Consolidated Bioprocess for Bioethanol Production from Lignocellulosic Biomass Using *Clostridium thermocellum* DSM 1237

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A consolidated bioprocessing (CBP) using *Clostridium thermocellum* DSM 1237 for bioethanol production in anaerobic bottles and a 3-L fermenter from biomass was investigated. The effects of key operational parameters including different carbon sources, temperature, and substrates on the metabolic performance of the strain were firstly evaluated. It was found that ethanol yield reached 0.60 g/L with a cell biomass of 0.80 g/g at the optimal temperature of 60 °C with 0.5% (w/v) cellobiose. Further experiments indicated that sugarcane bagasse (SCB) could be utilized to efficiently culture this strain. Ethanol yield reached 0.68 g/L (65.8% of theoretical yield) from alkali-pretreated SCB. In the subsequent 3-L fermenter trial, the maximum ethanol 0.86 g/L (83.3% of theoretical yield) was achieved, with enzymes enriched in both cellulase and xylanase. The CBP provided enzymes on-site and integrated hydrolysis and fermentation in one-step, which might be an effective approach for economic bioethanol production.

INTRODUCTION

The crisis of climate change coupled with accelerated energy consumption has driven the search for renewable and clean alternative fuels. Conversion of lignocellulosic biomass to ethanol has been considered as a potential solution to solve the energy crisis and environmental pollution problems. Bioethanol technologists aim to use agro-industrial residues as feedstocks that are highly abundant and easily available throughout the world (Lazar *et al*. 2019).

Lignocellulosic biomass is composed primary of cellulose, hemicellulose, and lignin. Its structure and composition are different according to the substrate species. The biomass recalcitrance (resistance to degradation) currently limits its utilization (Zabed *et al*. 2017). Generally, the process for bioethanol production from lignocellulosic biomass includes pretreatment, enzymatic hydrolysis, and fermentation (Zhao *et al*. 2019). The capital cost of pretreatment and production or purchase of enzymes remain the dominant cost hurdles to overcome. Although the expense of enzymes has dropped in the past decades, it still covers about half of the fuel ethanol production cost (Xu *et al*. 2018; Donato *et al*. 2019).

Consolidated bioprocessing (CBP) combines cellulose hydrolysis with fermentation of the reducing sugars into one process mediated by a single strain or

microbial consortium without the aid of commercial cellulase. The use of CBP has attracted increasing attention as an effective and potential solution to reduce ethanol production cost and simplify the operation process. The critical step of CBP is to obtain efficient fermentation strains. Research of CBP has focused on the development of new and effective microorganisms (Blume *et al*. 2013; Hannah *et al*. 2017).

C. thermocellum is an aerobic bacterium that was first described by Bayer *et al*. (1983) and Lamed *et al.* (1983), and its extensive study began shortly after it was proposed that it could be useful in the direct conversion of biomass into bioethanol.

Previous studies (Lynd *et al*. 2005; Gupta and Verma 2015; Parisutham *et al*. 2017) have demonstrated that this kind of strain has a high cellulose decomposition rate and the ability to grow at elevated temperatures (50 to 60 °C), that can reduce the risk of contamination and produce thermostable enzymes to facilitate the cellulose degradation. These are very important factors in industrial processes. *C. thermocellum* can produce cellulosomes and a variety of free enzymes to deconstruct the cell wall architecture. The released cellulosomes, or multienzyme complexes, can enhance synergistic actions between different enzymes and contribute to its high rate of cellulose hydrolysis (Olson *et al*. 2012; Chung *et al*. 2015; Cui *et al*. 2019). This thermophilic strain used hydrolysate and carried out fermentation to produce ethanol, hydrogen, and other organic acids. The use of thermophilic strains for the fermentation step at temperature above 50 °C could avoid the cooling energy consumption that is required when using yeast for ethanol production, and further improve the bioethanol production. Therefore, this anaerobic organism has become an attractive candidate for application in bioconversion of cellulose to bioethanol (Li and Zhu 2011; Akinosho *et al.* 2017).

