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Ethanol and Hydrogen Production with Thermophilic Bacteria from Sugars and Complex Biomass

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1. Introduction

The increase in carbon dioxide (CO_2) emissions has clearly much more profound effects on global climate than earlier anticipated. The main source of CO_2 is by combustion of fossil fuel but its concentration has increased from 355 ppm in 1990 to 391 ppm in 2011 (Mauna Loa Observatory: NOAA-ASRL, 2011). Production of biofuels from biomass has emerged as a realistic possibility to reduce fossil fuel use and scientists have increasingly searched for new economically feasible ways to produce biofuels. The term biofuel is defined as fuel produced from biomass that has been cultivated for a very short time; the opposite of fuel that is derived from fossil fuel biomass (Demirbas, 2009). Plants and autotrophic microorganisms fix gaseous CO_2 into volatile (sugars) and solid compounds (lignocellulose, starch) during growth. These compounds can thereafter be converted to biofuels which, by combustion, releases CO_2 back to atmosphere. This simplified way of carbon flow is not completely true, because growing, cultivating, harvesting and process conversion to biofuels will, in almost all cases, add more CO_2 to atmosphere although less as compared to fossil fuels.
There are several types of biofuels produced and used worldwide today. The most common are methane, ethanol (EtOH) and biodiesel but also, to a lesser extent, hydrogen (H_2), butanol and propanol. There are also several methods to produce biofuels, ranging from direct oil extraction from fat-rich plants or animal fat (biodiesel) to complex fermentations of various types of carbohydrate rich biomass (H_2, EtOH, butanol). Fermentation processes can be performed by both bacteria and yeasts. This overview mainly focuses on the production of EtOH and H_2 from biomass with thermophilic bacteria.

2. Production of EtOH and H_2 from biomass

EtOH as a vehicle fuel originated in 1908 when Henry Ford's famous car, Ford Model T was running on gasoline and EtOH or a combination of both (Gottemoeller & Gottemoeller, 2007). Biomass was however not used as a source for EtOH production until in the early thirties of the 20th century when Brazil started to extract sugar from sugarcane for EtOH production. During the World War II, EtOH production peaked at 77 million liters in Brazil (mixed to gasoline at 42%) (Nardon & Aten, 2008). After the war, cheap oil outcompeted the use of EtOH and it was not until the oil crisis in the mid 70's

that interest in EtOH rose again. The program "Pro-Alcool" was launched in 1975 to favour EtOH production from sugarcane. In US, there has been a steady increase in EtOH production from starch based plant material, e.g. corn, since the late 1970's (Nass et al., 2007). Perhaps the main reason for the increase in EtOH production is the discovery that methyl *tert*-butyl ether (MTBE), earlier used in gasoline as an additive, was contaminating groundwater, leading to search for alternative and more environmentally friendly source (Vedenov & Wetzsstein, 2008). Today, US and Brazil produce more than 65.3 billion liters of EtOH which corresponds for 89% of the world production (Renewable Fuel Association, 2010).

Production of EtOH from lignocellulose rich biomass has recently been focused upon. The main reason is the fact that EtOH production from starch and sugar based biomasses is in direct competition with food and feed production. This has been criticized extensively lately, because of the resulting rise in the prizes of food and feed products (Cha & Bae, 2011). Production of EtOH from sugars and starch is called first generation production, opposite to second generation production where lignocellulosic biomass is used. Lignocellulose is composed of complex biopolymers (lignin, cellulose and hemicelluloses) that are tightly bound together in plants. The composition of these polymers varies in different plants (cellulose, 36-61%; hemicellulose, 13-39%; lignin 6-29%) (Olsson & Hahn-Hagerdal, 1996). Of these polymers, only cellulose and hemicelluloses can be used for EtOH production. However, before fermentation, the polymers need to be separated by physiological, chemical or biological methods (Alvira et al., 2010). The most common method is to use chemical pretreatment, either weak acids or bases but many other methods are known and used today (see Alvira et al., 2010 and references therein). This extra pretreatment step has been one of the major factors for the fact that EtOH production from complex biomass has not been commercialized to any extent yet compared to first generation ethanol production. Also, after hydrolysis, expensive enzymes are needed to convert the polymers to monosugars which can only then be fermented to EtOH. Conventionally, most of the EtOH produced today is first generation EtOH but lately, especially after US launched their large scale investment programs (US Department of Energy, 2007), second generation of EtOH seems to becoming a reality within the next few years or decades.

The sugars available for fermentation after the pretreatment and hydrolysis of biomass (when needed) can be either homogenous like sucrose and glucose from sugarcane, and starch, respectively or heterogeneous when originating from lignocellulosic biomass. Thus, the main bulk of biomass used for EtOH production today are two types of sugars, the disaccharide sucrose and the monosugar glucose, both of whom can easily be fermented to EtOH by the traditional baker's yeast, *Saccharomyces cerevisae*. This microorganisms has many advantages over other known EtOH producing microorganisms. The most important are high EtOH yields (>1.9 mol EtOH/mol hexose), EtOH tolerance (> 12%), high robustness and high resistance to toxic inhibitors. However, the wild type yeast does not degrade any pentoses (Jeffries, 2006). The use of genetic engineering to express foreign genes associated with xylose and arabinose catabolism have been done with some success (van Maris et al., 2007) and a new industrial strain with xylose and arabinose genes was recently described (Sanchez et al., 2010). Also, no yeast has been reported to have cellulase or hemicellulase activity. The mesophilic bacterium *Zymomonas mobilis* is a highly efficient EtOH producer. The bacterium is homoethanolgenic, tolerates up to 12% EtOH and grows 2.5 times faster compared to yeasts (Rogers et al., 1982). The bacterium utilizes the Entner-

Doudoroff pathway with slightly higher EtOH yields than yeasts but lacks the pentose degrading enzymes. Many attempts have however been made to insert arabinose and xylose degrading genes in this bacterium (Deanda et al., 1996; Zhang et al., 1995). The company DuPont has recently started to use a genetically engineered *Z. mobilis* for cellulosic EtOH production (DuPont Danisko Cellulosic Ethanol LLC, 2011).

Especially, the lack of being able to utilize arabinose and xylose, both major components in the hemicellulosic fraction of lignocelluloses, has lead to increased interest in using other bacteria with broader substrate spectrum. Bacteria often possess this ability and are capable of degrading pentoses, hexoses, disaccharides and in some cases even polymers like cellulose, pectin and xylans (Lee et al., 1993; Rainey et al., 1994). The main drawback of using such bacteria is their lower EtOH tolerance and lower yields because of production of other fermentation end products like acetate, butyrate, lactate and alanine (Baskaran et al., 1995; Klapatch et al., 1994; Taylor et al. 2008). Additionally, most bacteria seem to tolerate much lower substrate concentrations although the use of fed batch or continuous culture may minimize that problem. On the opposite however, many bacteria show good EtOH production rates. The use of thermophilic microorganisms has especially gained increased interest recently. The main reasons are, as previously mentioned, high growth rates but also less contamination risk as well as using bacteria that can grow at temperatures where "self distillation" is possible, thus eliminating low EtOH tolerance and high substrate concentration problems. Also, the possibilty to use bacteria with the capacity to hydrolyze lignocellulosic biomass and ferment the resulting sugars to EtOH simultaneously is a promising method for EtOH production.

The production of H_2 is possible in several ways but today the main source of H_2 is from fossil fuels and, to a lesser extent, by electrolysis from water. H_2 is an interesting energy carrier and its combustion, opposite to carbon fuels, does not lead to emission of CO_2. Biological production of H_2 is possible through photosynthetic or fermentative processes (Levin et al., 2004; Rupprecht et al., 2006). This chapter will focus on biological H_2 production by dark fermentation by thermophilic bacteria only. Fermentative production of H_2 has been known for a long time and has the advantage over photosynthetic processes of simple operation and high production rates (Chong et al., 2009). Also, many types of organic material, e.g. wastes, can be used as substrates. Thus, its production possesses the use of waste for the production of renewable energy. Fermentative hydrogen production has though not been commercialized yet but several pilot scale plants have been started (Lee & Chung, 2010; Lin et al., 2010).

3. Physiology of thermophilic EtOH and H_2 producing bacteria

Thermophilic bacteria can degrade many carbohydrates and produce various end products, among them both EtOH and H_2. Figure 1 shows the carbon flow from glucose by fermentation by the use of Embden-Meyerhof pathway (EMP). The majority of microorganisms degrade hexoses through this pathway or the Entner-Douderoff pathway (ED). The degradation of glucose with EMP generates two NADH, two pyruvates, the key intermediate in most organisms, together with the formation of two ATP by substrate level phosphorylation. The ED pathway, however, is more restricted to Gram-negative bacteria and Archaea and generates only one mol of ATP, which explains its low distribution among anaerobic bacteria. Some bacteria, especially hyperthermophiles, are known to be able to use both pathways simultaneously (Moat et al., 2002; Siebers & Schönheit, 2005).

There are also some variations of the classical EMP among thermophilic microorganisms. Some archaea e.g. *Pyrococcus* and *Thermococcus* use ADP instead of ATP to transfer phosphate groups to hexoses in the preparation steps of the glycolysis. These bacteria also use ferredoxin-dependent glyceraldehyde-3-phosphate ferredoxin oxidoreductase (GAPOR) for converting glyceraldehyde-3-phosphate to 3-phosphoglycerate in one step (Chou et al., 2008). Thermophilic bacteria, however, use the common glyceraldehydes-3-phosphate dehydrogenase (GAPDH) and reduce glyceraldehydes-3-phosphate to 1,3-glycerate which is thereafter converted to 3-phosphoglycerate. Thus, both groups produce two molecules of ATP by substrate level phosphorylation but the archaea "sacrifice" one and use it to together with two molecules of AMP to produce two molecules of ADP, needed for hexose phosphorylation. Consequently, the amount of energy conserved in glucose to acetate conversion is 3.2 instead of the expected 4.0 ATP/glucose (Sapra et al., 2003).

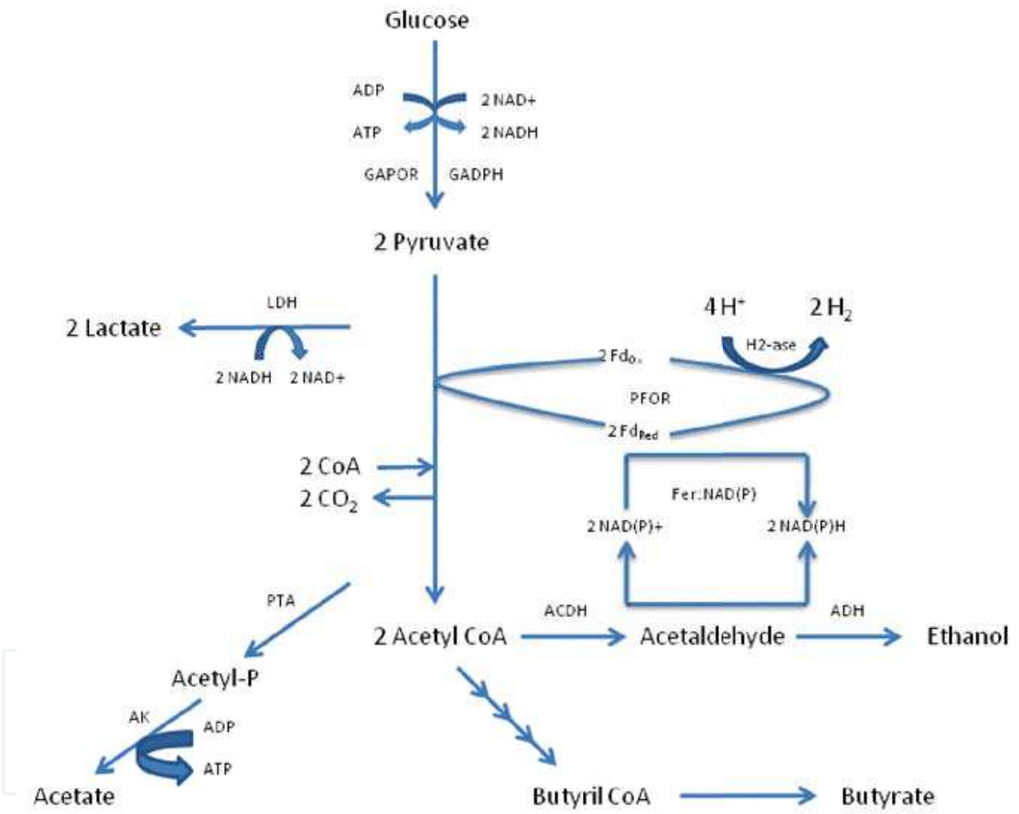

Fig. 1. Simplified scheme of glucose degradation to various end products by strict anaerobic bacteria. Enzyme abbreviations: ACDH, acetaldehyde dehydrogenase; ADH, alcohol dehydrogenase; AK, acetate kinase; Fer:NAD(P), ferredoxin:NAD(P) oxidoreductase; H_2-ase, hydrogenase; LDH, lactate dehydrogenase; PFOR, pyruvate:ferredoxin oxidoreductase; PTA, phosphotransacetylase.

Pyruvate is the end product of glycolysis and can be converted to fermentation products like H_2, EtOH and many more (Fig. 1). The carbon flow depends on the microorganisms involved and the environmental conditions. Pyruvate can e.g. be reduced to lactate by lactate dehydrogenase (LDH) but the most favorable pathway for anaerobic bacteria is to

oxidize pyruvate to acetyl-CoA and CO_2 by using pyruvate:ferredoxin oxidoreductase (PFOR) which can be converted to acetate with concomitant ATP synthesis from the acetyl-phosphate intermediate. Acetate is thus the oxidized product but the main advantage for the microorganism is the extra ATP produced. The electrons are transported to reduced ferredoxin which acts as an electron donor for hydrogenases and H_2 is produced as the reduced product. There are mainly two types of hydrogenases; NiFe hydrogenases and the FeFe hydrogenases. Recent overview articles have been published on the subject (Chou et al., 2008; Kengen et al., 2009). Acetyl Coenzyme A can also be converted to acetaldehyde by acetaldehyde dehydrogenase (ACDH) and further to EtOH by alcohol dehydrogenase.

Strict anaerobes can produce H_2 from two major breakpoints during degradation of glucose. Firstly, from a NAD(P)H by GAPDH and from pyruvate ferredoxin oxidoreductase (PFOR) (Jones, 2008). The principal H_2 pathway is through PFOR because of thermodynamics hindrance of reoxidizing NADH (Jones, 2008). It is a well known phenomenon that the low H_2 yields observed by mesophilic and moderate thermophilic bacteria are due to the fact that H_2 production from either ferredoxin or NAD(P)H are thermodynamically unfavorable (Jones, 2008; Hallenbeck, 2009). The redox potential of Fd_{red}/Fe_{ox} couple depends on the microorganism and temperature involved. In nature, high partial pressures of H_2 are relatively uncommon because of the activity of H_2 scavenging microbes, e.g. methanogens or sulfate reducing bacteria (Cord-Ruwisch et al., 1988). This results in a low partial pressure of H_2 which is favorable for a complete oxidation of glucose to acetate and CO_2. At high temperatures, the influence of the partial pressure of H_2 is less on the key enzymes responsible for H_2 production. This is the main reason why extremophilic bacteria have been reported to produce up to 4 moles of H_2 together with 2 moles of acetate in pure cultures and also for the fact that microorganisms growing at lower temperatures direct their end product formation to other reduced products. At lower temperatures, the NADH ferrodoxin oxidoreductase (NOR) that converts NADH to Fd_{red} is strongly inhibited. The E° is – 400 mV for Fd_{red}/Fd_{ox} couple but -320 mV for the $NADH/NAD^+$ couple (Jones, 2008; Hallenbeck, 2009). Therefore, at low temperatures, elevated H_2 concentrations inhibit H_2 evolution at much lower concentrations as compared to extreme temperatures. Mesophilic and moderate thermophilic bacteria respond to this by directing their reducing equivalents to other more favorable electron acceptors and consequently produce reduced products like EtOH, lactate, butyrate and alanine (Fig. 1).

Following are the main stoichiometry equations for the degradation of glucose to various end products by microorganisms with special focus on H_2 and EtOH production.

The amount of H_2 produced depends on the fermentation pathways used and end product formation. For example, if acetic acid is the final product the theoretical yield for one mole of glucose is four moles of H_2:

$$C_6H_{12}O_6 + 4\,H_2O \rightarrow 2CH_3COO^- + 4H_2 + 2HCO_3^- + 4H^+ \quad (1)$$

If on the other hand the final product is butyric acid, the theoretical yield of H_2 is only two moles of H_2 per mole of glucose:

$$C_6H_{12}O_6 + 2\,H_2O \rightarrow CH_3CH_2CH_2COO^- + 2H_2 + 2HCO_3^- + 3H^+ \quad (2)$$

The production of EtOH by *Saccharomyces cerevisae* and *Zymomonas mobilis* occurs according to:

$$C_6H_{12}O_6 + 4\ H_2O \rightarrow 2CH_3COH^- + 2HCO_3^- + 4H^+ \quad (3)$$

Bacteria however, usually produce a mixture of EtOH together with other end products. This results in lower EtOH yields and, in some cases, production of H_2. If lactate is the only end product, no H_2 is formed:

$$C_6H_{12}O_6 + 4\ H_2O \rightarrow \text{Lactate}^- + 2HCO_3^- + 4H^+ \quad (4)$$

4. Thermophilic anaerobic bacteria – classification and physiology

In recent years, thermophilic anaerobic bacteria have gained increased attention as potential EtOH and H_2 producing microorganisms. Depending on optimal growth temperatures, thermophilic bacteria can be divided into several categories, e.g. moderate thermophiles (T_{opt} between en 45 to 55°C), true thermophiles (T_{opt} between 55 to 75°C) and extremophiles with optimum temperature above 75°C (Brock, 1986). The ability of thermophiles to live at high temperatures is mainly due to their thermostable proteins; the cell membrane of thermophilic bacteria contains more saturated fatty acids which make it stiffer and more heat resistant as compared to mesophiles (Brock, 1986).

