



Effect of Thermal Hydrolysis Temperature on Hydrogen Fermentation of Food Waste: Focused on Lignocellulose and Energy Analysis

열가수분해 온도가 음식물 쓰레기의 수소 발효에 미치는 영향에 관한 연구: 리그노셀룰로오스와 에너지 분석을 중심으로

2023년 8월

서울대학교 대학원

건설환경공학부

김 준 현

# Effect of Thermal Hydrolysis Temperature on Hydrogen Fermentation of Food waste: Focused on Lignocellulose and Energy Analysis

# 지도 교수 김 재 영

# 이 논문을 공학석사 학위논문으로 제출함 2023년 8월

서울대학교 대학원

건설환경공학부

# 김 준 현

김준현의 공학석사 학위논문을 인준함

## 2023년 8월

위 원 장 남 경 필 (인) 부위원장 김 재 영 (인) 위 원 최용주 (인)

# Abstract

# Effect of Thermal Hydrolysis Temperature on Hydrogen Fermentation of Food waste: Focused on Lignocellulose and Energy analysis

Junhyeon Kim

Department of Civil and Environmental Engineering The Graduate School Seoul National University

Hydrogen is a potential clean energy that can replace fossil fuels due to its clean, renewable, and non-polluting characteristics. It offers higher energy efficiency compared to gasoline and diesel, making it an attractive option for various sectors, including transportation, industry, and residential areas. The researches on hydrogen production from organic wastes are increasing as organic wastes generation continues to rise. Among various methods of hydrogen production, hydrogen fermentation provides the advantage of simultaneous hydrogen production and waste disposal. It also yields volatile fatty acids (VFAs) widely used in different fields, while minimizing greenhouse gas emissions (CH4, CO2).

i

Organic wastes, including food waste and livestock manure, consist of 30-50 % (by dry wt.) lignocellulose. The presence of lignocellulose structures in these organic wastes can inhibit the biodegradation rate and hinder hydrolysis step, resulting in low biogas production. In order to solve this issue, various pretreatment methods, such as biological, mechanical, chemical, and thermal hydrolysis, have been applied prior to HF to enhance biodegradability by breaking down lignocellulose. Among these various pretreatments, thermal hydrolysis process (THP), which disintegrate lignocellulosic waste using steam of high temperature and pressure, has been widely applied due to its significant performance improvement.

THP exhibits certain drawbacks, including high heat and electricity requirements, as well as the potential generation of recalcitrant substances, such as melanoidins, through Maillard reaction and carbonization at high temperatures. Previous studies investigating the application of THP in HF primarily focused on improving hydrogen production. However, the energy balance of the HF process and the underlying mechanism of Maillard reaction, carbonization remained unknown. Further research is necessary to understand these aspects and address the knowledge gaps.

The main objective of this study is to investigate the effects of thermal hydrolysis temperature of food waste on hydrogen production and net energy gain. The investigation focuses on assessing the influence of THP on biogas production through biochemical hydrogen potential (BHP) test. Additionally, an analysis of structure change of lignocellulose was performed to enhance our

ii

understanding of how THP impacts HF. Furthermore, an energy analysis is performed to evaluate the net energy gain of HF depending on THP of food waste using continuous stirred-tank reactor (CSTR) test.

Food waste was treated with THP at temperatures of 120, 140, 160, and 180 °C. The highest hydrogen production was exhibited in the case of food waste treated at 140 °C, with a 3-fold increase compared with untreated food waste, as determined by BHP test. Decrease in cellulose crystallinity and the morphological change of lignocellulose indicated an improvement of accessibility for microorganisms, which resulted in hydrogen production increase. However, at temperatures of 160 and 180 °C, changes in functional groups and formation of humic-acid like substances were observed, suggesting the occurrence of Maillard reaction and carbonization, which can lead to a decrease in hydrogen production.

In CSTR test, food waste pretreated at 140  $^{\circ}$ C (THP HF) and untreated food waste (Control HF) were used as substrates. The application of THP contributed to the improvement of organic matter removal rate by 1.1-fold, and the enhancement of butyrate-toacetate ratio (Bu/AC) in THP HF, resulting in a 1.6-fold increase in hydrogen production. The application of THP led to significant alteration in the bacterial communities, contributing to enhance process stability. However, the net energy gain of THP HF was lower than Control HF, due to the higher energy input associated with THP.

In conclusion, THP proved to be effective in enhancing hydrogen production from food waste by increasing surface area through the depolymerization of lignocellulose, while also improving the stability of the reactor. However, at high temperatures, the production of refractory substances led to a decrease in hydrogen production. Despite the positive effect of THP on hydrogen production, the net energy gain decreased due to higher energy input required for pretreatment step. Nevertheless, the energy efficiency of THP HF can be improved by increasing the heat recovery rate of the THP process to 95 %.

**Keywords:** Hydrogen fermentation; Food waste; Thermal hydrolysis; Lignocellulose; Energy balance **Student Number:** 2021–26328

# Contents

1. Inti	coduction	1
1.1	Background	1
1.2	Objectives of the study	6
1.3	Scope of the study	7
о т <b>.</b>		0
2. Lite	erature review	9
2.1 H	Iydrogen fermentation	9
2.1.1	Deactivation of methanogens	10
2.	1.1.1 Heat-shock	10
2.	1.1.2 Acid and base treatment	11
2.1.2	Hydrogen fermentation of glucose	13
2.2 F	Pretreatment of organic wastes	15
2.2.3	l Thermal hydrolysis pretreatment (THP)	17
3. Ma	terials and methods	19
3.1 S	Substrate and inoculum	19
3.2 7	THP process	21
3.3 E	Biochemical hydrogen potential (BHP) test	22
3.3.3	l BHP test set-up	22
3.3.2	2 Kinetic study	24
3.4 C	Continuous stirred-tank reactor (CSTR) test.	25
3.5 A	Analytical methods	27

3.5.1 Proximate analysis	.27
3.5.2 Elemental analysis	.28
3.5.3 Water quality analysis	.28
3.5.4 Measurement of biogas production	.29
3.5.5 Structure change of lignocellulose	.30
3.5.6 Isolation of cellulose	.32
3.5.7 Analysis of melanoidins	.33
3.5.8 Microbial analysis	.35
3.5.9 Energy balance analysis	.36

4. Results and Discussion	40
4.1 Effect of THP on characteristics of food waste	40
4.1.1 Solid contents	40
4.1.2 Water quality and elements	41
4.2 Hydrogen production in BHP test	43
4.2.1 Hydrogen production	43
4.2.2 Kinetics of BHP test	44
4.2.3 VFAs production of BHP test	47
4.3 Structure of lignocellulose	49
4.3.1 Crystallinity of cellulose	49
4.3.2 Morphological change of lignocellulose	50
4.3.2.1 SEM analysis	50
4.3.2.2 BET analysis	52
4.3.3 Mechanism of Maillard reaction and carbonization	54

4.3.4 Composition of functional groups in food waste	56
4.4 Formation of humic-acid like substances	
4.5 Energy analysis of BHP test	61
4.6 CSTR test of HF	
4.6.1 Reactor performance	62
4.6.2 VFAs production of CSTR test	65
4.6.3 Microbial analysis	67
4.6.4 Energy analysis of CSTR test	71
4.6.5 Sensitivity analysis	72

5. Cor	clusions7	4
--------	-----------	---

# List of Figures

Fig 1.1 Chemical processes of anaerobic digestion	2
Fig 1.2 Structure of lignocellulose	3
Fig 1.3 Degradation of lignocellulose by pretreatment	4
Fig 1.4 Net energy gain of HF	5
Fig 2.1 Biochemical pathways of HF	10
Fig 2.2 The transition of spore-forming bacteria to vegetative cell by he	at–
shock	11
Fig 2.3 Pathway of HF using glucose (hexose) as substrate)	14
Fig 2.4 Formation of melanoidins by Maillard reaction	18
Fig 3.1 Schematic diagram of BHP test	23
Fig 3.2 Pictures of BHP test	23
Fig 3.3. Schematic diagram of CSTR reactor	26
Fig 3.4 Picture of laboratory-scale reactors for CSTR	26
Fig 3.5 Regions in EEM based on excitation and emission	34
Fig 3.6 Flow diagram of energy analysis	36
Fig 4.1 Solubilization of food waste by THP temperature	41
Fig 4.2 Hydrogen production from BHP test by THP temperature	43
Fig 4.3 Hydrogen production fitted with modified Gompertz model	45
Fig 4.4 Hydrogen production fitted with 1 <sup>st</sup> order kinetic model	45
Fig 4.5 XRD result of cellulose from food waste	49
Fig 4.6 SEM images of lignocellulose in food waste	51

Fig 4.7 FT-IR results of lignocellulose in food waste
Fig 4.8 C 1s XPS spectra of lignocellulose in food waste
Fig 4.9 The EEM of food waste60
Fig 4.10 Energy balance of BHP test61
Fig 4.11 Hydrogen production of CSTR reactors63
Fig 4.12 VS removal rate of CSTR reactors63
Fig 4.13 VFAs production of Control HF and THP HF66
Fig 4.14 Butyrate-to-acetate ratio (Bu/Ac) of Control HF and THP HF.66
Fig 4.15 The relative abundance of major bacteria phylum and genus of seed
sludge, Control HF, and THP HF69
Fig 4.16 Sensitivity analysis of CSTR test73

# List of Tables

Table 2.1 Main effects of pretreatment methods to substrate1	6
Table 3.1 Components used to make 1 kg of food waste2	0
Table 3.2 Humas kit used for each analysis item2	8
Table 3.3 Setting condition of GC-TCD	9
Table 3.4 Excitation-emission boundaries of five regions in EEM	4
Table 3.5 Parameters used for energy analysis	9
Table 4.1. Solids content of food waste by THP temperature4	0
Table 4.2 Water quality and elemental analysis of food waste by TH	Ρ
temperature4	2
Table 4.3 Parameters for kinetic study in BHP test	6
Table 4.4 VFAs production of BHP test	8
Table 4.5 BET surface area and volume of food waste by THP5	3
Table 4.6 Characterization of surface composition of food waste	8
Table 4.7 Hydrogen production of CSTR test from food waste6	4
Table 4.8 Alpha diversity indices of bacteria community of seed sludge an	d
CSTR reactors7	0
Table 4.9 Energy analysis of CSTR test7	1

# 1. Introduction

#### 1.1 Background

Hydrogen (H<sub>2</sub>) is one of a highly promising alternatives to fossil fuels, primarily due to is advantageous characteristics. Firstly, hydrogen is a clean, renewable and non-polluting energy source, as it only produces water as a by-product during combustion (Mohan and Pandey, 2013). Secondly, hydrogen exhibits high energy efficiency, boasting a net calorific value of 120 MJ/kg-H<sub>2</sub>, surpassing other fuels like gasoline (44 MJ/kg), diesel (43 MJ/kg), and natural gas (54 MJ/kg). Lastly, hydrogen finds application across a wide range of sectors, including transportation, industry, commerce, and residential areas.