While *C. thermocellum* has one of the fastest known rates of cellulose hydrolysis, the recalcitrance and low ethanol yield are its leading challenges for industrial-scale ethanol production. Although numerous examinations (Olson *et al.* 2015; An *et al*. 2018; Ghosh *et al*. 2018; Kothari *et al*. 2019) have obtained a detailed genetic and molecular scheme of the cellulolytic system in *C. thermocellum*, certain aspects of the strain's growth and metabolic characteristics have not been fully revealed. In many natural and industrial fermentation conditions, substrates availability is limited, and the organisms grow at sub-optimal rates. Growth performance affects the bioenergetics of bacterial growth and the final fermentation products.

Although microbial free enzymes can deconstruct biomass structures, many bacteria degrade cellulose *via* enzyme complexes called cellulosomes. The study of the performance and mechanisms of these macromolecular complexes for improving lignocellulosic biomass conversion efficiency using cellulosomes is an ongoing important research topic for commercial utilization of the efficient bacteria. The aim of the present work was to identify the cell-growth behaviors of the thermophilic anaerobic bacterium *C. thermocellum* DSM 1237 by optimizing its growth condition and evaluate its ethanol production performance.

In this study, cellulosic ethanol production by the cultivation of *C. thermocellum* DSM 1237 in anaerobic bottles and a 3-L fermenter with biomass as the substrate were investigated. Cell growth conditions of the strain, including different carbon sources, temperature, and different substrates, and its corresponding ethanol fermentation performance were evaluated. Based on the optimal conditions, the fermenter application tests were performed to achieve high concentration ethanol directly from cellulose using *C. thermocellum* DSM 1237.

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EXPERIMENTAL

Materials

Substrates

Guangxi Fenghao Sugar Co., Ltd. (Yulin, China) provided sugarcane bagasse (SCB). It was pre-milled, screened, and the fractions between 40- and 60-meshes were collected for alkali-pretreatment. The milled bagasse (1 g dry weight) was mixed with 20 mL of 0.5 M NaOH (or with 4% (w/v) peroxide to assist the NaOH), and incubated in a water bath at 80 °C for 2 h with agitation. After reaction, the treated bagasse was washed with tap water until neutrality and dried at 60 °C for the subsequent experiments. Other materials, such as corn and rice straw, were collected by Cofco Group Co. LTD located in Shenzhen Guangdong province.

All chemicals and reagents used in this study were ordered from Sigma Aldrich (Shanghai, China) and were of either analytical or molecular grade unless stated otherwise.

Microorganisms and growth medium

The *C. thermocellum* DSM 1237 was supported by the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) and used throughout this study. It was revived from -80 °C before the experiments.

Seed culture

A modified nutrient medium (Johnson *et al*. 1981) was used for the activation of the *C. thermocellum* culture with a composition of (per L): 2.60 g $MgCl_2 \cdot 6H_2O$, 1.30 g $(NH_4)_2SO_4$, 1.43 g KH_2PO_4, 5.50 g K_2HPO_4, 0.13 g $CaCl_2 \cdot 2H_2O$, 6.00 g Na$_2$-β-glycerol phosphate$\cdot 4H_2O$, 1.10 mL $FeSO_4 \cdot 7H_2O$ solution (0.1% w/v in 0.1g/L$(NH_4)_2SO_4$), 0.25 g L-Glutathione reduced, 4.50 g yeast extract, 0.5 mL Na-resazurin solution (0.1% w/v), 5.0 g cellobiose, and 1 mL resazurin.

This medium composition is the basic solution for activation of the *C. thermocellum*. The initial pH value of the medium was 7.0. After sterilization at 121 °C for 20 min, the nutrient medium was immediately transferred to an anaerobic chamber and left for a half day. It was then inoculated with 5% (v/v) *C. thermocellum* DSM1237, and cultured at 60 °C for 48 h. For a seed culture, *C. thermocellum* was repeatedly transferred over 4 generations continuously for approximately 48 h, and then used as the seed to initiate each fermentation experiment with 5% (v/v) inoculum size unless other noted.

Cell growth

The *C. thermocellum* DSM 1237 was grown anaerobically in a fresh CM3 medium (composition provided by DSMZ) containing (per L): 2.0 g yeast extract, 1.3 g $(NH_4)_2SO_4$, 1.5 g KH_2PO_4, 1.0 g $MgCl_2 \cdot 6\ H_2O$, 0.15 g $CaCl_2$, 0.5 g L-Glutathione reduced, and 1 mL resazurin, for the inoculant used in all the fermentation experiments. The medium pH value was adjusted to 7.0 with NaOH or HCl and was maintained under an anaerobic atmosphere.

