaro et al. 2011).

The principal investment for an algae biomass project may be split into the costs associated with the growth of these organisms, harvesting (steps as isolation of the biomass from the culture, dewatering and/or concentration of algae to facilitate further processing stages) and finally the extraction of algal oil (Singh and Gu 2010). The growth of microalga depends on a supply of carbon and light to carry out photosynthesis. Among the different types of metabolisms, controlled changes in environmental conditions can cause metabolic shifts affecting growth rate and lipid productivity (Amaro et al. 2011). Microalgae can grow photoautotrophically (in the light), heterotrophically (use of a substrate as carbon source) or photoheterotrophically (using simultaneously light and a substrate as carbon source), one organism capable of these three characteristic growths is *Spirulina* sp. (Chojnacka and Noworyta 2004).

The main advantages of producing biofuels from the culturing of microalgae systems are the high efficiency as it is evidenced from the high biomass yields per hectare when compared to lipid yields from conventional crops. The productivity of microalgae biomass can be estimated in 1.535 kg/m^3 d, if an average oil content as 30% (w/w dry biomass) is assumed, this yield would be 98.4 m^3per hectare, while this value for palm oil (which is the lipid producing crop with the highest productivity) is estimated in 4.8 m^3per hectare (Taparia et al. 2016). In addition, microalgae can be harvested all year round producing a continuous supply of oil, although harvesting and concentration stages may have a higher cost when compared with conventional oil-producing crops, the reliability of this process may counterbalance this disadvantage. Finally, the avoided use of freshwater resources in microalgae biofuel production is another feature which should not be disregarded (Schenk et al. 2008).

Farming is one of the largest commercial consumers of water, on average 20 mega litres of water/ha is required by the crop to fulfil evapotranspirational needs and account for losses during the course of irrigation (Shrivastava et al. 2011; Greenland et al. 2018). The requirements of water for producing ethanol as biofuel from sugarcane are estimated in 88 kg water/kg cane for a plant crop (Singh et al. 2018), which gives an idea of the relevance of this parameter when evaluating the efficiency in the use of resources. On this same line, Shi and co-workers (2017) evaluated the water needs for producing hydroprocessed ester and fatty acid (HEFA) jet fuel from rapeseed cultivation, indicating that the water footprint calculated for jet fuel production in North Dakota was 131–143 m^3 per GJ fuel. These data are strong arguments to favour the development of alternative production of lipid feedstock for biodiesel or drop-in biofuels. In fact, some experimental work is intended to the use of liquid

digestate as culture medium form microalgae systems, this is the case of Montero and co-workers (2018) who evaluated the cultivation of *Chlorococcum* sp. obtaining biomass productivities of 23.4 mg/L d although a high dilution proportion was used (5.6% v/v). In a similar approach, anaerobic digestates were tested under batch cultivation of *Chlorella* sp. for oil production. Pig farm digestate was found most suitable as the growth medium generating 0.95 g/L medium (dry biomass) (Chaiprapat et al. 2017).

A schematic representation of the process for obtaining lipids from microalgae cultures is shown in Fig. 10.5. Different culturing ponds and reactors systems have been developed in an attempt to increase volumetric biomass productivity and counteract the effect of light shading of high-density cultures. A full description of factors affecting microalgae growth and types of the different reactors under development can be found elsewhere (Bajpai 2019; Grobbelaar 2010; Ugwu et al. 2008). Previous to lipid extraction, the removal of chlorophyll is necessary, since this compound makes the oil more susceptible to photo-oxidation and decreases its storage stability (Park et al. 2014). In addition, the presence of chlorophyll can decrease the efficiency of transesterification and interfere with biodiesel quality characteristics thus the relevance of its removal as a key step in the commercial production of microalgae oil (Li et al. 2016).

The residual microalgae biomass is a fraction needing further valorisation. The use of microalgae as input for different bioconversion processes has been studied by several authors, considering the anaerobic digestion as a feasible option due to its high biogas production potential (Sialve et al. 2009). Biogas potential of *S. platensis* was studied by Varol and Ugurlu (2016) showing high volatile solid removal in batch studies (about 89–93%) achieved under initial total solids concentrations of 0.6–5%. Another way of valorising this biomass is by thermal methods, either pyrolysis for producing biocrudes, hydrothermal liquefaction or co-combustion with conventional fuels (Coimbra et al. 2019; Eboibi 2019; Mohammed et al. 2018).

In addition to microalgae, many other microorganisms like yeast, bacteria and fungi, have the ability to accumulate oils under special culture conditions. These microbial oils might become one important raw material for the fabrication process of biodiesel once the reduction in fermentation costs is attained by the use of wastes as substrates and the avoidance of sterilisation stages which are crucial to increase economic feasibility (Martínez et al. 2015). Many yeast species, such as *Cryptococcus albidus*, *Lipomyces lipofera*, *Lipomyces starkeyi*, *Rhodosporidium toruloides*, *Rhodotorula glutinis*, *Trichosporon pullulan* and *Yarrowia lipolytica*, were found to be able to accumulate oils under some cultivation conditions where parameters such as C/N ratio, temperature, pH, oxygen and concentration of trace elements and inorganic salts have a significant influence on the yields of oil accumulation (Li et al. 2008).

Lipid accumulating microorganisms have the capacity to store lipids to a content greater than 20%, with a similar triacylglycerol (TAG) structure of that of oil derived from plants (Ratledge 1993). Fungi have been studied for producing specific polyunsaturated fatty acids (PUFA), whereas oleaginous moulds have been cultivated for producing high-value PUFA because the oil accumulated is characterised by a higher

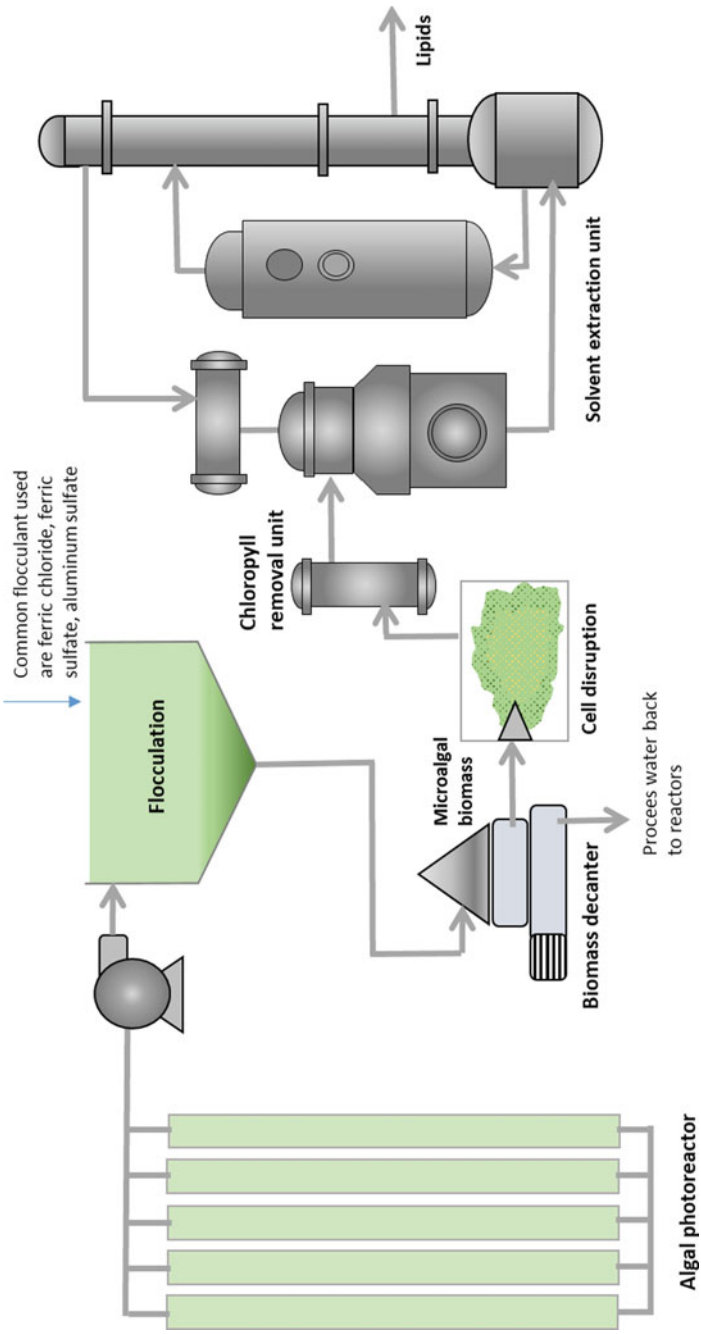

Fig. 10.5 Representation of microalgae lipid production process

level of unsaturation (Papanikolaou and Aggelis 2011). Yeast exhibit several advantages over other microbial sources, associated with high productivity because their duplication times are usually lower than 1 h, are much less affected than plants by season or climate conditions, and their cultures are more easily scaled up than those of microalgae since there are no constraints associated with the penetration of light into the reactor. Additionally, some oily yeasts have been reported to accumulate lipids up to 80% of their dry weight and can generate different types of oils from a variety of carbon sources or from low-quality lipids present in the culture media with the aim to increase their quality (Ageitos et al. 2011).

Some species of bacteria have the ability to accumulate oil, but the lipid composition is usually quite different from that of yeast strains. Bacteria usually produce complex lipids, such as polyhydroxyalkanoic acids, as a means of energy storage, and these compounds are deposited as insoluble inclusions in the cytoplasm. This accumulation process takes place when a carbon source is available in excess but there is also a deficiency of another nutrient (usually nitrogen) thus limiting the growth capacity. The accumulation of lipids for yeasts and some bacteria belonging to the actinomycetes group takes place mostly during the stationary phase of growth when proteins are not being synthesised with these organisms being highly affected by the type of carbon source and conditions applied (Martínez et al. 2015; Spiekermann et al. 1999).

