



## Short Communication

# Comparison of bio-hydrogen production yield capacity between asynchronous and simultaneous saccharification and fermentation processes from agricultural residue by mixed anaerobic cultures



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## ABSTRACT

Taken common agricultural residues as substrate, dark fermentation bio-hydrogen yield capacity from asynchronous saccharification and fermentation (ASF) and simultaneous saccharification and fermentation (SSF) was investigated. The highest hydrogen yield of 472.75 mL was achieved with corncob using ASF. Hydrogen yield from corn straw, rice straw, corncob and sorghum stalk by SSF were 20.54%, 10.31%, 13.99% and 5.92% higher than ASF, respectively. The experimental data fitted well to the modified Gompertz model. SSF offered a distinct advantage over ASF with respect to reducing overall process time (60 h of SSF, 108 h of ASF). Meanwhile, SSF performed better than ASF with respect to shortening the lag-stage. The major metabolites of anaerobic fermentation hydrogen production by ASF and SSF were butyric acid and acetic acid.

## 1. Introduction

Hydrogen is recognized as a potential energy substitute for fossil fuels due to its clean, renewable and high electrical-conversion efficiency, which plays an important role in future (Zhang et al., 2017). However, current hydrogen is mainly produced from fossil fuels, which causes environment pollution. In contrast, bio-hydrogen production is one of the potential solution because of its low cost, environmentally friendly, and sufficient supply (Zhi and Wang, 2014). Among the various biological hydrogen production processes, dark-fermentation can utilize various organic matters, and presents shorter fermentation period and higher hydrogen production rate (Khan et al., 2016; Alibardi and Cossu, 2015). Hence, dark-fermentation bio-hydrogen production has received increasing attention.

Given that lignocellulosic biomass can be degraded into sugars, and then utilized by fermentation microbes, the adoption of agricultural residues will significant increase the reserves of raw material for bio-hydrogen production. The annual yield of agricultural biomass is estimated at approximately  $5.0 \times 10^9$  tons in China (Zhang et al., 2014). The process of bio-hydrogen production from agricultural straw contains mainly three stages, pretreatment, enzymatic hydrolysis, and fermentation (Liu and Chen, 2016; Cantarella et al., 2004). Enzymatic hydrolysis is utilized to convert cellulose into sugars, and then fermented to hydrogen. The scheme is referred to as asynchronous saccharification

and fermentation (ASF) because that enzymatic hydrolysis and fermentation are performed sequentially. However, enzymatic hydrolysis can be conducted simultaneously in one reactor, i.e. simultaneous saccharification and fermentation (SSF) (Öhgren et al., 2007). The combination of enzymatic hydrolysis and fermentation would simplify the process and reduce cost (Öhgren et al., 2007). Simultaneous saccharification and fermentation also have the advantages of low contamination risk, short processing period, and continuous removal of hydrolysis end-products that inhibit enzymes, (Cantarella et al., 2004; Rodrigues et al., 2015; Sariapan and Reungsang, 2014). With these advantages, SSF of the pretreated lignocellulosic feedstock is considered to be an ideal integrated process for hydrogen production. Currently, SSF technology was mainly used in ethanol and lactic acid fermentation (Barros et al., 2016; Rodrigues et al., 2015), few literatures reported the differences between ASF and SSF processes during hydrogen production (Sariapan and Reungsang, 2014; Jiang et al., 2016). In this study, bio-hydrogen yield capacities through SSF and ASF were compared using different agricultural residues, including corn straw, wheat straw, rice straw, corncob and sorghum stalk. Hydrogen production rate, hydrogen concentration, and cumulative hydrogen production were analyzed to evaluate the two hydrogen production pathways of different straws.

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**Table 1**  
Composition of the agricultural residues.

	Corn straw	Wheat straw	Rice straw	Corn cob	Sorghum stalk
Cellulose/%	39.12 ± 0.68	35.10 ± 0.45	43.21 ± 0.58	40.20 ± 0.23	38.35 ± 0.62
Hemicellulose/%	30.95 ± 0.54	24.82 ± 0.36	24.81 ± 0.67	32.62 ± 0.57	21.45 ± 0.81
Lignin/%	10.73 ± 0.28	20.4 ± 1.74	17.81 ± 0.49	10.20 ± 0.82	17.25 ± 0.62
Moisture content/%	4.35 ± 0.21	6.58 ± 0.23	6.24 ± 0.15	6.56 ± 0.32	5.43 ± 0.18

## 2. Materials and methods

### 2.1. Raw material and pretreatment

Raw materials (corn straw, wheat straw, rice straw, corncob, and sorghum) were collected from a farm in Zhengzhou, Henan province, China. The straw materials were air-dried, and milled by a vegetation disintegrator through a 60-mesh sieve. The content of cellulose, hemicellulose and lignin in the raw materials were determined according to NREL method (Sluiter et al., 2004).