Consolidated Bioprocessing

Optimization of cell-growth conditions

Sterile CM3 medium (200 mL culture volume) was prepared for ten groups of parallel experiments (each group with 20 mL CM3 medium) under anaerobic conditions. The pH of the medium was adjusted to the optimal growth value of 7.0 just before inoculation. Freshly prepared *C. thermocellum* DSM 1237 was added with 5% (v/v) inoculation in the reaction. Fermentations were performed in 50-mL bottles with supplemented 0.5% (w/v) of cellobiose, xylose, and glucose, separately, as carbon sources at 60 °C for 72 h to investigate the effect of different carbon sources on the growth of the cell.

The optimization of temperature for cell growth was performed with the same procedure of the above tests using ten groups of parallel bottles with 0.5% (w/v) cellobiose as the carbon source. Cell growth and ethanol fermentation performance were assessed by varying the reaction temperature with 50 °C, 55 °C, 60 °C, 65 °C, and 70 °C, separately.

Consolidated bioprocessing with different substrates of raw SCB, alkali-pretreated SCB, and peroxide reinforced alkali-treated SCB, with corn and rice straw as carbon sources were conducted at 60 °C for 120 h at 0.3% (w/v) solids loading to produce ethanol by *C. thermocellum* DSM 1237. The fermentation process started with autoclaved different carbon sources in 50-mL anaerobic bottles. The carbohydrates consumption and the final ethanol yield were analyzed during the reaction.

Samples were collected at specific time points, and centrifuged at 4 °C, 12000 rpm for 10 min for cell biomass, ethanol, sugars, and acids analysis. All experiments were performed in triplicate, and the given numbers are the mean values.

Fermenter experiment

The bioreactor experiments were conducted with 8 g of alkali-treated SCB as the carbon source in 2.5 L of CM3 medium (pH 7.0) at 60 °C and 150 rpm in a 3-L controlled fermenter (Fig. 1). Before reaction, the system was autoclaved at 121 °C for 30 min to keep it in a sterile and anaerobic environment. After 12 h, 8% (v/v) prepared *C. thermocellum* DSM 1237 medium was inoculated to the system. Continuous nitrogen was fed for 5 min at the beginning of the reaction to exhaust other gases. The pH value was scrutinized by an on-line monitoring system (Shanghai Baixing Engineering, Shanghai, China). Samples were anaerobically withdrawn at each time interval from culture vessels and separated by centrifugation. The cell-free supernatant was stored at 4 °C for analysis.

Fig. 1. The 3-L fermenter

Analytical Methods

Chemical components of SCB before and after pretreatment, and corn and rice straw were determined according to the standardized methods of the National Renewable Energy Laboratory (NREL, Golden, CO, USA) (Sluiter *et al*. 2008). Sugars including cellobiose, glucose, and xylose were analyzed by high-performance liquid chromatography (HPLC) using a Shodex sugar SH-1011 column coupled with a refractive index detector (Wilford, Massachusetts, USA). Cellulase FPU activity and xylanase activity were measured by the IUPAC (International Union of Pure and Applied Chemistry) method (Ghose 1987). The protein concentration (biomass) was assayed with the Quick Start Bradford Protein Assay (Thermo Fisher Scientific, Waltham, America) with BSA as the standard (Bradford 1976). Ethanol concentration was measured with a gas chromatograph (Agilent 6820; Agilent Technologies Inc., California, America) equipped with a flame ionization detector (FID) and a fused-silica capillary column (DB-FFAP, 30 m $\times$ 0.25 mm $\times$ 0.25 µm). The surface morphology and characteristics of the different residual solids were observed by scanning electron microscopy (SEM). Images were taken using the model JEOL JSM- 4800 LV SEM (Japan Electronics Co. LTD, Tokyo, Japan) and performed at a beam accelerating voltage of 10 kV. Digital images were captured using 1280 $\times$ 960 resolution and 160 s dwell time. The substrate conversion efficiency was calculated by estimating consumed glucose/xylose equivalents and expressed as a percentage (%). Ethanol yield from substrate was calculated by grams of ethanol produced divided by grams of glucose/xylose equivalents consumed.

