Combined hydrogen and ethanol production from sugars and lignocellulosic biomass by *Thermoanaerobacterium* AK$_{54}$, isolated from hot spring

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**Abstract**

Combined biohydrogen and bioethanol (CHE) production from monosugars, polymeric carbohydrates and hydrolysates made from various lignocellulosic biomasses was investigated by strain AK$_{54}$, a saccharolytic, thermophilic ethanol and hydrogen producing bacterium isolated from a hot spring in Iceland. Optimum growth conditions for the strain were between pH 5.0–6.0 and at 65 °C. As determined by full 16S rRNA analysis, strain AK$_{54}$ belongs to the genus *Thermoanaerobacterium*, most closely affiliated with *Thermoanaerobacterium aciditolerans* (99.0%). Effect of increased initial glucose concentration on growth and end product formation was investigated and good correlations were observed between increased substrate loadings and end product formation of up to 50 mM where clear inhibition was shown. The ability to utilize various carbon substrates was tested with positive growth on xylose, glucose, fructose, mannose, galactose, sucrose and lactose. The major end products in all cases were ethanol, acetate, lactate, hydrogen and carbon dioxide. By lowering the partial pressure of hydrogen during glucose degradation, the end product formation was directed towards hydrogen, acetate and ethanol but away from lactate. Hydrogen and ethanol production from hydrolysates from biomass (7.5 g L$^{-1}$ (dw)); cellulose, newspaper, grass (*Phleum pratense*), barley straw (*Hordeum vulgare*), and hemp (*Cannabis sativa* L.) was investigated. The biomass was chemically (acid/alkali) and enzymatically pretreated. The highest ethanol production was observed from cellulose hydrolysates (24.2 mM) but less was produced from lignocellulosic biomasses. Chemical pretreatment of biomass hydrolysates increased hydrogen and ethanol yields substantially from barley straw, hemp and grass but not from cellulose or newspaper. The highest hydrogen was also produced from cellulose hydrolysates or 6.7 mol-H$_2$ g$^{-1}$ TS pretreated with alkali (12.2 mol-H$_2$ g$^{-1}$ glucose equivalents) but of the lignocellulosic biomass, highest yields were from grass pretreated with base (4.9 mol-H$_2$ g$^{-1}$ TS).

**Keywords:**

Biohydrogen, Bioethanol, Combined hydrogen and ethanol production, Hydrolysate, Lignocellulosic Biomass

1. Introduction

Currently, the world’s energy demand is focused on the use of fossil fuels which are inevitably depleting [1–3]. Fossil fuels have the big disadvantage that they are high in sulfur, nitrogen and metal content and its burning results in extensive amounts of SO$_2$ and NO$_x$ emissions to the atmosphere. Additionally, CO$_2$ is released which is considered to have undesirable climatic consequences. Energy consumption is growing at rising rates at the same time, leading to increased interest of renewable alternatives to fossil-based fuels. Biohydrogen and bioethanol are promising CO$_2$ neutral types of biofuels since they are derived from renewable sources [4–6]. Biohydrogen has a great potential as a clean, renewable energy carrier and has higher energy content as compared to hydrocarbon fuels and water as the sole end product after combustion [7–9]. Production of bioethanol as fuel has increased in recent years; in 2005 around 54.2 billion liters of ethanol was produced, mostly from sugar (Brazil) and starch (USA) [5]. Techniques used today for bioethanol production depend on limited supply of raw material leading to interest in fermentation of lignocellulosic biomass (e.g. wood, straw and grasses), namely second generation ethanol production [10,11]. Production of biohydrogen and bioethanol through microbial fermentation are well known processes but thermophiles have many advantages compared to mesophiles of microorganisms concerning fast growth rates and their ability to degrade a broad variety of substrates. Furthermore, many thermophiles produce fewer types of undesired end products compared to mesophiles [12,13]. High values of hydrogen produced per mol of glucose utilized have been reported by the hyperthermophiles *Caldicellulosiruptor saccharolyticus* and *Thermotoga elfii*; 3.3–4.0 mol-H$_2$ mol carbohydrate [14,15] and by enrichment culture isolated from Icelandic hot spring; 3.2 mol-H$_2$ mol-glucose$^{-1}$ in semi-continuous batch reactor and 2.10 mol-H$_2$ mol-glucose$^{-1}$ in batch culture [16,17]. The highest ethanol yield reported is for the thermophilic bacteria *Thermoanaerobacter ethanolicus*; 1.9 mol-EtOH mol-glucose$^{-1}$ degraded [18] but several strains are capable of yields reaching 1.5 mol-EtOH mol-glucose$^{-1}$ [19].

