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Research

Preliminary Assessment on Pretreatment Methods For Landfill Waste Utilization in Biohydrogen Production

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ABSTRACT

Landfill waste consists of a mixture of components that have high potential as a substrate for hosting various microorganisms' growth. Utilizing this waste as a fermentation substrate is seen as an economical solution for the management of the waste. Treating this waste is crucial to remove unnecessary components for the growth of specific organisms to ensure a high reaction yield. Fermentative hydrogen production from this waste specifically requires the hydrogen-consuming bacteria to be reduced. In this work, heat, ultraviolet (UV) radiation, acid, and alkaline pretreatment were conducted on the landfill waste. The changes in the reduced sugar content and appearance of bacterial colonies were observed and compared. Heat pretreatment at 65 °C was found to give among the best increase (74 – 88%) in reducing sugar content and reduction (50 – 85%) in the number of aerobic bacterial colonies detected. Global warming potential and eutrophication potential recorded from simulated heat pretreatment plant was comparable to other heat-based pretreatment reported by other researchers with a potential reduction in severity as the plant size increased.

Key words: Biohydrogen, landfill waste, pretreatment, value-added product

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INTRODUCTION

Vehicles and machines rely on fuel to operate. Unfortunately, fossil fuel, which is the most used fuel, has caused environmental issues including the generation of greenhouse gases, which trap the ambient heat and induce a greenhouse effect. Greenhouse gases include carbon dioxide (CO_2), sulfur oxides (SOx), methane (CH_4), and nitrous oxide (N_2O) (Stiling, 2009). The main by-product of fossil fuel combustion is CO_2, which occupies 73% of greenhouse gases. CO_2 concentration in the global atmosphere is increasing at an overwhelming rate with an average amount of 412.5 ppm in 2021, which is about a 12% increase from the average emission in the year 2000 (Rebecca, 2021). Due to its harmful effects, the production of CO_2 from the combustion of fossil fuels needs to be reduced. Replacing fossil fuels with hydrogen (H_2) will be a promising alternative that eliminates the emission of CO_2 into the environment (Kapdan & Kargi, 2006). Additionally, H_2 has a higher energy density, 2.75 times of hydrocarbon (Kapdan & Kargi, 2006).

Fermentative H_2 production utilizing various types of materials is one way to commercially produce H_2. Landfill waste is one of the high-potential materials for the purpose. Landfill waste contains a significant amount of sugar and is a good candidate for substrate in H_2 fermentation. Landfill waste consists mainly of food waste, yard waste, and other organic compounds (Norsa'adah *et al*., 2020). Hydrogen fermentation from food waste was reported to give H_2 yield in the range of 1.97 – 3.76 mol H_2/mol sugar (Han *et al*., 2016; Preethi *et al*., 2019), which were among the highest reported as compared to other types of substrates such as paper mill effluent and palm oil mill effluent (Preethi *et al*.,

2019). Utilizing this waste as the major substrate in the fermentation process could improve the whole lifecycle footprint of the waste. Hydrogen production relies on suitable microbial activities, other than the fermentation conditions such as types of substrate, pH, and temperature (Wong *et al*., 2014). The function and nature of the microbes will be the key factors for efficient H_2 production. From the molecular studies, H_2-producing bacteria (HPB) have been identified inside the landfill solid waste, unfortunately, H_2-consuming bacteria (HCB) can grow in the same medium (Wong *et al*., 2014).

Waste pretreatment to remove HCB while maintaining HPB can be conducted through physical and chemical methods (Wong *et al*., 2014). Physical pretreatments include methods of heat, ultrasonics, ultraviolet (UV) irradiation, aeration, and freezing and thawing (Baruah *et al*., 2018). pH pretreatment and, chemical activation and inhibition are among the methods of chemical pretreatment (Sołowski *et al*., 2020). It is crucial to choose suitable pretreatment methods and parameters as bacteria will act differently under different stresses applied and therefore affect the H_2 yield. For instance, acid pretreatment for seed sludge that contains HCB, *Propionibacterium granulosum*, enhances its growth and reduces H_2 production by 10.4 fold (Wong *et al*., 2014). In another study, acid pretreatment recorded better H_2 yield in the fermentation by using HPB, *Clostridium*, with 0.86 mol H_2/mol of glucose (Pachapur *et al*., 2019). Pretreatment of raw materials has been reported to improve the hydrolysis step in fermentative H_2 production in terms of overall yield, cost, time consumption, and environmental impact potential (Kucharska *et al*., 2018; Mohamed Usman *et al*., 2020).