With the annual increase in the production of organic wastes like food waste, swine manure, and sewage sludge, the effort to produce hydrogen from organic wastes is emerging. However, current operating plants primarily rely on reforming methane (CH<sub>4</sub>) obtained from anaerobic digestion to produce hydrogen, which entails several drawbacks. The reforming process necessitates the use of high-temperature steam, typically ranging from 700 to 1,000 °C, thereby requiring additional energy inputs. Moreover, this process can contribute to air pollution as it generates greenhouse gases, including carbon dioxide (CO<sub>2</sub>).

1

Hydrogen fermentation (HF) is one of environmentally friendly process among the hydrogen production methods as it can produce energy source (hydrogen) and solve waste disposal problems simultaneously (Lee et al., 2008). This method boasts several advantages, including low energy requirements, the production of acids (VFAs) which is highly valued in markets, and minimal greenhouse gas emissions compared to reforming process (Zappi et al., 2021). HF process (Fig 1.1) can be performed by preventing methanogenesis step through the deactivation of methanogens during anaerobic digestion (Xu, 2007). Methanogens can be deactivated with treatments (heat shock, chemical, biological, ultrasonication, etc.).



Fig 1.1 Chemical processes of anaerobic digestion (Najafpour, 2015)

Organic wastes (food waste, livestock manure, etc.) are classified as lignocellulosic substrates due to their lignocellulose content, which ranges from 30 – 50 % (by dry wt.) (Liu et al., 2020; Orlando and Borja, 2020; and Singh and Yadav, 2021). Lignocellulose is composed with lignin, cellulose, and hemicellulose, and the interconnected structure of lignin and hemicellulose acts as a physical barrier to cellulose degradation (C. Saha, 2009). This inherent lignocellulosic structure can inhibit the biodegradation rate of organic wastes and result in low biogas production. Therefore, various pretreatment methods (mechanical, biological, chemical, physical, etc.) are employed prior to anaerobic digestion to break down lignocellulose (Ariunbaatar et al., 2014). These pretreatments break the chemical bonds within lignocellulose and modify its structure, thereby enhancing its biodegradability and promoting biogas production (Wang et al., 2016).



Fig 1.2 Structure of lignocellulose (Wakerley et al., 2017)



Fig 1.3 Degradation of lignocellulose by pretreatment (Lee et al., 2014)

The thermal hydrolysis process (THP) is a physical pretreatment method used to disintegrate lignocellulosic waste by subjecting it to high temperature and pressure steam, thereby improving the performance of anaerobic digestion (Kim et al., 2013a). However, this method has the drawback of requiring high heat and electricity. Additionally, it is important to consider the formation of recalcitrant substances, such as melanoidins, through Maillard reactions and carbonization at high temperatures (Dwyer et al., 2008; Diez–Simon et al., 2019). As a result, it becomes necessary to optimize the pretreatment temperature of THP.

As THP requires high heat and electricity, energy balance plays a critical role in evaluating the sustainability of the process (Ghimire, 2015). HF, characterized by the production of volatile fatty acids and alcohols as end products, exhibits a low hydrogen production (Perera et al., 2010). Previous studies on HF with organic wastes have reported negative net energy gain values, indicating that the process consumes more energy than

it generates (Perera et al., 2010). By applying THP prior to HF, hydrogen production can increase, yet it comes at the expense of higher energy consumption.



Fig 1.4 Net energy gain of HF (FW, food waste; SS, sewage sludge; RS, rice slurry; and CM, cattle manure)

Therefore, it is necessary to investigate the performance improvement of hydrogen production from food waste by THP, and to determine the temperature of THP, which promotes the formation of recalcitrant substances. Additionally, it is crucial to analyze the energy balance of the process and examine the effect of thermal hydrolysis on structure change in lignocellulose, in order to provide a comprehensive understanding and discussion of these aspects.

### 1.2 Objectives of the study

The main objectives of this study are (1) to investigate the effect of thermal hydrolysis temperature on hydrogen production through structure change in lignocellulose, and (2) to assess the energy balance from food waste to hydrogen by hydrogen fermentation

By achieving these objectives, this study aims to provide insights into the structure change of lignocellulose by THP, as well as the conditions that can lead to the formation of recalcitrant substances. Furthermore, the feasibility of hydrogen fermentation applied with THP can be explored through energy analysis.

#### 1.3 Scope of the study

Among organic wastes, food waste was used to achieve the objective of this study. BHP test and CSTRs were operated under mesophilic (35  $^{\circ}$ C) to evaluate the effects of THP and its temperature on HF. Furthermore, a structure analysis of lignocellulose was performed to gain insights into the reasons underlying the observed performance improvement or recalcitrant formation. The specific scopes of this study are as follows:

(1) Lab-scale HF

Lab-scale BMP test and CSTRs were performed to assess the effect of THP on biogas production under mesophilic conditions. The temperature for enhancing hydrogen production was determined and proposed based on the experimental results.

#### (2) Structure analysis of lignocellulose

Structure change of lignocellulose was analyzed to gain a better understanding of how THP affects HF. XRD, SEM, and BET were performed to investigate the performance improvement resulting from THP. Additionally, FT–IR and XPS was utilized to explore the mechanisms of Maillard reaction and carbonization.

7

#### (3) Energy analysis of the process

The energy balance of the process was evaluated by calculating the net energy gain. The net energy gain was calculated by considering the energy consumed for THP and heating the fermenter, as well as the energy generated from hydrogen produced through HF.

## 2. Literature review

#### 2.1 Hydrogen fermentation

Anaerobic digestion is a biological process that decomposes organic materials by microorganisms in an environment with no oxygen, leading to the production of methane, carbon dioxide. Anaerobic digestion involves four sequential steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During hydrolysis step, complex organic molecules like carbohydrates, proteins and lipids are broken down into simpler compounds such as long-chain fatty acids, amino acids and monosaccharides. These intermediates undergo further fermentation in the acidogenesis and acetogenesis steps, resulting in the production of hydrogen and volatile fatty acids. Finally, in the methanogenesis step, hydrogen and volatile fatty acids are converted to methane.

HF, on the other hand, is a modified process of anaerobic digestion that specifically focuses on hydrogen production by preventing methanogenesis step. This is achieved by deactivating methanogens while preserving hydrogen-producing bacteria in the seed sludge. Various treatment methods, such as heat shock, acid treatment, base treatment, and ultrasonication are employed to inhibit methanogenesis and promote HF.



Fig 2.1 Biochemical pathways of HF (Ghimire, 2015)

#### 2.1.1 Deactivation of methanogens

#### 2.1.1.1 Heat-shock

Temperature is one of crucial factors that significantly influences the activation of microbial. High temperature can disrupt the chemical bonds present in cell walls and membranes, ultimately resulting in the dissolution of cell components. While hydrogen-producing bacteria, such as *Clostridium* and *Bacillus*, which are spore-forming bacteria, can survive by forming spore, non-spore forming bacteria like methanogens, lactic acid bacteria are inhibited (Wang and Yin, 2017). Spore forming bacteria activates by heat-shock and form vegetative cell through interaction with specific germinants, such as amino acids, sugars and purines (Valdez-Vazquez and Poggi-Varaldo, 2009; Fig 2.2).



Fig 2.2 The transition of spore-forming bacteria to vegetative cell by heatshock (Setlow, 2003)

#### 2.1.1.2 Acid and base treatment

pH is also a critical factor that can exerts a significant influence on microbial activation. The optimal pH range for the growth of methanogens is typically between 6.8–7.2 while hydrogen-producing bacteria can grow across a broader pH range of 5–9 (Pachapur et al., 2019). Methanogens can be effectively deactivated by treating to pH conditions ranging 2–4 or 10–12 for a duration of 24 hours (Wang and Yin, 2017). However, it is important to consider the potential drawbacks associated with the use of high concentrations of acid and base. These drawbacks include corrosion issues, increased maintenance cost, and the possibility of inhibiting hydrogen-producing bacteria by generating toxic chemicals such as chloroform and 2–bromoethanesulfonate (Wong et al., 2014; Pachapur et al., 2019).

#### 2.1.1.3 Other treatments

While methanogens are strictly anaerobic bacteria, it is worth noting that many hydrogen-producing bacteria such as *Enterobacter* spp. belongs to facultative anaerobic bacteria (Wang and Yin, 2017). Through aeration treatment, which involves introducing an appropriate amount of dissolved oxygen, hydrogen-producing bacteria can be activated while simultaneously deactivating methanogens.

Ultrasonication and microwave treatments have been employed to suppress methanogens and preserve hydrogen-producing bacteria, inducing the formation of spore through heat. Microwave treatment generates heat through the absorption of microwaves by cell components, resulting in the collapse of cell wall (Wang and Yin, 2017; Yanga and Wang, 2017). Ultrasonication treatment creates thousands of bubbles through pressure and directs them into the cell. The vibration and heat generated by cavitation lead to the collapse of cell wall (Elbeshbishy et al., 2010; Yanga and Wang, 2017; and Rajesh Banu et al., 2020).