In recent years, the search for valorising waste material and obtaining new sources for the production of biofuels has been intensive. In this regard, the valorisation of effluents obtained from palm oil mill has been studied by Louhasakul et al. (2016) using a novel approach for generating an extra source of biodiesel. These authors proposed the use of palm oil mill effluents (POME) which is a high organic (carbohydrate and proteins) content liquid stream also containing high amount of nutrients and mineral salts. This effluent was used as culture media of the marine yeast *Yarrowia lipolytica.* After the selection of strains, *Y. lipolytica TISTR 5151* was reported to produce lipids and cell-bound lipase at the highest levels of 1.64 ± 0.03 g/L and 3353 ± 27 U/L, respectively. The main relevance of the idea behind these type of experiments is the possibility of culturing a single organism for valorising a waste stream from palm oil production and perform the direct transesterification reaction using cell-bound lipase from the wet yeast cells and produce 40.9% of fatty acid methyl esters, without the need of costly procedures such as isolation, purification and immobilisation.

Another approach is the use of molasses for growing lipid accumulating organisms. Molasses is a by-product from the processing of cane or beet sugar and contains uncrystallised sugar and some sucrose. The use of this carbon source was proposed by Jiru and co-workers (2018) for obtaining lipids and evaluating also the quality of the resultant biodiesel from the transesterification reaction. In their study, *Rhodotorula kratochvilovae* (syn, *Rhodosporidium kratochvilovae*) SY89 was cultivated in a nitrogen-limited medium using molasses. The yeast was able to accumulate lipids to a content of 38.25 ± 1.10% on a cellular dry biomass basis corresponding to a lipid yield of 4.82 ± 0.27 g/L. Although these results are promising, they are still far from making the process feasible for an industrial application. Increasing concentration of

titers in the reactor is vital to scale the process and lower production costs. In this line, Matsakas et al. (2015) reported on the use of sweet sorghum at high solid concentrations as a feedstock for single cell oil production by *Rhodosporidium toruloides*. Sweet sorghum is considered an excellent carbon source for this process because it possesses high photosynthetic activity yielding high amounts of soluble and insoluble carbohydrates, requiring low fertilisation inputs and irrigation rates. These authors obtained a fermentation yield of 13.77 g/L (content of lipid in the culturing media) when using sweet sorghum juice (20% w/w enzymatically liquefied sweet sorghum).

Pilot-scale tests have also been performed using a reactor volume of 1 m^3. This scale of process had allowed also to assess the economic evaluation of the lipid recovery stage and performance of biodiesel in diesel engines. This study was reported by Soccol et al. (2017) and attained a lipid concentration in the reactor of 20.5 g/L using as substrate sugarcane juice and *Rhodosporidium toruloides* DEBB 5533 as lipid accumulating organism. Under conditions tested, the estimated final cost of microbial biodiesel produced was US$ 0.76/L, considering in this assessment, energy and steam demands in addition to raw materials and fermentation costs.

Another interesting approach for increasing the efficiency in the utilisation of resources associated with the fabrication of biodiesel is the use of crude glycerol as carbon source for transforming this chemical into lipids. Thus, crude glycerol can be further valorised without the need of processing through costly fractionation and distillation stages (Ma and Hanna 1999). This is the approach tested by Dobrowolski et al. (2016) using *Yarrowia lipolytica* A101 in fermenter obtaining from batch cultivation in a bioreactor a lipid content of 4.72 g/L. Although productivity is not as high as in reports described above, these results allow for a cascade valorisation of raw materials for increasing the productivity of the global biorefinery performance.

10.2.4 Biogas

The energy and climate policies in the EU and the introduction of various support schemes intended to promote the use of renewable resources have encouraged the installation of industrial biogas plants. In Europe, most of the modern anaerobic digesters provide electricity and heat in electricity-only plants, heat only or combined heat and power (CHP) plants (Scarlat et al. 2018). However, in many European countries, the treatment of wastes for producing heat and electricity may not be economically feasible due to the low organic content of some waste streams or to the low biochemical methane potential of some of these materials. Many of these plants, dedicated to the treatment of animal manures struggle to find suitable co-substrates compatible with the process to increase the biogas productivity of the digester which has a direct impact on revenues derived from electricity and heat generation. Co-digestion with animal manures has become in many cases the most adequate alternative for attaining profitability. It has been extensively reported the increase in biogas production when co-substrates such as agricultural wastes (Cuetos et al. 2011, 2013), food wastes (Li et al. 2013; Ormaechea et al. 2017; Zhang et al.

2017) and industrial wastes (Gómez et al. 2007; Nordell et al. 2016) are treated along with animal manures and similar increments have also been reported in the case of sewage sludge treatment (Gómez et al. 2006; Martínez et al. 2012; Oliveira et al. 2018).

Another option to increase economic feasibility of digestion plants is to identify and explore alternative products/chemicals in addition to the production of energy by adopting the biorefinery approach. The integration of different processes intended for biomass conversion to produce fuels, power and chemicals seems an interesting configuration to increase the industrial efficiency in the production of biomass-derived products (Sawatdeenarunat et al. 2016). The production of biogas can be proposed as a last step valorisation in the biorefinery concept. This is the idea presented by Uellendahl and Ahring (2010) who proposed the valorisation of the effluent from the ethanol fermentation when using pre-treated lignocellulosic biomass. The anaerobic digestion of this effluent showed no signs of toxicity to the anaerobic microorganisms. This idea was materialised in a commercial strategy under the BioGasol company in the field of renewable energy for the sustainable production of bioethanol based on lignocellulosic biomasses. Conversion of straw and other agricultural residues into ethanol, biogas, hydrogen and solid fuel with reuse of process water is possible with this complete valorisation scheme.

There is a vast experience in anaerobic digestion processes with several reports indicating the successful digestion of agricultural, food-industry wastes and those derived as by-products from other processes conforming multiple valorisation approach of the biorefinery concept, as it would be, the digestion of vinasses which are side-stream effluents from ethanol fermentation (Buitrón et al. 2019; Cabrera-Díaz et al. 2017; Martínez et al. 2018a, b). The implementation of vinasse biodigestion in sugarcane biorefineries has been studied by Longati et al. (2019) who reported a positive impact when evaluating introduction of this technology into ethanol type biorefinery. The use of biogas from vinasse for a standard first-generation ethanol plant can increase in 9.20% the surplus of electric energy yielded to the grid, which has a significant impact on the global energy balance of the process. Estimated values of methane yield from vinasses are 0.234–0.300 m^3 $CH_4/COD_{removed}$ (Júnior et al. 2016; Fuess et al. 2016) which gives a clear idea of the high potential of this effluent for producing bioenergy. Despite the significant improvements in both scientific and technological aspects related to anaerobic digestion of vinasse, pilot- to full-scale experiences are still scarce even though biomethane production in ethanol processing plants results in outstanding performance regarding electricity generation (Fuess and Zaiat 2018).

In a similar line, of valorising side streams from conventional biofuel production processes, is the use of crude glycerol as co-substrate in digesters treating either sewage sludge or manures. Different authors report increments of methane yield from 35 to 50% in average with the increase in glycerine ratio (Lobato et al. 2010; Fierro et al. 2016). Crude glycerol provides high organic load to digesters allowing for a significant increase in the biogas performance of reactors. Valorisation of this side stream from biodiesel production process can thus be carried out without the

need of further refining. However, there are limits to the use of glycerine as co-substrate since its presence in the digester alters the microbial flora and causes a preferential degradation of this readily biodegradable substrate leading to an incomplete stabilisation of the main feeding (animal manure) (Fierro et al. 2016; González et al. 2019). Another important issue is based on the fact that digestion is a process performed on sequential reactions, where the organic material is first acidified and then these short-chain fatty acids must be submitted to further degradation by the action or archaea microflora. Any unbalance associated with overloading of readily degradable material may cause fatty acid build-up leading to the decrease in biogas production by methanogenic inhibition.

Another approach where anaerobic digestion was proposed as the final valorisation step to be integrated into a biorefinery was that of Martínez et al. (2018a, b). The concept proposed by these authors considers the use of green biomass, where this material is first subjected to mechanical fractionation generating two fractions: one solid called press cake and another liquid known as green juice. The press cake is composed of lignocellulosic fibre material and residual proteins, which makes it a valuable feed, or it can either be used as lignocellulosic feedstock for biofuels and green chemical production. The green juice contains non-denatured proteins and free amino acids which can be valorised for producing protein concentrates, leading to a residual effluent called brown juice containing water-soluble carbohydrates, residual proteins and minerals which is suitable for anaerobic digestion. Therefore, the whole valorisation of biomass is attained producing a great diversity of green chemical products and energy.

It seems logical to consider conventional centralised waste treatment plants as centres for the transformation of organic materials and, therefore, grant these facilities greater status by converting them into biorefineries. Biomass such as lignocellulosic material and wastes can be valorised in conjunction. Wastes contain various high-value chemical substances and elements, including carbon sources in the form of carboxylic and other acids, carbohydrates, proteins and nutrients such as nitrogen (N), in the form of ammonium, phosphorus (P) and metals (Zacharof 2017). The use of recovered materials from waste is beneficial for the environment but also for the economy and the digestion process is capable of producing a valuable energy source and a stable form or organic matter suitable to be incorporated into different processes to benefit from the recovery of nutrients and organic compounds. For example, phosphate rock is a non-renewable natural resource with different applications including drinking water softening, feed and food additives and fertilisers. Mining phosphate rock is gradually becoming costlier (Zacharof 2017) and the depletion of this element is making imperative the search for the recovery of phosphorus from waste materials. This idea has been explored through the recovery of phosphorus from pig-slurry by a biological acidification step in the form of struvite (Daumer et al. 2010; Piveteau et al. 2017). By means of a lactic acid fermentation 60–90% of total phosphorus and total magnesium could be easily solubilised without interfering in a subsequent valorisation of the slurry by anaerobic digestion (Piveteau et al. 2017).

The production of biogas by means of anaerobic digestion also produces a digestate still needing final disposal. Digestates have been traditionally used as organic

amendments for crops, but the great size of many of these installations sometimes makes unfeasible the spreading during some seasons or even the whole year round, thus becoming a problem. One alternative recently proposed was the thermal treatment by pyrolysis (Feng and Lin 2017) where organic compounds presenting high water content are first introduced in an anaerobic digester for biogas recovery and subsequent pyrolysis of the slurry is attained for producing gases and oil which can be valorised as fuels along with a char fraction having interesting properties in agronomic application and for improving the performance of fermentation systems due to its capacity to act as carbon conductive material (Gómez et al. 2018; González et al. 2018). Pyrolysis is a thermal process where organic compounds are decomposed under inert atmosphere, generating light gaseous products (short-chain hydrocarbons) along with hydrogen, carbon monoxide and carbon dioxide. The characteristics of products and yields obtained for the different fractions are highly dependent on process conditions (temperature and reactor operation) and heating ramp (Tripathi et al. 2016).