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Hot springs are a potential source for hydrogen and ethanol producing microorganisms [16–19]. In this study, a thermophilic bacterium, efficient in ethanol (and hydrogen) production, is studied. Fermentation of various carbohydrates and hydrolysates from various complex biomasses investigated. Optimal conditions for ethanol and hydrogen production in terms of the partial pressure of hydrogen, temperature and pH were explored and kinetic parameters from glucose degradation were identified.

2. Materials and methods

2.1. Medium composition and strain identification

The strain was isolated from a hot spring (66°C, pH 5.3) in Grensdalur (South-West Iceland) and the isolation procedure has been published earlier [20]. The anaerobic medium used in all experiments was according to Orlygsson and Baldursson [21]. After isolation the isolate was analyzed for full 16S rRNA sequence analysis according to Vesteinsdottir et al. [22] and references therein. The strain was kept frozen in 30% glycerol until use in all experiments.

2.2. Determination of growth

Cell concentration was determined by measuring absorbance at 600 nm by Perkin Elmer spectrophotometer. Maximum (specific) growth rate (μmax) for each growth experiment was derived from the absorbance data (OD600). All experiments were done in duplicate.

2.3. Determination of pHopt and Topt

To determine the strain’s optimum pH for growth the pH was set to various levels in the range of 3.0–9.0 with increments of 1.0 pH unit by supplementing the medium with HCl or NaOH (1 M). For the pH determination the isolate was grown at 60°C. For the temperature determination the isolate was grown at pH 6.0 and temperature ranging from 30°C to 75°C. Optimal pH and temperature were thereafter used in all experiments performed. Experiments were done in 117.5 mL serum bottles with 50 mL liquid medium.

2.4. Effect of substrate concentration

To study the effect of substrate concentration, initial glucose concentration varied from 5 to 400 mM. Control samples did not contain glucose. Liquid samples were collected at the end of incubation time (5 days) to analyze final glucose concentration, pH and fermentation end products. Experiments were done in 59.0 mL serum bottles with 20.0 mL liquid medium.

2.5. Substrate spectrum utilization

The anaerobic medium used was supplemented with various filter sterilized substrates: glucose, fructose, galactose, mannose, xylose, ribose, arabinose, sucrose, lactose, lactate, formate, succinate, malate, pyruvate, oxalate, crotonate, glycerol, inositol, sorbitol, alanine, aspartate, glycine, glutamate, serine, threonine, histidine, cysteine (all 20 mM), starch, cellulose, xylan, pectin, casamino acids, peptone, beef extract, tryptone (all 2 g L−1). Growth was observed by increase in optical density which was measured at the beginning and at the end of incubation time (5 days). Fermentation end products were analyzed from experimental bottles where growth was detected. Experiments were done in 59.0 mL serum bottles with 20.0 mL liquid medium.

2.6. Growth kinetics of glucose degradation

Growth kinetic experiment was done for the strain using glucose (20 mM) as the sole carbon source. Growth was determined by measuring optical density as well as end product formation during time. Experiments were done in 117.5 mL serum bottles with 50.0 mL liquid medium.

2.7. Effect of liquid–gas volume ratio on hydrogen production

The influence of partial pressure of hydrogen (pH2) on hydrogen production was investigated using different media volumes from 5.0 mL to 90.0 mL in 117.5 mL serum bottles. Liquid samples were taken at the end of incubation time (5 days) to analyze fermentation end products and final glucose concentration.