12

#### 2.1.2 Hydrogen fermentation of glucose

In HF, carbohydrates, such as glucose, xylose, sucrose and molasses are highly favorable substrates due to its high biodegradability (Li and Fang, 2007). Among carbohydrates, many studies on HF using glucose have been reported (Fang and Liu, 2002; Hu and Chen, 2007; and Wang and Wan, 2008).

Several pathways for hydrogen production by HF have been proposed. Fig 2.3 illustrates the pathway of HF using glucose as substrate. During HF, VFAs (acetate, butyrate, propionate, etc.) are generated along with hydrogen production. Acetate and butyrate are known as products of the hydrogen-producing pathway, while propionate is a product of the hydrogen-consuming pathway (Hussy et al., 2003). Theoretically, acetate and butyrate fermentation (Eq. 1–2) with glucose can yield 4 mol and 2 mol of hydrogen, respectively, while propionate fermentation (Eq. 3) with glucose consumes 2 mol of hydrogen (Hussy et al., 2003; Łukajtis et al., 2018; and Nagarajan et al., 2022)

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2$$
 (Eq. 1)

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2CH_2COOH + 2H_2 + 2CO_2$$
(Eq. 2)

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$$
 (Eq. 3)



Fig 2.3 Pathway of HF using glucose (hexose) as substrate (Nagarajan et al., 2022)

#### 2.2 Pretreatment of organic wastes

Organic wastes, including livestock manure, food waste, and sewage sludge are abundant sources of complex and insoluble organic matters, such as lignocellulose (Krishna and Kalamdhad, 2014; Nguyen et al., 2021). The hydrolysis step is considered as the rate-limiting step in anaerobic digestion and HF of organic wastes (Ariunbaatar et al., 2014; Orlando and Borja, 2020), Pretreatments have been employed to improve the biodegradability and enhance biogas production by accelerating hydrolysis step (Carrère et al., 2010; Ariunbaatar et al., 2014; and Yang et al., 2017).

Various pretreatments methods, including biological, mechanical, chemical and thermal hydrolysis, have been utilized enhance the performance of the process. Biological and mechanical pretreatment can accelerate hydrolysis rate, facilitating the breakdown of complex organic compounds. On the other hand, chemical pretreatment and thermal hydrolysis are effective in not only accelerating the hydrolysis rate but also enhancing the biodegradability of the substrate. However, biological pretreatment is associated with drawbacks such as long treatment time and high cost of enzymes. Mechanical and chemical pretreatment can form inhibitors that may adversely affect process stability. Thermal hydrolysis requires substantial amount of heat and electricity, but it does not affect the stability of the process. The main effects and disadvantages of each pretreatments are summarized on Table 2.1

15

Pretreatment methods		Performance	Electrical	Main offects	Disadvantagos
		improvement	input	Main effects	Disadvantages
Biological	- Aerobic	Moderate	Moderate	– Accelerate hydrolysis rate	- Long treatment time
	- Anaerobic				
	- Enzymes				– High cost of enzymes
Mechanical	– Ultrasonic	Moderate	Moderate	- Accelerate hydrolysis rate	– High maintenance cost
	- Grinding				- Formation of inhibitors
Chemical	- Alkali	Moderate	_	- Accelerate hydrolysis rate	- Corrosion problem
	- Acid				- Formation of chemical
	– Ozonation			- Increase biodegradability	inhibitors
Thermal hydrolysis		Substantial	Substantial	- Accelerate hydrolysis rate	- High heat and electricity
				- Increase biodegradability	demand

Montgomery and Bochmann, 2014; Bhatt and Shilpa, 2015; Achinas et al., 2017; and Khan et al., 2022)

Table 2.1 Main effects of pretreatment methods to substrate (Carrère et al., 2010; Ariunbaatar et al., 2014;

#### 2.2.1 Thermal hydrolysis pretreatment (THP)

In order to enhance the biodegradability of substrates and biogas production in anaerobic digestion process, THP prior to anaerobic digestion has been extensively studied in previous researches. THP involves the decomposition organic compounds containing carbohydrates, proteins, fats, and nucleic acids into low molecular substances that are easily decomposed by microorganisms, using steam of high temperature and pressure (Kim et al., 2013a). Among various pretreatments, THP is widely recognized as the most effective form improving anaerobic digestion performance, without introducing any drawbacks that could impact stability of the process (Carrère et al., 2010). The numbers of thermal hydrolysis plants are increasing every year, with Cambi accounting for 53 % of the total thermal hydrolysis plants as of 2020 (Barber, 2020).

The effect of THP on HF is primarily influence by temperature, as high temperatures can lead to the generation of recalcitrant substances such as melanoidins through Maillard reaction and carbonization at high temperature (Dwyer et al., 2008; Diez–Simon et al., 2019). Studies on HF of food waste by Ding et al. (2017) and Wei et al, (2022) both identified an optimal temperature of 140 °C, resulting in a significant increase in hydrogen production by 23.9 and 242.6 % (by vol.), respectively (Ding et al., 2017; Wei et al., 2022). However, temperatures higher than 140 °C can lead to a decrease in hydrogen production due to the carbonization of food waste, which resulted in the formation of insoluble high–carbon hydrochar, and

reduced content of soluble carbohydrates (Li et al., 2013; Ding et al., 2017). Maillard reactions between soluble carbohydrates and proteins at high temperatures can also decrease the total carbohydrate content (Yeshanew et al., 2016). Therefore, if the conversion of soluble carbohydrates to melanoidins surpasses the solubilization of large molecular carbohydrate polymers at high temperature, the content of soluble carbohydrates decreases, leading to a decrease in hydrogen production decrease. (Ding et al., 2017).



Fig 2.4 Formation of melanoidins by Maillard reaction (Kroh et al., 2008; Echavarria et al., 2012)

## 3. Materials and methods

#### 3.1 Substrate and inoculum

The methodology for producing food waste followed the guidelines announced by the South Korean Ministry of Environment. The food waste was stored at -20 °C before thermal hydrolysis process (THP). The components used to generate the food waste were comprehensively listed in Table 3.1.

The inoculum was collected from an anaerobic digestion plant operating in steady state with swine manure and food waste in a ratio of 6:4 (by vol.). The large particles were removed with 500  $\mu$ m sieve. Subsequently, the inoculum was treated with heat at 100°C for 15 minutes via autoclave (VS-1221, Vision Scientific, Republic of Korea) to deactivate methanogens. The inoculum was stored at 4°C prior to being acclimated at the fermentation temperature (35 ± 1°C) for 24 hours to activate hydrogen-producing bacteria.

Category	Composition(g)	Ingredients (g)	Weight (g)
Grains	$160 \pm 30$	Rice	160
		Cabbage	80
<b>X</b> 7 ( 11	500 + 60	Potato	200
Vegetables	500 ± 60	Onion	200
		Radish	20
	140 + 20	Apple	70
Fruits	140 ± 30	Orange	70
	200 + 20	Pork	50
Meat and fish	200 ± 30	Mackerel	150

#### Table 3.1 Components used to make 1 kg of food waste

\* Water content adjusted to 80  $\,\pm\,$  5 % (by wet wt.)

#### 3.2 THP process

The THP process was carried out using 2 L stainless-steel autoclave (HR-S-2000, Ilshin Autoclave, Republic of Korea). THP temperature was varied at 120, 140, 160, and 180 °C to evaluate the effect of temperature on performance improvement and recalcitrant formation (Ding et al., 2017; Wei et al., 2022). In each trial, 1.5 L of food waste was loaded into the reactor, which was then sealed. The process was maintained for 30 minutes at a stirring speed of 120 rpm once the reactor reached the desired temperature. The pressure during the process was maintained as the saturated vapor pressure of water at the THP temperature by autoclave itself. After each trial, the reactor was cooled down to room temperature, and the pretreated substrates were stored at -20 °C before use.

The solubilization of food waste by THP temperature was quantified as the ratio between the increase in soluble fraction following THP and the initial particulate fraction. The solubilization with COD was calculated using Eq. 4 (Val de rio et al., 2011):

Solubilization (% by wet wt.) =  $\frac{COD_S - COD_{S0} (mg/L)}{COD_{P0} (mg/L)} \times 100$  (Eq. 4)

where  $COD_S$ : Soluble COD of hydrothermal treated substrate;

 $COD_{S0}$ : Soluble COD of raw substrate;

 $COD_{P0}$ : Particulate COD of raw substrate ( $COD_{T0} - COD_{S0}$ ); and  $COD_{T0}$ : Total COD of raw substrate

#### 3.3 Biochemical hydrogen potential (BHP) test

#### 3.3.1 BHP test set-up

In order to evaluate the hydrogen production under different thermal hydrolysis temperatures, BHP test was performed following the methodology recommended by Angelidaki et al. (2009). Specifically, 10 mL of inoculum and 90 mL of nutrient and trace elements were added to a 160 mL serum bottle. The substrate was added based on the substrate to inoculum ratio (SIR) of 1:2 (by VS) to maintain a consistent ratio of microorganisms to sample quantity (Angelidaki et al., 2009). The pH of the sample was adjusted to 5.5 using 1 M HCl and 1 M NaOH, which is optimal for the growth of hydrogen-producing bacteria (Fang and Liu, 2002; Tang et al., 2008). The serum bottle was then purged with N<sub>2</sub> gas for 2 minutes to create an anaerobic condition and sealed with a rubber stopper and aluminum cap. The BHP test was performed in quintuplicate and incubated at a temperature of  $35\pm1$  °C until hydrogen was no longer produced.



