Recent Studies on Thermophilic Anaerobic Bioconversion of Lignocellulosic Biomass

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Abstract

This paper reviews recent research developments in biological thermophilic lignocellulosic biomass conversion based on sixty four references published in the past 4 years (2009-2012). Bioconversion of hydrolysate and lignocellulosic biomass with or without pretreatment at thermophilic condition (with temperature higher than 50°C) to fermentation product like hydrogen, methane, ethanol and carboxylic acids was discussed in terms of the bioaugmentation techniques and microorganisms involved. pH control by dosing buffering chemicals ($CaCO_3$ or $KHCO_3$), co-culturing cellulolytic species (e.g. *Clostridium*) with fermentative species (e.g. *Thermoanaerobacter* or *Thermoanaerobacterium*) and co-digestion lignocellulosic biomass with nutrient sufficient feedstock like manure were the most popular bioaugmentation techniques in the studies reviewed. In addition, multi-fuel generation with bio-refinery methane production was testified to be more energy efficient than produce hydrogen or ethanol alone. This review may shed lights on the perspectives of scientific and technical challenges faced for the thermophilic anaerobic lignocellulose bioconversion.

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1 Introduction

Lignocellulosic biomass usually refers to plant biomass (agricultural and forestry), which generally consists of 30–56% cellulose, 10–27% hemicellulose and 3–30% lignin [1]. Lignocellulosic biomass is an ideal resource for biofuel production for its low cost and plentiful supply. A major doubt on using plant biomass as alternative energy source is how to convert it into biofuels (methanol, methane, hydrogen, etc.). Conversion of lignocellulosic biomass by microbial biochemical reactions has attracted worldwide attention as a potential low-cost green technology for renewable energy generation. Thermophiles or hyperthermophiles growing at elevated temperatures (generally from 50 to 85°C) were especially attractive because the higher temperature is thermodynamically favorable for biofuel production [2] and the contamination of unwanted microorganisms could be principally prevented [3].

Among the recent studies on thermophilic lignocellulose bioconversion, most processes are designed to produce hydrogen for its high energy content and environmental friendly by-product of combustion. Biofuel in the liquid form, like ethanol [4] and carboxylic acids, are also interested fermentation products for its prompt economic value as alternative and additive to fossil fuels or as chemical products. Additionally, many other studies also focus on converting lignocellulose into methane as it has superior feasibility in reactor scaling up and more complete carbon conversion of substrates.

Different from reviews focused solely on one type of fermentation product from biomass feedstock [5–7,2,8,9], this article aims to give a comprehensively reviewed on the thermophilic bioconversion of lignocellulosic biomass. As the research focus varies largely between the liquid and solid form of feedstock applied, this review were divided into two branches according to the forms of substrates adopted: one branch targets the fermentation of liquid hydrolysate (liquid fraction generated from pretreatment of lignocellulosic biomass) while the other one summarizes the studies using raw or pretreated solid lignocellulosic biomass as feedstock. At this time, research is continuing on both branches of thermophilic fermentation, as none of them have been fully optimized. Thus, in this review, we summarize the latest scientific and technical tactics that were testified to promote thermophilic lignocellulosic biomass conversion in terms

of substrate utilization, product yield as well as reactor stability. In addition, challenging issues of feedstock utilization and manipulation of microbial community was discussed to provide future perspectives of thermophilic lignocellulose bioconversion development.

2   Fermentation of hydrolysate (liquid fraction after pretreatment)

Hydrolysate is ought to be a promising resource for fermentation as it contains high concentration of degradable sugars extracted from the lignocellulosic biomass during pretreatment, however besides releasing reduced oligosaccharides from cellulose and hemicellulose, the pretreatment process simultaneously generates less degradable pentose and strong fermentation inhibitors, both of which become the major challenges for the consequent hydrolysate fermentation.

2.1   Tolerance to fermentation inhibitors

The most common fermentation inhibitors include toxic compounds of furfural, hydroxymethylfurfural (HMF) and phenols as well as fermentation products like volatile fatty acids (VFAs), especially acetate. Toxic compounds of furfural, HMF and phenols generated from chemical or physical explosion of polysaccharides and lignin content of the biomass might inhibit the microorganisms, while VFAs produced from the pretreatment would induce product repression effect that impedes the initiation of fermentation reaction.

As a result, tolerance to such fermentation inhibitors, usually expressed as the maximum bearable hydrolysate concentration for stable biofuel production develops into one of the important standards to evaluate the fermentation performance. Among the studies reviewed, the common bearable concentration is around 30% (v/v) of original hydrolysate solution. This means that hydrolysate must be diluted at least 3.3 times before applying to the anaerobic reactor, otherwise, overloading of hydrolysate will result in quick failure of the reactor indicated by lowered product yield, prolonged lag time as well as sudden pH drop [10,11]. Noticeably, there is only one study carried out by Kaparaju *et al.* [12] which successfully utilized 100% hydrolysate in continuous stirring reactor (CSTR) for thermophilic methane production by applying hydrolysate derived from hydrothermal pretreatment of wheat straw which contained no furfural and HMF and very low concentration of phenols (61 mg L^{-1}).

Chemical compositions of hydrolysate generated from various pretreatment processes were summarized in Table1. Although it is not within this paper's scope to review the relative merits of each pretreatment technology, the recalcitrance of hydrolysate, especially the content of fermentation inhibitors, depends greatly on the pretreatment

process from which it derives. It is interesting to notice that studies used enzymatic hydrolysis after chemical pretreatment generally reported a less toxic hydrolysate composition with low or none furfural, HMF and phenols content. But no study had clearly demonstrated the detoxification effect of enzymatic hydrolysis on hydrolysate preparation. In addition, feedstocks with similar chemical composition could produce hydrolysate with totally different fermentability when pretreated at same conditions, for example *Panagiotopoulos et al.* observed a different fermentability of barley straw and corn stalk hydrolysate which is in strong contrast to the similarity of the composition of these two feedstocks (Table 4) [13].

2.2   Strategies for detoxification of hydrolysate

In order to eliminate inhibitors in hydrolysate, Lee *et al.* (2011) demonstrated an effective approach to detoxify hardwood hydrolysate by dosing activated carbon at 2.5wt% which selectively removed 42% of formic acid, 14% of acetic acid, 96% of HMF and 93% of the furfural with only 8.9% of sugars loss [14].

In addition, reactor configuration was also proven to be an effective way to overcome the toxicity of hydrolysate. As demonstrated by Kaparaju *et al.* (2009) CSTR could achieve methane production of 297 mL CH_4 g^{-1} COD with 100% (v/v) hydrolysate at organic loading rate (OLR) of 1.9 g COD L^{-1} d^{-1} while up-flow sludge blanket (UASB) even with diluted hydrolysate (10% (v/v)) and an higher OLR (2.8 g COD L^{-1} d^{-1}) yielded less methane (267 mL CH_4 g^{-1} COD) [12].

Longer hydrolytic retention time (HRT) is another practical method to lower the inhibitory content for fermentation. Both UASB and anaerobic filter reactor (AF) gave sharply decreased and subsequently fluctuated hydrogen production at an HRT of 0.5 day while the original rate and yield were recovered when the HRT was increased back to 1 day [15]. Nevertheless, too long HRT might result in cell mass washout, for example, hydrogenogens were washed out at an HRT of 2.5 days for a 1-liter CSTR [15]. Hence, it is impractical to increase reactor tolerance to hydrolysate by solely prolonging HRT.

Co-digestion of toxic hydrolysate with nutrient-sufficient manure was used as an alternative approach to increase reactor's inhibitor tolerance capacity. Co-digestion of hydrolysate with pig manure (1:3 v/v) promoted UASB tolerance from 10% to 25%

hydrolysate, although methane yield of 219 mL CH_4 g^{-1} COD was slightly lower than 267 mL CH_4 g^{-1} COD without co-digestion [12].

2.3 Strains used for hydrolysate fermentation

Most of the studies preferred using pure cultures for thermophilic anaerobic hydrolysate fermentation as shown in Table 2 and Table 3 which summarize the inoculum seed sources. Comparing to the mixed culture, pure culture inoculum, especially those genetically modified strains, is favorable for its higher product yield and production rate. However, the efficiency of pure culture systems is sensitive to environmental change (pH and temperature variation) and very fragile to contamination by other microbial species. Fortunately, thermophilic condition provides an ideal environment for pure culture operation in which contamination is effectively prevented by the high temperature. For example, a reactor had been operated continuously for approximately 143 days, and no contamination was observed without using any agent to prevent bacterial contamination [16].

Some strains have shown satisfactory tolerance to fermentation inhibitors in hydrolysate. *Caldicellulosiruptor saccharolyticus* DSM 8903 showed normal growth on hydrolysate of NaOH retreated biomass up to a sugar concentration of 20 g L^{-1} (corresponding to around 32% hydrolysate) [17]. *Thermoanaerobacter* BG1L1 exhibited significant resistance to high levels of acetic acid (up to 10 g L^{-1}) and other metabolic inhibitors present in the hydrolysate [16].

