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ORIGINAL ARTICLE

Enhanced biohydrogen production from oat straw co-digested with cow dung / sewage sludge by combined aerobic digestion and anaerobic fermentation

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Abstract *- Hydrogen was produced from oat straw by combined aerobic and anaerobic fermentation with fungi and cow dung. With aerobic pre-digestion, the maximum hydrogen production rate reached 133 ml/g volatile suspended solids per hour. The maximum hydrogen yield was 71.5 ml/g straw in 6 days by biological process. The lignocellulosic conversion of oak-straw waste was 39%, with the complex component converting 68% of the hemi-cellulose and 61% of the cellulose, but only 34% of lignin conversion. Aerobic pre-digestion by Trichoderma viride and Saccharomyces cerevisiae was significantly effective for lignin degradation. Combining aerobic digestion and anaerobic fermentation is a promising low-cost efficient and environmentally friendly method, not only for hydrogen production, but also for converting straw biomass.*

Keywords *- Biohydrogen production; aerobic digestion; anaerobic fermentation; pretreatment; straw wastes*

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Introduction

Production of biofuels from renewable biomass is a potentially important fuel generation technique to reduce reliance on fossil fuels and provide a more sustainable alternative (Han and Shin, 2004). As hydrogen is a clean energy that could be produced from waste materials, such as organic waste crop straw (Ilgi and Fikret, 2006), some believe that hydrogen could replace fossil fuels as the next generation energy carrier (Fan et al., 2004; Verhelst, 2014).

Straw, a major lignocellulosic waste, is a promising source of potential candidates for energy production due to its content of approximately 70-80% carbohydrates, low cost, abundance and wide availability (Dererie et al., 2011). Harvesting of crops produces large amounts of straw annually, most of which is improperly discarded, thereby causing serious environmental problems, such as air pollution from burning (Dominguez-Escriba and Porcar, 2010). It is imperative to solve these problems by transforming these wastes into useful products. Producing hydrogen from straw could not only eliminate its environmental impacts, but also ensure a secure renewable energy supply (Kaparaju et al., 2009).

Considerable research in recent years has been focused on the conversion of biomass renewable resources to hydrogen (Pakarinen et al., 2008; Kim et al., 2012). Fan et al. (2006) successfully used wheat straw wastes pretreated with HCl to produce biohydrogen gas by cow dung compost. Li and Chen (2007) employed enzymatic hydrolysis and steam explosion to generate hydrogen by simultaneous saccharification and fermentation of corn straw with *Clostridium butyricum*. Wu et al. (2013) produced 189 ml of H_2 from 2 g dry barley straw after 7 days dark fermentation aided by ozone pretreatment.

Since straw is a complex polymeric substance mainly composed of cellulose, hemicellulose and lignin, successful composting of lignocellulosic straw requires effective pre-digestion in order to free cellulose and hemicellulose from lignin before the hydrogen is produced. Diverse pretreatment methods are used in hydrogen production, including physical, chemical, ozone and thermal options (Xin and Kumakura, 1992; Vrije et al., 2002; Li and Chen, 2007; Wu et al., 2013). Although some researchers have obtained high hydrogen production in relatively short periods of time, those treatments are complex, costly and place great demands on equipment (Antizar-Ladislao and Turrion-Gomez, 2008). In addition, the chemicals used in the treatments inevitably cause environmental pollution, deviating from the original intention of clean energy.