Fig 3.1 Schematic diagram of BHP test



Fig 3.2 Pictures of BHP test
## 3.3.2 Kinetic study

Modified Gompertz equation (Eq. 5) was utilized to evaluate the hydrogen production rate and lag phase time.

$$M = P \times exp \left[ -exp \left\{ \frac{R_m \times e}{p} \left( \lambda - t \right) + 1 \right\} \right]$$
(Eq. 5)

where M: Cumulative hydrogen production (mL-H<sub>2</sub>/g-VS);

- *P*: Hydrogen production potential (mL $-H_2/g-VS$ );
- $R_m$ : Maximum hydrogen production rate (mL-H<sub>2</sub>/g-VS/d);
- $\lambda$ : lag phase time (hours); and
- t: time over the period (hours)

 $1^{st}$  order kinetic model considered with lag phase (Eq. 6) was utilized to evaluate the hydrogen production rate constant

$$M = P \times [1 - \exp\{-k \times (t - \lambda)\}]$$
(Eq. 6)

where M: Cumulative hydrogen production (mL-H<sub>2</sub>/g-VS);

*P*: Hydrogen production potential  $(mL-H_2/g-VS)$ ;

- *k*: hydrolysis rate constant (hour<sup>-1</sup>);
- $\lambda$ : lag phase time (hours); and
- t: time over the period (hours)

# 3.4 Continuous stirred-tank reactor (CSTR) test

Two laboratory-scale reactors with a total volume of 3 L and a working volume of 2 L were operated for HF. The reactors were operated The slurry was stirred at 60 rpm and under a hydraulic retention time (HRT) of 5 days and an organic loading rate (OLR) of 10 g-VS/L/day with a stirring speed of 60 rpm. The temperature of reactor was maintained at a constant temperature of  $35\pm1$  °C by a mesophilic constant temperature chamber. The pH of the reactor was regulated using a pH controller (Bluelab, United Kingdom). The feeding of substrates and withdrawal of digestate was performed daily. In order to prevent the substrate from leaking out, the same volume of digestate was removed before feeding. One reactor was fed with untreated food waste (Control HF), while the other was fed with THP treated food waste (THP HF). The performance of the reactors was evaluated based on hydrogen production, volatile fatty acids (VFAs) generation, and organic matter reduction. Microbial analysis was conducted to compare the dominant species between the two reactors.



Fig 3.3. Schematic diagram of CSTR reactor



Fig 3.4 Picture of laboratory-scale reactors for CSTR

# 3.5 Analytical methods

#### 3.5.1 Proximate analysis

Proximate analysis was performed according to waste treatment test methods announced by the South Korean Ministry of Environment. Total solids (TS) was measured by drying the residue overnight in a 105  $^{\circ}$ C oven. Volatile solids (VS) was calculated by igniting the dried samples in a muffle furnace at 550  $^{\circ}$ C for 1 hour. The calculations for TS and VS were done using the following equations:

$$TS(\% by wet wt.) = \frac{W_{total} - W_{dis\hbar}}{W_{sample} - W_{dis\hbar}} \times 100$$
(Eq. 7)

where  $W_{sample}$ : Weight of wet sample and dish (mg);

W<sub>dish</sub>: Weight of dish (mg); and

Wtotal: Weight of dried residue and dish (mg)

$$VS(\% by wet wt.) = \frac{W_{total} - W_{volatile}}{W_{total} - W_{dish}} \times 100$$
(Eq. 8)

where  $W_{total}$ : Weight of dried residue and dish (mg);

W<sub>dish</sub>: Weight of dish (mg); and

*W*<sub>volatile</sub>: Weight of residue and dish after ignition (mg)

## 3.5.2 Elemental analysis

Elemental analysis was performed at the "National Center for Inter– university Research Facilities (NCRIF)" located in Seoul national university, Seoul, Republic of Korea. The contents of carbon (C), hydrogen (H), nitrogen (N), sulfur (S), and oxygen (O) were determined using elemental analyzer (Flash 2000, Thermo Fisher Scientific, USA)

#### 3.5.3 Water quality analysis

Water qualities of substrates, inoculum and effluents were measured with water quality analyzing kit (Humas, Republic of Korea). The items of water quality analysis were total and soluble chemical oxygen demand (TCOD, SCOD), total and soluble T–N, and ammonium ion (NH<sub>4</sub><sup>+</sup>). The samples used for analyzing SCOD, soluble T–N, and NH<sub>4</sub><sup>+</sup> were filtered using a 0.45  $\mu$ m syringe filter before the analysis.

Item	Analysis	Method	Range (mg/L)
HS-COD-MR	TCOD, SCOD	Dichromate	50-1,500
HS-TN(CA)-H	total T–N, soluble T–N	Chromotropic acid	10-100
HS-NH3(N)-H	Ammonium ion	Nessler	2-60

Table 3.2 Humas kit used for each analysis item

### 3.5.4 Measurement of biogas production

During the BHP test, the pressure in the bottle was measured with the instrument consisting of T-valve, pressure gauge, and a syringe needle. The volume of the biogas was determined by applying the ideal gas equation under steady state (25 °C, 1 atm). The biogas volume during the CSTR test was measured using a wet gas meter. Additionally, the composition of the biogas was analyzed using gas chromatography (GC) equipped with a thermal conductivity detector (TCD).

Item	Conditions
Model	ACME 6100 (Younglin, Republic of Korea)
Detector type	Thermal Conductivity Detector (TCD)
Column	8/100 Porapak N (Agilent Technologies, USA) 10 ft × 1/8 in. × 2.1 mm SS
Oven temp.	40 °C
Detector temp.	120 °C
Injector temp,	120 °C
Carrier gas	Helium (He)

Table	3.3	Setting	condition	of	GC-	TCD
1 abic	0.0	Setting	condition	<b>U</b> 1	00	100

### 3.5.5 Structure change of lignocellulose

In order to investigate the effect of thermal hydrolysis temperature on the structure of lignocellulose, X-ray diffraction (XRD), scanning electron microscopy (SEM), Brunauer Emmett Teller (BET), X-ray photoelectron spectroscopy (XPS), and Fourier transform infrared (FT-IR) analyses were conducted.

XRD was used to analyze changes in cellulose crystallinity, with SmartLab (Rigaku, Japan) operating at 40 kV and 40 mA using Cu–K $\alpha$  radiation within the  $2\theta$  range of  $10-50^{\circ}$ . In order to quantify the crystallinity of cellulose, the crystallinity index was calculated using the method developed by Segal et al. (1959) (Segal et al., 1959; Yang et al., 2009; and Yu et al., 2023).

$$CrI(\%) = \frac{I_{Cr} - I_{Am}}{I_{Cr}} \times 100$$
 (Eq. 9)

where CrI: Crystallinity index;

 $I_{Cr}$ : Intensity of peak at crystalline area; and

 $I_{Am}$ : Intensity of peak at amorphous area

SEM was utilized to observe the morphological changes of lignocellulose within the substrates, and JSM-7800F Prime (JEOL ltd., Japan) was used for SEM analysis. Prior to analysis, lignocellulose was isolated through benzene/ethanol (2:1 by vol.) solution.

BET was conducted using BELSORP mini X (MicrotracBEL Corp., Japan) to determine the surface area of the samples.

XPS was performed to identify the composition of functional groups based on their binding energy using ESCALAB250 (Thermo Fisher Scientific, USA) with AlKαX-rays (1486.7 eV) radiation. XPS analysis took place at "Cooperative Center for Research Facilities (CCRF)" located in Kookmin university, Seoul, Republic of Korea.

FT-IR spectroscopy was performed using TENSOR27 (Bruker, Belgium) to identify the change of functional groups within the wavelength range of 4,000-400 cm<sup>-1</sup>.

## 3.5.6 Isolation of cellulose

Cellulose was isolated using the method described by Galiwango et al. (2019). The extracts were removed by treating the sample with benzene/ethanol (2:1 by vol.) solution. Subsequently, 5 grams of the extract-free sample were weighed into a 250 mL beaker, and 200 mL of 0.5 M HCl was added. The mixture was heated at 100 °C and stirred for 2 h, and the resulting residue was filtered and washed with deionized water. After air drying overnight, the residue was treated with 0.5 M NaOH at 100 °C for 2 h under constant stirring. The resulting cellulose was filtered, washed with 0.5 M NaOH, and air dried overnight. Finally, bleaching was performed using 15, 20, and 10 % (by wt.) solutions of acetic acid, hydrogen peroxide, and sulfuric acid, respectively, at 75 °C for 15 min (Galiwango et al., 2019).

### 3.5.7 Analysis of melanoidins

The Excitation-Emission Matrix (EEM) spectroscopy was conducted with fluorescence spectrometer (Cary Eclipse, Agilent technologies, USA). The scanning range for excitation and emission was 200-400, 250-550 nm, respectively, with 5 nm increments. The slits of excitation and emission were set for 5 nm, and the scanning speed was 9,600 nm/min (Kim et al., 2023). All samples were diluted to a dissolved organic carbon (DOC) concentration of 5 mg-C/L with deionized water to compare the intensity of melanoidins based on an equal amount of organic matters. The intensity of the sample was standardized to Raman unit using the Raman peak of deionized water as the fluorescence intensity can be affected by temperature (Goletz et al., 2011). The matrix is divided into five regions based on excitation and emission wavelengths, as depicted in Fig 3.5. These regions, denoted as Region I, II, III, IV, and V, correspond to tyrosine-like aromatic protein, tryptophan-like aromatic protein, fulvic acid-like substance, soluble microbial byproduct, and humic acid-like substance, respectively (Park and Snyder, 2018).

33



Fig 3.5 Regions in EEM based on excitation and emission (exact boundaries are defined in Table 3.4)

Turne			Region		
туре	Ι	П	Ш	IV	V
Excitation (nm)	200-250	200-250	200-250	250-400	250-400
Emission (nm)	280-330	330-380	380-550	280-380	380-550

Table 3.4 Excitation-emission boundaries of five regions in EEM

# 3.5.8 Microbial analysis

Microbials samples for taxonomic profiling were extracted from the digestate of CSTR reactors. The extraction of microbials was performed using a 500  $\mu$ m filter. Subsequently, sequencing was conducted at CJ Bioscience, Inc. (Seoul, Republic of Korea), employing the MiSeq sequencing system (Illumina, CA, USA), following the manufacturer' s instructions. The abundance of bacterial communities was determined as a percentage of the total number of valid reads in each sample, with a threshold of set at a minimum of 1 %.