The use of char derived from pyrolysis has been studied for improving the performance of anaerobic digestion, thus reporting on a better stabilisation of the microbial system when inhibitors are present either by adsorbing onto the carbon surface the toxic compounds or by offering protecting sites to the microflora (Martínez et al. 2018b). In addition, the effect of char has been also evaluated for assessing the performance of high-loaded systems, indicating that the presence of this material accelerated the degradation rate of substrates up to 86% and favours the selective colonisation of functional microbes (*Methanosarcina* and *Methanosaeta*) (Luo et al. 2015).

Figure 10.6 shows a schematic representation of the valorisation of manures and lignocellulosic biomass integrated in a biorefinery concept for biomass conversion into biofuels. In this scheme, it also considered the treatment of pyrolysis water obtained from condensation reactions. The thermal conversion of biomass in a pyrolysis process yields in addition to oxygenated biooils, water derived from the initial content of the material and that formed through the thermal transformation process. Biooils and water form a miscible phase due to the oxygen content of the oily phase. The water thus obtained in this process needs further treatment. It is estimated that the water content of biooils may be as high as 52% of the total oil fraction (Abnisa et al. 2013; Mullen and Boateng 2011). The treatment of this aqueous phase by anaerobic digestion has been attempted by Hübner and Mumme (2015) reporting a removal of organic content of about 63.4% (measured as chemical oxygen demand, COD) and having a significant effect on the degradability of this liqueur, the temperature of the pyrolysis process. In addition to the use of char for enhancing the digestion process, this scheme is also considered the agronomic application of this material. Char addition to different crops has been reported (Rondon et al. 2007; Rosas et al. 2015) to favour carbon sequestration and enhance nutrient retention reducing thus run-off and the number of fertilisations needed because of the better use of fertilisers by plants (Van Zwieten et al. 2010) and causing a modification of the soil ecological niche in the long term (Hardy et al. 2019).

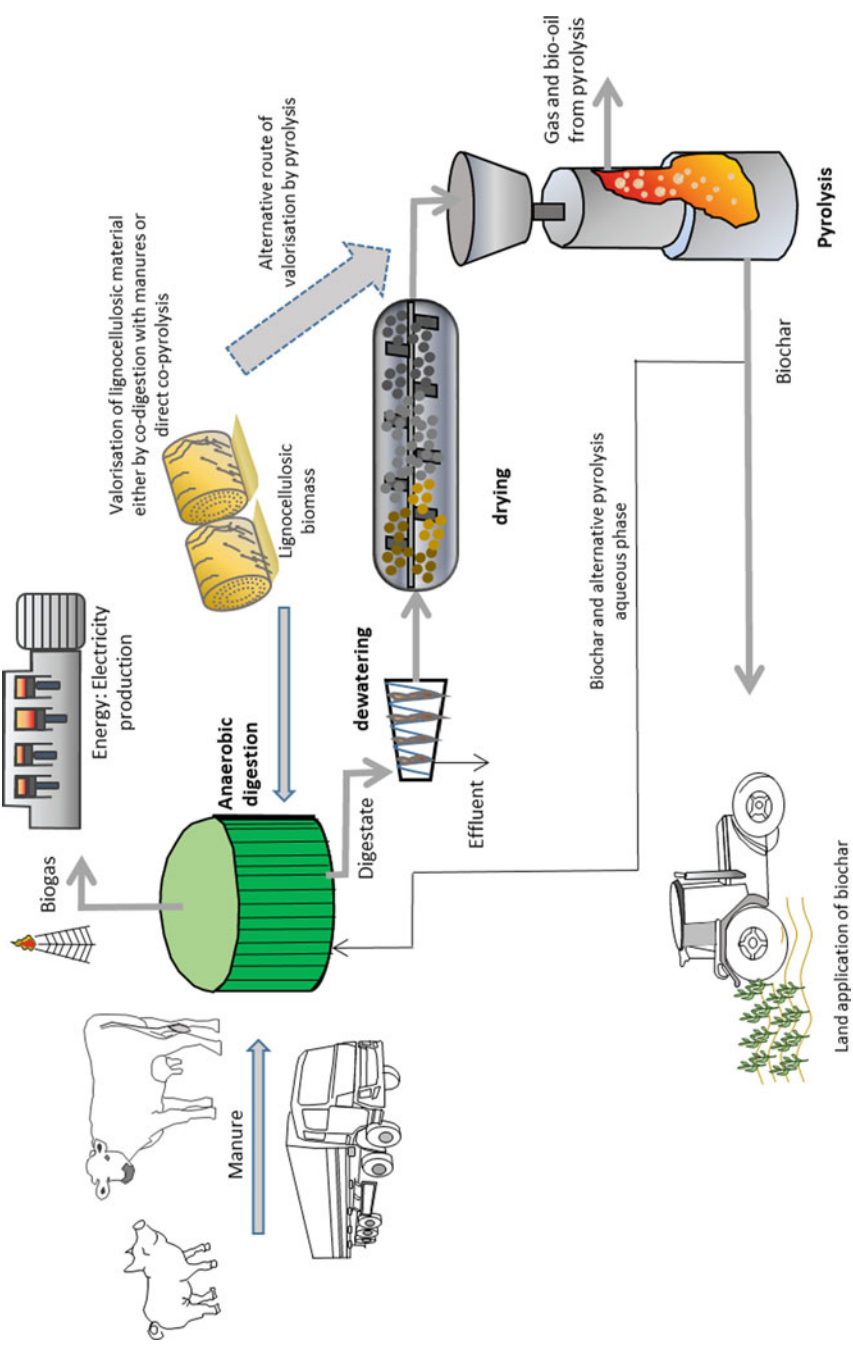

Fig. 10.6 Valorisation of manures and lignocellulosic biomass for producing biogas and pyrolysis valuable products from the combined pyrolytic treatment of digestates

10.2.5 Hydrogen

Biological production of hydrogen can be attained by different ways as it is direct and indirect biophotolysis and photofermentation (light-dependent methods), bioelectrochemical systems (BES) and dark fermentation process (no light-dependent methods) (Martínez et al. 2019a, b). Photofermentation is the biological process of converting organic molecules to H_2 and CO_2 in the presence of light, in anaerobic, nitrogen-limited conditions. Photosynthetic purple non-sulphur (PNS) bacteria as *Rhodobacter sphaeroides* and other PNS bacteria can produce hydrogen using a variety of organic compounds (Sagir et al. 2017). The limitation of nitrogen forces the bacteria to 'dump' the excess energy and reducing power through the production of hydrogen (Koku et al. 2002).

Two enzymes namely, nitrogenase and hydrogenase play an important role in biohydrogen production. Photofermentation by PNS bacteria can attain a significant increase in hydrogen yields of the biological process by optimisation of growth conditions and immobilisation of active cells (Basak and Das 2007). PNS bacteria have the ability to use light energy in a wide range of absorption spectra (522–860) nm without evolving oxygen which might cause inactivation of the system. Energy from light enables these organisms to overcome the thermodynamic barrier in the conversion of organic acids into hydrogen (Kumar et al. 2019; Miyake et al. 1982; Basak and Das 2009). Their ability to assimilate different types of carbon sources has led to the development of hydrogen-producing systems as a single stage, using glucose and sucrose, along with hydrocarbon-rich substrates as black strap, and beet molasses with hydrogen yields reported in a single-stage configuration in the range of 9–14 mol H_2/mol substrate (Abo-Hashesh et al. 2013; Keskin and Hallenbeck 2012; Sagir et al. 2017) using *Rhodobacter capsulatus*. Immobilised systems, on the other hand, can attain higher conversions of acid substrates, as it is the case of the use of lactic acid using a polyurethane foam reactor for the retention of *Rhodobacter sphaeroides* GL_{-1}. These organisms evolved hydrogen at a rate of 0.21 mL H_2/h mL foam and a conversion of 86% of lactic acid (Fedorov et al. 1998). These yields can be further improved by the alternation of light–dark periods. Sargsyan et al. (2015) reported on the effect of dark periods in hydrogen evolution when culturing *Rhodobacter sphaeroides* MDC 6522 from Armenian mineral springs. These authors reported that at inoculation of bacteria, illumination after 24 h dark period in comparison with continuous illumination can be used for enhancing H_2 yields, reporting values of 3–8 mmol H_2/g DW (cell dry weight) based on different alternating light–dark periods.

Another approach for increasing hydrogen productivity of reactors is the use of acid organic compounds as substrates for the photofermentative process. The acids can be derived from a previous fermentative hydrogen-producing system. Thus, in this two-stage configuration the fermentative hydrogen reactor evolves biogas composed mainly of hydrogen and CO_2, along with short-chain fatty acids (mainly C2 and C4 species) in the effluent stream which is subsequently treated in a second

photofermentation stage. In general, the fermentative production process is considered to be ineffective due to the low-conversion rate of substrate into hydrogen. Pure cultures of *Enterobacter*, *Bacillus* and *Clostridium* are known for producing hydrogen from soluble sugars and starch (Hawkes et al. 2002). In the case of *Clostridium*, the fermentative process gives the higher yields due to the ability of these organisms for re-oxidising the NADH generated during glycolysis but even though this conversion only yields 33% of the theoretical value (12 mol H_2/mol glucose) (Hallenbeck 2009; Moreno and Gómez 2012). This process has similarities with those at industrial scale such as the acidogenic stage of anaerobic digestion and acetone–butanol (solvent) production by clostridia (Hawkes et al. 2002).