Thermophilic bacteria are capable of adapting to environmental conditions and are able to thrive in geothermal areas although the temperature might be slightly higher than the optimum growth temperature. Geothermal areas offer stability in heat and are thus favorable habitats for thermophilic bacteria (Brock, 1986; Kristjansson & Alfredsson, 1986). Generally, most known thermophilic species are obligate or facultative anaerobes since geothermal areas have low oxygen concentrations (Amend & Shock, 2001). Less variety seems to be of strict anaerobic, heterotrophic thermophilic bacteria (see review of Wagner & Wiegel, 2008 and references therein).

4.1 Thermophilic EtOH and H_2 producing bacteria

There are relatively few genera of thermophiles that include bacteria with good H_2 and EtOH producing capacities. Among good EtOH producers are bacteria that belong to the genera of *Clostridium, Thermoanaerobacter* and *Thermoanaerobacterium* but good H_2 producers are the extremophiles like *Caldicellulosiruptor* and *Thermotoga* and the archaeon *Thermococcus* and *Pyrococcus*. It varies to a great extent how much data is available in literature concerning pure culture studies of individual species on biofuel production. Much data is not on the efficiency of these bacteria to produce H_2 and EtOH but merely on phylogenetic status and basic physiological properties. Also, the data on biofuel production properties from these bacteria on hydrolysates from lignocellulosic biomass is scarce but more is known on yields from monosugars. Below, the discussion will be on the major phylogenetic and physiological characteristics of most of the "good" EtOH and H_2 producing thermophiles known today. Later chapters deal with H_2 and EtOH production rates and yields from both sugars and from complex lignocellulosic biomasses by these bacteria and more.

4.1.1 *Clostridium*

The genus *Clostridium* belongs to the family Clostridiaceae, order Clostridiales, class Clostridia and phylum Firmicutes. These bacteria are spore forming and often present in environments which are rich in plant decaying material. It is thus not surprising that many species are capable of polymer hydrolyzation and this is one of the main reasons for

extensive research on biofuel production from complex biomass by these bacteria (Canganella & Wiegel, 1993; Carreira & Ljungdahl, 1993). Several cellulose-degrading enzymes form a structure called cellulosome, located and embedded on the external surface of the cell membrane (Demain et al., 2005). The genus contains a very diverse group of bacteria as shown by a phylogenetic analysis of Collins and co-workers where *Clostridium* species were compared both within species belonging to the genus and to related taxa (Collins, et al., 1994). This investigation and others lead to the conclusion that more than half of the species currently assigned to the genus *Clostridium* are in fact not closely related to the type species *C. butyricum* and should therefore not be included in the newly defined genus *Clostridium*. The genus contains more than 200 validly described species but only about 15 are thermophilic. Two of those thermophilic Clostridia, *C. thermocellum* and *C. thermohydrosulfuricum* (now *Thermoanaerobacter thermohydrosulfuricum*) have attracted the most attention and the cellulosome of *C. thermocellum* has been characterized extensively (Demain et al., 2005). Among other well known thermophilic Clostridia are *C. thermobutyricum* (Wiegel et al., 1989), *C. thermosucciongenes* (Drent et al., 1991) and *C. clariflavum* (Shiratori et al., 2009) and several others.

4.1.2 *Thermoanaerobacterium*

Thermoanaerobacterium together with genus *Thermoanaerobacter* falls within clusters V, VI and VII in phylogenetic interrelationships of *Clostridium* species (Collins et al., 1994). The genus was first described in 1993 when two thermophilic, xylan degrading strains were isolated from Frying Pan Springs in Yellowstone National Park (Lee et al., 1993). They were compared with other xylan degrading bacteria and new taxonomic assignments were proposed thereafter. Today the genus consists of nine validly described species; *T. acidítolerans*, *T. aotearoense*, *T. saccharolyticum*, *T. thermosaccharolyticum*, *T. thermosulfurigenes*, *T. xylanolyticum, T. fijiensis, T. polysaccharolyticum and T. zeae* (German Collection of Microorganisms and Cell Cultures and references therein). Most *Thermoanaerobacterium* species have been isolated from hot springs or leachate of waste from canning factories. *Thermoanaerobacterium* species are known for their abilities to convert carbohydrates to various end products like acetate, EtOH, lactate, H_2 and CO_2. Some species have shown promising EtOH and H_2 production capacity but production of mixed end products limit their use (Ren et al., 2008; 2009; 2010; Romano et al., 2010; Sveinsdottir et al., 2010). *T. saccharolyticum* has however been genetically engineered and both acetate and lactate formation has been knocked out (Shaw et al., 2008). According to the description, members of this genus reduce thiosulfate to elemental sulfur while members of *Thermoanaerobacter* reduce thiosulfate to H_2S (Lee et al., 1993).

4.1.3 *Thermoanaerobacter*

Bacteria within this genus were originally classified within the genus *Clostridium* because of close phylogenetic relationship and physiological properties. These bacteria use the classical EMP pathway for sugar degradation and produce EtOH, acetate and lactate as major end products (Lee et al., 1993). Most species have broad substrate range and can degrade both pentoses and hexoses. The genus consists of 24 species (subspecies included) originating from various environments like hot springs and oil fields (Collins et al., 1994; Larsen et al., 1997; Lee et al., 1993; German Collection of Microorganisms and Cell Cultures and references therein). Most species produce EtOH and H_2 as well as lactate, and in some cases alanine as end products. The type species, *Thermoanerobacter ethanolicus* and several other

species within the genus has been extensively studied for EtOH production (Fardeau et al., 1996; Georgieva & Ahring, 2007; Georgieva et al., 2008a. b; Lacis & Laword 1988a,b; Lamed & Zeikus, 1980a,b). H_2 production is usually low compared to EtOH by *Thermoanaerobacter* although *Thermoanaerobacter tengcongensis* has been described to produce up to 4 moles of H_2 from one mole of glucose under nitrogen flushed fermentor systems (Soboh et al., 2004).

4.1.4 *Caldicellulosiruptor*

The genus *Caldicellulosiruptor* was first proposed in 1994 by Rainey and co-workers on the basis of physiological characteristics and phylogenetic position of a strain they isolated, *Caldicellulosiruptor saccharolyticus* (Tp8T 6331) (Rainey et al., 1995). Today the genus holds nine different species; *C. acetigenus*, *C. bescii*, *C. hydrothermalis*, *C. kristjanssonii*, *C. kronotskyensis*, *C. lactoaceticus*, *C. obsidiansis*, *C. owensensis* and *C. saccharolyticus* (German Collection of Microorganisms and Cell Cultures and referenses therein). All species are extremely thermophilic, cellulolytic, non-spore-forming anaerobes that have been isolated from geothermal environments such as hot springs and lake sediments (Rainey et al., 1994; Yang et al., 2010). *Caldicellulosiruptor* species have a relatively broad substrate spectrum capable to utilize e.g. cellulose, cellobiose, xylan and xylose. Extreme thermophiles, have been shown to have superior H_2 production yields and rates compared to mesophiles and produce few other byproduct besides acetate. This makes *Caldicellulosiruptor* species excellent candidates for H_2 production. *C. saccharolyticus* and *C. owensis* have been extensively studied for H_2 production from sugar and hydrolysates from lignocellulosic biomass (Kadar et al., 2004; Vrije et al., 2007; Zeidan & van Niel, 2010).

4.1.5 *Thermotoga*

The genus of *Thermotoga* was first described in 1986 when a unique extremely thermophilic bacteria was isolated from geothermally heated sea floors in Italy and the Azores (Huber et al., 1986). Today, nine different species have been identified; *T. elfii*, *T. hyphogea*, *T. lettingae*, *T. maritima* (type species), *T. naphthophila*, *T. neapolitana*, *T. petrophila*, *T. subterranean* and *T. thermarum* (German Collection of Microorganisms and Cell Cultures and references therein). These species are extremophiles, growing at temperatures that are highest reported for bacteria. All are strictly anaerobic and the cells are rod-shaped with an outer sheethlike structure called toga. (Huber et al., 1986; Jannasch et al., 1988). Most species have been isolated from deep environments, high temperature and pressure environments like oil reservoirs, often rich of sulfur-compounds. Most of them are thus able to reduce either elemental sulfur, thiosulfate or both. Members of *Thermotoga* ferment sugars to mainly acetate, CO_2 and H_2 like *Caldicellulosiruptor* species. Only three species have been reported producing traces of EtOH. Most strains have shown the property of reducing pyruvate to alanine from sugar fermentation and *T. lettingae* produces alanine from methanol (in the presence of elemental sulfur or thiosulfate) (Balk et al., 2002). Other special feature within the genus is the ability of *T. lettingae* to degrade xylan at 90°C and its property of methanol metabolism (Balk et al., 2002). Hydrogen production has been extensively studied for *T. elfi*, *T. maritima* and *T. neapolitana* (d'Ippolito et al., 2010; Nguyen et al., 2008a,b; van Niel et al., 2002).

4.1.6 Other thermophilic bacteria producing H_2 and EtOH

Apart from the above mentioned genera the capacity to produce EtOH and H_2 has been reported for many other genera. Examples are species within *Caloramator, Caldanaerobacter,*

Caldanerobius and the archaeon *Thermococcus* and *Pyrococcus*. Some species within these genera will be discussed in later chapters.

5. Production of EtOH by thermophilic bacteria

The interest in EtOH production by thermophilic bacteria originates shortly after the oil crisis in the mid 70's of the twentieth century. Earliest reports on EtOH production from sugars include work on *Thermoanaerobacter brockii* and *Clostridium thermocellum* (Ben Bassat et al., 1981; Lamed et al., 1980; Lamed & Zeikus, 1980a, 1980b) but later on other *Thermoanaerobacter* species, e.g. *T. finnii,* (Faredau et al., 1996), *T. thermohydrosulfuricus* (Lovitt et al., 1984; Lovitt et al., 1988), *T. mathrani* (Larsen et al., 1997) and *Thermoanaerobacterium* species (Koskinen et al., 2008a; Sveinsdottir et al., 2009; Zhao et al., 2009, 2010). It was however not until recently that the use of thermophilic bacteria for EtOH production from lignocellulosic biomass arises. The earliest reports on EtOH production of more complex nature are from 1981 on starch (Ben Bassat et al., 1981) and 1988 on avicel (Lamed et al., 1988). The first study on lignocellulosic biomass (hemicellulose fraction of birch- and beechwood) was in 1983 by *Thermoanaerobacter ethanolicus* and several other thermophilic bacteria (Wiegel et al., 1983). Following chapters are divided into two main subchapters; 1) studies of EtOH production from sugars both in batch and continuous cultures with either pure or cocultures of thermophilic bacteria and 2) studies of EtOH production from lignocellulosic biomass by mixed or pure cultures of thermophilic bacteria.

5.1 Production of EtOH from sugars

Although it has been known for a long time that thermophilic bacteria produce EtOH from various carbohydrates it was not until 1980 the first papers appeared in literature with the focus on EtOH production. Earlier investigations include work on *Thermoanaerobacter brockii, Thermoanaerobacter thermohydrosulfuricus* and *Clostridium thermocellum* (Ben Bassat et al., 1981; Lamed & Zeikus, 1980a; 1980b; Lovitt et al., 1984). Ethanol yields by *T. brockii* were only moderate or between 0.38 (Lamed & Zeikus, 1980b) to 0.44 mol EtOH mol glucose^{-1} equivalents (Ben Bassat et al., 1981). In the latter investigation the focus was mostly on the effects of additional acetone and H_2 on end product formation. Much higher yields were later observed by *Thermoanaerobacter thermohydrosulfuricus*, or 0.9 to 1.9 mol EtOH mol glucose^{-1}. (Lovitt et al., 1984; 1988), also with the main focus on the effect of solvents on EtOH production, e.g. EtOH tolerance. *Thermoanerobacter ethanolicus* was described in 1981 (Wiegel & Ljungdahl., 1981) showing extremely good yields of ethanol from glucose (1.9 mol EtOH mol glucose^{-1}). Later this strain has been extensively studied by Lacis and Lawford (Lacis and Lawford 1988a, 1988b, 1989, 1991). Early observation was on high EtOH yields on xylose at low substrate (4.0 g L^{-1}) concentrations. The yields were 1.30 and 1.37 mol EtOH mol xylose^{-1} in batch and continuous cultures, respectively (Lacis & Lawford, 1988a) but only at low substrate concentrations. At higher concentrations (27.5 g L^{-1}) the yields lowered to 0.6 mol EtOH mol xylose^{-1}. Further studies by using xylose limiting continuous cultures, indicated that EtOH yields were more dependent on length of cultivation than upon growth rate and higher yields were presented (1.43 mol mol xylose^{-1}) (Lacis & Lawford, 1988b, 1989). Later data from this strain on glucose showed lower EtOH yields and the direction of the carbon flow was towards lactate formation by increasing substrate concentrations (Lacis & Lawford, 1991). *Thermoanerobacter ethanolicus* JW200 showed also very good EtOH yields from xylose and glucose at low (10 g L^{-1}) substrate concentrations, or

1.45 and 1.95 mol, respectively (Carreira et al., 1982). A mutant strain was later developed (JW200Fe(4)) that showed similar yields but at higher (30 g L^{-1}) substrate concentrations (Carreira et al., 1983). Other investigations on this species on sucrose showed between 1.76 to 3.60 mol EtOH mol $sucrose^{-1}$ with high substrate concentrations (15 to 30 g L^{-1}) (Avci et al., 2006). Recent study on *Thermoanerobacter ethanolicus* strain interestingly shows that the addition of external acetate increases EtOH yields from xylose, glucose and cellobiose (He et al., 2010). EtOH yields on xylose were 1.0 mol EtOH mol $glucose^{-1}$ without any acetate added but increased to 1.17 by adding 150 mM of acetate. Similar increase was observed on glucose, or from 1.16 to 1.34 mol EtOH mol $glucose^{-1}$ without and with added acetate, respectively. It has been suggested that acetate may disrupt energy production through accelerated fermentation (Russel, 1992) which may lead to lower biomass production and higher end product formation. Fardeau et al. (1996) investigated the effect of thiosulfate as electron acceptor on sugar degradation and end product formation by *Thermoanaerobacter finnii*. This strain shows good EtOH yields on xylose or 1.76 mol EtOH mol $xylose^{-1}$ which is actually higher than the theoretical yield (1.67) from this sugar. Yields on glucose were however lower or, 1.45 mol EtOH mol $glucose^{-1}$. Not surprisingly, the addition of thiosulfate shifted end product formation towards acetate with higher cell yield and lower EtOH production. A study of bacteria isolated from Icelandic hot spring shows that a *Thermoanerobacter* sp. AK33 showed good EtOH yields on monosugars (Sveinsdottir et al., 2009). Glucose and xylose fermentations resulted in 1.5 and 0.8 mol EtOH from one mole of glucose and xylose, respectively. *Thermoanaerobacterium* AK17, isolated from Icelandic hot spring, has been extensively studied for EtOH production (Koskinen et al., 2008a; Orlygsson & Baldursson, 2007; Sveinsdottir et al., 2009). This strain produces 1.5 and 1.1 mol EtOH from one mole of glucose and xylose, respectively. A moderate thermophile, *Paenibacillus* sp. AK25 has also been shown to produce 1.5 mol EtOH mol $glucose^{-1}$ (Sveinsdottir et al., 2009).

One of the main drawbacks for the use of thermophilic bacteria for EtOH production from biomass is their low tolerance towards EtOH. Several studies have been done with *Clostridium thermosaccharolyticum* (Baskaran et al., 1995; Klapatch et al., 1994) and *Thermoanaerobacter* sp. (Georgieva et al., 2008b) to increase EtOH tolerance. The highest EtOH tolerance is by a mutant strain of *Thermoanaerobacter ethanolicus*, or 9% (wt/vol) at 69°C (Carriera & Ljungdahl, 1983) but later studies with JW200 Fe(4), one of its derivatives, show much less tolerance (Hild et al., 2003). Georgieva and co-workers published very high EtOH tolerance (8.3%) for *Thermoanerobacter* BG1L1, a highly efficient xylose degrader in continuous culture studies (Georgieva et al., 2008b). *Thermoanerobacter thermohydrosulfuricus* degrades various pentoses and hexoses as well as starch to high concentrations of EtOH (Ng et al., 1981). By transferring the parent strain (39E) to successively higher concentrations of EtOH, an alcohol tolerant strain (39EA) was obtained (Lovitt et al., 1984). The mutant strain grows at 8% EtOH concentrations (wt/vol) at 45°C but only up to 3.3% at 68°C. The parent strain produces 1.5 mol EtOH mol $glucose^{-1}$ without any addition of EtOH but the yield lowered to 0.6 mol at 1.5% initial EtOH concentrations. The mutant strain showed lower EtOH yields without any addition of EtOH, or 0.9 mol EtOH mol $glucose^{-1}$ but the yields did not decrease to any extent by increasing initial EtOH concentrations up to 4%. Further experiments with the wild type also indicated the role of H_2 production and its influence on EtOH production (Lovitt et al., 1988). Thus, by changing the gas phase from nitrogen to H_2 or carbon monoxide, EtOH yields increased from 1.41 mol EtOH mol $glucose^{-1}$ to 1.60 and 1.90 mol, respectively.