RESULTS AND DISCUSSION

Cell-growth and Ethanol Fermentation Performance

Different carbon sources

The protein secretion that influences the cell-growth of anaerobic bacteria, such as *C. thermocellum*, *C. cellulolyticum*, as well as other cellulase subunits, is dependent on available carbon sources (Cho *et al*. 2010).

To achieve high density of biomass to promote substrate conversion, *C. thermocellum* DSM 1237 was separately cultured using cellobiose, glucose, and xylose as its carbon sources (Table 1). It was found that cultures containing cellobiose produced more cells, as indicated by the determined higher biomass. The maximum biomass (0.32 g/g DM) and ethanol (0.55 g/L) increased 146.15% and 19.57%, respectively, when using cellobiose as carbon sources compared to the glucose (biomass 0.13 g/g DM and ethanol 0.46 g/L), based on observations after 72 h fermentation in CBP at 60 °C. After reaction, the corresponding residual sugars in 0.5% (w/v) of the cellobiose system was 1.22 g/L, with nearly 75.6% substrate conversion efficiency. In contrast, the lowest biomass, 0.09 g/g DM, was observed when xylose was used in fermentation with its high residual sugar of 3.39 g/L. The incomplete utilization of sugars was evident with an increased accumulation of glucose and xylose, leading to a lower ethanol yield.

Similar to ethanol and cell biomass, a remarkable variation in acetic acid concentration from 0.61 g/L to 0.33 g/L was observed under various carbon sources (Table 1). The high acetic acid was one of the major soluble metabolites produced in fermentation, and it might have noticeable influence on ethanol production. The specific mechanism needs further discussion. Subsequent enzymes detections found that the system was rich in

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both cellulase and xylanase activities with 0.18 FPU/g and 0.12 IU/g solids, respectively, which was important for efficient degradation of the substrate.

Table 1. Maximum Growth, Metabolites, and Residual Sugars in CBP from Different Carbon Sources

Item	Cellobiose	Glucose	Xylose
Biomass (g/g DW)	0.32	0.13	0.09
Residual sugars (g/L)	1.22	2.85	3.39
Ethanol concentration (g/L)	0.55	0.46	0.35
Acetic acid (g/L)	0.60	0.61	0.33
Cellulase (FPU/g)	0.18	0.04	0.02
Xylanase (IU/g)	0.12	0.07	0.04

Maximum growth: When cell biomass achieved highest value; Metabolites: The strain`s metabolites include ethanol, acetic acid, cellulose, and xylanase.

The obtained high ethanol and biomass in CBP indicated that *C. thermocellum* DSM 1237 had improved growth performance with cellobiose as its carbon source (Table 1). Therefore, the subsequent temperature optimization process was carried out with cellobiose as the carbon source based on the above results.

Temperature optimization
The influence of temperature for the substrate fermentation of *C. thermocellum* DSM 1237 was investigated at the suitable carbon nutrients. As shown in Fig. 2, increasing temperature from 50 °C to 60 °C could enhance cell density (Fig. 2A). The maximum biomass 0.80 g/g DM and ethanol yield 0.60 g/L were obtained under the optimal temperature of 60 °C, and the corresponding residual sugars were 1.51 g/L with 69.9% substrate conversion efficiency. When the temperature was higher than 60 °C, a dramatic drop in ethanol concentration was observed. Further increase in temperature beyond 60 °C did not increase either cell biomass or ethanol production; rather, it would induce high concentration of acetic acid production (Fig. 2B). Despite the low ethanol production, the bacteria were still able to grow at temperature of 70 °C with only 0.13 g/g cell biomass obtained (Fig. 2A). This lowest growth and ethanol production suggested that the upper-limit of temperature for the strain was below 70 °C, which agreed with the previous reports where the highest temperature of 70 °C was suggested for the growth of *C. thermocellum* strains upon most occasions (Akinosho *et al*. 2014).

The detected temperature classified this bacterium as thermophilic and indicated that it was a strong candidate for cellulosic ethanol production. High temperature could reduce the contamination risk in the CBP process and would produce stable enzymes for motivating their utilization to efficient metabolize the cellulosic biomass. Growth of *C. thermocellum* at 60 °C was also observed in other studies (Koeck *et al*. 2015; Singh *et al*. 2017). To facilitate the subsequent lignocellulosic biomass fermentation process, *C. thermocellum* DSM 1237 was cultured at 60 °C with different substrates to improve its metabolism efficiency to achieve high ethanol concentrations.

