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Full Length Research Paper

Biological hydrogen production from acid-pretreated straw by simultaneous saccharification and fermentation

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Hydrogen is currently produced in large amounts in the chemical industry, including that from the steam reforming of fossil fuels. Hydrogen must be produced sustainably to be economically feasible. This could be achieved from water through electrolysis powered by photosynthetic or other renewable energy, or by gasification or pyrolysis of biomass. It may also be possible to develop a cost-effective and reliable technology to produce hydrogen directly from renewable biomass or organic waste products through anaerobic fermentation. Lignocellulosic biomass contains approximately 70 to 80% carbohydrates. If properly hydrolyzed, these carbohydrates can serve as an ideal feedstock for fermentative hydrogen production. In this research, batch tests of biohydrogen production from acid-pretreated wheat straw were conducted to analyze the effects of various associated bioprocesses. The objective of the pretreatment phase was to investigate the effects of various sulfuric acid pretreatments on the conversion of wheat straw to biohydrogen. When sulfuric acid-pretreated solids at a concentration of 2% (w/v) were placed in an oven for 90 min at 120ºC, they degraded substantially to fermentative gas. Therefore, wheat that is pretreated under the evaluated conditions is suitable for hydrolysis and fermentation in a batch test apparatus. Different conditions were evaluated in the tests, which were conducted in accordance with standard batch test procedures (DIN 38414 S8): fresh straw, pretreated straw and simultaneous saccharification and fermentation (SSF). The SSF method proved to be the most effective and economical way to convert wheat straw to biohydrogen. The hydrogen yield by this method was 1 mol- H_2/mol-glucose, which resulted from 5% carbon degradation (η_C gas) or the equivalent of 64% of the hydrogen volume that was produced in the reference test (glucose equivalent test). This method also proved to have the shortest lag phase for gas production. H_2 yields observed in this study are higher than the H_2 yields reported in other studies (the use of mixed culture inocula resulted in a slightly higher yield).

Key words: Biohydrogen, wheat straw, acid pretreatment, simultaneous saccharification and fermentation (SSF), bioenergy.

INTRODUCTION

Ongoing fossil fuel use to meet global energy needs is causing critical environmental problems all over the world. Concerns related to these problems include energy, economic and political crises and effects on human, animal and plant health. Since the oil crisis in 1973, considerable progress has been made in the search for alternative energy sources (Momirlan and Veziroglu, 2002). Modern bioenergy has received increased attention in the past decade because it not only provides an effective energy source from a technical point of view but also utilizes resources that can be sustainably obtained around the globe (Silveria, 2005).

It is well known that biomass residues such as crop stalks are persistent environmental pollutants. The annual global yield of biomass residue exceeds 220 billion tons, which equals the energy of 60 to 80 billion

tons of crude oil (Fan et al., 2006). Cellulosic biomass is a promising resource due to its abundance and low cost; it has been used in a variety of fermentation processes to produce biofuels like ethanol and hydrogen (Ladisch et al., 1983; Lechner et al., 2006). Hawkes et al. (2002) suggested that hydrogen production from fermentable biomass has an advantage over ethanol production because the microorganisms that produce hydrogen can use a wider range of cellulosic hydrolysates than the yeast on which ethanol production is chiefly based.

Hydrogen, an entirely carbon-free fuel with a high combustion enthalpy (141.9 kJ/g), is considered a feasible alternative to fossil fuels, and the technology for using hydrogen as a transport fuel is already well established. Hydrogen is a promising, ideal fuel of the future that offers many social, economic and environmental benefits. In the long term, it has the potential to reduce the global dependence on imported oil and also to decrease carbon and other emissions from the transportation sector. The idea of a post-fossil fuel, hydrogen-based economy began to gain mainstream interest only in the last decade (Kotay and Das, 2007).

