学 位 論 文 内 容 の 要 旨	
学位論文題目	Development of Bio-Hydrogen Production Process from Wood Material as a Renewable Feedstock
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Fossil fuel energy crisis and awareness of climate change are concerned recently; the development of clean alternative energy sources is now significantly interested worldwide. Bio-hydrogen (H_2) produced from lignocellulosic biomass is one of promising candidate due to its clean, CO_2-free, and high efficiency energy carrier to meet the future needs. However, lignocellulosic materials require pretreatment and hydrolysis prior using as a feedstock in dark fermentation.

The waste from disposable wooden chopsticks (DWC) was represented as wood waste and investigated in this study. A thermophilic anaerobic mixed culture obtained from a hot spring was used as inoculum for fermentative H_2 production, and its optimum initial pH and temperature were determined to be 7.0 and 50°C, respectively. During the fermentation, acetic and butyric acids were also produced along with H_2 production, thus it could be concluded that acetate-butyrate fermentation occurred during H_2 production by mixed culture used in this study. Moreover, microbial community of the thermophilic anaerobic mixed culture had been characterized, *Thermoanaerobacterium* spp. were the H_2-producing bacteria mainly present in the fermentation process.

The feasibility of using DWC as a feedstock for fermentative H_2 production was established in this study. H_2 production from DWC may be enhanced by removing lignin and increasing the porosity of the material prior to enzymatic hydrolysis. Alkaline pretreatment, used to delignify DWC waste, were investigated in this study. The effects of NaOH concentration, temperature and retention time were examined and it was found that pretreatment time had no effect on lignin removal or carbohydrate released in enzymatic hydrolysate. The highest percentage of lignin removal (41%) was obtained with 2% NaOH at 100°C, correlated with the highest sugar released (67 mg/g pretreated DWC) in the enzymatic hydrolysate. Moreover, the surface structure of the pretreated DWC was changed from smooth to rough in comparison to raw DWC. These evidences supported that alkaline pretreatment degraded lignin mainly from

the cellulosic biomass, and this allowed enzyme to obtain easier access to cellulose molecules during enzymatic hydrolysis. Most of inhibitors (phenolic compounds) were released into the pretreatment liquid phase, so that the total phenolics content in the enzyme hydrolysate was much lower. Thus, detoxification was not required after pretreatment; hydrolysate could be used directly as a feedstock for H_2 production in this study. The enzymatic hydrolysate was then used as substrate for H_2 production by anaerobic mixed culture enriched from hot spring and yielded 8 mL H_2/g pretreated DWC (6.4 mL H_2/g DWC).

According to H_2 production yield was low; it might due to the fact that low sugar concentration was obtained in enzymatic hydrolysate. So that the enzymatic hydrolysis step needed to be improved the efficiency by optimizing its condition. In order to optimize the condition of the enzymatic hydrolysis of alkaline pretreated DWC, response surface methodology is an efficient experimental tool used to determine the optimal conditions of numerous variables. In the present study, cellulase dosage, β-glucosidase dosage, Tween 80, and hydrolysis time, were found to have a significant effect on enzymatic hydrolysis based on the Plackett-Burman design (PBD). Those factors were subsequently investigated on the optimal levels by a central composite design (CCD), which was determined at 36 FPU/g pretreated DWC of cellulase, 53 CBU/g pretreated DWC of β-glucosidase, 0.4 g/g pretreated DWC of Tween 80 for 105 h. Under optimal conditions, glucose and reducing sugar yielded at 121.7 and 435.8 mg/g pretreated DWC, respectively. Reducing sugar yield was increased 4.6 fold after optimization of enzymatic hydrolysis condition.

In order to enhance H_2 production from enzymatic hydrolysate by a thermophilic anaerobic mixed culture, optimization of a medium composition was employed in this section. Seven medium components were screened using PBD, three out of seven components (yeast extract, phosphate buffer, and $FeSO_4 \cdot 7H_2O$) were found to have a significant effect on H_2 production. These components subsequently optimized their concentration via response surface methodology using CCD. The maximum H_2 production was achieved at 1,949 mL H_2/ L pretreated DWC hydrolysate under optimum conditions using 6.74 g/L yeast extract, 29.62 mM phosphate buffer and 0.05 g/L $FeSO_4 \cdot 7H_2O$. In addition, the results found that fermentation pathway was possibly shifted from acetate to acetate-ethanol type fermentation when the concentration of yeast extract was increased together with increasing the concentration of

yield approximately 40 mL H_2/g pretreated DWC (32 mL H_2/g DWC). The results in this study show a potential application using DWC as a renewable feedstock for fermentative H_2 production. Furthermore, this process could be applied on other hardwood waste i.e. wood chips, bark form forest residue; saw dust from furniture factory and wooden waste from demolishing construction, etc. Even though, the cost operation was considered and found relatively high in this study since the initial phase of development of H_2 production process from wood material was just investigated by this work. However, there are a feasibility to be improved the fermentative H_2 production process by several aspects and efforts in order to be more applicable in practice.

