Bioaugmentation with an acetate-type fermentation bacterium *Acetobacteroides hydrogenigenes* improves methane production from corn straw

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HIGHLIGHTS

- *A. hydrogenigenes* is an acetate-type bacterium with high hydrogen yields.
- Bioaugmentation potential of *A. hydrogenigenes* was investigated.
- Addition of *A. hydrogenigenes* improved methane yield by 19–23% from corn straw.
- *A. hydrogenigenes* could increase cellulose and hemicelluloses removal rates.

ARTICLE INFO

ABSTRACT

The effect of bioaugmentation with an acetate-type fermentation bacterium in the phylum *Bacteroidetes* on the anaerobic digestion of corn straw was evaluated by batch experiments. *Acetobacteroides hydrogenigenes* is a promising strain for bioaugmentation with relatively high growth rate, hydrogen yields and acetate tolerance, which ferments a broad spectrum of pentoses, hexoses and polyoses mainly into acetate and hydrogen. During corn straw digestion, bioaugmentation with *A. hydrogenigenes* led to 19–23% increase of the methane yield, with maximum of 258.1 mL/g-corn straw achieved by 10% inoculation (control, 209.3 mL/g-corn straw). Analysis of lignocellulosic composition indicated that *A. hydrogenigenes* could increase removal rates of cellulose and hemicelluloses in corn straw residue by 12% and 5%, respectively. Further experiment verified that the addition of *A. hydrogenigenes* could improve the methane yields of methyl cellulose and xylan (models for cellulose and hemicelluloses, respectively) by 16.8% and 7.0%.

1. Introduction

Anaerobic digestion (AD) has been widely applied for the treatment of wastewater and biodegradable solid wastes, such as municipal sewage sludge, kitchen waste and animal manures. Agricultural residues are abundant and sustainable renewable natural resources in nature, which are attractive feedstock for anaerobic digestion due to its abundance, low production costs, high polysaccharide content and methane yield. However, currently the efficiency of anaerobic technology in treating lignocellulosic biomass to methane is limited. Since methane production are attributed to the activity and stability of the anaerobic microbial populations. Amounts of attention on improvement strategies for anaerobic digestion have presently been paid to design and optimize the complex microbial consortia responsible for lignocellulosic biomass degradation. Bioaugmentation is a feasible strategy for pollutant removal and site remediation, which introduces specific microorganisms to the local population. The technology has been applied in agriculture and in wastewater treatment for many years. Currently, the most successful cases of bioaugmentation occur in confined systems, such as bioreactors in which the conditions can be controlled to favor survival and prolonged activity of the exogenous microbial population (Fantroussi and Agathos, 2005; Gentry et al., 2004).

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Lignocellulosic biomass mainly consists of lignin (5–25%) and carbohydrate polymers (cellulose, 35–45%; hemicelluloses, 25–40%), which are associated with each other (Klinke et al., 2003). Under methanogenic conditions, lignin is resistant to anaerobic degradation, while cellulose and hemicelluloses could be converted into more readily fermentable mono-, di- and oligo-saccharides. Therefore, enhancing the degradation efficiency of the cellulose and hemicelluloses is one of the key factors to facilitate methane production. Stable anaerobic digestion of organic waste is accomplished by four important stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Cellulose is hydrolyzed into glucose, and hemicelluloses is hydrolyzed into pentoses (xylose and arabinose), hexoses (mannose, glucose and galactose) and sugar acids by hydrolytic-fermentative bacteria (Hendriks and Zeeman, 2009). Pentoses and hexoses are further degraded mainly via five fermentation pathways: acetate-type, butyrate-type, propionate-type, ethanol-type and lactate-type fermentation, with the products of propionate, butyrate, acetate, ethanol, lactate, H_2, etc. (Jayasinghearachchi et al., 2009; McDonald and Edwards, 1976). During AD process, the conversion rates of volatile fatty acids (VFAs) to methane varies in the order of acetate > ethanol > butyrate > propionate (Wang et al., 2009). Moreover, acetate is normally the most concentrated of carboxylic acids but is less inhibitory to methanogens than propionate and butyrate (Mösche and Jördening, 1999). Compared to other fermentation pathways, hydrogen producing acetate-type fermentation has advantage for methane yield as the equation described: $C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CO_2 + 2CH_3COOH$, since its products of H_2/CO_2 and acetate could be directly converted into methane by methanogens. It is therefore obvious that acetate-type fermentation bacteria seem to be promising microorganisms for increasing methane production. Currently, most investigations are focused on cellulolytic or hemicellulolytic bacteria as enhanced microorganisms for anaerobic digestion, and no studies on hydrogen producing, acetate-type microorganisms for the bioaugmentation of lignocellulosic biomass as sole feed stock (Akila and Chandra, 2010; Lü et al., 2013; Nielsen et al., 2007; Peng et al., 2014; Weiβ et al., 2010).