Ethanol production based on different substrates
Thermophilic anaerobic bacteria are known for their ability to break down plant cell walls using a complex enzyme named cellulosome. Cellulosomes are able to degrade crystalline cellulose and produce soluble sugars that might be transformed into bioethanol

after fermentation. The one-step conversion process using the specific anaerobic strain of *C. thermocellum* DSM1237 to directly realize the conversion of biomass to ethanol, would reduce the operating cost of cellulosic ethanol.
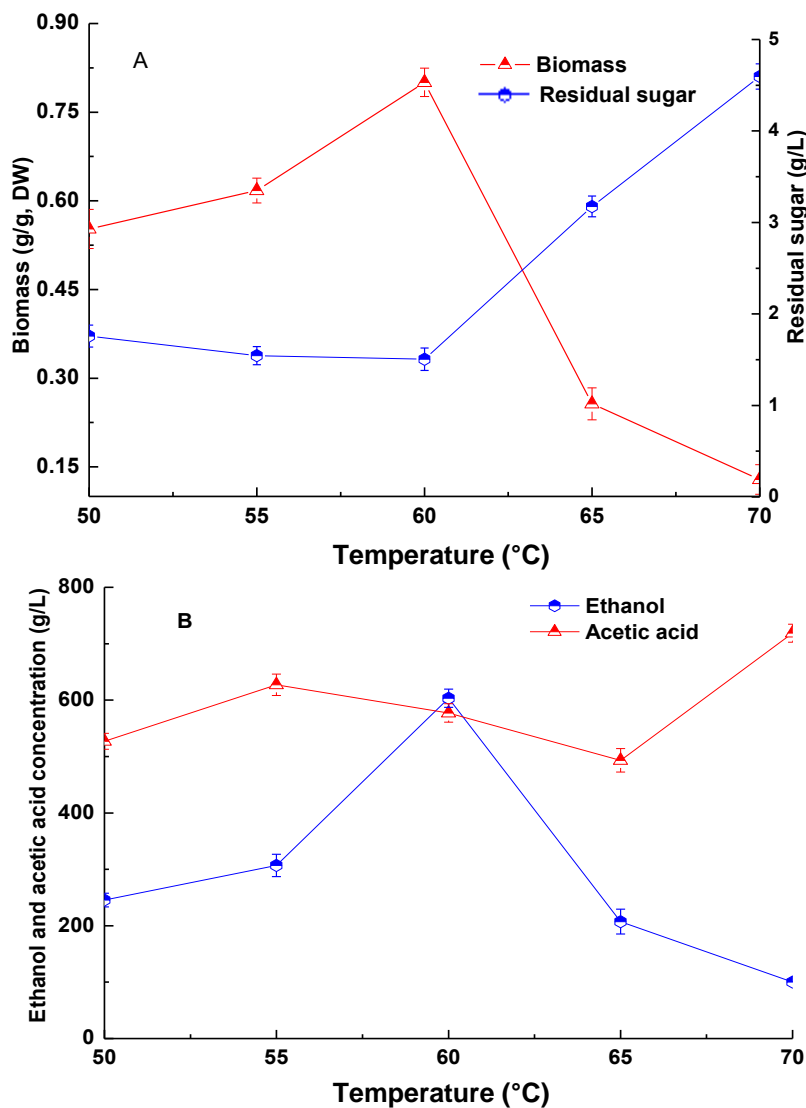

Fig. 2. Effect of different temperatures on (A) biomass residual sugar and (B) ethanol and acetic acid concentration

Based on the above optimized cell growth conditions, SCB, alkali-treated SCB, peroxide enhanced alkali-treated SCB, and corn and rice straw were selected as fermentation substrates to investigate ethanol production performance of *C. thermocellum* DSM1237 under anaerobic conditions at 60 °C. According to compositional analysis using NREL protocol, the SCB used in this study was composed of glucan, xylan, and lignin in percentages of 41.90%, 24.68%, and 21.35%, respectively. After pretreatment, the composition was dramatically changed, especially for reinforced pretreatment. Peroxide-assisted alkali-treated SCB removed almost 97.85% lignin, which made cellulose totally exposed and facilitated the subsequent microbial metabolism (Table 2, Fig. 3). The remaining solids are mainly glucan and xylan, and its stubborn chemical structures and

chemical bonds are broken during reinforced alkali-pretreatment, which facilitate the enzyme to degrade the substrate.