For the pure culture system used in hydrolysate fermentation, aside from the tolerance to inhibitors, the pentose (especially xylose) utilization capacity is another important parameter to evaluate the fermentation ability of the strain. As shown in Table1, the amount of xylose was equivalent to that of easily degradable glucose for most hydrolysate. The present of glucose in the substrate was reported to be adversary to the uptake of xylose by microorganisms. For instance, the presence of 50% glucose remarkably reduced the utilization rate of xylose from around 0.29 g L^{-1} h^{-1} to 0.09 g L^{-1} h^{-1} by *Thermoanaerobacterium thermosaccharolyticum* W16. The strain was able to produce hydrogen from xylose at 10.7 mmol H_2 L^{-1} h^{-1} (yield of 2.19 mol H_2 mol^{-1} xylose) which was comparable to that from glucose of 12.9 mmol H_2 L^{-1} h^{-1} (yield of 12.42 mol H_2 mol^{-1} glucose) [18].

Since some strains are glucose preferred, like *Thermoanaerobacterium thermosaccharolyticum* W16 [18,19] and *Thermotoga neapolitana* [20], while some others preferred xylose in the contrary, for example *Caldicellulosiruptor saccharolyticus* DSM 8903 [21,17,22], co-culture strategy has been used to promote xylose utilization. Co-culturing *Caldicellulosiruptor saccharolyticus* with the enriched compost microflora resulted in fast and simultaneous consumption of both glucose and xylose in the medium with a relatively high specific hydrogen production rate of 40 mmol H_2 g^{-1} cell dry weight h^{-1} and high volumetric productivity of 2.5 mmol-H_2 $L^{-1}h^{-1}$ [21].

2.4   Optimal conditions

The optimal operation conditions for thermophilic anaerobic hydrolysate fermentation are summarized in Table 2 and Table 3. On one hand, fermentative hydrogen yield from hydrolysate, on average around 9 mmol H_2 g^{-1} sugar digested, is generally lower than the common hydrogen yield based on hexose fermentation of 11 - 22 mmol H_2 g^{-1} sugar digested (equivalent to 2 - 4 mol-H_2 mol-hexose^{-1}). This lowered yield is caused by the complex sugar composition and inhibitors in the hydrolysate as mentioned above. On the other hand, some studies showed great potential of thermophilic anaerobic hydrogen production from hydrolysate. It was reported the maximum hydrogen yield of 18.9 mmol H_2 g^{-1} sugar digested (equivalent to 86% of theoretical hydrogen yield from hexose) in batch experiment using *Caldicellulosiruptor saccharolyticus* pure culture to digest Miscanthus hydrolysate (10 g sugar L^{-1}) [20]. Furthermore, when fermenting the synthetic hydrolysate prepared using glucose and xylose at 1:1 ratio, co-culture of *Caldicellulosiruptor saccharolyticus* and compost microflora gave very high hydrogen yield of 21.1 mmol H_2 g^{-1} sugar digested, which was 96% of theoretical hydrogen yield from hexose [21]. Zeidan and Van Niel's study had proven that with a proper detoxification process, high hydrogen yield could be obtained from thermophilic hydrolysate fermentation even the hydrolysate contained relatively high concentration of pentose like xylose (Table 1). In addition, pilot scale reactors (with volume of 1 liter or more) had been successfully set up for continuous hydrogen production from hydrolysate. The highest bearable ORL was 3.9 g sugar L^{-1} d^{-1} with 20% hydrolysate in UASB which produced hydrogen at 33.6 mmol H_2 L^{-1} d^{-1} with yield of 8.8 mmol H_2 g^{-1} sugar digested [15]. Hydrolysate was also used as feedstock for thermophilic anaerobic methane and

ethanol production as well. The highest methane yield of 16.8 mmol CH_4 g^{-1} COD digested was obtained in CSTR digesting 100% wheat straw hydrolysate [12]. Crespo *et al.* reported the highest ethanol yield of 0.46 g ethanol g^{-1} sugar digested using *Caloramator boliviensi* as inoculum to digest 20% sugarcane bagasse hydrolysate.

It is difficult to compare the product yield of thermophilic anaerobic fermentation among different studies as many of them did not contain information about the substrate utilization percentage which makes it impossible to put the yield number into the same unit. So the yield in this review is expressed in two different units, i.e. mmol product g^{-1} sugar **digested** and mmol product g^{-1} sugar **added**. The highest yield mentioned above is based on the unit of mmol product g^{-1} sugar digested which is more representative for the whole picture, yet it may not cover all the studies in the area. The major reason for lack of substrate utilization information maybe lie in the fact that the cost of lignocellulosic biomass feedstock is too low to arouse sufficient attention to how much is left-over when operating the reactor. In addition, the insoluble characteristics of lignocellulosic substrate also lead to inconveniency in quantification. However the information about feedstock utilization condition is very important to evaluate the process performance not only because it provides standardized yield quantification but also because adequate substrate conversion percentage is crucial to ensure the sustainability of reactors as leftover hydrolysate will bring about substrate inhibition of the microorganisms and uninterrupted accumulation of solid feedstock will cause mixing difficulty and physical clogging to the reactor.

3   Fermentation of solid lignocellulosic biomass with/without pretreatment

3.1   Lignocellulosic biomass feedstock

Various types of lignocellulosic biomass have been used as feedstock for thermophilic fermentation studies. Their chemical compositions were summarized in Table 4. Among the various biomass resources, rice straw, wheat straw, and corn stover are considered as the most abundant and have the highest potential for biofuel production[23]. Agricultural residues (straw, stalk and stover of farm crops) have relatively higher content of cellulose with less hemicellulose and lignin than forestry residues (wood stems) (Table 4). Aside from the sources of lignocellulosic materials from agricultural or forestry left-over, a list of potential bioenergy crops is being developed. Different from most plants assimilating CO_2 first into C3 compound, energy crops like perennial grasses utilizing CO_2 following the productive C4 photosynthesis pathway which was reported to have higher maximum efficiencies of light, nitrogen and water than C3 [24]. Switchgrass, napiergrass and *Miscanthus* were the most common energy crops in the studied reviewed. Switchgrass is favored for its higher cellulose content which indicates a greater potential for fermentative biofuel production, nevertheless, napiergrass with higher hemicellulose and lower lignin content shows better accessibility for the pretreatment and hydrolysis process prior. One thing to be noticed is that in stark contrast to the long history of domestication of food crops to maximize productivity, until recently, minimal effort has been directed towards optimizing potential energy crops for the generation of biofuels [24]. In addition, purified cellulose such as microcrystalline cellulose (α-cellulose, Sigma-cellulose and Avicel), filter paper (with >98% cellulose content) and Carboxymethyl Cellulose (CMC) were broadly utilized in the fermentation studies for the purpose of simplifying the system, or standardizing the product yield, or as a control.

3.2   Affecting parameters of lignocellulosic biomass fermentation

In this section, the anaerobic thermophilic lignocellulosic biomass fermentation is categorized according to the target fermentation products, namely hydrogen, methane, ethanol and carboxylic acids. Among the 41 reviewed research work on anaerobic thermophilic fermentation of solid lignocellulosic biomass, 16 studies were mainly focused on lignocellulosic hydrogen production while methane, ethanol and carboxylic acids production were respectively investigated in 6, 6 and 4 studies. Another 6 studies

investigated the multi-stage process combining two or three fermentation processes for biofuel production, while the rest 5 studied other aspects of fermentation other than yield of products.

3.2.1 Lignocellulosic hydrogen production

3.2.1.1 Effect of Temperature

Clearly, temperature is a crucial operational parameter because it largely determines the microbial community developing in the reactor. In the reviewed studies, the effect of thermophilic condition was often compared to mesophilic condition. Thermophilic condition with fermentation temperature above 50°C was in general reported to be beneficial for lignocellulosic hydrogen production because optimal temperature of hydrogenase for the reversible conversion of protons to molecular hydrogen was in the range from 50 to 70°C [25]. Furthermore, higher cellulase activity (7.2 IU g^{-1}fungal pretreated cornstalk) was also observed under 55°C than 35°C [26]. In addition, hydrogen production rates showed a significant (P< 0.05) promotion at elevated fermentation temperature [27].

As thermophilic biomass-degrading consortia are not as common as those mesophilic ones in nature, a general approach adopted requires certain steps of enrichment under the thermophilic condition. Hence, the temperature effect on such enrichment process was investigated extensively. Carver *et al.* (2002) found that enrichment at 60°C showed a shortened lag time and 10 folder higher H$_2$ yield than enrichment conducted at 55°C. Simultaneously, the methane formation, which is common in 55°C, was eliminated after enriched at 60°C [27]. Enrichment at 60°C from rumen fluid also showed increased hydrogen production while enrichment at 52°C failed after 3 batches [28]. However, the enrichment of thermophilic compost in another study also conducted by Nissilä's group, following similar approach, showed just opposite result that all enrichments of the pretreated (at 80°C for 20 min) compost community failed at 60°C while enrichment at 52°C showed the highest hydrogen yield [29]. Thus, there seems currently no standardized protocol to ensure successful enrichment of thermophilic cellulolytic consortia for lignocellulosic hydrogen production and the effectiveness of the enriched community was principally unable to control.

