Bio-hydrogen production from cellulosic biomass by continuous dark fermentation

著者	Samir Ibrahim Gadow
学位授与機関	Tohoku University
学位授与番号	環博第80号
URL	http://hdl.handle.net/10097/58202

	サミール エブラヒム ガードウ
氏名	Samir Ibrahim Gadow
授与学位	博士（環境科学）
学位記番号	
学位授与年月日	平成 25 年 9 月 25 日
学位授与の根拠法規	学位規則第 4 条第 1 項
研究科，専攻の名称	東北大学大学院環境科学研究科（博士課程）環境科学専攻
学位論文題目	Bio-hydrogen production from cellulosic biomass by continuous dark fermentation
指導教員	東北大学教授　李　玉友
論文審査委員	主査　東北大学教授　李　玉友　　東北大学教授　境田　清隆 東北大学教授　西村　修 （工学研究科）

論 文 内 容 要 旨

Energy is one of the vital inputs to the socioeconomic development of any country. Compared to other current fuel to energy conversion technologies, the higher efficiency of the conversion of hydrogen to electrical energy due to its high calorific value makes hydrogen a potential substitute for fossil fuels. Besides being energy efficient, it is also carbon-free, non-polluting, and recyclable. Over 95% of world's hydrogen demand is now being derived from fossil fuels. This presents a major problem since the same amount of CO_2 as is formed in the combustion of fossil fuels is released. Biological processes have the potential to generate hydrogen from renewable and/or recyclable feed-stocks. Therefore, this study provides a sustainable solution to generate hydrogen from cellulosic materials which consider a major pollutant from different human activity sectors as a sustainable and clean energy. The effect of temperature on the hydrogen fermentation of cellulose was evaluated by a continuous experiment using a mixed culture without pretreatment. The experiments were conducted at three different temperatures, which were mesophilic (37±1°C), thermophilic (55±1°C) and hyper-thermophilic (80±1°C), with an influent concentration of cellulose of 5 g/l and a hydraulic retention time (HRT) of 10 days. A stable hydrogen production was observed at each condition. At 37±1°C, the maximum hydrogen yield was 0.6 mmol H_2/g cellulose. However, at 55±1 °C and 80±1°C, the maximum hydrogen yields were 15.2 and 19.02 mmol H_2/g cellulose, respectively. While 26% of the biogas was methane under the mesophilic temperature, no methane gas was detected under both the thermophilic and hyper-thermophilic temperatures. The results show that operational temperature is a key to sustainable bio-hydrogen production and that the thermophilic and hyper- thermophilic conditions produced better results than mesophilic condition. In industry, it is important for systems to be able to cope with such unexpected interruptions as maintenance and power failure. Therefore, the previous systems were operated for 164 days to investigate the effect of temperature and temperature shock on the cellulosic-dark hydrogen fermentation by mixed microflora. During steady state condition, the sudden decreases in the fermentation temperature occurred twice in each condition for 24 h. The results show that the 55±1 and 80±1°C presented stable hydrogen yields of 12.28 and 9.72 mmol /g cellulose, respectively. However, the 37±1°C