Utilizing microbial consortia to convert lignocelluloses has been proposed as a highly efficient approach, as it avoids feedback regulation and metabolite repression problems (Soundar and Chandra, 1987), while being claimed to have remarkable advantages of simplicity, low cost, harmlessness and low equipment requirements

(Mshandete et al., 2005). Previous studies indicated that some microorganisms can be used to help degrade the cell walls of straw. *Phanerochaete chrysosponium* was used as lignin-degrading fungus in early years (Glenn and Gold, 1983; Gold and Alic, 1993). Kausar et al. (2010) selected *Trichoderma viride* as cellulolytic fungi and *Aspergillus niger* as ligninolytic fungi to digest rice straw and obtained a significant decrease in the straw cellulose and hemicellulose contents. The yeast *Pachysolen tannophylus* was also employed in single-batch bioconversion of wheat straw to ethanol (Zayed and Meyer, 1996). It has been reported that hydrogen can be produced by glucose fermentation through three metabolic pathways, including oxidative decarboxylation of pyruvic acid to acetyl-CoA, oxidation of NADH (nicotinamide adenine dinucleotide, reduced form) to NAD^+ (oxidized form) and acetogenesis by hydrogen-producing acetogens (Zhang et al., 2012). However, the exact or dominant pathways of hydrogen production in the anaerobically activated fermentation process have not yet been identified. Limited research has been reported that uses these fungi to improve hydrogen productivity in straw anaerobic fermentation.

The focus of this research is to develop safe, economic and sustainable techniques for converting straw into biohydrogen with high efficiency by utilizing oat straw waste through a novel fermentation process combining aerobic bio-digestion and anaerobic fermentation with fungi and cow dung. Hydrogen production conditions were also optimized, including temperature, inoculum size and inoculum source (cow dung or sewage sludge).

Material and Methods

2.1. Experimental apparatus

To verify our hypothesis, bench batch scale experimentation was adopted. Simple equipment and apparatus were utilized in the biohydrogen production process. An autoclave reactor, microwave, centrifuge and oven were needed for pretreatment, as well as an incubator for aerobic digestion and an orbital shaker for anaerobic fermentation. Details of the processes are described in Sections 2.3 to 2.5.

2.2. Raw materials

The oat straw waste used as substrate was obtained from a farm in Richmond, a suburb of Vancouver, Canada.

For aerobic fermentation, two fungal species were tested: (1) *Trichoderma viride* from School of Architecture & Environment in Sichuan University, Chengdu, China; and (2) *Saccharomyces cerevisia* from the Life Sciences Institute at the University of British Columbia, Vancouver, Canada.

For anaerobic fermentation, cow dung used as digesting microflora was obtained in a suburb of Vancouver, Canada; mesophilic and thermophilic sludge were collected from the Lulu Island Waste Water Treatment Plant and the Annacis Island Waste Water Treatment Plant, respectively, both in greater Vancouver.

The initial physical and chemical characteristics of oat straw, cow dung and sewage sludge are summarized in supplemental information, Table S1 and S2.

2.3. Pretreatment of the materials

The oat straw waste was chopped into 1-2 cm pieces, then milled and sieved through a 2.0 mm screen, and dried at 70 ± 1 °C for 4 hours. The moisture content was measured as the weight loss of 1 g of oat straw (Zhu, 2005). The cellulose, hemicellulose and lignin contents were measured following the methods described by Goering and Van Soest (1970).

The two microorganism species were enriched and domesticated before aerobic fermentation at 30 ± 1 °C by shaking at 150 rpm for 36 h in the medium, modified as proposed by Mandels and Weber (1969): 22 g Ammonium tartrate; 20 g glucose; 20 g KH_2PO4; 8.7 g $MgSO_4$; 1.0 g $CaCl_2$; 0.6 g NaCl; 0.35 g $MnSO_4$; 60 mg $FeSO_4$; 110 mg $CoCl_2$; 60 mg $ZnSO_4$; 95 mg $CuSO_4$; 6 mg H_3BO_3; 6 mg Na_2MoO_4 and 100 mg VB1 in 1L deionized water.