2.8. Pretreatment of biomass and hydrolysate preparation

Hydrolysates (HL) were made from different biomasses: Whatman filter paper (cellulose), hemp (Cannabis sativa) – leaves and stem fibers, newspaper with ink, barley straw (Hordeum vulgare) and grass (Phleum pratense). Hydrolysate, made from Whatman paper, was used as a control sample as it consists of 99% cellulose. The preparation of the hydrolysates was according to Sveinsdottir et al. [19] yielding a final dry weight of 25 g L−1. Chemical pretreatment consisted of either acid (0.75% H2SO4) or base (0.75% NaOH) before autoclaving for 30 min (121°C). Control samples were not chemically pretreated, only heated. After autoclaving, the pH of the slurry was adjusted to 5.0 by adding either HCl or NaOH. Enzymatic hydrolysis was done by adding two enzymes to each bottle, Celluclast and Novozyme 188 (0.1 mL g−1 dw; 70 and 25 U g−1 of Celluclast and Novoyme 188, respectively), and incubated in water bath at 45°C for 68 h. After adjusting the pH to the pH optimum of the strain, hydrolysates were collected by filtering the liquid into sterile bottles, using 45 μm filters.

2.9. Fermentation of hydrolysates

Fermentation of hydrolysates by strain AK4 was done in 7.0 mL anaerobic medium in 117.5 mL serum bottles. The medium was supplemented with 3.0 mL of each hydrolysate (total liquid volume 10.0 mL) giving a final hydrolysate concentration of 7.5 g L−1. Control sample contained no hydrolysate.

2.10. Analytical methods

Hydrogen, ethanol and volatile fatty acids were measured by gas chromatograph as previously described [21]. Determination of glucose and lactate was performed by using Shimadzu high performance liquid chromatography (HPLC) according to Almarsdottir et al. [23]. Hydrogen was measured directly from the headspace of the experimental bottles and values are corrected for different liquid and gas head space used in the experimental set up.

3. Results

3.1. Phylogenetic characteristics of the strain

The result of the analysis of the complete sequence of 16S rRNA (1427 nt) is presented as a phylogenetic tree (Fig. 1), showing the position of strain AK4 as a member within the genus Thermoanaerobacterium. The closest relative is Thermoanaerobacterium aciditolerans (DSM 16487) with 99.0% homology.
3.2. Temperature and pH ranges

The strain had a relatively narrow temperature growth range of 55.0 °C to 70.0 °C with optimal temperature being 65.0 °C (maximum growth rate: 0.642 h⁻¹). No growth was observed below 55.0 °C or above 70.0 °C. The pH optimum was between 5.0 and 6.0. Below pH 4.0 and above pH 7.0 no growth was observed. The maximum growth rate ($\mu_{\text{max}}$) at pH 5.0 was 0.614 h⁻¹. At pH 6.0 the growth rate was slightly lower (0.574 h⁻¹).

3.3. Effect of substrate concentration

Table 1 shows a good correlation between low initial glucose concentrations (5.0–20.0 mM) and end product formation. However, at higher glucose loadings a clear inhibition is observed resulting in lower amounts of end product formation, insufficient glucose degradation and lowering of the pH.

At glucose concentration of 50 mM, only 14.7 mM were degraded and at higher concentrations only small fraction of the

Table 1

<table>
<thead>
<tr>
<th>Initial glucose (mM)</th>
<th>End product formation (mmol L⁻¹)</th>
<th>End glucose (mM)</th>
<th>End pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
<td>Acetate</td>
<td>Lactate</td>
</tr>
<tr>
<td>0</td>
<td>3.1 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td>6.7 ± 0.2</td>
<td>2.1 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>10</td>
<td>11.4 ± 0.3</td>
<td>3.1 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>20</td>
<td>20.4 ± 0.3</td>
<td>4.1 ± 0.1</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>50</td>
<td>11.4 ± 0.3</td>
<td>3.1 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>100</td>
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<td>4.1 ± 0.1</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>200</td>
<td>12.5 ± 0.4</td>
<td>3.1 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>400</td>
<td>11.7 ± 0.5</td>
<td>3.1 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
</tbody>
</table>

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glucose was degraded. Highest hydrogen accumulation was achieved when the glucose concentration was 20 mM (22.3 mmol H₂ L⁻¹). Ethanol production decreases from 20.4 mM to 11.4 mM when glucose concentration is increased from 20 to 50 mM. Further increase of glucose does not result in increased end products.

