

Biohydrogen Production from Acid Pre-treated Grass

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Abstract: Hydrogen is an ideal energy resource. It is an attractive alternative energy source since it generates a huge amount of energy with only water as a reaction product. Biohydrogen is gaining popularity among renewable energy sources due of its high energy conversion to usable power and low pollution production. Grass waste has been identified as a suitable substrate among biomass residues for biohydrogen production. In this study, HCl acid was used to pretreat grass waste. Pretreatment process was applied to enhance hydrogen fermentation of grass waste. This study aims to gain the knowledge on how acid pre-treated grass (4% HCl) feasible for dark fermentation. Automatic Methane Potential Test System (AMPTS II) was used for Biochemical Hydrogen Potential (BHP) test. Biochemical Hydrogen Potential (BHP) test was used to determine the hydrogen yield from acid pre-treated grass (4% HCl). The hydrogen production potential and the hydrogen production from acid pre-treated grass (4% HCl), were 46.62 ml H₂/ g VS and 180.9 mL respectively. The Modified Gompertz Modelling was not appropriate for assessing the kinetic of hydrogen production from acid pre-treated grass (4% HCl), due to the difference between laboratory data and Modified Gompertz Model which was exceeded 50%.

Keywords: Biohydrogen, Grass Waste, Acid Pretreatment

1. Introduction

In recent years, there has been increasing in research into alternative fuels in order to reduce reliance on fossil fuels, mostly through supplying local energy supplies. Hydrogen (H₂), is an interesting alternative energy because it generates a large quantity of energy while only producing water as a reaction product [1]. Hydrogen is interesting due to its high conversion efficiency to useful power, low pollutant emission, and high energy density [2]. However, in order for hydrogen to be considered as a

suitable alternative for fossil fuels, it must be produced from renewable sources other than fossil fuels. Fermentative hydrogen production has the advantage of producing hydrogen in mild conditions while also allowing residual biomass to be utilised. The dark fermentation method is more appealing than photofermentative methods because it has the ability to utilise organic wastes and has higher production rates [3].

Lignocellulosic biomass is a most abundant organic materials that can be used to produce bioenergy and biofuels in a sustainable production, such as hydrogen production [4]. Lignocellulosic biomass including grass waste has been suggested as a possible dark fermentation substrate among biomass wastes. the rigid structure of pure lignocellulosic biomass prohibits microbes from consuming it, limiting the efficiency of grass waste hydrogen production [5]. The pretreatment of lignocellulosic biomass to hydrolyse the integrated heterogeneous and crystalline structure is required for the conversion process of lignocellulosic biomass to hydrogen [6]. The effectiveness of acid pretreatment methods for increasing hydrogen fermentation of lignocellulosic biomass including grass waste has been proven in which acid pre-treated biomass solubilised well, as well as an increase in biomass roughness [7]. In this study, hydrogen fermentation from grass waste using acid pretreatment was evaluated. Biohydrogen production potential (BHP) was run in the Automatic Methane Potential Test (AMPTS II). BHP assay was performed can obtain the accumulated hydrogen production and hydrogen yield data. Kinetic analysis modified Gompertz model was used to evaluate the cumulative hydrogen production progress from batch tests. Moreover, the kinetic analysis modified Gompertz model will be compared to laboratory approach to evaluate the fit of model to the laboratory testing.

The aim of this research is to characterise the acid pre-treated grass and analyse the hydrogen yield and kinetics from the digestion of acid pre- treated grass. Solids, complex organic compounds, and cellulose are the characteristics that have been identified from the grass samples. Then, in order to determine the appropriate methods and procedures, each of these characteristics measurement method have been identified.

2. Materials and Methods

2.1 Grass waste

Grass waste was used as substrate for hydrogen fermentation in this study. The grass waste, was collected at rugby field, UTHM campus. After the collection, they were dried to the sunlight until it dry, before grinded using a blender until the particles become fine [5]. Then, they were dried again in the oven for 3 hours at 105°C.

2.2 Acid pretreatment of grass waste

For acid pretreatment process on grass waste, 1.0 g of dried grass was mixed with 20 mL of HCL aqueous solution at 4% (w/v) concentrations. The acid (HCL) pretreatment at 4% resulted in the highest hydrogen production. Then, the pretreated grass was boiling for 30 min [5]. Next, the mixture was neutralised to pH 7 by added the dilute sodium hydroxide (NaOH) at the concentration of 4% (w/v).

