Venkata Mohan S, Lalit Babu V, Sarma PN (2008g) Effect of various pretreatment methods on anaerobic mixed microflora to enhance biohydrogen production utilizing dairy wastewater as substrate. Biores Technol 99:59–67
Venkata Mohan S, Mohankrishna G, Sarma PN (2008h) Integration of acidogenic and methanogenic processes for simultaneous production of biohydrogen and methane from wastewater treatment. Int J Hydrogen Energy 33:2156–2166
Venkata Mohan S, Veer Raghuvulu S, Dinakar P, Sarma PN (2009a) Integrated function of microbial fuel cell (MFC) as bio-electrochemical treatment system associated with bioelectricity generation under higher substrate load. Biosen Bioelect 24:2021–2027
Venkata Mohan S, Srikanth S, Sarma PN (2009b) Non-catalyzed microbial fuel cell (MFC) with open air cathode for bioelectricity generation during acidogenic wastewater treatment. Bioelectrochemistry 75:130–135
Venkata Mohan S, Srikanth S, Veer Raghuvulu S, Mohanakrishna G, Kiran Kumar A, Sarma PN (2009c) Evaluation of the potential of various aquatic eco-systems in harnessing bioelectricity through benthic fuel cell: effect of electrode assembly and water characteristics. Biores Technol 100:2240–2246
Venkata Mohan S, Mohanakrishna G, Velvizhi G, Lalit Babu V, Sarma PN (2010a) Bio-catalyzed electrochemical treatment of real field dairy wastewater with simultaneous power generation. Biochem Eng J 51:32–39
Venkata Mohan S, Mohanakrishna G, Sarma PN (2010b) Composite vegetable waste as renewable resource for bioelectricity generation through non-catalyzed open-air cathode microbial fuel cell. Biores Technol 101:970–976
Venkata Mohan S, Mohanakrishna G, Srikanth S (2011a) Biohydrogen Production from Industrial Effluents (Chapter 22). In: Pandey A, Larroche C, Ricke SC, Dussap C-G, Gnansounou E (eds) Biofuels: alternative feedstocks and conversion processes, Academic Press, Elsevier, Burlington, pp 499–524 (ISBN: 978-0-12-385099-7)
Venkata Mohan S, Mohanakrishna G, Chiranjeevi P (2011b) Sustainable power generation from floating macrophytes based ecological microenvironment through embedded fuel cells along with simultaneous wastewater treatment. Biores Technol 102:7036–7042
Virdis B, Rabaey K, Yuan Z, Keller J (2008) Microbial fuel cells for simultaneous carbon and nitrogen removal. Water Res 42:3013–3024
Wagner RC, Regan JM, Oh SE, Zuo Y, Logan BE (2009) Hydrogen production from swine wastewater. Water Res 43:1480–1488
Wang X, Cheng S, Feng Y, Merrill MD, Saito T, Logan BE (2009) The use of carbon mesh anodes and the effect of different pretreatment methods on power production in microbial fuel cells. Environ Sci Technol 43:6870–6874
Yazdi RH, Christy AD, Dehority BA, Morrison M, Yu Z, Tuovinen OH (2007) Electricity generation from cellulose by rumen microorganisms in microbial fuel cells. Biotechnol Bioeng 97:1398–1407
You S, Zhao Q, Zhang J, Jiang J, Zhao S (2006) A microbial fuel cell using permanganate as the cathodic electron acceptor. J Power Sources 162:1409–1415
Zhang JN, Zhao QL, You SJ, Jiang JQ, Ren NQ (2008) Continuous electricity production from leachate in a novel upflow air-cathode membrane-free microbial fuel cell. Water Sci Technol 57:1017–1021
Zhang Y, Min B, Huang L, Angelidaki I (2009a) Generation of electricity and analysis of microbial communities in wheat straw biomass-powered microbial fuel cells. Appl Environ Microbiol 75:3389–3395
Zhang C, Li M, Liu G, Luo H, Zhang R (2009b) Pyridine degradation in the microbial fuel cell. J Hazard Mater 172:465–471
Zhu X, Ni J (2009) Simultaneous processes of electricity generation and p-nitrophenol degradation in a microbial fuel cell. Electrochem comm 11:274–277
Zuo Y, Xing D, Regan JM, Logan BE (2008) Isolation of the exoelectrogenic bacterium ochrobactrum anthropi YZ-1 by using a U-tube microbial fuel cell. Appl Environ Microbiol 74:3130–3137

Chapter 15
Biomethanation Potential of Biological and Other Wastes

J. C. Costa, D. Z. Sousa, M. A. Pereira, A. J. M. Stams and M. M. Alves

Abstract Anaerobic technology has been traditionally applied for the treatment of carbon rich wastewater and organic residues. Anaerobic processes can be fully integrated in the biobased economy concept for resource recovery. After a brief introduction about applications of anaerobic processes to industrial wastewater treatment, agriculture feedstock and organic fraction of municipal solid waste, the position of anaerobic processes in biorefinery concepts is presented. Integration of anaerobic digestion with these processes can help in the maximisation of the economic value of the biomass used, while reducing the waste streams produced and mitigating greenhouse gases emissions. Besides the integration of biogas in the existing full-scale bioethanol and biodiesel production processes, the potential applications of biogas in the second generation lignocellulosic, algae and syngas-based biorefinery platforms are discussed.

J. C. Costa · D. Z. Sousa · M. A. Pereira · A. J. M. Stams · M. M. Alves (✉)
IBB—Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, 4710-057 Braga, Portugal
e-mail: madalena.alves@deb.uminho.pt

J. C. Costa
e-mail: carloscosta@deb.uminho.pt

D. Z. Sousa
e-mail: dianasousa@deb.uminho.pt

M. A. Pereira
e-mail: alcina@deb.uminho.pt

A. J. M. Stams
e-mail: fons.stams@wur.nl

A. J. M. Stams
Laboratory of Microbiology, Wageningen University, Dreijenplein 10 6703 HB Wageningen, The Netherlands

V. K. Gupta and M. G. Tuohy (eds.), *Biofuel Technologies*,
DOI: 10.1007/978-3-642-34519-7_15, © Springer-Verlag Berlin Heidelberg 2013

15.1 Introduction

We are presently living in the transition from a linear economy (cradle to grave) founded on abundant fossil resources to a circular biobased economy concept (cradle to cradle), where waste and by-products should re-enter the cycle of production and the energy carried should derive from renewable sources. In the circular economy's thinking, biorefinery concepts based on a variety of bioresources, by-products and (bio) wastes are emerging. There is a huge opportunity for anaerobic digestion (AD) as multi-functional process that integrates environmental protection, renewable energy production, nutrients and water recycling (Fig. 15.1). Anaerobic conversion processes should be the core of any treatment process of biodegradable waste or carbon rich wastewater, though complemented with appropriate post-treatment processes either biological or physicochemical (Van Lier and Lettinga 1999). The wide range of feedstocks suitable for biogas production includes animal waste, municipal sludge, industrial wastewater and organic fractions of municipal solid waste (OFMSW), as well as aquatic and terrestrial energy crops whenever available for energy production. One of the main advantages of AD is the possibility of recovering renewable energy in the form of biogas, which is a versatile energy carrier that can be used for electricity production, heating purposes, vehicle and jet fuel and replacement of natural gas by injection of upgraded biogas in the natural gas grid. In addition, biogas may be considered as starting compound for biotechnological production of chemicals. On the other hand, organic waste stabilisation and nutrient redistribution are, besides energy production, objectives of any AD plant (Fig. 15.1).

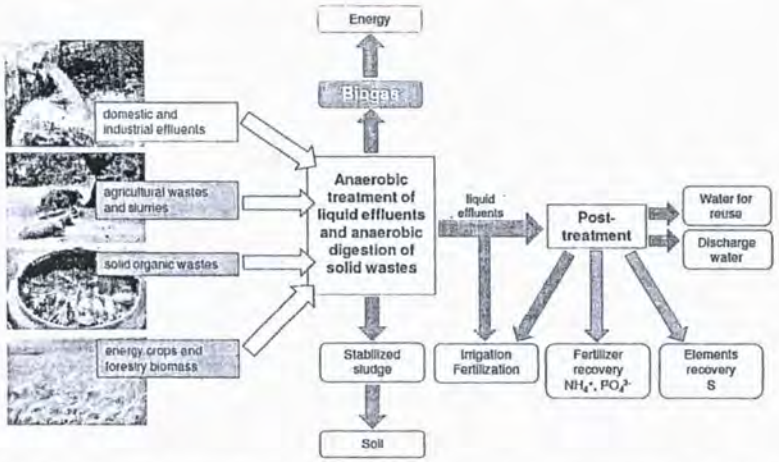

Fig. 15.1 The role of anaerobic processes for sustainable bio-resources recovery (adapted from van Lier and Lettinga 1999)

15.2 Current Applications of Anaerobic Processes

15.2.1 The Biochemical Process of Methane Production

Anaerobic digestion of complex organic substrates proceeds through a series of parallel and sequential steps, with several groups of microorganisms involved. Figure 15.2 depicts the main pathways of the anaerobic degradation of complex substrates under methanogenic conditions. AD starts with the hydrolysis of complex substrates to simpler compounds. Complex substrates, such as carbohydrates, proteins and lipids, are normally present in the form of suspended compounds or colloidal matter and, before transport through the cellular membrane, need to be transformed into smaller molecules. Such process takes place during the hydrolysis step, in which these complex compounds are hydrolysed into their basic building units. This step is aided by extracellular enzymes (hydrolases), which are excreted by fermentative bacteria. Carbohydrates are converted into soluble sugars (saccharides) by cellulases, amylases, xylanases and other hydrolytic enzymes; proteins are degraded via peptides and amino acids by proteases and peptidases; and, lipids are transformed into long chain fatty acids (LCFA) and glycerol by lipases. In the case of complex particulate substrates, hydrolysis of biopolymers can be the rate limiting step in the whole degradation process (Masse et al. 2002; Van Lier et al. 2001; Vavilin et al. 1996). An efficient hydrolysis step is important to make complex substrates accessible for the anaerobic microbial communities, optimising the methanogenic potential of the (waste) water to be treated.