3.2.1.2 Effect of pretreatment

Studies of the pretreatment process counted for a big proportion of lignocellulosic hydrogen researches. However, minimal effort had been put into optimizing pretreatment process for thermophilic fermentation. Among reviewed recent studies, only three of them involved the effect of pretreatment on thermophilic lignocellulosic hydrogen production. A pretreatment method combining 10% ammonia and 1.0% dilute sulfuric acid was reported to be able to increase digestibility of rice straw by hyperthermophile *Thermotoga neapolitana* from 29% to 85.4% with improved hydrogen yield from 2.3 to 2.7 mmol H_2 g^{-1} straw [30]. A so-called Microwave-assisted acid pretreatment (MAP) showed obvious advantages of short duration and high efficiency of lignocellulosic hydrolysis as demonstrated by the smaller particle size and larger specific surface area of treated corn stover. [31] Likely lime pretreatment was also testified to promote thermophilic hydrogen yield from cornstalk by 38.1% [32].

3.2.1.3 Effect of fermentation substrate

Thermophilic cellulosic hydrogen production was affected by the type and amount of substrate applied in the fermentation. Among sweet sorghum, sugarcane bagasse, wheat straw, maize leaves and silphium, wheat straw was reported as the best substrate for *Caldicellulosiruptor saccharolyticus* in terms of H_2 production capacity. The strain was able to produce hydrogen from wheat straw at 44.7 L H_2 kg^{-1} dry biomass and H_2 yield of 3.8 mol H2 mol^{-1} glucose [33,13]. One the other hand, co-digestion of cellulose with microalgal biomass like *Dunaliella tertiolecta* or *Chlorella vulgaris* would increase cellulosic hydrogen yield. Higher yield of 7.7 mmol H_2 g^{-1} volatile solids (VS) was obtained when cellulose was co-digested with *D. tertiolecta* [34]. Interestingly, co-substrate of yeast extract exhibited a significant stimulation of cellulose degradation and hydrogen production from filter paper that the extent of cellulose utilization and hydrogen production by co-culture of *Clostridium thermocellumand* and *Clostridium thermopalmarium* displayed a linear relationship with the logarithm of the yeast extract concentration, and the optimal weight ratio of yeast extract to cellulose was 1:1 [35].

Substrate loading also influenced the hydrogen production. Chen *et al.* (2012) reported that increasing substrate loading from 30 to 90 g TS L^{-1} improved hydrogen yield but further to 120 g TS L^{-1} resulted in decreased hydrogen yield [36]. Meanwhile,

although *Clostridium thermocellum* grew 22% slower at low-cellulose concentrations (with growth rate of 0.15 h^{-1}) than at high-cellulose concentrations, the maximum specific rate of H$_2$ production (6.41±0.13 mmol H$_2$ g^{-1} dry cell h^{-1}) obtained during the exponential phase from low-carbon cultures was about 37% higher than that obtained from high-carbon cultures [37].

3.2.1.4 *Effect of pH*

pH is one of the well-known affecting factors of fermentation process. Acidic condition was observed to be adverse to thermophilic cellulosic hydrogen production. Chen *et al.* (2012) found that pH < 6.0 was unsuitable for thermophilic cellulose hydrolyzing microorganisms and no hydrogen was generated with initial pH lower than 5.5. Similarly, Xia *et al.* (2012) reported pH lower than pH 6.0 would induce the grow of *Thermoanaerobacterium* over the more cellulolytic *Clostridium* species and thus resulted in process failure of the semi-batch reactor. Optimal initial pH was reported to be pH 6.5 with hydrogen yield of 1.01 mmol H$_2$ g^{-1} TS added for heat pretreated sludge inoculums [36] (Table 5). Slightly higher optimal pH of pH 7.3 was reported for enriched rumen fluid [28]. Likewise, increase buffering capacity of the reaction medium was proven to be favorable to maintain efficient cellulose degradation especially at high cellulosic substrate loading, for example, at a load of filter paper of 9 g L^{-1}, increasing the alkali KHCO$_3$ concentration from 0 to 60 mM promoted cellulose utilization by 7 times from 1.23 g L^{-1} (equivalent to 13.5% substrate conversion) to 8.59 g L^{-1} (equivalent to 94.3% substrate conversion) [35].

3.2.1.5 *Mathematical model*

Attempt had been made to use mathematical model to predict thermophilic lignocellulosic hydrogen yield and evaluate influence of operational parameters. Both thermodynamic equation and continuum particle distribution model (CPDM) was applied to predict hydrogen yield.

3.2.1.5.1 Model based on thermodynamic equation

Based on acids and hydrogen yield in the batch experiment, Forrest *et al.* (2011) use thermodynamic formula Eq. (1) and (2) to calculate the theoretical energy selectivity (γ, kJ/g) of the reaction in batch test and then applying the result to predict hydrogen yield

using formula Eq. (3) in continuous MixAlco™ process which shared similar fermentation conditions, including substrate, buffer and incubation temperature, with batch tests.

$$\Delta G_c = \sum_j Acid_j \times \left[\sum_i (x_i \times \Delta G_{ji})\right] + H_2 \times \Delta G_{H_2} \tag{1}$$

$$\gamma \equiv \frac{\Delta G_c}{\Delta VS} \tag{2}$$

$$H_2(mol) = \frac{\gamma \times (\Delta VS) - \sum_j Acid_j \times \left[\sum_i (\chi_i \times \Delta G_{ji})\right]}{\Delta G_{H_2}} \tag{3}$$

ΔG_c : the total Gibbs free energy change from catabolic reactions (kJ)

$Acid_i$: the carboxylic acid of Type j (mol)

x_i : the mol fraction of sugar of Type i (e.g., mol hexose/mol total sugars)

ΔG_{ji} : the Gibbs free energy of reaction for Type j acid from Type i sugar (kJ/mol)

ΔG_{H_2}: the Gibbs free energy of reaction for hydrogen (kJ/mol)

γ: the energy selectivity of a system (kJ/g VS digested)

ΔVS: amount of VS digested (g)

The above thermodynamic calculation was able to predict hydrogen production from paper fermentation to within 11% and from bagasse fermentation to within 21% of the actual production by an enriched mixed thermophilic cellulolytic culture [38].

3.2.1.5.2 CPDM
In addition, the concept of continuum particles was used in the continuum particle distribution model (CPDM) to describe the thermophilic lignocellulosic acids production. A "continuum particle" is defined as 1 g of initial volatile solids with a composition identical to the biomass being fed to the fermentor. Eq. (4) is the governing equation deployed in the CPDM method. It relates the specific reaction rate ($\hat{r}_{pred}$) with acetic acid equivalent concentration (Aceq) and substrate conversion (x). The detailed description and implementation of the CPDM model could be found in [39].

$$\hat{r}_{pred} = \frac{e(1-x)^f}{1+g(\varphi \cdot \text{Aceq})^h} \tag{4}$$

$$\text{Aceq } (g/L) = 60.05 \times [\alpha \ (mol/L)] \tag{5}$$

$$\alpha \ (mol/L) = acetic \ (mol/L) \tag{6}$$
$$+1.75 \times propionic \ (mol/L)$$
$$+2.5 \times butyric \ (mol/L)$$
$$+3.25 \times valeric \ (mol/L)$$
$$+4.0 \times caprioc \ (mol/L)$$
$$+4.75 \times heptanoic \ (mol/L)$$

$\hat{r}_{pred}$: the reaction rate per continuum particle

x : fraction of conversion of Volatile Solids (VS)

e ,f, g, h : empirical constants which need to be determined by the least square method in batch test.

φ: the ratio of total grams of carboxylic acids to total grams of acetate; it is introduced to avoid inhibitory effect of higher acids that would overestimate the specific rate

Aceq: mass total acids concentration in term of acetic acids mass equivalent on a mass basis

α: molar total acids concentration in terms of acetic acids molar equivalent on a mass basis

The CPDM was reported to be able to predict substrate conversion and total acid production with averagely around 10% error in semi-continuous batch reactors and the MixAlco process [39,40]. In addition, CPDM optimizations show that high conversion (> 80%) and total acid concentration of 21.3 g L^{-1} were possible with 300 g substrate L^{-1}, 30 days liquid residence time, and 3 g L^{-1} day^{-1} solid loading rate [39].