Organisms	Sugar	Cultivation method	Sugar conc. (gL^{-1})	Ethanol yield (mol EtOH mol $sugar^{-1}$)	Temp. (°C)	Reference
T. brockii	Cellobiose	Batch	10.0	0.38	60	Lamed & Zeikus (1980)
T. brockii	Glucose	Batch	5.0	0.44	nd	Ben Bassat et al. (1981)
T. ethanolicus	Glucose	Batch	8.0	1.90	72	Wiegel & Ljungdahl. (1981)
T. ethanolicus	Glucose	Batch	20.0	1.90	68	Carreira et al. (1983)
T. thermohydrosulfuricus	Glucose	Batch	5.0	1.60	60	Lovitt et al. (1984)
T. thermohydrosulfuricus	Glucose	Batch	5.0	0.90	60	Lovitt et al. (1984)
T. thermohydrosulfuricus	Glucose	Batch	10.0	1.40-1.90	60	Lovitt et al. (1988)
T. ethanolicus	Xylose	Batch	4.0-27.5	0.60-1.30	60	Lacis & Lawford (1988a)
T. ethanolicus	Xylose	Con	4.0	1.37	60	Lacis & Lawford (1988a)
T. ethanolicus	Xylose	Con	4.0	1.43	60	Lacis & Lawford (1988b)
T. ethanolicus	Xylose	Con	4.0	1.37	68	Lacis & Lawford (1989)
T. ethanolicus	Xylose	Con	4.0	1.37	67-69	Lacis & Lawford (1991)
T. ethanolicus	Xylose	Con	20.0	1.06	67-69	Lacis & Lawford (1991)
T. finnii	Glucose	Batch	NA	1.45	60	Fardeau et al. (1996)
T. finnii	Xylose	Batch	NA	1.76	60	Fardeau et al. (1996)
C. thermocellum	Cellobiose	Batch	2.6	1.60	60	Knutson et al. (1999)
T. ethanolicus	Xylose	Con	4.0	1.30	69	Hild et al. (2003)
T. ethanolicus	Sucrose	Batch	15-30	1.80-3.60	65	Avci et al. (2006)
T. thermohydrosulfuricus	Sucrose	Batch	15-30	1.10 - 3.00	65	Avci et al. (2006)
Thermoanaerobacter ap 65-2	Sucrose	Batch	15-30	1.30-3.20	65	Avci et al. (2006)
Thermoanaerobacter BG1L1	Xylose	Con	10.0	1.28	70	Georgieva at al. (2008)
Enrichment cultures	Glucose	Batch	18.0	0.10-1.70	50-78	Koskinen et al. (2008)
Coculture	Glucose	Con	12.6-25.2	1.37	60	Koskinen et al. (2008a)
Thermoanaerobacterium AK17	Glucose	Batch	3.6	1.50	60	Sveinsdottir et al. (2009)
Thermoanaerobacterium AK17	Xylose	Batch	3.0	1.10	60	Sveinsdottir et al. (2009)
Thermoanaerobacter Ak33	Glucose	Batch	3.6	1.50	70	Sveinsdottir et al. (2009)
Thermoanaerobacter Ak33	Xylose	Batch	3.0	0.80	70	Sveinsdottir et al. (2009)
Paenibacillus AK25	Glucose	Batch	3.6	1.50	50	Sveinsdottir et al. (2009)
Paenibacillus AK25	Xylose	Batch	3.0	0.90	50	Sveinsdottir et al. (2009)
Mixed culture	Glucose	Batch	5.0	1.53	70	Zhao et al. (2009)
Mixed culture	Xylose	Batch	2.0	1.60	70	Zhao et al. (2010)
Enrichment cultures	Glucose	Batch	9.0	1.34	50-75	Orlygsson et al. (2010)
Enrichment cultures	Xylose	Batch	7.5	1.30	50-75	Orlygsson et al. (2010)
T. ethanolicus	Xylose	Batch	5.0	1.00-1.20	65	He et al. (2010)
T. ethanolicus	Glucose	Batch	5.0	1.20-1.30	65	He et al. (2010)

Table 1. EtOH production from sugars by defined and mixed cultures of thermophilic bacteria. Cultivation was either in batch or continuous (con). EtOH yields as well as substrate concentrations and incubation temperature are also shown.

Recent studies with mixed cultures (batch) were conducted on glucose (Zhao et al., (2009) and xylose (Zhao et al., 2010) where various environmental parameters were optimized for both EtOH and H_2 production. The main bacterial flora, originating from biohydrogen reactor operated at 70°C and fed with xylose and synthetic medium, was identified as various species of *Thermoanaerobacter, Thermoanaerobacterium* and *Caldanaerobacter*. Highest yields observed to be 1.53 and 1.60 mol EtOH mol $glucose^{-1}$ and $xylose^{-1}$ respectively.
Several efforts have recently been made to enrich for new ethanologenic thermoanerobes. Two surveys have been done from Icelandic hot springs where several interesting bacteria were isolated with EtOH yields of > 1.0 mol EtOH from one mol glucose and xylose (Koskinen et al., 2008; Orlygsson et al., 2010).

5.2 Production of EtOH from complex biomass

Production of EtOH from lignocellulosic biomass has gained increased interest in recent years. The type of biomass used has varied to a great extent, e.g. wheat straw, barley straw, hemp, grass, paper and more. Also, the type of pretreatment used is different from one experiment to another. Most data is on biomass pretreated with dilute sulfuric acid or with alkaline pretreatment. The concentration of hydrolysates made from the biomass is also very broad, mostly varying from 0.2 % (w/v) to 15% (w/v). Finally, either pure or mixed cultures are used and either batch or continuous mode. The maximum yield of EtOH from glucose fermentation is 0.51 g EtOH g glucose^{-1}. This corresponds to 2 mol EtOH/mol hexose or 11.1 mM g^{-1}. Considering the complex structure of lignocellulosic biomass, it is not surprising that EtOH yields are usually considerable lower from such substrates (Table 2). Earliest available data on thermophilic bacteria using polymeric biomass originates from studies on *Thermoanaerobacter ethanolicus* and *Clostridium thermocellum* on hemicellulose from birch- and beechwood (Wiegel et al., 1983). These early reports showed promising results but highest yields were observed from the mutant strain *T. ethanolicus*, 4.5 mM g^{-1} xylose equivalent used. Three strains of *Clostridium thermocellum* produced between 1.40 to 2.60 mM EtOH g avicel^{-1} (Lamed et al., 1988). Higher yields (5.0 mM g^{-1} and 5.5 mM g^{-1}) by this bacterium were shown on the same substrate by others (Ahn et al., 1996; Lynd et al., 1989). Rani and co-workers studied EtOH production from both cellulose and lignocellulosic biomass by *C. thermocellum* (Rani et al., 1998). EtOH yields on avicel and Whatman paper was up to 7.2 and 8.0 mM g^{-1} EtOH, respectively. Similar yields were obtained from paddy straw, sorghum stover and corn stubs, pretreated with alkali. The highest yields of EtOH production from cellulosic biomass by *C. thermocellum* are from filter paper, 8.2 mM g^{-1} substrate (Balusu et al., 2004; 2005). In all studies mentioned above with *C. thermocellum* the concentration of cellulose was below 8.0 g L^{-1}. Lin and co-workers recently investigated degradation of napier grass and cellulose (avicel) by *C. thermocellum* and a mixed enrichment culture (Lin et al., 2010). They used from 2.0 to 40.0 g L^{-1} substrate concentrations. The pure culture produced merely 0.72 mM g^{-1} avicel but up to 3.87 mM g^{-1} Napier grass. The mixed culture produced between 0.7-0.9 mM g^{-1} Napier grass and 0.4–5.7 mM g^{-1} avicel. A dramatic decrease in yields was observed by increasing substrate concentrations.

Ahring and co-workers (Ahring et al., 1996) investigated the potential of five thermoanaerobes for EtOH production from the hemicelluloses fraction of wheat straw hydrolysates. Three of the strains produced only minor amounts of EtOH from xylan but *Thermoanaerobacterium saccharolyticum* HG8 and strain A3 produced 6.30 and 5.43 mM g xylan^{-1}, respectively. Strain A3 was further investigated on hydrolysates made from wheat straw, pretreated with wet oxidation. EtOH yields were lower as compared to xylan, or 2.61 mM g wheat straw^{-1} pretreated without oxygen.

Thermoanerobacter mathranii was isolated in 1993 from Hveragerdi in Iceland (Larsen et al., 1997) and has been adapted by Ahring et al., (1996). The strain has been investigated for EtOH production capacity on wet oxidized wheat straw (Ahring et al., 1999). By using very high substrate concentrations (60 g L^{-1}) and wet oxidation with different amounts of sodium carbonate the amount of total sugars released varied from 3.5 to 9.9 g L^{-1}. A fermentation of the strain on undiluted hydrolysate by the strain resulted in the production of approximately 9 mM of EtOH, or 1.3 mM g sugar^{-1}. This strain was also investigated for the effects of inhibitory compounds and hydrolysate concentration on the fermentation of wheat straw hydrolysates (Klinke et al., 2001). The main outcome was that the addition of

hydrolysate to a medium containing 4 g L xylose^{-1} did not inhibit EtOH production and it produced 5.5 mM g xylose^{-1}. Increased concentrations of aromatic compounds and hydrolysates however, severely inhibited EtOH production by the strain. Wheat straw hydrolysates have also been investigated by other thermophilic bacteria (Sommer et al., 2004) but with lower EtOH yields.

Organisms	Biomass	Cultivation method	Substr. conc. (gL^{-1})	Ethanol yield (mM g sugar^{-1})	Temp. (°C)	Reference
T. ethanolicus	Wood hydrolysate	Batch	8.0	3.30-4.50	70	Wiegel et al. (1983)
C. thermocellum (3 strains)	Avicel	Batch	20.0	1.40-2.60	60	Lamed et al. (1988)
C. thermocellum	Avicel	Batch	2.5	5.00	60	Lynd et al. (1989)
C. thermocellum	Wood hydrolysate	Batch	4.8	3.10	60	Lynd et al. (1989)
C. thermocellum	Avicel	Con	5.0	5.48	60	Ahn et al. (1996)
C. thermocellum	Avicel	Batch	5.0	3.66	60	Ahn et al. (1996)
C. thermocellum	Whatman paper	Batch	8.0	7.20-8.00	60	Rani et al. (1997)
C. thermocellum	Avicel	Batch	8.0	6.50-7.20	60	Rani et al. (1997)
C. thermocellum	Paddy straw	Batch	8.0	6.10-8.00	60	Rani et al. (1997)
C. thermocellum	Sorghum stover	Batch	8.0	4.80-8.10	60	Rani et al. (1997)
C. thermocellum	Corn stubs	Batch	8.0	4.60-7.80	60	Rani et al. (1997)
Thermophilic strain A3	Xylan	Batch	10.0	5.43	70	Ahring et al. (1996)
T. saccharolyticum	Xylan	Batch	10.0	6.30	60	Ahring et al. (1996)
Thermophilic strain A3	Wheat straw	Batch	60.0 (10.0)*	2.61	70	Ahring et al. (1996)
T. mathranii	Wheat straw	Batch	60.0 (6.7)*	2.61	70	Ahring et al. (1999)
T. mathranii	Wheat straw	Batch	60.0	5.30	70	Klinke et al. (2001)
Several	Wheat straw	Batch	30.0	0.30-0.50	70	Sommer et al. (2004)
Several	Wheat straw	Batch	60.0	0.20-0.40	70	Sommer et al. (2004)
C. thermocellum	Filter paper/Corn steep liq.	Batch	45.0/8.0	8.18	60	Balusu et al. (2005)
T. ethanolicus	Beet molasses	Batch	40.0 (19.5)*	4.81	65	Avci et al. (2006)
T. thermohydrosulfuricus 70-1	Beet molasses	Batch	40.0 (19.5)*	2.95	65	Avci et al. (2006)
Thermoanaerobacter sp. 65-2	Beet molasses	Batch	40.0 (19.5)*	7.25	65	Avci et al. (2006)
Thermoanaerobacter BG1L1	Corn stover	Batch	25.0-150.0	8.50-9.20	70	Georgieva et al. (2007)
Thermoanaerobacter BG1L1	Wheat straw	Batch	30.0-120.0	8.50-9.20	70	Georgieva et al. (2008)
Thermoanaerobacter BG1L1	Corn stover	Con	25.0-150.0	8.50-9.20	70	Georgieva et al. (2008)
Clostridium thermocellum	Avicel	Batch	300-700**	0.70	60	Chinn et al. (2008)
T. ethanolicus	Been card HL	Batch	10.0	1.80	60	Miyazaki et al. (2008)
Clostridium sp.	Been card HL	Batch	10.0	0.85	60	Miyazaki et al. (2008)
Thermoanaerobacterium sp.	Been card HL	Batch	10.0	0.90	60	Miyazaki et al. (2008)
Thermoanaerobacterium AK17	Cellulose	Batch	7.5	5.81	60	Sveinsdottir et al. (2009)
Thermoanaerobacterium AK17	Grass	Batch	7.5	2.91	60	Sveinsdottir et al. (2009)
Thermoanaerobacterium AK17	Paper	Batch	7.5	2.03	60	Sveinsdottir et al. (2009)
Mixed	Napier grass	Batch	2.0-40.0	0.70-0.90	60	Lin et al. (2010)
Mixed	Avicel	Batch	2.0-40.0	0.40-5.70	60	Lin et al. (2010)
C. thermocellum	Napier grass	Batch	2.0-40.0	0.80-3.90	60	Lin et al. (2010)
C. thermocellum	Avicel	Batch	10.0	0.70	60	Lin et al. (2010)
Mixed *(C. thermocellum)*	Banana waste	Batch	10.0-100.0	5.50-9.20	60	Harish et al. (2010)

Table 2. EtOH production from lignocellulosic biomass by defined and mixed cultures of thermophilic bacteria. Cultivation was either in batch or continuous (con). EtOH yields given in mM/g substrate degraded as well as substrate concentrations and incubation temperature are also shown. * = sugar concentration, ** = 30 to 50% as hydrolysate.

Fermentation of beet molasses by three thermophilic *Thermoanaerobacter* species (*T. ethanolicus*, *Thermoanaerobacter* sp. and *T. thermohydrosulfuricus*) were recently investigated

(Avci et al., 2006). The concentration of sugars were 19.5 g L^{-1} and and fermentation resulted in yields between 3.0 (*T. thermohydrosulfuricus*) and 7.26 mM g^{-1} (*Thermoanaerobacter* sp.). The highest reported EtOH yields reported from complex biomass are by *Thermoanaerobacter* BG1L1 on corn stover and wheat straw (Georgieva & Ahring, 2007; Georgieva et al., 2008a). The biomass was pretreated with acid or wet oxidation and EtOH yields were up to 9.2 mM g^{-1} for biomass hydrolysates.

Studies on *Thermoanaerobacterium* sp and *Clostridium* sp. on been curd refuse hydrolysates were investigated by Miyazaki and co-workers (Miyazaki et al., 2008) with emphasis on cooperation between aerobic cellulose degrading *Geobacillus* with the anaerobes. EtOH yields in this study were relatively low, or between 0.72 to 1.80 mM g substrate^{-1}. Studies on EtOH production by *Thermoanaerobacterium* sp. AK17, isolated from Icelandic hot spring, on various types of lignocellulosic biomass were reported recently (Sveinsdottir et al., 2009). Batch culture studies on 7.5 g L^{-1} of cellulose, grass and newspaper, pretreated with heat and enzymes, showed EtOH yields of 2.0 (paper), 2.91 (grass) to 5.81 (cellulose) mM/g biomass. Optimization experiments were recently done on this strain where EtOH yields on grass and cellulose were increased to 4.0 and 8.6 mM g^{-1}, respectively. The main environmental factors concerning increasing EtOH yields were the use of acid/alkali for pretreatment and by lowering the substrate concentration from 7.5 to 2.5 g L^{-1} (unpublished results).

6. Production of H_2 from thermophilic bacteria

H_2 production from various organic materials by fermentation has been known for a long time. Firstly, the focus was mainly on facultative mesophilic bacteria within the genera of e.g. *Enterobacter*, *Citrobacter* and strict anaerobes like the typical acetate/butyrate fermentative *Clostridia*. There are numerous publications which focus on mesophilic bacteria that will not be dealt with in this paper. It has not been until relatively recently that H_2 production by thermophiles has gained increased interest and in the past three years there has been an explosion of number of publications within this field of research. Thermophilic bacteria have many advantages as compared to mesophiles concerning H_2 production, however, have remained less studied. High temperatures favor the stoichiometry of H_2 production resulting in higher H_2 yields compared to mesophilic systems (van Groenestijn et al., 2002; van Niel et al., 2003). Furthermore, thermophilic fermentation results in less variety of end products as compared to those of mesophilic fermentation (van Niel et al., 2003). The discussion below is divided into production of H_2 from sugars and from other biomass.

6.1 Production of H_2 from sugars

Pure cultures are, for the most part, used to study effects of environmental factors affecting commercial H_2 production. Several studies on H_2 production on sugars, using pure thermophilic cultures have been reported. The most common are dealing with bacteria belonging to the genera of *Thermoanaerobacterium*, *Caldicellulosiruptor* and *Thermotoga*. Table 3 summarizes studies using pure cultures for H_2 production from sugars.