In this work, several pretreatments were chosen namely heat, UV, and pH pretreatment, to prepare the waste material for H_2 production. Available works on landfill waste pretreatment for H_2 production are still lacking. The data is important to gauge the potential of this raw material as well as to explore a new waste management approach. The changes in the reduced sugar content and microbial colony count of the samples before and after pretreatment were compared. A simple life cycle assessment was conducted to evaluate the sustainability of the chosen pretreatment method for large-scale applications.

MATERIALS AND METHODS

Waste collection and preparation

The solid waste was collected from Gading Senggara Jabor-Jerangau Integrated Landfill, Kuantan. Sample collection was done from several points and characterized by the age of the waste. Fresh waste is generally around 1 – 2 weeks old. Rotten waste is typically more than 6 weeks old, with most components having decomposed. Upon arrival at the laboratory, any inorganic solid portions (i.e., metal & plastic) were separated from the waste. The waste sample was then ground, producing sludge, and stored in a chiller until use. For pH measurement, 1 g of sample was taken and diluted in 9 mL of distilled water.

Pretreatment

For heat pretreatment, 15 g of the sample were placed in the 60 mL vials and submerged in the water bath at different temperatures including 65 °C, 70 °C, 80 °C and 90 °C, each for 15, 30, and 60 min. UV radiation pretreatment was conducted by adding 15 g of sample in the 50 mL beaker and placing it under the UV light inside the laminar flow for 10, 15, and 20 min. Acid pretreatment was done at pH 3, 4, and 5 using 1 M of Hydrochloric Acid (HCl). The alkaline pretreatment was done at pH 10, 11, and 12 using 1 M of Sodium Hydroxide (NaOH). The pretreatment was conducted for 1 week at 4 °C and room temperature. After completion, the mixture was neutralized with distilled water and stored in the chiller.

DNS analysis

The treated sample (1 g) was added to 9 mL of distilled water in a centrifuge tube. 1 mL of the solution was then transferred into the new test tube and 1 mL of DNS was added. The test tubes were then vortexed and put inside the boiling water bath for 10 min. After cooling down the mixture, 10 mL of distilled water was added to each of the tubes. The absorbance reading of the samples was measured at 540 nm by using a spectrophotometer. The analysis was also conducted for untreated samples.

Spread plate and colony count method

Nutrient agar was used for the spread plate method. Serial dilution of sample was done by putting 1 g of sample into 9 mL of distilled water and continued until 10^{-11} of dilution factor was achieved. The diluted sample (0.1 mL) was pipetted onto the nutrient agar and spread by using a sterile L-shape spreader. The plates were then sealed with parafilm and kept in the incubator at 37 °C for 24 h. The colonies growing on the plates were observed.

Catalase test

By using a sterile inoculation loop, a small amount of each colony that appeared on the plate

was transferred onto a clean glass slide. Two drops of hydrogen peroxide (3%) were added and bubble formation was observed.

Pretreatment simulation

A simple simulation of a batch pretreatment plant was conducted using SuperPro Designer V9.5 (Intelligen, Inc., Scotch Plains, NJ, US) with gate-to-gate system boundary involving waste preparation and pretreatment (Figure 1). The functional unit of 1 kg reducing sugar produced was applied. The pretreatment requires heat input, which then generates a small stream of the gas mixture that is vented out from the pretreatment reactor. The environmental impacts were assessed as being primarily based on the vented gas and onsite combined heat and power (CHP) generation that uses natural gas as fuel. Autoclave and acid pretreatment were simulated for comparison, which was reported to give a high H_2 yield (Wu & Chang, 2007; Hu *et al*., 2014). It was assumed that the autoclave and acid pretreatment gave high reducing sugar yield equivalent to those of 65 °C heat pretreatment, due to the lack of experimental data on the effect of these pretreatments on landfill waste.