In anaerobic crop residues treatment, a high abundance of *Bacteroidetes* organisms are frequently detected; they are considered to be of high relevance for the degradation of complex polysaccharides (Qiao et al., 2013; Wang et al., 2010; Yan et al., 2012). In this study, growth, hydrogen production characteristics, and bioaugmentation potential of a hydrogen producing acetate-type bacterium *Acetobacteroides hydrogenigenes* in the phylum *Bacteroidetes* for AD of corn straw were described.

2. Methods

2.1. Raw material and substrates

Corn straw used in this study was naturally harvested and sun-dried, smashed to approximately 1–3 mm pieces using a grinder. The straw was dried at 105 °C for 12 h to a constant weight before experiments, which contained 93.3% total solids (TS), of which 36%, 30% and 6% were cellulose, hemicelluloses and lignin respectively. Kraft lignin (KL), methyl cellulose (MC) and xylan were purchased from SIGMA.

2.2. Inoculum, bioaugmentation microorganisms and preparation of cell suspension

Bioaugmentation was performed in batch experiments inoculated with adapted biogas slurry taken from a full-scale mesophilic (35 °C), anaerobic completely stirred tank reactor (CSTR) (volume,

500 m^3) feeding with untreated corn straw as the sole feedstock for more than one year (Qiao et al., 2013). Biogas slurry (2 L) was suspended with 2 L sterile distilled water in a 20 L tank. Then, solid content was separated from slurry by filter cloth. To assure that solid content in solution were dominated by bacteria, solution obtained then be filtered by 315 micron cartridge. The suspension solution was then centrifuged at 8000 rpm for 10 min, and the pellets were collected and resuspended in blank medium without substrate. The total solid (TS) and volatile solid (VS) content of methanogenic inoculum were 5% and 60%, respectively. The medium used for cultivation, hydrogen production characteristics and bioaugmentation was prepared as described previously (Sekiguchi et al., 2000).

A. hydrogenigenes (DSM 24657) used for bioaugmentation was originally isolated from a reed swamp (Su et al., 2014). *Terrimicrobium sacchariphilum* (JCM 17479) and *Clostridium pascui* (DSM 10365) were used as control bacteria: *T. sacchariphilum* was originally isolated from rural rice paddy field (Qiu et al., 2014), and *C. pascui* was obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) (Wilde et al., 1997). Main characteristics of the three anaerobic bacteria were listed in Table S1. *A. hydrogenigenes* and *T. sacchariphilum* were maintained on 10 mM glucose medium, and growth was observed within 2–4 days of incubation at 37 °C. Glutamate (2 mM) and yeast extract (0.01%) were used to cultivate *C. pascui* at 37 °C. For bioaugmentation experiment, the cells were harvested at logarithmic phase. The bacterial suspensions were centrifuged at 8000 rpm for 15 min, and the pellets were collected and resuspended in blank medium.

2.3. Evaluation of growth and hydrogen production characteristics of A. hydrogenigenes

Growth and hydrogen production characteristics of *A. hydrogenigenes* were evaluated by batch experiment. Metabolism of glucose by *A. hydrogenigenes* in pure culture and co-culture with a hydrogenotrophic methanogen *Methanospirillum hungatei* (DSM 864) was determined with 3 mM glucose and 0.01% yeast extract (inoculum size, 5%, v/v). The influence of glucose concentrations on the growth of *A. hydrogenigenes* in pure culture and co-culture with *M. hungatei* was checked in the range of 1.2–38 g/L glucose. The effect of acetate on the growth of pure culture of *A. hydrogenigenes* was checked in the medium containing 1.3 mM glucose. Initial sodium acetate (10, 20, 50, 100, 150, 200 mM) was added respectively. The control test was conducted without sodium acetate addition. To test the effect of hydrogen on the growth of *A. hydrogenigenes* in pure culture and co-culture with *M. hungatei*, 101 kPa mixture of hydrogen, carbon dioxides and nitrogen ($H_2/CO_2/N_2 = 3/1/1$, v/v) was used to sparge the headspace of the serum vials containing 3 mM glucose medium at the start of the experiments. Except otherwise described, all experiments were conducted with 60-mL (liquid volume, 20 mL) serum vials in triplicate at 37 °C, with exponential-phase pure culture of *A. hydrogenigenes* grown on glucose medium as the inoculum (10%, v/v). The growth of cells was determined visually by observing turbidity and the increase in OD$_{600}$, monitoring glucose depletion, hydrogen and acetate production. The growth rate of cells was evaluated based on the production of hydrogen (or methane for the co-cultures).

2.4. Bioaugmentation experiments

To investigate the bioaugmentation potential of *A. hydrogenigenes* on corn straw biodegradation (TS, 1%), batch experiments were carried out in 300 mL (liquid volume, 120 mL) serum vials under an atmosphere of $N_2{:}CO_2$ (80:20, v/v) at 37 °C (pH$_{25°C}$ 7.0) with anaerobic slurry as inoculum (inoculum size, 10%, v/v)

without shaking. Furthermore, to investigate the role of A. hydrogenigenes during corn straw degradation, methyl cellulose (MC), kraft lignin (KL), and xylan were used as models for cellulose, lignin and hemicelluloses, respectively. The effect of A. hydrogenigenes on anaerobic degradation of MC, KL and xylan was designed in a similar manner to that of corn straw digestion. Except that the experiment was carried out in 60 mL (liquid volume, 20 mL) serum vials. Each experiment was performed in triplicate. The TS, contents of cellulose, hemicelluloses and lignin of corn straw were measured before and after the degradation assay. Methane production was measured periodically.