presented low hydrogen yield of 3.56 mmol/g cellulose and methane yield of 5.4 mmol/g cellulose. The reactor performance under 55±1°C or 80±1°C appeared to be more resilient to the sudden decreases in the fermentation temperature than 37±1°C. The experimental analysis results indicated that the changing in soluble by-products could explain the effect of temperature and temperature shock, and the thermophilic temperature is expected having a better economic performance for cellulosic-hydrogen fermentation. Based on our previous results, a long-term continuous thermophilic (55±1°C) cellulosic-hydrogen fermentation using a continuous stirred tank reactor (CSTR) by anaerobic mixed microflora was carried out. The results show that the system reached a steady state condition after 60 days. A stable hydrogen yield of 10.9±0.23 mmolH_2/g cellulose was maintained for 190 days with acetate, butyrate and ethanol as main soluble byproducts. The microbial community structures during the steady state were analyzed by 16S rRNA gene cloning and sequencing in the CSTR. The cloning results show that a total of 21 OTUs were detected from a total 104 clones. According to analysis results, the 70% of the microbial community were able to hydrolyze the cellulose effectively such as *Pedobacter* sp., *T. thermosaccharolyticum*, *Enterobacter cloacae* and *clostridium* sp. In particular, *T. thermosaccharolyticum* generates hydrogen from cellulose effectively because it has β-Glucosidase, which is an important enzyme in the cellulase enzyme system. It also plays an important role in hydrolyzing cellobiose to fermentable glucose. The purified β-Glucosidase enzyme is reported to be stable over a pH range of 5.2–7.6, had a 1 h half-life at 68°C, and its optimal activity is at pH 6.4 and 70°C. The phylogenetic tree in Figure 3 illustrates that twenty one OTUs were affiliated with the nine strains of *T. thermosaccharolyticum, Enterobacter cloacae, Aeromonas salmonicida, Pedobacter* sp, *Delftia* sp., *Clostridium* sp., *Flavobacterium* sp., *Parabacteroides* sp., and *Acidovorax sp.* The eight OTUs form a cluster with 100% bootstrap value with the five known species of *Thermoanaerobacterium, Clostridium, Aeromonas and Delftia.* A cluster (76% bootstrap value) appeared with nine OUTs with the two known species of *Enterobacter* and *Acidovorax.* The rest of OUTs form a cluster with 100% bootstrap value with the three known species of *Pedobacter, Flavobacterium,* and *Parabacteroides.* The experimental results show that the cellulolytic bacteria (*Thermoanaerobacterium, Clostridium, Enterobacter* and *Flavobacterium*) and none cellulosic bacteria (*Aeromonas, Delftia, Parabacteroides and Acidovorax*) improved the system performance to produce hydrogen and degrade the cellulose by co-metabolism effects. A total of twenty six batch experiments were conducted to investigate the activity of thermophilic H_2 producing mixed microflora to the temperature variation. The experiment results show that the cellulosic-hydrogen mixed microflora utilized the cellulose or glucose in a wide range of temperature from 35 to 65 °C, with a sharp increase was found with the maximum activity of 521.4mL H_2/g VSS·d at 55 °C. However, the performance of HPB was negatively affected by increasing fermentation temperature after 55 °C and completely inhibited at 70 °C. The activation energy for cellulose and glucose were estimated at 103 and 98.8 kJ/mol, respectively. A continuous stirred tank reactor was used for the dark hydrogen fermentation of cellulose by mixed microflora at hyper-thermophilic temperature (70±1°C) for 240 days. A total of twenty six batch experiments were conducted to investigate the effect of temperature on the activity of cellulosic-hydrogen producing bacteria. The results show that the system reached a steady state condition after 90 days. A stable hydrogen yield of 7.07±0.23 mmolH_2/g cellulose was maintained for 150 days with acetate, butyrate, ethanol and propionate as main soluble byproducts. The cloning results show that a total of 6 OTUs were detected from a total 100 clones. Eighty five clones of cellulose-degrading mixed culture were composed of microbes closely affiliated to genus *clostridium*. The dominant cellulolytic bacterium was *Thermoanaerobacterium thermosaccharolyticum*. This explains the ability of this microbial community to degrade cellulose effectively to hydrogen gas. These results supported the data in the literature: the *T. thermosaccharolyticum* generates hydrogen from cellulose effectively because it has β-Glucosidase, which is an important enzyme in the cellulase enzyme system. It also plays an important role in hydrolyzing cellobiose to fermentable glucose. The purified β-Glucosidase enzyme is reported to be stable over a pH range of 5.2–7.6, had a 1 h half life at 68°C, and its optimal activity is at pH 6.4 and 70°C. This explains why the optimal