2.3 Inoculum

Anaerobically digested sludge is used as the inoculum source in this study. The inoculum sludge was taken from the full-scale anaerobic digester that used to treat palm oil mill effluent (POME). On the way back to the UTHM campus, the anaerobically digested sludge sample was stored in container with the ice cube. The samples were then kept refrigerated at 4°C to avoid biodegradation [8]. Before the fermentation or biohydrogen potential (BHP) test, the inoculum undergoes pretreatment process by heating to 100°C for 15 minutes to kill the hydrogen producing bacteria [9].

2.4 Analytical method

The measurements of Total Solid (TS) and Volatile Solid (VS) were carried out based on Standard Methods (2540 G) [10]. Chemical oxygen demand (COD) and was analysed using the HACH Method 8000 [11]. For the grass waste either in form of grass waste or acid pretreated grass, the organic matter was reported in form of soluble COD (SCOD) [12]. The pH value was also recorded by using the calibrated pH meter (Eutech PHTEST30/Oakton 35634-30, waterproof pH Testr 30, USA). Biochemical Hydrogen Potential (BHP) test was used to determine the hydrogen yield during acid pre-treated grass (4% HCl) hydrogen fermentation process. The Automatic Methane Potential Test System (AMPTS II) was used to conduct the BHP test.

2.5 Biochemical Hydrogen Potential (BHP) test

BHP test determine amount of hydrogen that can be produced when a certain substrate is biodegraded under fermentative conditions [13]. The BHP test was performed in a mesophilic condition which was the temperature of 35°C [13]. The reactors were incubated with intermittent shaking at 120 rpm [14]. While, the substrate to inoculum ratio (S/I) of 2.7 [14]. The BHP was conducted by using the Automatic Methane Potential Test System (AMPTS II) [15].

The batch assay was prepared using 500 mL Duran bottle with 400 mL of working volume [14]. There are 2 bottles duplicate blank reactors, which contain only inoculum samples, and 3 bottles of triplicate sample reactors, which are mixture of inoculum and substrate. Based on VS, substrate (acid pretreated grass) and inoculum (anaerobically digested sludge) are mixed at a 2.7 ratio of substrate to inoculum. Table 1 show the mass of substrate and inoculum.

Table 1: Mass of substrate and inoculum

Reactor ID	Inoculum (g)	Substrate (g)
BHP Blank 1	191.4	-
BHP Blank 2	191.4	-
BHP Sample 1	191.4	208.6
BHP Sample 2	191.4	208.6
BHP Sample 3	191.4	208.6

The optimal pH for hydrogen production is 5.5 [16]. Therefore, in this study the pH adjustment was conducted to achieve pH 5.5 by using NaOH. The nitrogen (N₂) gas were flushed for 3 minutes into the all reactors to remove the oxygen (O₂) contained in the reactors. Then, all the Duran bottles were inserted into the sample incubation unit (Part A). Incubate the batch reactors at 35°C with an orbital shaking of 120 rpm [14]. The gas production measurements were performed daily and recorded the hydrogen produced. The cumulative hydrogen production curve is automatically updated by the AMPTS II software.

The stop time criterion (ST) is obtained when the hydrogen production curve becomes plateau (FT). Besides, when the coefficient of variation between the last three recordings of cumulative hydrogen is less than 5%, it also can be used as a stop condition [14]. Figure 1 show the AMPTS II, which consist of three units, where sample incubation unit (Unit A), CO₂ fixing (Unit B), and gas volume measuring (Unit C).

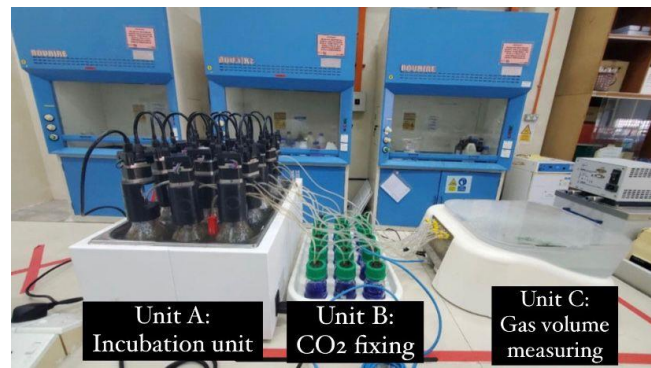


Figure 1: Automatic Methane Potential Test System (AMPTS II) test.