The combined approach for increasing the productivity of hydrogen is then based on the ability of the dark fermentative process (denomination based on the lack of needing light for evolving this gas) for assimilating not only carbohydrate but also cellulosic compounds along with the ability of operating using mixed cultures and wastewaters and solid wastes as substrates (Li and Fang 2007). Table 10.3 presents some results reported by different authors for enhancing hydrogen production using

Table 10.3 Hydrogen yields obtained from two-stage processes considering dark fermentation and subsequent photofermentation

Substrate	Dark fermentation	Photofermentation	Yield from two-stage	References
Sucrose	Mixed culture 3.67 mol H_2/mol sucrose (360 mL H_2/L h)	*R. sphaeroides* SH2C 4.06 mol H_2/mol sucrose	3.67 mol H_2/mol sucrose	Tao et al. (2007)
Olive mill wastewater (OMW)	Mixed culture	*R. sphaeroides* O.U.001	29 – 35 L H_2/L_{OMW}	Eroğlu et al. (2006)
Glucose	*Enterobacter cloacae* 1.86 mol H_2/mol glucose	*R. sphaeroides* O.U.001 1.5–1.72 mol H_2/mol acetic acid	2.78 mol H_2/mol glucose*	Nath et al. (2005)
Molasses	Thermophile *Caldicellulosiruptor saccharolyticus* (72 ºC) 2.1 mol H_2/mol hexose	*R. capsulatus* (DSM1710) 3.71 mol H_2/mol hexose	5.81 mol H_2/mol hexose	Özgür et al. (2010)
Palm oil mill effluent (POME)	*Clostridium butyricum* LS2 0.784 mL H_2/mL POME (21 mL H_2/h)	*Rhodopseudomonas palustris* 26 mL H_2/h	3.064 mL H_2/mL POME	Mishra et al. (2016)
Glucose	Microaerobic dark fermentative process	*R. capsulatus* JP91	7.8 mol H_2/mol glucose	Sağır et al. (2018)

*Calculated based on data reported

a two-stage configuration. Other approaches include the combination of photofermentation by PNS bacteria as second stage of effluents derived from a thermophilic dark fermentation process of Miscanthus hydrolysate by *Thermotoga neapolitana.* However, in this case, the need of additional steps for coupling the two processes such as centrifugation, dilution, buffer addition, pH adjustment and sterilisation may lead to a significant increase in installation costs of this alternative when implemented at industrial scale (Uyar et al. 2009).

Other processes for producing hydrogen involve the utilisation of hydrogen protons and electrons derived from water photolysis. This feature is characteristic of green algae and cyanobacteria. The water photolysis process can be divided into indirect and direct pathways (Oey et al. 2016). In the indirect pathway, solar energy is first converted into carbohydrates which are then used as substrates for hydrogen production. This process is mediated by nitrogenases and hydrogenases enzymes depending on the Cyanobacteria species, whereas hydrogenases are exclusively used by microalgae (Dutta et al. 2005; Oncel et al. 2015). On the contrary, in direct photolysis, which has only been reported in microalgae, the process involves the use of electrons derived from the light-driven water splitting reaction of photosystem II to directly evolve hydrogen using hydrogenase as mediated enzyme (Melis et al. 2000). Many species of green algae have been reported to produce hydrogen by photolysis such as *Chlorella sorokiniana*, *Chlorella vulgaris*, *Scenedesmus obliquus* with *Chlamydomonas reinhardtii* one of the most studied organisms (Mortensen and Gislerød 2016; Rashid et al. 2013; Senger and Bishop 1979; Yadav et al. 2017).

The industrial feasibility of this process is, however, associated with its performance under the use of solar light, thus the relevance of carrying out studies under outdoor conditions which can be susceptible of contamination by other cultures. Because the hydrogen-producing hydrogenase is very sensitive to oxygen, the process of hydrogen production by microalgae must be performed in a two-stage configuration: under oxygenic photosynthesis for generation of the required algal biomass, followed by hydrogen biosynthesis under anaerobic conditions, this idea was explored by Geier et al. (2012) reporting 19.8–48.0 mL H_2/L reactor when light was set at 200 μmol photons/m^2 s but when increasing photosynthetically active radiation under outdoor cultivation only a maximum of 10% of the hydrogen amounts produced by cells grown under laboratory conditions was reached, indicating that further research will be required to investigate the effect of high irradiances and temperatures at midday along with carbon source content. A similar approach was tested by Xu et al. (2017) when adding a fermentative bacterium to the algae to enhance H_2 production without limiting electron resources, in this study *Chlamydomonas reinhardtii* cc849 was co-cultured with *Azotobacter chroococcum* to improve yields. Maximum production was in the range of 68–149 μmol H_2/mg Chl was reported in the co-culture at 100–200 μE/m^2 s of light intensity, values much higher to that of the pure algae culture (28 μmol H_2/mg Chl).

Another process which has been subject to intensive research in recent years is the production of hydrogen by bioelectrochemical systems (BES). This category includes microbial fuel cells (MFCs), microbial electrolysis cells (MECs) and microbial electrosynthesis cells. In these processes, electrochemically active bacteria grow attached

on electrodes and degrade organic matter present in wastewater while producing either electricity, gas fuels and other value-added chemicals becoming a low energy-intensive technology capable of reducing the high energy demand of conventional waste treatment systems (Li et al. 2018; Khan et al. 2018).

MECs have been directly proposed for producing hydrogen from carbohydrate-rich effluent streams or as a second stage of the dark fermentative process to overcome the theoretical barrier associated with this process and improve its industrial feasibility by attaining the complete conversion of the organic compounds. In this line, the productivity of MECs has been evaluated using mixtures of volatile fatty acids as substrate in an attempt of coupling the biolectrochemical process to the fermentative one. The highest production rate reported by Rivera et al. (2015) was 81 mL H_2/L day when testing different acid concentrations ranging from 400 to 1200 mg/L measured as chemical oxygen demand. However, when lactic acid is also produced as a deviation of the dark fermentation process, a negative effect may be observed in the hydrogen yield of the MEC system. Moreno and co-workers (2015) reported on a decrease in hydrogen yield from 70 to 10 mL H_2/L day when the proportion of a dark fermentation effluent derived from the treatment of cheese whey (rich in lactic acid) was increased in the influent stream of the second stage MEC.

Because the dark fermentation process and anaerobic digestion are characterised by high organic loadings, additional research is needed to attain success in coupling any of these treatments to either BES or photofermentation systems. Many approaches consider the dilution of effluents obtained from dark fermentation to make it suitable for the subsequent stage, but these intermediary pre-treatments lead to additional costs which may suppose an excessive burden at large-scale implementation. Another important aspect is the negative effect on hydrogen yields that exert the presence of lactic acid bacteria due to the production of antimicrobial peptides, in this line Rosa et al. (2016) reported on maximum hydrogen yields of 1.7–2.1 L H_2/L day when using cheese whey as substrate but indicating also a severe decline when lactic acid bacterial proliferated in the reactor.

Some other attempts to gain stability on performance consider the coupling in a single reactor allowing for balance between different microbial cultures as it is the combination of MEC and anaerobic microflora or allowing the natural growth of anaerobic competitors in traditional MEC and dark fermentative systems. In the first case, the production of upgraded biogas can be obtained by coupling MEC and anaerobic digestion in a single chamber. Bo et al. (2014) reported on the enhancement of CH_4 yield (which was increased 2.3 times, whereas COD removal rate was tripled). The integrated process was capable of transforming the unwanted CO_2 component of biogas into CH_4 on the anode by the dominant microbes, hydrogenotrophic electromethanogens, using the hydrogen gas in situ generated. A similar idea was tested by Yin et al. (2016) in this case by co-culturing *Geobacter* with *Methanosarcina* in an AD–MEC coupled system, reporting a significant increase in organic matter removal thanks to the ability of co-existence of *Methanosarcina* and *Geobacter* in the biofilm, thus the first one obtaining electrons transferred from *Geobacter* and then reducing carbon dioxide into methane.

The production of biohythane follows a similar approach. This gas is a mixture of biogas enriched with hydrogen, either by the coupling of gases independently produced in biological transformation processes or by allowing the competition of methanogens in traditional MEC and dark fermentative processes. The presence of hydrogen in biogas allows for increasing the energy content of this mixture, and avoids the need of a separate installation for storing and upgrading a pure H_2 gaseous stream. An example of this approach is the integration of microalgae systems for producing hydrogen and the subsequent valorisation of the residual microalgae biomass through dark fermentation followed by conventional digestion process evaluated by Lunprom et al. (2019). These authors used *Chlorella* sp. biomass pre-treated by acid and thermal methods for obtaining yields of 12.5 mL H_2/g VS (volatile solid) and 81 mL CH_4/g VS.

A similar configuration with the same aim of producing hythane was studied by Farhat et al. (2018) using a standard H_2-CH_4 producing system in a two-phase configuration for treating waste materials, but operating in the acidification and H_2 fermentative phase as an anaerobic sequencing batch reactor, allowing for high microbial biomass retention but low hydraulic residence time and operating the subsequent methanogenic phase as standard continuously stirred tank reactor. The novelty in this case was based on the introduction of the gaseous stream generated in the first phase into the second methanogenic phase to enrich biogas, obtaining thus a fuel stream with 8% H_2, 28.5% CO_2 and 63.5% CH_4.

The conversion of H_2 and CO_2 into methane is greatly dependent on the predominant microflora present in the anaerobic reactor. The introduction of a H_2 stream into an anaerobic reactor digesting sewage sludge was evaluated by Martínez et al. (2019b) with the aim of calculating the efficiency of energy production from a MEC hydrogen-producing system for treating wastewaters and the enrichment of biogas derived from the conventional digester, when the hydrogen stream is introduced into the methanogenic reactor. These authors reported an increase in biogas production but not in methane content due to the enrichment of homoacetogenic groups along with other acetogenic microorganisms which produced acetate from hydrogen. Bacteria utilised hydrogen (transferred from the gas phase) and CO_2 to produce acetate, which was subsequently consumed by acetoclastic methanogens, thus the content of biogas was not modified, and CO_2 concentration was kept about 40% in average after hydrogen gas addition.

Several approaches in coupling different biological processes have been studied in an attempt of increasing conversion yields of organic materials, in particular wastes, for producing fuels, valuable products and energy. The most studied and used at an industrial scale is the anaerobic digestion processes for the production of methane. However, such bioconversion has limited net energy yields. The biorefinery concept is then based on the coupling of several steps for increasing the global efficiency. In recent years, a novel approach which is based on the multitude of studies regarding the ability of microorganisms for the direct transfer of electrons is electrofermentation. This technology has attracted much interest due to its ability to boost the microbial metabolism through extracellular electron transfer during fermentation. It has been studied on various acetogens and methanogens, where the enhancement in the biogas

yield reached up to twofold (Kumar et al. 2018) probably becoming in the near future one of the alternatives for increasing economy feasibility of biorefineries.