To ensure the quality of the hydrogen product, the cow dung compost and sludge were heat-treated by boiling for 30 min to inactivate H_2-consuming bacteria and to enrich spore-forming H_2 producers (Chang et al., 2011). The sludge was centrifuged at 1000 rpm to reduce the water content. The heat-treated sewage sludge and cow dung were sieved through a 2.0-mm screen in order to remove impurities such as sand, hay residue and dust particles. The TS and VS of sludge and cow dung were analyzed according to standard methods (APHA, 1998), and the chemical oxygen demand (COD) according to SFS 5504 (Finnish Standard Association, 1988). Soluble chemical oxygen demand (SCOD) from the sludge and cow dung was analyzed after the leaching test, which was modified from SFS-EN 12457-4 (Finnish Standard Association, 2002). The pH was determined with a Metrohm 774 pH-meter (Metrohm, Switzerland).

2.4. Aerobic digestion

For aerobic digestion, 6 g of raw oat straw and two microorganism species, *S. cerevisiae* and *T. viride*, were inoculated together with 1 ml of each strain into 250 ml Erlenmeyer flasks containing 100 ml of medium, as described above in section 2.3. The culture was incubated at 28 ±1 °C for 72 h with shaking at 150 rpm. The straw was then washed with deionized water three times and dried at 105 ± 1 °C for 24 h before anaerobic fermentation.

For comparison, two pretreatment methods, chemical and physical, were tested on this substrate. Chemical treatment involved mixing the suspension containing ground oat straw waste with a particle size less than 0.3 mm and dilute 2% H_2SO_4, which was put into the

sterilizer at 121 ± 0.5 °C and 103.4 ± 0.05 kPa (Bao et al., 2009). Physical treatment involved mixing straw waste with 2% $FeCl_3$ to a concentration of 25 g/L and then putting it into a microwave oven and heating at 160 °C for 19 min (Lü and Zhou, 2011). To further control the hydrogen production and avoid pollutants leaving the production system, the two groups of straw treated by these two methods were finally washed with deionized water until their pH reached 6-7 without color. This is to ensure that no soluble substrate (i.e. soluble pollutants) resulted from acid. They were then dried in an oven at 65 °C prior to use in the subsequent anaerobic fermentation.

2.5. Anaerobic fermentation

The anaerobic experiments were performed with 150 ml serum vials as batch reactors containing the mixture of the compost/sludge, 6 g of the pretreated or raw oat straw, and 60 ml of nutrient stock solution. These vials were infused with nitrogen to remove oxygen from the headspace of the reactors and keep the environment anaerobic. The bottles were incubated in an orbital shaker, with a rotation speed of 90 rpm to provide better contact among substrates. Each liter of nutrient stock solution contained 4 g yeast extract, 12.4 g of KH_2PO_4; 0.1 g of $MgSO_4.7H_2O$; 0.01 g of NaCl; 0.01 g of $Na_2MoO_4.2H_2O$; 0.01 g of $CaCl_2.2H_2O$; 0.015 g of $MnSO_4.7H_2O$ and 0.0278 g of $FeCl_2$. The above method was modified from Lay et al. (1999). The volume of biogas was determined using glass syringes of 5 to 50 ml.

Biological hydrogen production appears to be usually accompanied by the formation of volatile fatty acids (VFAs) and alcohol, whereas both of these are the main by-products in the metabolism of hydrogen fermentation (Fan et al., 2006). In this research, VFAs and alcohol were measured as the major by-products of the aerobic fermentation. After 6 days of fermentation, cultures were taken for measurement of reducing sugars, VFAs analysis, and ethanol concentration. The reducing sugar concentration was estimated using the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). The ethanol content was determined by gas chromatography (GC, Hewlett Packard HP6890 series system), as described by Krishna et al. (1999). The concentrations of the VFAs were analyzed using the same GC with a flame ionization detector (FID) and a capillary column (HP_FFAP, 30 m × 0.32 mm × 0.53 μm). The temperature of the injection port was 180 °C, detector at 250 °C, oven temperature program with programmed column temperature from 80-200 °C at 15 °C/min. Helium was the carrier gas at a flow rate of 1-2 ml/min.

The residue of the straw was collected to determine the cellulose, hemicellulose and lignin contents by the methods described above.