2.6 Kinetic analysis

The performance of hydrogen production of acid pre-treated grass used in this study was described by using kinetic analysis Modified Gompertz model (Eq 1). Furthermore, kinetic analysis was utilised to analyse the anaerobic digester's efficiency [17]. This model can be used to model the cumulative hydrogen production at any given time (H), hydrogen production potential (P), lag phase (λ) and hydrogen production rate for a cumulative hydrogen production time (R_m). The sum square of errors between the experimental data and model results was minimised to estimate constants. The calculations were done with Microsoft Excel version 2016 [13]. In this study, Excel Solver was used as a statistical tool to estimate hydrogen production potential (P), lag phase (λ) and maximum hydrogen production rate (R_m) from the non-linear graphs analysis which fit the experimental data set [18].

$$H = P \cdot \exp\left\{-\exp\left[\frac{R_m \cdot e}{P}(\lambda - t) + 1\right]\right\} \quad (\text{Eq. 1})$$

3 Results and Discussion

3.1 Characterisation of substrate and inoculum

The characterisation was conducted on different sample, including anaerobically digested sludge, and acid pre-treated grass. The TS refers for the organic content of the substrate, while the VS refers for the biodegradable and refractory of volatile solids [19]. The results were tabulated in Table 2. In this study, the acid pre-treated grass contained 87490 mg/L of TS and 68360 mg/L of VS. Deng et al., (2019) observed that acid pretreated grass silage contained 55500 mg/L and 51100 mg/L of TS and VS respectively. VS/TS ratio of acid pre-treated grass in this study was 78%. In other study, VS/TS ratio of acid pre-treated grass was 92% [10] Soluble chemical oxygen demand (SCOD) concentration of acid pre-treated grass was 713 mg/L. While, Yang & Wang, (2018b) measured SCOD of 463.9 mg/L for acid pre-treated grass.

While, for anaerobically digested sludge used in this study, contained 40900 mg/L of TS and 28640 mg/L of VS. Yang & Wang, (2018b) found that TS and VS concentration in the anaerobically digested sludge of 18868 mg/L and 10693 mg/L respectively. The anaerobically digested sludge in this study contained 19236 mg/L of COD content. While, according to Choong et al., (2018), the COD content for anaerobically digested sludge was 32580 mg/L.

Table 2: Concentration of the selected parameters (N=2)

Parameter	Anaerobically digested sludge	Acid pre-treated grass
TS (mg/L)	40900	87490
VS (mg/L)	28640	68360
VS/TS	0.70	0.78
COD (mg/L)	19236	-
Soluble COD (SCOD) (mg/L)	-	713

3.2 Cumulative biohydrogen production

Hydrogen production readings were taken daily until its reading show are smaller than 5% continuously for 3 days [14]. In this study, hydrogen production reading were taken up to day 15. The reading showed similarities for 3 days, thus the hydrogen production rate will be less than 5%. Table 3 shows the net hydrogen production from acid pre-treated grass. The final reading of hydrogen production of blank and sample reactors were 27.4 mL and 208.3 mL respectively. While the final net value was 180.9 mL Net cumulative hydrogen is calculated by subtracting the value of hydrogen from sample reactors with each blank reactors [22].