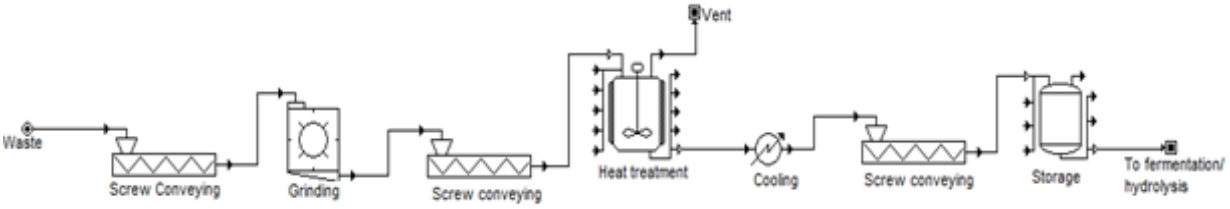

Fig. 1. System boundary applied in the pretreatment simulation.

Life-cycle impact assessment

The CO_2 emission from the CHP plant was calculated based on Equation 1, with 44/12 being the ratio of molecular weights of CO_2 and carbon (EPA, 2016). The emitted methane (CH_4) and nitrous oxide (N_2O) were estimated based on Equation 2 (EPA, 2016). The emission factors used in the calculation were 1.0 g CH_4/MMBtu and 0.1 g N_2O/MMBtu. The carbon content, hydrogen content, and higher heating value (HHV) of the natural gas were assumed to be 75.85%, 24.15%, and 49.18 MJ/kg respectively.

The environmental impact assessment from the pretreatment plant was conducted based on global warming potential (GWP) (Equation 3) and eutrophication potential (EP) (Equation 4). Only emissions that were generated from the plant were considered for the assessment of specific environmental impact potential. All aqueous waste streams were assumed to be appropriately treated, causing no environmental impacts. The impact calculation and assessments were also done with the aid of the Tool for Reduction and Assessment of Chemical and Other Environmental Impacts (TRACI) 2.1 database (Bare *et al*., 2012).

Equation 1:

$$\text{Emission } (CO_2) = \text{Mass}_{\text{fuel}} \text{ (kg)} \times \text{Fuel carbon content (\%)} \times (44/12)$$

Equation 2:

$$\text{Emission } (CH_4 \text{ and } N_2O) = \text{Mass}_{\text{fuel}} \text{ (kg)} \times \text{HHV}_{\text{fuel}} \text{ (MJ/kg)} \times \text{Emission factor (g/MMBtu)}$$

Equation 3:

$$\text{GWP(kg } CO_2\text{eq./L)} = CO_2 \text{ (kg/L)} + (CH_4\text{(kg/L)} \times 25) + (N_2O \text{ (kg/L)} \times 298)$$

Equation 4:

$$\text{Impact potential} = \sum \text{Characterization factor} \times \text{Mass of emitted chemicals}$$

RESULTS AND DISCUSSION

The sludge waste before the pretreatment was characterized by the presence of reducing sugar, starch, and pH values. The fresh waste sample was slightly acidic (pH 4.67) while the rotten waste was slightly basic (pH 8.18). The Benedict's and Iodine test has shown positive results indicating the presence of reducing sugar and starch in the waste. Further quantification of the reducing sugar through DNS assay determined the promisingly high initial sugar content in fresh waste (20.8 mg/mL) as compared to rotten waste (8.9 mg/mL).