#### 3.5.9 Energy balance analysis

In order to perform a quantitative evaluation and comparison of the net energy gain of the process with and without THP, an energy balance analysis of the process was conducted. The net energy gain ( $E_{net}$ ) was calculated by accounting for the energy consumed by the THP process, energy loss from the hydrogen fermenter, and the energy produced from hydrogen. The input substrate amount was assumed to be 1 ton. The energy flow diagram used for the analysis is presented in Fig 3.6.



Fig 3.6 Flow diagram of energy analysis ((a) without THP and (b) with THP)

The input energy  $(E_{in})$  for the process with THP  $(E_{in,THP})$  was defined as the energy consumed for THP process, while the process without THP  $(E_{in,untreated})$  was the energy required to heat the substrate to the temperature of HF. It was assumed that 85 % of heat is recovered during the THP process and both processes is assumed that additional 5 % of energy is consumed due to heat loss during HF. Input energy was calculated using the equation proposed by He et al. (2017) (He et al., 2017):

$$E_{in,THP} = C_{p,feed} (T_{THP} - T_{feed}) - \emptyset C_{p,feed} (T_{THP} - T_{HF}) + 5 \% C_{p,feed} (T_{HF} - T_{feed})$$
(Eq. 10)

where  $E_{in,THP}$ : Energy input of the process with THP (MJ/ton);

 $C_{p,feed}$ : Specific heat capacity of substrate (kJ/kg/°C);  $T_{THP}$ : Temperature of THP (°C);  $T_{feed}$ : Temperature of substrate (°C); and  $T_{HF}$ : Temperature of hydrogen fermenter (°C)

 $E_{in,untreated} = C_{p,feed} (T_{HF} - T_{feed}) + 5 \% C_{p,feed} (T_{HF} - T_{feed})$ (Eq. 11) where  $E_{in,untreated}$ : Energy input of the process without THP (MJ/ton)

Energy output  $(E_{out})$  is calculated by multiplying the hydrogen production, density of hydrogen, and the lower heating value of hydrogen (Perera et al., 2010):

$$E_{out} = G \rho_{H_2} \left( LHV_{H_2} \right) \tag{Eq. 12}$$

where  $E_{out}$ : Energy output by hydrogen (MJ/ton);

G: Hydrogen production (mL-H<sub>2</sub>/g-VS);

 $ho_{H_2}$ : Density of hydrogen (kg/L); and

 $LHV_{H_2}$ : Lower heating value of hydrogen (MJ/kg-H<sub>2</sub>)

The net energy gain  $(E_{net})$  of the process was determined by subtracting consumed energy  $(E_{in})$  from produce energy  $(E_{out})$ , following the equation:

$$E_{net} = E_{out} - E_{in} \tag{Eq. 13}$$

where  $E_{net}$ : Net energy gain (MJ/ton)

The parameters used for calculating energy analysis are summarized in Table 3.5.

A sensitivity analysis was conducted to assess the influence of process variables, including heat recovery rate and heat loss rate, on the net energy gain of the process. During this analysis, variations in heat loss, TS of food waste, and hydrogen production were varied with a range of -20 to +20 % from the experimental value (assumed value), while heat recovery rate was varied within the range of -20 to +17 %, representing the upper limit where the heat recovery rate reaches 100 %.

Parameters	Value	Units	References
Heat recovery by heat exchanger ( <b>ø)</b>	85	%	He et al., 2017
Density of hydrogen $( ho_{H_2})$	$8.9 \times 10^{-5}$	kg-H <sub>2</sub> /L	
Lower heating value of hydrogen $(LHV_{H_2})$	120	MJ/kg-H <sub>2</sub>	Perera et al., 2010
Heat capacity of food waste ( <b>C<sub>p,feed</sub></b> )	3.34	kJ/kg/℃	Lewis, 2009
Feed temperature $(T_{feed})$	25		
Fermentation temperature $(T_{HF})$	35	C	Assumed

Table 3.5 Parameters used for energy analysis

# 4. Results and Discussion

# 4.1 Effect of THP on characteristics of food waste

# 4.1.1 Solid contents

The table presented in Table 4.1 shows the solid contents of food waste at different THP temperatures. The TS and VS of the food waste remained relatively constant, except for the food waste treated at 180 °C, which showed a decrease of approximately 26 % (by dry wt.) in both TS and VS. This phenomenon can be attributed to the solubilization of primary organics (proteins, carbohydrates, and VFAs) and the subsequent evaporation of volatile compounds as a result of high temperature (Li et al., 2016; Liu et al., 2020).

THP temperature (°C)	TS (% by wet wt.)	VS (% by wet wt.)
Untreated	$25.12~\pm~0.54$	$24.21 \pm 0.55$
120	$21.78~\pm~0.10$	$20.89 \pm 0.07$
140	$23.46~\pm~0.80$	$22.64 \pm 0.84$
160	$21.60~\pm~0.44$	$20.56 \pm 0.45$
180	$18.70~\pm~0.36$	$17.84 \pm 0.37$

Table 4.1. Solids content of food waste by THP temperature

### 4.1.2 Water quality and elements

Soluble T-N and ammonium ion increased with increasing THP temperature. Soluble T-N and ammonium ion can increase as proteins decompose to amino acids by THP (Barber, 2016; Carrère et al., 2016; and Xu et al., 2020). The increase of soluble T-N and ammonium ion indicates that decomposition of proteins was enhanced at higher THP temperature.

Solubilization of food waste was observed to increase with THP temperature up to a maximum at 160  $^{\circ}$ C (Fig 4.1), but decreased at 180  $^{\circ}$ C. In addition, the carbon content of food waste increased from 50 to 59  $^{\circ}$  (by dry wt.) when treated at 180  $^{\circ}$ C. This suggests that at 180  $^{\circ}$ C, carbonization occurred, leading to the generation of insoluble compounds that reduced solubilization (Yeshanew et al., 2016)



Fig 4.1 Solubilization of food waste by THP temperature

THP	COD	(g/L)	T−N (g	g-N/L)	$\mathrm{NH_4}^+\mathrm{-N}$	Elen	nental ar	nalysis (%	by dry	wt.)
temperature (℃)	Total	Soluble	Total	Soluble	(g-N/L)	С	Н	0	Ν	S
Untreated	$237.03 \pm 16.94$	$\begin{array}{r} 80.46 \pm \\ 2.08 \end{array}$	$\begin{array}{r} 7.52 \ \pm \\ 0.51 \end{array}$	2.30 ± 0.39	0.62 ± 0.00	49.96	7.11	31.50	4.31	0.20
120	$175.91 \pm 13.04$	$\begin{array}{r} 77.54 \\ 2.84 \end{array}$	$\begin{array}{c} 5.89 \ \pm \\ 0.53 \end{array}$	$2.58 \pm 0.29$	$\begin{array}{c} 0.77 \hspace{0.1cm} \pm \\ 0.14 \end{array}$	48.49	6.98	31.32	4.39	0.20
140	$161.84 \pm 23.63$	$\begin{array}{r} 103.29 \hspace{0.1 cm} \pm \\ \hspace{0.1 cm} 2.93 \end{array}$	$\begin{array}{r} 4.48 \ \pm \\ 0.37 \end{array}$	$\begin{array}{c} 2.55 \hspace{0.1cm} \pm \\ 0.22 \end{array}$	1.69 ± 0.10	50.80	7.30	31.70	3.77	0.16
160	$\begin{array}{r}148.62 \\ 7.40\end{array}$	$\begin{array}{r}121.83  \pm \\  0.79 \end{array}$	$\begin{array}{c} 5.06 \\ \pm \\ 0.78 \end{array}$	3.80 ± 0.23	2.04 ± 0.05	50.95	7.07	30.33	4.45	0.19
180	139.00 ± 22.04	$\begin{array}{r} 88.36 \pm \\ 4.38 \end{array}$	6.00 ± 0.24	$\begin{array}{c} 4.67 \\ 0.28 \end{array}$	2.78 ± 0.09	58.51	7.55	22.79	5.25	0.15

Table 4.2 Water quality and elemental analysis of food waste by THP temperature

# 4.2 Hydrogen production in BHP test

## 4.2.1 Hydrogen production

Fig 4.2 illustrates the hydrogen production of food waste treated at various thermal hydrolysis temperature. As temperature increases to 140 °C, the highest hydrogen production of 96.73 mL-H2/g-VS, which was 3-fold of untreated (36.67 mL-H2/g-VS), was observed at 140 °C. However, when the temperature increases above 140 °C up to 180 °C, hydrogen production began to decrease. At 180 °C, hydrogen production decreased to 11.81 mL-H<sub>2</sub>/g-VS, which is 32.21 % (by vol.) of untreated. The decrease of hydrogen production is attributed to the formation of recalcitrant substances (e.g., melanoidins) by Maillard reaction and carbonization at high temperatures (Dywer et al., 2008; Diez-Simon et al., 2019).



Fig 4.2 Hydrogen production from BHP test by THP temperature

### 4.2.2 Kinetics of BHP test

The kinetic parameters obtained from fitting modified Gompertz model and 1<sup>st</sup> order kinetic model to BHP results are summarized in Table 4.3. The maximum hydrogen production rate ( $R_m$ ) exhibited a similar trend with the BHP results. The highest value of  $R_m$ , 8.08 mL-H<sub>2</sub>/g-VS/h, was observed in food waste treated at 140 °C, which was 5-fold of untreated. This indicates that THP at 140 °C improved digestibility of food waste.

The lag phase  $(\lambda)$  is the time taken by microorganisms to adapt to the environment (Ashah et al., 2020). The lowest lag phase was observed at 160 °C, while the largest was at 140 °C, with values of 9.66, 22.08 hours, respectively. These results suggest that there is no clear correlation between the lag phase and hydrogen production in HF using food waste by thermal hydrolysis temperature.

The hydrolysis rate constant (k) represents the velocities of degradation and biogas production (Li et al., 2016). The k value showed no significant change by thermal hydrolysis temperature (p > 0.05). This indicates THP does not have an effect on hydrolysis rate when using food waste as a substrate.

Modified Gompertz model exhibited a higher range of  $R^2$  values (0.90 – 0.99) compared to the 1<sup>st</sup> order kinetic model (0.80 – 0.92), indicating that modified Gompertz model provided a better fit to the experimental data than 1<sup>st</sup> order kinetic model.