10.3 Conclusion and Future Prospects

The biorefinery concept admits a diversity of technologies and biological transformations with the aim of producing green compounds. The conversion of biomass into chemicals and energy, however, possess several restrictions associated with the availability of sugars and cellulosic components. Pre-treatments favour the access of microbials to organic compounds but introduce a high demand of energy in the global process which should be carefully evaluated. As experience of performance, it can be used the one obtained from the installation and operation of conventional digestion processes and ethanol plants. These plants have not been a focus of success in all territories installed, and those dealing with the production of cellulosic ethanol have been through serious financial problems. Digestion plants, on the contrary, are well known for the high amount of subsidies or government incentives needed to attain economic feasibility. To favour the production of renewable energies and the treatment of wastes, digestion is at this moment the best environmental option and it is also considered as the best technological alternative regarding its energy demand. The lessons obtained from these two processes should be used as basis for evaluating future complex technologies if the biorefinery concept is to become a reality.

There is a great need on evaluating pilot-scale plants close to an industrial configuration in order to establish energy demands and costs of installation along with operation at a commercial scale. Several reports deal with laboratory scale with volumes of millilitres or litres, but there is an urgent need for obtaining reliable data at higher scale (m^3) for an extended time of evaluation to test microbial stability of the biological process and determining process conditions to avoid microbial shifts. Sterilisation needs and aseptic conditions (which are a common feature of biological processes operating with pure cultures and genetically modified microorganism) are usually against plant profitability, thus becoming an additional factor to be assessed if the aim is to transform the current economy into a green economy.

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References

Abnisa F, Arami-Niya A, Daud WW, Sahu JN (2013) Characterization of bio-oil and bio-char from pyrolysis of palm oil wastes. BioEnergy Res 6:830–840

Abo-Hashesh M, Desaunay N, Hallenbeck PC (2013) High yield single step conversion of glucose to hydrogen by photofermentation with continuous cultures of Rhodobacter capsulatus JP91. Bioresour Technol 128:513–517

Ageitos JM, Vallejo JA, Veiga-Crespo P, Villa TG (2011) Oily yeasts as oleaginous cell factories. Appl Microbiol Biotechnol 90:1219–1227

Ahring BK, Westermann P (2007) Coproduction of bioethanol with other biofuels. In: Biofuels, pp 289–302. Springer, Berlin.

Amaro HM, Guedes AC, Malcata FX (2011) Advances and perspectives in using microalgae to produce biodiesel. Appl Energy 88:3402–3410

Amoah J, Kahar P, Ogino C, Kondo A (2019) Bioenergy and biorefinery: feedstock, biotechnological conversion and products. Biotechnol J 1800494

Amorim HV, Lopes ML, de Castro-Oliveira JV, Buckeridge MS, Goldman GH (2011) Scientific challenges of bioethanol production in Brazil. Appl Microbiol Biotechnol 91:1267

Annamalai N, Rajeswari MV, Balasubramanian T (2016) Endo-1, 4-β-glucanases: role, applications and recent developments. In: Microbial enzymes in bioconversions of biomass, pp 37–45. Springer, Cham.

Apiwatanapiwat W, Murata Y, Kosugi A, Yamada R, Kondo A, Arai T, Rugthaworn Ps, Mori Y (2011) Direct ethanol production from cassava pulp using a surface-engineered yeast strain co-displaying two amylases, two cellulases, and β-glucosidase. Appl Microbiol Biotechnol 90:377–384

Aresta M, Dibenedetto A, Dumeignil F (eds) (2012) Biorefinery: from biomass to chemicals and fuels. Walter de Gruyter

Aro EM (2016) From first generation biofuels to advanced solar biofuels. Ambio 45:24–31

Bajpai P (2018) Bioconversion of hemicelluloses. In: Biotechnology for pulp and paper processing, pp 545–560. Springer, Singapore

Bajpai P (2019) Cultivation of third generation biofuel. In: Third generation biofuels, pp 17–28. Springer, Singapore

Basak N, Das D (2007) The prospect of purple non-sulfur (PNS) photosynthetic bacteria for hydrogen production: the present state of the art. World J Microbiol Biotechnol 23:31–42

Basak N, Das D (2009) Photofermentative hydrogen production using purple non-sulfur bacteria *Rhodobacter sphaeroides* OU 001 in an annular photobioreactor: a case study. Biomass Bioenergy 33:911–919

Basso LC, De Amorim HV, De Oliveira AJ, Lopes ML (2008) Yeast selection for fuel ethanol production in Brazil. FEMS Yeast Res 8:1155–1163

Bilal M, Asgher M, Iqbal HM, Ramzan M (2017) Enhanced bio-ethanol production from old newspapers waste through alkali and enzymatic delignification. Waste Biomass Valori 8:2271–2281

Blakey S, Rye L, Wilson CW (2011) Aviation gas turbine alternative fuels: a review. Proc Combust Inst 33:2863–2885

Bo T, Zhu X, Zhang L, Tao Y, He X, Li D, Yan Z (2014) A new upgraded biogas production process: coupling microbial electrolysis cell and anaerobic digestion in single-chamber, barrel-shape stainless steel reactor. Electrochem Commun 45:67–70

Buitrón G, Martínez-Valdez FJ, Ojeda F (2019) Biogas production from a highly organic loaded winery effluent through a two-stage process. BioEnerg Res 1:8

Cabrera-Díaz A, Pereda-Reyes I, Oliva-Merencio D, Lebrero R, Zaiat M (2017) Anaerobic digestion of sugarcane vinasse through a methanogenic UASB reactor followed by a packed bed reactor. Appl Biochem Biotechnol 183:1127–1145

Capolupo L, Faraco V (2016) Green methods of lignocellulose pretreatment for biorefinery development. Appl Microbiol Biotechnol 100:9451–9467

Chaiprapat S, Sasibunyarat T, Charnnok B, Cheirsilp B (2017) Intensifying clean energy production through cultivating mixotrophic microalgae from digestates of biogas systems: effects of light intensity, medium dilution, and cultivating time. BioEnerg Res 10:103–114

Cherubini F, Jungmeier G (2010) LCA of a biorefinery concept producing bioethanol, bioenergy, and chemicals from switchgrass. Int J Life Cycle Ass 15:53–66

Chojnacka K, Noworyta A (2004) Evaluation of *Spirulina* sp. growth in photoautotrophic, heterotrophic and mixotrophic cultures. Enzyme Microb Technol 34:461–465

Coimbra RN, Escapa C, Otero M (2019) Comparative thermogravimetric assessment on the combustion of coal, Microalgae Biomass and Their Blend. Energies 12:2962

Cuetos MJ, Fernández C, Gómez X, Morán A (2011) Anaerobic co-digestion of swine manure with energy crop residues. Biotechnol Bioproc E 16:1044

Cuetos MJ, Gómez X, Martínez EJ, Fierro J, Otero M (2013) Feasibility of anaerobic co-digestion of poultry blood with maize residues. Bioresour Technol 144:513–520

Dakota. https://www.dakotagold.com/distillers-grains

Dashko S, Zhou N, Compagno C, Piškur J (2014) Why, when, and how did yeast evolve alcoholic fermentation? FEMS Yeast Res 14:826–832

Daumer ML, Picard S, Saint-Cast P, Dabert P (2010) Technical and economical assessment of formic acid to recycle phosphorus from pig slurry by a combined acidification–precipitation process. J Hazard Mater 180:361–365

Díez-Antolínez R, Hijosa-Valsero M, Paniagua-García AI, Garita-Cambronero J, Gómez X (2018) Yeast screening and cell immobilization on inert supports for ethanol production from cheese whey permeate with high lactose loads. PLoS ONE 13(12):e0210002. https://doi.org/10.1371/journal.pone.0210002

Dobrowolski A, Mituła P, Rymowicz W, Mirończuk AM (2016) Efficient conversion of crude glycerol from various industrial wastes into single cell oil by yeast Yarrowia lipolytica. Bioresour Technol 207:237–243

Dong C, Wang Y, Wang H, Lin CSK, Hsu HY, Leu SY (2019) New generation urban biorefinery toward complete utilization of waste derived lignocellulosic biomass for biofuels and value-added products. Energy Proc 158:918–925

Du C, Lin SKC, Koutinas A, Wang R, Webb C (2007) Succinic acid production from wheat using a biorefining strategy. Appl Microbiol Biotechnol 76:1263–1270

Dürre P (1998) New insights and novel developments in clostridial acetone/butanol/isopropanol fermentation. Appl Microbiol Biotechnol 49:639–648

Dutta D, De D, Chaudhuri S, Bhattacharya SK (2005) Hydrogen production by cyanobacteria. Microb Cell Fact 4:36

Eboibi BE (2019) Impact of time on yield and properties of biocrude during downstream processing of product mixture derived from hydrothermal liquefaction of microalga. Biomass Conv Bioref 9:379–387

Eggeman T, Verser D (2006) The importance of utility systems in today's biorefineries and a vision for tomorrow. Appl Microbiol Biotechnol 130:361–381

Eroğlu E, Eroğlu İ, Gündüz U, Türker L, Yücel M (2006) Biological hydrogen production from olive mill wastewater with two-stage processes. Int J Hydrog Energ 31:1527–1535

European biogas association report. https://european-biogas.eu/wp-content/uploads/2017/12/Statistical-report-of-the-European-Biogas-Association-web.pdf

Ezeji TC, Qureshi N, Blaschek HP (2004) Acetone butanol ethanol (ABE) production from concentrated substrate: reduction in substrate inhibition by fed-batch technique and product inhibition by gas stripping. Appl Microbiol Biotechnol 63:653–658

Farhat A, Miladi B, Hamdi M, Bouallagui H (2018) Fermentative hydrogen and methane co-production from anaerobic co-digestion of organic wastes at high loading rate coupling continuously and sequencing batch digesters. Environ Sci Pollut R 25:27945–27958

Farrokh P, Sheikhpour M, Kasaeian A, Asadi H, Bavandi R (2019) Cyanobacteria as an eco-friendly resource for biofuel production: a critical review. Biotechnol Progr. https://doi.org/10.1002/btpr.2835

Fava F, Totaro G, Diels L, Reis M, Duarte J, Carioca OB, Poggi-Varaldo HM, Ferreira BS (2015) Biowaste biorefinery in Europe: opportunities and research & development needs. New Biotechnol 32:100–108

Fedorov AS, Tsygankov AA, Rao KK, Hall DO (1998) Hydrogen photoproduction by *Rhodobacter sphaeroides* immobilised on polyurethane foam. Biotechnol Lett 20:1007–1009