Products formed during the hydrolysis step are further converted inside the bacterial cells in a process known as acidogenesis (or fermentation). Acidogenesis

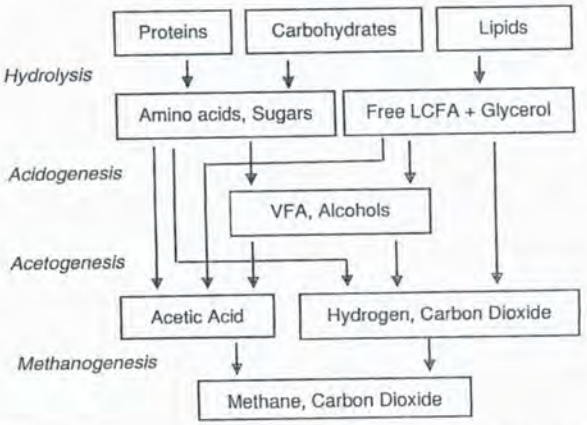

Fig. 15.2 Simplified representation of the anaerobic digestion process (Pereira 2003 adapted from Gujer and Zehnder 1983)

is the first energy yielding step during anaerobic digestion and consists in the degradation of soluble substrates, without the presence of an external electron acceptor. Main substrates for acidogenesis include soluble saccharides, amino acids and glycerol and results in the formation of acetate, propionate, butyrate, carbon dioxide, hydrogen and other organic products, such as lactate and alcohols (Harper and Pohland 1986). Soluble sugars are largely converted into acetate and hydrogen, but formation of propionate, butyrate, lactate and ethanol occurs as well. LCFA degradation requires an external electron acceptor for oxidation, and therefore is covered in the acetogenesis section. Nevertheless, hydrogenation of unsaturated fatty-acids might take place during the acidogenesis step. Normally, the bacteria responsible for the hydrolysis also ferments the resulting monomers (Schink 1997). In general, these bacteria have a short doubling time, and therefore acidogenesis is not regarded as a limiting step in the process of anaerobic digestion (Gujer and Zehnder 1983; Mosey 1983).

Fermentation products (short chain fatty acids and alcohols) and LCFA (resulting from lipid hydrolysis) can be further oxidised to acetate by obligate hydrogen producing acetogens in the acetogenic step. Fatty acids oxidation is coupled to the reduction of hydrogen ions or bicarbonate, functioning as external electron acceptors, to form hydrogen and formate, respectively. Under standard conditions (Temperature of 0 °C and Pressure of 1 atm), these reactions are thermodynamically unfavourable, and the complete conversion of the substrates only proceeds when hydrogen and formate concentration is kept low (Schink and Stams 2006; Stams and Plugge 2009). This is achieved by syntrophic association with hydrogen and formate-utilising microorganisms.

In the presence of inorganic electron acceptors other than protons and CO_2, competition for different substrates may occur. Such is the case of wastewaters containing sulfate, in which sulfate-reducing bacteria can compete with syntrophic acetogenic bacteria for electrons resulting from fatty-acids, and with methanogens for electrons resulting from hydrogen and acetate (Stams et al. 2005).

Methanogenesis is the production of methane and, in various environments, is the final step in the degradation of organic matter. This highly specialised process is carried out by methanogenic archaea, which metabolise the end products of the previous reactions (mainly hydrogen, carbon dioxide, formate, methanol, methylamines, and acetate) to form methane. In anaerobic bioreactors, this process mainly occurs through two pathways: (1) carbon dioxide reduction (hydrogenotrophic methanogenesis (Boone et al. 1989; Schink 1997)), and (2) acetate dissimilation (acetoclastic methanogenesis (Jetten et al. 1992)) (Fig. 15.2). Several authors have reported methanogenesis as being the rate-limiting conversion in the whole anaerobic digestion process in bioreactors (Fang et al. 1995; Huang et al. 2003).

15.2.2 Anaerobic Digestion of Slurries and Biowaste

Anaerobic digestion is already an effective and mature technology to produce renewable energy carriers from organic waste, to reduce odour and pathogen levels in manure and produce a biofertilizer, to reduce greenhouse gas emission from a farmstead and to treat food waste/by-products (Cantrell et al. 2008; Mata-Alvarez et al. 2000; Weiland 2010). According to data from the EurObserv'ER (2010) between 2000 and 2009, the biogas produced in Europe increased about five times. However, there are large differences of biogas technology implementation in Europe. For instance, in Germany, 51.5 ton oil equivalent (TOE) were produced per 1,000 inhabitants as biogas primary energy in 2009, whereas in Portugal only 2.2 TOE/1,000 inhabitants were produced in the same period. The average of the European Union countries was 16.7 TOE/1,000 inhabitants.

In theory, all types of biowaste can be used for biogas production. The composition of the biogas and the biogas productivity depends on the feedstock, on the reactor type and organic loading rate applied, and on the microbial consortium activity. Table 15.1 presents some data on the biogas yields for some types of waste and raw materials. There is a long tradition of anaerobic sewage sludge and animal manure treatment. Presently, agricultural applications are mainly based in co-digestion of manure with available co-substrates such as harvest residues, top and leaves of sugar beets, organic wastes from agriculture related activities, food waste, collected municipal biowaste from households and energy crops (Weiland 2010). The advantages of co-digestion are (Cecchi et al. 1996; Mata-alvarez et al. 2000; Murto et al. 2004; Neves 2009):

- Dilution of toxic substances coming from any of the substrates involved, including, possible detoxification of some xenobiotics, based on co-metabolism process;
- Improved nutrient balance reducing micro and macronutrient deficiency;
- Improving process stability;
- The use of a co-substrate can also help to establish the required moisture contents of the digester feed. Better handling and digestibility can be achieved by mixing solid waste with diluted waste;
- In addition, economic advantages can be significant, derived from the fact of sharing equipment.

Table 15.1 Average biogas yields of several substrates (adapted from Weiland 2010)

	Substrate	m^3 biogas t^{-1} feedstock
Agricultural wastes	Cow manure	25
	Pig manure	30
Agricultural raw materials	Grass	100
	Fodder beets	110
	Wheat corn	630
Non-agricultural wastes	Biowaste	120
	Food residues	240
	Used grease	800

15.2.3 Anaerobic Wastewater Treatment

The application of anaerobic technology for industrial wastewater treatment is also established (Rajeshwari et al. 2000; Angenent et al. 2004). High-rate anaerobic wastewater treatment technology has become a standard for a certain range of industrial wastewaters. Thousands of full-scale installations are in operation worldwide, treating mainly wastewater containing readily degradable organic pollutants such as Volatile Fatty Acids (VFA) and carbohydrates. Reliable technologies, such as the upflow anaerobic sludge blanket (UASB), the expanded granular sludge bed (EGSB) and the internal circulation (IC) reactors, promoted the confidence in AD technology. Recently, the inverted anaerobic sludge blanket (IASB) reactor (Alves et al. 2007) was developed for the treatment of effluents with high content of lipids and the proof of concept demonstrated an efficient treatment capacity for an extremely concentrated slaughterhouse effluent. The first full-scale reference is presently under construction.

15.3 The Role of Biogas in Biorefinery Platforms

15.3.1 Introduction

According to the International Energy Agency (IEA) Bioenergy Task 42, "biorefinery is the sustainable processing of biomass into a spectrum of marketable products (food, feed, materials, chemicals) and energy (fuels, power, heat)".

The integration of AD technology within a biobased economy is a logical and intuitive step now. Biorefineries for the production of chemicals and biofuels from vegetable biomass have been in focus in the recent years (Langeveld et al. 2010). Special attention has been given to the development and optimisation of processes for the production of ethanol and biodiesel, which are presently done at full scale in several countries. Brazil and the United States have well established and growing economy based on ethanol production. Second generation ethanol from lignocellulosic biomass is emerging and will be a mature technology in the near future. Biodiesel production from vegetable oils is representative in countries such as Germany and France. Integration of AD with these processes can help in the maximisation of the economic value of the biomass used, while reducing the waste streams produced and mitigating greenhouse gases emissions. Besides, other products such as compost can be produced and further recycled for agriculture purposes or for vegetable biomass growth.

15.3.2 Biogas Opportunities in Bioethanol Production

First generation bioethanol, derived from starch crops like corn and wheat, or sugar crops like sugar cane and sugar beet, has been rapidly adopted as a primary

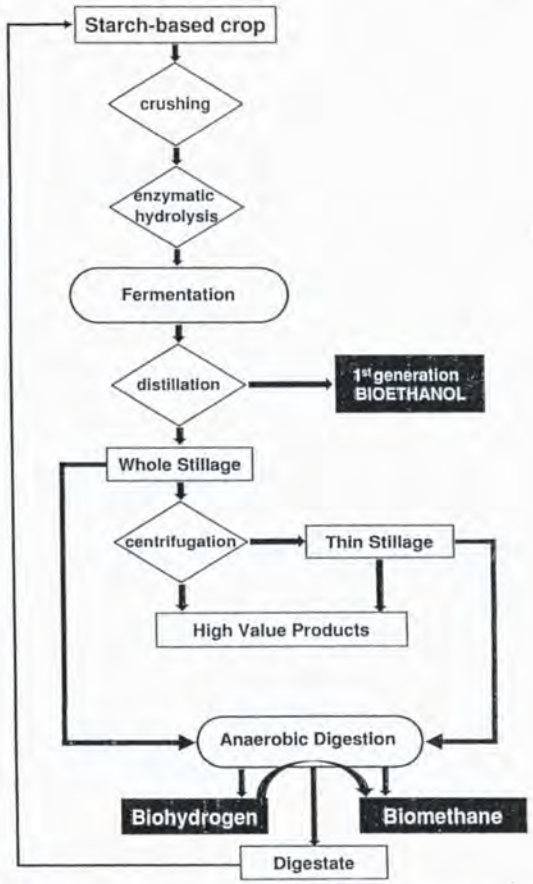

Fig. 15.3 Biorefinery concept for the production of bioethanol from sugar-based crops, including an anaerobic digestion step for the energetic valorisation of the by-products

transportation fuel in the United States and Brazil. Total world production grew from 17.1 billion litres in 2000 to 86.9 billion litres in 2010, with the United States as the top producer with 50 billion litres, mainly corn-derived ethanol, followed by Brazil with 26 billion litres using sugarcane as primary feedstock. The European Union produced 4.4 billion litres of ethanol in 2010, accounting for 5.1 % of world's production (Lichts 2010).