2.4.1. Bioaugmentation of A. hydrogenigenes into the biogas slurry to enhance corn straw biodegradation

To determine the bioaugmentation potential of hydrogen producing acetate-type fermentation bacterium on corn straw biodegradation, A. hydrogenigenes was used as an enhanced organism. A propionate-type bacterium T. sacchariphilum and an anaerobic amino acids fermenting bacterium C. pascui were used as the control bacteria. A total volume of 200 mL of pure cultures were collected by centrifugation at 8000 rpm for 15 min at 35 °C, and resuspended in blank medium to give the same OD_{600}. The cell suspension was then inoculated into two kinds of bottles containing medium supplemented with either (i) corn straw and biogas slurry, or (ii) biogas slurry with no addition of corn straw as control test, respectively (inoculum size: 10%, v/v). In addition, two other sets of control experiments were performed to evaluate corn straw degradation without bioaugmentation and endogenous methanogenic activity of the slurry itself: (i) corn straw and biogas slurry, (ii) biogas slurry. In the calculation of methane production, the methane yields were corrected by subtracting the amount of methane formed in the control experiments accordingly. Effect of different inoculation ratios (5%, 10% and 20%) of A. hydrogenigenes on methane production from corn straw was designed in a similar manner as described above.

2.4.2. Effect of A. hydrogenigenes on anaerobic degradation of MC, KL and xylan

To investigate the mechanism of A. hydrogenigenes on the bioaugmentation of corn straw degradation, batch experiments were performed with three models of cellulose, lignin and hemicelluloses of corn straw as sole substrates. Bioaugmentation of methyl cellulose (MC), kraft lignin (KL), and xylan (each 0.1%, w/w) degradation by A. hydrogenigenes was tested similarly with that of the corn straw (see Section 2.4.1.). The inoculum size of A. hydrogenigenes was set in 10% (v/v).

2.5. Analytical methods

The contents of cellulose, hemicelluloses and lignin before and after corn straw digestion were measured according to Georing and Van Soest (1970). SCOD, TS, VS and pH were measured according to the standard methods (SEPA, 2002). Glucose concentration was determined by a glucose biosensor with a glucose oxidase-immobilized membrane (SBA-40D, Biology institute of Shandong Academy of sciences, Shandong province, China). The gas composition and acetate concentration were analyzed by a gas chromatograph as described previously (Yuan et al., 2011).

2.6. Data analysis

Data were presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Duncan's multiple range tests were performed for mean separations. SPSS for windows (SPSS Inc. Chicago, version 16.0) was used for all statistical analysis and P values <0.05 were considered significant.

3. Results and discussion

3.1. Evaluation of growth and hydrogen production characteristics of A. hydrogenigenes

A. hydrogenigenes is an anaerobic, hydrogen producing bacterium belonging to Bacteroidetes isolated from a reed swamp (Su et al., 2014). The main characteristics of A. hydrogenigenes and control strains were summarized in Table S1. A. hydrogenigenes could degrade various substrates, including hexoses, pentoses, oligose, polyoses, tryptone and yeast extract. Prior to bioaugmentation study, growth and hydrogen production characteristics of A. hydrogenigenes were investigated to determine its potential for bioaugmentation. Initial substrate and products concentrations play an important role on the growth and production rates of the hydrogen, and high substrate or products concentrations may have an inhibition on fermentation (Argun et al., 2009; Fabiano and Perego, 2002; Jayasinghearachchi et al., 2009). Therefore, the effects of glucose, liquid and gaseous end products on the growth and hydrogen production of A. hydrogenigenes were determined by varying their initial concentrations in the medium.

A. hydrogenigenes produced acetate and hydrogen as the main fermentation products from glucose (1 mol of glucose was converted to approximately 1.9 mol of acetate and 4.2 mol of hydrogen, electron recovery: 99%) (Fig. 1a and b). The actual degradation and products formation were nearly equivalent to the theoretical stoichiometry (Equation: $C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CO_2 + 2CH_3COOH$). The influence of initial glucose concentration on the growth of A. hydrogenigenes by pure culture and co-culture was shown in Fig. 1. Although A. hydrogenigenes produced hydrogen, its growth was not stimulated in co-cultivation with M. hungatei, suggesting that the growth of the strain may be slightly affected by hydrogen produced (Fig. 1a and b). However, the presence of M. hungatei could considerably increase the efficiency of glucose degradation in a certain concentration range: i.e., 1.2–1.5 g/L glucose was almost completely degraded by the co-culture, while 60% degraded by pure culture (Fig. 1c and d). The strain grew well and kept a high hydrogen (or methane) yield within a wide range of initial glucose concentrations for both pure culture and co-culture (up to 38 g/L) within one month of incubation, and the final pH range was 6.4–6.8 (Fig. 1c and d). The degradation rates of glucose became lower with the increase of glucose concentration. The maximal hydrogen yield was 571 mL/g-glucose (equivalent to 4.6 mol H_2/mol-glucose) at initial glucose concentration of 1.5 g/L with pH 7.0. The observed hydrogen yield was 343 mL/g glucose (2.8 mol H_2/mol-glucose), which was corresponding to 70% of the theoretical yield with 3.8 g/L initial glucose.