Feng Q, Lin Y (2017) Integrated processes of anaerobic digestion and pyrolysis for higher bioenergy recovery from lignocellulosic biomass: a brief review. Renew Sustain Energy Rev 77:1272–1287

Fernando S, Adhikari S, Chandrapal C, Murali N (2006) Biorefineries: current status, challenges, and future direction. Energy Fuel 20:1727–1737

Fierro J, Martinez EJ, Rosas JG, Fernández RA, López R, Gómez X (2016) Co-digestion of swine manure and crude glycerine: Increasing glycerine ratio results in preferential degradation of labile compounds. Water Air Soil Pollut 227:78

FitzPatrick M, Champagne P, Cunningham MF, Whitney RA (2010) A biorefinery processing perspective: treatment of lignocellulosic materials for the production of value-added products. Bioresour Technol 101:8915–8922

Formanek J, Mackie R, Blaschek HP (1997) Enhanced butanol production by *Clostridium beijerinckii* BA101 grown in semidefined P2 medium containing 6 percent maltodextrin or glucose. Appl Environ Microbiol 63:2306–2310

Fuess LT, Zaiat M (2018) Economics of anaerobic digestion for processing sugarcane vinasse: applying sensitivity analysis to increase process profitability in diversified biogas applications. Process Saf Environ 115:27–37

Fuess LT, Kiyuna LSM, Garcia ML, Zaiat M (2016) Operational strategies for long-term biohydrogen production from sugarcane stillage in a continuous acidogenic packed-bed reactor. Int J Hydrog Energ 41:8132–8145

Geier SC, Huyer S, Praebst K, Husmann M, Walter C, Buchholz R (2012) Outdoor cultivation of *Chlamydomonas reinhardtii* for photobiological hydrogen production. J Appl Phycol 24:319–327

Gogoi H, Nirosha V, Jayakumar A, Prabhu K, Maitra M, Panjanathan R (2018) Paper mill sludge as a renewable substrate for the production of acetone-butanol-ethanol using Clostridium sporogenes NCIM 2337. Energy Source Part A 40:39–44

Goldemberg J (2008) The Brazilian biofuels industry. Biotechnol Biofuels 1:6

Gómez X, Cuetos MJ, Cara J, Moran A, García AI (2006) Anaerobic co-digestion of primary sludge and the fruit and vegetable fraction of the municipal solid wastes: conditions for mixing and evaluation of the organic loading rate. Renew Energy 31:2017–2024

Gómez X, Cuetos MJ, García AI, Morán A (2007) An evaluation of stability by thermogravimetric analysis of digestate obtained from different biowastes. J Hazard Mater 149:97–105

Gómez X, Meredith W, Fernández C, Sánchez-García M, Díez-Antolínez R, Garzón-Santos J, Snape CE (2018) Evaluating the effect of biochar addition on the anaerobic digestion of swine manure: application of Py-GC/MS. Environ Sci Pollut R 25:25600–25611

Gonçalves BC, Silva MB (2018) Green microalgae as substrate for producing biofuels and chlorophyll in biorefineries. In: Sustainable biotechnology-enzymatic resources of renewable energy, pp 439–461. Springer, Cham

González J, Sánchez M, Gómez X (2018) Enhancing anaerobic digestion: the effect of carbon conductive materials. C 4:59

González R, Smith R, Blanco D, Fierro J, Gómez X (2019) Application of thermal analysis for evaluating the effect of glycerine addition on the digestion of swine manure. J Therm Anal Calorim 135:2277–2286

Green Car Congress (2019). https://www.greencarcongress.com/2019/07/20190722-poet.html

Greenland SJ, Dalrymple J, Levin E, O'Mahony B (2018) Improving agricultural water sustainability: strategies for effective farm water management and encouraging the uptake of drip irrigation. In: The Goals of Sustainable Development, pp 111–123. Springer, Singapore

Grobbelaar JU (2010) Microalgal biomass production: challenges and realities. Photosynth Res 106:135–144

Hallenbeck PC (2009) Fermentative hydrogen production: principles, progress, and prognosis. Int J Hydrog Energy 34:7379–7389
Han Q, Jin Y, Jameel H, Chang HM, Phillips R, Park S (2015) Autohydrolysis pretreatment of waste wheat straw for cellulosic ethanol production in a co-located straw pulp mill. Appl Biochem Biotechnol 175:1193–1210
Hardy B, Sleutel S, Dufey JE, Cornelis JT (2019) The long-term effect of biochar on soil microbial abundance, activity and community structure is overwritten by land management. Front Environ Sci 7:110
Hawkes FR, Dinsdale R, Hawkes DL, Hussy I (2002) Sustainable fermentative hydrogen production: challenges for process optimisation. Int J Hydrog Energy 27:1339–1347
Hijosa-Valsero M, Paniagua-García AI, Díez-Antolínez R (2018) Industrial potato peel as a feedstock for biobutanol production. New Biotechnol 46:54–60
Hill J, Nelson E, Tilman D, Polasky S, Tiffany D (2006) Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. P Natl Acad Sci USA 103:11206–11210
Hübner T, Mumme J (2015) Integration of pyrolysis and anaerobic digestion–use of aqueous liquor from digestate pyrolysis for biogas production. Bioresour Technol 183:86–92
Ito T, Nakashimada Y, Senba K, Matsui T, Nishio N (2005) Hydrogen and ethanol production from glycerol-containing wastes discharged after biodiesel manufacturing process. J Biosci Bioeng 100:260–265
Jiang Y, Guo D, Lu J, Dürre P, Dong W, Yan W, Zhang W, Ma J, Jiang M, Xin F (2018a) Consolidated bioprocessing of butanol production from xylan by a thermophilic and butanologenic *Thermoanaerobacterium* sp. M5. Biotechnol Biofuels 11:89
Jiang Y, Zhang T, Lu J, Dürre P, Zhang W, Dong W, Zhou J, Jiang M, Xin F (2018) Microbial co-culturing systems: butanol production from organic wastes through consolidated bioprocessing. Appl Microbiol Biotechnol 102:5419–5425
Jin Q, Neilson AP, Stewart AC, O'Keefe SF, Kim YT, McGuire M, Wilder G, Huang H (2018) Integrated approach for the valorization of red grape pomace: production of oil, polyphenols, and acetone–butanol–ethanol. ACS Sustain Chem Eng 6:16279–16286
Jiru TM, Steyn L, Pohl C, Abate D (2018) Production of single cell oil from cane molasses by *Rhodotorula kratochvilovae* (syn, *Rhodosporidium kratochvilovae*) SY89 as a biodiesel feedstock. Chem Cent J 12:91
Joshi SM, Waghmare JS, Sonawane KD, Waghmare SR (2015) Bio-ethanol and bio-butanol production from orange peel waste. Biofuels 6:55–61
Júnior ADNF, Koyama MH, de Araújo Júnior MM, Zaiat M (2016) Thermophilic anaerobic digestion of raw sugarcane vinasse. Renew Energy 89:245–252
Kádár Z, Szengyel Z, Réczey K (2004) Simultaneous saccharification and fermentation (SSF) of industrial wastes for the production of ethanol. Ind Crop Prod 20:103–110
Kamm B, Kamm M (2004) Principles of biorefineries. Appl Microbiol Biotechnol 64:137–145
Keskin T, Hallenbeck PC (2012) Hydrogen production from sugar industry wastes using single-stage photofermentation. Bioresour Technol 112:131–136
Khan N, Khan MD, Sultana S, Khan MZ, Ahmad A (2018) Bioelectrochemical systems for transforming waste to energy. In: Modern age environmental problems and their remediation, pp 111–128. Springer, Cham
Kiyoshi K, Furukawa M, Seyama T, Kadokura T, Nakazato A, Nakayama S (2015) Butanol production from alkali-pretreated rice straw by co-culture of Clostridium thermocellum, and Clostridium saccharoperbutylacetonicum. Bioresour Technol 186:325–328
Koku H, Eroğlu I, Gündüz U, Yücel M, Türker L (2002) Aspects of the metabolism of hydrogen production by *Rhodobacter sphaeroides*. Int J Hydrog Energy 27:1315–1329
Kołtuniewicz AB, Dąbkowska K (2016) Biorefineries–factories of the future. Chem Process Eng 37:109–119
Kour D, Rana KL, Yadav N, Yadav AN, Rastegari AA, Singh C et al (2019a) Technologies for biofuel production: current development, challenges, and future prospects. In: Rastegari AA,