Typical steps on current sugar-based ethanol technology include milling of the sugar cane (or sugar beet crops) to extract the juice, fermentation of sugar to ethanol by yeast and distillation of ethanol (Fig. 15.3). For starch (cereal) based crops, similar procedures are performed, with an additional hydrolysis step to break down the polymers into simple C6 sugars (Fig. 15.4). Both processes produce large amounts of by-products, namely:

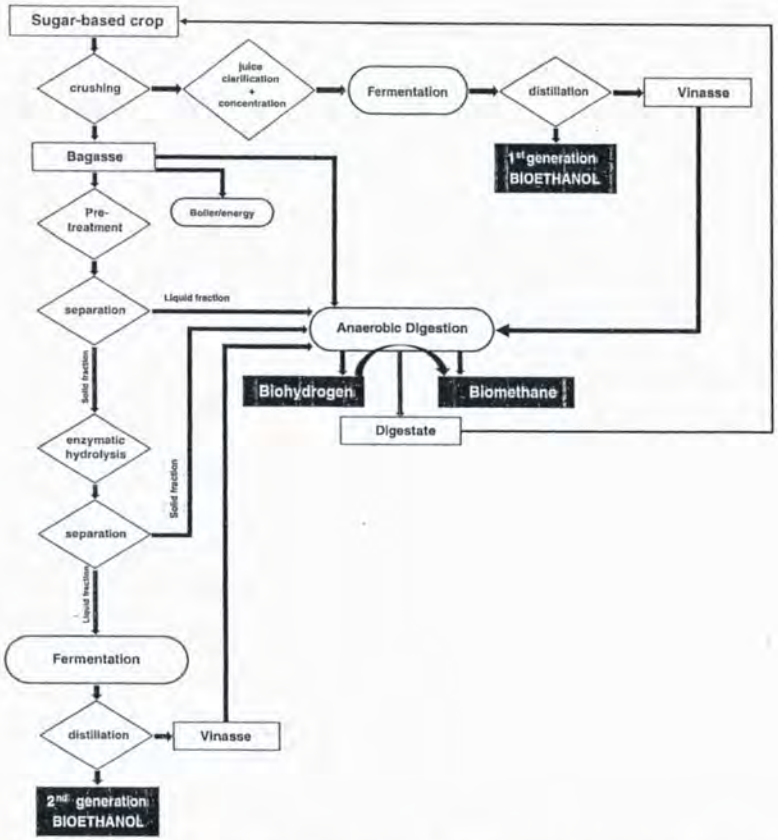

Fig. 15.4 Biorefinery concept for the production of bioethanol from starch-based crops, including an anaerobic digestion step for the energetic valorisation of the by-products

- Bagasse: the biomass left over after sugarcane has been crushed and the juice extracted. Approximately 240 kg are generated per ton of sugarcane (Dias et al. 2009);
- Vinasse: the effluent obtained after ethanol is distilled from the fermented sugar mixture,
- Whole stillage: the effluent obtained after ethanol is distilled from the fermented cereal mixture. Up to 20 litres per litre of ethanol produced depending on the feedstock used (van Haandel and Catunda 1994).

In a traditional corn-to-ethanol process, the whole stillage is centrifuged to produce wet cake (solid fraction) and thin stillage (liquid fraction). A significant

fraction (around 50 %) of the thin stillage is recycled as backset to the second stage of the liquefaction process and the remaining part is evapourated to syrup. The syrup is then mixed with the centrifuged solids to produce distiller's dried grains with soluble (DDGS) that are sold as livestock feed (Eskicioglu et al. 2011). DDGS processing (centrifuging, evaporation and drying) is energy demanding. It can account for approximately 35 % of electricity and 30 % of natural gas consumption of a bioethanol plant (Meredith 2003). Currently, it is still profitable to process whole stillage and sell as animal feed, but as fuel demand increases, the risks of DDGS market saturation increase as well.

Anaerobic digestion has long been considered an alternative approach to handle ethanol by-products, reduce the environmental impact of the generated wastewaters and improve the net energy balance ratio of the process (Figs. 15.3 and 15.4) (Plugge et al. 2009). Research has primarily been focused on thin stillage because it is the largest wastewater contributor. In the 1980s, mesophilic studies on corn thin stillage reported promising performances with methane yields of 250–370 L CH_4 kg^{-1} chemical oxygen demand (COD) removed that could replace about 60 % of the daily energy requirement of the bioethanol plant (Stover et al. 1984). In recent studies, AD of corn thin stillage was evaluated at thermophilic conditions. Whole stillage exits the distillation column at above 55 °C, and thus heating demand to achieve thermophilic digestion is not so high and can provide improved efficiency and economics. Schaefer and Sung (2008) reported methane yields ranging between 600 and 700 L CH_4 kg^{-1} volatile solids (VS) removed during AD of thin corn stillage in thermophilic continuous stirred-tank reactor (CSTR) operated at 30, 20 and 15 days hydraulic retention time (HRT). It was estimated that natural gas consumption at corn derived ethanol plants could be reduced by 43–59 % with this level of methane production. In another study, thin corn stillage was treated in thermophilic sequencing batch reactors (SBR) to produce 254 L CH_4 kg^{-1} TCOD fed with a 10 day HRT (Agler et al. 2008). These authors also estimated that the methane generated would reduce natural gas consumption in conventional dry grind ethanol plants by 51 %, improving the net energy balance ratio of ethanol from 1.26 (conventional) to 1.70. Lee and co-workers (2011) demonstrate that mesophilic anaerobic digestion might provide a more attractive option for enhancing the net energy gain in the existing corn-to-ethanol industry. Thin corn stillage treatment in a mesophilic CSTR at 25 day HRT rendered a methane yield of 271 L CH_4 kg^{-1} COD fed, which if incorporated in a corn-to-ethanol plant could increase the net energy balance ratio to 1.80.

Mesophilic or thermophilic digestion of whole corn stillage has only recently been studied. Biochemical methane potential (BMP) batch assays indicated significant methane potential for whole corn stillage at concentrations ranging from 6.35 to 50.8 g TCOD L^{-1}. Specific methane yields ranging between 401 and 458 L CH_4 kg^{-1} VS added and between 429 and 693 L CH_4 kg^{-1} VS added were obtained at mesophilic and thermophilic condition, respectively (Eskicioglu et al. 2011). However, continuous flow experiments with the full-strength whole corn stillage (254 g TCOD L^{-1}) at organic loading rates of 4.25, 6.30 and

9.05 g TCOD L^{-1} d^{-1} indicated unstable performance under thermophilic conditions and at mesophilic temperatures. Only at 60 day HRT was stable.

In sugarcane-based ethanol plants (Fig. 15.3), the bagasse generated is presently used directly as a solid biofuel to co-generate heat and electricity which is used in the plant and also sold to the electricity grid (Amorim et al. 2011). The vinasse produced (around 12 L for each litre of ethanol distilled (Amorim et al. 2011)), rich in minerals, such as potassium, calcium, magnesium, nitrogen and phosphorus, has been used as a fertilizer in the sugarcane fields. However, the increasing volume of vinasse is saturating the soil and threatening the quality of the ground water. It is not economically feasible to transport the vinasse over longer distances, and therefore a solution to this environmental problem should be found on location. AD is a viable approach to treat cane vinasse (Blonkaja et al. 2003; Peréz-Garcia et al. 2005; Seth et al. 1995; Souza et al. 1992) and contributes for the production of renewable energy.

Bagasse combustion and AD of vinasse are presently implemented at some distilleries at full scale (van Haandel 2005). By using steam turbines fuelled with bagasse combustion, electric power can be generated at a rate of 1 MWh per m^3 of produced alcohol. Anaerobic digestion can be applied to vinasse to produce enough biogas for 0.5 MWh/m^3 of alcohol, bringing total electric power production from subproducts to 1.5 MWh/m^3 of alcohol (van Haandel 2005).

Nowadays, bagasse is also generally recognised as a promising feedstock for cellulosic ethanol production, i.e. second generation (2G) bioethanol (derived from the non-food component of biomass) (Fig. 15.3), and it is expected that biofuel produced in this way will have less impact on the environment. Most processes and technologies for 2G bioethanol are still under development in different research activities and pilot/demo plants but are not yet on the market (IEA 2010). The challenge for biorefineries in the future is to use all side- and by-products from industry processes as well as crop residues.

Production of bioethanol, methane and heat from sugarcane bagasse in a biorefinery concept has been recently evaluated by Rabelo and co-workers (2011). Four different biofuel production scenarios showed that 63–65 % of the energy produced by bagasse combustion could be recovered by combining ethanol production with the combustion of lignin and hydrolysis residues, and AD of pretreatment liquors, whereas only 32–33 % of the energy was recovered by bioethanol production alone (Rabelo et al. 2011). The possibility of using wheat straw for the production of bioethanol (from cellulose), biohydrogen (from hemicellulose) and biogas (from effluents of bioethanol and biohydrogen production) was also evaluated (Kaparaju et al. 2009). Fermentation of cellulose, obtained from hydrothermal pre-treatment of wheat straw and enzymatic hydrolysis, yielded 0.41 g ethanol g^{-1} glucose, while dark fermentation of the hydrolysate produced 178.0 mL H_2 g^{-1} sugars, and the effluents from both bioethanol and biohydrogen processes produced methane with the yields of 0.324 and 0.381 m^3 kg^{-1} VS added, respectively. Six different wheat straws to biofuel production scenarios were further evaluated showing that either use of wheat straw for biogas production or multi-fuel production were energetically most efficient processes

compared to the production of mono-fuel such as bioethanol when fermenting C6 sugars alone (Kaparaju et al. 2009). Other studies focused on the evaluation of whole-crop biorefinery concept. For instance, Luo and co-workers (2011) investigated the utilisation of the whole rapeseed plant (seed and straw) for multiple biofuels production. An ethanol yield of 0.15 g^{-1} ethanol g^{-1} dry straw was obtained after combined alkaline peroxide and steam pre-treatment. Methane alone or hydrogen and methane were produced, in batch, from the individual process by-products (rapeseed cake, glycerol, hydrolysate and stillage) at similar energy yields (11–15 kJ g^{-1} VS). In continuous operation, only the two stage hydrogen and methane fermentation could work stably at an organic loading rate up to 6 g COD L^{-1} d^{-1} with average yields of 45 mL H_2 g^{-1} VS and 347 mL CH_4g^{-1} VS (Luo et al. 2011). The energy recovery efficiency from rapeseed plant increased from 20 % in the conventional biodiesel process to 60 % in the biorefinery concept, by utilisation of the whole rapeseed plant for biodiesel, bioethanol, biohydrogen and methane production.

15.3.3 Biogas Opportunities in Biodiesel Production

First generation biodiesel is produced from vegetable oils of oleaginous plants (e.g. rapeseed, soybean, sunflower, palm oil, etc.) by transesterification processes or cracking (Nigam and Singh 2011). The competition with agricultural land raised ethical issues and new generations of biodiesel appeared: the second generation from non-edible vegetable oil (e.g. jatropha) and from wastes (e.g. animal fat), and the third generation of biodiesel from algae (Rittmann 2008). Globally, there are more than 350 oil-bearing crops identified as potential sources for biodiesel production (Atabani et al. 2012). From the available techniques for oil conversion in biodiesel, transesterification of oil with alcohol in the presence of a catalyst is the most used and technically feasible (Marchetti et al. 2007).

The costs associated with biodiesel are a limiting factor for their utilisation. In future biorefinery concepts, by-products from the cultivation of energy crops should be used to produce other biofuels and/or added value products. For instance, although biodiesel could be the ultimate economical product in a biorefinery, the by-products from this process can also be utilised for the production of methane and hydrogen in anaerobic digesters, and consequently improving the energy and economic balance of these production systems (Borjesson and Mattiasson 2008). Besides, the excess energy can be sold to the public electricity grid, and the AD digestate may be used as fertilizer for the production of new biomass crops. The main biodiesel derived by-products are:

- Crude glycerol: it occurs in vegetable oils at a level of approximately 10 % (w/w). The make-up of crude glycerol varies depending on the parent feedstock and the biodiesel production process. Crude glycerol generated by the most usual method of homogeneous base-catalysed transesterification, and separated from biodiesel

by settling, contains approximately 50–60 % of glycerol, 12–16 % of alkalies (especially in the form of alkali soaps and hydroxides), 15–18 % of methyl esters, 8–12 % of methanol and 2–3 % of water (Kocsisova and Cvengroš 2006). COD of crude glycerol can exceed 1,000 g L^{-1} and the pH goes over 9.