Thermotoga neopolitana was first described by Jannasch and co-workers (1988) but earliest data of H_2 production is from 2002 where the bacterium produced 2.0 ml L^{-1} h^{-1} on glucose in batch cultures (van Ootegehem et al., 2002). H_2 production capacity from glucose by this species has since then been investigated in detail by others (Eriksen et al., 2008; d'Ippolito et

al., 2008; Nguyen et al., 2008, 2010; Munro et al., 2009) showing yields between 1.84 to 3.85 mol H_2 mol glucose^{-1}. Xylose can also be used by the bacterium with good yields, or 2.20 mol H_2 mol xylose^{-1} (Nguyen et al., 2010b). Most studies reported on H_2 production by *T. neopolitana* have been conducted in batch experiments with relatively low sugar concentrations (5 to 7 g L^{-1}). The only experiment in continuous culture is reported by d'Ippolito et al., (2010) on glucose but very high yields were reported (3.85 mol H_2 mol glucose^{-1}). Other studies on species within the genus have been on *T. elfii* (van Niel et al., 2002) and *T. maritima* (Nguyen et al., 2008; Schröder et al., 1994) with H_2 yields varying from 1.67 to 4.00 (maximum) mol H_2 mol glucose^{-1}.

Organisms	Substrate	Cultivation method	Biomass conc. (g L^{-1})	Volumetric H_2 productivity (mL L^{-1} h^{-1})	H_2 yield (mol H_2 mol glu^{-1} equiv.)	Temp. (°C)	Reference
P. furiosus	Maltose	Con	0.22	5.5-22.0	2.90	98	Schicho et al. (1993)
T. maritima	Glucose	Batch	0.1	6.9	4.00	80	Schroder et al. (1994)
T. elfii	Glucose	Con	10.0	0.6	3.30	65	van Niel et al. (2002)
C. saccharolyticus	Sucrose	Con	10.0	0.6	3.30	70	van Niel et al. (2002)
T. neapolitana	Glucose	Batch	5.0	0.6	N/A	70	Van Ooteghem et al. (2002)
T. tengcongensis	Glucose	Con	4.5	N/A	4.00	75	Soboh et al. (2004)
C. saccharolyticus	Glucose	Batch	1.7	N/A	2.50	70	Kadar et al. (2004)
C. saccharolyticus	Xylose	Batch	1.6	11.3	2.70	70	Kadar et al. (2004)
C. saccharolyticus	Xyl/Glu	Batch	1.0	9.2	2.40	70	Kadar et al. (2004)
C. saccharolyticus	Glucose	Con	4.0	2.5	3.60	70	Vrije et al. (2007)
T. thermosaccharolyticum	sucrose	Batch	20.0	3.0	2.53	60	O-Thong et al. (2008)
T. thermosaccharolyticum	Glucose	Batch	10.0	1.6	2.42	60	Ren et al. (2008)
T. thermosaccharolyticum	Xylose	Batch	10.0	1.6	2.19	60	Ren et al. (2008)
T. neapolitana	Glucose	Batch	5.0	N/A	2.40	80	Eriksen et al. (2008)
T. neapolitana	Glucose	Batch	7.5	N/A	1.84	80	Nguyen et al. (2008a)
T. maritima	Glucose	Batch	7.5	N/A	1.67	80	Nguyen et al. (2008a)
T. neapolitana	Glucose	Batch	2.5	0.1	3.85	77	Munro et al. (2009)
C. thermocellum	Cellobiose	Batch	1.1	N/A	1.73	60	Levin et al. (2006)
C. saccharolyticus	Glucose	Con	10.0	N/A	3.00	70	Willquist et al. (2009)
T. neapolitana	Glucose	Batch	7.0	N/A	3.24	77	Nguyen et al. (2010b)
T. neapolitana	Xylose	Batch	4.0	N/A	2.20	77	Nguyen et al. (2010b)
T. thermosaccharolyticum	Xylose	Batch	12.2	N/A	2.37	60	Cao et al. (2010)
T. neapolitana	Glucose	Con	5.0	6.3	3.85	80	d'Ippolito et al. (2010)
C. owensis	Glucose	Con	10.0	1.9	3.80	70	Zeidan & van Niel (2010)
C. owensis	Xylose	Con	10.0	1.4	2.70	70	Zeidan & van Niel (2010)
C. thermolacticum	Lactose	Batch	10.0	N/A	1.80	58	Collet et al. (2003)
Clostridium AK14	Glucose	Batch	3.6	N/A	2.21	50	Almarsdottir et al. (2010)
Clostridium AK14	Xylose	Batch	3.0	N/A	2.55	50	Almarsdottir et al. (2010)

Table 3. H_2 production from sugars by pure cultures of thermophilic bacteria. Cultivation was either in batch or continuous (con). Volumetric H_2 production rates, H_2 yields as well as substrate concentrations and incubation temperature are also shown.

Species belonging to genus *Caldicellulosiruptor* have been intensively investigated for H_2 production. *C. saccharolyticus* grown on sucrose showed good yields in continuous culture, or 6.6 mol H_2 mol sucrose^{-1} (= 3.3 mol H_2 mol hexose^{-1}) (van Niel et al., 2002) and between 2.5 and 3.0 mol H_2 for one mole of xylose and glucose in batch (Kadar et al., 2004; Willquist et al., 2009). Higher yields were observed in continuous culture, or 3.6 as well as high H_2 production rates (Vrije et al., 2007). Recently *C. owensis* has also been shown to be a good H_2 producer both in continuous culture with H_2 yields of 3.8 and 2.7 from glucose and xylose,

respectively (Zeidan & van Niel, 2010). Hydrogen production from glucose (4.5 g L^{-1}) in batch by *Thermoanaerobacter tengcongensis* has been investigated (Soboh et al., 2009). The culture was continuously flushed with N_2 to keep the partial pressure of H_2 low. This resulted in higher growth rates but due to high N_2 flushing rates H_2 could not be quantified. However, glucose was almost completely converted to acetate and since no external electron acceptor was added, it was assumed that 4.0 mol H_2 were formed per mol glucose degraded. Other thermophilic bacteria that have been investigated for H_2 production capacity are e.g. *Clostridium* sp. (Almarsdottir et al., 2010 ; Levin et al., 2006), *Thermoanerobacterium saccharolyticum* (Cao et al., 2010; Kadar et al., 2004) and *Pyrococcus furiosus* (Schicho et al., 1993).

In practice it may not be feasible to use pure cultures for H_2 production in large scale production facilities. Therefore, a more attention has recently been upon the use of mixed culture studies for H_2 production, often with sugars as model substrates.

Origin	Substrate	Cultivation method	Biomass conc. (g L^{-1})	Volumetric H_2 productivity (mL L^{-1} h^{-1})	H_2 yield (mol H_2 mol glu^{-1} equiv.)	Temp. (°C)	Reference
Mixed	Glucose	Con	4.9	N/A	2.47	70	Kotsopoulus et al. (2005)
Compost	Lactose	Fed-batch	2.0	N/A	3.70	55	Calli et al. (2008)
Compost	Xylose	Fed-batch	2.0	N/A	1.70	55	Calli et al. (2008)
Natural anaerobic mixed culture	Xylose	Batch	20.0	N/A	0.80	55	Lin et al. (2008)
Anaerobic culture fromhot spring	Glucose	Batch	4.5	N/A	1.16	52	Karadag et al. (2009)
Household solid waste	Xylose	Batch	0.5	N/A	1.62	70	Kongjan et al. (2009)
Household solid waste	Xylose	Con	1.0	2.6	1.61	70	Kongjan et al. (2009)
Cow manure	Glucose	Con	5.0	50.8	3.32	75	Yokoyama et al. (2009)
Mixed	Xylose	Con	6.0	3.4	2.60	70	Zeidan et al. (2010)
Mixed	Glucose	Batch	2.0	N/A	1.58	70	Zhao et al. (2009)
Mixed	Xylose	Batch	2.0	N/A	1.84	70	Zhao et al. (2010)
Sediments-rich samples from hot springs	Glucose	Batch	10.0	N/A	1.71	60	Hniman et al. (2010)
Sediments-rich samples from hot springs	Xylose	Batch	10.0	N/A	1.57	60	Hniman et al. (2010)
Anaerobic culture from hot spring	Glucose	Con	9.0	N/A	1.10	37	Karadag & Puhakka (2010)
Enrichment cultures from hot springs	Glucose	Batch	18.0	N/A	2.10	59	Koskinen et al. (2008a)
Mixed	Glucose	Con	3.6	6.1	0.80	60	Koskinen et al. (2008b)
Enichment culture from hot spring	Glucose	Batch	5.9	N/A	3.20	60	Koskinen et al. (2008c)
Enichment culture from hot spring	Glucose	Con	18.0	N/A	2.74	58	Koskinen et al. (2008c)

Table 4. H_2 production from sugars by mixed cultures of thermophilic bacteria. Cultivation was either in batch or continuous (con). Volumetric H_2 production rates, H_2 yields as well as substrate concentrations and incubation temperature are also shown.

The origin of bacteria used in such studies are from e.g. compost, hot springs, manure or anaerobic digestion systems (Calli et al., 2008; Hniman et al., 2010; Karadag et al., 2009; Karadag & Puhakka, 2010; Lin et al., 2008; Zhao et al., 2009; Zhao et al., 2010). Available data from such experiments are presented in Table 4. Although the yields of H_2 production are usually lower as compared to pure culture studies, very high yields have indeed been obtained. An example of this is from the study of xylose and lactose, fed batch fermentation with bacteria from compost. Yields on lactose were 3.70 mol H_2 mol $lactose^{-1}$ (Calli et al., 2008). Glucose fermentation in continuous culture with bacteria from manure resulted in 3.32 mol H_2 mol $glucose^{-1}$ (Yokoyama et al., 2009). Enrichment culture from Icelandic geothermal hot spring produced H_2 of up to 3.20 mol H_2 mol $glucose^{-1}$ in batch assay (Koskinen et al., 2008c). A continuous culture study showed H_2 yields of 2.74 mol H_2 mol $glucose^{-1}$. The enrichment culture was dominated by strains closely affiliated with *Thermobrachium celere.*

6.2 Production of H_2 from complex biomass

Available data on H_2 production from complex biomass has exploded in the last three years. Complex biomass, such as food waste and lignocellulosic agricultural residues has been used for thermophilic biohydrogen production in both laboratory and pilot scale. The discussion below will be divided according to H_2 production from different types of biomass.

6.2.1 Agricultural wastes and energy crops

Several studies have been done with various corn straw as substrate both in pure (Ivanova et al., 2009) and mixed (Kongjan & Angelidaki, 2010; Kongjan et al., 2010) cultures. Mixed cultures, originating from methanogenic sludge from a potato factory were used in continuous cultures (UASB, CSTR, AF) with hemicellulose rich wheat straw (Kongjan & Angelidaki, 2010). The highest H_2 production yields of 9.5 mmol H_2 g sugar^{-1} (1.70 mol H_2 mol glucose^{-1}) was achieved in the UASB reactor. The reactors were fed with hydrolysates that contained 4.4% (TS), mainly xylose. The hydrolysate prepared with hydrothermal pretreatment was diluted prior to inoculation to 25% (v/v). The main conclusion from this study was that reactor configuration is of great importance for enhancing and stabilizing H_2 production. In another study on this substrate the focus was on the importance of hydrolysate concentrations (Kongjan et al., 2010). High hydrolysate concentrations strongly inhibited H_2 production. Batch culture trials on 5% hydrolysate concentrations showed highest yield or 14.1 mmol H_2 g sugar^{-1} (2.55 mol H_2 mol hexose^{-1} equivalent) but CSTR-reactor that ran on 20% HL showed considerable lower yields or 7.9 mmol H_2 mol sugar^{-1} (1.43 mol H_2 mol glucose^{-1} equivalent). Phylogenetic analysis of the mixed cultures showed presence of *Caldanaerobacter subterraneus*, *Thermoanaerobacter subterraneus* and *Thermoanaerobacterium thermosaccharolyticum*.

Caldicellulosiruptor saccharolyticus has been used for H_2 production from hemicellulose-rich pine tree wood shavings, maize leaves, wheat straw, sugarcane bagasse and the sweet sorghum bagasse without chemical pretreatment in batch (Ivanova et al., 2009). The highest yields of 3.8 mol H_2 mol glucose $^{-1}$ equivalents was achived from wheat straw hydrolysates. The maize leaves were used both unpretreated and pretreated with cellulase-producing aerobic bacteria, *Bacillus amyloliquefaciens*. The pretreatment greatly improved the H_2 yields. Unpretreated maize leaves yielded 1.53 mol H_2 mol glucose^{-1} while pretreated leaves yielded 3.67 mol H_2 mol glucose^{-1}. Lower yields were obtained from other biomass. *Thermotoga neapolitana* produced 2.3 to 2.7 mmol H_2 g korean rice straw^{-1} (0.41 to 0.49 mol H_2 mol hexose^{-1} equivalent) from untreated and thermally ammonia or dilute sulfuric acid pretreatment, respectively (Nguyen et al., 2010b). Fermentation of hydrolysates from Miscanthus hydrolysates by *Caldicellulosiruptor saccharolyticus* and *Thermotoga elfi*, pretreated by alkali, resulted in 3.4 and 3.2 mol H_2 mol glucose^{-1} equivalent, respectively (de Vrije et al., 2009).

Corn stover and corn stover cornstalk have been investigated for H_2 production capacity by many (Cao et al., 2009; Datar et al., 2007; Liu et al., 2008b; Liu & Cheng, 2010; Ren et al., 2010). Pure culture studies on *Thermoanaerobacterium thermosaccharolyticum* on corn stover hydrolysates showed maximum of 2.7 mol H_2 mol glucose^{-1} equivalent diluted corn stover hydrolysates that contained a mixture of glucose, xylose and arabinose (total sugar concentration, 10 g L^{-1}) (Ren et al., 2010). Pretreatment consisted of mincing with hammer mill, drying and enzymatic hydrolysis. The bacterium showed classical acetate/butyrate fermentation and yields were similar as on equal amounts of pure sugars. Earlier reports on

the production capacity of this bacterium on corn stover pretreated with acid showed similar yields, or 2.24 mol H_2 mol glucose^{-1} (Cao et al., 2009). From a study of Liu and Cheng (2010), corn stover was pretreated with microwave assisted strategy and the resulting biomass hydrolysate fermented with mixed thermophilic microflora from a anaerobic digester. H_2 production capacity was however modest, or 1.53 mol H_2 mol glucose^{-1} equivalents

Culture	Feedstock	Cultivation method	Biomass conc. (g L^{-1})	Volumetric H_2 productivity (mL L^{-1} h^{-1})	H_2 yield (mol H_2 mol glu^{-1} equiv.)	Temp. (°C)	Reference
Mixed	Cellulose wastewater	Batch	5.0	ND	0.82	55	Liu et al. (2003)
C. saccharolyticus	Paper sludge	Batch	8.4	91.8	3.70	70	Kadar et al. (2004)
C. thermocellum	Delignified wood fibers	Batch	0.1-4.5	ND	1.00-2.30	60	Levin et al. (2006)
C. thermocellum 27405	Cellulose	Batch	0.1-4.5	ND	0.80-2.00	60	Levin et al. (2006)
C. thermocellum 27405	Whatman paper	Batch	0.1-4.5	ND	0.80-1.90	60	Levin et al. (2006)
Thermotoga neapolitana	Microcrystalline cellulose	Batch	5.0	ND	1.00-2.20	80	Nguyen et al. (2008b)
C. thermocellum	Dried distillers grain	Batch	5.0	5.1	1.27	60	Magnusson et al. (2008)
C. thermocellum	Barley hulls	Batch	5.0	2.0	1.24	60	Magnusson et al. (2008)
C. thermocellum	Cellulose	Batch	1.1	5.1	0.76	60	Magnusson et al. (2008)
C. thermocellum	Contaminated barley hulls	Batch	5.0	5.4	1.18	60	Magnusson et al. (2008)
Coculture	Cellulose	Batch	5.0	ND	1.80	60	Liu et al. (2008b)
T. thermosaccharolyticum	Corn stover	Batch	6.4-12.2	ND	2.24	60	Cao et al. (2009b)
T. thermosaccharolyticum	Miscanthus hydrolysate	Batch	10.0	282.2	3.40	72	Vrije et al. (2009)
Thermotoga neapolitana	Miscanthus hydrolysate	Batch	14.0	275.5	3.20	80	Vrije et al. (2009)
Mixed	Napier grass	Batch	10.0	ND	1.20	55	Lo et al. (2009)
Coculture	Cellulose (filter paper)	Batch	9.0	ND	1.36	55	Geng et al. (2010)
C. saccharolyticus	Wheat straw	Batch	20.0	ND	3.80	70	Ivanova et al. (2009)
C. saccharolyticus	Sweet sorghum plant	Batch	30.0	ND	1.75	70	Ivanova et al. (2009)
C. saccharolyticus	Sugarcane bagasse	Batch	15.0	ND	2.30	70	Ivanova et al. (2009)
C. saccharolyticus	Maize leaves	Batch	8.0	ND	3.67	70	Ivanova et al. (2009)
Mixed	Oil palm trunk hydrolysate	Batch	10.0	ND	1.94	60	Hniman et al. (2010)
Mixed	Corn stover	Batch	13.3	ND	1.53	55	Liu & Cheng (2010)
Clostridium AK14	Cellulose	Batch	5.0	ND	1.10-1.20	50	Almarsdottir et al. (2010)
Clostridium AK14	Hemp stem	Batch	5.0	ND	0.60-0.70	50	Almarsdottir et al. (2010)
Clostridium AK14	Hemp leaf	Batch	5.0	ND	0.20-0.40	50	Almarsdottir et al. (2010)
Clostridium AK14	Grass	Batch	5.0	ND	0.80-0.90	50	Almarsdottir et al. (2010)
Clostridium AK14	Paper	Batch	5.0	ND	0.10-0.40	50	Almarsdottir et al. (2010)
Clostridium AK14	Barley straw	Batch	5.0	ND	0.70-0.80	50	Almarsdottir et al. (2010)
Mixed	Wheat straw	Con	3.9*	34.2	1.70	70	Kongjan & Angelidaki (2010)
Mixed	Wheat straw	Con	3.9*	10.1	1.51	70	Kongjan & Angelidaki (2010)
Mixed	Wheat straw	Con	3.9*	20.6	1.00	70	Kongjan & Angelidaki (2010)
Mixed	Wheat straw	Batch	0.8-3.9*	ND	1.20-2.60	70	Kongjan et al. (2010)
Mixed	Wheat straw	Con	3.1*	7.7	1.42	70	Kongjan et al. (2010)
Mixed	Wheat straw	Batch	50.0	ND	2.54	70	Kongjan et al. (2010)
T. thermosaccharolyticum W16	Corn stover	Batch	10*	250.9	2.70	60	Ren et al. (2010)
Thermotoga neapolitana	Korean rice straw	Batch	10*	31.8	0.41	75	Nguyen et al. (2010b)
Thermotoga neapolitana	Korean rice straw	Batch	10.0	112.4	0.49	75	Nguyen et al. (2010b)

Table 5. H_2 production from agricultural wastes and energy crops. Cultivation was either in batch or continuous (con). Volumetric H_2 production rates, H_2 yields as well as substrate concentrations and incubation temperature are also shown. * = concentrations of sugars.