The gas composition (H_2, CH_4 and CO_2) was analyzed with a gas chromatograph (Fisher-Hamilton Gas Partitioner) equipped with a thermal conductivity detector (TCD) and a 6-foot stainless column packed with Porapak Q (80/100 mesh). The operating temperatures of the injection port at ambient temperature. Helium was the carrier gas at a flow rate of 20 ml/min.

All the experiments were performed in duplicate and the average values are reported. The experimental plan is summarized in Fig. 1.

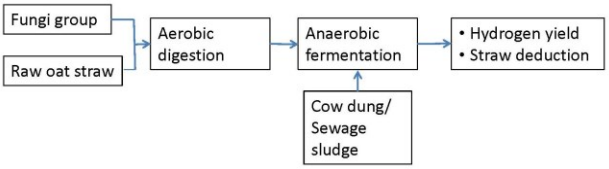

Fig. 1. Summary of the fermentation process.

2.6. Analytical methods and data analysis

The cumulative hydrogen production data were fitted to a modified Gompertz equation (Fan and Chen, 2004), which was a suitable model for describing the progress of cumulative hydrogen production in a batch experiment:

$$H_2 = P \cdot \exp\left\{-\exp\left[\frac{Rm \cdot e}{P}(\lambda - t) + 1\right]\right\}$$

where H_2 is the cumulative hydrogen production (ml), P is the hydrogen production potential (ml), t is the reaction time (d) Rm is the maximum hydrogen production rate (ml/h), λ is the lag-phase time (h) and e is 2.718. Origin 6.0 analysis software was used to fit the equation and determine P, Rm and λ. The hydrogen yield (ml/g straw) was obtained by dividing P by the dry weight of straw used for fermentation. The maximum specific hydrogen production rate (ml/g VSS) was obtained by dividing Rm by the volatile suspended solids (VSS) obtained by subtracting ash from dry straw.

Analysis of variance (ANOVA) was used to estimate the statistical parameters. Two additional confirmation experiments were later conducted to verify the validity of the statistical experimental strategies.

3. Results and Discussion

3.1. Effects of aerobic pre-digestion on hydrogen production

Because chemical and physical pretreatments are often used when utilizing cellulosic materials (Antizar-Ladislao and Turrion-Gomez, 2008), the same substrate was used to compare the effects of aerobic pre-digestion with chemical and physical pretreatment on hydrogen fermentation. Hydrogen fermentation from un-pretreated straw was used as a control. Table 1 presents the kinetic parameters for hydrogen production with the three types of pretreatment. Though hydrogen yields of both chemical and physical pretreated fermentation were a little higher than for aerobic pre-digested fermentation, the lag stages of the two former were much longer and the maximum specific hydrogen production rate was less than that of the latter. Moreover, the aerobic digestion

may have broken lignin barriers in cell walls for improved substrate conversion and shortened the start time of anaerobic fermentation. Therefore, aerobic digestion by *T. viride* and *S. cerevisiae* is a promising pretreatment method for hydrogen production from oat straw, and the anaerobic fermentation conditions were optimized in the following experiments.

Table 1. Kinetic parameters for hydrogen production with different pretreatments (initial pH: 7.0 ± 0.2, S:L[a]= 1:10, cow dung inoculum mass: 5 g, temperature: 65 ± 1 ℃).

Pretreatment type	Hydrogen yield (ml/g)	R_m[b]	λ(h)[c]	R^2[d]
Aerobic bio-digestion	64	130	7	0.99485
Acid pre-hydrolysis	65	93	11	0.99395
Microwave	67	102	9	0.99532
No pretreatment	31	35	20	0.99743

[a] S:L is the ratio of straw mass to stock solution volume (g/ml).
[b] Rm is the maximum hydrogen production rate (ml/h).
[c] λ is lag-phase time (h).
[d] R^2 is the coefficient of determination.