Hydrogen and carbon dioxide are the primary composition in the biogas. Hydrogen partial pressure (pH_2) in the gas phase is one of the key factors affecting hydrogen production. In general, hydrogen synthesis pathways are sensitive to hydrogen concentrations and are subject to end-product inhibition (Wang et al., 2007). The effect of pH_2 on A. hydrogenigenes in pure culture and co-culture with M. hungatei was investigated. Glucose could be completely degraded at high initial hydrogen partial pressure (>40 mmol/L culture) by both cultures, however, the pure culture needed a longer lag period of 1–2 days before glucose consumption compared to the co-culture with M. hungatei (Fig. 2a and b). Compared with no hydrogen addition, both cultures needed a longer lag times of 3–4 days for growth when hydrogen presented (Figs. 1a, b, and 2a, b). These results further confirmed that A. hydrogenigenes can tolerate high hydrogen partial pressure. Moreover, the concentration of liquid product of acetate may also impact hydrogen producing bacteria. The effect of acetate on the growth of A. hydrogenigenes is illustrated in Fig. 2c. The addition of acetate up to 50 mM only had a little inhibition (<24%) on the growth and
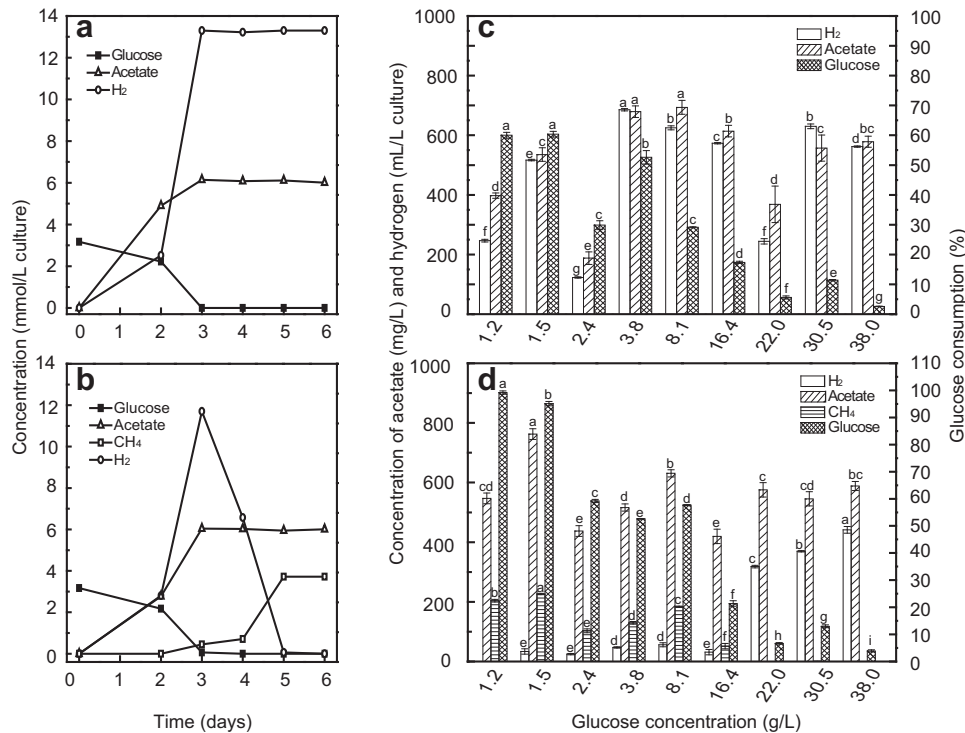
Fig. 1. Metabolism of glucose by *A. hydrogenigenes* in pure culture and co-culture with *M. hungatei*: (a and b) time course of 3 mM glucose degradation; (c and d) effect of different initial glucose concentrations. Significant differences are indicated by different letters ($p < 0.05$).

glucose consumption of *A. hydrogenigenes*. 100 and 200 mM acetate inhibited glucose degradation by 35% and 11%, respectively. The high tolerance was important for maintenance microbe activity in the bioaugmentation system when high levels of saccharides, hydrogen or acetate present. It has been reported that the range of acetate concentration tends to be 0.1–100 mM in AD process (Kovács et al., 2004). *A. hydrogenigenes* had the potential for bioaugmentation with relatively high growth rate, hydrogen yields and products tolerance.