3.4. Substrate utilization

Table 2 shows the end product formation from substrates the strain could degrade. Of the carbon sources tested, strain AK54 only utilized the sugars. Degradation of all substrates resulted in ethanol and acetate as the main soluble metabolites while lactate was produced in smaller extent. Additionally, hydrogen and carbon dioxide were not produced. Fermentation of 20 mM of various C₅, C₆ and C₁₂ sugars showed that most ethanol and hydrogen were produced from the disaccharide lactose or 41.4 mM and 31.8 mmol L⁻¹, respectively. From glucose, ethanol and hydrogen production were 23.7 and 22.6 mmol L⁻¹, respectively. This corresponds to 1.03 and 1.02 mol-EtOH and mol-H₂ for every mol of glucose degraded (end product formation on yeast extract subtracted). Other end products were lactate and CO₂.

3.5. The dynamics of glucose degradation

Glucose was completely degraded in 24 h. The doubling time was 1.01 h ($μ_{max} = 0.684$ h⁻¹). The main end products produced were ethanol (23.1 mM) and hydrogen (17.2 mmol L⁻¹). The ethanol and hydrogen production rates were 2.5 mM EtOH h⁻¹ and 2.1 mmol H₂ L⁻¹ h⁻¹, respectively.

### Table 2

<table>
<thead>
<tr>
<th>Substrates</th>
<th>End product formation (mmol L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>Xylose</td>
<td>14.6 ± 0.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>23.7 ± 0.5</td>
</tr>
<tr>
<td>Galactose</td>
<td>31.7 ± 0.2</td>
</tr>
<tr>
<td>Fructose</td>
<td>19.3 ± 0.4</td>
</tr>
<tr>
<td>Mannose</td>
<td>25.3 ± 0.3</td>
</tr>
<tr>
<td>Lactose</td>
<td>41.4 ± 0.6</td>
</tr>
<tr>
<td>Sucrose</td>
<td>13.6 ± 0.1</td>
</tr>
</tbody>
</table>

3.6. Effect of partial pressure of hydrogen on hydrogen production

To investigate the influence of the partial pressure of hydrogen (pH₂) on hydrogen production, the strain was cultivated on glucose (20 mM) using different liquid/gas (L–G) volume ratios. Fig. 3 shows that the strain produces 1.8 mol-H₂ mol-glucose⁻¹ (45.0% of the theoretical yield) at the lowest L–G ratio, dropping to 0.95 mol-H₂ mol-glucose⁻¹ (23.8%) at the highest ratio.

The L–G ratio clearly affects the production of other volatile end products. Ethanol and acetate production decreases with increasing media volume compared to gas head space and hydrogen yields decrease but the flow of electrons is directed towards the production of lactate. Glucose was completely degraded in all cases. By using the fermentation data from the lowest and highest L–G the following equations are observed.

1.0 glucose $→$ 1.25 EtOH + 0.66 Acetate + 0.09 Lactate + 1.80 H₂ + 1.91 CO₂ (low L–G) (1)

1.0 glucose $→$ 0.70 EtOH + 0.41 Acetate + 0.45 Lactate + 0.95 H₂ + 1.11 CO₂ (high L–G) (2)

3.7. End product formation from hydrolysates

Strain AK54 was cultivated on hydrolysates (7.5 g L⁻¹) made from different types of complex biomass. The experiments were done in 10 mL hydrolysate media in 117.5 mL serum bottles, thus with L–G ratio of 0.09. Highest ethanol and hydrogen production was observed from cellulose but the glucose released by enzyme hydrolysis was not completely degraded, neither in hydrolysate without chemical pretreatment or in acid/base pretreated hydrolysates (Table 3).