3.2 Optimization of anaerobic fermentation process
3.2.1 Effects of inoculum source on H_2 production

Table 2. Kinetic parameters for hydrogen production by inoculum source (initial pH: 7.0; S:L[a]=1:10, inoculum mass: 5 g; temperature: 65 ± 1 ℃ for cow dung and thermophlic sludge, and 36 ± 1 ℃ for mesophilic sludge).

Inoculum	Hydrogen yield (ml/g)	R_m[b]	λ(h)[c]	R^2[d]
Cow dung	65	127	10	0.99534
Thermophilic sludge	60	101	7	0.99498
Mesophilic sludge	9	5	20	0.99876

[a] S:L is the ratio of straw mass vs. stock solution volume (g/ml).
[b] Rm is the maximum hydrogen production rate (ml/h).
[c] λ is lag-phase time (h).
[d] R^2 is the coefficient of determination.

Considering their low cost and availability, mesophilic and thermophilic sludge and cow dung were added into the anaerobic fermentation system separately to compare their decomposition effects. Table 2 shows that fermentation with cow dung produced the most abundant hydrogen and the highest specific hydrogen production rate compared to the two kinds of sludge. However, the shortest lag stage was obtained by the fermentation with the inoculum of thermophilic sludge. The reason for this may lie in the fact that the thermophilic sludge taken from waste water treatment plant had already acclimated before the experiment so that it could adapt more quickly to the fermentation than the cow dung sampled from a dairy farm, in which microorganisms needed time to adjust to unfavorable fermentation conditions (Li and Chen, 2007). Unfortunately, little hydrogen was detected in the fermentation with mesophilic sludge, indicating that this kind of sludge may not be a good microflora source for the production of hydrogen. Therefore, our results showed that hydrogen fermentation was more favorable in cow dung than in sludge.

3.2.2 Temperature
Table 3 summarizes the kinetic parameters for hydrogen fermentation at temperatures ranging from 50 to 70 °C. The results show that the hydrogen yield and maximum specific hydrogen production rate increased with increasing temperature, up to 70 °C, at which hydrogen production slowed slightly, indicating that microorganisms were inhibited at too high temperature. Furthermore, the efficiency of hydrogen production at 65 °C was much greater than at other temperatures. However, there was not much difference between lag-phases in fermentations at different temperatures.

Table 3. Kinetic parameters for hydrogen production at different temperatures (initial pH 7.0 ± 0.2; S:L[a]=1:10; inoculum, cow dung).

Temperature (± 1 ℃)	Hydrogen yield (ml/g)	R_m[b]	λ(h)[c]	R^2[d]
50	50	48	13	0.99763
55	59	60	8	0.99534
60	62	96	7.5	0.99658
65	70	129	7	0.99498
70	65	117	7.5	0.99876

[a] S:L is the ratio of straw mass vs. stock solution volume (g/ml).
[b] Rm is the maximum hydrogen production rate (ml/h).
[c] λ is lag-phase time (h).
[d] R^2 is the coefficient of determination.

3.2.3 Inoculum size
Cow dung compost was added at (w/w) ratios of mass of compost to substrate mass from 0.5 to 1.5. The results showed that the compost concentration clearly affected the hydrogen yield. Table 4 demonstrates that both the hydrogen yield and maximum specific hydrogen production rate increased as the inoculum ratio increased from 0.5 to 1 and decreased when inoculum size was larger.

The shortest lag stage was obtained at an inoculum ratio of 1. It is apparent that up to a point, having more microorganisms allows them to use the substrate more completely and grow more quickly. For a large inoculum ratio, microorganisms would contact materials fully. But

excessive inoculum would result in the accumulation of harmful chemicals and a drop in pH in the reactor, inhibiting the growth of hydrogen-producing bacteria (Fan et al., 2006).

The partial pressure of hydrogen in the batch reactor initially rose with increasing inoculum concentration. It is known that alcohol is produced from decomposition of reducing sugar to provide ATP (adenosine triphosphate, energy storage form in creatures) for the microflora and is a media product of hydrogen production. When the partial pressure of hydrogen reached a certain level in the reactor headspace, however, the microorganisms switched to alcohol production, inhibiting hydrogen production (Fan et al., 2004). As a result, the optimum inoculum ratio was 1:1.