Table 3: Accumulated hydrogen production

Day	Average		Net (mL)
	Blank reactor (mL)	Sample reactor (mL)	
0	0.0	0.0	0.0
1	8.5	76.7	68.2
2	14.0	92.1	78.1
3	15.3	101.6	86.4
4	16.6	107.8	91.3
5	18.4	112.5	94.1
6	20.7	121.9	101.3
7	21.4	134.0	112.6
8	22.1	148.1	126.0
9	22.8	161.4	138.6
10	23.6	175.0	151.5
11	24.2	186.8	162.6
12	27.4	208.3	180.9
13	27.4	208.3	180.9
14	27.4	208.3	180.9

Figure 2, showed the accumulated hydrogen production curve. The acid pre-treated grass (substrate) degraded the microbes rapidly in day 1. The hydrogen production curve showed the consistent curve at day 12 with maximum cumulative hydrogen production of 180.9 mL from the digestion of acid pre-treated grass (4% HCl) Previous study found that, the maximum hydrogen production achieved at 40 hours with 72.21 mL [1]. Table 4 showed the hydrogen production rate for acid pre-treated grass.

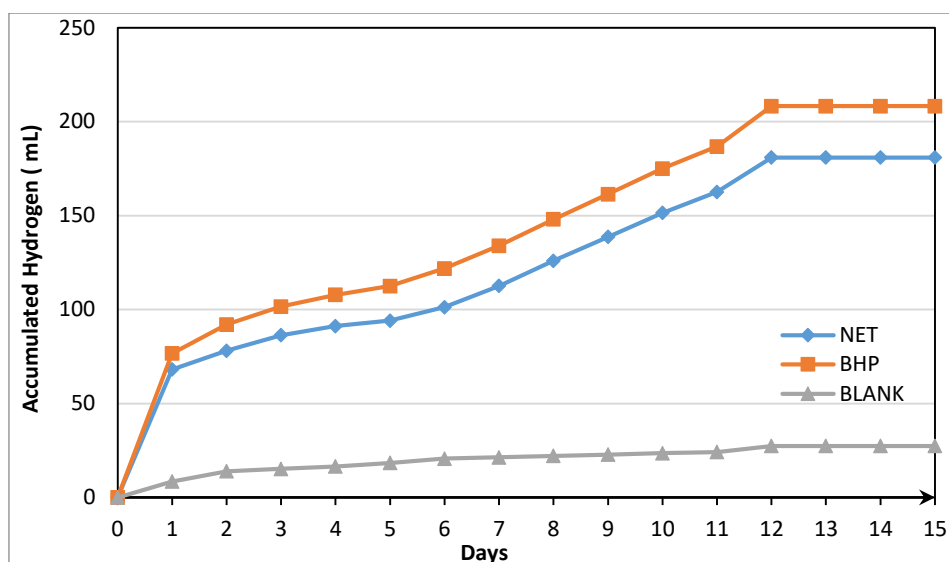


Figure 2: Accumulated hydrogen production for net, BHP and blank

Table 4: Hydrogen production rate

Day	Hydrogen production rate (ml H ₂ /g VS day)
1	17.57
2	2.56
3	2.13
4	1.25
5	0.74
6	1.83
7	2.93
8	3.45
9	3.26
10	3.31
11	2.86
12	4.73
13	0.00
14	0.00
15	0.00

3.3 Hydrogen yield

In this study, hydrogen yield was defined as the cumulative hydrogen production per grams dry grass waste added, which is the most commonly used index for evaluating hydrogen fermentation efficiency [20]. The hydrogen production potential of acid pre-treated grass (4% HCl) was 46.62 ml H₂/g VS.

Figure 3 showed the hydrogen yield curve from acid pre-treated grass (4% HCl) by the batch fermentation process. The curve showed the hydrogen yield increase before the constant on the day 12.