3.2. Effect of bioaugmentation of A. hydrogenigenes into the biogas slurry to enhance corn straw biodegradation

To assess the effect of bioaugmentation with hydrogen-producing acetate-type bacterium *A. hydrogenigenes* into the biogas slurry, batch experiments were performed with corn straw (TS, 1%) as the sole carbon source. A propionate-type bacterium *T. sacchariphilum* and an anaerobic amino acids fermenting bacterium *C. pascui* were selected as the control bacteria. Corn straw was converted to methane within 40 days of fermentation (Fig. 3a). Methane yields from corn straw with *A. hydrogenigenes* addition were significantly higher ($p < 0.05$) than that with control bacteria addition at days 11, 27 and 35. Addition of *A. hydrogenigenes* led to a significant increase in methane yield by 18.4%. The specific methane yield was 234.6 mL/g-corn straw around day 27 in the presence of *A. hydrogenigenes*. However, with addition of control bacteria of propionate-type fermentation bacterium *T. sacchariphilum* or amino acids degrading bacterium *C. pascui*, the methane yields were 200.4 and 205.7 mL/g-corn straw respectively, which were almost the same as that of the control (198.1 mL/g-corn straw). Based on the above results, it can be concluded that bioaugmentation with hydrogen-producing, acetate-type fermentation bacterium *A. hydrogenigenes* can improve methane yield from AD of corn straw.

In the AD process, hydrogen plays an important role in complex microbiological food chain, which is related to biogas production

and process stability. Hydrogen below a certain concentration makes biogas formation become thermodynamically possible. Furthermore, methanogens need a good supply of hydrogen to carry out redox reaction (Kovács et al., 2004). Recently, several researches focused on improvement of biogas production by bioaugmentation with hydrogen-producing cellulolytic or hemicellulolytic bacteria (Bagi et al., 2007; Kovács et al., 2013). Bagi reported that mesophilic *Enterobacter cloacae* and thermophilic *Caldicellulosiruptor saccharolyticus* improved the biogas yield obviously from AD of mixtures of pig slurry, sludge and dried plant biomass inoculated with natural biogas-generating ecosystem in batch experiment. The results demonstrated that only the *in situ* generated H_2 rather than the extra supplemented H_2 was responsible for the intensification. Addition of *C. saccharolyticus* increased 60–70% biogas yield in 15 m³ anaerobic, thermophilic, complete mixing fermentor fed with pig manure slurry as substrate (Bagi et al., 2007). Kovács demonstrated that *E. cloacae* and *C. saccharolyticus* increased biogas yield 33–47% and 40–42%, respectively in 5 L reactor fed with pig slurry and chopped sweet sorghum (Kovács et al., 2013). Combined results from this study with previous studies, it can be assumed that bioaugmentation with hydrogen-producing bacterium will be a feasible strategy for improving methane production from waste treatment.

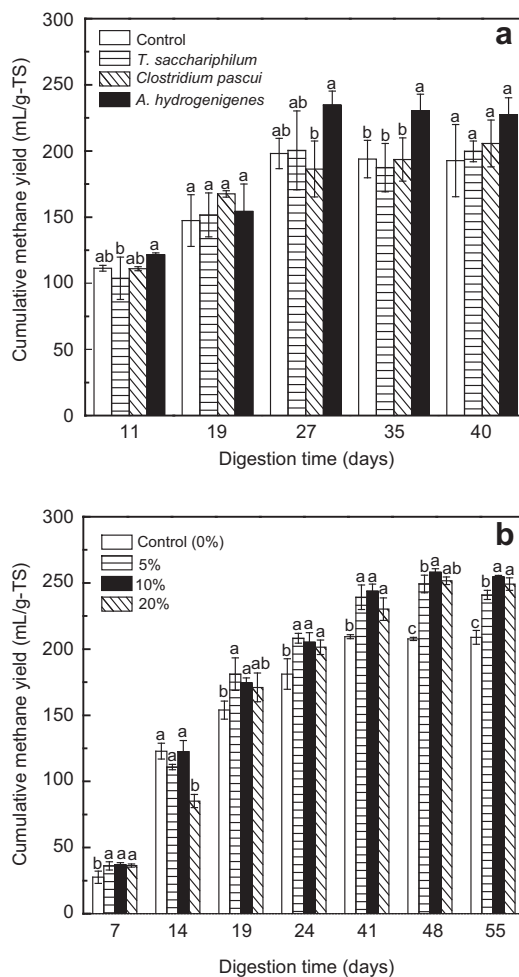### 3.3. Effect of inoculation ratios of A. hydrogenigenes on methane production from corn straw