- Biodiesel processing wastewater: water is used at the end of the biodiesel production chain to remove impurities such as excess of oil and methanol, residual catalyst, soap and glycerol. A large amount of wastewater is generated in this process, from 0.2 to 1.2 L per litre of biodiesel produced. This wastewater has a high pH value of approximately 9 and a COD content of hundreds of grams per litre, which is particularly attributed to methanol, glycerol and oil and grease (Phukingngam et al. 2011).
- Crop waste after oil extraction (cake): it refers to the remaining biomass (aquatic and terrestrial energy crop) after the oil extraction for biodiesel production. This waste still hold some lipids. Traces of solvent, salts and pigments are other examples of elements that may be found in the waste.

Wastes and by-products from the biodiesel industry still contain high energetic potential. To optimize the energetic balance of both aquatic and terrestrial crops used for biodiesel production, an anaerobic digestion process can be included in a biorefinery structure to convert the wastes and by-products in methane and hydrogen (Fig. 15.5).

Glycerol is the main by-product of biodiesel production (by the transesterification process). The crude glycerol possesses very low value because of the impurities contained. As the demand and production of biodiesel grow exponentially, the huge amounts of glycerol produced and subsequent destination is a problematic issue associated with biodiesel manufacturing. Usually, crude glycerol is refined and channelled to markets in the pharmaceutical and cosmetic industries (Demirbas 2009). When refined to a chemically pure substance, it would be a very valuable by-product. Purifying it to that stage is costly and generally out of the range of economic feasibility for the majority of small/medium biodiesel facilities. Currently, some biodiesel producing companies from the European Union are facing problems in getting rid of excess glycerol, as disposal is also quite expensive (Luo et al. 2011). Studies have been conducted to investigate alternative glycerol utilisation routes such as production of ethanol, 1, 3-propanediol and other high value products (Silva et al. 2009). Also, the AD of crude glycerol to produce methane and hydrogen is being explored to make biodiesel more competitive. However, high contents of COD and possible accumulation of fatty acids, the presence of methanol, inorganic salts, unreacted mono-, di-and triglycerides and methyl esters and the lack of nitrogen represents severe disadvantages for AD since these characteristics can inhibit the process.

The co-digestion of crude glycerol with a complementary substrate is the most used technique to overcome these problems, by decreasing the C:N ratio or by diluting the waste. For example, the co-digestion of potato processing wastewater with glycerol increased the methane per litre of wastewater treated by a factor of 1.5 (Ma et al. 2008). Adding glycerol to manure can increase significantly the

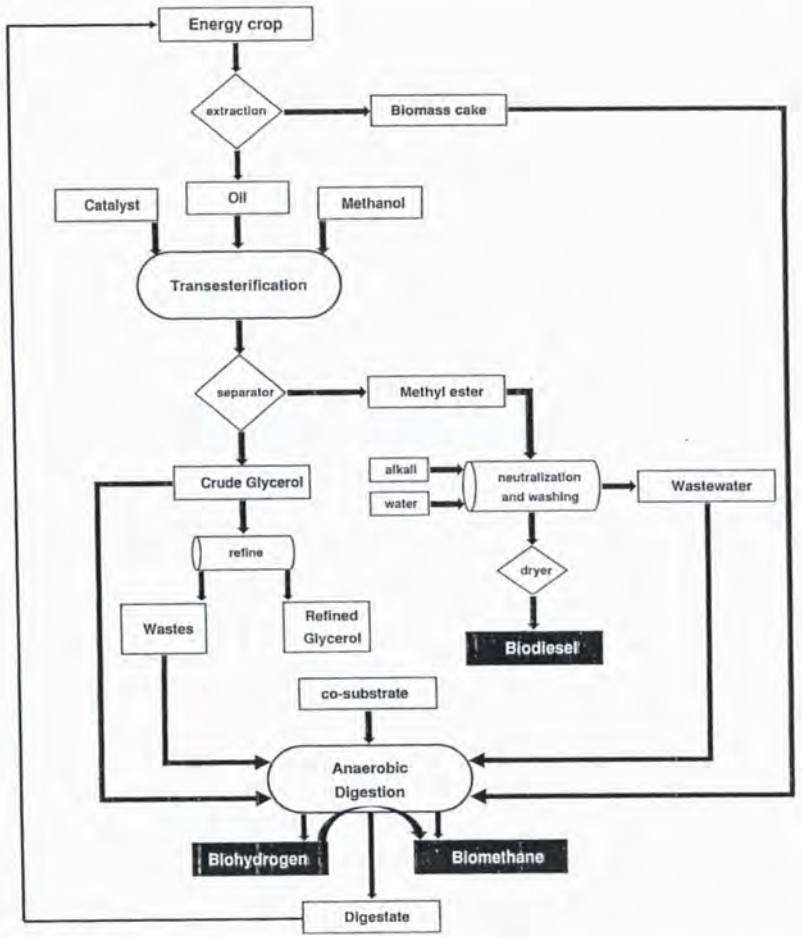

Fig. 15.5 Biorefinery concept for production of biodiesel, including an anaerobic digestion step for the energetic valorisation of wastes and by-products

methane production. Under mesophilic conditions, the addition of 4 % glycerol to screened manure increased biogas production by up to 400 %, and at thermophilic conditions, using sonicated mixtures of ground cattle manure with 6 % added glycerol, 0.35 m^3 CH_4 kg^{-1} COD removed were obtained (Castrillón et al. 2011). Amon and co-workers (2006) showed that the addition of crude glycerol (6 %) to a mixture of maize silage, pig manure and rapeseed meal, increased methane production from 570 to 680 L CH_4 kg^{-1} VS. Mesophilic anaerobic treatment of crude

glycerol as an only organic substrate is feasible, although the specific inhibition effects and requirements resulting from the nature and composition of the substrate can cause difficulties (Kolesarova et al. 2011). Glycerol should be carefully added to an anaerobic co-digestion facility because if certain threshold values are exceeded, severe damages can be done to the process and partial or complete inhibition can be caused. Different limits were reported, from 1 to 6 % of glycerol (Amon et al. 2006; Fountoulakis et al. 2010; Holm-Nielsen et al. 2008). At those limits no VFA accumulation was observed as signal of organic overloading. The different limits may be explained by the different characteristics of the co-substrate, mainly related with their nitrogen content and respective C:N ratio imposed in the anaerobic reactor.

Besides the traditional vegetable oil and animal fat, other substrates have been used to produce biodiesel, such as used-cooking oils. Also, biodiesel from microalgae is gaining market relevance, although mainly in research and development stage (see Sect. 15.3.4). According to the EN14214 (2008), the biodiesel obtained by the catalysed transesterification process requires purification, which generates large amounts of highly polluted wastewater. This wastewater has pH values in the range of 9.2–10.8, COD from 168 to 300 g L^{-1} and fat content of 18–22 g L^{-1} (Jaruwat et al. 2010). A combination of acidification—electrocoagulation with a subsequent AD step to efficiently purify wastewater derived from biodiesel manufacturing was developed (Siles et al. 2011). The anaerobic biodegradability of acidified—electrocoagulated wastewater was found to be 98 %, while the methane yield coefficient reached 297 L CH_4 kg^{-1} COD removed (1 atm, 0 °C). Also, the anaerobic co-digestion of glycerol and wastewater derived from biodiesel manufacturing, in which COD was found to be 1,054 and 428 g L^{-1}, respectively, was studied in batch laboratory-scale reactors at mesophilic temperature (Siles et al. 2010). Wastewater biodegradability was found to be near 100 %, while the methane yield coefficient was 310 L CH_4 kg^{-1} COD removed (1 atm, 25 °C).

The AD of the energy crop fraction not rich in oil and the remaining fraction after oil extraction are potentially good candidates for valorisation in an AD process. The high nitrogen content of lipid-free cake may be problematic for the process if ammonia exceeds inhibitory values (0.1–1.1 g N L^{-1}). Though the rapeseed cake can be efficiently degraded with a methane yield of 378 L CH_4 kg^{-1} VS, corresponding to 82 % of the theoretical value (Luo et al. 2011). Gunaseelan (2009) examined two integrated biorefineries schemes for the energetic valorisation of *Jatropha curcus*: (1) convert plant pruning, fruit hulls and de-oiled seed cake to methane concomitantly with oil to biodiesel, and (2) convert the seeds, plant pruning and fruit hulls entirely to methane. According to the author, the first scheme would produce 90 GJ ha^{-1} y^{-1} (with 54 GJ from the oil) and the second alternative would produce 97 GJ ha^{-1} y^{-1}. These results were obtained based on batch assays; therefore, conclusions should be drawn carefully.

As conclusion is possible to state that anaerobic co-digestion technology could be readily integrated into existing biodiesel facilities, thus establishing true biorefineries and revolutionising the biodiesel industry by dramatically improving its economics (Yazdani and Gonzalez 2007). Besides, AD could help circumvent the

disadvantages of chemical catalysis such as low product specificity, use of high pressure and/or temperatures, inability to use crude glycerol with high levels of contaminants, etc. For instance, for 1 ha rapeseed plant per year, 1,230 kg biodiesel and 627 kg ethanol could be obtained, but also, 27.4 kg hydrogen and 1,626 kg methane can be achieved by anaerobic co-digestion of the by-products (Luo et al. 2011).