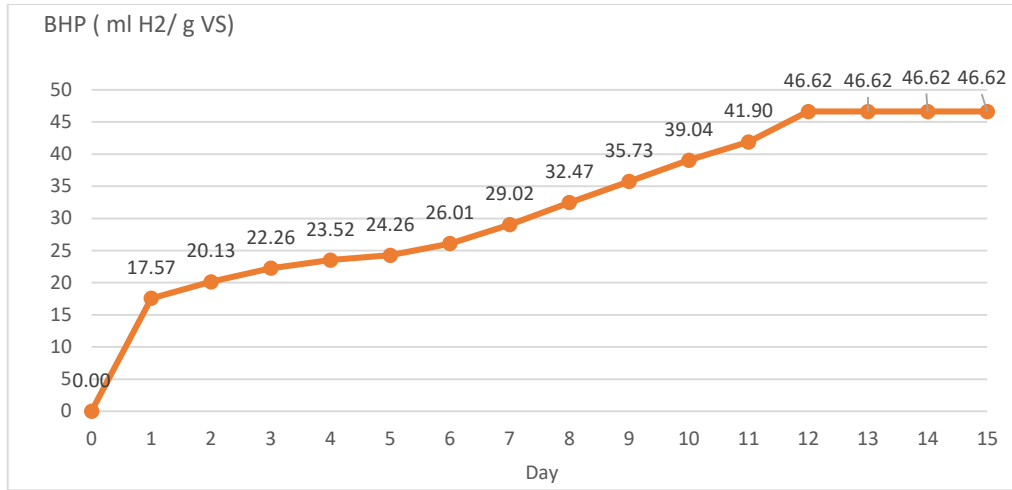


Figure 3: Hydrogen production potential curve

3.4 Kinetic analysis by Modified Gompertz Model

The experimental data was used to do a kinetic analysis based on the cumulative hydrogen production [23]. Table 4 show the value of kinetic parameters. Due to the hydrogen production potential (P), the difference value between laboratory testing and Modified Gompertz model, was 12.42%. This indicated that the Modified Gompertz model was not appropriate for evaluating the kinetic of hydrogen production of acid pre-treated grass because the difference was exceeded 50%. According to Gadhamshetty et al., (2010), the difference of laboratory approach with Modified Gompertz Model cannot exceed 50%.

Table 4: Kinetic parameters

Parameter	Laboratory	Modified Gompertz	Difference (%)
Hydrogen production potential, P (ml H ₂ /g VS)	46.62	40.83	12.42
Maximum hydrogen production rate, R _m (ml H ₂ /g VS/day)	17.57	6.28	64.26

Figure 4 showed the graph of the hydrogen yield observed from laboratory data and Modified Gompertz Modelling. From this figure it is obvious that Modified Gompertz was not best fit to the laboratory data.

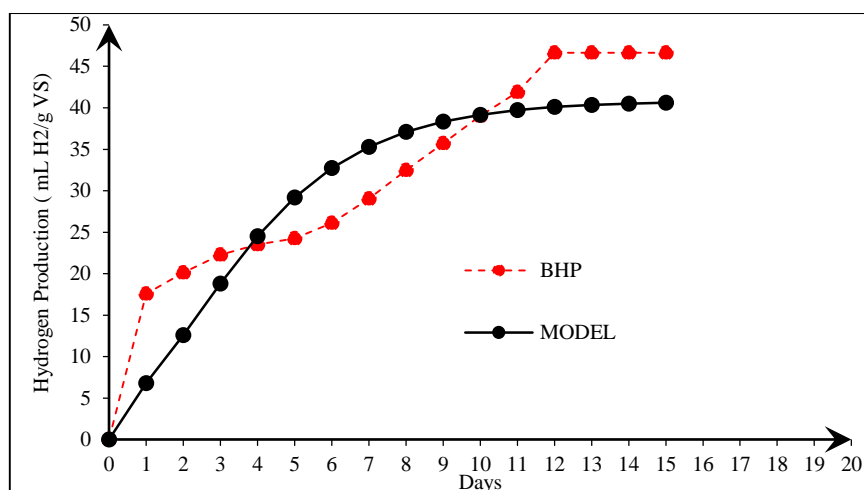


Figure 4: Curve of hydrogen production potential observed from laboratory and Modified Gompertz Model

4 Conclusion

This study investigated the characteristics of acid pre-treated grass and analysed hydrogen yield and kinetics from the digestion of acid pre-treated grass (4% HCl) was successfully characterized for TS, VS and COD test. This study showed that the acid pretreatment (4% HCl) on the grass waste is possible for hydrogen production. The dark fermentation (BHP) at batch mode for acid pre-treated grass (4% HCl) resulted the ultimate hydrogen production potential and cumulative hydrogen production of 46.62 ml H₂/ g VS and 180.9 mL respectively. The Modified Gompertz model was used to conduct the kinetic analysis study. However, The Modified Gompertz Modelling was not appropriate for assessing the kinetic of hydrogen production from acid pre-treated grass (4% HCl), due to the difference between laboratory data and Modified Gompertz Model which was exceeded 50%.

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