Hydrogen is currently produced in large amounts in the chemical industry, including from the steam reforming of fossil fuels. Hydrogen must be produced sustainably to be economically feasible. This could be achieved from water through electrolysis powered by photosynthetic or other renewable energy, or by gasification or pyrolysis of biomass. It also may be possible to develop a cost-effective and reliable technology to produce hydrogen directly from renewable biomass or organic waste products through anaerobic fermentation (Hawkes et al., 2007). Strategies for H_2 production from plant sources essentially follow two major routes: Photochemical conversion of sunlight and dark fermentative processes. Although many scientific issues still require research, the fermentative path currently appears to be closer to practical utilization. The benefits of this approach include the low cost of biomass and the fact that the byproducts of agricultural food production can be used as feedstock (Fan et al., 2006; Pattra et al., 2008; Kyazze et al., 2008). Many substrates have been used for fermentative hydrogen production; glucose, sucrose and starch have been the most widely used. In recent years, however, a few studies have begun to use organic wastes as a substrate for hydrogen production (Kapdan and Kargi, 2006). Unused, lignocellulosic waste biomass from forestry, agriculture, and municipal sources is a potential feedstock for the synthesis of biofuels like hydrogen (H_2), which could replace fossil fuels and reduce greenhouse gas emissions (Das and Veziroglu, 2001; Hallenbeck, 2005; Levin et al., 2007). Lignocellulosic biomass refers to plant biomass that is composed of cellulose, hemicellulose, and lignin. Cellulose consists of linear, highly ordered chains of glucose and is one of the most abundant biopolymers on the planet (Levin et al., 2009). Li et al. (2007), Zhang et al. (2007) and Ivanova et al. (2009) investigated mesophilic microbial communities that convert lignocellulosic biomass like wheat straw and corn stalks into biohydrogen. Still, research into the conversion of cellulosic biomass to biohydrogen is limited. In general, it is difficult to directly convert raw crop stalk wastes into biohydrogen gas by microbe anaerobic fermentation due to the complex chemical composition of the waste, which incorporates cellulose, hemicellulose, lignin, protein, and fat. Although the complex structures of lignocellulosic biomasses are not ideal for fermentative hydrogen production, some pretreatment methods allow this biomass to be easily used by hydrogen-producing bacteria (Wang et al., 2009). Fan et al. (2006) reported the conversion of wheat straw wastes that were pre-treated with dilute HCl by microwave heating into biohydrogen. Zhang et al. (2007) used corn stalks for hydrogen production after pretreatment with 0.5% NaOH at 36°C. Interesting questions remain about the ideal environmental conditions (e.g., pretreatment conditions, substrate concentration and acid concentration) for the subsequent fermentation of the resulting fractions.

Pretreatment is an important tool for practical cellulose conversion processes. It alters the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars. Pretreatment also removes lignin and hemicellulose, reduces cellulose crystallinity, and increases the porosity of the materials (Mosier et al., 2005). Successful biological conversion of biomass to hydrogen is strongly dependent on processing the raw materials to produce feedstock that can be fermented by the microorganisms. Three main biofuel fermentation processes are widely used in research on bioethanol production from lignocellulosic biomass materials: Separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and direct microbial conversion (DMC) (Levin et al., 2009). To the best of our knowledge, the current study is the first report of biohydrogen production from wheat straw residues under various bioprocess systems.

MATERIALS AND METHODS

Hydrogen-producing microflora

The seed sludge of mixed culture was obtained from a continuously stirring tank reactor that was producing hydrogen by fermenting waste sugar at 36±1°C and pH 5.2 to 5.4. The reactor had been operating continuously for four months and produced a biogas composed of more than 60% hydrogen.

Raw material

The wheat straw used in this study was collected at a farm in the outskirts of Mönchengladbach, Germany. A straw bale was transported to the laboratory site at Duisburg-Essen University in Essen. The sampled biomass, which primarily contained stalk,

straw and wheat residues, was sealed in a plastic bag at room temperature before pretreatment. The main ingredients of the sample were 44.33% carbon and 0.434% nitrogen on a dry-weight basis. The volatile solid mass of the fresh straw was about 93% of the total solid. Mechanical treatment of the sample was performed for size reduction using a FRITSCH mill equipped with a 1-cm sieve.

Acid pretreatment and enzyme hydrolysis

Acid pretreatment of wheat straw was performed in a convection oven at 90 and 120°C with a solids ratio of 10% (w/v). Sulfuric acid concentration was 2% (w/v).

The enzyme mixture Methaplus® (Cowatech, Burglengenfel, Germany) was tested in different bioconversion processes. Methaplus is an enzyme mixture of cellulases, xylanases, and ß-glucanases that is produced especially for use in biogas plants. Recommended dosages (retrieved from http://www.cowatec.de/e/methaplus.htm) for the tests were 100 g/Mg TS and 0.1 mg/g VS (Krupp and Widmann, 2009).