temperatures range was 60-70°C when using such mixed microflora. *Thermoanaerobacter tengcongensis* was detected with a 10% abundance in the microbial community structure. This organism had a unique combination of hydrogenases, a ferredoxin-dependent [NiFe] hydrogenase and an NADH-dependent Fe-only hydrogenase for H_2 formation [40]. Additionally, *T. tengcongensis* growth occurred at temperatures between 50 and 80 °C, with an optimum around 75 °C; at pH values between 5.5 and 9.0. The ethanol produced during cellulose fermentation could be attributable to a group of anaerobic bacteria known as *Acetobacterium* and several *Clostridia* such as *Moorella sp*, which can grow autotrophically on H_2 and CO_2 with acetate production by the acetyl-CoA pathway. In fact, Moorella *thermoacetica* was detected in the microbial community with 99% similarity and 10% abundance. This behavior of *Moorella sp.* has been confirmed by different reports where, when, isolated from a mud sample, it produced acetate and ethanol from H_2 and CO_2 at a thermophilic temperature at pH 5. Our cloning results show also the genus *Enterobacter* could grew at these conditions and that hydrogen was generated from cellulose hydrolysate. The phylogenetic tree in Figure 4 illustrates that five OTUs were affiliated with the family *Thermoanaerobacteriaceae*, which belongs to order *Thermoanaerobacteriales* of class *Clostridia*. The five OTUs form a cluster with 90% bootstrap value with the three known species of *Thermoanaerobacterium* and *Moorella*. The OTU-D_A11, OUT-C_G11 and OUT-C_E07 have 98% similarity with *T. thermosaccharolyticum* and *T. saccharolyticum*. The optimum growth for all *Thermoanaerobacterium* species was reported at 55–70 ∘C and at pH values of 5.2–7.8. Several species of *Thermoanaerobacterium*, including *T. thermosaccharolyticum*, *T. saccharolyticum*, *Enterobacter sp*, are known of their H_2 producing capabilities and ability to degrade cellulose. The cellulosic-hydrogen producing bacteria were able to utilize the cellulose or glucose within a wide range of fermentation temperatures (45-80°C) to produce hydrogen. The activation energy for cellulose and glucose were estimated at 133.2 and 117.7 kJ/mol, respectively. Our results suggest that thermophilic or hyper-thermophilic bacteria play an important role in increasing the effectiveness of cellulose degradation to hydrogen energy, those systems are considered to have a good economic performance, and therefore has potential for future large-scale. A seventy eight standardized batch experiments were used to determine hydrogen producing activity for three different anaerobic mixed microflora, which taken from three different running acidogenic reactors operated under 37+1°C, 55+1°C and 70+1°C, using cellulose and glucose as sole of carbon source. Analysis of 16S rRNA sequences showed that the cellulolytic hydrogen-producing bacteria were close to *Enterobacter* genus in mesophilic culture and *Thermoanaerobacterium* genus in thermophilic and hyper-thermophilic cultures. The MC was able to utilize the cellulose to produce methane gas within a temperature range between 25 to 45 °C and hydrogen gas from 35 to 60 °C with maximum hydrogen producing activity obtained at 55 °C. The TC utilized the cellulose in a wide range of temperature from 35 to 65 °C, with a sharp increase was found between 50 and 55°C and maximum activity of 521.4mL H_2/g VSS·d at 55 °C. However, the performance of HPB was negatively affected by increasing fermentation temperature after 55 °C and completely inhibited at 70 °C. The HC was able to utilize the cellulose within a wide range of fermentation temperatures (45-80 °C) with maximum activity of 489.4 mL H_2/g VSS·d at 65 °C. the activation energy is estimated using regression (R^2, 0.98 and 0.92) to be 118.7 and 86.8 kJ/mol by MCDB, (R^2, 0.93 and 0.91) to be 103 and 98.8 kJ/mol by TCHPB and (R^2, 0.93 and 0.94) to be 133.2 and 117.7 kJ/mol by HCHPB for cellulose and glucose, respectively. It is possible that fermentative hydrogen production systems may be made more economical by combining different processes such as anaerobic oxidation of fermentation byproducts (Acetate and Ethanol). The microorganisms are needed with hydrogen productivity by microbial design strategies. To realize the potential of fermentative hydrogen production from organic wastes, an enriched microbial culture of hydrogen producers needs to be developed. In addition, the following technical barriers must be addressed; Understanding the competitive between hydrogen producers and hydrogen consumers bacteria in the mixed culture environment; Understanding the biological pathways relevant to hydrogen production; Improved microbial design by operation parameters to overcome biological limitations; Characterization, identification and isolation of hydrogen producing microbes capable of fermenting organic waste/wastewater