The amounts of end products produced from cellulose are in good correlation to what was observed from glucose in earlier experiments (Tables 1 and 2; Fig. 2). Ethanol production from grass hydrolysates without chemical pretreatment was 68% of the ethanol produced cellulose hydrolysates. Pretreatment of grass however increased end product formation significantly and reached similar levels as for cellulose hydrolysates. Additionally, no glucose was detected at the end of fermentation in the grass samples and the same was true for all other samples except for the cellulose hydrolysates. Other hydrolysate samples gave lower end product yields, highest observed on hemp stem hydrolysates but lowest on hemp leaves. Pretreatment however, enhanced end product for-
mation in very different manner, depending on the type of biomass. The most profound increase was observed for barley straw (ethanol increases e.g. for more than 100%) but only slightly higher values were observed on paper hydrolysates pretreated with acid and lower values were observed on the alkali pretreated paper hydrolysates.

4. Discussion

Hydrogen and ethanol producing bacteria have gained much attention in recent years because of increased interest in renewable energy sources. High values of hydrogen produced per mol of glucose utilized have been reported from the pure cultures of hyper-thermophiles [14] and thermophilic enrichment cultures [16,17]. The highest ethanol yield reported is by the thermophilic bacteria *T. ethanolicus*, 1.9 mol-ETOH mol-glucose⁻¹ [18] but several strains are capable of yields reaching 1.5 mol-ETOH mol-glucose⁻¹ [19].

Strain AK54 was isolated from Icelandic hot spring with glucose as carbon source. Ethanol and hydrogen production capability by the strain on mono- and disaccharides and hydrolysates from various complex biomasses was studied in batch cultures. Phylogenetic studies on the strain reveals that the bacterium belongs to the genus *Thermoanaerobacterium* (Fig. 1), most closely affiliated with *T. aciditolerans* (99.0%), a well known ethanol and hydrogen producer. This bacterium has been reported to have similar low pH optimum as strain AK54 [24]. Other strains closely related to strain AK54 (0.5–1.7% difference within the strains) are *Clostridium thermoanamylolyticum, Thermoanaerobacterium oateae*, *Thermoanaerobacterium islandicum* and *Thermoanaerobacterium thermosulfurigenes*. All have similar phenotypic characteristics as strain AK54 and most of them have been reported to produce ethanol and hydrogen [20,25,26].

Environmental factors like high initial substrate concentration and the partial pressure of hydrogen can play an important role in both ethanol and hydrogen production [12,27–30]. Fermenting microorganisms can have limited tolerance towards increased substrate loadings [31] as was clearly observed in present study when strain AK54 was cultivated on different initial concentrations of glucose varying from 5 to 400 mM. Results show that end product formation increases proportionally when glucose concentration is 5–20 mM (Fig. 2). Above 20 mM concentration, a no further increase is observed which could possibly be explained by substrate inhibition [32] or inhibition by the low pH caused by accumulation of acetate and lactate. Hydrogen production is also sensitive to hydrogen concentrations and are subject to end product inhibition [33]. Various headspace liquid media volumes containing 20 mM glucose in 117.5 mL serum bottles were tested in order to investigate the effect of hydrogen partial pressure on the H₂ production. The culture containing 5 mL liquid (highest gas volume headspace) showed the highest hydrogen yields (45.0%) but the culture with 90 mL liquid (lowest gas volume) showed only 24% hydrogen yields. Similarly, both ethanol and acetate decreased with less gas headspace whereas lactate increased. It is well known that thermophilic bacteria shift their end product formation upon changes in partial pressure of hydrogen. This has been reported for *Caldercellulosiruptor saccharolyticus* and *Caldercellulosiruptor owensis* where increased pH₂ led to increased lactate formation [15,33]. Other bacteria produce ethanol to dispose excess of reducing equivalents like *Thermoaerobacter tengcongensis* [34]. Obviously, strain AK54 does not produce more ethanol under high pH₂ but dispose excess electrons to lactate formation.

