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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 & Wetszstein, 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 homoethanologenic, 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 \textit{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 possibility 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 \textit{H}_2 is possible in several ways but today the main source of \textit{H}_2 is from fossil fuels and, to a lesser extent, by electrolysis from water. \textit{H}_2 is an interesting energy carrier and its combustion, opposite to carbon fuels, does not lead to emission of \textit{CO}_2. Biological production of \textit{H}_2 is possible through photosynthetic or fermentative processes (Levin et al., 2004; Rupprecht et al., 2006). This chapter will focus on biological \textit{H}_2 production by dark fermentation by thermophilic bacteria only. Fermentative production of \textit{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 \textit{H}_2 producing bacteria

Thermophilic bacteria can degrade many carbohydrates and produce various end products, among them both EtOH and \textit{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-Doudoroff 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 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).

![Simplified scheme of glucose degradation to various end products by strict anaerobic bacteria.](image)

Pyruvate is the end product of glycolysis and can be converted to fermentation products like H₂, 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 CO₂ 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 H₂ 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 H₂ 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 H₂ pathway is through PFOR because of thermodynamics hindrance of reoxidizing NADH (Jones, 2008). It is a well known phenomenon that the low H₂ yields observed by mesophilic and moderate thermophilic bacteria are due to the fact that H₂ production from either ferredoxin or NAD(P)H are thermodynamically unfavorable (Jones, 2008; Hallenbeck, 2009). The redox potential of Fd_red/Fd_ox couple depends on the microorganism and temperature involved. In nature, high partial pressures of H₂ are relatively uncommon because of the activity of H₂ scavenging microbes, e.g. methanogens or sulfate reducing bacteria (Cord-Ruwisch et al., 1988). This results in a low partial pressure of H₂ which is favorable for a complete oxidation of glucose to acetate and CO₂. At high temperatures, the influence of the partial pressure of H₂ is less on the key enzymes responsible for H₂ production. This is the main reason why extremophilic bacteria have been reported to produce up to 4 moles of H₂ 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 ferredoxin 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 H₂ concentrations inhibit H₂ 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 H₂ and EtOH production. The amount of H₂ 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 H₂:

\[
C₆H₁₂O₆ + 4 H₂O \rightarrow 2CH₃COO⁻ + 4H₂ + 2HCO₃⁻ + 4H⁺ \tag{1}
\]

If on the other hand the final product is butyric acid, the theoretical yield of H₂ is only two moles of H₂ per mole of glucose:

\[
C₆H₁₂O₆ + 2 H₂O \rightarrow CH₃CH₂CH₂COO⁻ + 2H₂ + 2HCO₃⁻ + 3H⁺ \tag{2}
\]

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

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C₆H₁₂O₆ + 4 H₂O \rightarrow 2CH₃COH + 2HCO₃⁻ + 4H⁺  \hspace{1cm} (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 H₂. If lactate is the only end product, no H₂ is formed:

C₆H₁₂O₆ + 4 H₂O \rightarrow \text{Lactate}⁻ + 2HCO₃⁻ + 4H⁺  \hspace{1cm} (4)

4. Thermophilic anaerobic bacteria – classification and physiology

In recent years, thermophilic anaerobic bacteria have gained increased attention as potential EtOH and H₂ producing microorganisms. Depending on optimal growth temperatures, thermophilic bacteria can be divided into several categories, e.g. moderate thermophiles (T_{opt} between 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 H₂ producing bacteria

There are relatively few genera of thermophiles that include bacteria with good H₂ and EtOH producing capacities. Among good EtOH producers are bacteria that belong to the genera of *Clostridium*, *Thermoanaerobacter* and *Thermoanaerobacterium* but good H₂ 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 H₂ 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 H₂ producing thermophiles known today. Later chapters deal with H₂ 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
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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. thermohydrosulphuricum* (now *Thermoanaerobacter thermohydrosulphuricum*) 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. thermosuccinigenes* (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. aciditolerans*, *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$_2$S (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, *Thermoanaerobacter 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; Lacs & Laword 1988a.b; Lamed & Zeikus, 1980a,b). H₂ production is usually low compared to EtOH by *Thermoanaerobacter* although *Thermoanaerobacter tengcongensis* has been described to produce up to 4 moles of H₂ 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. owensis* and *C. saccharolyticus* (German Collection of Microorganisms and Cell Cultures and references 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 H₂ production yields and rates compared to mesophiles and produce few other byproduct besides acetate. This makes *Caldicellulosiruptor* species excellent candidates for H₂ production. *C. saccharolyticus* and *C. owensis* have been extensively studied for H₂ 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. subterranea* 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 sheath like 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, CO₂ and H₂ 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. elfii*, *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 H₂ and EtOH

Apart from the above mentioned genera the capacity to produce EtOH and H₂ has been reported for many other genera. Examples are species within *Caloramator*, *Caldanaerobacter*,

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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), also with the main focus on the effect of solvents on EtOH production, e.g. EtOH tolerance. *Thermoanaerobacter 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). *Thermoanaerobacter ethanolicus* JW200 showed also very good EtOH yields from xylose and glucose at low (10 g L$^{-1}$) substrate concentrations, or


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.


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.