A coculture of *Clostridium thermocellum* and *Thermoanaerobacterium thermosaccharolyticum* grown on hydrolysate made from 5 g L^{-1} of corn stalk and corn cob powder (no pretreatment), resulted in 1.80 mol H_2 mol glucose^{-1} (Liu et al., 2008b). *Clostridium* AK14 was used to degrade hemp (both stem and leaf), grass, paper and straw (Almarsdottir et al., 2010). Highest yields were observed on grass pretreated with 0.75% sulfuric acid and

enzymes, or 6.23 mol H_2 g VS^{-1}. Pretreatment with either alkali or acid increased H_2 in most cases substantially.

Several studies of H_2 production form cellulose have been conducted (Almarsdottir et al., 2010; Geng et al., 2010; Levin et al., 2006; Liu et al., 2003; Liu et al., 2008b; Nguyen et al., 2008). Various sources of cellulose have been used, e.g. wastewater (Liu et al., 2003) Whatman filter paper (Almarsdottir et al., 2010; Geng et al., 2008), microcrystalline cellulose (Liu et al., 2008b; Nguyen et al., 2008b). Hydrogen yields from these studies (all batch) varied from 0.95 to 2.32 mol H_2 mol $glucose^{-1}$ equivalent. In some studies the focus was on different pretreatment methods used. Studies with pure cultures of *Clostridium* AK14, a moderate thermophilic bacterium showed similar results (1.17 mol H_2 mol $glucose^{-1}$ equivalent) from Whatman paper whether it was only enzymatically pretreated or pretreated with both enzymes and weak acid or alkali (Almarsdottir et al., 2010). Hydrogen production from microcrystalline cellulose by *Thermotoga neapolitana* increased however from 1.59 to 2.20 mol H_2 mol $glucose^{-1}$ equivalent by using ionic liquid pretreatment (Nguyen et al., 2008b). The influence of substrate concentrations on H_2 yields from degradation of cellulosic substrates by *Clostridium thermocellum* were investigated by Levin et al., (2006). Highest yields were observed on delignified wood fibers at 0.1 g L^{-1}, 2.32 mol H_2 mol $glucose^{-1}$ equivalents. At 4.5 g L^{-1} yields dramatically decreased to less than 1 mol H_2 mol $glucose^{-1}$. Other reports on H_2 production from lignocellulosic biomass presented in Table 5 include studies on paper sludge (Kadar et al., 2004), oil palm trunk hydrolysate (Hniman et al., 2010), Napier grass (Lo et al., 2009) and barley hulls (Magnusson et al., 2008) and are not discussed in detail in this overview.

6.2.2 Starch and mixed biomass

Several studies of H_2 production from starch have been done, both with pure soluble starch and a starch based biomass. Akutsu and co-workers used mixed cultures from five different kinds of sludge as inocula to produce hydrogen from starch in CSTR-reactors without any pretreatment (Akutsu et al., 2008). The highest H_2 production yields (2.30 mol H_2 mol $glucose^{-1}$ equivalent) was obtained with thermophilically digested waste activated sludge as inocula. Phylogenetic analysis showed the presence of *Thermoanaerobacterium* in all reactors. *Janthinobacterium* and aerobic bacteria of the genus *Flavobacterium* were also detected. Two other studies by Akutsu and co-workers focused on the effects of different factors on H_2 production from starch (Akutsu et al., 2009a, 2009b). In the first study (Akutsu et al., 2009b) the effects of substrate concentrations (10-70 g L^{-1}) on H_2 production were investigated in continuous cultures using a mixed culture originating from thermophilic acidogenic sludge treating potato waste. The H_2 yields varied from 1.84 to 2.82 mol H_2 mol $glucose^{-1}$ at 70 and 20 g L^{-1} substrate concentrations, respectively. The maximum H_2 production rate was 182 ml $L^{-1}h^{-1}$. In the other study (Akutsu et al., 2009a), the effects of hydrolic retention time, pH and substrate concentrations were further investigated. Hydrogen production rate was gradually increased from 62 to 167 ml H_2 $L^{-1}h^{-1}$ by lowering the HRT from 40 h to 6h but on the other hand, maximum H_2 yields were obtained at 48 h HRT, or 1.68 mol H_2 mol $glucose^{-1}$ equivalent. Additionally, H_2 production diminished greatly when pH was higher than 6.0 or lower than 4.7 indicating the importance of pH for H_2 production (Akutsu et al., 2009a). Study of starch degradation and H_2 production in repeated batch by extreme mixed cultures, originating from cow manure showed H_2 yields of 1.73 mol H_2 mol $glucose^{-1}$ (Yokoyama et al., 2007). The main emphasis was on the phylogenetic analysis of the microbiological community and presence of various *Caldanaerobacter* species was observed.

Culture	Feedstock	Cultivation method	Biomass conc. (g L^{-1})	Volumetric H_2 productivity (mL L^{-1} h^{-1})	H_2 yield (mol H_2 mol glu^{-1} equiv.)	Temp. (°C)	Reference
T. kodakaraensis	Starch	Con	0.5	6.7	3.30	85	Kanai et al. (2005)
Mixed	Food waste	Con	6.9	ND	2.50-2.80	55	Chu et al. (2008)
Mixed	Soluble starch	Batch*	6.3	ND	1.73	75	Yokoyama et al. (2007)
Mixed	Starch	Con	10.0	42.4-70.8	1.40-2.30	55	Akutsu et al. (2008)
C. saccharolyticus	Sweet sorghum	Batch	2.0	nd	2.63	72	Ivanova et al. (2009)
Mixed	Starch	Con	60.0	nd	1.68	55	Akutsu et al. (2009a)
Mixed	Starch	Con	15.0-70.0	nd	1.84-2.82	55	Akutsu et al. (2009b)
Mixed	Wheat starch	Batch	20.0	7.4	2.40	55	Cakir et al. (2010)
T. neapolitana	Algal starch	Batch	5.0	44.6-227.0	1.80-2.50	75	Nguyen et al. (2010c)
C. saccharolyticus	Carrot pulp	Batch	10.0	351.7	2.80	72	Vrije et al. (2010)
T. neapolitana	Carrot pulp	Batch	10.0	280.0	2.70	80	Vrije et al. (2010)
Mixed	Rice winery wastewater	Con	10.0 (COD)	158.3	2.14	55	Yu et al. (2002b)
Mixed	Food waste	Con	25.0 (sugars)	ND	0.60-1.80	55	Shin et al. (2004)
Mixed	Food waste	Con	14.1 (VSS)	16.7-41.7	1.00-2.40	55	Shin & Youn (2005)
Mixed	POME	Batch	85.0 (COD)	24.2	2.53	60	O-Thong et al. (2008)
Mixed	Household solid waste	Batch	0.5	ND	0.30-2.00	70	Liu et al. (2008a)
Mixed	Household solid waste	Batch*	10.0 (VS)	ND	0.82	70	Liu et al. (2008b)
Mixed	Kitchen waste	Batch	23.7 (VSS)	ND	0.88	55	Lee et al. (2008)
Mixed	Cheese whey (lactose rich)	Con	Variable	12.5 - 329.1	ND	55	Azbar et al. (2009)
Mixed	Cheese whey wastewater	Batch	21.3	ND	1.55	55	Azbar et al. (2009)
Mixed	Pig slurry	Con	45.0 (TS)	3.8	ND	70	Kotsopoulos et al. (2009)
Mixed	Kitchen waste	Con	60.5	66.7	0.23	55	Wang et al. (2009)
Mixed	POME	Con	7.0-8.4 (VSS)	379.2	2.17	60	Prasertsan et al. (2009)
Mixed	Crude Palm Oil + sucrose	Batch	24.0	ND	2.50	55	Ismail et al. (2009)
Mixed	Vegetable kitchen waste	Con*	10.0	41.7	1.70	55	Lee et al. (2010)

Table 6. H_2 production from starch and mixed biomass. Cultivation was either in batch or continuous (con). Volumetric H_2 production rates, hydrogen yields as well as substrate concentrations and incubation temperature are also shown.
* = Repeated batch, ** = Semicontinuous

Cakir and co-workers compared hydrogen production from ground wheat starch under mesophilic (37°C) and thermophilic conditions (55°C) with mixed microflora from a heat-treated anaerobic sludge (Cakir et al., 2010). The starch was pretreated with sulfuric acid and heat in order to convert it to soluble sugars. The highest H_2 yield was 2.40 mol H_2 mol glucose^{-1}, obtained under the thermophilic conditions. The hyperthermophilic *Thermotoga neapolitana* was used by to produce hydrogen from green algal biomass (Nguyen et al., 2010c). Starch is a major accumulated constituent of algal biomass and therefore makes a good potential feedstock for both EtOH and H_2 production. Two different pretreatments were used to disrupt the algal cell wall (sonication and MeOH exposure) and two other to improve starch conversion to H_2 (HCl + heat and enzymes). All methods gave good effect on H_2 production but the highest H_2 yield (2.5 mol H_2 mol glucose^{-1}) was obtained with enzymatic hydrolysis (Nguyen et al., 2010c).
Many types of different food waste biomass have been used to produce H_2, almost exclusively with mixed cultures from various seed sludge. Lee and co-workers have done two different studies on H_2 production from high vegetable kitchen waste (Lee et al., 2008 and Lee et al., 2010). No pretreatment was used in either study. In the first study, a series of batch fermentation tests were conducted at four different pH levels to observe the effects of

pH on the H_2 production. Hydrogen yields from different pH levels were all similar, the highest obtained at pH 7.0 (0.49 mmol H_2 g COD^{-1}) except for pH 5.5 (the lowest pH level), where there was no H_2 production at all (Lee et al., 2008). The main bacteria present belong to the genus *Clostridium*. In the other investigation much higher yields were obtained, or 1.7 mmol H_2 g COD^{-1} and the predominant species was closely affiliated to *Thermoanaerobacterium thermosaccharolyticum* (Lee et al., 2010). Recent study of H_2 production from kitchen waste with mixed cultures from various sources showed good production rates (66.7 ml L^{-1} h^{-1}) but much lower yields (0.23 mol H_2 mol $glucose^{-1}$ equivalent) (Wang et al., 2009). A continuous culture study on H_2 production from food waste by the use of mixed culture originating from anaerobic waste water treatment plant resulted in maximum of 2.8 mol H_2 mol $hexose^{-1}$ (Chu et al., 2008). Other studies with food waste include e.g. continuous culture (CSTR) studies by Shin et al., (2004) and Shin &Youn (2005) at sugar concentration of 25 g L^{-1}. Clearly the effects of substrate concentrations are important but higest yields (1.8 mol H_2 mol $hexose^{-1}$) were obtained at 8 g VS/L (Shin et al., 2004). Maximum H_2 production rate and yield occurred at 8 g VSL^{-1} d^{-1}, 5 days HRT and pH 5.5 (Shin & Youn, 2005). Hydrogen production from household solid waste by using extreme-thermophilic (70°C) mixed culture resulted in 2 mol H_2 mol $hexose^{-1}$ (Liu et al., 2008a) and 0.82 mol H_2 mol $hexose^{-1}$ (Liu et al., 2008b).

Other studies on various mixed substrates include pig slurry (Kotsopoulous et al., 2009), rice winery wastewater (Yu et al., 2002), palm oil effluent (POME) (Ismail et al., 2010; O'Thong et al., 2008; Prasertsan et al., 2009), and cheese whey (Azbar et al., 2009a, 2009b), and are presented in Table 6. Fewer studies have been done using pure microbial cultures producing H_2 from complex biomass. *Caldicellulosiruptor saccharolyticus* and *Thermotoga neapolitana* showed good H_2 yields from carrot pulp hydrolysate, or 2.8 and 2.7 mol H_2 mol $hexose^{-1}$, respectively (de Vrije et al., 2010). *Thermococcus kodakaraensis* KOD1 showed very high H_2 yields on starch (3.3 mol H_2 mol $hexose^{-1}$) in continuous culture in a gas lift fermentor with dilution rate of 0.2 h^{-1} (Kanai et al., 2005).

7. Pros and cons of using thermophiles for biofuel production

The use of thermophilic bacteria for production of H_2 and EtOH has several pros and cons compared to the use of mesophilic bacteria, phototrophic bacteria and yeasts. It is possible to compare the use of different microorganisms by looking at several factors of both practical and economical point of view. Historically, yeasts have been and still are, the microorganisms most widely used for EtOH production from homogenous material like sucrose and glucose. The main reason for this are e.g. very high yields, few end products and high EtOH tolerance. However, wild type yeasts do not have degradation genes for pentose and polymer degradation and genetic engineering studies have not yet delivered stable organisms for large scale production. The main benefits of using bacteria for biofuel production is their broad substrate spectrum and they may therefore be a better choice for EtOH production from more complex biomass e.g. agricultural wastes (Taylor et al., 2008). The main drawback of the use bacteria for biofuel production is their low EtOH tolerance and more diverse end product formation. This is the main reason for no commercialized large scale plants have been built yet. Thermophilic bacteria are often very tolerant towards various environmental extremes. Apart from growing at higher temperatures, often with higher growth rates, many are acid and salt tolerant which may be of importance when various mixed substrates are used. In general bacteria tolerate lower EtOH concentrations as

compared to yeasts and elevated substrate concentrations may inhibit growth. This may possible be solved by either using fed batch or continuous cultures or by „self distillation" of EtOH.

H_2 production by mesophilic bacteria has been known for a long time. The main drawback of using mesophilic bacteria is the fact that H_2 production is inhibited at relatively low partial pressures of H_2 resulting in a change of carbon flow away from acetate (and H_2) towards e.g. EtOH and lactate. Extremophilic bacteria are less phroned towards this inhibition and much higher H_2 concentrations are needed before a change in the carbon flow occurs. H_2 production by photosynthesis has gained increased interest lately but H_2 production rates are much slower as compared to bacteria and a need for large and expensive reactors inhibit its practical use. Additionally, fermentation is not dependent on light and can be runned continuously.

Furfural and hydroxymethylfurfural (HMF) are furan derivatives from pentoses and hexoses, respectively and are among the most potent inhibitory compounds generated from acid hydrolysis of lignocellulosic biomass. Most microorgansisms are more sensitive to furfural than HMF but usually inhibition occurs at concentrations above 1 g L^{-1}. Sensitivity of thermophilic bacteria towards these compounds seem to be similar as compared to yeast (de Vrije et al., 2009; Cao et al., 2010).

8. Genetic engineering of thermophiles – state of the art

The main hindrance of using thermophilic bacteria is low tolerance to EtOH and the production of other end products like acetate and lactate. Several efforts have been done to enhance EtOH tolerance for thermophiles. Most of these studies were performed by mutations and adaptation to increased EtOH concentrations (Lovitt et al., 1984,1988; Georgieva et al., 1988) and has already been discussed. Elimination of catabolic pathways leading to other end products by genetic engineering has only got attention in the past few years.

The first report on genetic engineering on thermophilic bacteria to increase biofuel production is on *Thermoanaerbacterium saccharolyticum* (Desai et al., 2004). The L-lactate dehydrogenase (LDH) was knocked out leading to increased EtOH and acetate production on both glucose and xylose and total elimination of lactate production. The wild type strain produced 8.1 and 1.8 mM of lactate from 5 g L^{-1} of glucose and xylose, respectively. Later study of the same species resulted in elimination of all acid formation and generation of homoethanolic strain. This strain uses pyruvate:ferredoxin oxidoreductase to convert pyruvate to EtOH with electron transfer from ferredoxin to NAD(P) but this is unknown by any other homoethanolgenic microbes who use pyruvate decarboxylase. The strain produces 37g L^{-1} of EtOH which is the highest yields reported so far for a thermophilic anaerobe (Shaw et al., 2008).

Two *Geobacillus thermoglucosidasius* strains producing mixed acids from sugar fermentation with relatively low EtOH yields were recently genetically engineered to increase yields (Cripps et al., 2009). The authors developed an integration vector system that led to the generation of stable gene knockouts but the wild type strains had shown problems of genetic instability. They inactivated lactate dehydrogenase and to deal with the excess carbon flux they upregulated the expression of PDH (pyruvate dehydrogenase) to make it the sole fermentation pathway. One of their mutants (TM242) produced EtOH from glucose at more than 90% of the maximum theoretical yields (Cripps et al., 2009).