44



Fig 4.3 Hydrogen production fitted with modified Gompertz model



Fig 4.4 Hydrogen production fitted with 1<sup>st</sup> order kinetic model

THP		Modified Gompertz equation			1 <sup>st</sup> Order kinetic equation		
temperature (℃)	$(mL-H_2/g-VS)$	$R_m$ (mL-H <sub>2</sub> /g-VS/h)	λ (h)	$R^2$	k (h <sup>-1</sup> )	λ (h)	$R^2$
Untreated	$36.67 \pm 18.19$	$1.66 \pm 0.15$	$\begin{array}{r} 19.82 \ \pm \\ 0.93 \end{array}$	0.99	0.033 ± 0.008	$\begin{array}{r} 5.43 \pm \\ 3.50 \end{array}$	0.82
120	$23.83 \pm 14.37$	$0.64 \pm 0.17$	$\begin{array}{r}10.81 \ \pm \\5.49\end{array}$	0.90	0.030 ± 0.006	$4.33 \pm 3.25$	0.85
140	96.73 ± 24.94	$8.08 \pm 0.22$	$\begin{array}{r} 22.08 \ \pm \\ 0.15 \end{array}$	0.99	0.036 ± 0.009	$\begin{array}{r} 5.03 \ \pm \\ 3.59 \end{array}$	0.80
160	$47.59 \pm 5.69$	$1.32~\pm~0.13$	9.66 ± 1.99	0.99	$\begin{array}{c} 0.035 \ \pm \\ 0.005 \end{array}$	$\begin{array}{rrr} 3.56 & \pm \\ & 2.12 \end{array}$	0.92
180	$11.81 \pm 4.02$	$0.89~\pm~0.17$	$\begin{array}{r}19.71 \ \pm \\ 1.32\end{array}$	0.99	$\begin{array}{c} 0.037 \hspace{2pt} \pm \\ 0.009 \end{array}$	$\begin{array}{r} 4.48 \ \pm \\ 3.19 \end{array}$	0.83

Table 4.3 Parameters for kinetic study in BHP test

#### 4.2.3 VFAs production of BHP test

During HF, various volatile fatty acids (VFAs) are produced, with acetate and butyrate being the metabolic products of the hydrogenproducing pathways, and propionate being the metabolic product of the hydrogen-consuming pathway of glucose. The butyrate to acetate ratio (Bu/Ac) and concentration of propionate are effective indicator of hydrogen production. Higher Bu/Ac and lower propionate concentration indicate higher efficiency of hydrogen production (Tang et al., 2008; Kim et al., 2013b; and Cheng et al., 2016). With increasing THP temperature, Bu/Ac increased and showed maximum value of 1.00 at 140 °C, and then decreased at higher temperature. Propionate concentration was also lowest in 140 °C with a value of 10.88 mg/L. Food waste treated at 140 °C resulted in the highest Bu/Ac and lowest propionate concentration, leading to highest hydrogen production.

THP	Ţ				
temperature (°C)	Ac	Bu	Pro	TVFAs	Bu/Ac
Untreated	59.81	36.96	13.07	178.25	0.62
120	61.46	45.39	11.08	168.89	0.74
140	55.66	55.59	10.88	164.91	1.00
160	67.95	61.23	18.50	230.27	0.90
180	41.92	37.31	15.21	158.02	0.89

Table 4.4 VFAs production of BHP test (Ac: acetate, Bu: butyrate, Pro: propionate; and TVFAs: total VFAs)

# 4.3 Structure of lignocellulose

### 4.3.1 Crystallinity of cellulose

Cellulose have both amorphous and crystalline areas while, lignin and hemicellulose only have amorphous area (Ahmed et al., 2022). Crystallinity of cellulose was examined by XRD. Fig 4.5 displays the XRD spectra of cellulose isolated from food waste. The peaks exhibit the same pattern as those of commercial cellulose, indicating successful isolation of cellulose from food waste. THP treatment resulted in a decrease in the crystallinity of the isolated cellulose by about 13.8 % at 140 °C (33.7 %) compared to untreated (47.5 %). A reduction in crystallinity implies an increase in surface area and improved accessibility of cellulose to microorganisms (Yang et al., 2009; Galiwango et al., 2019; and Podgorbunskikh et al., 2019), leading to enhanced hydrogen production.



Fig 4.5 XRD result of cellulose from food waste

### 4.3.2 Morphological change of lignocellulose

## 4.3.2.1 SEM analysis

The SEM images of lignocellulose isolated from food waste after thermal hydrolysis at a magnification of x5,000 are presented in Fig 4.6. The isolation of lignocellulose was carried out using a benzene/ethanol (2:1 by vol.) solution for 6 hours. The images indicate that the rough surface of lignocellulose became smooth due to THP treatment, which is consistent with the findings of Arelli et al. (2020) who treated food waste at 120 °C for 80 min (Arelli et al., 2020). The morphological changes observed after the pretreatment can be attributed to the application of high temperature and pressure, which leads to the breakage of lignocellulose by enhancing the contact between substrates and microorganisms, leading to an increase in hydrogen production (Veluchamy and Kalamdhad, 2017; Gandhi et al., 2018; and Arelli et al., 2020).



Fig 4.6 SEM images of lignocellulose in food waste ((a) untreated, (b) 120  $^{\circ}$ C, (c) 140  $^{\circ}$ C, (d) 160  $^{\circ}$ C, and (d) 180  $^{\circ}$ C)

### 4.3.2.2 BET analysis

The BET surface area and pore volume of food waste treated at different THP temperatures are presented in Table 4.5. The results indicate that the surface area and pore volume of THP treated food waste increased compared to untreated food waste. This increase in surface area and pore volume can be attributed to the formation of micropores and mesopores by thermal hydrolysis (Wang et al., 2022). The enhanced surface area is known to positively influence the biodegradability of the material, leading to an increase in hydrogen production (Chinaglia et al., 2018). Specifically, in the case of food waste treated at 120 and 140 °C, the surface area increased by 1.7 and 3.6-fold, respectively indicating a corresponding increase in hydrogen production due to the expanded surface area. However, the relationship between surface area and THP temperature did not exhibit a consistent trend, as observed in 140, 160, and 180 °C.

THP temperature	Surface area	Pore volume	Pore diameter
(°C)	$(\times 10^{-2} \mathrm{m^2/g})$	$(\times 10^{-4}  \text{cm}^3/\text{g})$	(nm)
Untreated	4.65	3.58	30.80
120	7.82	8.49	43.45
140	17.13	8.44	19.71
160	10.94	12.10	44.23
180	15.71	7.46	18.98

Table 4.5 BET surface area and volume of food waste by THP

### 4.3.3 Mechanism of Maillard reaction and carbonization

Fig 4.7 shows the FT-IR spectra of food waste by THP temperature. The broad peak at  $3,200 - 3,400 \text{ cm}^{-1}$  corresponds to the O-H bond in hydroxyl and carboxyl groups (Ryu et al., 2022). The intensity of this peak decreased in food waste treated at 160 and 180 °C, indicating dehydration occurred (Elaigwu and Greenway, 2016). A peak at 1,709 cm<sup>-1</sup> attributed to the C=O bond in ketones, aldehyde, ester, and carboxyl groups appeared at temperatures above 160  $\,^{\circ}$ C and grew in strength at 180  $\,^{\circ}$ C. The increase in peak of C=O bond at THP process can be attributed to the formation of melanoidins (Mohsin et al., 2018). Peaks at 572 and 1,625 cm<sup>-1</sup> correspond to C-H and C=C bonds in the aromatic structure of lignin, while the peak at 860 cm<sup>-1</sup> represents the C-H bond in cellulose (Yang et al., 2009; Ryu et al., 2022). The decrease of these peaks in food waste treated at 180  $^\circ$ C indicates the decomposition of lignin and cellulose (Song et al., 2019; Kubovský et al., 2020). Additionally, the disappearance of the peak at 1,077cm<sup>-1</sup>, corresponding to the C–O bond from esters, phenols, and aliphatic alcohols shows that decarboxylation occurred (Elaigwu and Green way, 2016). Considering that dehydration occurs in Maillard reaction and that both dehydration and decarboxylation are typical reactions observed during carbonization, it is reasonable to anticipate the occurrence of Maillard reaction and the formation of melanoidins at 160 and 180 °C, while carbonization occurred at 180 ℃

54



Fig 4.7 FT-IR results of lignocellulose in food waste ((a) 4,000-2,000 cm<sup>-1</sup>, (b) 2,000-400 cm<sup>-1</sup>)

### 4.3.4 Composition of functional groups in food waste

The surface composition of the food waste at different THP temperatures was analyzed using XPS. Fig 4.8 presents the accurately peak-fitted C 1s spectra obtained from the analysis. The XPS survey revealed the presence of three C-containing species in the form of distinct peaks: C-C/C-H (aliphatic structure) with a binding energy of 285.0 eV; C-O (ether or alcohol groups) with 286.4 eV; and C=O (ketones and aldehydes) with 288.6 eV, as reported in previous studies (Parshetti et al., 2014; Tang et al., 2023). The positions of these peaks remained unchanged by THP, indicating that the functional groups were unaffected by pretreatment. However, the composition of these species showed notable changes. The composition of C-C/C-H increased, which can be attributed to the depolymerization of lignocellulose into aliphatic structures. Conversely, the composition of C-O decreased, indicating the dissociation of glycosidic bonds and hydroxyl structures by degradation of cellulose and hemicellulose (Zhan et al., 2022; Zeng et al., 2023). The composition of C=O, known for its high binding energy, decreased at 120 °C but exhibited an increasing trend at high temperatures. The increase of C=O composition is attributed to the formation of melanoidins by Maillard reaction (Lin et al., 2020). This corresponds with the findings from FT-IR, where a peak at 160  $^{\circ}$ C was observed and grew in strength at 180 °C. This result suggests the formation of melanoidins at high temperatures.