3.2.1.6 Microbial communities

Nowadays, great research interest are devoted into seeking answers to the crucial microbiologic questions of "who is doing what" and "how to control them" in various

functional microbial communities. Without exception, communities involved in thermophilic lignocellulosic hydrogen production were extensively studied from characterization of community structure of mixed fermentation consortia to optimization of isolated strains. A co-culture of hydrolytic and fermentative bacteria seems crucial for many lignocellulose degrading systems. Chen *et al.* (2012) observed an association of hydrolytic bacteria *Clostridium pasteurianum* and *Clostridium stercorarium* with fermentative bacteria of *Thermoanaerobacterium saccharolyticu*m in the repeated-batch reactor inoculated with sludge from municipal wastewater treatment plant. This association led to the microbial hydrolysis and fermentation of raw rice straw avoiding the pretreatment step [36]. Similar community was enriched from compost pile as revealed by pyrosequencing analysis that the enriched TC60 consortium (11 OTUs) was predominated by *Thermoanaerobacter* (49%), *Clostridium* spp.(30%) and *Clostridium thermocellum* (21%) [27]. Again enrichment from compost pile by Nissilä *et al.* (2011) also showed that a bacterium closely related to *Thermoanaerobium thermosaccharolyticum* was mainly responsible for hydrogen production and bacteria closely related to *Clostridium cellulosi* and *Clostridium stercorarium* were responsible for cellulose degradation [29]. Slightly different from compost and sludge source, enrichment of rumen fluid showed a sole dominance of *Clostridium stercorarium* subsp. *Leptospartum* [28]. Briefly, no matter what source inoculation applied, the superior ability of genus *Clostridium* in thermophilic biomass degradation was well demonstrated by its dominance in the enriched thermophilic cellulolytic cultures. And the microbial co-existence of fermentative *Thermoanaerobacterium* or *Thermoanaerobacter* with cellulolytic *Clostridium* indicated a positive function of microbial co-culture on thermophilic lignocellulosic hydrogen production.

Such effect of co-culturing hydrolytic and fermentative strain was further investigated with isolated strains. For example, Li and Liu (2012) reached 94.1% higher hydrogen yield of 68.2 mL H_2 g^{-1}cornstalk with co-culture of *Clostridium thermocellum* and *Clostridium thermosaccharolyticum* [41]. And the volumetric hydrogen production was promoted by 2.4 times as compared to mono-culture by the co-cultivation of fermentative *Clostridium thermopalmarium* with cellulolytic *Clostridium thermocellumand* at ratio 0.05:1 [35]. In addition, it is interesting to notice that strains of

genus *Thermoanaerobacterium* though had been found to co-exist with cellulolytic *Clostridium* in several enriched thermophilic consortia, was seldom used in co-culture study on solid lignocellulose fermentation, in spite quite many hydrolysate studies use it.

3.2.1.7 Optimal conditions

The optimal conditions for anaerobic thermophilic lignocellulosic hydrogen production were summarized in Table 5. Again the discrepancy of yield calculation, make it difficult to compare fermentation performance among literatures. However, general trend of hydrogen yield could still be observed that the simplified fermentation environment tended to have higher hydrogen production yield than those of real reactor system. Simplification either by inoculating pure culture or using purified cellulosic substrate like α-cellulose or filter paper resulted in higher hydrogen yield with the highest yield of 21.1 mmol H_2 g^{-1} glucose equivalent digested obtained by applying *Caldicellulosiruptor saccharolyticus. DSM8903* to ferment wheat straw [33]. In reactors treating complex lignocellulosic substrate, the highest reported hydrogen yield was 8.5 mmol H_2 g^{-1} glucose equivalent digested when digesting pretreated corn stover [31]. Additionally, higher hydrogen yield may be achieved by enlarging reactor size, for example, hydrogen yield in the 8-liter CSTR reached 74.9 mL H_2 g^{-1}cornstalk which was 9.8% higher than that in the 125 mL anaerobic bottle [41]. Similar hydrogen yield increase was also demonstrated in 100-liter CSTR as compared to 125 mL batch test [42]. The most likely reason for such phenomenon is that the larger reactor tends to have better mixing and mass transfer which will promote both the hydrolysis and hydrogen generation reaction.

3.2.2 Lignocellulosic ethanol production

The approach of co-culturing was wildly used in the studies of lignocellulosic ethanol production. Strains from g*enus* of *Clostridium* and *Thermoanaerobecter* were most often used in this approach. Ethanol production by co-culture of *Clostridium themocellum* and *Clostridium thermolacticum* was up to 2-fold higher than mono-culture of each strain, especially with microcrystalline cellulose as substrate [43]. Similarly co-culture *Thermoanaerobacter* strain X514 with *Clostridium thermocellum* promoted the ethanol production by 440% [44].

Genetic modification of ethanol-producing strain is another effective way to promote ethanol yield. By deleting the genes for both lactate dehydrogenase (Ldh) and phosphotransacetylase (Pta), the stable strain evolved after 2,000h showed 4.2-fold increase in ethanol yield over the wild-type strain [45]. In addition, Yao and Mikkelsen (2010) had successfully modified the metabolism of *Thermoanaerobacter mathranii* towards enhanced ethanol generation by eliminating the *Idh* gene and expressing a heterologeous gene *gldA* [46].

Several other interesting observations on lignocellulosic ethanol production were reported in literature. It was reported by Xu and Tschirner (2011) that higher initial ethanol level favored the ethanol production for both mono-culture and co-culture of *Clostridium themocellum* and *Clostridium thermolacticum*. At the optimized condition, the ethanol production from microcrystalline cellulose increased from 1.0 g L^{-1} at an initial ethanol level of 0 g L^{-1} to 3.8 g L^{-1} at an initial ethanol level of 4 g L^{-1} [43]. Unexpectedly, acetate also showed an stimulatory effect on ethanolic fermentation by *Thermoanaerobacter ethanolicus 39E*, enhancing ethanol production by up to 394% whereas lactate was in general inhibitory to ethanolic fermentation [47]. In addition, the presence of a complete vitamin B12 biosynthesis pathway in *Thermoanaerobacter* strain X514 was testified to be associated with the higher ethanolic fermentation efficiency as compared to another strain 39E in which the B12 biosynthesis pathway was incomplete. The significance of the vitamin B12 bio-synthesis capacity was further supported by the observation of improved ethanol production in strain 39E (by 203%) following the addition of exogenous vitamin B12 [44].

3.2.3   Lignocellulosic carboxylic acids production

Carboxylic acids like acetic acid are another type of targeting products for lignocellulosic fermentation because it could be chemically converted into a wide variety of chemicals and fuels. Increase fermentation temperature from 40°C to 55°C resulted in higher total acids yield but lower substrate conversion ratio [48,49]. In addition, the continuum particle distribution model (CPDM) was reported to be able to predict substrate conversion and total acid production with averagely around 10% error in semi-continuous batch reactor and the MixAlco process [39,40]. Another interesting phenomenon found by Budhavaram and Fan (2009) was that the buffering of $CaCO_3$ which could result

in 10 g L^{-1} more lactic acid production in LB medium was ineffective when a so-called "lean" solution with limited phosphate and nutrient was used as the fermentation medium [48]. However the authors failed to provide any tentative explanation of the observed negative effect of pH control.

3.2.4 Lignocellulosic methane production

Stable cellulose-utilizing thermophilic methanogenic community had been enriched from several sources including compost, digestion sludge, soil and so forth [50–52]. Co-existence of thermophilic cellulose degrader with methanogens had been commonly observed in these communities. The most common thermophilic cellulose degraders reported were affiliated to the genus of *Clostridium*. The importance of *Clostridium* species in the lignocellulosic methane production had been validated in two studies in both of which the sudden drop of *Clostridium* proportion in the community either induced by pH drop or by substrate alteration resulted in significant performance declination of the reactor [50,53]. Additionally, co-digestion of lignocellulosic waste with nitrogen-rich waste like palm oil mill effluent (POME) [52] could effectively improve the methane yield, for example, methane yield of 276–340 mL CH$_4$ g^{-1} VS-added from co-digesting palm empty fruit bunches with POME was 25-32% higher than that from digesting fruit bunches alone.

3.2.5 Multiple stage process

Besides the one-stage fermentation studies mentioned above, fermentation pipeline in which the effluent or residues of the first stage was further fermented in subsequent reactors was developed to improve the overall energy efficiency. Such multiple-stage strategy contains more than one fermentation process carried out in separated reactors. From the perspective of biorefinery, a final stage of bio-methane production was usually adopted to refine the effluent or residues of the biohydrogen or bioethanol production. A roughly 30% more energy recovery could be added up to overall process by the refinery stage of bio-methane generation [54–58]. In addition, multiple-stage process enabling the production of multiple biofuels could benefit the subsequent utilization of the fuels, for example, addition of a small amount of hydrogen to methane would significantly improve the efficiency and stability of combustion in motors because hydrogen has eight times faster flame speed than methane and the addition of hydrogen serves to reduce

air/fuel ratio (lambda) as well. However, directly fermenting pretreated wheat straw into bio-methane showed higher energy output (10452 MJ Ton^{-1} of dry matter) than firstly fermenting into ethanol or hydrogen (around 8000 MJ ton^{-1} of dry matter). Furthermore combustion of the straw had the highest energy output of around 1600 MJ ton^{-1} of dry matter [59]. Despite low energy output from production of biofuels as compared to incineration of lignocellulosic biomass, the advantage of mitigating world-wide fossil fuel shortage by generating liquid fuel alternatives and negligible environmental damage of the fermentation process serves to explain the importance of conversion of biomass to biofuels. Finally, by comparing different technological scenarios, *Dias et al.* claimed that integrating first and second generation ethanol production technique leaded to better economic results for bioethanol plant, especially when advanced hydrolysis technologies and pentoses fermentation were included [60].