Reducing sugar content

Reducing the sugar content of the treated waste was determined to investigate the effect of the pretreatment on the sugar content in the waste. Ideally, a pretreatment must not degrade any available sugar to be used as a substrate for the following fermentation process. Figure 2 and Figure 3 summarize the results of reducing sugar content for the fresh and rotten waste, respectively. For fresh waste, heat pretreatment at all temperatures showed an increase in reducing sugar concentration, while UV, acid, and alkaline pretreatment showed a decrease in reducing sugar. This might indicate that UV, acid, and alkaline pretreatment are too harsh for the waste, which ends up degrading most of the sugar content in the waste. The sugar content of the waste was coming from discarded organic materials, with only small fractions of cellulosic. Therefore, harsh pretreatment might not be required. Heat pretreatment at 65 – 80 °C given almost the same reducing sugar concentration. While at 90 °C pretreatment, less reducing sugar was detected might be due to the high temperature that denaturing any available bacteria, thus reducing the available enzymes responsible for hydrolyse complex compounds into sugar (suppressing effect) (Baghchehsaraee *et al*., 2008).

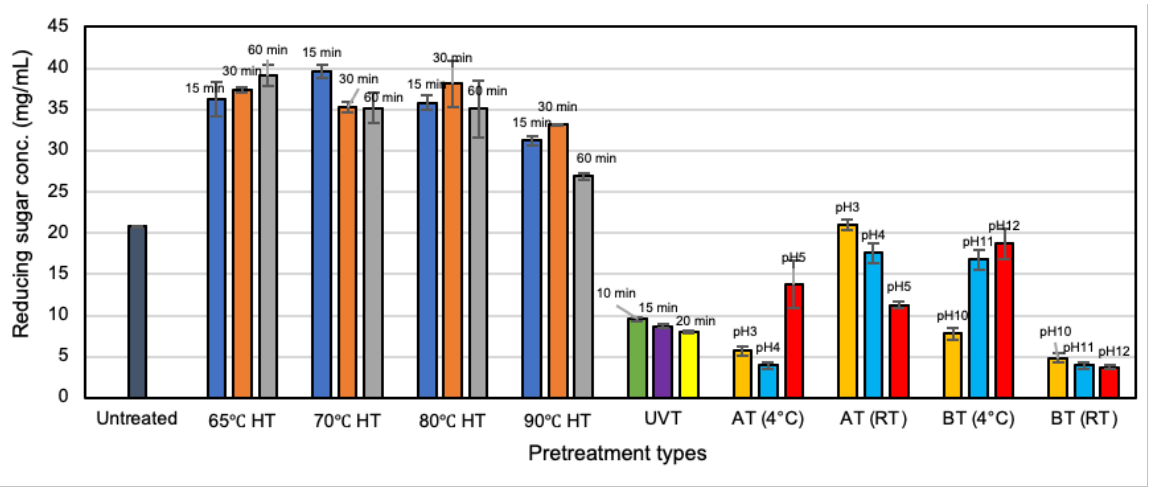

Fig. 2. Reducing sugar content in fresh waste. HT, heat pretreatment; UVT, ultraviolet radiation pretreatment; AT, acid pretreatment; BT, alkaline pretreatment; RT, room temperature.

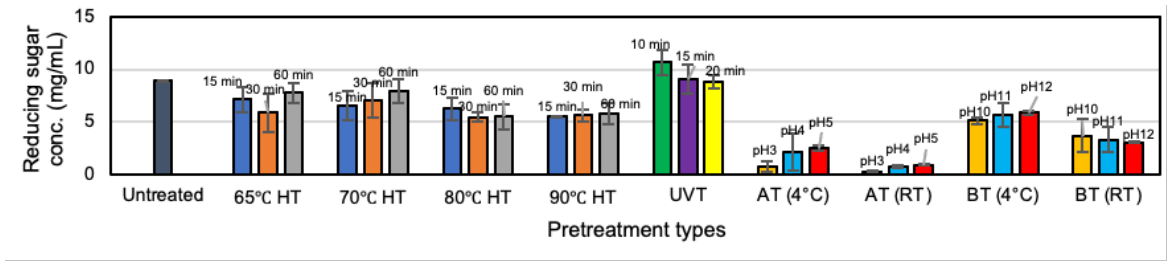

Fig. 3. Reducing sugar content in rotten waste. HT, heat pretreatment; UVT, ultraviolet radiation pretreatment; AT, acid pretreatment; BT, alkaline pretreatment; RT, room temperature.