### 2.2. Microorganisms and media

The strains were originally screened from sludge (a municipal wastewater treatment plants), cow dung, pig manure, and camel dung. Then, the mixture were cultured in 2 L glass bottle with stopper at 35 °C. For test studies, the initial bacterial fluid was heat-treated at 100 °C for 10 min, and then, the bacteria was cultured in medium that contained the following components: soya peptone (5 g L<sup>-1</sup>), pancreatic peptone (15 g L<sup>-1</sup>) and NaCl (5 g L<sup>-1</sup>). The augmented period was conducted for 4 cycles that lasted for almost 16 d. The major identified species were *Enterococcus*, *Sporanaerobacter*, *Para clostridium* and *Clostridium\_sensu\_stricto\_1* by 16S rDNA (Vezzulli et al., 2013). The optimal fermentation condition of the strains (40 °C, pH 5.5) is close to enzymatic hydrolysis (50 °C, pH 4.8) (Zhang et al., 2016).

### 2.3. Procedures of ASF and SSF

For ASF process, enzymatic hydrolysis experiments were conducted in 200 mL glass reactors loaded with 5 g of raw material, 100 mL citric acid buffer (pH 4.8), and 0.75 g cellulase (10 FPU/mg). The experiments were performed at 50 °C and 150 rpm for 48 h, and then, the pH was adjusted to 5.5 with 5 M KOH. Forty-five milliliter inoculum and 55 mL formulated nutrient solution (soya peptone 5 g L<sup>-1</sup>, pancreatic peptone 15 g L<sup>-1</sup> and NaCl 5 g L<sup>-1</sup>) were inoculated to start the fermentation.

Compared with ASF, SSF made enzymatic hydrolysis and fermentation conduct simultaneously. Argon was used to purge the reactors to ensure no oxygen exists, and the reactors were incubated 40 °C for 60 h. The volume and composition of produced gas were measured every 12 h. Each experiment was repeated three times to guarantee the reproducibility of the experimental results.

### 2.4. Instruments and methods

All pH were measured by pH meter (PHS-3C, Shanghai, China). Oxidation-Reduction Potential (ORP) was measured by ORP meter (SX712, Shanghai, China).

Hydrogen content in produced gas sample was determined by a gas chromatograph (Agilent, 6820 GC-14B) using nitrogen as carrier with a flow rate of 40 mL/min. The settled temperatures of injection port, column oven, and detector were 100 °C, 80 °C, and 150 °C, respectively.

Liquid samples were centrifuged at 10,000 rpm for 15 min, and then, filtered with 0.45-μm drainage membrane filter for analyzing volatile fatty acid (VFA) concentration. Concentration of VFA (including acetic acid, butyrate and propionic) were analyzed by Agilent 6820 GC

(Agilent Technologies, USA) equipped with a 30 × 0.320 mm × 1.0 μm capillary column (DB-FFAP) and a flame ionization detector (FID).

### 2.5. Analytical methods

The cumulative hydrogen production data from each batch experiment were fitted to a modified Gompertz equation (Zhang et al., 2015), the modified Gompertz Eq. (1) as follows:

$$P(t) = P_{\max} \exp \left\{ -\exp \left[ \frac{r_m e}{P_{\max}} (\lambda - t) + 1 \right] \right\} \quad (1)$$

where,  $P(t)$  is cumulative hydrogen production (mL TS),  $P_{\max}$  is the maximum hydrogen production potential (mL TS),  $r_m$  is the maximum hydrogen production rate (mL h<sup>-1</sup> TS),  $\lambda$  is the lag phase time (h),  $t$  is the incubation time (h),  $e$  is 2.718. The model parameters were determined using the Origin 9.0 software (OriginLab).

The kinetic equation of hydrogen production rate ( $v(t)$ ) can be obtained by differentiating the Eq. (1):

$$v(t) = \frac{dP(t)}{dt} = r_m \exp \left\{ 2 + \frac{r_m}{P_{\max}} (\lambda - t) - \exp \left[ \frac{r_m e}{P_{\max}} (\lambda - t) + 1 \right] \right\} \quad (2)$$

## 3. Results and discussion

### 3.1. Composition of raw material

The cellulose, hemicellulose and lignin contents of the raw material were measured, and the results were listed below (Table 1). The mentioned compositions of the agricultural residues were found to fit the normal range (Song et al., 2015; Zhang et al., 2014).

### 3.2. Analysis of bio-hydrogen yield from ASF

The cumulative hydrogen production from different materials by ASF, and the degradation of reducing sugar are shown in Fig. 1a. During 24 h, the concentration of reducing sugar decreased obviously, because that most reducing sugar was converted into hydrogen. The accumulated hydrogen production increased greatly at this stage. The modified Gompertz equation was employed to fit the cumulative hydrogen production data from each batch experiment, and the kinetic parameters are shown in Table 2. The fitting efficiency between measured cumulative hydrogen production data and those fitted Gompertz equation was high ( $R^2 \geq 0.99$ ; Table 2), indicating a strong correlation between them. The  $\lambda$  of different substrate was within the 1.71–2.63 h range, it demonstrates the anaerobic bacteria can adapt to the environment quickly (Yin and Wang, 2016). According to Table 2, the  $P_{\max}$  of corncob is higher than others, indicating corncob is a better fermentation material, which is similar to previous studies (Zhang et al., 2014). The hydrogen yield of wheat straw is the lowest due to its high lignin content and lowest reducing sugar concentration. The hydrogen production rate curves fitted by Eq. (2) (Fig. 1b), the maximum hydrogen production rate appears in 6–8 h, with corncob as substrate reached the maximum 27.92 mL h<sup>-1</sup>. The peak period of hydrogen production is concentrated in 18 h, during the period, the cumulative hydrogen production accounts for 80–90% of total hydrogen

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