56



Fig 4.8 C 1s XPS spectra of lignocellulose in food waste ((a) untreated, (b) 120  $^{\circ}$ C, (c) 140  $^{\circ}$ C, (d) 160  $^{\circ}$ C, and (d) 180  $^{\circ}$ C)

	Composition of functional groups (%)			
(°)	С-С/С-Н	С-О	C=0	
(0)	(285.0 eV)	(286.4 eV)	(288.6 eV)	
Untreated	13.36	79.44	7.20	
120	69.52	26.36	4.12	
140	74.22	23.62	5.16	
160	46.35	47.89	5.76	
180	64.41	28.38	7.22	

Table 4.6 Characterization of surface composition of food waste

# 4.4 Formation of humic-acid like substances

In order to investigate the formation of humic-acid like substance, which is recalcitrant substance including melanoidins, an EEM was conducted. Fig 4.9 illustrates the EEM of food waste treated at different THP temperatures. In the case of food waste treated at 120 °C, soluble microbial products (soluble carbohydrates and proteins) were observed. These Soluble microbial products are considered as biodegradable compounds, which disappeared under high temperature and pressure (Liu et al., 2018a; Xu et al., 2019). On the other hand, the formation and accumulation of humic-acid like substances were particularly observed at 160 and 180 °C. This observation is consistent with the results obtained from the FT-IR and XPS analyses. Given that melanoidins are known to be recalcitrant substances that can hinder hydrogen production (Dwyer et al., 2018; Diez-Simon et al., 2019), it is expected that the formation of melanoidins at 160 and 180 °C contributed to a decrease in hydrogen production.


Fig 4.9 The EEM of food waste ((a) untreated, (b) 120  $^{\circ}$ C, (c) 140  $^{\circ}$ C, (d) 160  $^{\circ}$ C, and (e) 180  $^{\circ}$ C)

## 4.5 Energy analysis of BHP test

The net energy gain of hydrogen production was calculated based on the results of the BHP test. Among various THP temperatures, untreated, 140, and 160 °C showed positive net energy gain. The net energy gain of food waste treated at 140 °C (146 MJ/ton) was 2.4-fold of untreated (60 MJ/ton). Therefore, treating food waste at a THP temperature of 140 °C is the most energy-efficient for HF. However, it should be noted that the BHP test was conducted under optimal conditions for microorganisms, and the conditions will be different with full-scale plant. Therefore, energy analysis conducted with CSTR is needed. Based on the result of energy analysis, in CSTR test, food waste pretreated at 140 °C and untreated food waste was used as substrates.



Fig 4.10 Energy balance of BHP test

## 4.6 CSTR test of HF

#### 4.6.1 Reactor performance

Two CSTRs were operated at a temperature of  $35 \pm 1$  °C in this study. Initially, both reactors were fed with untreated food waste for acclimatization, and once acclimatized, THP treated food waste was introduced into the THP HF. Following substrate adaptation, the CSTR test was conducted for 4 HRTs, which is about 20 days. Fig 4.11 and 4.12 depict the hydrogen production and VS removal rate of Control HF and THP HF. The hydrogen production of THP HF exhibited a significant increase of approximately 1.6fold compared to Control HF. This result corresponds with the findings obtained from the BHP test, confirming the efficacy of thermal hydrolysis pretreatment in enhancing hydrogen production of food waste by decomposing non-degradable organic matters (Appels et al., 2008; Ariunbaatar et al., 2014). Moreover, VS removal ratio of the THP HF increased by 4.9 % (by wet wt.), indicating that the decomposition of organic matters through THP improved the biodegradability of food waste, leading to an enhancement in hydrogen production. However, the hydrogen production obtained in this study were not as high as those reported in studies for food waste (Table 4.7), while VS removal ratio was higher. Further research is required to investigate the reasons behind the observed discrepancy between hydrogen production and VS removal rate.



Fig 4.11 Hydrogen production of CSTR reactors



Fig 4.12 VS removal rate of CSTR reactors

HRT	- 11	FW	Fermenter	H <sub>2</sub> yield	VS removal	Reference	
	рн	pretreatment	temperature (°C)	(mL/g-VS)	(% by wet wt.)		
5 d	$5.5 \pm 0.1$	_	$25 \pm 1$	$12.9~\pm~1.9$	$52.5~\pm~2.1$	This study	
		THP	$50\pm1$	$21.1 \pm 2.7$	$57.4~\pm~2.0$		
24 h	_	Base	$35\pm1$	62.6 ± 4.4 -		Kim and Shin, 2008	
2 d	$5.5 \pm 0.5$	_	27	180	16	Elbeshbishy et al.,	
		Ultrasonication	57	247	16	2011	
5 d	5.5	_	$37 \pm 2$	$60.7~\pm~6.2$	$47.0~\pm~5.1$	Siddiqui et al., 2011	
2 d	5.5	_	$34\pm1$	$20.2~\pm~0.2$	$53.2~\pm~6.5$	Redondas et al., 2012	
3 d	5.5	_	$37 \pm 0.1$	$12.6 \pm 5.0^{1)}$	$23.5~\pm~4.0$	Baldi et al., 2019	
5 d	$5.5 \pm 0.1$	_	$55\pm1$	$125 \pm 0.8$	$13.9^{2}$	Shin and Youn, 2005	
5 d	$5.0 \pm 0.2$	_	55	$70.7 \pm 55$	15.5	Algapani et al., 2016	

Table 4.7 Hydrogen production of CSTR test from food waste

1) mL-H<sub>2</sub>/g-VS/d; 2) COD removal rate

#### 4.6.2 VFAs production of CSTR test

During HF, a variety of VFAs are produced. Acetate and butyrate formation have been found to positively correlate with hydrogen production, while propionate and lactate formation has shown to hinder hydrogen production (Sivagurunathan et al., 2016; Ding et al., 2017). In CSTR test, acetate, butyrate, caproate, and valerate were detected as products, while propionate was not formed and only small amounts of lactate were produced. These findings indicate the absence of propionate and lactate inhibition on hydrogen production. The total concentration of VFAs in Control HF was approximately 1.3-fold of THP HF, and the concentrations of acetate and butyrate were also higher in Control HF compared to the THP HF by 2.0fold and 1.1-fold, respectively. As mentioned in section 4.2.3, the butyrateto-acetate ratio (Bu/Ac) has been shown to have better correlation with hydrogen production. In the CSTR test, Bu/Ac in THP HF was higher than that in Control HF, with values of 1.3 and 0.5, respectively. The enhance hydrogen production observed in THP HF, compared to the Control HF, can be attributed to the higher Bu/Ac.



Fig 4.13 VFAs production of Control HF and THP HF



Fig 4.14 Butyrate-to-acetate ratio (Bu/Ac) of Control HF and THP HF

### 4.6.3 Microbial analysis

A microbial analysis was conducted to investigate the community abundance of the seed sludge and CSTR reactors. The dynamics of bacteria phyla are depicted in Fig 4.15(a). In the seed sludge, *Firmicutes* and *Bacteroidetes* were the dominant phyla, accounting for relative abundances of 30.73 and 40.48 %, respectively. The findings align with previous studies on HF, where *Firmicutes* and *Bacteroidetes* were also identified as dominant phyla, ranging from 6 to 40% and 11 to 50%, respectively (Im et al., 2012; Yang et al., 2019; and Yang and Wang, 2019). *Firmicutes* and *Bacteroidetes* play essential roles in the decomposition of organic matter, including the production of VFAs and ammonia through protein decomposition (Liu et al., 2016). Interestingly, CSTR test exhibited a decrease in *Bacteroidetes* abundance, while Actinobacteria increased from 2.15 % in seed sludge to 43.26 and 64.09 % in Control HF and THP HF, respectively, becoming the dominant phylum. Actinobacteria are known for their efficient breakdown of complex organic matters and their contribute to lignin degradation through the production of lignin-degrading enzymes (Jaenicke et al., 2011; Wirth et al., 2012). Additionally, *Firmicutes* and *Actinobacteria* were consistently identified as the predominant phyla across all reactors, indicating the potential for organic matter hydrolysis (Liu et al., 2018b).

The stability of CSTR reactors can be understood by examining the dynamic profiles of bacteria at the genus level (Fig 4.15(b)). In the THP HF, *Olsenella* and *Bifidobacteirum* were the dominant genera, while

*Caproiciproducens* and *Bifido bacterium* prevailed in the Control HF. The abundance of *Olsenella*, belonging to the *Actinobacteria* phylum, significantly increased from 5.36 % in Control HF to 41.50 % in THP HF by the application of THP. The higher proportion of *Olsenella* content corresponds to relatively high carbohydrate removal, resulting in an elevated hydrogen production (Li et al., 2011). Bifidobacterium accounted for 37.33 % in Control HF, where as it comprised 22.28 % in THP HF. Bifidobacterium is capable of fermenting lactic acid by degrading starch into small molecules (Duangmanee et al., 2007; Cheng et al., 2008). In an environment with high lactic acid concentrations, the hydrogen fermentation process can shift to lactic acid fermentation, resulting in a reduction of hydrogen production (Bundhoo 2016; Canto-Robertos and Mohee, et al.. 2023). *Caproiciproducens*, a microbe that ferments caproic acid, was more abundant in Control HF, comprising 28.49 % compared to 5.21 % in THP HF. The fermentation of caproic acid consumes 4 mol-H<sub>2</sub>/mol-glucose, which can result in a lower hydrogen production (Xia et al., 2016). Consequently, THP HF demonstrated higher hydrogen production due to the higher relative abundance of Olsenella, and lower relative abundance of Bifidobacterium and Caproiciproducens.