Yadav AN, Gupta A (eds) Prospects of renewable bioprocessing in future energy systems, pp 1–50. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-030-14463-0_1
Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA et al. (2019b) Agriculturally and industrially important fungi: current developments and potential biotechnological applications. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi. Perspective for value-added products and environments, vol 2, pp 1–64. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-030-14846-1_1
Kumar H, Christopher LP (2017) Recent trends and developments in dissolving pulp production and application. Cellulose 24:2347–2365
Kumar P, Chandrasekhar K, Kumari A, Sathiyamoorthi E, Kim BS (2018) Electro-fermentation in aid of bioenergy and biopolymers. Energies 11:343
Kumar S, Sharma S, Thakur S, Mishra T, Negi P, Mishra S et al (2019) Bioprospecting of microbes for biohydrogen production: current status and future challenges. In: Molina G, Gupta VK, Singh BN, Gathergood N (eds) Bioprocessing for biomolecules production. Wiley, USA, pp 443–471
Li C, Fang HH (2007) Fermentative hydrogen production from wastewater and solid wastes by mixed cultures. Crit Rev Environ Sci Technol 37:1–39
Li X, Mupondwa E (2014) Life cycle assessment of camelina oil derived biodiesel and jet fuel in the Canadian Prairies. Sci Total Environ 481:17–26
Li A, Antizar-Ladislao B, Khraisheh M (2007) Bioconversion of municipal solid waste to glucose for bio-ethanol production. Bioproc Biosyst Eng 30:189–196
Li Q, Du W, Liu D (2008) Perspectives of microbial oils for biodiesel production. Appl Microbiol Biotechnol 80:749–756
Li Q, Cai H, Hao B, Zhang C, Yu Z, Zhou S, Chenjuan L (2010) Enhancing clostridial acetone-butanol-ethanol (ABE) production and improving fuel properties of ABE-enriched biodiesel by extractive fermentation with biodiesel. Appl Biochem Biotechnol 162:2381–2386
Li Y, Zhang R, Liu X, Chen C, Xiao X, Feng L, He Y, Liu G (2013) Evaluating methane production from anaerobic mono-and co-digestion of kitchen waste, corn stover, and chicken manure. Energy Fuel 27:2085–2091
Li T, Xu J, Wu H, Wang G, Dai S, Fan J, He H, Xiang W (2016) A saponification method for chlorophyll removal from microalgae biomass as oil feedstock. Mar Drugs 14:162
Li Z, Fu Q, Kobayashi H, Xiao S (2018) Biofuel production from bioelectrochemical systems. In: Bioreactors for microbial biomass and energy conversion, pp 435–461. Springer, Singapore
Lobato A, Cuetos MJ, Gómez X, Morán A (2010) Improvement of biogas production by co-digestion of swine manure and residual glycerine. Biofuels 1:59–68
Longati AA, Lino AR, Giordano RC, Furlan FF, Cruz AJ (2019) Biogas production from anaerobic digestion of vinasse in sugarcane biorefinery: a techno-economic and environmental analysis. Waste Biomass Valori 1:19
Louhasakul Y, Cheirsilp B, Prasertsan P (2016) Valorization of palm oil mill effluent into lipid and cell-bound lipase by marine yeast *Yarrowia lipolytica* and their application in biodiesel production. Waste Biomass Valori 7:417–426
Lunprom S, Phanduang O, Salakkam A, Liao Q, Imai T, Reungsang A (2019) Bio-hythane production from residual biomass of *Chlorella* sp. biomass through a two-stage anaerobic digestion. Int J Hydrog Energy 44:3339–3346
Luo C, Lü F, Shao L, He P (2015) Application of eco-compatible biochar in anaerobic digestion to relieve acid stress and promote the selective colonization of functional microbes. Water Res 68:710–718
Ma F, Hanna MA (1999) Biodiesel production: a review1. Bioresour Technol 70:1–15
Mabee WE, Saddler JN (2006) The potential of bioconversion to produce fuels and chemicals. In Annual meeting-pulp and paper technical association of Canada, June 2006, vol 92, No C, p 185. Pulp and Paper Technical Association of Canada, 1999
Maiti S, Gallastegui G, Sarma SJ, Brar SK, Le Bihan Y, Drogui P, Buelna G, Verma M (2016) A re-look at the biochemical strategies to enhance butanol production. Biomass Bioenergy 94:187–200

Martínez EJ, Fierro J, Sánchez ME, Gómez X (2012) Anaerobic co-digestion of FOG and sewage sludge: study of the process by Fourier transform infrared spectroscopy. Int Biodeterior Biodegrad 75:1–6

Martínez E, Raghavan V, González-Andrés F, Gómez X (2015) New biofuel alternatives: integrating waste management and single cell oil production. Int J Mol Sci 16:9385–9405

Martínez EJ, Rosas JG, González R, García D, Gómez X (2018) Treatment of vinasse by electrochemical oxidation: evaluating the performance of boron-doped diamond (BDD)-based and dimensionally stable anodes (DSAs). Int J Environ Sci Technol 15:1159–1168

Martínez EJ, Rosas JG, Sotres A, Morán A, Cara J, Sánchez ME, Gómez X (2018) Codigestion of sludge and citrus peel wastes: evaluating the effect of biochar addition on microbial communities. Biochem Eng J 137:314–325

Martínez EJ, Blanco D, Gómez X (2019a) Two-stage process to enhance bio-hydrogen production. In: Improving biogas production, pp 149–179. Springer, Cham

Martínez EJ, Sotres A, Arenas CB, Blanco D, Martínez O, Gómez X (2019) Improving anaerobic digestion of sewage sludge by hydrogen addition: analysis of microbial populations and process performance. Energies 12:1228

Matsakas L, Bonturi N, Miranda EA, Rova U, Christakopoulos P (2015) High concentrations of dried sorghum stalks as a biomass feedstock for single cell oil production by *Rhodosporidium toruloides*. Biotechnol Biofuels 8:6

Melis A, Zhang LP, Forestier M, Ghirardi ML, Seibert M (2000) Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga *Chlamydomonas reinhardtii*. Plant Physiol 122:127–135

Menon V, Rao M (2012) Trends in bioconversion of lignocellulose: biofuels, platform chemicals & biorefinery concept. Prog Energy Combust 38:522–550

Metzger P, Largeau C (2005) *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. Appl Microbiol Biotechnol 66:486–496

Mishra P, Thakur S, Singh L, Ab Wahid Z, Sakinah M (2016) Enhanced hydrogen production from palm oil mill effluent using two stage sequential dark and photo fermentation. Int J Hydrog Energy 41:18431–18440

Miyake J, Tomizuk N, Kamibayashi A (1982) Prolonged photo-hydrogen production by *Rhodospirillum rubrum*. J Ferment Technol 60:199–203

Mohammed IY, Abba Z, Matias-Peralta HM, Abakr YA, Fuzi SFZM (2018) Thermogravimetric study and evolved gas analysis of new microalga using TGA-GC-MS. Biomass Conv Bioref 8:669–678

Montero E, Olguín EJ, De Philippis R, Reverchon F (2018) Mixotrophic cultivation of *Chlorococcum* sp. under non-controlled conditions using a digestate from pig manure within a biorefinery. J Appl Phycol 30:2847–2857

Moreno R, Gómez X (2012) Dark fermentative H2 production from wastes: effect of operating conditions. J Environ Sci Eng A 1:936

Moreno R, Escapa A, Cara J, Carracedo B, Gómez X (2015) A two-stage process for hydrogen production from cheese whey: integration of dark fermentation and biocatalyzed electrolysis. Int J Hydrog Energy 40:168–175

Mortensen LM, Gislerød HR (2016) The growth of *Chlorella sorokiniana* as influenced by CO 2, light, and flue gases. J Appl Phycol 28:813–820

Mullen CA, Boateng AA (2011) Production and analysis of fast pyrolysis oils from proteinaceous biomass. BioEnergy Res 4:303–311

Nakayama S, Kiyoshi K, Kadokura T, Nakazato A (2011) Butanol production from crystalline cellulose by cocultured *Clostridium thermocellum* and *Clostridium saccharoperbutylacetonicum* N1–4. Appl Environ Microbiol 77:6470–6475

Nath K, Kumar A, Das D (2005) Hydrogen production by *Rhodobacter sphaeroides* strain OU 001 using spent media of *Enterobacter cloacae* strain DM11. Appl Microbiol Biot 68:533–541

Nonato R, Mantelatto P, Rossell C (2001) Integrated production of biodegradable plastic, sugar and ethanol. Appl Microbiol Biot 57:1–5

Nordell E, Nilsson B, Påledal SN, Karisalmi K, Moestedt J (2016) Co-digestion of manure and industrial waste–the effects of trace element addition. Waste Manag 47:21–27
Oey M, Sawyer AL, Ross IL, Hankamer B (2016) Challenges and opportunities for hydrogen production from microalgae. Plant Biotechnol J 14:1487–1499
Oliveira JV, Duarte T, Costa JC, Cavaleiro AJ, Pereira MA, Alves MM (2018) Improvement of biomethane production from sewage sludge in co-digestion with glycerol and waste frying oil, using a design of experiments. BioEnergy Res 11:763–771
Oncel SS, Kose A, Faraloni C (2015) Genetic optimization of microalgae for biohydrogen production (Chap. 25). In: Kim S-K (ed) Handbook of marine microalgae. Academic Press, Boston, pp 383–404
Ormaechea P, Castrillón L, Marañón E, Fernández-Nava Y, Negral L, Megido L (2017) Influence of the ultrasound pretreatment on anaerobic digestion of cattle manure, food waste and crude glycerine. Environ Technol 38:682–686
Özgür E, Afsar N, de Vrije T, Yücel M, Gündüz U, Claassen PA, Eroglu I (2010) Potential use of thermophilic dark fermentation effluents in photofermentative hydrogen production by *Rhodobacter capsulatus*. J Clean Prod 18:S23–S28
Papanikolaou S, Aggelis G (2011) Lipids of oleaginous yeasts. Part I: biochemistry of single cell oil production. Eur J Lipid Sci Technol 113:1031–1051
Park JY, Choi SA, Jeong MJ, Nam B, Oh YK, Lee JS (2014) Changes in fatty acid composition of *Chlorella vulgaris* by hypochlorous acid. Bioresour Technol 162:379–383
Patle S, Lal B (2007) Ethanol production from hydrolysed agricultural wastes using mixed culture of *Zymomonas mobilis* and *Candida tropicalis*. Biotechnol Lett 29:1839–1843
Pena R, Lú-Chau TA, Lema JM (2012) Use of white-rot fungi for valorization of stillage from bioethanol production. Waste Biomass Valori 3:295–303
Pereira LG, Chagas MF, Dias MO, Cavalett O, Bonomi A (2015) Life cycle assessment of butanol production in sugarcane biorefineries in Brazil. J Clean Prod 96:557–568
Piveteau S, Picard S, Dabert P, Daumer ML (2017) Dissolution of particulate phosphorus in pig slurry through biological acidification: a critical step for maximum phosphorus recovery as struvite. Water Res 124:693–701
Qureshi N, Blaschek HP (2001) Recent advances in ABE fermentation: hyper-butanol producing *Clostridium beijerinckii* BA101. J Ind Microbiol Biotechnol 27:287–291
Qureshi N, Liu S, Hughes S, Palmquist D, Dien B, Saha B (2016) Cellulosic butanol (ABE) biofuel production from sweet sorghum bagasse (SSB): impact of hot water pretreatment and solid loadings on fermentation employing *Clostridium beijerinckii* P260. BioEnergy Res 9:1167–1179
Rana KL, Kour D, Sheikh I, Yadav N, Yadav AN, Kumar V et al (2019) Biodiversity of endophytic fungi from diverse niches and their biotechnological applications. In: Singh BP (ed) Advances in endophytic fungal research: present status and future challenges, pp 105–144. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-030-03589-1_6
Rashid N, Lee K, Han JI, Gross M (2013) Hydrogen production by immobilized *Chlorella vulgaris*: optimizing pH, carbon source and light. Bioproc Biosyst Eng 36:867–872
Rastegari AA, Yadav AN, Yadav N (2020) New and Future Developments in Microbial Biotechnology and Bioengineering: Trends of Microbial Biotechnology for Sustainable Agriculture and Biomedicine Systems: Diversity and Functional Perspectives. Elsevier, Amsterdam
Rastegari AA, Yadav AN, Gupta A (2019) Prospects of renewable bioprocessing in future energy systems. Springer International Publishing, Cham
Ratledge C (1993) Single cell oils—have they a biotechnological future? Trends Biotechnol 11:278–284
Refaat AA (2011) Biodiesel production using solid metal oxide catalysts. Int J Environ Sci Technol 8:203–221
Rivera I, Buitrón G, Bakonyi P, Nemestóthy N, Bélafi-Bakó K (2015) Hydrogen production in a microbial electrolysis cell fed with a dark fermentation effluent. J Appl Electrochem 45:1223–1229