One of the main reasons of increased interest on thermophiles concerning the production of biofuels is their broad substrate spectrum capacity. When lignocellulosic biomass is hydrolyzed the main sugars are glucose and xylose [35], both of which strain AK54 [34]. Other strains closely related to strain AK54 (0.5–1.7% difference within the strains) are *Clostridium thermoanamylolyticum, Thermoanaerobacterium oateae*, *Thermoanaerobacterium islandicum* and *Thermoanaerobacterium thermosulfurigenes*. All have similar phenotypic characteristics as strain AK54 and most of them have been reported to produce ethanol and hydrogen [20,25,26].

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One of the main reasons of increased interest on thermophiles concerning the production of biofuels is their broad substrate spectrum capacity. When lignocellulosic biomass is hydrolyzed the main sugars are glucose and xylose [35], both of which strain AK54 degrades. The strain degrades all sugars tested except for arabinose and ribose but was not capable of degrading any of the polymeric substances tested. Many of the other bacteria within *Thermoaerobacterium* are however capable of degrading pectin and xylan, e.g. *T. aciditolerans* [24]. End product formation was the same as from glucose but highest ethanol and hydrogen concentrations were observed from the disaccharide lactose.

Hydrogen production from lignocellulosic biomass has got increased attention recently. Several studies on thermophilic bacteria growing on untreated wastewater cellulose have shown yields between 0.82 and 1.24 mol-H₂ mol-glucose⁻¹ equivalents [36,37]. Co-culture studies of *Clostridium thermocellum* and *Thermoanaerobacterium saccharolyticum* on hydrogen production from microcrystalline cellulose resulted in 1.8 mol-H₂ mol-glucose⁻¹ equivalents [38]. Other studies on pretreated hydrolysates from lignocellulosic biomass have shown higher yields. Lalaurette et al. [39] showed hydrogen yields of 1.64 mol-H₂.
mol-glucose\(^{-1}\) equivalent from corn stover hydrolysates (pretreated with dilute sulfuric acid) by \textit{C. thermocellum}. Mixed culture studies (35 and 50 °C) on the same biomass pretreated with steam explosion and dilute sulfuric acid resulted in 2.84 mol-H\(_2\) mol-glucose\(^{-1}\) equivalents [40]. Acid hydrolysis of corn stover and subsequent fermentation of the hydrolysate by \textit{T. saccharolyticum} resulted in 2.24 mol-H\(_2\) mol-glucose\(^{-1}\) [41]. Fermentation of hydrolysates from Miscanthus by \textit{C. saccharolyticus} and \textit{Thermotoga neutrophilica}, pretreated by alkali, resulted in 3.4 and 3.2 mol-H\(_2\) mol-glucose\(^{-1}\) equivalent, respectively [42].

It was decided to use 7.5 L dw\(^{-1}\) of biomass hydrolysates in present investigation. Assuming a 100% hydrolysis of cellulose, 463.3 mM of glucose would be expected in control hydrolysates. This is slightly higher compared to glucose values analyzed in the cellulose hydrolysates and is clearly too high for a complete degradation by strain AK\(_{54}\) since between 6.9 and 15.9 mM of glucose were not degraded. This inhibition of high substrate correlates well with the results obtained with different initial glucose concentrations (Table 1). Additionally, the hydrolysis is not so successful in cellulose hydrolysates pretreated with acid as compared to base and untreated sample since only 29.5 mM of glucose were analyzed in the hydrolysate. The strain produced ca. 1 mol-EtOH mol-glucose\(^{-1}\) (mean values from all three cellulose hydrolysates; control subtracted). Other end products were acetate, lactate and hydrogen giving the stoichiometry (glucose values used are values analyzed after enzyme treatment and after fermentation):

\[
\begin{align*}
1.00 \text{ Glucose} & \rightarrow 0.99 \text{ EtOH} + 0.55 \text{ Acetate} + 0.20 \text{ Lactate} \\
& + 1.96 \text{ H}_2 + 1.54 \text{ CO}_2
\end{align*}
\]