Table 4. Kinetic parameters for hydrogen production with different inoculum sizes (mass ratio of inoculum vs. straw). (Initial pH, 7.0; S:L[a]＝1:10; inoculum, cow dung; T=temperature, 65 ±1 ℃).

Inoculum size (Inoculum/ Straw)	Hydrogen yield (ml/g)	R_m [b]	λ(h)[c]	R^2 [d]
0.50	59	70	10.5	0.99534
0.75	62	119	8.5	0.99658
1.00	69	130	7.5	0.99498
1.25	65	122	8.0	0.99879
1.50	60	110	8.9	0.99765

[a] S:L is the ratio of straw mass vs. stock solution volume (g/ml)..
[b] Rm is the maximum hydrogen production rate (ml/h).
[c] λ is lag-phase time (h).
[d] R^2 is the coefficient of determination.

at 7 h to 31.5 ml H_2 /g straw at 10 h, while CO_2 fluctuated from 5.7 to 5.0 ml/g straw. The maximum hydrogen yield of 71.5 ml H_2/g straw was observed at 144 h, with a maximum hydrogen production rate of 133 ml H_2/g VSS per hour. The hydrogen content in the biogas was 61.5%, whereas CO_2 accounted for just 14.4%, and there was no significant methane observed in this study.
Compared with the control, the pH of the medium decreased significantly (from 7.0 to 5.0) with the progress of hydrogen evolution and straw decomposition (Table 5), which can be explained by the VFA results. In addition, there was considerable water production during the fermentation, which is necessary for microorganisms to contact with materials fully so as to use the substrate completely and grow quickly to a logarithmic phase. The optimum pH for hydrogen production appeared to be in the 5-5.5 range.

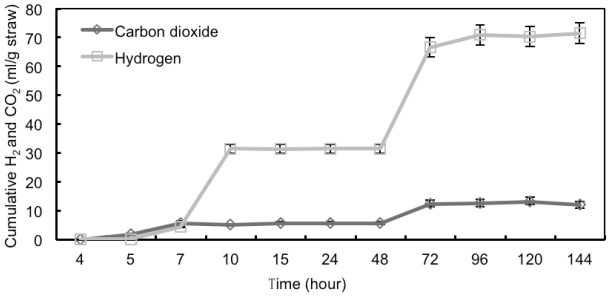

Fig. 2. Development of cumulative hydrogen and carbon dioxide yield during the conversion of the substrate to biohydrogen under the condition of aerobic bio-digestion.

Hydrogen production was accompanied by the formation of VFAs throughout the straw fermentation (Table 5). All the by-products by aerobic pre-digested hydrogen fermentation were noticeably higher than for fermentation without aerobic pre-digestion (control). During the anaerobic fermentation period, acetate was the main VFA product at 5061.2 mg/l, while propionate and butyrate reached maximum yields of only 202.4 and

Table 5. Soluble metabolites produced from aerobic pre-digested straw with un-pretreated straw as the control in hydrogen fermentation (initial pH＝7).

Straw	pH	HAc (mg/L)	HBu (mg/L)	HPr (mg/L)	Isovaleric acid	Valeric acid	EtOH (mg/L)
Control	6.5	415.4	7.8	9.5	0	0	719
Aerobic pre-digested	5.0	5061.2	202.4	303.4	270.4	1.3	550

3.3 Biodegradation effects of combined aerobic digestion and anaerobic fermentation of oat straw
In this paper, VFAs and alcohol were selected as the main byproducts of the composts consuming the substrate. Fig. 2 shows the changes in accumulative hydrogen and carbon dioxide yield during the conversion of the anaerobic pre-digested oat straw wastes to biohydrogen by cow dung compost. As shown in Fig. 2, hydrogen began to evolve after 4 h of cultivation. The hydrogen yield increased rapidly from 4.3 ml H_2 /g straw

303.4 mg/l at 144 h of fermentation, respectively. Our findings also showed that there was some iso-valeric (270.4 mg/l) and a little valeric acid 1.3 mg/l produced in the metabolic path of microbial digestion toward hydrogen production.