Unpromisingly low sugar level was detected in the rotten waste after the pretreatment with most of the pretreatment recorded a decreased value compared to the untreated waste. The structural characteristics of the waste were believed to be the cause for the obtained results. In most of the rotten waste, it is believed that the content of carbon was low as it has been consumed by microorganisms in the growing process. Additionally, this has resulted in fewer microbial populations and, therefore, fewer enzyme production to hydrolyze any remaining carbohydrate.

Colony-forming unit (CFU) and catalase test of bacteria grown from the waste

The spread plate method for CFU counting and the catalase test were conducted only on fresh waste treated using HT due to the promising reduction of sugar content recorded. Untreated waste was recorded with far higher CFU than treated waste (Table 1). Most of the colonies grown on the plate were assumed to be aerobic (HCB) due to the incubation at aerobic conditions. HT pretreatment for 65 °C recorded among the lowest CFU might indicate the suitability of the pretreatment in reducing the HCB colonies.

Table 1. Colony count and catalase test result of bacteria grown from fresh waste

Pretreatment	No. of colony (CFU/mL)	Catalase test	
		Result	Remarks
Untreated	17.1×10^{12}	+	Rapid formation of bubbles
65 °C (15 min)	8.5×10^{12}	+	A small amount of bubbles
65 °C (30 min)	7.6×10^{12}	+	
65 °C (60 min)	2.6×10^{12}	+	
70 °C (15 min)	7.0×10^{12}	+	A small amount of bubbles, slow rate of formation
70 °C (30 min)	7.4×10^{12}	+	
70 °C (60 min)	6.9×10^{12}	+	High amount of bubbles, slow rate of formation
80 °C (15 min)	3.5×10^{12}	-	no bubble
80 °C (30 min)	11.0×10^{12}	-	A small amount of bubbles and, a very slow rate of formation
80 °C (60 min)	13.6×10^{12}	-	
90 °C (15 min)	13.2×10^{12}	-	
90 °C (30 min)	14.9×10^{12}	-	
90 °C (60 min)	10.1×10^{12}	-	no bubble

Comparing the colony features between untreated and treated waste, most of them had a similar appearance, which is cream, white, and some orange-colored colonies. The shapes of the colonies were round and punctiform. All colonies had raised elevation. For the colonies margin, most of the treated samples grew entire and undulate margin and some of the samples grew entire margin colonies.

The catalase test was then conducted in the same set of experiments. In general, a positive catalase test given by rapid formation of bubbles indicates the presence of hydrogen-consuming bacteria (HCB), while catalase-negative, given by no or slow formation of bubbles indicates the presence of hydrogen-producing bacteria (HPB). Formation of bubbles by catalase negative (may also be false positive) is possible by reaction of other enzymes such as peroxidase, which is often present in facultative anaerobes such as HPB *Citrobacter* sp., *Enterobacter* sp. and certain *Bacillus* sp. (Su *et al*., 2018). Overall, treated waste gave a lower bacterial count compared to those untreated, indicating that HT could reduce the growth of aerobic bacteria in the waste.

HT at 80 °C and 90 °C showed a different trend in the bacterial count, in which at most pretreatment times, the colony of bacterial counted was higher than that of the other pretreatment temperature and time. A high temperature (80 – 90 °C) might allow the growth of certain types of microorganisms, particularly the endospore-forming and archaea. This is in agreement with previous work by Wong *et al*. (2014), who reported that high temperatures (80 – 95 °C) have protected HPBs such as *Clostridium* sp. from heat damage due to sporulation. Although the catalase test results were negative at this condition (80 – 90 °C HT), to conclusion that the bacteria grown on the plate was anaerobic (HPB), is still vague since the plates were incubated in aerobic conditions. However, it could be concluded that the HT applied can reduce the number of aerobic bacteria (HCB) which often reduces the yield in fermentative H_2 production (Han *et al*., 2016).