A strain of *Thermoanaerobacter mathranii* was genetically engineered to improve the EtOH production (Yao & Mikkelsen, 2010). A strain that had already had the *ldh* gene deleted to eliminate an NADH oxidation pathway (Yao & Mikkelsen, 2010) was used. The results obtained indicated that using a more reduced substrate such as mannitol, shifted the carbon balance towards more reduced end products like EtOH. In order to do that without having to use mannitol as a substrate they expressed an NAD^+-dependent GLDH (glycerol dehydrogenase) in this bacterium.

A possible approach to increase H_2 yields is to convert more of the substrate to H_2 by altering metabolism by genetic engineering. Studies on either maximizing yields of existing pathways or metabolic engineering of new pathways have been published (Hallenbeck & Gosh, 2010). Genetic manipulation and metabolic flux analysis are well developed and have been suggested to be applied to biohydrogen (Hallenbeck & Benemann, 2002; Vignais et al., 2006). However, no study on genetic engineering on thermophilic bacteria considering H_2 production has been published to our knowledge. So far, the main emphasis has been on the mesophilic bacteria *E.coli* and *Clostridium* species.

Fermentative bacteria often possess several different hydrogenases that can operate in either proton reduction or H_2 oxidation (Hallenbeck & Benemann, 2002). Logically, inactivation of H_2 oxidation would increase H_2 yields. This has been shown for *E. coli* where elimination of *hyd*1 and *hyd*2 led to a 37% increase in H_2 yield compared to the wild type strain (Bisaillon et al., 2006).

Studies on metabolically engineering Clostridia to increase H_2 production have been published. One study showed that by decreasing acetate formation by inactivate *ack* in *Clostridium tyrobutyricum*, 1.5-fold enhancement in H_2 production was observed; yields from glucose increased from 1.4 mol H_2-mol glucose^{-1} to 2.2 mol H_2-mol glucose^{-1} (Liu et al., 2006).

9. Conclusion

Many bacteria within the genera *Clostridium, Thermoanaerobacter, Thermoanaerobacterium, Caldicellulosiruptor* and *Thermotoga* are good H_2 and/or EtOH producers. Species within *Clostridium* and *Caldicellulosiruptor* are of special interest because of their ability to degrade cellulose and hemicelluloses. Highest EtOH yields on sugars and lignocelluloses hydrolysates are 1.9 mol EtOH mol glucose^{-1} and 9.2 mM g biomass^{-1} (corn stover and wheat straw) by *Thermoanaerobacter thermohydrosulfuricus* and *Thermoanaerobacter* species, respectively. Highest H_2 yields on sugars and lignocelluloses hydrolysates are 4 mol H_2 mol glucose^{-1} and 3.7 mol H_2 mol glucose^{-1} equivalent (from wheat straw) by *Thermotoga maritima* and *Caldicellulosiruptor saccharolyticus*, respectively. Clearly many bacteria within these genera have great potential for EtOH and hydrogen production, especially from complex lignocellulosic biomass. Recent information in genome studies of thermoanaerobes has led to experiments where *Thermonanaerobacterium* and *Thermoanaerobacter* species have been genetically engineered to make them homoethanolgenic. Thus, the greatest drawback of using thermophilic bacteria for biofuel production, their mixed end product formation, can be eliminated but it remains to see if these strains will be stable for upscaling processes.

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11. References

Ahn, H.J. & Lynd, L.R. 1996. Cellulose degradation and ethanol production by thermophilic bacteria using mineral growth medium. *Applied Biochemistry and Biotechnology,* 57: 599-604.

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

Ahring, B.K.; Licht, D.; Schmidt, A.S.; Sommer, P. & Thomsen, A.B. 1999. Production of ethanol from wet oxidised wheat straw by *Thermoanaerobacter mathranii*. *Bioresource Technology,* 68: 3-9.

Akutsu, Y.; Lee, D.-Y.; Chi, Y.-Z.; Li, Y.-Y.; Harada, H. & Yu, H.-Q. 2009a. Thermophilic fermentative hydrogen production from starch-wastewater with bio-granules. *International Journal of Hydrogen Energy,* 34: 5061-5071.

Akutsu, Y.; Li, Y.; Harada, H. & Yu, H. 2009b. Effects of temperature and substrate concentration on biological hydrogen production from starch. *International Journal of Hydrogen Energy,* 34: 2558-2566.

Akutsu, Y.; Li, Y.; Tandukar, M.; Kubota, K. & Harada, H. 2008. Effects of seed sludge on fermentative characteristics and microbial community structures in thermophilic hydrogen fermentation of starch. *International Journal of Hydrogen Energy,* 33: 6541-6548.

Almarsdottir, A.R.; Taraceviz, A.; Gunnarsson, I. & Orlygsson, J. 2010. Hydrogen production from sugars and complex biomass by *Clostridium* species, AK14, isolated from Icelandic hot spring. *Icelandic Agricultural Sciences,* 23: 61-71.

Alvira, P.; Tomas-Pejo, E.; Ballesteros, M. & Negro, M. J. 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresource Technology,* 101: 4851-4861.

Amend & Shock. 2001. Energetics of overall metabolic reactions of thermophilic and hyperthermophilic Archea and Bacteria. *FEMS Microbiology Reviews*,25: 175-243.

Avci, A. & Donmez, S. 2006. Effect of zinc on ethanol production by two thermoanaerobacter strains. *Process Biochemistry,* 41: 984-989.

Azbar, N.; Dokgoez, F.T.; Keskin, T.; Eltem, R.; Korkmaz, K.S.; Gezgin, Y.; Akbal, Z.; Oencel, S.; Dalay, M.C.; Goenen, C. & Tutuk, F. 2009a. Comparative evaluation of bio-hydrogen production from cheese whey wastewater under thermophilic and mesophilic anaerobic conditions. *International Journal of Green Energy,* 6: 192-200.

Azbar, N.; Dokgoz, F.T.C.; Keskin, T.; Korkmaz, K.S. & Syed, H.M. 2009b. Continuous fermentative hydrogen production from cheese whey wastewater under thermophilic anaerobic conditions. *International Journal of Hydrogen Energy,* 34: 7441-7447.

Balk, M.; Weijma, J. & Stams, A.J.M. 2002. *Thermotoga lettingae* sp nov., a novel thermophilic, methanol-degrading bacterium isolated from a thermophilic anaerobic reactor. *International Journal of Systematic and Evolutionary Microbiology,* 52: 1361-1368.

Balusu, R.; Paduru, R.M.R.; Seenayya, G. & Reddy, G. 2004. Production of ethanol from cellulosic biomass by *Clostridium thermocellum* SS19 in submerged fermentation - screening of nutrients using plackett-burman design. *Applied Biochemistry and Biotechnology,* 117: 133-141.

Balusu, R.; Paduru, R.R.; Kuravi, S.K.; Seenayya, G. & Reddy, G. 2005. Optimization of critical medium components using response surface methodology for ethanol production from cellulosic biomass by *Clostridium thermocellum* SS19. *Process Biochemistry,* 40: 3025-3030.

Bisaillon, A.; Turcot, J. & Hallenbeck, P.C. 2006. The effect of nutrient limitation on hydrogen production by batch cultures of *Escherichia coli*. *International Journal of Hydrogen Energy*, 30: 1504-1508.

Baskaran, S., Hogsett, D.A L. & Lynd, L.R. 1994. Ethanol-production using thermophilic bacteria – growth-medium formulation and product tolerances. *Abstracts of Papers of the American Chemical Society, 207*, 172-BTEC.

Ben-Bassat, A.; Lamed, R. & Zeikus, J.G. 1981. Ethanol-production by thermophilic bacteria – metabolic control of end product formation in *Thermoanaerobium brockii*. *Journal of Bacteriology,* 146: 192-199.

Brock, T.D. 1986. Introduction: an overview of the thermophiles, In: *Thermophiles: General Molecular and Applied Microbiology*, T.D., Brock, (Ed.), pp. 1-16, John Wiley and Sons, ISBN 0471820016, New York, USA.

Cakir, A.; Ozmihci, S. & Kargi, F. 2010. Comparison of bio-hydrogen production from hydrolyzed wheat starch by mesophilic and thermophilic dark fermentation. *International Journal of Hydrogen Energy,* 35: 13214-13218.

Calli, B.; Schoenmaekers, K.; Vanbroekhoven, K. & Diels, L. 2008. Dark fermentative H_2 production from xylose and lactose – Effects of on-line pH control. *International Journal of Hydrogen Energy, 33:* 522-530.

Canganella, F. & Wiegel, J. 1993. The potential of thermophilic Clostridia in biotechnology. In: *The Clostridia and Biotechnology,* D.R. Woods, (Ed.), pp. (391-429), Butterworths Publishers, ISBN 0750690046, Stoneham, MA, USA.

Cao, G.-L.; Ren, N.; Wang, A.; Guo, W.; Yao, J.; Feng, Y. & Xhao, Q. 2010. Statistical optimization of culture condition for enhanced hydrogen production by *Thermoanaerobacterium thermosaccharolyticum* W16. *Bioresource Technology,* 101: 2053-2058.

Cao, G.-L.; Ren, N.; Wang, A.; Lee, D.; Guo, W.; Liu, B.; Feng, Y. & Zhao, Q. 2009. Acid hydrolysis of corn stover for biohydrogen production using *Thermoanaerobacterium thermosaccharolyticum* W16. *International Journal of Hydrogen Energy,* 34: 7182-7188.

Cao, G.-L.; Ren, N-Q.; Wang, A-J.; Guo, W-Q.; Xu, J-F. & Liu, B-F. 2009. Effect of lignocellulose-derived inhibitors on growth and hydrogen production by *Thermoanaerobacterium thermosaccharolyticum* W16. *International Journal of Hydrogen Energy,* 35: 13475-13480.

Carreira, L.H.; Ljungdahl, L. G.; Bryant, F.; Szulcynski, M. & Wiegel, J. 1982. Control of product formation with *Thermoanaerobacter ethanolicus*: enzymology and physiology. In: *Proc. 4th International Symposium on Genetics of Industrial Microorganisms*, pp. 351-355, Kodansha Ltd., Tokyo, Japan,

Carreira, L.H.; Wiegel, J. & Ljungdahl, L.G. 1983. Production of ethanol from bio-polymers by anaerobic, thermophilic, and extreme thermophilic bacteria. I. Regulation of carbohydrate utilization in mutants of *Thermoanaerobacter ethanolicus*. *Biotechnology and Bioengineering,* Symph.13, pp. 183-191.

Carreira, L.H. & Ljungdahl, L.G. 1993. Production of ethanol from biomass using anaerobic thermophilic bacteria, In: *Liquid fuel developments*, D.L. Wise, (Ed.), pp. 1-28, CRC Press, ISBN 0849360943, Boca Raton, Fl, USA.

Cha, K.S. & Bae, J.H. 2011. Dynamic impacts of high oil prices on the bioethanol and feedstock markets. *Energy policy*, 39: 753-760.

Chinn, M.S.; Nokes, S.E. & Strobel, H.J. 2008. Influence of moisture content and cultivation duration on *Clostridium thermocellum* 27405 end-product formation in solid substrate cultivation on avicel. *Bioresource Technology,* 99: 2664-2671.

Chong, M-L.; Sabaratnam, V.; Shirai, Y. & Hassan, M.A. 2009. Biohydrogen production from biomass and industrial wastes by dark fermentation. *International Journal of Hydrogen Energy,* 34: 3277-3287.

Chou, C-J.; Jenney, Jr. F.E.; Adams, M.W.W. & Kelly, R.M. 2008. Hydrogensis in hyperthermophilic microorganisms: implications fo biofuels. *Metabolic Engineering*, 10: 394-404.

Chu, C.;Li, Y.; Xu, K.; Ebie, Y.; Inamori, Y. & Kong, H. 2008. A pH- and temperature-phased two-stage process for hydrogen and methane production from food waste. *International Journal of Hydrogen Energy,* 33: 4739-4746.

Collet, C.; Schwitzguebel, J.P. & Peringer, P. 2003. Improvement of acetate production from lactose by growing *Clostridium thermolacticum* in mixed batch culture. *Journal of Applied Microbiology,* 95: 824-831.

Collins, M.D.; Lawson, P.A.; Willems, A.; Cordoba, J.J.; Fernandezgarayzabal, J. & Garcia, P. 1994. The phylogeny of the genus *Clostridium* - proposal of 5 new genera and 11 new species combinations. *International Journal of Systematic Bacteriology,* 44: 812-826.

Cord-Ruwisch, R.; Seitz, H. & Conrad, R. 1988. The capacity of hydrogenotrophic anaerobic bacteria to compete for traces of hydrogen depends on the redox potential of the terminal electron acceptor. *Archives of Microbiology*, 149: 350-357.

Cripps, R.E.; Eley, K.; Leak, D.J.; Rudd, B.; Taylor, M.; Todd, M.; Boakes, S.; Martin, S. & Atkinson, T. 2009. Metabolic engineering of *Geobacillus thermoglucosidasius* for high yield ethanol production. *Metabolic Engineering,* 11: 398-408.

Datar, R.; Huang, J.; Maness, P.; Mohagheghi, A.; Czemik, S. & Chornet, E. 2007. Hydrogen production from the fermentation of corn stover biomass pretreated with a steam-explosion process. *International Journal of Hydrogen Energy,* 32: 932-939.

Demain, A. L.; Newcomb, M. & Wu, J. H. 2005. Cellulase, Clostridia, and Ethanol. *Microbiology and molecular biology reviews*, 69: 124-154.

Demirbas, A. 2009. Political, economic and environmental impacts of biofuels: A review. *Applied Energy*, 86: 108-117.

Deanda, K.; Zhang, M.; Eddy, C. & Picataggio, S. 1996. Deveolpment of an arabinose-fermenting *Zymomonas mobilis* strain by metabolic pathway engineering. *Applied and Environmental Microbiology*, 62: 4465-4470.

Desai, S.G.; Guerinot, M.L. & Lynd, L.R. 2004. Cloning of L-lactate dehydrogenase and elimination of lactic acid production via gene knockout in *Thermoanaerobacterium saccharolyticum* JW/SL-YS485. *Applied Microbiology and Biotechnology,* 65: 600-605.

d'Ippolito, G.; Dipasquale, L.; Vella, F.M.; Romano, I.; Gambacorta, A.; Cutignano, A. & Fontana, A. 2010. Hydrogen metabolism in the extreme thermophile *Thermotoga neapolitana*. *International Journal of Hydrogen Energy,* 35: 2290-2295.

Drent, W.J.; Lahpor, G.A.; Wiegant, W.M. & Gottschal, J.C. 1991. Fermentation of inulin by *Clostridium thermosuccinogenes* sp. nov., a thermophilic anaerobic bacterium isolated from various habitats. *Applied and Environmental Microbiology*, 57: 455-462.

DuPont Danisko Cellulosic Ethanol LLC. 2011. Fermentation, In: *Technology*, (April 4, 2011), Available from http://www.ddce.com/technology/fermentation.html

Eriksen, N.T.; Nielsen, T.M. & Iversen, N. 2008. Hydrogen production in anaerobic and microaerobic *Thermotoga neapolitana*. *Biotechnology Letters,* 30: 103-109.

Fardeau, M.L.; Faudon, C.; Cayol, J.L.; Magot, M.; Patel, B.K.C. & Ollivier, B. 1996. Effect of thiosulphate as electron acceptor on glucose and xylose oxidation by *Thermoanaerobacter finnii* and a *Thermoanaerobacter* sp isolated from oil field water. *Research in Microbiology,* 147: 159-165.

Geng, A.; He, Y.; Qian, C.; Yan, X. & Zhou, Z. 2010. Effect of key factors on hydrogen production from cellulose in a co-culture of *Clostridium thermocellum* and *Clostridium thermopalmarium*. *Bioresource Technology,* 101: 4029-4033.

Georgieva, T.I. & Ahring, B.K. 2007. Evaluation of continuous ethanol fermentation of dilute-acid corn stover hydrolysate using thermophilic anaerobic bacterium *Thermoanaerobacter* BG1L1. *Applied Microbiology and Biotechnology,* 77: 61-68.

Georgieva, T.I.; Mikkelsen, M.J. & Ahring, B.K. 2008a. Ethanol production from wet-exploded wheat straw hydrolysate by thermophilic anaerobic bacterium *Thermoanaerobacter* BG1L1 in a continuous immobilized reactor. *Applied Biochemistry and Biotechnology,* 145: 99-110.

Georgieva, T.I.; Mikkelsen, M.J. & Ahring, B.K. 2008b. High ethanol tolerance of the thermophilic anaerobic ethanol producer *Thermoanaerobacter* BG1L1. *Central European Journal of Biology*, 2: 364-377.

German Collection of Microorganisms and Cell Cultures. Available from: http://www.dsmz.de/

Goettemoeller, J., & Goettemoeller. A. 2007. *Sustainable Ethanol: Biofuels, Biorefineries, Cellulosic Biomass, Flex-Fuel Vehicles, and Sustainable Farming for Energy Independence*, Prairie Oak Publishing, ISBN 9780978629304, Maryville, Missouri.

Hallenbeck, P.C. 2009. Fermentative hydrogen production: Principles, progress and prognosis. *International Journal of Hydrogen Energy,* 34: 7379-7389.

Hallenbeck, P.C. & Benemann, J.R. 2002. Biological hydrogen production; fundamentals and limiting processes. *International Journal of Hydrogen Energy,* 27: 1185-1193.

Hallenbeck, P.C. & Ghosh, D. 2010. Improvements in fermentative biological hydrogen production through metabolic engineering. *Journal of Environmental Management*, doi:10.1016/j.jenvman.2010.07.021 (in press).