Batch test set-up

Batch test results depend mainly on the inoculum microbial activity and the correct determination and interpretation of the accumulated gas amount (VDI 2004). Therefore, the batch tests were conducted with the equipment described in DIN 38414 S8 (Krupp and Widmann, 2009). The batch tests took place under mesophilic conditions in 0.5-L fermentation vessels in a tempered climate chamber. The test samples were manually mixed once a day, and the amount of produced gas was measured by the liquid replacement method in eudiometers. The temperature in the climate chamber was set to 35°C, as is common in anaerobic batch tests (DIN 38414 S8). The typical test duration was seven days, including the lag-phase. As an additional modification, the inserted inoculum/substrate amount was calculated according to Ochs (2005); the proportion of inoculum to substrate was fixed to 1:1 in relation to VS. Usually, 1 g VS substrate and 1 g VS inoculum in the form of CSTR biohydrogen-producing sludge were inserted into the test vessels, which were filled to 300 g with tap water. To improve the fermentation conditions, 1 ml nutrient was added to each system. Mineral nutrients were added at the following concentrations (modified based on Hussy et al., 2002): NH_4Cl 2600 mg, K_2HPO_4 250 mg, KH_2PO_4 250 mg, $MgCl_2$. $6H_2O$ 320 mg, $FeSO_4$. $7H_2O$ 86 mg, $CoCl_2$. $6H_2O$ 15 mg, $MnCl_2$. $4H_2O$ 15 mg, (NH_4) $6Mo_7O_{24}$. $4H_2O$ 14 mg, $Na_2B_4O_7$. $10H_2O$ 12 mg, $NiCl_2$. $6H_2O$ 49 mg, $ZnCl_2$ 23 mg, $CuCl_2$. $2H_2O$ 10 mg and $CaCl_2$. $2H_2O$ 66 mg. The solution was stored as a 13-fold concentrate and acidified to pH < 2. During all tests, a nutrient dilution according to Hussy et al. (2003) was provided in addition to the feeding substrate to ensure a proper supply of trace elements, nitrogen and phosphorous.

The fermentation yields of the batch processes were evaluated according to DIN 38414 S8. The daily volume of produced gas of each trial was estimated and converted to standard volume (0°C, 1013 hPa). The degree of degradation ($\eta_{C,\ gas}$) was calculated by dividing the carbon weight from fermented gas (produced in CO_2 form) by the carbon weight of the input substrate to demonstrate the degradability of input material to fermentation gas (Krupp and Widmann, 2009).

Analytical methods

The produced gas was collected in special bags, and its volume was measured by the liquid replacement method in eudiometers. The volumes of gas produced daily in each trial were converted to standard volumes. The hydrogen content of the produced gas was determined off-line daily with a Conthos 2 process gas analyzer based on thermal conductivity (LFE, Maintal, Germany). Amounts of CO_2, CH_4 and O_2 were measured off-line daily with a GA94 infrared gas analyzer (Geotechnical Instruments/Ansyco, Karlsruhe, Germany). The amounts of organic acid were also measured daily as equivalents of acetic acid by the Hach-Lange cuvette test LCK 365 (50 to 2500 mg/L). Chemical Oxygen Demand (COD) was determined photometrically using Hach-Lange cuvette tests according to DIN 38409 H41. COD was determined photometrically by measuring the color change by potassium dichromate dilution with an MDA photometer ISIS 9000, type LPG 282 (Hach-Lange Dusseldorf, Germany). Total solids (TS) and volatile solids (VS) of the sludge inocula and input and output test samples were determined by standard methods DIN38414 S2 and S3. Carbon and nitrogen were determined with a vireo MAX CN analyzer (Elementar Analysis Systems, Hanau, Germany). Dissolved organic carbon (DOC) levels were determined with a DIMATOC 2000 DOC analyzer (DIMATEC Analysentechnik GmbH, Essen, Germany). To obtain Total organic carbon (TOC), the samples were filtered using a <45-µm celluloseacetate filter (Whatman, Brentford, UK) before analysis.