Robinson PH, Karges K, Gibson ML (2008) Nutritional evaluation of four co-product feedstuffs from the motor fuel ethanol distillation industry in the Midwestern USA. Anim Feed Sci Technol 146:345–352

Rondon MA, Lehmann J, Ramírez J, Hurtado M (2007) Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. Biol Fert Soils 43:699–708

Rosa PRF, Gomes BC, Varesche MBA, Silva EL (2016) Characterization and antimicrobial activity of lactic acid bacteria from fermentative bioreactors during hydrogen production using cassava processing wastewater. Chem Eng J 284:1–9

Rosas JG, Gómez N, Cara J, Ubalde J, Sort X, Sánchez ME (2015) Assessment of sustainable biochar production for carbon abatement from vineyard residues. J Anal Appl Pyrol 113:239–247

Sagir E, Ozgur E, Gunduz U, Eroglu I, Yucel M (2017) Single-stage photofermentative biohydrogen production from sugar beet molasses by different purple non-sulfur bacteria. Bioproc Biosyst Eng 40:1589–1601

Sağır E, Yucel M, Hallenbeck PC (2018) Demonstration and optimization of sequential microaerobic dark-and photo-fermentation biohydrogen production by immobilized *Rhodobacter capsulatus* JP91. Bioresour Technol 250:43–52

Sahu S, Pramanik K (2018) Evaluation and optimization of organic acid pretreatment of cotton gin waste for enzymatic hydrolysis and bioethanol production. Appl Biochem Biotechnol 186:1047–1060

Sargsyan H, Gabrielyan L, Hakobyan L, Trchounian A (2015) Light–dark duration alternation effects on Rhodobacter sphaeroides growth, membrane properties and bio-hydrogen production in batch culture. Int J Hydrog Energy 40:4084–4091

Saritha M, Arora A, Choudhary J, Rani V, Singh S, Sharma A, Sharma S, Nain L (2016) The role and applications of xyloglucan hydrolase in biomass degradation/bioconversion. In: Microbial enzymes in bioconversions of biomass, pp 231–248. Springer, Cham

Sawatdeenarunat C, Nguyen D, Surendra KC, Shrestha S, Rajendran K, Oechsner H, Xie L, Khanal SK (2016) Anaerobic biorefinery: current status, challenges, and opportunities. Bioresour Technol 215:304–313

Scarlat N, Dallemand JF, Fahl F (2018) Biogas: developments and perspectives in Europe. Renew Energy 129:457–472

Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, Kruse O, Hankamer B (2008) Second generation biofuels: high-efficiency microalgae for biodiesel production. BioEnergy Res 1:20–43

Schieb PA, Lescieux-Katir H, Thénot M, Clément-Larosière B (2015) Prospects for the Bazancourt-Pomacle biorefinery between now and 2030. In: Biorefinery 2030, pp 81–100. Springer, Berlin

Senger H, Bishop NI (1979) Observations on the photohydrogen producing activity during the synchronous cell cycle of *Scenedesmus obliquus*. Planta 145:53–62

Sharma HK, Xu C, Qin W (2019) Biological pretreatment of lignocellulosic biomass for biofuels and bioproducts: an overview. Waste Biomass Valori 10:235–251

Shi R, Ukaew S, Archer DW, Lee JH, Pearlson MN, Lewis KC, Shonnard DR (2017) Life cycle water footprint analysis for rapeseed derived jet fuel in North Dakota. ACS Sustain Chem Eng 5:3845–3854

Shrivastava AK, Srivastava Arun K, Soloman S (2011) Sustaining sugarcane productivity under depleting water resources. Curr Sci India 101:748–754

Sialve B, Bernet N, Bernard O (2009) Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol Adv 27:409–416

Singh J, Gu S (2010) Commercialization potential of microalgae for biofuels production. Renew Sustain Energy Rev 14:2596–2610

Singh V, Johnston DB, Naidu K, Rausch KD, Belyea RL, Tumbleson ME (2005) Comparison of modified dry-grind corn processes for fermentation characteristics and DDGS composition. Cereal Chem 82:187–190

Singh SP, Pathak J, Sinha RP (2017) Cyanobacterial factories for the production of green energy and value-added products: An integrated approach for economic viability. Renew Sustain Energy Rev 69:578–595

Singh AK, Kumari VV, Gupta R, Singh P, Solomon S (2018) Efficient irrigation water management in sugarcane through alteration of field application parameters under subtropical India. Sugar Tech 20:21–28

Soccol CR, Neto CJD, Soccol VT, Sydney EB, da Costa ESF, Medeiros ABP, de Souza Vandenberghe LP (2017) Pilot scale biodiesel production from microbial oil of *Rhodosporidium toruloides* DEBB 5533 using sugarcane juice: performance in diesel engine and preliminary economic study. Bioresour Technol 223:259–268

Spiekermann P, Rehm BHA, Kalscheuer R, Baumeister D, Steinbüchel AA (1999) Sensitive, viable-colony staining method using Nile red for direct screening of bacteria that accumulate polyhydroxyalkanoic acids and other lipid storage compounds. Arch Microbiol 171:73–80

Stadler T, Chauvet JM (2018) New innovative ecosystems in France to develop the bioeconomy. New Biotechnol 40:113–118

Statista. https://www.statista.com/statistics/271472/biodiesel-production-in-selected-countries/

Tao Y, Chen Y, Wu Y, He Y, Zhou Z (2007) High hydrogen yield from a two-step process of dark-and photo-fermentation of sucrose. Int J Hydrog Energy 32:200–206

Taparia T, MVSS M, Mehrotra R, Shukla P, Mehrotra S (2016) Developments and challenges in biodiesel production from microalgae: a review. Biotechnol Appl Biochem 63:715–726

Tran HTM, Cheirsilp B, Hodgson B, Umsakul K (2010) Potential use of *Bacillus subtilis*, in a co-culture with *Clostridium butylicum*, for acetone–butanol–ethanol production from cassava starch. Biochem Eng J 48(2):260–267

Tripathi M, Sahu JN, Ganesan P (2016) Effect of process parameters on production of biochar from biomass waste through pyrolysis: a review. Renew Sustain Energy Rev 55:467–481

Uellendahl H, Ahring BK (2010) Anaerobic digestion as final step of a cellulosic ethanol biorefinery: biogas production from fermentation effluent in a UASB reactor—pilot-scale results. Biotechnol Bioeng 107:59–64

Ugwu CU, Aoyagi H, Uchiyama H (2008) Photobioreactors for mass cultivation of algae. Bioresour Technol 99:4021–4028

Uyar B, Schumacher M, Gebicki J, Modigell M (2009) Photoproduction of hydrogen by *Rhodobacter capsulatus* from thermophilic fermentation effluent. Bioproc Biosyst Eng 32:603–606

van Dyk S, Su J, Mcmillan JD, Saddler J (2019) Potential synergies of drop-in biofuel production with further co-processing at oil refineries. Biofuel Bioprod Bior 13:760–775

Van Zwieten L, Kimber S, Morris S, Chan KY, Downie A, Rust J, Joseph S, Cowie A (2010) Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. Plant Soil 327:235–246

Varol A, Ugurlu A (2016) Biogas production from microalgae (*Spirulina platensis*) in a two stage anaerobic system. Waste Biomass Valori 7:193–200

Villar A, Arribas JJ, Parrondo J (2012) Waste-to-energy technologies in continuous process industries. Clean Technol Envir 14:29–39

Wang YR, Chiang YS, Chuang PJ, Chao YP, Li SY (2016) Direct in situ butanol recovery inside the packed bed during continuous acetone-butanol-ethanol (ABE) fermentation. Appl Microbiol Biotechnol 100:7449–7456

Xu L, Cheng X, Wu S, Wang Q (2017) Co-cultivation of *Chlamydomonas reinhardtii* with *Azotobacter chroococcum* improved H_2 production. Biotechnol Lett 39:731–738

Xue C, Wang Z, Wang S, Zhang X, Chen L, Mu Y, Bai F (2016) The vital role of citrate buffer in acetone–butanol–ethanol (ABE) fermentation using corn stover and high-efficient product recovery by vapor stripping–vapor permeation (VSVP) process. Biotechnol Biofuels 9:146

Yadav AN, Kumar R, Kumar S, Kumar V, Sugitha T, Singh B et al (2017) Beneficial microbiomes: biodiversity and potential biotechnological applications for sustainable agriculture and human health. J Appl Biol Biotechnol 5:45–57

Yadav AN, Singh S, Mishra S, Gupta A (2019) Recent advancement in white biotechnology through fungi. Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham

Yadav AN, Rastegari AA, Yadav N (2020) Microbiomes of extreme environments: biodiversity and biotechnological applications. CRC Press, Taylor & Francis, Boca Raton, USA

Yin Q, Zhu X, Zhan G, Bo T, Yang Y, Tao Y et al (2016) Enhanced methane production in an anaerobic digestion and microbial electrolysis cell coupled system with co-cultivation of *Geobacter* and *Methanosarcina.* J Environ Sci 42:210–214

Zacharof MP (2017) Grape winery waste as feedstock for bioconversions: applying the biorefinery concept. Waste Biomass Valori 8:1011–1025

Zhang J, Loh KC, Lee J, Wang CH, Dai Y, Tong YW (2017) Three-stage anaerobic co-digestion of food waste and horse manure. Sci Rep-UK 7:1269

Zverlov VV, Berezina O, Velikodvorskaya GA, Schwarz WH (2006) Bacterial acetone and butanol production by industrial fermentation in the Soviet Union: use of hydrolyzed agricultural waste for biorefinery. Appl Microbiol Biotechnol 71:587–597