Ethanol began to be produced after 4.5 h of cultivation and increased to 719 mg/l at 144 h, significantly higher than the control. When the reaction reached a quasi-steady state, the production of VFAs and ethanol reached

a plateau. Hydrogen production stopped when the available substrate was consumed, and the ethanol, acetate and butyrate were left in the batch reactor as significant by-products. At this time, acetate accounted for 75-80% of total VFAs, resulting in an acidic environment of pH~5 as indicated earlier, whereas the amounts of propionate and butyrate were very low.
This result is similar to that found for biohydrogen fermentation from glucose (Fan et al., 2002), which also generates acetate as the main VFA component, but with higher levels of butyrate. It implied that hydrogen fermentation from aerobic pre-digested oat straw was directed by acidogenic pathways and was essentially acetate-type fermentation. In order for microorganisms to convert oat straw into biohydrogen efficiently, it is necessary for the activity of the propionate and butyrate producers to be suppressed. Under the aerobic pre-digestion and optimum conditions of hydrogen production, the metabolic pathways may be different from those used in biohydrogen fermentation reported as butyrate-type (Chen, et al., 2005). Other compounds produced in the process of fermentation, such as isovaleric and valeric acids, etc., might have some influence on the metabolic pathway.

Table 6 presents the percentage composition of oat straw and its aerobic pre-digested fermentation residue. The conversion of the substrate was 39%, while the conversions of hemi-cellulose and cellulose were 68% and 61%, respectively. More importantly, the conversion of lignin was a remarkable 34%, which shows that aerobic pre-digestion by *T. viride* and *S. cerevisiae* was significantly helpful for lignin degradation.

Table 6. Conversion of substrate in aerobic pre-digested straw after hydrogen fermentation.

Conversion (%)	Substrate	Hemi-cellulose	Cellulose	Lignin
Control	11	29	28	1
Aerobic pre-digested	39	68	61	34

In summary, our results indicate that it is effective to enhance biohydrogen production from oat straw co-digested with cow dung/sewage sludge by combined aerobic and anaerobic fermentation. There is almost no material cost due to utilizing waste-to-resource incorporating natural processes; there is no need to use extreme pressure, i.e. the pretreatment pressure in this process is ~103 kPa, whereas aerobic digestion and anaerobic fermentation are operated at atmospheric pressure; nor is a high temperature required, i.e. the pretreatment temperature in this process is ~120 °C, whereas aerobic digestion (<30 °C) and anaerobic fermentation are operated at (<70 °C). Other processes, e.g. Change et al (2011), use 150 °C for pretreatment, consuming much more energy; Dererie et al. (2011) employed enzyme for pretreatment, which is much more expensive; Li and Chen (2007) pretreated straw at the high pressure of 1.5 MPa.

4. Conclusions

Compared with chemical and physical pretreatment, combined aerobic digestion and anaerobic fermentation has the advantages of a short lag-stage and high hydrogen production rate. The hydrogen content in the biogas was 61.5%, with 14.4% CO_2, negligible methane and maximum hydrogen yield of 71.5 ml H_2/g straw. In addition, the results showed that this method had positive effects on the conversion rate of oat straw (39%) and the conversion of lignin (34%). Therefore, this technology could be a low cost, efficient and environmentally friendly biological method, not only for hydrogen production, but also for straw biomass conversion.

5. Recommendations

Our hypothesis and assumptions have been verified using bench batch scale trials (~1 L). The next step is to conduct pilot trials before application, e.g. at a scale of 100-500 L, where we do take advantage of a continuous process to increase productivity by removing the by-products in the anaerobic fermentation, such as iso-valeric and valeric acid and supply more active microflora and nutrients.

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