Kinetic modeling

The cumulative volume of H_2 production in the batch experiments followed the modified Gompertz equation:

$$H = P\ exp\left\{-\exp\left[\frac{R_m\ e}{P}(\gamma - t) + 1\right]\right\}$$

where H is the cumulative H_2 production (ml), λ is the lag time (h), P is the H_2 production potential (mL), R_m is the maximum H_2 production rate (ml/h), and e is 2.718281828 (Fang and Li, 2006).

RESULTS AND DISCUSSION

The most important information provided by the batch tests is the amount of produced biogas and its composition, which allows identification of the best method for preparing the agricultural lignocellulosic material for degradation by the biohydrogen-producing bacteria. To make the methods comparable, the results were first standardized by determining the hydrogen production efficiency and calculating the degree of degradation based on carbon input and dioxide output.

Effects of different bioprocess methods on bio-H_2 yield

After three iterations of each of the tests were executed in a randomized order, analysis of variance (Table 1) was used to test the hypothesis that significant variation existed between the different treatments ($p<.01$).

This result indicates that the selected biofuel production system has a significant effect on biohydrogen production. To standardize the tests, glucose, which was expected to be completely digested and therefore to demonstrate the ultimate capability of the digested

Table 1. Different bioprocess results for biohydrogen production in batch tests.

Bioprocess systems	Cumulative H_2 yield	Cumulative H_2 yield from glucose	Yield	$\eta_{c, gas}$	Glucose equivalence
Unit	NmL/gVS	NmL/g VS	mol H_2/mol glucose	% C input	%
Fresh straw [a]	5.69	194.64	0.04	0.98	2.9
Pretreatment only	37.11	194.64	0.29	1.44	19
SSF	125.11	194.64	1	4.88	64

[a] Comparison of mean (Duncan test at the 0.05 level of probability).

sludge, was also used under the same environmental conditions.

A Duncan average comparison table was used to identify significant differences among the selected methods (Table 1). This table revealed that all of the methods differed considerably in terms of both hydrogen production and carbon degradation. Duncan's test can identify significant differences between group means in an analysis of variance setting. Table 1 shows hydrogen production and degrees of degradation based on the amount of CO_2 in the gas phase and the glucose equivalence of the test substrates. For the tested series, the mean value for hydrogen production from glucose of 194.64 NmL/g VS was used. The hydrogen concentration in the produced biogas was calculated to be between 57.2 and 63.9%. For simplification, an average hydrogen concentration in the biogas of 60% was assumed for all tests.

As shown in Table 1, fresh wheat straw without pretreatment (designated the first test method) did not undergo any degradation by fermentative bacteria, which means that the bacteria were not able to produce gas from fresh straw. The next method (pretreatment only) involved pretreating the wheat straw samples and supplying the prepared substrate with hydrogen-producing bacteria. Results showed that pretreatment alone did not yield an interesting result. However, the degradation of cellulosic substances resulted in the production of 37.11 NmL H_2/gVS gas plus 1.44% carbon degradation from the solid to the gas phase. The remarkable contrast between this method and the previous one indicates the positive effect of pretreatment.

The SSF method had a significant ($p \leq 0.05$) effect on biohydrogen production and carbon degradation. It proved to be the most effective and economical way to convert wheat straw to biohydrogen. By mixing cellulose and glucose in the same reactor, glucose was rapidly removed during hydrolysis before it inhibited cellulase enzymes. The optimum temperature selected for the reaction (37 to 38°C) represented a compromise between the optimum temperatures for the enzymes in hydrolysis and the bacteria in dark fermentation.

Effect of SSF method on biohydrogen production

Cumulative hydrogen data depicted in Figure 1 were correlated with the Gompertz equation and the constants were determined by regression analysis. Table 2 summarizes the Gompertz equation coefficients for different bioprocess methods concentrations. As presented in Table 2, the highest cumulative hydrogen (362 ml) and the formation rate (11.86 ml h^{-1}) were obtained with SSF method. However, the lowest lag phase (8 h) was obtained with pretreatment only method.