4   Conclusion

Thermophilic fermentation process although be regarded as a promising technology to promote lignocellulose hydrolysis and fermentation efficiency, is still at its infantile stage with a number of aspects requiring systematic evaluation and optimization. Based on reviews of the recent studies on the thermophilic anaerobic lignocellulose bioconversion, major knowledge gaps include:

First, complete lignocellulosic substrate conversion is still practically impossible no matter utilizing hydrolysate or after-pretreated solid residues, except for reactors with simplified systems or purified substrates. Even though the adversity of the unutilized substrate to the overall fermentation process had been observed in many scenarios, the reason for such reaction cease was rarely addressed or paid attention to in most studies.

Second, information of microbial community structure is still quite limited and rather static. Little quantitative microbial characterization of the fermentative communities could be provided to facilitate the essential stages of reactor operation such as reactor start-up, failure recovery etc. The introduction of the emerging Next Generation Sequencing (NGS) technology into the dynamic monitoring of reactor communities would help setting up the knowledge linkage between variation of microbial structure and reactor performance.

Last but not the least, standardized performance evaluation measure is needed. The expression of product yield is inconsistent among studies, which causes difficulty to the systematic comparison of fermentation performance among studies. In some cases, the product yield was simply expressed in the unit of product per mass of substrate added while in other cases it was defined as product per mass of substrate converted. Given the lignocellulosic substrate was not fully converted in most of the case; these two units were hardly consistent and impossible to compare.

Based on the above review, it is clear that converting lignocellulose into biofuels at thermophilic condition is a very feasible solution to produce biofuels from lignocellulosic materials. While, concern remains on how to establish an applicable and affordable thermophilic lignocellulose conversion process. pH control by dosing buffering

chemicals ($CaCO_3$ or $KHCO_3$), co-culturing cellulolytic species (e.g. *Clostridium*) with fermentative species (e.g. *Thermoanaerobacter* or *Thermoanaerobacterium*) and co-digesting lignocellulosic biomass with nutrient sufficient feedstock like manure were the most applicable bioaugmentation strategies in the studies reviewed. Another considerable pathway is to develop a multiple-stage process for multi-fuel output like ethanol-CH_4 or H_2-CH_4 from raw lignocellulosic waste. Aside from the scientific research, a throughout economic assessment is also essential in order to put this entire blueprint into reality.

Table 1 Chemical composition hydrolysate derived from various pretreatment and hydrolysis technologies.

Source biomass	Pretreatment and hydrolysis	TS %	VS%	Furfural (mg L^{-1})	HMF (mg L^{-1})	Phenols (mg L^{-1})	Total sugars (g L^{-1})	Glucose (g L^{-1})	Xylose (g L^{-1})	Arabinose (g L^{-1})	VFA (g L^{-1})	Others (g L^{-1})	Reference
Sugarcane bagasse	Steam explosion	-	-	500	100	-	25.2	1.5	22.2	-	4.1	1.5 cellobiose	[61]
Wheat straw	Hydrothermal pretreatment	4.4	3.3	250	140	140	15.5	2.9	11.3	1.3	0.7	-	[11]
Wheat straw	Hydrothermal pretreatment	12.0	10.2	N.D.	N.D.	61	84.5	10.3	6.9	N.D.	0.18	75.6 lignin	[10]
Hardwood chip	Autohydrolysis	-	-	780	130	N.D.	3.62	0.36	1.96	0.68	10.36	-	[14]
Corn stover	Acid pretreatment	-	-	410	-	40	11.84	1.85	9.11	0.88	1.35	-	[19]
Corn stover	Dilute acid pretreatment	30	-	3800	600	-	83.1	15.6	67.5	-	13.7	-	[62]
Corn stover	Acid pretreatment and enzyme hydrolysis	-	-	-	-	-	31.5	17.3	12.6	1.9	-	-	[18]
Sweet sorghum bagasse	Alkaline pretreatment and enzymatic hydrolysis	-	-	N.D.	N.D.	N.D.	61.9	31.2	13.2	1.6	4.3	15.9 di and/or oligosugars	[17]
Miscanthus	Alkaline and acid pretreatment and enzymatic hydrolysis	-	-	N.D.	N.D.	-	38.3	26.8	10.3	1.2	3.7	-	[20]

-: information not stated in the paper
N.D.: not detected

Table 2 Optimal conditions for thermophilic hydrogen and methane production from lignocellulosic hydrolysate.

| Process Parameter | | | | | | | | | | Performance parameters at optimal conditions | | | | | | Ref |
| Inoculum seed | | Feedstock | | | Reactor | | Tep | pH | Conv[4] (%) | Hydrogen production | | | Methane production | | | |
Source	Ino[1]	type	Conc[2] (g L^{-1})	OLR (g L^{-1} d^{-1})	Conf[3]	HRT				Yield (mmol g^{-1} sugar)	Rate (mmol L^{-1} d^{-1})	Cont[5] (%)	Yield (mmol g^{-1} COD)	Rate (mmol L^{-1} d^{-1})	Cont[5] (%)	
Thermoan-aerobacterium	-	30% Cellulose hydrolysate	7.9 ^{S}	N/A	Batch	N/A	65	5.0-6.0^{ini}	76	13.9 ^{d}	-	-	N.D.	N.D.	N.D.	63
Thermoan-aerobacterium	-	30% grass hydrolysate	7.9 ^{S}	N/A	Batch	N/A	65	5.0-6.0^{ini}	100	8.4 ^{d}	-	-	N.D.	N.D.	N.D.	
Caldicellulosiruptor saccharolyticus	10% (v/v)	Sweet sorghum bagasse hydrolysate	10.0 ^{S}	N/A	Batch	N/A	72	6.8^c	97	14.4 ^{d}	244.8	-	N.D.	N.D.	N.D.	17
Thermoanaerobacterium thermosaccharolyticum	4% (v/v)	Corn stover hydrolysate	11.8 ^{S}	N/A	Batch	N/A	60	7.0	100	12.4 ^{d}	146.8	-	N.D.	N.D.	N.D.	19
Co-culture of *Caldicellulosiruptor saccharolyticus* and compost microflora	15% (v/v) at 1:1	Synthetic hydrolysate with 1%(w/v) 1:1 glucose:xylose	10.0 ^{S}	N/A	Batch	N/A	70	6.5^c	100	12.8 ^{d}	540	-	N.D.	N.D.	N.D.	21
Co-culture of *Caldicellulosiruptor saccharolyticus* and *C. kristjanssoniiat*	15% (v/v) at 1:1	Synthetic hydrolysate with 1%(w/v) 1:1 glucose:xylose	10.0 ^{S}	N/A	Batch	N/A	70	6.5^c	100	**21.1 ^{d}**	408	-	N.D.	N.D.	N.D.	
Caldicellulosiruptor saccharolyticus	-	Miscanthus hydrolysate	10.0 ^{S}	N/A	Batch	N/A	70	-	100	**18.9 ^{d}**	302.4	-	N.D.	N.D.	N.D.	20
Thermotoga neapolitana	-	Miscanthus hydrolysate	14.0 ^{S}	N/A	Batch	N/A	80	-	100	17.8 ^{d}	295.2	-	N.D.	N.D.	N.D.	
Anaerobic digestion sludge	0.52g TS/l	Ground wheat hydrolysate	20.0 ^{S}	N/A	Batch	N/A	55	7.0-5.5^c	87.5	9.9 ^{d}	38.2	-	N.D.	N.D.	N.D.	64
Anaerobic digestion sluge	1.5g TS/l	Ground wheat hydrolysate	4.2 ^{S}	N/A	Batch	N/A	55	7.0-5.5^c	95	6.35 ^{d}	62.4	-	N.D.	N.D.	N.D.	
Reactor sludge	20% (v/v)	Wheat straw 5% hydrolysate	0.8 ^{S}	N/A	Batch	N/A	70	-	97.1	13.36 ^{d}	7.7	17	N.D.	N.D.	N.D.	11

Reactor sludge	20% (v/v)	Wheat straw 20% hydrolysate	3.1 ^{S}	1.03	CSTR	3d	70	5.5-5.2 ^{c}	98	8.06 ^{d}	8.5	37	N.D.	N.D.	N.D.	
Reactor sludge	42% (v/v)	Wheat straw 20% hydrolysate	3.9 ^{S}	3.9	AF $^{4)}$	1d	70	5.4	94.3	5.5 ^{d}	20.2	32.6	N.D.	N.D.	N.D.	
Reactor sludge	45% (v/v)	Wheat straw 20% hydrolysate	3.9 ^{S}	3.9	UASB	1d	70	5.1	98.1	8.8 ^{d}	33.6	43	N.D.	N.D.	N.D.	15
Reactor sludge	-	Wheat straw 20% hydrolysate	3.9 ^{S}	1.3	CSTR	3d	70	5.3	97.1	7.9 $^{a/}$	9.9	41.5	N.D.	N.D.	N.D.	
Reactor sludge treating cow manure	100% (v/v)	Wheat straw 100% hydrolysate	38.0 ^{COD}	1.9	CSTR	20d	55	7.4	72	N.D.	N.D.	N.D.	16.8 ^{d}	23.1	58.9	
Thermophilic granule	39% (v/v)	Wheat straw 10% hydrolysate	5.6 ^{COD}	2.8	UASB	2d	55	7.2	71	N.D.	N.D.	N.D.	15.4 ^{d}	30.6	66.9	12
Thermophilic granule	39% (v/v)	Co-digestion, Wheat straw 10% hydrolysate and pig manure (1:3 v/v)	17.1 ^{COD}	8.53	UASB	2d	55	7.9	71	N.D.	N.D.	N.D.	12.6 ^{d}	38.2	65	
Thermophilic granules	39% (v/v)	Wheat straw 25% hydrolysate	34.2 ^{COD}	17.1	UASB	48h	55	6.8 ^{c}	76	N.D.	N.D.	N.D.	8.3 ^{d}	108.2	63.7	10
Thermophilic granules	75% (v/v)	Wheat straw hydrolysate	11.3 ^{COD}	N/A	Batch	N/A	55	-	-	N.D.	N.D.	N.D.	13.2 mmol g^{-1} VS ^{a}	8.5	-	