He, Q.; Lokken, P.M.; Chen, S. & Zhou, J. 2009. Characterization of the impact of acetate and lactate on ethanolic fermentation by *Thermoanaerobacter ethanolicus*. *Bioresource Technology,* 100: 5955-5965.

Hild, H.M.; Stuckey, D.C. & Leak, D.J. 2003. Effect of nutrient limitation on product formation during continuous fermentation of xylose with thermoanaerobacter ethanolicus JW200 fe(7). *Applied Microbiology and Biotechnology,* 60: 679-686.

Hniman, A., O-Thong, S. & Prasertsan, P. 2010. Developing a thermophilic hydrogen-producing microbial consortia from geothermal spring for efficient utilization of xylose and glucose mixed substrates and oil palm trunk hydrolysate. *International Journal of Hydrogen Energy*, In Press.

Huber, R.; Langworthy, T.A.; Konig, H.; Thomm, M.; Woese, C.R. & Sleytr, U.B. 1986. *Thermotoga maritima* sp. nov., represents a new genus of unique extremely thermophilic eubacteria growing up to 90 °C. *Archives of Microbiology,* 144: 324-333.

Ismail, I.; Hassan, M.A.; Rahman, N.A.A. & Soon, C.S. 2010. Thermophilic biohydrogen production from palm oil mill effluent (POME) using suspended mixed culture. *Biomass & Bioenergy,* 34: 42-47.

Ivanova, G.; Rakhely, G. & Kovács, K.L. 2009. Thermophilic biohydrogen production from energy plants by *Caldicellulosiruptor saccharolyticus* and comparison with related studies. *International Journal of Hydrogen Energy,* 34: 3659-3670.

Jannasch, H.W.; Huber, R.; Belkin, S. & Stetter, K.O. 1988. *Thermotoga neapolitana* sp. nov. of the extremely thermophilic, eubacterial genus *Thermotoga*. *Archives of Microbiology,* 150: 103-104.

Jeffries, T.W. 2006. Engineering yeasts for xylose metabolism. *Current Opinion in Biotechnology,* 17: 320–326

Jones, P. 2008. Improving fermentative biomass-derived H_2-production by engineered microbial metabolism. *International Journal of Hydrogen Energy,* 33: 5122-5130.

Kádár, Z.; de Vrijek, T.; van Noorden, G.E.; Budde, M.A.W.; Szengyel, Z. & Reczey, K. 2004. Yields from glucose, xylose, and paper sludge hydrolysate during hydrogen production by the extreme thermophile *Caldicellulosiruptor saccharolyticus*. *Applied Biochemistry and Biotechnology,* 113-116: 497-508.

Kanai, T.; Imanaka, H.; Nakajima, A.; Uwamori, K.; Omori, Y.; Fukui, T.; Atomi, H. & Imanaka, T. 2005. Continuous hydrogen production by the hyperthermophilic archaeon, *Thermococcus kodakaraensis* KOD1. *Journal of Biotechnology,* 116: 271-282.

Karadag, D.; Makinen, A.E.; Efimova, E. & Puhakka, J.A. 2009. Thermophilic biohydrogen production by an anaerobic heat treated-hot spring culture. *Bioresource Technology,* 100: 5790-5795.

Karadag, D. & Puhakka, J.A. 2010. Direction of glucose fermentation towards hydrogen or ethanol production through on-line pH control. *International Journal of Hydrogen Energy,* 35: 10245-10251.

Kengen, S.W.M.; Gorrissen, H.P.; Verhaart, M.; van Niel, E.W.J.; Claassen, P.A.M. & Stams, A.J.M. 2009. Biological hydrogen production by anaerobic microorganisms. In: *Biofuels*, W. Soetaert; E.J. Vandamme, (Ed.), pp. 197-221, John Wiley & Sons, ISBN 9780470026748, Chichester, UK.

Klapatch, T.R.; Hogsett, D.A.L.; Baskaran, S.; Pal, S. & Lynd, L.R. 1994. Organism development and characterization for ethanol production using thermophilic bacteria. *Applied Biochemistry and Biotechnology,* 45-46: 209-223.

Klinke, H.B.; Thomsen, A.B. & Ahring, B.K. 2001. Potential inhibitors from wet oxidation of wheat straw and their effect on growth and ethanol production by *Thermoanaerobacter mathranii*. *Applied Microbiology and Biotechnology,* 57: 631-638.

Knutson, B.L.; Strobel, H.J.; Nokes, S.E.; Dawson, K.A.; Berberich, J.A. & Jones, C.R. 1999. Effect of pressurized solvents on ethanol production by the thermophilic bacterium *Clostridium thermocellum*. *Journal of Supercritical Fluids,* 16: 149-156.

Kongjan, P. & Angelidaki, I. 2010. Extreme thermophilic biohydrogen production from wheat straw hydrolysate using mixed culture fermentation: Effect of reactor configuration. *Bioresource Technology,* 101: 7789-7796.

Kongjan, P.; Min, B. & Angelidaki, I. 2009. Biohydrogen production from xylose at extreme thermophilic temperatures (70 °C) by mixed culture fermentation. *Water Research,* 43: 1414-1424.

Kongjan, P.; O-Thong, S.; Kotay, M.; Min, B. & Angelidaki, I. 2010. Biohydrogen production from wheat straw hydrolysate by dark fermentation using extreme thermophilic mixed culture. *Biotechnology and Bioengineering,* 105: 899-908.

Koskinen, P.E.P.; Beck, S.R.; Orlygsson, J. & Puhakka, J.A. 2008a. Ethanol and hydrogen production by two thermophilic, anaerobic bacteria isolated from Icelandic geothermal areas. *Biotechnology and Bioengineering,* 101: 679-690.

Koskinen, P.E.P.; Lay, C.; Beck, S.R.; Tolvanen, K.E.S.; Kaksonen, A.H.; Orlygsson, J.; Lin, C.Y. & Puhakka, J.A. 2008b. Bioprospecting thermophilic microorganisms from Icelandic hot springs for hydrogen and ethanol production. *Energy & Fuels,* 22: 134-140.

Koskinen, P.E.P.; Lay, C.; Puhakka, J.A.; Lin, P.; Wu, S.; Orlygsson, J. & Lin C,Y. 2008c. High-efficiency hydrogen production by an anaerobic, thermophilic enrichment culture from an Icelandic hot spring. *Biotechnology and Bioengineering,* 101: 665-678.

Kotsopoulos, T.A.; Zeng, R. J. & Angelidaki, I. 2006. Biohydrogen production in granular up-flow anaerobic sludge blanket (UASB) reactors with mixed cultures under hyper-thermophilic temperature (70 °C). *Biotechnology and Bioengineering,* 94: 296-302.

Kotsopoulos, T.A.; Fotidis, I.A.; Tsolakis, N. & Martzopoulos, G.G. 2009. Biohydrogen production from pig slurry in a CSTR reactor system with mixed cultures under hyper-thermophilic temperature (70 °C). *Biomass & Bioenergy,* 33: 1168-1174.

Kristjansson, J.K. & Alfredsson, G.A. 1986. Life in Icelandic Hot Springs, *Natturufraedingurinn*, 56: 49-68.

Lacis, L.S. & Lawford, H.G. 1991. *Thermoanaerobacter ethanolicus* growth and product yield from elevated levels of xylose or glucose in continuous cultures. *Applied and Environmental Microbiology,* 57: 579-585.

Lacis, L.S. & Lawford, H.G. 1989. Analysis of the variation in ethanol yield from glucose or xylose with continuously grown *Thermoanaerobacter ethanolicus*. *Applied Biochemistry and Biotechnology,* 20-21: 479-490.

Lacis, L.S. & Lawford, H.G. 1988a. Effect of growth-rate on ethanol-production by *Thermoanaerobacter ethanolicus* in glucose-limited or xylose-limited continuous culture. *Biotechnology Letters,* 10: 603-608.

Lacis, L.S. & Lawford, H.G. 1988b. Ethanol-production from xylose by *Thermoanaerobacter ethanolicus* in batch and continuous culture. *Archives of Microbiology,* 150: 48-55.

Lamed, R., Su, T.M. & Brennan, M.J. 1980. Effect of stirring on ethanol-production by *Clostridium thermocellum*. *Abstracts of Papers of the American Chemical Society, 180*(AUG), 44-MICR.

Lamed, R. & Zeikus, J.G. 1980a. Ethanol-production by thermophilic bacteria - relationship between fermentation product yields of and catabolic enzyme-activities in *Clostridium thermocellum* and *Thermoanaerobium brockii*. *Journal of Bacteriology,* 144: 569-578.

Lamed, R. & Zeikus, J.G. 1980b. Glucose fermentation pathway of *Thermoanaerobium brockii*. *Journal of Bacteriology,* 141: 1251-1257.

Lamed, R. J.; Lobos, J.H. & Su, T.M. 1988. Effects of stirring and hydrogen on fermentation end products of *Clostridium thermocellum*. *Applied Environmental Microbiology,* 54: 1216-1221.

Larsen, L.; Nielsen, P. & Ahring, B.K. 1997. *Thermoanaerobacter mathranii* sp nov, an ethanol-producing, extremely thermophilic anaerobic bacterium from a hot spring in Iceland. *Archives of Microbiology,* 168: 114-119.

Lee, Y-W. & Chung, J. 2010. Bioproduction of hydrogen from food waste by pilot-scale combined hydrogen/methane. *International Journal of Hydrogen*, 35: 11746-11755.

Lee, Y.E.; Jain, M.K.; Lee, C.Y.; Lowe, S.E. & Zeikus, J.G. 1993. Taxonomic distinction of saccharolytic thermophilic anaerobes - description of *Thermoanaerobacterium xylanolyticum* gen-nov, sp-nov, and *Thermoanaerobacterium saccharolyticum* gen-nov, sp-nov - reclassification of *Thermoanaerobium brockii*, *Clostridium thermosulfurogenes*, and *Clostridium thermohydrosulfuricum* E100-69 as *Thermoanaerobacter brockii* comb-nov, *Thermoanaerobacterium thermosulfurigenes* comb-nov, and *Thermoanaerobacter thermohydrosulfuricus* comb-nov, respectively - and transfer of *Clostridium thermohydrosulfuricum* 39E to *Thermoanaerobacter ethanolicus*. *International Journal of Systematic Bacteriology,* 43: 41-51.

Lee, Z.; Li, S.; Kuo, P.; Chen, I.; Tien, Y.; Huang, Y.; Chuang, C-P.; Wong, S-C. & Cheng, S-S. 2010. Thermophilic bio-energy process study on hydrogen fermentation with vegetable kitchen waste. *International Journal of Hydrogen Energy,* 35: 13458-13466.

Lee, Z.; Li, S.; Lin, J.; Wang, Y.; Kuo, P. & Cheng, S. 2008. Effect of pH in fermentation of vegetable kitchen wastes on hydrogen production under a thermophilic condition. *International Journal of Hydrogen Energy,* 33: 5234-5241.

Levin, D.B.; Pitt, L. & Love, M. 2004. Biohydrogen production: prospects and limitations to practical application. *International Journal of Hydrogen Energy*, 29: 173-185.

Levin, D.B.; Islam, R.; Cicek, N. & Sparling, R. 2006. Hydrogen production by *Clostridium thermocellum* 27405 from cellulosic biomass substrates. *International Journal of Hydrogen Energy,* 31: 1496-1503.

Lin, C., Wu, C., Wu, J., & Chang, F. 2008. Effect of cultivation temperature on fermentative hydrogen production from xylose by a mixed culture. *Biomass & Bioenergy,* 32: 1109-1115.

Lin, C-W,; Wu, C-H,; Tran, D-T,; Shih, M-C,; Li, W-H. & Wu C-F. 2010. Mixed culture fermentation from lignocellulosic materials using thermophilic lignocellulose-degrading anaerobes. *Process Biochemistry*, 46: 489-493.

Lin C-Y, Wu S-Y, Lin P-J, Chang J-S, Hung C-H, Lee K-S, Lay C-H, Chu C-Y, Cheng C-H, Chang AC, Wu J-H, Chang F-Y, Yang L-H, Lee C-W. & Lin Y-C. A pilot scale high-rate biohydrogen production system with mixed microflora. *International Journal of Hydrogen Energy*, in press.

Liu, C. & Cheng, X. 2010. Improved hydrogen production via thermophilic fermentation of corn stover by microwave-assisted acid pretreatment. *International Journal of Hydrogen Energy,* 35: 8945-8952.

Liu, D.W.; Zeng, R.J. & Angelidaki, I. 2008. Enrichment and adaptation of extreme-thermophilic (70 °C) hydrogen producing bacteria to organic household solid waste by repeated batch cultivation. *International Journal of Hydrogen Energy,* 33: 6492-6497.

Liu, D.; Min, B. & Angelidaki, I. 2008. Biohydrogen production from household solid waste (HSW) at extreme-thermophilic temperature (70 °C) - influence of pH and acetate concentration. *International Journal of Hydrogen Energy,* 33: 6985-6992.

Liu, H.; Zhang, T. & Fang, H.H.P. 2003. Thermophilic H-2 production from a cellulose-containing wastewater. *Biotechnology Letters,* 25: 365-369.

Liu, X.; Zhu, Y. & Yang, S.T. 2006. Construction and Characterization of *ack* Deleted Mutant of *Clostridium tyrobutyricum* for Enhanced Butyric Acid and Hydrogen Production. *Biotechnology Pregress*, 22: 1265-1275.

Liu, Y.; Yu, P., Song, X. & Qu, Y. 2008b. Hydrogen production from cellulose by co-culture of *Clostridium thermocellum* JN4 and *Thermoanaerobacterium thermosaccharolyticum* GD17. *International Journal of Hydrogen Energy,* 33: 2927-2933.

Lo, Y. C.; Huang, C.; Fu, T.; Chen, C. & Chang, J. 2009. Fermentative hydrogen production from hydrolyzed cellulosic feedstock prepared with a thermophilic anaerobic bacterial isolate. *International Journal of Hydrogen Energy,* 34: 6189-6200.

Lovitt, R.W.; Longin, R. & Zeikus, J.G. 1984. Ethanol production by thermophilic bacteria: physiological comparison of solvent effects on parent and alcohol-tolerant strains of *Clostridium thermohydrosulfuricum*. *Applied and Environmental Microbiology*, 48: 171-177.

Lovitt, R.W.; Shen, G.J. & Zeikus, J.G. 1988. Ethanol-production by thermophilic bacteria - biochemical basis for ethanol and hydrogen tolerance in *Clostridium thermohydrosulfuricum*. *Journal of Bacteriology,* 170: 2809-2815.

Lynd, L.R.; Grethlein, H.E. & Wolkin, R.H. 1989. Fermentation of cellulosic substrates in batch and continuous culture by *Clostridium thermocellum*. *Applied and Environmental Microbiology,* 55: 3131-3139.

Magnusson, L.; Islam, R.; Sparling, R.; Levin, D. & Cicek, N. 2008. Direct hydrogen production from cellulosic waste materials with a single-step dark fermentation process. *International Journal of Hydrogen Energy,* 33: 5398-5403.

Miyazaki, K.; Irbis, C.; Takada, J. & Matsuura, A. 2008. An ability of isolated strains to efficiently cooperate in ethanolic fermentation of agricultural plant refuse under initially aerobic thermophilic conditions: Oxygen deletion process appended to consolidated bioprocessing (CBP). *Bioresource Technology,* 99: 1768-1775.

Moat, A.G.; Foster, J.W. & Spector, M.P. 2002. Central pathways of carbohydrate metabolism, In: *Microbial Physiology 4th ed*., A.G. Moat; J.W. Foster & M.P. Spector, (Ed.), pp. 350-367, Wiley-Liss Inc., ISBN 0-471-39483-1, New York, USA.

Munro, S.A.; Zinder, S.H. & Walker, L.P. 2009. The fermentation stoichiometry of *Thermotoga neapolitana* and influence of temperature, oxygen, and pH on hydrogen production. *Biotechnology Progress,* 25: 1035-1042.

Nardon L. & Aten K. 2008. Beyond a better mousetrap: A cultural analysis of the adoption of etahnol in Brazil. *Journal of World Business*, 43: 261-273.

Nass, L.L.P.; Pereira, A.A. & Ellis, D. 2007. Biofuels in Brazil: An overview, *Crop Science,* 47: 2228-2237.

Ng, T.K.; Ben-Bassat, A. & Zeikus, J.G. 1981. Ethanol production by thermophilic bacteria: Fermentation of cellulosic substrates by cocultures of *Clostridium thermocellum* and *Clostridium thermohydrosulfuricum*. *Applied Environmental Microbiology*, 41: 1337-1343.

Nguyen, T.A.D.; Kim, J.P.; Kim, M.S.; Oh, Y.K. & Sim, S.J. 2008a. Optimization of hydrogen production by hyperthermophilic eubacteria, *Thermotoga maritima* and *Thermotoga neapolitana* in batch fermentation. *International Journal of Hydrogen Energy,* 33: 1483-1488.

Nguyen, T.D.; Han, S.J.; Kim, J.P.; Kim, M.S.; Oh, Y.K. & Sim, S.J. 2008b. Hydrogen production by the hyperthermophilic eubacterium, *Thermotoga neapolitana*, using cellulose pretreated by ionic liquid. *International Journal of Hydrogen Energy,* 33: 5161-5168.

Nguyen, T.D.; Han, S.J.; Kim, J.P.; Kim, M.S. & Sim, S.J. 2010a. Hydrogen production of the hyperthermophilic eubacterium, *Thermotoga neapolitana* under N_2 sparging condition. *Bioresource Technology,* 101: S38-S41.