博士論文審査結果の要旨及びその担当者

論文提出者氏名	Samir I.M. Gadow（サミール I.M. ガードウ）
論　文　題　目	Bio-hydrogen production from cellulosic biomass by continuous dark fermentation (暗条件での連続式発酵によるセルロース系バイオマスからのバイオ水素生産)
論文審査担当者	主査　教授　李　玉友 教授　境田　清隆　　教授　西村　修 （工学研究科）

論文審査結果の要旨

近年、再生可能エネルギーの開発と応用が注目を集めるようになった。バイオマスからグリーンな生物燃料を生成する技術は世界的に活発に研究されており、とりわけ暗条件での水素発酵が重要な研究課題の一つとなっている。セルロースは自然界に大量に存在するバイオマス資源の一つであり、水素発酵の重要な原料である。本論文は、環境微生物の代謝機能を利用してセルロースから水素を効率よく生成する技術を開発することを目的として、一連の室内実験を行うことで、セルロースの水素発酵に及ぼす温度の影響、基質濃度の影響、温度ショックの影響を検討したとともに、中温、高温、超高温の３つの温度範囲における水素生成活性、最適温度および微生物群集構造について研究した。全論文が８章からなる。

第１章は総論であり、バイオマスエネルギーの課題を提起し、本研究の意義と目的について説明している。

第２章「文献レビュー」では、バイオマスエネルギー開発における水素生産を取り上げ、水素発酵の原理および関連微生物の研究進展についてレビューし、セルロースから水素発酵を行うための技術課題について整理した。

第３章「セルロースの水素発酵に及ぼす温度の影響」では、完全混合型反応槽を３つ組み立て、それぞれ中温（37℃）、高温（55℃）および超高温（80℃）の条件で連続実験を行い、３つの温度条件における水素発酵反応の化学量論式を把握したとともに、高温と超高温条件での水素発酵の優位性を明らかにした。これらの知見はセルロースの水素発酵の応用設計に基礎的データを提供するものである。

第４章「セルロースの連続式水素発酵に及ぼす温度ショックの影響」では、 中温（37℃）、高温（55℃）および超高温（80℃）の条件で水素発酵の連続運転を行っている反応槽に、それぞれ一時的停電による温度ショックを２度と与えて反応系への影響およびその回復過程を検討した。その結果、温度が高いほどショック影響が強く、回復に時間がかかること、また同じ温度条件では２度目のショック影響が低かったことを明らかにした。これは全く新しい知見として高く評価できる。

第５章「高温水素発酵バイオリアクターの長期運転性能と微生物群集構造の特徴」では、連続式水素発酵バイオリアクターを高温（55℃）条件で 240 日間以上長期運転を行い、水素発酵の安定性を実証したとともに、安定状態における水素生成収率をはめて、高温反応系における微生物群集構造の特徴解析や活性度を明らかにした。

第６章「超高温水素発酵バイオリアクターの長期運転性能と微生物群集構造の特徴」では、連続式水素発酵バイオリアクターを高温（70℃）条件で 240 日間以上長期運転を行い、水素発酵の安定性を実証したとともに、安定状態における水素生成収率をはめて、超高温反応系における微生物群集構造の特徴解析や活性度を明らかにした。

第７章「異なる嫌気性混合培養系における水素生成活性および微生物群構造の比較」では、中温（37℃）、高温（55℃）および超高温（70℃）条件でそれぞれ長期安定運転を実現した連続式水素発酵バイオリアクターにおける混合微生物系の群集構造をクロニング法で解析したとともに、それぞれの微生物系によるグルコースとセルロースの代謝活性を把握して水素発酵のための最適温度を提示した。

第８章「総括」では、本論文の主な成果を総括し、水素発酵の応用展望を述べている。

以上のように、本論文は、セルロースからの水素発酵を異なる温度条件で実施して、それぞれの条件における発酵性能を把握したとともに、中温、高温、超高温の３つの温度範囲にわたり最適温度および微生物群集構造も明らかにした。これらの研究成果はバイオマスのエネルギー利用に活用でき、環境科学と技術の発展に寄与するところが少なくない。

よって、本論文は博士(環境科学)の学位論文として合格と認める。