[1] Ino: inoculation information
[2] Conc: substrate loading concentration
[3] Conf: configuration of the reactor
[4] Conv: substrate conversion percentage
[5] Cont.: content in the gas phase
[6] AF: anaerobic filter reactor
^{S}: substrate concentration in unit of g sugar L^{-1}
^{COD}: substrate concentration in unit of g COD L^{-1}

^{ini}: pH was controlled at the beginning of the reaction
^{c}: pH was controlled at constant value throughout the reaction
^{a}: product yield calculated based on feedstock added
^{d}: product yield calculated based on feedstock digested
-: information not stated in the paper
N/A: parameter not applicable due to technological difference
N.D.: not detected

Table 3 Optimal conditions for thermophilic ethanol and carboxylic acids production from lignocellulosic hydrolysate

Process Parameter									Performance parameters at optimal conditions					Ref
Inoculum seed		Feedstock			Reactor		Tep	pH	Conv[4] (%)	Ethanol production		Carboxylic acids production		
Source	Ino [1]	Type	Conc [2] (g sugar L^{-1})	OLR (g L^{-1} d^{-1})	Conf[3]	HRT				Yield	Rate (g $L^{-1}d^{-1}$)	Yield	Rate (g $L^{-1}d^{-1}$)	
Caloramator boliviensis	14.7% (v/v)	20% Sugarcane bagass hydrolysate	8.9	9.12g/ld	Packed bag reactor	23h	60	70-7.5^c	100	0.46 g g^{-1} sugar^d	4.27	0.27 g g^{-1} sugar^d	2.46	61
Thermoan-aerobacterium	-	30% Cellulose hydrolysate	7.9	N/A	Batch	N/A	65	5.0-6.0^{ini}	76	0.33 g g^{-1} glucose^d	-	0.23 g g^{-1} glucose^d	-	63
Thermoan-aerobacterium	-	30% grass hydrolysate	7.9	N/A	Batch	N/A	65	5.0-6.0^{ini}	100	0.3 g g^{-1} glucose^d	-	0.25 g g^{-1} glucose^d	-	63
Thermoanaerobacterium saccharolyticum	10%(v/v)	100% Hardwood hydrolysate after enzyme hydrolysis	13.7	N/A	Batch	N/A	51	>5.8^c	94	7.1 g L^{-1}	-	-	-	14
Thermoanaerobacter strain AK5	2% (v/v)	20% Cellulose hydrolysate	4.5	N/A	Batch	N/A	65	-	-	0.25 g g^{-1} biomass^d	-	-	-	65
Thermoanaerobacter BG1L1	OD=0.9-1	20% Wheat straw hydrolysate	11.8	5.9g/ld	FBR[5]	2d	70	7.0^c	92	0.42 g g^{-1} sugar^d	2.28	-	-	16
Thermoanaerobacter BG1L1	OD=0.9-1	21%Corn stover hydrolysate	17.2	8.6g/ld	FBR[5]	2d	70	7.0^c	95	0.42 g g^{-1} sugar^d	3.43	-	-	62

[1] Ino: inoculation information
[2] Conc: substrate loading concentration
[3] Conf: configuration of the reactor
[4] Conv: substrate conversion percentage
[5] FBR: fluidized bed reactor

^{ini}: pH was controlled at the beginning of the reaction
^{c} : pH was controlled at constant value throughout the reaction
^{a}: product yield calculated based on feedstock added
^{d}: product yield calculated based on feedstock digested
-:   information not stated in the paper
N/A: information not available due to technological difference
N.D.: not detected

Table 4 Chemical composition of common lignocellulosic feedstock.

Biomass	Cellulose	Hemicellulose	Lignin	Ash	Reference
Rice straw	41.5	19.6	22.8	10.9	30
Wheat straw	33-40	20-25	12-20	-	66
Barley straw	37.2	24.6	16.1	6.4	13,33
Corn stalk	36.7	26.2	16.9	4.9	13,33
Corn stalk	36.5	31.3	11.9	-	26
Corn stalk	31.1	27.0	14.4	-	42
Corn stover	36.5	31.3	11.9	-	31
Corn stover	40.3	20.8	19.1	-	19
Corn stover	37.6	21.5	19.1	-	18
Hardwood stem	20-25	45-50	20-25	-	67
Softwood stem	27-30	35-40	25-35	-	67
Sugarcane baggase	40-45	30-35	20-30	-	68
Sweet sorghum bagasse	38.5	21.4	17.6	3.7	17
Switchgrass	42.6	27.3	21.0	-	69
Switchgrass	31.0-46.2	15.2-22.5	17.8	-	70
Napiergrass	20-28	29-42	3-4%	-	70
Napiergrass	31.5	24.5	4.2	-	71
Filter paper	>98	-	-	-	35

-: information not stated in the paper

Table 5 Optimal conditions for the thermophilic hydrogen and methane production from lignocellulosic biomass

| Process Parameters for optimal conditions | | | | | | | | | | Performance parameters at optimal conditions | | | | | | Ref |
| Inoculum seed | | Feedstock | | | Reactor | | Tep | pH | Conv[4] | Hydrogen production | | | Methane production | | | |
Source	Ino[1]	type	Conc[2]	OLR	Conf[3]	HRT				Yield	Rate (mmol L^{-1} d^{-1})	Cont[5]	Yield	Rate (mmol L^{-1} d^{-1})	Cont[5]	
C. thermocellum ATCC 27405	10%(v/v)	α-cellulose	1 g L^{-1}	N/A	Batch	N/A	60	7.2^{ini}	83%	10.6mmol g^{-1} glucose equivalent^d	11.7	-	N.D.	N.D.	N.D.	37
C. thermocellum 7072	10%(v/v)	Corn stalk	30 g TS L^{-1}	N/A	CSTR	N/A	55	7.4	63.5%	2.5 mmol g^{-1} cornstalk^d	23.0	-	N.D.	N.D.	N.D.	42
Clostridium sp. TCW1	-	Filter paper	5.0 g L^{-1}	N/A	Batch	N/A	60	7.0^{ini}	99.7%	11.3 mmol g^{-1} filter paper^d	56.6	36.7%	N.D.	N.D.	N.D.	72
Thermotoga neapolitana DSM 4359	-	Pretreated rice straw	10 g L^{-1}	N/A	Batch	N/A	75	7.5^{ini}	85.4%	2.7 mmol g^{-1} straw^d	109.8	28.1%	N.D.	N.D.	N.D.	30
Caldicellulosiruptor saccharolyticus. DSM8903	3%(v/v)	Wheat straw	10 g L^{-1}	N/A	Batch	N/A	70	7.2^{ini}	-	**21.1 mmol g^{-1} glucose equivalent^d**	-	-	N.D.	N.D.	N.D.	33
C. thermocellum DSM 7072 and *C. thermosaccharolyticum* DSM 869	12.5% (v/v) at 1:0.25	Autoclave pretreated cornstalk	10 g L^{-1}	-	CSTR	-	55	-	43.7%	3.1 mmol g^{-1} cornstalk^d	4.6	-	N.D.	N.D.	N.D.	41
C. thermocellum DSM 1237 and *C.thermopalmarium* DSM 1237	10%(v/v) at 1:0.05	Filter paper	9 g L^{-1}	N/A	Batch	N/A	55	6.9^{ini}	90%	7.6 mmol g^{-1} glucose equivalent^d	-	-	N.D.	N.D.	N.D.	35
Enriched compost	2% (v/v)	Avicell	5 g L^{-1}	N/A	Batch	N/A	52	7.0^{ini}	58.3%	13.3 mmol g^{-1} hexose^d	1.9	57%	N.D.	N.D.	N.D.	29
Reactor sludge	25%(v/v)	pretreated and raw cornstalk (1:5 w/w)	13.3 g TS L+	N/A	Batch	N/A	55	-	41.6%	4.8 mmol g^{-1}TS^d	6.3	--	N.D.	N.D.	N.D.	26
Enriched consortium TC60 from compost	10%(v/v)	*D. tertiolecta* and cellulose (2:1 w/w)	4 g VS L^{-1}	N/A	Batch	N/A	60	7.0^{ini}	-	7.7 mmol g^{-1}VS^a	28.2	-	N.D.	N.D.	N.D.	34
Marine sediment	-	Office paper with chicken manure	97.5 g VS L^{-1}	N/A	Batch	N/A	55	7.0^{ini}	64%	2.2 mmol g^{-1} VS^d	3.2	-	N.D.	N.D.	N.D.	38