Nguyen, T.D.; Kim, K.; Kim, M.S. & Sim, S.J. 2010b. Thermophilic hydrogen fermentation from korean rice straw by thermotoga neapolitana. *International Journal of Hydrogen Energy,* 35: 13392-13398.

Nguyen, T.D.; Kim, K-R.; Nguyen, M-T.; Kim, M.S.; Kim, D. & Sim S.J. 2010c. Enhancement of fermentative hydrogen production from green algal biomass by *Thermotoga neaolitana* by various pretreatment methods. *International Journal of Hydrogen Energy,* 35: 13035-13040.

Mauna Loa Observatory: NOAA-ASRL. March 8, 2011. Atmospheric CO_2, In: *CO_2Now.org,* March 10, 2011, Available from http://co2now.org/

Olsson, L. & Hahn-Hagerdal, B. 1996. Fermentation of lignocellulosic hydrolysates for ethanol production. *Enzyme and Micorbial Technology,* 18: 312-331.

Orlygsson, J. & Baldursson, S.R.B. 2007. Phylogenetic and physiological studies of four hydrogen-producing thermoanaerobes from Icelandic geothermal areas. *Icelandic Agricultural Sciences,* 20: 93-105.

Orlygsson, J.; Sigurbjornsdottir, M.A. & Bakken, H.E. 2010. Bioprospecting thermophilic ethanol and hydrogen producing bacteria from hot springs in Iceland. *Icelandic Agricultural Sciences,* 23: 73-85.

O-Thong, S.; Prasertsan, P.; Intrasungkha, N.; Dhamwichukorn, S. & Birkeland, N. 2008. Optimization of simultaneous thermophilic fermentative hydrogen production and COD reduction from palm oil mill effluent by thermoanaerobacterium-rich sludge. *International Journal of Hydrogen Energy,* 33: 1221-1231.

O-Thong, S.; Prasertsan, P.; Karakashev, D. & Angelidaki, I. 2008. Thermophilic fermentative hydrogen production by the newly isolated *Thermoanaerobacterium thermosaccharolyticum* PSU-2. *International Journal of Hydrogen Energy,* 33: 1204-1214.

Prasertsan, P.; O-Thong, S. & Birkeland, N. 2009. Optimization and microbial community analysis for production of biohydrogen from palm oil mill effluent by thermophilic fermentative process. *International Journal of Hydrogen Energy,* 34: 7448-7459.

Rainey, F.A.; Donnison, A.M.; Janssen, P.H.; Saul, D.; Rodrigo, A.; Bergquist, P.L.; Daniel, R.M.; Stackebrandt, E. & Morgan, H.W. 1994. Description of *Caldicellulosiruptor-saccharolyticus* gen-nov, sp-nov - an obligately anaerobic, extremely thermophilic, cellulolytic bacterium. *FEMS Microbiology Letters,* 120: 263-266.

Rani, K.S.; Swamy, M.V. & Seenayya, G. 1998. Production of ethanol from various pure and natural cellulosic biomass by *Clostridium thermocellum* strains SS21 and SS22. *Process Biochemistry,* 33: 435-440.

Rani, K. S.; Swamy, M.V. & Seenayya, G. 1997. Increased ethanol production by metabolic modulation of cellulose fermentation in *Clostridium thermocellum*. *Biotechnology Letters,* 19: 819-823.

Ren, N.; Cao, G.; Guo, W.; Wang, A.; Zhu, Y.; Liu, B. & Xu, J-F. 2010. Biological hydrogen production from corn stover by moderately thermophile *Thermoanaerobacterium thermosaccharolyticum* W16. *International Journal of Hydrogen Energy,* 35: 2708-2712.

Ren, N.; Cao, G.; Wang, A.; Lee, D.; Guo, W. & Zhu, Y. 2008. Dark fermentation of xylose and glucose mix using isolated *Thermoanaerobacterium thermosaccharolyticum* W16. *International Journal of Hydrogen Energy,* 33: 6124-6132.

Ren, N.; Wang, A.; Cao, G.; Xu, J. & Gao, L. 2009. Bioconversion of lignocellulosic biomass to hydrogen: Potential and challenges. *Biotechnology Advances,* 27: 1051-1060.

Renewable Fuels Association. 2010. Annual Industry Outlook, In: *Climate of Opportunity,* (April 2, 2011), Available from
http://www.ethanolrfa.org/pages/annual- industry-outlook

Rogers, P.L.; Lee., K.J.; Skotnicki, M.L. & Tribe, D.E. 1982. Ethanol production by *Zymomonas Mobilis*. *Advances in Biochemical Engineering*, 23: 37-84.

Romano, I.; Dipasquale, L.; Orlando, P.; Lama, L.; d'Ippolito, G.; Pascual, J. & Gambacorta, A. 2010. *Thermoanaerobacterium thermostercus* sp nov., a new anaerobic thermophilic hydrogen-producing bacterium from buffalo-dung. *Extremophiles,* 14: 233-240.

Rupprecht, J.; Hankamer, B.; Mussgnug, J. H.; Ananyev, G.; Dismukes, C. & Kruse, O. 2006. Perspectives and advances of biological H_2 production in microorganisms. *Applied Microbiology and Biotechnology*, 72: 442-449.

Russel, J.B. 1992. Another explanation for the toxicity of fermentation of acids at low pH: anion accumulation versus uncoupling. *Journal of Applied Bacteriology*, 73: 363-370.

Sanchez, R.G.; Karhumaa, K.; Fonseca, C.; Nogue, V.S.; Almeida, J.R.M.; Larsson, C.U.; Bengtsson, O.; Bettinga, M.; Hahn-Hagerdal, B. & Gorwa-Grauslund, M.F. 2010. Improved xylose and arabinose utilization by an industrial recombinant *Saccharomyces cerevisae* strain using evolutionary engineering. *Biotechnology for Biofuels*, 3: 1-11.

Schroder, C.; Selig, M. & Schonheit, P. 1994. Glucose fermentation to acetate, CO_2 and H_2 in the anaerobic hyperthermophilic eubacterium *Thermotoga maritima* - involvement of the Embden-Meyerhof pathway. *Archives of Microbiology,* 161: 460-470.

Schicho, R. N.; Ma, K.; Adams, M.W.W. & Kelly, R. M. 1993. Bioenergetics of sulfur-reduction in the hyperthermophilic archaeon *Pyrococcus furiosus*, *J. Bacteriol.* 175: 1823-1830.

Sapra, R.; Bagraman, K. & Adams, M.W.W. 2003. A simple energy-conserving system: Proton reduction to proton translocation. *Proceedings of the National Academy of Sciences of the United States of America*. 100: 7545-7550.

Shaw, A.J.; Podkaminer, K.K.; Desai, S.G.; Bardsley, J.S.; Rogers, S.R.; Thorne, P.G.; Hogsett, D.A. & Lynd, L.R. 2008. Metabolic engineering of a thermophilic bacterium to produce ethanol at high yield. *Proceedings of the National Academy of Sciences of the United States of America*. 105: 13769-13774.

Shin, H. S. & Youn, J. H. 2005. Conversion of food waste into hydrogen by thermophilic acidogenesis. *Biodegradation,* 16: 33-44.

Shin, H.S.; Youn, J.H. & Kim, S.H. 2004. Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis. *International Journal of Hydrogen Energy,* 29: 1355-1363.

Shiratori, H.; Sasaya, K.; Ohiwa, H.; Ikeno, H.; Ayame, S.; Kataoka, N.; Miya, A.; Beppu, T. & Ueda, K 2009. *Clostridium clariflavum* sp nov and *Clostridium caenicola* sp nov., moderately thermophilic, cellulose-/cellobiose-digesting bacteria isolated from methanogenic sludge. *International Journal of Systematic and Evolutionary Microbiology,* 59: 1764-1770.

Siebers, B. & Schönheit, P. 2005. Unusual pathways and enzymes of central carbohydrate metabolism in Archaea, *Current Opinion in Microbiology*, 8: 695-705.

Soboh, B.; Linder, D. & Hedderich, R. 2004. A multisubunit membrane-bound [NiFe] hydrogenase and an NADH-dependent Fe-only hydrogenase in the fermenting bacterium *Thermoanaerobacter tengcongensis*, *Microbiology*, 150: 2451-2461.

Sommer, P.; Georgieva, T. & Ahring, B.K. 2004. Potential for using thermophilic anaerobic bacteria for bioethanol production from hemicellulose. *Biochemical Society Transactions,* 32: 283-289.

Sveinsdottir, M.; Baldursson, S.R.B. & Orlygsson, J. 2009. Ethanol production from monosugars and lignocellulosic biomass by thermophilic bacteria isolated from Icelandic hot springs. *Icelandic Agricultural Sciences,* 22: 45-58.

US Department of Energy. February 28, 2007. Biorefinery Grant Announcement, In: *US Department of Energy; News Archive,* (April 3, 2001), Available from http://www.energy.gov/news/archives/4828.htm

Taylor, M.P.; Eley, K.L,; Martin, S,; Tuffin, M,; Burton, S.G. & Cowan, D.A. (2008). Thermophilic ethanologenesis: future prospects for second-generation bioethanol production. *Trends in Biotechnology*, 27: 398-405.

van Groenestijn, J.W.; Hazewinkel, J.H.O.; Nienoord, M. & Bussmann, P.J.T. 2002. Energy aspects of biological hydrogen production in high rate bioreactors operated in the thermophilic temperature range. *International Journal of Hydrogen Energy,* 27: 1141-1147.

van Maris, A.J.; Winkler, A.A.; Kuyper, M.; de Laat, W.T.; van Dijken, J.P. & Pronk, J.T. 2007. Development of efficient xylose fermentation in *Saccharomyces cerevisae*: xylose isomerase as a key component. *Adv. Biochem. Eng. Biotechnol.* 108: 179-204.

van Niel, E.W.J.; Budde, M.A.W.; de Haas, G.G.; van der Wal, F.J.; Claasen, P.A.M. & Stams, A.J.M. 2002. Distinctive properties of high hydrogen producing extreme thermophiles, *Caldicellulosiruptor saccharolyticus* and *Thermotoga elfii*. *International Journal of Hydrogen Energy,* 27: 1391-1398.

van Niel, E.W.J.; Claassen, P.A.M. & Stams, A.J.M. 2003. Substrate and product inhibition of hydrogen production by the extreme thermophile, *Caldicellulosiruptor saccharolyticus*. *Biotechnology and Bioengineering,* 81: 255-262.

van Ooteghem, S.A.; Beer, S.K. & Yue, P.C. 2002. Hydrogen production by the thermophilic bacterium *Thermotoga neapolitana*. *Applied Biochemistry and Biotechnology,* 98-100: 177-189.

Vedenov, D. & Wetzstein, M. 2008. Toward an optimal U.S. ethanol fuel subsidy. *Energy Economics,* 30: 2073-2090.

Vignais, P.M.; Magnin, J.-P. & Willison, J.C. 2006. Increasing biohydrogen production by metabolic engineering, *International Journal of Hydrogen Energy*, 31: 1478–1483.

Vrije, G. J. de.; Mars, A.E.; Budde, M.A.W.; Lai, M.H.; Dijkema, C.; Waard, P. de. & Classen, P.A.M. 2007. Glycolytic pathway and hydrogen yield studies of the extreme thermophile *Caldicellulosiruptor saccharolyticus*. *Applied Microbiology and Biotechnology*, 74: 1358-1367.

Vrije, T. de.; Bakker, R.R.; Budde, M.A.W.; Lai, M.H.; Mars, A.E. & Claassen, P.A.M. 2009. Efficient hydrogen production from the lignocellulosic energy crop miscanthus by the extreme thermophilic bacteria *Caldicellulosiruptor saccharolyticus* and *Thermotoga neapolitana*. *Biotechnology for Biofuels,* 2: 12.

Vrije, T. de.; Budde, M.A.W.; Lips, S.J.; Bakker, R.R.; Mars, A.E. & Claassen, P.A.M. 2010. Hydrogen production from carrot pulp by the extreme thermophiles *Caldicellulosiruptor saccharolyticus* and *Thermotoga neapolitana*. *International Journal of Hydrogen Energy,* 35: 13206-13213.

Wagner, I.D. & Wiegel, J. 2008. Diversity of Thermophilic anaerobes. *Incredible Anaerobes: From Physiology to Genomics to Fuels*, 1125: 1-43.

Wang, Y.; Li, S.; Chen, I. & Cheng, S. 2009. Starch hydrolysis characteristics of hydrogen producing sludge in thermophilic hydrogen fermentor fed with kitchen waste. *International Journal of Hydrogen Energy,* 34: 7435-7440.

Wiegel, J. & Lungdahl, L.G. 1981. *Thermoanaerobacter ethanolicus* gen. nov., spec. nov., a new extreme thermophilic, anaerobic bacterium. *Archives of Microbiology*, 128: 343-348.

Wiegel, J.; Carreira, L. H.; Mothershed, C. P. & Puls, J. 1983. Production of ethanol from biopolymers by anaerobic, thermophilic, and extreme thermophilic bacteria. II. *Thermoanaerobacter ethanolicus* JW200 and its mutants in batch cultures and resting cell experiments. *Biotechnology and Bioengineering,* 13: 193-205.

Wiegel, J.; Kuk, S.U. & Kohring, G.W. 1989. *Clostridium thermobutyricum* sp. nov., a moderate thermophile isolated from a cellulolytic culture, that produces butyrate as the major product. *International Journal of Systematic Bacteriology,* 39: 199-204.

Willquist, K.; Claassen, P.A.M. & van Niel, E.W.J. 2009. Evaluation of the influence of CO_2 on hydrogen production by *Caldicellulosiruptor saccharolyticus*. *International Journal of Hydrogen Energy,* 34: 4718-4726.

Yao, S. & Mikkelsen, M.J. 2010. Metabolic engineering to improve ethanol production in *Thermoanaerobacter mathranii*. *Applied Microbiology and Biotechnology,* 88: 199-208.

Yang, S.; Kataeva, I.; Wiegel, J.; Yin, Y.; Dam, P.; Xu, Y.; Westpheling, J. & Adams, M.W.W. 2010. Classification of '*Anaerocellum thermophilum*' strain DSM 6725 as *Caldicellulosiruptor bescii* sp. nov. *International Journal of Systematic and Evolutionary Microbiology,* 60: 2011-2015.

Yokoyama, H.; Moriya, N.; Ohmori, H.; Waki, M.; Ogino, A. & Tanaka, Y. 2007. Community analysis of hydrogen-producing extreme thermophilic anaerobic microflora enriched from cow manure with five substrates. *Applied Microbiology and Biotechnology,* 77: 213-222.

Yokoyama, H.; Ohmori, H.; Waki, M.; Ogino, A. & Tanaka, Y. 2009. Continuous hydrogen production from glucose by using extreme thermophilic anaerobic microflora. *Journal of Bioscience and Bioengineering,* 107: 64-66.

Yu, H. Q.; Zhu, Z. H.; Hu, W. R. & Zhang, H. S. 2002. Hydrogen production from rice winery wastewater in an upflow anaerobic reactor by using mixed anaerobic cultures. *International Journal of Hydrogen Energy,* 27: 1359-1365.

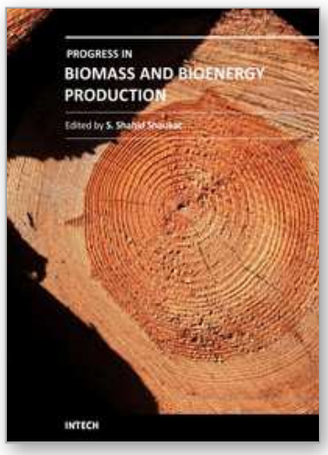
Zeidan, A.A. & van Niel, E.W.J. 2010. A quantitative analysis of hydrogen production efficiency of the extreme thermophile *Caldicellulosiruptor owensensis* OLT. *International Journal of Hydrogen Energy,* 35: 1128-1137.

Zeikus, J.G.; Hegge, P.W. & Anderson, M.A. 1979. *Thermoanaerobium brockii* gen. nov. and sp. nov., a new chemoorganotrophic, caldoactive, anaerobic bacterium. *Archives of Microbiology*, 122: 41-48.

Zhang, M.; Franden, M.A.; Newman, M.; Mcmillan, J.; Finkelstein, M. & Picataggio, S. 1995. Promising ethanologens for xylose fermentation—scientific note. *Appl Biochem Biotechnol,* 51-52: 527–536.

Zhao, C.; Karakashev, D.; Lu, W.; Wang, H. & Angelidaki, I. 2010. Xylose fermentation to biofuels (hydrogen and ethanol) by extreme thermophilic (70 °C) mixed culture. *International Journal of Hydrogen Energy,* 35: 3415-3422.

Zhao, C.; O-Thong, S.; Karakashev, D.; Angelidaki, I.; Lu, W. & Wang, H. 2009. High yield simultaneous hydrogen and ethanol production under extreme-thermophilic (70 °C) mixed culture environment. *International Journal of Hydrogen Energy,* 34: 5657-5665.



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Alternative energy sources have become a hot topic in recent years. The supply of fossil fuel, which provides about 95 percent of total energy demand today, will eventually run out in a few decades. By contrast, biomass and biofuel have the potential to become one of the major global primary energy source along with other alternate energy sources in the years to come. A wide variety of biomass conversion options with different performance characteristics exists. The goal of this book is to provide the readers with current state of art about biomass and bioenergy production and some other environmental technologies such as Wastewater treatment, Biosorption and Bio-economics. Organized around providing recent methodology, current state of modelling and techniques of parameter estimation in gasification process are presented at length. As such, this volume can be used by undergraduate and graduate students as a reference book and by the researchers and environmental engineers for reviewing the current state of knowledge on biomass and bioenergy production, biosorption and wastewater treatment.

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