Table 2. Chemical Composition of Different Substrates Before and After Pretreatment

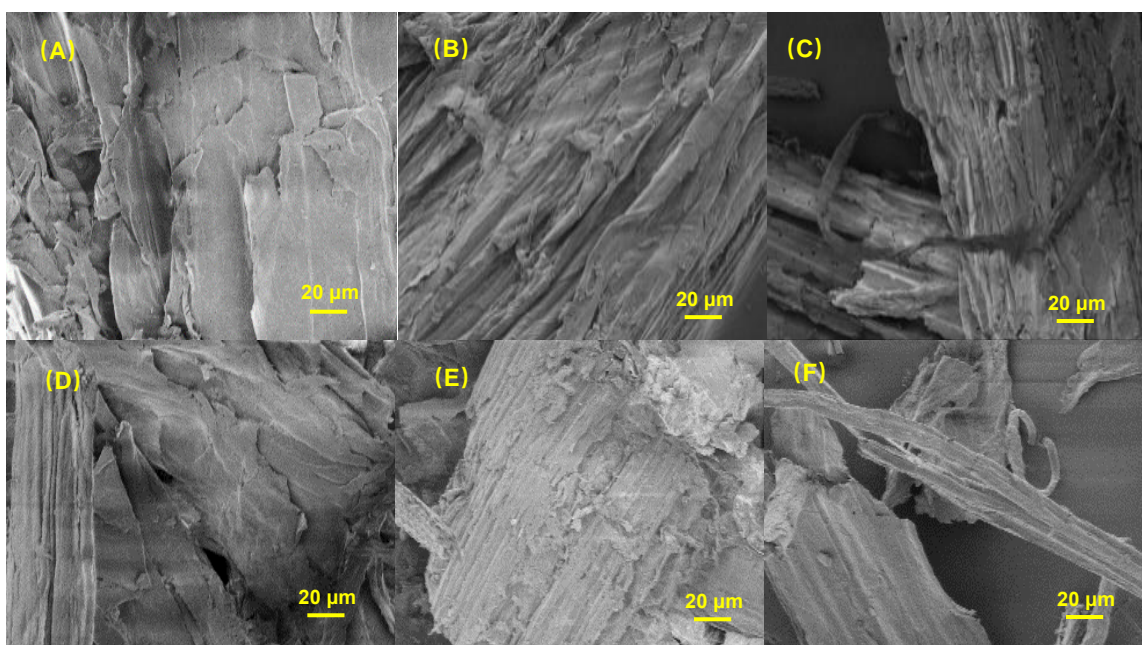| Samples	Glucan (%)	Xylan (%)	Lignin (%)	Other Components (%)
SCB	41.90	24.68	21.35	12.07
Alkali-treated SCB	64.41	24.95	6.03	4.63
Peroxide-assisted SCB	69.52	23.67	0.46	4.35
Corn straw	40.19	34.41	14.28	11.12
Rice straw	38.47	24.10	26.10	11.33

Fig. 3. Scanning electron micrographs of (A) raw, (B) alkali-pretreated, (C) peroxide-assisted alkali-pretreated SCB, (D) raw, (E) alkali-pretreated, and (F) peroxide-assisted alkali-pretreated SCB after fermentation

Culture of the strain was monitored for cell growth over a 120 h fermentation (Table 3). It was found that cell biomass reached 0.75 g/g DM with final ethanol concentration of 0.64 g/L (65.5% theoretical conversion efficiency) using alkali-treated SCB as the substrate, and the final substrate conversion rate was 64%. When the assisted step of peroxide was added to alkali pretreatment, the highest biomass of 0.88 g/g DM and almost 0.68 g/L ethanol with a theoretical conversion efficiency of 65.8% and substrate conversion rate of approximately 69% were achieved having the reinforced treated SCB as the substrate. For the untreated SCB, corn, and rice straw, the final ethanol yield were relatively lower than the pretreated SCB for their comparatively low enzyme activities produced in reactions in a short time (Table 3).