To further investigate the effect of inoculation ratios of *A. hydrogenigenes* on methane yields, *A. hydrogenigenes* at 5–20% ratios were evaluated by methane yields, TS removal rates, SCOD, cellulose, hemicelluloses and lignin in corn straw. As shown in Fig. 3b, there were significant differences between non-bioaugmentation and bioaugmentation treatments ($p < 0.05$). The maximum methane yields in inoculation ratios of 5%, 10%, and 20% of *A. hydrogenigenes* were 249.2, 258.2, and 251.7 mL/g-corn straw respectively,
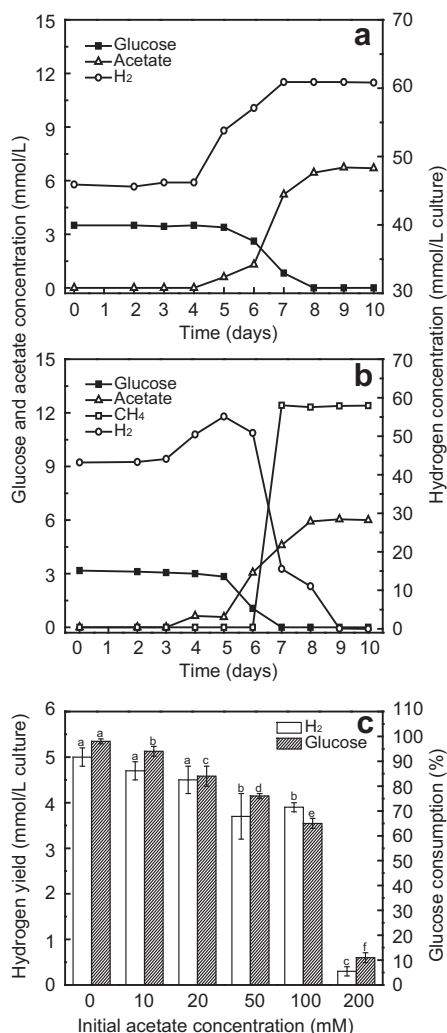
Fig. 2. Effects of hydrogen partial pressure (a and b) and different initial acetate concentrations (c) on the growth and metabolism of *A. hydrogenigenes*: (a and c) pure culture; (b) co-culture with *M. hungatei*. Significant differences are indicated by different letters ($p < 0.05$).

Fig. 3. Effect of bioaugmentation on the cumulative methane yield from anaerobic digestion of corn straw: (a) with *A. hydrogenigenes* and control bacteria *T. sacchariphilum* and *Clostridium pascui*; (b) different inoculum sizes of *A. hydrogenigenes*. Significant differences are indicated by different letters ($p < 0.05$).

increasing by 19–23% compared to that of the control (209.3 mL/g-corn straw). The methane production with 10% inoculum of *A. hydrogenigenes* was significantly higher ($p < 0.05$) than that of 5% and 20% inoculums at the end of fermentation (48–55 days), suggesting that the methane yield by bioaugmentation was not basically proportional to inoculation ratios of enhanced microorganisms. Fotidis used "microbiological domino effect" to explain the function of bioaugmentation bacteria, indicating that bioaugmentation bacteria are sufficient to establish a change in the microbial community though with a relative low abundance (Fotidis et al., 2014). Table 1 showed the values of TS removal efficiency and SCOD after degradation. The TS removal rates with 5%, 10% and 20% inoculation ratios of *A. hydrogenigenes* were 47.0%, 47.1% and 47.2%, which were 5.1% higher than that of the control (44.7%), but the experimental standard deviation (SD) was high. SCOD with 5%, 10% and 20% inoculation ratios of *A. hydrogenigenes* were 1423.8, 1152.5 and 1498.8 mg/L, which was 8–29% lower than that of the control (1628.8 mg/L). Combined with the values of methane yield, TS removal rate and SCOD, it can be hypothesized that hydrogen-producing acetate-type fermentation bacterium *A. hydrogenigenes* improved methane production of corn straw by conversion hydrolysates of cellulose and hemicelluloses to more H_2 and acetate, which then be utilized by methanogens to methane.

To evaluate which components of corn straw contributed to the improvement of methane production with bioaugmentation, lignocellulosic composition of corn straw after digestion was measured (Table 1). The cellulose removal rates with addition of *A. hydrogenigenes* were 76.4% (5% inoculum), 75.4% (10% inoculum) and 71.5% (20% inoculum), which was 5–12% significantly higher than that of the control (68.1%). The hemicelluloses removal rates with *A. hydrogenigenes* addition were 64.3% (5% inoculum), 65.9% (10% inoculum) and 66.9% (20% inoculum) respectively, which was 0.5–5.0% higher than that of the control (63.9%). *A. hydrogenigenes* ferments soluble C5 and C6 sugars, di- and oligosaccharides to acetate and hydrogen, which could promote cellulose and hemicelluloses fermentation.

3.4. Effect of A. hydrogenigenes on methane production from MC, KL and xylan

A. hydrogenigenes could improve methane production through increasing the degradation of hydrolysates of corn straw. To further demonstrate the effect of *A. hydrogenigenes* on AD of cellulose, hemicelluloses and lignin, batch experiments were conducted with kraft lignin (KL), methyl cellulose (MC) and xylan as model substrates. Xylan is the dominant component of hemicelluloses in agricultural plants such as straw (Ebringerová and Heinze, 2000). Kraft lignin was usually utilized as model of lignin component

Table 1
Effect of *A. hydrogenigenes* on TS removal efficiency, SCOD and removal rates of cellulose and hemicelluloses from corn straw.