15.3.4 Biogas Opportunities from Algae

Currently, biodiesel from vegetable oils and bioethanol from starch or sugar crops are the most technically feasible and commercialised alternative renewable biofuels. Algae should be seen as a promising source for bioenergy production in the future, since it has several advantages over other energy crops, including high yields and growth rates, the capacity to capture CO_2, and do not compete with food crops for arable land (Table 15.2). Two major drawbacks are still associated with the production and transformation of algae to bioenergy, i.e. the quantity of nutrients required and the high costs associated with dewatering. The inclusion of

Table 15.2 Advantages and disadvantages of algae biomass as energy crop

Advantages	Disadvantages
High photon conversion efficiency (high biomass yields per hectare)	Costs of cultivation
Produced all year round	Supply of CO_2 for high efficiency production
High growth rates	Harvesting process
Numerous species	High sodium concentration in marine species
Load on freshwater source is reduced (can utilise salt and wastewater streams)	Presence of sand
Do not compromise food production (improved land use efficiency)	High content of nitrogen and phosphate
Help in bio-fixation of waste CO_2 (CO_2-neutral fuel production)	Low C/N ratio
Assimilate nutrients and produce dissolved oxygen	
Does not require herbicides or pesticides application	
Valuable co-products such as proteins and residual biomass (fertilizer)	
Biochemical composition can be mutated to increase the yield	
Low lignin content	
Releases low amounts of H_2S	
Produces non-toxic and highly biodegradable biofuels	
Double credits under new EU directives	

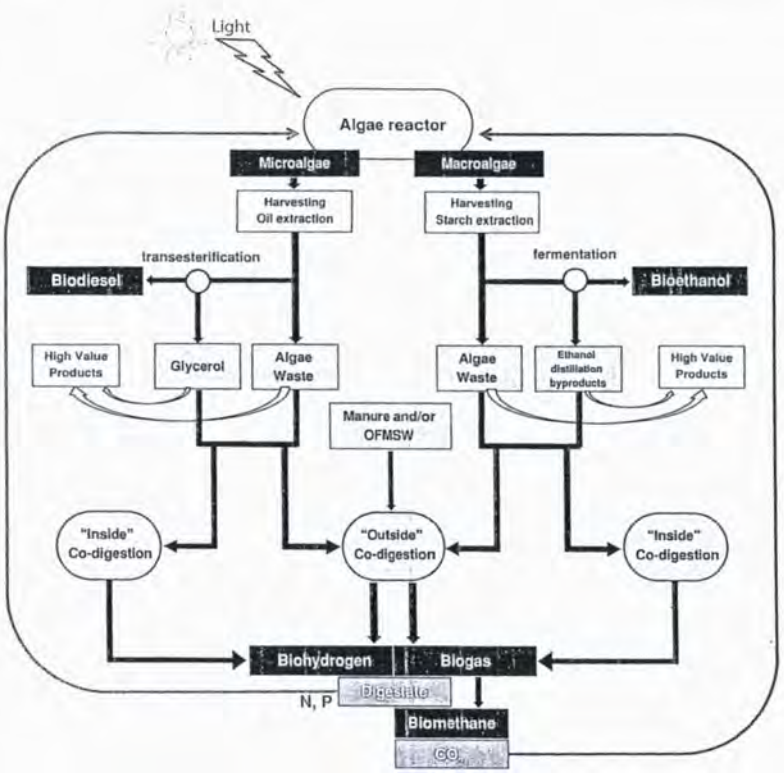

Fig. 15.6 Algae-based biorefinery showing the several biofuels produced and the potential role of anaerobic co-digestion

an AD process, in a biorefinery concept (Fig. 15.6), may help in overcoming both problems since it may provide the necessary nutrients (and CO_2) for the biomass cultivation, and supply the energy needed for a positive economical balance.

Algae are already used for the production of several high value products, including pigments, antioxidants, fatty acids, vitamins, pharmaceutical products and protein-rich feed for both animal and human consumption. Regarding the biofuels market, algae are the only feedstock potentially capable of completely replace the fossil fuel dependency. In the last three or four decades, many public and private investors, like ExxonMobil, have become interested and millions of dollars have been invested worldwide. Research in algae-biomass for biodiesel, bioethanol, biogas and hydrogen is a hot topic nowadays, although there is not yet production of biofuel from algae at a commercial scale (Demirbas 2009; Rusten and Sahu 2011).

Both micro (photosynthetic cells mostly unicellular) and macroalgae (multicellular, fast growing, marine and freshwater plant-like) have the necessary characteristics to be used as biomass for biofuel production. Microalgae can have up to 80 % of the dry weight in lipids, being therefore a potential good energy crop for biodiesel production. The yields of oil and fuels can be much higher (10–100 times) than terrestrial energy crops. However, unlike terrestrial energy crops, extensive drying is required before the biofuels production as the presence of water will inhibit several downstream processes, such as lipid extraction and transesterification.

The extraction of lipids from microalgae generates a by-product, mainly composed of proteins and polysaccharides, which can go up to 60 % of the total biomass. There is an increased demand for protein-rich substances available for human food and animal feed. However, in a more integrated approach, microalgae residues can be fermented to produce bioethanol and/or biogas and have further high-value products extracted in a biorefinery type concept (Fig. 15.6). Also, a two-step AD biorefinery may be a good alternative, with the consecutive production of hydrogen and methane. Mussgnug and co-workers (2010) observed an increase of 23 % in the methane yield from *Chlamydomonas reinhardtii* after H_2 production, compared with the AD of fresh microalgae.

There is no substantial knowledge about the production of bioethanol from microalgae and/or microalgae wastes but there are several research studies exploring their methane potential (Table 15.3). The methane yield is very dependent on the algae species, values of 187 and up to 387 L CH_4 kg^{-1} VS were obtained with *Scenedesmus obliquus* and *Chlamydomonas reinhardtii*, respectively. Regarding the biohydrogen production, 66 L H_2 kg^{-1} VS were obtained with the residues of *Scenedesmus* after transesterification and pre-treatment with NaOH at 100 °C (Yang et al. 2011).

Macroalgae are more suitable for bioethanol or biogas production, due to the high carbohydrates content that can go up to 60 %, depending on the species, the season and place of cultivation. In contrast, the low content in lipids makes the biodiesel production unfeasible. Currently, no commercially credible assessment of the economic feasibility of macroalgae cultivation for biofuel production has been published. However, several research studies report the BMP of macroalgae (Table 15.3). Species from the genera *Gracilaria*, *Laminaria*, *Ulva* and *Sargassum* are the most studied and with highest methane potential. Currently, the vast majority of macroalgae are collected for human consumption and for hydrocolloid production.

The use of macroalgae resources is improbable to support a stand-alone biogas or bioethanol process. However, it is possible to introduce these processes into an existing facility where other biomass raw materials can be processed, such as co-digestion with manure or the OFMSW (Fig. 15.6). Couple algae production and wastewater treatment seems a very promising approach to two big markets, fuels production and wastewater treatment. In fact, wastewaters derived from municipal, agricultural and industrial activities potentially provide cost-effective and sustainable means of algae-biomass growth for subsequent biofuels production

Table 15.3 Macro and microalgae species and respective biochemical methane potential (BMP)

	%CH_4	L CH_4 kg^{-1} VS	Conditions	Pre-treatment	Reference
Microalgae					
Arthrospira platensis	61	293	38 °C/batch	–	Mussgnug et al. (2010)
Chlamydomonas reinhardtii	66	387	38 °C/batch	–	Mussgnug et al. (2010)
Chlamydomonas reinhardtii	–	310	38 °C/batch	24 h @ 105 °C	Mussgnug et al. (2010)
Chlamydomonas reinhardtii	–	476	38 °C/batch	H_2 production	Mussgnug et al. (2010)
Chlorella sp.	–	245	35 °C/CSTR (HRT = 15d)	Dried, transesterification, add glycerol	Ehimen et al. (2011)
Chlorella sp.	68	302	35 °C/CSTR (HRT = 15d)	Dried, transesterification	Ehimen et al. (2011)
Chlorella kessleri	65	218	38 °C/batch		Mussgnug et al. (2010)
Chlorella kessleri	–	159	38 °C/batch	24 h @ 105 °C	Mussgnug et al. (2010)
Chlorella vulgaris	–	240	35 °C/CSTR (HRT = 28d)	–	Ras et al. (2011)
Dunaliella salina	64	323	38 °C/batch	–	Mussgnug et al. (2010)
Euglena gracilis	67	325	38 °C/batch	–	Mussgnug et al. (2010)
Scenedesmus obliquus	62	178	38 °C/batch	–	Mussgnug et al. (2010)
Macroalgae					
Enteromorpha sp.	–	154 ± 7	37 °C/batch	–	Costa et al. (2012)
Gelidium amanssii	–	239	35 °C/batch	Ethanol production (fermentation residue)	Park et al. (2012)
Gelidium amanssii	–	283	35 °C/batch	Ethanol production (saccharification residue)	Park et al. (2012)
Gracilaria sp.	–	280–400	35 °C/batch	–	Bird et al. (1990)
Gracilaria sp.	–	182 ± 23	37 °C/batch	–	Costa et al. (2012)
Laminaria sp.	–	260–280	–	–	Chynoweth (2005)

(continued)

Table 15.3 (continued)

	%CH_4	L CH_4 kg^{-1} VS	Conditions	Pre-treatment	Reference
Laminaria digitata	–	500	–	–	Morand and Briand (1999)
Laminaria digitata	–	219	35 °C/batch	–	Adams et al. (2011)
Macrocystis	–	390–410	–	–	Chynoweth (2005)
Ulva sp.	59	110	35 °C/batch	–	Briand and Morand (1997)
Ulva sp.	55	94	35 °C/batch	Washed	Briand and Morand (1997)
Ulva sp.	49	145	35 °C/batch	Dried	Briand and Morand (1997)
Ulva sp.	52	177	35 °C/batch	Ground dried	Briand and Morand (1997)
Ulva sp.	54	203	35 °C/CSTR (HRT = 15d)	Grounded	Briand and Morand (1997)
Ulva sp. (hydrolysis juice)	–	313–330	BFBR (HRT = 2.5–5d)	Pre-hydrolysed	Morand and Briand (1999)
Ulva sp.	–	127	35 °C/batch	–	Otsuka and Yoshino (2004)
Ulva sp.	–	180	35 °C/batch	Washed, dried, grounded	Otsuka and Yoshino (2004)
Ulva sp.	–	148	35 °C/batch	Centrifuged and grounded	Peu et al. (2011)
Ulva sp.	–	196 ± 9	37 °C/batch	–	Costa et al. (2012)
Sargassum fluitans	–	143–182	–	–	Gunaseelan (1997)
Sargassum pteropleuron	–	119–171	–	–	Gunaseelan (1997)
Sargassum sp.	–	260–380	–	–	Chynoweth (2005)
Sargassum spp.	–	120–190	35 °C/batch	–	Bird et al. (1990)
Seaweed	44	120	37 °C/batch	–	Nkemka and Murto (2010)
Seaweed (leachate)	62	120	37 °C/batch	Pre-hydrolysed	Nkemka and Murto (2010)

(Pittman et al. 2011). AD may be used to convert the algae-biomass in bioenergy (biogas and hydrogen), and algae may act as a remediation agent by removing nutrients and sequestering CO_2, making an in situ biomethane upgrade. It was already reported that the anaerobic co-digestion of *Ulva* sp. with waste activated sludge seems to have a positive synergetic effect on the sludge biodegradability rate, with an increase up to 26 % (Costa et al. 2012).