The surface physical structure changes of raw, alkali, peroxide-assisted alkali, and fermented SCB substrates were analyzed by SEM (Fig. 3). *C. thermocellum* in CBP

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partially changed the surface structures in substrate, especially for the fermented SCB (Fig. 3E and F). The untreated SCB had a compact and smooth morphology (Fig. 1A). Alkali-treated samples showed a rough structure and an obvious gully because of the lignin removal (Fig. 1B). After peroxide reinforced alkali-pretreatment, some smaller fragments were produced (Fig. 1C). These changes improved microbial metabolism efficiency, which was in accordance with the ethanol yield obtained (Table 3), suggesting that most of the substrate had been sufficiently hydrolyzed. It can additionally be found from the residual sugar's concentration (Table 3).

Table 3. Ethanol Fermentation in CBP with Different Substrates

Item	SCB	Alkali-SCB	Alkali+4%H_2O_2	Corn Straw	Rice Straw
Biomass (g/g DW)	0.34	0.75	0.88	0.36	0.18
Residual sugars (g/L) (Cellobiose)	0.46	0.62	0.51	0.12	0.19
(Glucose)	0.18	0.33	0.23	1.16	0.04
(Xylose)	0.00	0.13	0.19	0.11	0.01
Ethanol concentration (g/L)	0.41	0.64	0.68	0.54	0.48
Acetic acid (g/L)	1.52	0.48	0.68	0.60	0.53
Cellulase (FPU/g)	0.006	0.01	0.12	0.01	0.11
Xylanase (IU/g)	0.001	0.039	0.071	—	—

The probable reasons for the noted observations might be that lignin is a key contributor to biomass recalcitrance with cellulases. It influences biomass conversion during CBP. As shown in Table 2, alkali-pretreated SCB could effectively remove much more lignin (71.8%) and retain most of cellulose in the residues of the raw materials, especially for peroxide-assisted alkali pretreatment. The typically used enzymes for ethanol production are cellulases isolated from fungi, in contrast, *C. thermocellum* contains at least 70 enzymes that can alter plant cell wall components and modify the accessibility of the enzymes to cellulose (Munir and Levin 2016). Lignin removal could be beneficial to biomass deconstruction. High molecular weight of lignin has been found to donate electrons to lytic polysaccharide monooxygenases, which is a group of copper-dependent enzymes present in fungi, *C. thermocellum*, and are involved in glycosidic bond cleavage that promote hydrolysis and subsequent fermentation (Westereng *et al*. 2016). Although structure changes could be observed during controlled CBP (Fig. 3), additional research is still needed to pinpoint its degradation mechanism.

Fermenter Application

To improve ethanol production from SCB in large-scale fermentation using *C. thermocellum* DSM 1237, a 3-L fermenter trial was conducted under controlled operation parameters at 60 °C for 56 h (Fig. 4).

The highest ethanol concentration of 0.86 g/L with a theoretical conversion efficiency of 83.3% from alkali-treated SCB (8 g DM) was achieved in CBP using *C. thermocellum* DSM 1237. The results obtained from the large-scale fermenter operation was comparatively higher than that obtained with the 50-mL bottle tests (ethanol yield 0.68 g/L). This may be caused by more sugars and enzymes that are produced in CBP with supplemented agitation, which accelerate the substrate mixing, heat, and energy transfer during fermentation, and thus result in the high metabolites (Fig. 4). The corresponding by-