【学位論文内容の要旨】

　近年，化石燃料の使用にともなう二酸化炭素の排出によって地球温暖化が大きな問題となっており，それゆえにクリーンエネルギーである水素に注目が集まっている．水素の生産方法としては種々あるが，最近注目されているのはバイオマスを原料としたリニューアブルな水素生産である．水素はエネルギーとして使用する際には水しか排出しないものの，その生産時には必然的に二酸化炭素が副生する．しかしながら，水素生産にバイオマスを原料とすることで，その副生する二酸化炭素さえもカーボンニュートラルとすることができ，極めて持続可能的にエネルギーを得ることができる．様々なバイオマスの中で，最も利用が進んでいないのが木質系バイオマスであり，本研究ではそれ（本研究では割り箸）を対象に，水素生産の方法としては嫌気性微生物による生物学的水素発酵を用いた水素生産プロセスの開発を行った．

　本論文の構成と内容は以下の通りである．
　第1章では，研究の背景，目的および論文の構成について述べている．
　第2章では，従来の研究についてまとめた．
　第3章では，水素発酵に適する微生物を大分県別府温泉の温泉湧出口付近の底泥からスクリーニングし，その最適水素発酵条件を求めた．実験結果から，最適条件は初期 pH が 7.0 で，培養温度が 50℃であることが明らかとなった．また，優占する水素発酵細菌は *Thermoanaerobacterium* spp.であることが分子生物学的手法により明らかとなった．次に，木質系バイオマス（本研究では割り箸）はそのままでは水素発酵できないことから，その前処理方法の最適化（コストを含む）を行い，2%の NaOH，100℃で 30 分の処理条件であることを明らかにした．上記の結果を用いて，水素発酵を行ったところ 8 mL-H_2/g-pretreated -DWC（廃棄割り箸）という低い結果となり，いまだ十分な水素収率が得られていないことがわかった．そこで以下の章では，加水分解をより効率的に行う方法（第4章），水素発酵をより効率的に行う方法（第5章）について検討した．
　第4章では，酵素による加水分解を一層向上させるために，その最適条件の検討を統計学的解析手法により行った．これにより酵素による加水分解は最大で 4.6 倍向上した．また，この解析手法により得られ

たモデルにより，コストを縮減しながら可能な限り加水分解の効率を低下させない条件が得られた．

　第5章では，第4章で得られた割り箸の加水分解液を用いた水素発酵条件（培地の組成条件）の最適化を第4章と同様に統計学的解析手法により行った．この結果から，培地の組成の中で酵母エキス，リン酸バッファー，硫酸鉄（Ⅱ）の3つが大きく水素生産に影響することが明らかとなり，その最適化により水素生産量は約5倍向上した．第4章と同様に，この解析手法により得られたモデルにより，コストを縮減しながら可能な限り水素生産量を低下させない培地組成条件が得られた．

　以上から，本研究の実施により木質バイオマスに適する水素生産プロセスが開発できたと考えられる．

　第6章の結論では，本論文を総括しその成果と今後の研究課題について述べている．

　公聴会には，学内外から25名の参加があり，活発な質疑応答がなされた．公聴会での主な質問内容は，①第4章では酵素の添加量の最適化を行っているが、過剰に酵素を添加すると加水分解効率はどうなるのか，②界面活性剤（TWEEN80）は加水分解にどの様な影響を与えるのか，③本プロセスのランニングコストは化石燃料から水素を生産する場合と比較してどうか，④本研究ではなぜ加水分解が困難な木質系バイオマスを対象としたのか（他にも分解しやすいバイオマスがあるのではないか），⑤最適化手法として用いた統計学的手法についてもう少し詳しく説明して欲しい，など多数であった．

　以上のいずれの質問に対しても発表者から的確で具体的な回答がなされた．

　以上より，本研究は独創性，信頼性，有効性，実用性および完成度ともに非常に優れており，博士（学術）の学位論文に十分値するものと判断した．