The biogas produced in the AD step may serve as the primary source of energy for the production and conversion of the algae-biomass. Moreover, the CO_2 generated from the combustion of biogas or from the purification to biomethane, and the nutrient-rich digestate formed during the AD, can be recycled in a closed-loop to produce algae-biomass (Fig. 15.6). Two main bottlenecks can be identified in the AD of algae biomass. First, the biochemical composition and the nature of the cell wall may decrease their biodegradability. Then, the high cellular protein content, impaired after the oil extraction in the case of microalgae, imposes a low C/N ratio (around 6:1), far from the ideal for anaerobic digestion, and may potentially result in the production of toxic ammonia concentrations (Sialve et al. 2009). The co-digestion of microalgae residues with a nutrient-deficient co-substrate, such as glycerol, the major by-product generated in the biodiesel industry, has the potential to improve the overall energy recovered as methane. It was reported that a C/N ratio of 12.4 increased the methane production by more than 50 % when co-digesting the microalgae residues with glycerol, compared with the methane production obtained by digesting the residues alone (Ehimen et al. 2011). It is important to state that glycerol is a versatile chemical with more than 1,000 known commercial applications; however, this market has becoming saturated due to the strong growth in biodiesel production.

In conclusion, we can say that microalgae have high oil content but are difficult to cultivate and harvest and macroalgae present low-cost cultivation and harvesting possibilities but are low in lipids. In a biorefinery all routes should be explored, either to produce biofuels or high-value products. Therefore, it seems very attractive the integration of an AD step in an algae-based biorefinery since it seems the logical answer for the two major drawbacks previously detected, generates energy that can balance the unfavourable energetic bill, and can provide the nutrients and carbon dioxide needed for the biomass growth. Concomitantly, algae may be seen as a bioremediation agent to remove nutrients and capture CO_2 in a wastewater treatment plant.

15.3.5 Biogas from Syngas

Anaerobic digestion is suitable for converting virtually all organic materials to methane. However, some more recalcitrant substrates, such as lignocellulosic biomass or other dry wastes (plastic and rubber, etc.), demand (thermo) chemical pre-treatments, which are often costly and do not always substantially improve methane production. Gasification of all kind of compact biomass/wastes, followed by a biological process for the conversion of the resulting syngas (mixture of CO, CO_2 and H_2) to methane, would be a feasible and promising alternative for the valorisation of recalcitrant materials.

Coal gasification has been traditionally used to produce syngas, which can be further used in thermochemical catalytic processes to produce fuels, such as methane (Fig. 15.7a). However, syngas bioconversion (Fig. 15.7b) has several advantages over catalytic processes: it can operate at milder temperatures and pressures, a fix CO/H_2 ratio is not required, there is less susceptibility to the impurities in the gas, and it does not require any costly pre-treatment of the feed gas or costly metal catalysts (Abubackar et al. 2011; Henstra et al. 2007). Syngas direct conversion to methane can be accomplished by various methanogens, such as *Methanosarcina* and *Methanothermobacter* species (Eqs. 15.1 and 15.2) (Daniels et al. 1977; O'Brien et al. 1984; Rother and Metcalf 2004).

$$4CO + 2H_2O \rightarrow 3CO_2 + CH_4 \quad (15.1)$$

$$CO + 3H_2 \rightarrow CH_4 + H_2O \quad (15.2)$$

Alternatively, a two-step process could be designed in which:

1. CO is firstly converted into acetate by acetogenic carboxydotrophic bacteria (Eqs. 15.3 and 15.4) or to H_2 by bacteria able to perform the water shift reaction (Eq. 15.5):

$$4CO + 4H_2O \rightarrow CH_3COO^- + 2HCO_3^- + 3H^+ \quad (15.3)$$

$$2CO + 2H_2 \rightarrow CH_3COO^- + H^+ \quad (15.4)$$

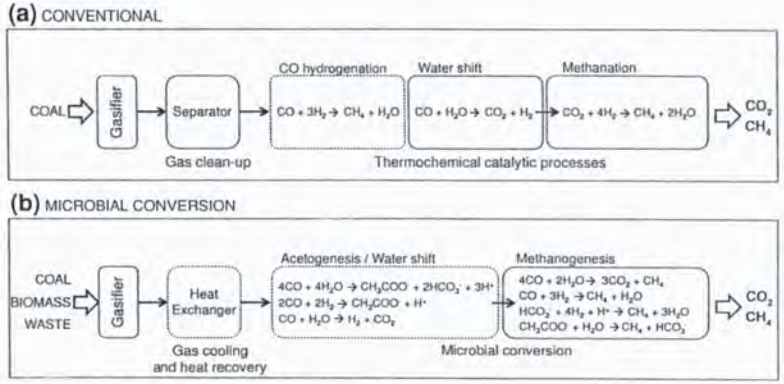

Fig. 15.7 Conventional (thermochemical) **a** and microbiological **b** routes for methane production from syngas deriving from coal, biomass or recalcitrant wastes (adapted from Basu et al. 1993)

$$CO + H_2O \rightarrow H_2 + CO_2 \quad (15.5)$$

2. Acetate and H_2 are further converted into methane by acetoclastic (Eq. 15.6) and hydrogenotrophic (Eq. 15.7) methanogens:

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad (15.6)$$

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \quad (15.7)$$

Hydrogenotrophic methanogens can utilise H_2 and CO_2 initially present in syngas for producing methane as well.

The capability of CO conversion to acetate has been identified in several bacteria from different taxa, e.g. *Clostridium*, *Peptostreptococcus*, *Moorella* and *Desulfotomaculum* species (Henstra et al. 2007). H_2 production via water shift reaction has been shown to occur in anaerobic bacteria, as for example *Rhodospirillum rubrum* (Kerby et al. 1995), *Rhodopseudomonas palustris* (Jung et al. 1999), *Carboxydothermus hydrogenoformans* (Svetlitchnyi et al. 2001), *Carboxydibrachium pacificum* (Sokolova et al. 2001), *Carboxydocella thermoautotrophica* (Sokolova et al. 2002), *Thermincola carboxydiphila* (Sokolova et al. 2005), etc.

Thus far, most of the studies on syngas anaerobic conversion have focused on the utilisation of pure cultures of microorganisms and strongly directed to ethanol production (Abrini et al. 1994; Cotter et al. 2009; Kundiyana et al. 2011). Fu and Mazzella (1990) described the potential of using pure/defined-cultures to convert CO and H_2 into methane. These authors developed a two-stage process for continuous syngas conversion: in the first stage, a small amount of syngas feed was used by *Peptostreptococcus productus* to produce acetate, which would be used in the second stage bioreactor for stimulating culture growth. The second stage employed a co-culture of *Rhodospirillum rubrum* and *Methanobacterium formicicum* for combined water shift and methanation of the remaining syngas feed.

Syngas conversion to methane by anaerobic mixed cultures is practically unexplored, and few reports are available on this subject (Guiot et al. 2011; Sipma et al. 2003). Sipma and co-workers (2003) tested seven anaerobic sludges from wastewater treatment reactors for their ability to convert CO at 30 and 55 °C. All the tested sludges could convert CO in the assays at mesophilic temperature, with a CO depletion rate between 0.14 and 0.62 mmol CO day^{-1}. Conversion of CO at 55 °C was achieved by five of the tested sludges and CO depletion rates varied between 0.73 and 1.32 mmol CO day^{-1}. Methane and/or acetate and methane and/or H_2 were the main products deriving from CO conversion during incubation at mesophilic and thermophilic conditions, respectively. Continuous CO conversion to methane, using a closed-loop 30 L gas-lift reactor, has been shown by Guiot et al. (2011). A maximum CO conversion of 75 % was obtained for a CO partial pressure of 0.6 atm and a gas recirculation ratio of 1:20. Under these conditions, methane yield (CH_4/CO) was approximately 95 % and other metabolites accumulated only at trace concentrations.

15.4 Future Prospects

Anaerobic digestion is an established technology with thousands of known applications worldwide. In a circular biobased economy concept (cradle to cradle), the reuse of all waste streams to produce valuable products and/or fuels should be mandatory. AD represents a relatively cheap technology, that integrated in biodiesel and/or bioethanol facilities, in a biorefinery concept, can represent a significant milestone in the economic viability of those technologies by using their (waste) water and by-products to generate biofuels (biomethane and/or biohydrogen) and a biofertilizer.

Several topics still need optimisation to definitively make AD of energy crops and biofuels production by-products economically feasible. Development of efficient and economically viable pre-treatments to improve the biodegradation of more recalcitrant feedstocks is urgent to increase the yield of AD processes. Biogas produced in AD plants is primarily composed of methane and CO_2, but it contains traces of other gases (NH_3, H_2S, etc.). To be used as a vehicle fuel or injected in the natural gas grid it has to be upgraded and compressed. Diverse technologies have been developed during the past years (water scrubbing, carbon molecular sieves, membranes, etc.). These techniques are costly and independent from the AD process, therefore suitable and costly technologies still to be developed.

Algae are the only crop capable of replacing the fossil fuel dependency, even though all the potential of algae-based biorefineries, it is still in the beginning of its development and many research and development is needed to achieve the desired efficiency and competitiveness. Genetic and metabolic engineering is likely to play an important role in improving microalgae strains to increase the lipids content and the easiness of extraction. The possibility to release valuable biochemical molecules using enzymatic hydrolysis from microalgae without dewatering the culture could have a major impact on the energetic needs for algae biofuels production. In fact, drying the algal biomass consumes about 69 % of the input energy (Jones and Mayfield 2011; Sander and Murthy 2010). Algae are a remediation agent that can be used in wastewater treatment. Couple algae-biomass production, nutrients removal, CO_2 sequestration and biogas production may represent an important milestone in the bioenergy goals, since the market of wastewater treatment is immense. However, an appropriate technology for biomass harvesting must be developed to bridge these technologies.

Concerning the syngas platform biorefinery, a significant challenge for the effective utilisation of syngas biologically is clearly the modest gas-liquid mass-transfer rates of the conventional gas–liquid contacting technologies (e.g. stirred tank reactors, airlift reactors or bubble columns) and the low solubility of the major syngas components in the aqueous culture medium (Bredwell et al. 1999). In fact, microbial syngas conversion depends strongly on the mass transfer of syngas to water (van Kasteren et al. 2005). One way of addressing this issue, and a future challenge in syngas fermentation, is the improvement of the volumetric mass transfer coefficient (kLa) for syngas-components and the development of appropriate bioreactor design.

In the future, biogas production will be based on a wide range of aquatic and terrestrial energy crops that will grow with sustainable and versatile methods. Organic waste, by-products from the food, agriculture and biorefinery industry will be naturally included in the several AD plants available worldwide.

