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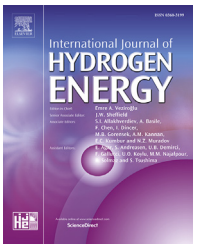

# Enhanced hydrogen and volatile fatty acid production from sweet sorghum stalks by two-steps dark fermentation with dilute acid treatment in between

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ABSTRACT

This study investigated the potential of hydrogen and volatile fatty acid coproduction from two steps dark fermentation with dilute acid treatments of the residual slurry after 1st step fermentation. Sweet sorghum stalks (SS) was used as substrate along with *Clostridium thermosaccharolyticum* as production microbe. Residual lignocelluloses after 1st step fermentation were treated for 1 h by sulfuric acid concentration of 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5% (w/v) with different reaction temperature of 120, 90 and 60 °C were studied. The optimum severity conditions for the highest yield of products found from the treatment acid concentration of 1.5% (w/v) at 120 °C for 10 g/L of substrate concentration. Experimental data showed that two-step fermentation increased 76% hydrogen, 84% acetic acid and 113% of butyric acid production from single step. Maximum yields of hydrogen, acetic acid and butyric acid were 5.77 mmol/g-substrate, 2.17 g/L and 2.07 g/L respectively. This two-step fermentation for hydrogen and VFA production using the whole slurry would be a promising approach to SS biorefinery.



## Introduction

The conversion of lignocelluloses into biofuels and commodity chemicals is currently one of the most attractive research interests in the world. However, the obstacle to disrupt the complex structure of biomass is a challenge in fermentation process [1]. Effective pretreatment is necessary to break the recalcitrance of lignocelluloses to enhance the hydrolysis efficiency [2–4]. Pretreatment is a process to remove recalcitrance of lignocelluloses for effective biological conversion [5]. The most important parameters for the biological conversion of lignocellulosic materials are cellulose crystallinity and degree of polymerization, lignin and hemicellulose protection, accessible surface area and the biomass swelling capacity [6–8]. The most effective and widely used pretreatments

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techniques are alkali and acid pretreatments. Lignin and hemicellulose can be removed or modified by alkaline pretreatments and resulting in increase of the porosity of lignocelluloses [9] but in acid pretreatment, the glycosidic bonds and glucuronosyl linkages in hemicellulose can be cleaved to soluble monomeric sugars [10]. The properties of cellulose contents remaining after acid treatment also has changed to lower degree of polymerization, reduced crystallinity and increased surface area. Hence accessibility of cellulose has increased, which is more suitable for fermentation. Most studied dilute acid pretreatment for a variety of lignocelluloses is sulfuric acid [11,12].

Sweet sorghum stalks are carbohydrate-rich biomass which contains both non-structural (sucrose, glucose, fructose) and structural (hemicellulose and cellulose) carbohydrate [13]. In liquid state dark fermentation process, it is not difficult to produce hydrogen from non-structural carbohydrate (soluble sugar) in sweet sorghum stalks. However, most parts of structural carbohydrate remained as un-degraded and unavailable for microbial consumption during fermentation [14]. It is therefore a challenge how to deal with this lignocellulosic fraction of sweet sorghum stalks for enhanced hydrogen production.

Sweet sorghum stalks contain more soluble sugar and less lignin compares to other lignocelluloses [14]. Due to low lignin content dilute acid treatment was chosen to degrade hemicellulose into monomeric sugars and can soften the lignin structure, transform the other components of biomass, improving cellulose conversion [11,15,16]. Acid pretreatment degrades pentose and hexose to Furfural and Hydroxyl Methyl Furfural (HMF) respectively at severe conditions of low pH and may inhibit the fermentation process [10,17–19]. Since acid pretreatments of raw sweet sorghum stalks can degrade some pentose and hexose sugars and produce toxic inhibitors, dilute acid treatment was done with residual biomass after consumption of soluble sugar in 1st step. Acid treatment after 1st step fermentation decrease the chance to produce less inhibitory compounds by losing soluble sugar presents in sweet sorghum stalks and may reduce the obstacle from inhibition.