This is in good relation with fermentation of pure glucose showed earlier and the high hydrogen yields (1.96 mol-H\(_2\) mol-glucose\(^{-1}\) equivalents or 12.2 mol-H\(_2\) TS cellulosic\(^{-1}\)) can be explained by low L–G ratio in the experimental set up. The hydrogen yields on lignocellulosic biomass varied between 0.4 and 4.9 mol-H\(_2\) TS\(^{-1}\) from hemp leaves (no pretreatment) and grass (alkali pretreatment), respectively.

It has been reported that pretreatment is important when wheat straw is used for ethanol production [43]. This was clearly observed in present study on barley straw where the ethanol production increased from 5.4 mM (only heat pretreatment) to 17.9 and 19.4 mM (control subtracted from values in Table 3) where H\(_2\)SO\(_4\) and NaOH were used, respectively. The pretreatment also affected ethanol production from grass where it increased from 15.1 mM to 24.9 mM (2.0–3.3 mM g\(^{-1}\) grass HL) when H\(_2\)SO\(_4\) was used. In other HL’s, the pretreatment had less effect, especially on paper where ethanol production was similar in all cases, varying from 9.2 to 10.9 mM. Several investigations on ethanol production by thermophilic bacteria from lignocellulosic biomass have been published recently. \textit{C. thermocellum} produced between 4.6 and 8.1 mM ethanol g\(^{-1}\) dw of alkali pretreated paddy straw, sorghum stover and corn stubs [44], \textit{Thermoanaerobacter mathranii} produced 5.3 mM g\(^{-1}\) dw of wheat straw, pretreated with alkaline wet oxidation [45] and \textit{Thermoanaerobacterium AK17} 2.9 mM g\(^{-1}\) dw of grass hydrolysates (without chemical pretreatment) [19], later optimized to 5.5 mM g\(^{-1}\) dw (pretreated with acid) [46]. The highest ethanol yields reported from lignocellulosic biomass are by \textit{Thermoanaerobacter BG1L1}, 9.2 mM g\(^{-1}\) corn stover and wheat straw but these values are from sugar equivalents consumed in the process [47].

There are mainly two methods to produce ethanol from lignocellulosic biomass, i.e. hydrolysis-fermentation and hydrolysis-gasification [48]. The main drawback of using lignocellulosic biomass for hydrolysis and fermentation is the low ethanol yield mainly due to the high content of lignin present and high cost of pretreatment. Additionally, pentose sugars are often substantial proportion of the hemicellulosic fraction of the hydrolysates produced. The use of broad substrate spectrum thermophilic bacteria can however increase yields substantially. Gasification of lignocellulosic biomass renders carbon monoxide and hydrogen which can be both biologically and chemically converted to ethanol. The main advantages of using gasification for ethanol production from lignocellulosic biomass is reduced cost and almost complete biomass conversion. However, yields are still relatively low and commercialization of both processes is still under development.

5. Conclusion

An anaerobic thermophilic bacterium was isolated from an Icelandic hot spring. Batch culture experiments show that the strain produces both ethanol and hydrogen from various sugars present in lignocellulosic biomasses but its production capacity is dependent on substrate concentration and the partial pressure of hydrogen. Maximum hydrogen production from glucose was achieved using L–G ratio of 0.04 and inhibition in end product formation was observed at glucose loadings above 20 mM. Clearly the major disadvantage of thermophilic bacteria for a large scale ethanol production from lignocellulosic biomass is their intolerance towards ethanol and high substrate concentrations. This drawback can however possible to overcome by using fed-batch or continuous cultures. Thus, strain AK\(_{54}\) seems to be a reasonable good ethanol and hydrogen producer and is interesting to test further in systems where both fuel types (ethanol and hydrogen) are product targets.

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