References

Abrini J, Naveau H, Nyns EJ (1994) *Clostridium autoethanogenum* sp nov, an anaerobic bacterium that produces ethanol from carbon monoxide. Arch Microbiol 161:345–351

Abubackar HN, Veiga MC, Kennes C (2011) Biological conversion of carbon monoxide: rich syngas or waste gases to bioethanol. Biofuel Bioprod Bior 5:93–114

Adams JMM, Toop TA, Donnison IS, Gallagher JA (2011) Seasonal variation in *Laminaria digitata* and its impact on biochemical conversion routes to biofuels. Bioresour Technol 102:9976–9984

Agler M, Garcia M, Lee E, Schilicher M, Angenent L (2008) Thermophilic anaerobic digestion to increase the net energy balance of corn grain ethanol. Environ Sci Technol 42:6723–6729

Alves MM, Picavet MA, Pereira MA, Cavaleiro AJ, Sousa DZ (2007) Novel anaerobic reactor for the removal of long chain fatty acids from fat containing wastewater. Patent number WO/2007/058557

Amon T, Amon B, Kryvoruchko V, Bodiroza V, Pötsch E, Zollitsch W (2006) Optimising methane yield from anaerobic digestion of manure: effects of dairy systems and of glycerin supplementation. Int Congr Ser 1293:217–220

Amorim HV, Lopes ML, Oliveira JVC, Buckerige MS, Goldman GH (2011) Scientific challenges of bioethanol production in Brazil. Appl Microbiol Biot 91:1267–1275

Angenent LT, Karim K, Al-Dahhan MH, Wrenn BA, Domínguez-Espinosa R (2004) Production of bioenergy and biochemicals from industrial and agricultural wastewater. Trends Biotechnol 22:477–485

Atabani AE, Silitonga AS, Badruddin IA, Mahlia TMI, Masjuki HH, Mekhilef S (2012) A comprehensive review on biodiesel as an alternative energy resource and its characteristics. Renew Sust Energ Rev 16:2070–2093

Basu R, Klasson KT, Clausen EC, Gaddy JL (1993) Biological conversion of synthesis gas. Bioreactor studies. Topical Report. Foster Wheeler USA Corporation, DOE contract no. DE-AC21-86MC23077

Bird KT, Chynoweth DP, Jerger DE (1990) Effects of marine algal proximate composition on methane yields. J Appl Phycol 2:207–213

Blonskaja V, Menert A, Vilu R (2003) Use of two-stage anaerobic treatment for distillery waste. Environ Res 7:671–678

Boone DR, Johnson RL, Liu Y (1989) Diffusion of the interspecies electron carriers H_2 and formate in methanogenic ecosystems and its implications in the measurement of Km for H_2 or formate uptake. Appl Environ Microb 55:1735–1741

Borjesson P, Mattiasson B (2008) Biogas as a resource-efficient vehicle fuel. Trends Biotechnol 26:7–13

Bredwell MD, Srivastava P, Worden RM (1999) Reactor design issues for synthesis-gas fermentations. Biotechnol Prog 15:834–844

Briand X, Morand P (1997) Anaerobic digestion of Ulva sp. 1. Relationship between Ulva composition and methanisation. J Appl Phycol 9:511–524

Cantrell KB, Ducey T, Ro KS, Hunt PG (2008) Livestock waste-to-bioenergy generation opportunities. Bioresour Technol 99:7941–7953

Castrillón L, Fernández-Nava Y, Ormaechea P, Marañón E (2011) Optimization of biogas production from cattle manure by pre-treatment with ultrasound and co-digestion with crude glycerin. Bioresour Technol 102:7845–7849

Cecchi F, Pavan P, Mata-Alvarez J (1996) Anaerobic co-digestion of sewage sludge: application to the macroalgae from the Venice lagoon. Resour Conserv Recy 17:57–66

Chynoweth DP (2005) Renewable biomethane from land and ocean energy crops and organic wastes. HortSci 40:283–286

Costa JC, Gonçalves PR, Nobre A, Alves MM (2012) Biomethanation potential of macroalgae Ulva spp and Gracilaria spp and in co-digestion with waste activated sludge. Bioresour Technol 114:320–326

Cotter JL, Chinn MS, Grunden AM (2009) Influence of process parameters on growth of Clostridium ljungdahlii and Clostridium autoethanogenum on synthesis gas. Enzyme Microb Technol 44:281–288

Daniels L, Fuchs G, Thauer RK, Zeikus JG (1977) Carbon monoxide oxidation by methanogenic bacteria. J Bacteriol 132:118–126

Demirbas A (2009) Progress and recent trends in biodiesel fuels. Energ Convers Manage 50:14–34

Dias MOS, Ensinas AV, Nebra SA, Maciel Filho R, Rossell CEV, Maciel MRW (2009) Production of bioethanol and other bio-materials from sugarcane bagasse: integration to conventional bioethanol production process. Chem Eng Res Des 87:1206–1216

Ehimen EA, Sun ZF, Carrington CG, Birch EJ, Eaton-Rye JJ (2011) Anaerobic digestion of microalgae residues resulting from the biodiesel production process. Appl Energ 88:3454–3463

EN14214 (2008) Automative Fuels. Fatty acid methyl esters (FAME) for diesel engines. Requirements and test methods

Eskicioglu C, Kennedy KJ, Marin J, Strehler B (2011) Anaerobic digestion of whole stillage from dry-gring corn ethanol plant under mesophilic and thermophilic conditions. Bioresour Technol 102:1079–1086

EurObserv'ER (2010) Biogas Barometer—November 2010. Systèmes Solaires, le journal des énergies renouvelables, 200:104–119. http://www.eurobserv-er.org/pdf/baro200b.pdf

Fang HHP, Chui HK, Li YY (1995) Anaerobic degradation of butyrate in a UASB reactor. Bioresour Technol 51:75–81

Fountoulakis MS, Petousi I, Manios T (2010) Co-digestion of sewage sludge with glycerol to boost biogas production. Waste Manage 30:1849–1853

Fu RK, Mazzella G (1990) Evaluation of biological conversion of coal-derived synthesis gas. Topical report. University of Arkansas, University of Arkansas, DOE contract no. DE-FG21-90MC27225

Guiot SR, Cimpoia R, Carayon G (2011) Potential of wastewater-treating anaerobic granules for biomethanation of synthesis gas. Environ Sci Technol 45:2006–2012

Gujer W, Zehnder AJB (1983) Conversion processes in anaerobic digestion. Water Sci Technol 15:127–167

Gunaseelan VN (1997) Anaerobic digestion of biomass for methane production: a review. Biomass Bioenerg 13:83–114

Gunaseelan VN (2009) Biomass estimates, characteristics, biochemical methane potential, kinetics and energy flow from Jatropha curcus on dry lands. Biomass Bioenerg 33:589–596

Harper SR, Pohland FG (1986) Recent developments in hydrogen management during anaerobic biological wastewater treatment. Biotechnol Bioeng 28:585–602

Henstra AM, Sipma J, Rinzema A, Stams AJM (2007) Microbiology of synthesis gas fermentation for biofuel production. Curr Opin Biotechnol 18:200–206

Holm-Nielsen JB, Lomborg CJ, Oleskowicz-Popiel P, Esbensen KH (2008) Online near infrared monitoring of glycerol-boosted anaerobic digestion processes: evaluation of process analytical technologies. Biotechnol Bioeng 99:302–313

Huang JS, Jih CG, Lin SD, Ting WH (2003) Process kinetics of UASB reactors treating non-inhibitory substrate. J Chem Technol Biotechnol 78:762–772

IEA (2010) Sustainable production of second-generation biofuels. OECD/IEA, Paris. http://www.iea.org/papers/2010/second_generation_biofuels.pdf. Cited 24 May 2012

Jaruwat P, Kongjao S, Hunsom M (2010) Management of biodiesel wastewater by the combined processes of chemical recovery and electrochemical treatment. Energ Convers Manage 51:531–537

Jetten MSM, Stams AJM, Zehnder AJB (1992) Methanogenesis from acetate: a comparison of the acetate metabolism in *Methanothrix soehngenii* and *Methanosarcina* spp. FEMS Microbiol Rev 88:181–197
Jones CS, Mayfield SP (2011) Algae biofuels: versatility for the future of bioenergy. Curr Opin Biotech 23:346–351
Jung GY, Jung HO, Kim JR, Ahn Y, Park S (1999) Isolation and characterization of Rhodopseudomonas palustris P4 which utilizes CO with the production of H2. Biotechnol Lett 21:525–529
Kaparaju P, Serrano M, Thomsen AB, Kongjan P, Angelidaki I (2009) Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept. Bioresour Technol 100:2562–2568
Kerby RL, Ludden PW, Roberts GP (1995) Carbon monoxide dependent growth of Rhodospirillum rubrum. J Bacteriol 177:2241–2244
Kocsisová T, Cvengroš J (2006) G-phase form methyl ester production—splitting and refining. Pet Coal 48:1–5
Kolesárová N, Hutňan M, Špalková V, Kuffa R, Bodík I (2011) Anaerobic treatment of biodiesel by-products in a pilot scale reactor. Chem Pap 65:447–453
Kundiyana DK, Wilkins MR, Maddipati P, Huhnke RL (2011) Effect of temperature, pH and buffer presence on ethanol production from synthesis gas by Clostridium ragsdalei. Bioresour Technol 102:5794–5799
Langeveld JWA, Dixon J, Jaworski JF (2010) Development perspectives of the biobased economy: a review. Crop Sci 50:S142–S151
Lee P-H, Bae J, Kim J, Chen W-H (2011) Mesophilic anaerobic digestion of corn thin stillage: a technical and energetic assessment of the corn-to-ethanol industry integrated with anaerobic digestion. J Chem Technol Biot 86:1514–1520
Lichts FO (2010) Industry Statistics: 2010 World Fuel Ethanol Production. Renewable Fuels Association. Available via http://www.ethanolrfa.org/pages/statistics#E. Cited 19 Feb 2012
Luo G, Talebnia F, Karakashev D, Xie L, Zhou Q, Angelidaki I (2011) Enhanced bioenergy recovery from rapeseed plant in a biorefinery concept. Bioresour Technol 102:1433–1439
Ma J, Van Wambeke M, Carballa M, Verstraete W (2008) Improvement of the anaerobic treatment of potato processing wastewater in a UASB reactor by co-digestion with glycerol. Biotechnol Lett 30:861–867
Marchetti JM, Miguel VU, Errazu AF (2007) Possible methods for biodiesel production. Renew Sust Energ Rev 11:1300–1311
Masse L, Masse DI, Kennedy KJ, Chou SP (2002) Neutral fat hydrolysis and long-chain fatty acid oxidation during anaerobic digestion of slaughterhouse wastewater. Biotechnol Bioeng 79:43–52
Mata-Alvarez J, Macé S, Llabres P (2000) Anaerobic digestion of organic solid wastes an overview of research achievements and perspectives. Bioresour Technol 74:3–16
Meredith J (2003) Understanding energy use and energy users in contemporary ethanol plants. In: Jacques KA, Lyons TP, Kelsall DR (eds) The alcohol textbook, 4th edn. Nottingham University Press, Nottingham, pp 355–361
Morand P, Briand X (1999) Anaerobic digestion of Ulva sp. 2. Study of Ulva degradation and methanisation of liquefaction juices. J Appl Phycol 11:165–177
Mosey FE (1983) Mathematical modeling of the anaerobic digestion process: regulatory mechanisms for the formation of short-chain volatile acids from glucose. Water Sci Technol 15:209–232
Murto M, Björnsson L, Mattiasson B (2004) Impact of food industrial waste on anaerobic co-digestion of sewage sludge and pig manure. J Environ Manage 70:101–107
Mussgnug JH, Klassen V, Schlüter A, Kruse O (2010) Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. J Biotechnol 150:51–56
Neves L (2009) Anaerobic co-digestion of organic wastes. University of Minho, Braga, Portugal. http://hdl.handle.net/1822/9875