Inoculation ratios	TS removal efficiency (%)[b]	SCOD (mg/L)[b]	Lignocellulosic composition (%)[b]			Degradation (%)[b]	
			Hemicelluloses	Cellulose	Lignin	Hemicellulose	Cellulose
Initial point[a]	–	–	27.5 ± 2.0a	31.0 ± 2.6a	10.3 ± 0.2b	–	–
0%	44.7 ± 0.3a	1628.8 ± 146.7a	18.0 ± 1.9b	17.9 ± 0.8b	17.5 ± 0.8a	63.9 ± 3.5a	68.1 ± 1.2c
5%	47.0 ± 2.6a	1423.8 ± 397.8a	17.9 ± 0.5b	13.4 ± 1.6c	16.1 ± 1.3a	64.3 ± 0.7a	76.4 ± 2.5a
10%	47.1 ± 2.7a	1152.5 ± 21.2a	17.1 ± 1.7b	13.9 ± 1.6c	16.8 ± 1.5a	65.9 ± 3.3a	75.4 ± 3.4ab
20%	47.2 ± 1.0a	1498.8 ± 203.3a	16.6 ± 2.0b	16.1 ± 0.6bc	17.1 ± 2.3a	66.9 ± 4.7a	71.5 ± 1.2bc

[a] The mixture of cornstalk and slurry before anaerobic fermentation.
[b] Data are shown as mean ± standard deviation. Different letters in a single column indicate significantly difference between different inoculation ratios at $p < 0.05$.
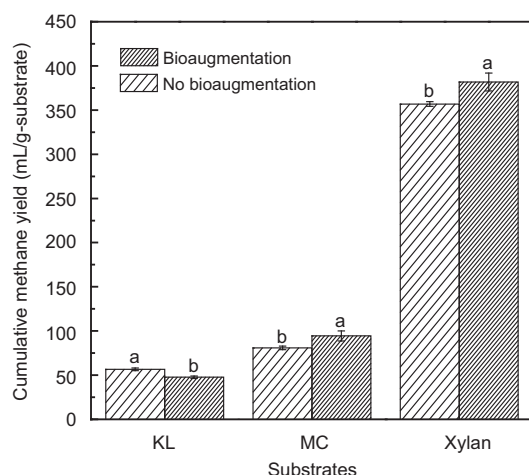
Fig. 4. Methane production from anaerobic degradation of kraft lignin (KL), methyl cellulose (MC) and xylan with addition of *A. hydrogenigenes*. Significant differences are indicated by different letters ($p < 0.05$).

for anaerobic degradation (Wu and He, 2013). Significantly differences were observed ($p < 0.05$) in methane yield between non-bioaugmentation and bioaugmentation of MC, KL and xylan degradation (Fig. 4). The maximum methane production of MC with bioaugmentation was 94.4 mL/g-MC, which was 16.8% higher in comparison with that of the control (80.9 mL/g-MC). Methane production from xylan with *A. hydrogenigenes* addition reached 381.8 mL/g-xylan, 7.0% higher than that of the control (356.8 mL/g-xylan). In contrast, the addition of *A. hydrogenigenes* did not improve methane yield from KL degradation. These results demonstrated that the addition of acetate-type bacterium *A. hydrogenigenes* could improve methane yields of MC and xylan but not lignin. The bioaugmentation results of MC, xylan and KL degradation were in accordance with that of corn straw degradation, suggesting that hydrogen-producing acetate-type fermentation bacterium *A. hydrogenigenes* could enhance methane production from cellulose and hemicelluloses.

This study described the effect of bioaugmentation with a hydrogen-producing acetate-type fermentation bacterium in the phylum *Bacteroidetes* on the AD of corn straw by adapted biogas

Table 2
Comparison of bioaugmentation on methane or biogas production from organic wastes with described species.

Main characteristics of enhanced microorganisms				Application in anaerobic digestion				
Species name	Substrates range	Main products	T (T_{opt} °C)	Feedstock	T (°C)	Reactor type	Increase of methane yield (%)[b]	References
Clostridium thermocellum (DSM 2360)	Cellulose, starch, sugars	Acetate, butyrate, ethanol and H_2	38–72 (43–65)	Microalgae	55	Batch	17–24	Lü et al. (2013)
Clostridium cellulolyticum (DSM 5812)	Cellulose, sugars	Acetate, ethanol, lactate, formate and H_2	25–45 (32–35)	Wheat straw	37	Batch	8–13	Peng et al. (2014)
Clostridium sp. (DSM 15427)	Xylan, sugars	Acetate, formate, lactate and H_2	5–50 (20)	Cattle manure	20	Batch	72[b]	Akila and Chandra (2010)
Caldicellulosiruptor lactoaceticus (DSM 9545)	Cellulose, xylan, starch, pectin, sugars	Lactate, acetate, H_2 and ethanol	50–78 (68)	Manure and its fractions	68/55[a]	Batch	10–24	Nielsen et al. (2007)
Caldicellulosiruptor saccharolyticus (DSM 8903)	Cellulose, glycogen, starch, xylan, sugars	Acetate, lactate and trace amounts of ethanol	45–80 (70)	Dried green biomass and tubers, sludge, pig manure slurry	55	Batch	ND	Bagi et al. (2007)
				Pig slurry and sweet sorghum	55	Reactor (5 L)	40–42[b]	Kovács et al. (2013)
Enterobacter cloacae (DSM 16657)	Sugars	Acid and H_2	(30–35)	Dried green biomass and tubers, sludge, pig manure slurry	35	Batch	ND	Bagi et al. (2007)
				Pig slurry and sweet sorghum	35	Reactor (5 L)	33–47[b]	Kovács et al. (2013)
Acetobacteroides hydrogenigenes (DSM 24657)	Pectin, starch, tryptone, yeast extract, sugars	Acetate and H_2	25–40 (37)	Corn straw	37	Batch	19–23	This study