Feasible way of producing biohydrogen in the dark fermentation processes are using fermentative microbes like *Clostridium* sp., *Enterobacter* sp., and others [20]. *C. thermosaccharolyticum* is a hydrogen producing bacterium, which can convert soluble sugar to hydrogen and volatile fatty acid (VFA) from lignocellulosic biomass [21,22]. In the 1st step of fermentation, *C. thermosaccharolyticum* was used in raw sweet sorghum stalks to completely utilize the soluble sugars. The residual solids after this step were treated with dilute sulfuric acid and used the whole slurry as a substrate for 2nd step of fermentation. Same strain *C. thermosaccharolyticum* was used in both steps to ensure maximum conversion of biomass to hydrogen and the byproducts acetic acid and especially commercially more valuable butyric acid [23–25]. Though in previous study *C. thermocellum* was used for cellulose degradation it produced only acetic acid along with hydrogen and still, there were huge undegraded structural carbohydrates [14,25]. There has been no report of two-step dark fermentation including the acid treatment of residual biomass in between.

The objective of this study was to ensure maximum conversion of biomass to hydrogen and volatile fatty acid without losing the soluble sugars present in sweet sorghum stalks. In the 1st step fermentation, *C. thermosaccharolyticum* strain consumed soluble sugar and the residual biomass was treated with dilute acid to produce more monomeric sugar along with some inhibitors. The severity of acid treatment was also evaluated. The same strain was reused in 2nd step fermentation after 1st step instead of fresh inoculum to minimize costs. This hydrogen producing strain produced commercially important butyric acid along with hydrogen.

## Experimental methodology

### Materials and chemicals

Sweet sorghum stalks raw materials were collected from Inner Mongolia, China (Sonid Youqi Farm). The sample was dried at 60 °C, ground to ≤1 mm powder and stored for all experiments. The contents of cellulose, hemicellulose, lignin and total soluble sugar in sweet sorghum stalks were 22.11, 19.15, 8.11 and 32.50 (% w/w, on dry weight basis), respectively. All analytical grade chemicals were purchased from Beijing Chemical Reagents Company (Beijing, China).

### Microorganism and culture conditions

*C. thermosaccharolyticum* DSM572 was collected from the Deutsche Sammlung von Mikroorganismen and Zellkulturen (DSMZ). The media for cultivation of this strain was modified CM4. The medium (pH 7) contained (g/L) 1.5-$KH_2PO_4$, 3.8-$K_2HPO_4 \cdot 3H_2O$, 4.7-$(NH_4)_2SO_4$, 1.6-$MgCl_2 \cdot 6H_2O$, 0.013-$CaCl_2$, 5.0-yeast extract, 1.25 × $10^{-3}$-$FeSO_4 \cdot 7H_2O$, 1.0 × $10^{-3}$-resazurin and 0.5-L-cysteine HCl. The *C. thermosaccharolyticum* was cultured into a fresh CM4 medium 24 h before used as inoculums. 10 g/L of sucrose were used as carbon sources for preparation of *C. thermosaccharolyticum* inoculum.

### 1st step fermentation

Anaerobic digestion experiments for hydrogen and VFA production using sweet sorghum stalk were performed in 135 mL glass bottle with media working volume of 50 mL. In the 1st step fermentation process, 0.5 g of powdered sweet sorghum stalk samples were taken into the bottle and 50 mL of prepared media was added into it. Rubber stopper and screw cap were used to seal the bottle. The fermentation bottle was degassed by a vacuum pump and again gassed and degassed three times with nitrogen gas to ensure anaerobic environment inside the bottle. The bottles were autoclaved for 20 min at 115 °C and inoculums of *C. thermosaccharolyticum* ($OD_{600}$ 1.10–1.19) were added into it under the sterile condition and kept at 55 °C for fermentation. Inoculums were 10% of total media volume.

### Dilute acid treatment and 2nd step fermentation

Residual solids after 1st step fermentation were separated by decantation of the top liquid part. Different concentration of

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