Nigam PS, Singh A (2011) Production of liquid biofuels from renewable resources. Prog Energ Combust 37:52–68
Nkemka VN, Murto M (2010) Evaluation of biogas production from seaweed in batch tests and in UASB reactors combined with the removal of heavy metals. J Environ Manage 91:1573–1579
O'Brien JM, Wolkin RH, Moench TT, Morgan JB, Zeikus JG (1984) Association of hydrogen metabolism with unitrophic or mixotrophic growth of Methanosarcina barkeri on carbon monoxide. J Bacteriol 158:373–375
Otsuka K, Yoshino A (2004) A fundamental study on anaerobic digestion of sea lettuce. OCEANS '04 MTS/IEEE TECHNO-OCEAN '04, Conference proceedings, 3:1770–1773
Park J-H, Yoon J-J, Park H-D, Lim DJ, Kim S-H (2012) Anaerobic digestibility of algal bioethanol residue. Bioresour Technol 113:78–82
Pereira MA (2003) Anaerobic biodegradation of long chain fatty acids—biomethanisation of biomass-associated LCFA as a challenge for the anaerobic treatment of effluents with high lipid-LCFA content. University of Minho, Braga, Portugal. http://hdl.handle.net/1822/4650
Pérez-García M, Romero-García LI, Rodríguez-Cano R, Sales-Márquez D (2005) Effect of pH influent conditions in fixed-film reactors for anaerobic thermophilic treatment of wine-distillery wastewater. Water Sci Technol 51:183–189
Peu P, Sassi J-F, Girault R, Picard S, Saint-Cast P, Béline F, Dabert P (2011) Sulphur fate and anaerobic biodegradation potential during co-digestion of seaweed biomass (*Ulva* sp.) with pig slurry. Bioresour Technol 102:10794–10802
Phukingngam D, Chavalparit O, Somchai D, Ongwandee M (2011) Anaerobic baffled reactor treatment of biodiesel-processing wastewater with high strength of methanol and glycerol: reactor performance and biogas production. Chem Pap 65:644–651
Pittman JK, Dean AP, Osundeko O (2011) he potential of sustainable algal biofuel production using wastewater resources. Bioresour Technol 102:17–25
Plugge CM, Van Lier JB, Stams AJM, Jeison D (2009) Microbial energy production from biomass. In: Rabaey K, Angenent L, Schroder U, Keller J (eds) Bioelectrochemical systems. IWA Publishing, London, pp 17–38
Rabelo SC, Carrere H, Maciel Filho R, Costa AC (2011) Production of bioethanol, methane and heat from sugarcane bagasse in a biorefinery concept. Bioresour Technol 102:7887–7895
Rajeshwari KV, Balakrishnan M, Kansal A, Lata K, Kishore VVN (2000) State-of-the-art of anaerobic digestion technology for industrial wastewater treatment. Renew Sust Energ Rev 4:135–156
Ras M, Lardon L, Sialve B, Bernet N, Steyer J-P (2011) Experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris*. Bioresour Technol 102:200–206
Rittmann BE (2008) Opportunities for renewable bioenergy using microorganisms. Biotechnol Bioeng 100:203–212
Rother M, Metcalf WW (2004) Anaerobic growth of *Methanosarcina acetivorans C2A* on carbon monoxide: an unusual way of life for a methanogenic archaeon. Proc Natl Acad Sci USA 101:16929–16934
Rusten B, Sahu AK (2011) Microalgae growth for nutrient recovery from sludge liquor and production of renewable bioenergy. Water Sci Technol 64:1195–1201
Sander K, Murthy GS (2010) Life cycle analysis of algae biodiesel. Int J Life Cycle Assess 15:704–714
Schaefer SH, Sung S (2008) Retooling the ethanol industry: thermophilic anaerobic digestion of thin stillage for methane production and pollution prevention. Water Environ Res 80:101–108
Schink B (1997) Energetics of syntrophic cooperation in methanogenic degradation. Microbiol Mol Biol Rev 61:262–280
Schink B, Stams AJM (2006) Syntrophism among prokaryotes. Prokaryotes 2:309–335
Seth R, Goyal SK, Handa BK (1995) Fixed film biomethanation of distillery spentwash using low cost porous media. Resour Conserv Recy 14:79–89
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

Siles JA, Martín MA, Chica AF, Martín A (2010) Anaerobic co-digestion of glycerol and wastewater derived from biodiesel manufacturing. Bioresour Technol 101:6315–6321
Siles JA, Gutiérrez MC, Martín MA, Martín A (2011) Physical–chemical and biomethanization treatments of wastewater from biodiesel manufacturing. Bioresour Technol 102:6348–6351
Silva GP, Mack M, Contiero J (2009) Glycerol: a promising and abundant carbon source for industrial microbiology. Biotechnol Adv 27:30–39
Sipma J, Lens PNL, Stams AJM, Lettinga G (2003) Carbon monoxide conversion by anaerobic bioreactor sludges. FEMS Microbiol Ecol 44:271–277
Sokolova TG, Gonzalez JM, Kostrikina NA, Chernyh NA, Tourova TP, Kato C, Bonch-Osmolovskaya EA, Robb FT (2001) *Carboxydobrachium pacificum* gen nov, sp nov., a new anaerobic, thermophilic, CO-utilizing marine bacterium from Okinawa Trough. Int J Syst Evol Micr 51:141–149
Sokolova TG, Kostrikina NA, Chernyh NA, Tourova TP, Kolganova TV, Bonch-Osmolovskaya EA (2002) *Carboxydocella thermautotrophica* gen. nov., sp nov., a novel anaerobic, CO-utilizing thermophile from a Kamchatkan hot spring. Int J Syst Evol Micr 52:1961–1967
Sokolova TG, Kostrikina NA, Chernyh NA, Kolganova TV, Tourova TP, Bonch-Osmolovskaya EA (2005) *Thermincola carboxydiphila* gen nov, sp nov, a novel anaerobic, carboxydotrophic, hydrogenogenic bacterium from a hot spring of the Lake Baikal area. Int J Syst Evol Micr 55:2069–2073
Souza ME, Fuzaro G, Polegato AR (1992) Thermophilic anaerobic digestion of vinasse in pilot plant UASB reactor. Water Sci Technol 25:213–222
Stams AJM, Plugge CM (2009) Electron transfer in syntrophic communities of anaerobic bacteria and archaea. Nat Rev Microbiol 7:568–577
Stams AJM, Plugge CM, De Bok FAM, Van Houten BHGW, Lens P, Dijkman H, Weijma J (2005) Metabolic interactions in methanogenic and sulfate-reducing bioreactors. Water Sci Technol 52:13–20
Stover EL, Gomathinayagam G, Gonzalez R (1984) Use of methane from anaerobic treatment of stillage for fuel alcohol production. 39th industrial water conference. Purdue University, West Lafayette, Indiana: Butterworth, Boston, pp 95–104
Svetlitchnyi V, Peschel C, Acker G, Meyer O (2001) Two membrane-associated NiFeS-carbon monoxide dehydrogenases from the anaerobic carbon-monoxide-utilizing eubacterium Carboxydothermus hydrogenoformans. J Bacteriol 183:5134–5144
Van Haandel AC (2005) Integrated energy production and reduction of the environmental impact at alcohol distillery plants. Water Sci Technol 52:49–57
Van Haandel AC, Catunda PFC (1994) Profitability increase of alcohol distilleries by the rational use of byproducts. Water Sci Technol 29:117–124
Van Kasteren JMN, van der Waall WR, Guo J, Verberne R (2005) Bio-ethanol from syngas. Eindhoven University of Technology (TU/e), I. &. E, Ed
Van Lier JB, Letting G (1999) Appropriate technologies for effective management of industrial and domestic waste waters: the decentralised approach. Water Sci Technol 40:171–183
Van Lier JB, Tilche A, Ahring BK, Macarie H, Moletta R, Dohanyos M, Pol LWH, Lens P, Verstraete W (2001) New perspectives in anaerobic digestion. Water Sci Technol 43:1–18
Vavilin VA, Rytov SV, Lokshina LY (1996) A description of hydrolysis kinetics in anaerobic degradation of particulate organic matter. Bioresour Technol 56:229–237
Weiland P (2010) Biogas production: current state and perspectives. Appl Microbiol Biot 85:849–860
Yang Z, Guo R, Xu X, Fan X, Li X (2011) Thermo-alkaline pretreatment of lipid-extracted microalgal biomass residues enhances hydrogen production. J Chem Technol Biotechnol 86:454–460
Yazdani SS, Gonzalez R (2007) Anaerobic fermentation of glycerol: a path to economic viability for the biofuels industry. Curr Opin Biotech 18:213–219

Chapter 16
Production of Bioethanol from Biomass: An Overview

Óscar J. Sánchez and Sandra Montoya

Abstract This chapter analyzes the main research trends on production of fuel ethanol from lignocellulosic materials. The main features of different pretreatment and detoxification methods are presented. The importance of process integration to simplify the overall process and improve the conversion of biomass into ethanol is discussed. Strategies for microbial strain development are disclosed in the framework of such integrated processes like simultaneous saccharification and co-fermentation and consolidated bioprocessing. The main challenges to fully develop the biomass-to-ethanol process are highlighted. Finally, the need of integrating the research efforts on molecular techniques and process integration is recognized.

16.1 Lignocellulosic Biomass as Feedstock

The biomass is organic matter made by living organisms that contain energy stored from the sun. The radiant energy from sunlight is absorbed by plants. This energy is converted into chemical energy in the form of glucose, starch or cellulose, through photosynthesis. The energy contained in the biomass (bioenergy) can be released and used by means of its combustion. Thus, the woody biomass is employed by many rural communities all around the world for heating and cooking. The biomass can also be burned in boilers to produce heat and electricity (solid biofuels). In addition, it can be transformed into liquid biofuels that, in turn,

Ó. J. Sánchez (✉) · S. Montoya
Institute of Agricultural Biotechnology, Universidad de Caldas, Calle 65 No. 26-10, Manizales, Colombia
e-mail: osanchez@ucaldas.edu.co

V. K. Gupta and M. G. Tuohy (eds.), *Biofuel Technologies*,
DOI: 10.1007/978-3-642-34519-7_16,