Figure 1 illustrates the effects of different bioprocess methods on the cumulative amount of hydrogen produced in batch tests. Hydrogen production from wheat straw stopped after two days, while sludge produced the same yield in five days. Adding straw to sludge as a carbon resource therefore reduces the lag phase of bacterial growth. Moreover, the microorganisms in the hydrogen CSTR sludge were not able to decompose wheat straw structures. Figure 1 depicts the significant effect of the selected pretreatment method on the hydrogen yield of fresh straw. The produced hydrogen amount was insufficient for a commercial scale. The SSF method demonstrated the best hydrogen yield. The highest conversion of carbon from the solid to the gas phase was also achieved by the SSF bioprocess method; its high hydrogen production capacity is demonstrated by its 64% glucose equivalence and 5% carbon degradation.

Comparison with other work

Extensive research has been conducted on the production of hydrogen and other biofuels from saccharides and starches. In recent years, this work has tended to focus on lignocellulosic sources, especially agricultural waste materials. Nevertheless, we could find very few studies on the production of biohydrogen using pretreatment methods and other biofuel production systems. Here, the efficiency of our selected method is compared with the results from other available studies. Notably, the majority of other work has used pure cultures rather than mixed cultures and did not mention the hydrogen yield of the applied sludge or its corresponding glucose equivalent hydrogen yield.

According to Table 3, H_2 yields observed in this study are higher than the H_2 yields reported in other studies. As indicated, the use of mixed culture inocula resulted in a slightly higher yield. The highest hydrogen yield was

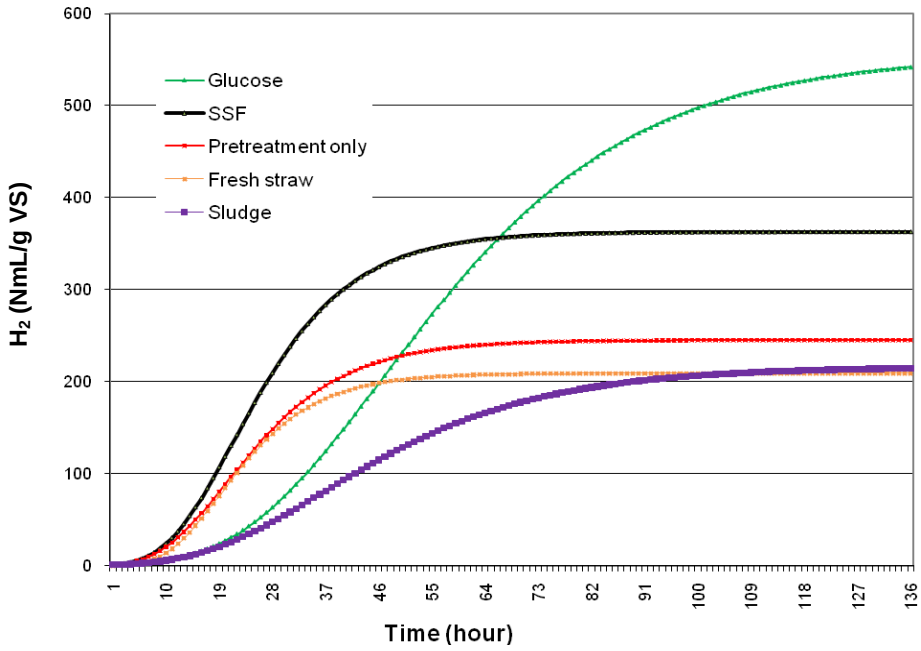

Figure 1. Effect of different cumulative bioprocess on cumulative H_2 production.

Table 2. Gompertz equation coefficient for different bioprocess methods.

Bioprocess	P (ml)	R_m (ml h^{-1})	λ (h)	R^2
Sludge	216	3.86	15	0.992
Fresh straw	220	8.43	9	0.995
Pretreatment only	245	8.01	8	0.993
SSF	362	11.86	9	0.996

obtained in this study from wheat straw (141 ml/g VS). This yield corresponded to a glucose equivalence of nearly 65%, which is comparable with study results reporting hydrogen production from lignocellulosic materials by dark fermentation (mixed culture). Zhang et al. (2007) studied hydrogen production from corn stalks by dark fermentation and obtained a maximum hydrogen yield of 149 ml/g VS using pure cultures. Results ho used acid pretreatments and mixed cultures at the same temperatures. These previous studies produced biohy drogen from corn stalks, wheat straw and corncobs, respectively.