Environmental impact potential

The simulated pretreatment plant revealed the energy requirement in the pretreatment to be in the range of 34 – 123 MJ/kg reducing sugar (Table 2), which was in agreement with the other pretreatment utilizing heat such as in autoclave pretreatment (92.93 MJ/L product) (Mahmud & Rosentrater, 2019).

However, considering the small system boundary applied in this study, it is expected that the energy consumption will be much lower at a larger system boundary due to higher available sugar obtained after the hydrolysis process, and even lower energy consumption will be recorded per every unit of H_2 produced. An increase in the temperature of the processing was directly proportional to the energy consumption. The same pattern was observed on the simulated autoclave pretreatment (285.37 MJ/kg reducing sugar) and acid pretreatment (21.80 MJ/kg reducing sugar), relative to its temperature requirement, 121 °C and 35 °C respectively.

Table 2. Energy requirement and environmental impact potential of the simulated plant

Pretreatment	Energy requirement (MJ/kg reducing sugar)	GWP (kg CO_2 eq./kg reducing sugar)	EP (kg N eq./kg reducing sugar)
65°C (15 min)	35.77	0.435	0.344
65°C (30 min)	34.82	0.422	0.334
65°C (60 min)	34.15	0.413	0.326
70°C (15 min)	41.83	0.398	0.315
70°C (30 min)	46.26	0.448	0.354
70°C (60 min)	46.35	0.449	0.355
80°C (15 min)	68.22	0.441	0.349
80°C (30 min)	64.59	0.414	0.328
80°C (60 min)	69.46	0.450	0.356
90°C (15 min)	107.71	0.505	0.400
90°C (30 min)	102.14	0.476	0.377
90°C (60 min)	123.24	0.587	0.465
Acid (Wu & Chang, 2007)	21.80	0.391	0.310
Autoclave (Hu *et al*., 2014)	285.37	0.958	0.709

GWP – Global warming potential

EP – Eutrophication potential

The calculated GWP of the HT was promisingly low (Table 2) in comparison to autoclave pretreatment simulated in this work (0.958 kg CO_2 eq./kg reducing sugar) and previous work (12.23 kg CO_2 eq./L product) (Mahmud and Rosentrater, 2019). The eutrophication potential for HT was slightly high because it was normalized to a small denominator amount but also due to slightly high nitrogen content in the waste materials that were emitted during the pretreatment. Acid pretreatment recorded low GWP due to the low steam requirement in the process. However, it recorded an additional environmental impact of acidification potential of about 0.05 kg SO_2 eq./kg reducing sugar due to the emission of chemicals used. This is in agreement with findings from a previous work, which mention that chemical-based pretreatment recorded higher total emissions compared to those of chemical-free options (Rodrigues Gurgel da Silva *et al*., 2019). The same work recorded a high CO_2 emission of extensive heat-based pretreatment including liquid hot water pretreatment (200 °C) and steam explosion pretreatment (220 °C), equivalent to those of other pretreatment approaches although in these heat-based pretreatments, the product yield was much lower.

CONCLUSION

Heat pretreatment (HT) was proven as a promising waste utilization approach for biohydrogen production. More importantly, HT at lower temperatures recorded better pretreatment performance. It provides a mild reaction effect which will preserve the available sugar in the waste and reduce the HCB, while the HPB that is often spore-forming will be retained. Additionally, HT does not require the use of any chemicals, thus reducing the total emissions. The environmental impact potential recorded from HT was promisingly low compared to autoclave pretreatment and comparable to the no-heat pretreatment approach, acid pretreatment. This provides preliminary knowledge on the performance of HT for future study towards its real application in biohydrogen production. Emphasis should be made on the optimization work of HT at the lower temperature ranges (65 – 70 °C) including the effect on enzyme hydrolysis and fermentation rate, as well as its overall environmental footprint.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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