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products of acetic acid was increasing with the prolongation of the incubation time. The accumulated acids in fermentation have an inhibitory influence on the formation of ethanol. The effect of acids concentration on ethanol yield will be further discussed in future work.
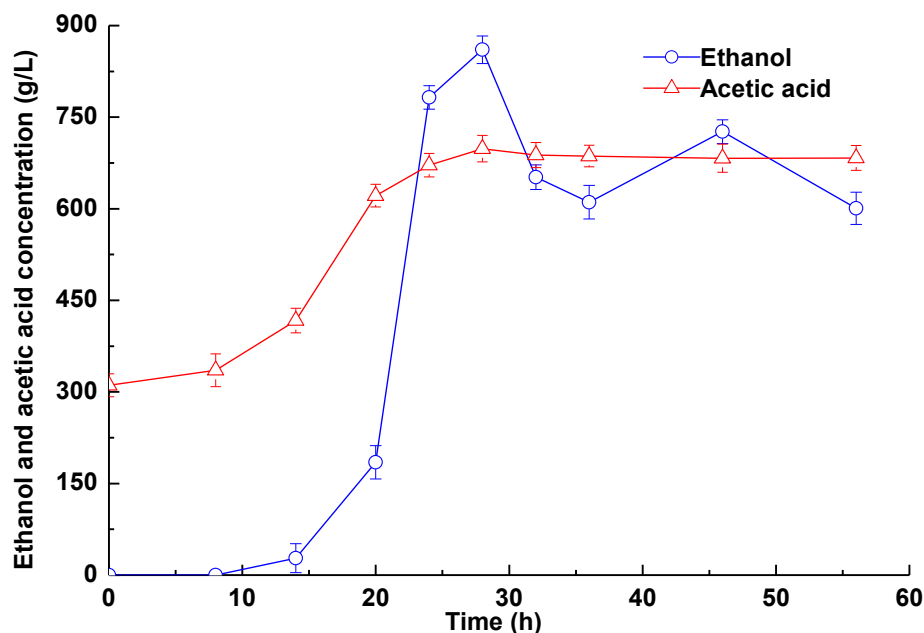

Fig. 4. Ethanol and acetic acid production from alkali-treated SCB in a 3-L fermenter through CBP

During CBP, ethanol production is a final critical factor, and previous studies of the maximum ethanol yield achieved in published experiments are listed in Table 4. Higher ethanol yield usually corresponds to methods that are more complex. Strain adaptability and compatibility are important modifying factors for efficient bioethanol conversion from lignocellulosic biomass and must be investigated case-by-case for each application.

Table 4. Comparison of Maximum Ethanol in CBP with Cellulosic Substrates

Organism	Substrate/ Biomass	Final Ethanol Concentration	Theoretical Yield	Reference
C. thermocellum DSM 1237	Alkali-treated SCB	0.27 g/g SCB 0.42 g/g glucose	83.30%	This work
Clostridium strain AK1	Cellulose hydrolysates from various biomass	0.16 to 0.34 g/g	66.75%	Olson (2015)
C. thermocellum/ C. thermocellum co-culture	Crystalline cellulose	Up to 4.19 g/L	75%	Scully and Orlygsson (2015)
Thermoanaerobacter sp. DBT-IOC-X2	Glucose	0.32 g/g	83.57%	Singh *et al.* (2018b)
C. thermocellum strain AG553	Crystalline cellulose	22.4 g/L	75%	Tian *et al.* (2016)
C. thermocellum ATCC 31924	crystalline cellulose	0.30 g/g cellulose	59.71%	Singh *et al.* (2018a)

Ethanol conversion from lignocellulosic biomass begins with the intense hydrolysis of substrate structures to obtain high fermentable sugars. Using one-step conversion approach by thermophilic microorganisms can reduce the production costs of cellulosic ethanol. These bacteria can withstand high temperatures, which could reduce contamination risk and produce thermostable enzymes. These all were crucial factors in industrial-scale ethanol production. The 3-L fermenter application using CBP from SCB with *C. thermocellum* DSM 1237 achieved higher (83.3%) theoretical ethanol yield compared with the related published works under relatively simple operation procedures (Table 4), which was vital for the subsequent scale-up trials.

CONCLUSIONS

1. Cell biomass of 0.80 g/g DM and ethanol yield of 0.60 g/L were obtained in growth media containing 0.5% (w/v) cellobiose at 60 °C after 72 h of fermentation. The strain was capable to degrade cellulosic biomass, the highest cell biomass of 0.82 g/g DM and ethanol yield of 0.68 g/L were achieved when using peroxide-assisted alkali-pretreated SCB as the substrate, at 60 °C for 120 h.

2. In the subsequent 3-L fermenter application, the maximum ethanol yield of 0.86 g/L with theoretical conversion efficiency of 83.3% was achieved. Exploitation of efficient strains in the highly integrated process of CBP might be the most promising strategy for cellulosic bioethanol production.

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