Ino	Conc	Feedstock	Loading	Rate	Conf	HRT	Temp	pH	Conv	Product yield					Cont	Ref
Winery	25%(v/v)	Pretreated corn stover	-	N/A	Batch	N/A	55	7.0^{ini}	37.6%	**8.5 mmol g^{-1} glucose equivalent^{d}**	-	-	N.D.	N.D.	N.D.	[31]
sludge from municipal wastewater treatment plant	42%(v/v)	Rice straw size < 0.297mm	90g TS L^{-1}	N/A	Batch	N/A	55	6.5^{ini}	-	1.01 mmol g^{-1} TS^{a}	-	-	N.D.	N.D.	N.D.	[36]
Cow rumen fluid	2% (v/v)	Avicell	5 g L^{-1}	N/A	Batch	N/A	60	7.3^{ini}	21%	10.7 mmol g^{-1} hexose^{d}	0.28	-	N.D.	N.D.	N.D.	[28]
Sludge from thermophilic biogas plant	-	Co-digest EFB[6] with POME[7]	46g VS L^{-1}	N/A	Batch	N/A	55	-	91%	ND	ND	ND	16.0 mmol g^{-1} VS^{a}	-	-	[52]
Hoggery	30% (wt)	Fungi pretreated rice straw	75 g TS L^{-1}	N/A	Batch	N/A	55	-	-	ND	ND	ND	4.4 mmol g^{-1} TS^{a}	5.7	-	[73]
Reactor sludge	32.5% (v/v)	Slurry effluent from ethanol production	-	3.5 kg m^{-3} d^{-1}	UASB	2.1h	53	7.8^{c}	68%	ND	ND	ND	13.9 mmol g^{-1} VS^{a}	-	-	[54]
C.thermocellum GCD7 and *C. thermosacchara* GSC2 *Methanoculleus thermophilicus* GML1; *Methanotrix* GMK2 and *Methanosarcina thermophila* GMH7	10% (v/v)	Freeze explosion pretreated stimulated OFMSW[8]	10 g VS L^{-1}	N/A	Batch	N/A	55	9.0^{ini}	-	4.2mmol g^{-1} VS^{a}	68.0	55%	-	-	<0.1%	[55]
Cow manure	30g/l	Effluent from hydrogen batch	-	2.6g VFA $L^{-1}d^{-1}$	UASB	5d	55	7.2^{c}	-	-	-	-	21.2 mmol g^{-1} VS^{a}	11.0	78.6%,	

Table 6 Optimal conditions for the thermophilic ethanol and carboxylic acids production from lignocellulosic biomass

Process Parameter									Performance parameters at optimal conditions					Ref
Inoculum seed		Feedstock			Reactor				Conv[4)]	Ethanol production		Carboxylic acids production		
Source	Ino[1)]	Type	Conc[2)]	OLR (g VS L^{-1} d^{-1})	Conf[3)]	HRT	Tep	pH		Yield (g/ g^{-1} substrate)	Rate (g L^{-1} d^{-1})	Yield (g g^{-1} VS)	Rate (g L^{-1} d^{-1})	
Clostridium thermocellum	1% (v/v)	Pretreated Napiergrass	20 g L^{-1}	N/A	Batch	N/A	60	7.0[ini]	100%	0.0476 [d]	-	-	-	71
C.thermocellum	1% (v/v)	Avicel	10 g L^{-1}	N/A	Batch	N/A	60	7.0[ini]	100%	0.0332 [d]	-	-	-	
Co-culture of *C. themocellum* and *C thermolacticum.*	10% (v/v) (1:1)	Microcrystalline cellulose with ethanol 4g/l	10 g L^{-1}l	N/A	Batch	N/A	57	9.0	99%	0.38 [d]	1.9	-	-	43
Co-culture of *C.thermocellum* and *T. Saccharolyticum*	20% (v/v) (1:1)	Avicel	92 g L^{-1}	N/A	batch	N/A	55	6.3[ini]	90%	0.46 [d]	6.2	N.D.	N.D.-	45
Enriched culture from compost of Napiergrass and sheep dung	1% (v/v)	Pretreated Napiergrass	40 g L^{-1}	N/A	Batch	N/A	60	7.0[ini]	100%	0.04 [d]	0.027	-	-	71
Enriched culture from compost of Napiergrass and sheep dung	1% (v/v)	Avicel	10 g L^{-1}	N/A	Batch	N/A	60	7.0[ini]	100%	0.108 [d]	0.013	-	-	71
Marine sediment	-	Lime pretreated water hyacinths with chicken manure at (80:20 wt)	100 g VS L^{-1}	N/A	batch	N/A	40	7.0[ini]	56%	-	-	0.53 [d]	1.1	49
Marine microorganisms	-	Lime treated bagasse co-digested with chicken manure (80:20 wt)	60.6 g VS L^{-1}	2.58	SBR[5)]	23.5d	55	6.6	59.5%	-	-	0.79 [d]	-	40
Marine sediment		Pretreated sugarcane bagasse and chicken manure (80:20 wt)	84.4 g VS L^{-1}	3.26	MaxAlco	25.9d	55	6.0[c]	59%	-	-	0.31 [d]	-	39

[1)]   Ino: inoculation information
[2)]   Conc: substrate loading concentration
[3)]   Conf: configuration of the reactor
[4)]   Conv: substrate conversion percentage
[5)]   SBR: sequential batch reactor
[ini]:   pH was controlled at the beginning of the reaction

[c]:   pH was controlled at constant value throughout the reaction
[d]:   product yield calculated based on feedstock digested
-:   information not provided in the study
N/A: parameter not applicable due to technological difference
N.D.: not detected

Reference:

1. L. R. Lynd, P. J. Weimer, W. H. Van Zyl, and I. S. Pretorius, *Microbiol. Mol. Biol. Rev.*, 2002, **66**, 506–577.
2. S. Raj, S. Talluri, and L. Christopher, *BioEnergy Res.*, 2012, **5**, 515–531.
3. S. E. Blumer-Schuette, I. Kataeva, J. Westpheling, M. W. Adams, and R. M. Kelly, *Curr. Opin. Biotechnol.*, 2008, **19**, 210–217.
4. M. P. Taylor, K. L. Eley, S. Martin, M. I. Tuffin, S. G. Burton, and D. A. Cowan, *Trends Biotechnol.*, 2009, **27**, 398–405.
5. C. Li and H. H. P. Fang, *Critical Reviews in Environmental Science and Technology*, 2007, **37**, 1–39.
6. N. Ren, A. Wang, G. Cao, J. Xu, and L. Gao, *Biotechnol. Adv.*, 2009, **27**, 1051–1060.
7. F. Talebnia, D. Karakashev, and I. Angelidaki, *Bioresour. Technol.*, 2010, **101**, 4744–4753.
8. M.-L. Chong, V. Sabaratnam, Y. Shirai, and M. A. Hassan, *Int J Hydrogen Energy*, 2009, **34**, 3277–3287.
9. C.-L. Cheng, Y.-C. Lo, K.-S. Lee, D.-J. Lee, C.-Y. Lin, and J.-S. Chang, *Bioresour. Technol.*, 2011, **102**, 8514–23.
10. P. Kaparaju, M. Serrano, and I. Angelidaki, *Appl. Energy*, 2010, **87**, 3779–3783.
11. P. Kongjan, S. O-Thong, M. Kotay, B. Min, and I. Angelidaki, *Biotechnol. Bioeng.*, 2010, **105**, 899–908.
12. P. Kaparaju, M. Serrano, and I. Angelidaki, *Bioresour. Technol.*, 2009, **100**, 6317–23.
13. I. a Panagiotopoulos, R. R. Bakker, M. A. W. Budde, T. de Vrije, P. A. M. Claassen, and E. G. Koukios, *Bioresour. Technol.*, 2009, **100**, 6331–8.
14. J. M. Lee, R. a. Venditti, H. Jameel, and W. R. Kenealy, *Biomass Bioenergy*, 2011, **35**, 626–636.
15. P. Kongjan and I. Angelidaki, *Bioresour. Technol.*, 2010, **101**, 7789–7796.
16. T. I. Georgieva, M. J. Mikkelsen, and B. K. Ahring, *Appl. Biochem. Biotechnol.*, 2008, **145**, 99–110.
17. I. a. Panagiotopoulos, R. R. Bakker, T. de Vrije, E. G. Koukios, and P. a. M. Claassen, *Int J Hydrogen Energy*, 2010, **35**, 7738–7747.
18. N. Ren, G. Cao, A. Wang, D. J. Lee, W. Guo, and Y. Zhu, *Int J Hydrogen Energy*, 2008, **33**, 6124–6132.
19. G. Cao, N. Ren, A. Wang, D.-J. Lee, W. Guo, B. Liu, Y. Feng, and Q. Zhao, *Int J Hydrogen Energy*, 2009, **34**, 7182–7188.
20. T. De Vrije, R. R. Bakker, M. A. Budde, M. H. Lai, A. E. Mars, and P. A. Claassen, *Biotechnol. Biofuels*, 2009, **2**, 12.
21. A. a. Zeidan and E. W. J. Van Niel, *Int J Hydrogen Energy*, 2009, **34**, 4524–4528.
22. A. L. VanFossen, M. R. A. Verhaart, S. M. W. Kengen, and R. M. Kelly, *Appl. Environ. Microbiol.*, 2009, **75**, 7718–7724.
23. S. Kim and B. E. Dale, *Biomass Bioenergy*, 2004, **26**, 361–375.
24. E. M. Rubin, *Nature*, 2008, **454**, 841–845.
25. Koesnandar, N. Nishio, A. Yamamoto, and S. Nagai, *J. Ferment. Bioeng.*, 1991, **72**, 11–14.
26. X.-Y. Cheng and C.-Z. Liu, *Applied Energy*, 2012, **91**, 1–6.
27. S. M. Carver, M. C. Nelson, R. Lepistö, Z. Yu, and O. H. Tuovinen, *Bioresour. Technol.*, 2012, **104**, 424–31.
28. M. E. Nissilä, H. P. Tähti, J. a. Rintala, and J. a. Puhakka, *Int J Hydrogen Energy*, 2011, **36**, 1482–1490.
29. M. E. Nissilä, H. P. Tähti, J. a Rintala, and J. a Puhakka, *Bioresour. Technol.*, 2011, **102**, 4501–6.