ND, not determined.
[a] Manure was pretreated with enhanced bacterium at 68 °C, then incubated at 55 °C for anaerobic digestion.
[b] Biogas yield.

slurry. The results showed that the addition of *A. hydrogenigenes* led to a higher methane yield from corn straw or methyl cellulose and xylan (models for cellulose and hemicelluloses, respectively). A proposed mechanism is that hydrogen-producing acetate-type fermentation bacterium promotes more hydrolysates of lignocellulosic biomass, such as C5 and C6 sugars to acetate and hydrogen, and provides a source of energy for the methanogens. The microbiology of anaerobic digestion is complex and delicate. Overall, anaerobic digestion involves four critical steps (hydrolysis, acidogenesis, acetogenesis and methanogenesis) comprised of a sequence of reactions. These complex reactions transpire via a consortium of microorganisms which includes enzyme-secreting, fermentative, H_2-consuming, H_2-producing, acetogenic, CO_2-reducing, and acticlastic methanogenic bacteria (Appels et al., 2008; Chynoweth and Isaacson, 1987). Organic materials must be decomposed to solutes capable of being actively or passively transported across cell membranes before they can be microbially metabolized. Hydrolysis of complex organic material is regarded as the rate-limiting steps. Presently, most researches have focused on the enrichment of cellulolytic consortia and bioaugmentation for hydrolysis and pretreatment of cellulosic biomass. Comparative bioaugmentation potential of *A. hydrogenigenes* and some species used as enhanced microorganisms are shown in Table 2. Among the fermentative anaerobes, genera *Clostridium*, *Caldicellulosiruptor* as well as *Enterobacter* have been well known as cellulose and hemicelluloses degrading bacteria and extensively studied for bioaugmentation from various organic wastes. *Clostridium thermocellum* is an anaerobic cellulolytic and hydrogenogenic bacterium, capable of improving methane production by 17–24% from thermophilic microalgae digestion (Lü et al., 2013). *Clostridium cellulolyticum*, a cellulolytic bacterium was able to increase methane yield from mesophilic wheat straw digestion by 8–13% (Peng et al., 2014). A psychrotrophic xylanolytic bacterium *Clostridium* sp. was found to increase biogas yield by 72% from cattle manure at 20 °C (Akila and Chandra, 2010). *Caldicellulosiruptor lactoaceticus*, an anaerobic thermophilic cellulose and xylan utilizing bacterium was used to pretreat manure, and improved methane production by 10–24% (Nielsen et al., 2007). These results indicated that cellulolytic and hemicelluloses degrading bacteria can be effective agents for improving methane production by 8–47% (Table 2). The current results are comparable with previous studies, bioaugmentation with acetate-type fermentation bacterium *A. hydrogenigenes* led to 19–23% increase of methane yield. Although different fermentation routes are possible, acetate-type fermentation bacterium plays an important role for its abilities to transform soluble sugars into hydrogen and acetate, which can be directly converted to biogas by methanogens. A well-functioning, stable digester had a very low dissolved hydrogen concentration and converts most of the organic substrate to acetic acid (Massé and Droste, 2000). Combined previous studies with this study, it could be assumed that hydrogen producing acetate-type bacterium seems to be an effective procedure for improving methane production from lignocellulosic materials. The fate and dynamics of *A. hydrogenigenes* in the AD process is unclear and require further investigations for future applicability on an industrial scale reactor.

4. Conclusions

Principal conclusions are summarized as follows:

(1) *A. hydrogenigenes* is a hydrogen-producing acetate-type bacterium with a wide range of growth, substrate utilization, relatively high growth rate, high hydrogen yields, substrate and products tolerance. The maximal hydrogen yield was 571 mL/g-glucose (equivalent to 4.6 mol H_2/mol glucose) at initial glucose concentration of 1.5 g/L.

(2) Bioaugmentation with *A. hydrogenigenes* could increase 19–23% methane yield of corn straw degradation. The maximum methane yields of 258.1 mL/g-corn straw was achieved by 10% inoculum. *A. hydrogenigenes* could improve the methane yields from methyl cellulose and xylan (models for cellulose and hemicelluloses, respectively) by 16.8% and 7.0%.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2014.12.022.

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