Table 3 shows the efficiencies and pretreatment methods used in other research on lignocellulosic materials. The efficiencies of these methods are substantially different from those of the SSF method. An analysis of different pretreatment methods shows that the effect of temperature on the deconstruction of lignocellulosic materials is not significant without the application of chemicals.

The methods described in this study should be further developed for the methodological accumulation, preservation, size reduction, pretreatment and chemical deconstruction of lignocellulosic waste materials. It is essential to upgrade biohydrogen production systems, including reactors that are specially designed to produce biohydrogen from lignocellulosic materials, by utilizing different substrates and comparing them with other methods, especially SSF.

Conclusions

Experiments revealed that the pretreatment, hydrolysis and fermentation of wheat straw facilitate its use by different methods of biohydrogen production in batch tests. Fresh straw and pretreated straw were applied along with the SSF bioprocess method to produce biohydrogen in standard batch tests (DIN 38414 S8). Pretreatment with 2% (w/v) sulfuric acid for a 90-min residence time at 120°C proved to be the most effective treatment. Significant differences were also observed between bioprocess methods; for example, the degradation of fresh straw to fermentation gas was less than 1%. The SSF method was found to be an effective and economical way to convert wheat straw to

Table 3. Comparison of yields with other works.

Inoculum		Substrate	Pretreatment method	Reactor type	T (°C)	Max. yield (mL H_2 /g VS)	Reference
Mix culture	Cow dung compost	Wheat straw	HCl	Batch	36	62.8	Fan (2007)
Pure culture	*Clostridium paraputrificum*	Corn stalk	Enzyme	Batch	55	132	Yuan (2009)
Pure culture	_	Corn stalk	0.2% HCl boiled 30 min	Batch	36	149.69	Zhang (2007)
Pure culture	Clostridium sp.	Corn stalk	Bio-pretreatment 15 day	Batch	36	176	Fan (2008)
Mix culture	Dairy manure	Corncob	1% HCl + 100°C 30 min	Batch	36	107.9	Pan (2009)
Mix culture	cow dung compost	Wheat straw	No pretreatment	Batch	36	1	Fan (2007)
Pure culture	*Clostridium butyricum*	Corn straw	No pretreatment	Batch	35	9	Li (2007)
Pure culture	C. saccharolyticus	Maize leaves	No pretreatment	Batch	70	18	Ivanova (2009)
Pure culture	_	Corn stalk	No pretreatment	Batch	36	3	Zhang (2007)
Pure culture	*C.m butyricum*	Corn straw	1.5 MPa 10 min	Batch	35	68	Li (2007)
Pure culture	C. saccharolyticus	Maize leaves	130°C 30 min	Batch	70	42	Ivanova (2009)
Pure culture	C. saccharolyticus	Sweet sorghum plant	130°C 30 min	Batch	70	32.4	Ivanova (2009)
Pure culture	C. saccharolyticus	Sugarcane bagasse	130°C 30 min	Batch	70	19.6	Ivanova (2009)
Pure culture	C. saccharolyticus	Silphium trifoliatum leaves	130°C 30 min	Batch	70	10.3	Ivanova (2009)
Pure culture	C. *saccharolyticus*	Wheat straw	130°C 30 min	Batch	70	49	Ivanova (2009)
Pure culture	_	Corn stalk	0.5% NaOH	Batch	36	57	Zhang (2007)
Mix culture	Anaerobic sludge	Corn Stover	220°C 3 min	Batch	35	49	Datar (2007)
Mix culture	Anaerobic sludge	Corn stover	1.2% HCl + 200°C 1 min	Batch	35	66	Datar (2007)
Mix culture	CSTR H_2 sludge	Wheat straw	No pretreatment	Batch	36	6.4	Current study
Mix culture	CSTR H_2 sludge	Wheat straw	2% H_2SO_4+120°C 90 min	Batch	36	41.9	Current study
Mix culture	CSTR H_2 sludge	Wheat straw	2% H_2SO_4+120°C 90 min	Batch (SSF)	36	141	Current study

biohydrogen; it achieved 1 mol H_2/mol glucose hydrogen yield, 64% glucose equivalence and 5% carbon degradation ($\eta_{C\ gas}$) and proved to have the shortest lag phase during gas production of all of the test runs.

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