30. T.-A. D. Nguyen, K.-R. Kim, M. S. Kim, and S. J. Sim, *Int J Hydrogen Energy*, 2010, **35**, 13392–13398.
31. C. Liu and X. Cheng, *Int J Hydrogen Energy*, 2010, **35**, 8945–8952.
32. G.-L. Cao, W.-Q. Guo, A.-J. Wang, L. Zhao, C.-J. Xu, Q. Zhao, and N.-Q. Ren, *Int J Hydrogen Energy*, 2012, **37**, 13161–13166.
33. G. Ivanova, G. Rákhely, and K. L. Kovács, *Int J Hydrogen Energy*, 2009, **34**, 3659–3670.
34. S. M. Carver, C. J. Hulatt, D. N. Thomas, and O. H. Tuovinen, *Biodegradation*, 2010, **22**, 805–14.
35. A. Geng, Y. He, C. Qian, X. Yan, and Z. Zhou, *Bioresour. Technol.*, 2010, **101**, 4029–33.
36. C.-C. Chen, Y.-S. Chuang, C.-Y. Lin, C.-H. Lay, and B. Sen, *Int J Hydrogen Energy*, 2012, 1–7.
37. R. Islam, N. Cicek, R. Sparling, and D. Levin, *Appl. Microbiol. Biotechnol.*, 2009, **82**, 141–8.
38. A. K. Forrest, M. E. Wales, and M. T. Holtzapple, *Bioresour. Technol.*, 2011, **102**, 9823–9826.
39. Z. Fu and M. T. Holtzapple, *Appl. Biochem. Biotechnol.*, 2010, **162**, 561–578.
40. Z. Fu and M. T. Holtzapple, *Appl. Microbiol. Biotechnol.*, 2011, **90**, 1669–79.
41. Q. Li and C.-Z. Liu, *Int J Hydrogen Energy*, 2012, **37**, 10648–10654.
42. X. Y. Cheng and C. Z. Liu, *Energy Fuels*, 2011, 1714–1720.
43. L. Xu and U. Tschirner, *Bioresour. Technol.*, 2011, **102**, 10065–10071.
44. Q. He, C. L. Hemme, H. Jiang, Z. He, and J. Zhou, *Bioresour. Technol.*, 2011, **102**, 9586–9592.
45. D. A. Argyros, S. A. Tripathi, T. F. Barrett, S. R. Rogers, L. F. Feinberg, D. G. Olson, J. M. Foden, B. B. Miller, L. R. Lynd, D. A. Hogsett, and N. C. Caiazza, *Appl. Environ. Microbiol.*, 2011, **77**, 8288–8294.
46. S. Yao and M. J. M. J. Mikkelsen, *Appl. Microbiol. Biotechnol.*, 2010, **88**, 1–10.
47. Q. He, P. M. Lokken, S. Chen, and J. Zhou, *Bioresour. Technol.*, 2009, **100**, 5955–5965.
48. N. K. Budhavaram and Z. Fan, *Bioresour. Technol.*, 2009, **100**, 5966–72.
49. A. K. Forrest, J. Hernandez, and M. T. Holtzapple, *Bioresour. Technol.*, 2010, **101**, 7510–5.
50. Y.-Q. Tang, P. Ji, J. Hayashi, Y. Koike, X.-L. Wu, and K. Kida, *Appl. Microbiol. Biotechnol.*, 2011, **91**, 1447–61.
51. Q. Zhang, J. He, M. Tian, Z. Mao, L. Tang, J. Zhang, and H. Zhang, *Bioresour. Technol.*, 2011, **102**, 8899–8906.
52. S. O-Thong, K. Boe, and I. Angelidaki, *Appl. Energy*, 2012, **93**, 648–654.
53. Y. Xia, L. Cai, T. Zhang, and H. H. P. Fang, *Int J Hydrogen Energy*, 2012, **37**, 13652–13659.
54. H. Uellendahl and B. K. Ahring, *Biotechnol. Bioeng.*, 2010, **107**, 59–64.
55. G. Kvesitadze, T. Sadunishvili, T. Dudauri, N. Zakariashvili, G. Partskhaladze, V. Ugrekhelidze, G. Tsiklauri, B. Metreveli, and M. Jobava, *Energy*, 2011, **37**, 94–102.
56. X.-Y. Cheng and C.-Z. Liu, *Bioresour. Technol.*, 2012, **104**, 373–379.
57. O. M. Pakarinen, H. P. Tähti, and J. a. Rintala, *Biomass Bioenergy*, 2009, **33**, 1419–1427.
58. P. Kongjan, S. O-Thong, and I. Angelidaki, *Bioresour. Technol.*, 2010, **102**, 4028–35.
59. P. Kaparaju, M. M. Serrano, A. B. A. B. Thomsen, P. Kongjan, and I. Angelidaki, *Bioresour. Technol.*, 2009, **100**, 2562–8.
60. M. O. S. Dias, T. L. Junqueira, O. Cavalett, M. P. Cunha, C. D. F. Jesus, C. E. V. Rossell, R. M. Filho, and A. Bonomi, *Bioresour. Technol.*, 2011, **103**, 161–152.
61. C. F. Crespo, M. Badshah, M. T. Alvarez, and B. Mattiasson, *Bioresour. Technol.*, 2012, **103**, 186–191.
62. T. I. Georgieva and B. K. Ahring, *Appl. Microbiol. Biotechnol.*, 2007, **77**, 61–8.
63. M. A. Sigurbjornsdottir and J. Orlygsson, *Appl. Energy*, 2011.
64. S. Ozmihci, F. Kargi, and A. Cakir, *Int J Hydrogen Energy*, 2011, **36**, 2111–2117.
65. H. Brynjarsdottir, B. Wawiernia, and J. Orlygsson, *Energy Fuels*, 2012, **26**, 4568–4574.
66. S. Prasad, A. Singh, and H. C. Joshi, *Resources, Conservation and Recycling*, 2007, **50**, 1–39.
67. P. McKendry, *Bioresour. Technol.*, 2002, **83**, 37–46.
68. F. Peng, J.-L. Ren, F. Xu, J. Bian, P. Peng, and R.-C. Sun, *J. Agric. Food Chem.*, 2009, **57**, 6305–6317.

69. N. K. Pessani, H. K. Atiyeh, M. R. Wilkins, D. D. Bellmer, and I. M. Banat, *Bioresour. Technol.*, 2011, **102**, 10618–10624.

70. S.-J. S. J. Yang, I. Kataeva, S. D. S. D. Hamilton-Brehm, N. L. N. L. Engle, T. J. T. J. Tschaplinski, C. Doeppke, M. Davis, J. Westpheling, and M. W. W. M. W. W. Adams, *Appl. Environ. Microbiol.*, 2009, **75**, 4762.

71. C.-W. Lin, C.-F. C.-H. Wu, D.-T. Tran, M.-C. Shih, and W.-H. Li, *Process Biochem.*, 2010, **46**, 489–493.

72. Y.-C. Lo, C.-Y. Huang, C.-L. Cheng, C.-Y. Lin, and J.-S. Chang, *Bioresour. Technol.*, 2011, **102**, 8384–8392.

73. L. Lianhua, L. Dong, S. Yongming, M. Longlong, Y. Zhenhong, and K. Xiaoying, *Int J Hydrogen Energy*, 2010, **35**, 7261–7266.