Fig 4.15 The relative abundance of major bacteria (a) phylum and (b) genus of seed sludge, Control HF, and THP HF

Popotor	Total	Good's coverage	OTUs	Chaol	Channon	Simpson
	reads	of library (%)		Clia01	Shannon	Shipson
Seed sludge	50,263	99.66	1,138	1,188.54	4.81	0.020
Control HF	33,471	99.87	204	217.89	2.28	0.188
THP HF	36,102	99.90	120	140.52	1.93	0.230

Table 4.8 Alpha diversity indices of bacteria community of seed sludge and CSTR reactors

#### 4.6.4 Energy analysis of CSTR test

The net energy gain  $(\mathbf{E}_{net})$ , energy generation  $(\mathbf{E}_{out})$  by hydrogen, and energy consumption  $(\mathbf{E}_{in})$  in CSTR test are summarized in Table 4.9.  $\mathbf{E}_{in}$ increased from 35.07 to 87.68 MJ/ton, while  $E_{out}$  increased from 33.45 to 54.53 MJ/ton in THP HF, corresponding to the increased hydrogen production achieved through THP application. However,  $E_{net}$  of THP HF was lower compared to Control HF. The contrast in results between CSTR and BHP tests can be attributed to the decrease in  $E_{out}$  observed in the CSTR test. Despite having the same  $E_{in}$ , the reduced hydrogen production in CSTR test, compared to the BHP test, resulted in a decrease in  $E_{out}$ . The decreased  $E_{out}$  in the CSTR test, coupled with the significantly higher  $E_{in}$ due to the application of THP in the THP HF, contributed to the observed differences between the two tests. Therefore, considering the higher  $E_{net}$ of Control HF compared to THP HF, Control HF demonstrated greater energy efficiency from an energy balance perspective in the CSTR test. Further research is necessary to enhance net energy gain by reducing the energy input required for THP of increasing the hydrogen production.

Ponotor	E <sub>in</sub>	E <sub>out</sub>	$E_{net}$	
Reactor	(MJ/ton)	(MJ/ton)	(MJ/ton)	
Control HF	35.07	$33.45 \pm 4.91$	$-1.62 \pm 4.91$	
THP HF	87.68	$54.53~\pm~7.04$	$-33.14 \pm 7.04$	

Table 4.9 Energy analysis of CSTR test

### 4.6.5 Sensitivity analysis

The results of the sensitivity analysis, depicting the influence of parameter variations on energy analysis, are illustrated in Fig 4.16. The heat recovery rate, initially assumed to be 85 %, exhibited a significant impact on net energy gain. As the heat recovery rate increased 68 to 100 % (representing -20 to 17 % variation of initial value), the net energy gain exhibited a substantial change, ranging from -92.76 to 17.54 MJ/ton. Similarly, the TS of food waste also had a considerable impact on net energy gain following the heat recover rate. A change in the TS of food waste from -20 to 20 % of the initial value led to variations in net energy gain from -48.57 to -17.93 MJ/ton.

Consequently, enhancing the heat recovery rate emerges as an effective approach to improve net energy gain, with the potential to achieve a positive net energy gain when the heat recovery rate reaches 95 %. Additionally, the TS of food waste also emerged as an important factor impacting net energy gain.



Fig 4.16 Sensitivity analysis of CSTR test

# 5. Conclusions

This study investigates the effects of thermal hydrolysis temperature of food waste on hydrogen production and net energy gain. Biochemical hydrogen potential (BMP) test was conducted to evaluate hydrogen production. Among the temperatures, food waste treated at 140  $\,^{\circ}$  showed the highest hydrogen production, representing a 2.6-fold increase compared to untreated food waste. The increase was attributed to the reduced crystallinity of cellulose from 47.5 % of untreated food waste to 33.7 % of 140 °C treated food waste, and the destruction of lignocellulose surface. These resulted in the surface area increase through the depolymerization of lignocellulose, which can enhance the availability for microorganisms. However, at THP temperature of 160 and 180 °C, the formation of C=O bonds indicated the occurrence of Maillard reaction and the decrease of C-O, C-H, and C=C bonds suggested carbonization. These reactions generated recalcitrant substances containing C=O bonds, which exhibit high binding energy, leading to decrease in hydrogen production. Continuous stirred-tank reactor (CSTR) test was conducted using untreated food waste (Control HF) and food waste treated at 140  $\,^{\circ}$ C (THP HF) as substrates. THP HF exhibited higher hydrogen production, representing a 1.6-fold increase compared to Control HF. Moreover, THP HF demonstrated a 1.1-fold increase in volatile solids (VS) removal rate (57.40% by wet wt.), indicating enhanced organic matter degradation. VFAs analysis revealed a higher butyrate to acetate ratio (Bu/Ac) in THP HF indicating increased hydrogen production efficiency compared to the Control HF. Microbial analysis revealed that *Olsenella*, a carbohydrate-degrading microorganism, was the dominant genus in THP HF (41.50 % of total genera), while *Bifidobacterium*, which can shift hydrogen fermentation to lactate fermentation, dominated in Control HF (37.33 % of total genera). High relative abundance of *Olsenella* and low relative abundance of *Bifidobacterium* have been associated with a significant increase in hydrogen production. Despite the higher hydrogen production observed in THP HF, the net energy gain was lower compared to Control HF due to higher energy input associated with THP. This result indicated that, in terms of energy balance, THP HF is less efficient than Control HF, and the application of THP did not have an impact on net energy gain. However, an increased heat recovery rate of THP process to 95 % could lead to enhanced energy efficiency of THP HF.

## References

- Achinas, S., Achinas, V., & Euverink, G. J. W. (2017). A Technological Overview of Biogas Production from Biowaste. *Engineering*, 3(3), 299–307. https://doi.org/10.1016/J.ENG.2017.03.002
- Ahmed, E., Zeitoun, A., Hamad, G., Zeitoun, M. A. M., Taha, A., Korma, S. A., & Esatbeyoglu, T. (2022). Lignocellulosic Biomasses from Agricultural Wastes Improved the Quality and Physicochemical Properties of Frying Oils. *Foods*, 11(19). https://doi.org/10.3390/foods11193149
- Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guwy, A. J., Kalyuzhnyi, S., Jenicek, P., & Van Lier, J. B. (2009). Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays. *Water Science and Technology*, 59(5), 927–934. https://doi.org/10.2166/wst.2009.040
- Appels, L., Baeyens, J., DegrIve, J., & Dewil, R. (2008). Principles and potential of the anaerobic digestion of waste-activated sludge. In *Progress in Energy and Combustion Science* (Vol. 34, Issue 6, pp. 755–781). https://doi.org/10.1016/j.pecs.2008.06.002
- Arelli, V., Juntupally, S., Begum, S., & Anupoju, G. R. (2020). Significance of pretreatment in enhancing the performance of dry anaerobic digestion of food waste: An insight on full scale implementation strategy with theoretical analogy. *Processes*, 8(9). https://doi.org/10.3390/PR8091018
- Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., & Lens, P. N. L. (2014). Pretreatment methods to enhance anaerobic digestion of organic solid waste. In *Applied Energy*

(Vol. 123, pp. 143–156). Elsevier Ltd. https://doi.org/10.1016/j.apenergy.2014.02.035

- Ashah, M. A., Lutpi, N. A., Wong, Y. S., Ong, S. A., & Malek, M. A. (2020). Study on biohydrogen production using different type of carrier materials in attached growth system. *IOP Conference Series: Earth and Environmental Science*, 476(1). https://doi.org/10.1088/1755-1315/476/1/012105
- Barber, W. P. F. (2016). Thermal hydrolysis for sewage treatment: A critical review. In Water Research (Vol. 104, pp. 53–71). Elsevier Ltd. https://doi.org/10.1016/j.watres.2016.07.069

Barber, 2020 (Sludge Thermal Hydrolysis\_ Application and Potential).

- Bhatt, S. M., & Shilpa. (2014). Lignocellulosic feedstock conversion, inhibitor detoxification and cellulosic hydrolysis – A review. *Biofuels*, 5(6), 633–649. https://doi.org/10.1080/17597269.2014.1003702
- Bundhoo, M. A. Z., & Mohee, R. (2016). Inhibition of dark fermentative bio-hydrogen production: A review. *International Journal of Hydrogen Energy*, 41(16), 6713– 6733. https://doi.org/10.1016/j.ijhydene.2016.03.057
- Canto-Robertos, M., Quintal-Franco, C., Ponce-Caballero, C., Vega-De Lille, M., & Moreno-Andrade, I. (2023). Inhibition of hydrogen production by endogenous microorganisms from food waste. *Brazilian Journal of Chemical Engineering*, 40(1), 137–150. https://doi.org/10.1007/s43153-022-00235-5
- Carrère, H., Antonopoulou, G., Affes, R., Passos, F., Battimelli, A., Lyberatos, G., & Ferrer,
  I. (2016). Review of feedstock pretreatment strategies for improved anaerobic digestion: From lab-scale research to full-scale application. In *Bioresource Technology* (Vol. 199, pp. 386–397). Elsevier Ltd.

https://doi.org/10.1016/j.biortech.2015.09.007

- Carrère, H., Dumas, C., Battimelli, A., Batstone, D. J., Delgenlls, J. P., Steyer, J. P., & Ferrer, I. (2010). Pretreatment methods to improve sludge anaerobic degradability: A review. In *Journal of Hazardous Materials* (Vol. 183, Issues 1–3, pp. 1–15). https://doi.org/10.1016/j.jhazmat.2010.06.129
- Cheng, H. H., Whang, L. M., Chung, M. C., & Chan, K. C. (2016). Biological hydrogen and methane production from bagasse bioethanol fermentation residues using a two– stage bioprocess. *Bioresource Technology*, *210*, 49–55. https://doi.org/10.1016/j.biortech.2015.12.084
- Cheng, C. H., Hung, C. H., Lee, K. S., Liau, P. Y., Liang, C. M., Yang, L. H., Lin, P. J., & Lin, C. Y. (2008). Microbial community structure of a starch–feeding fermentative hydrogen production reactor operated under different incubation conditions. *International Journal of Hydrogen Energy*, 33(19), 5242–5249. https://doi.org/10.1016/j.ijhydene.2008.05.017
- Chinaglia, S., Tosin, M., & Degli–Innocenti, F. (2018). Biodegradation rate of biodegradable plastics at molecular level. *Polymer Degradation and Stability*, 147, 237–244. https://doi.org/10.1016/j.polymdegradstab.2017.12.011
- C. Saha, B. (2009). Lignocellulose Biodegradation and Applications in Biotechnology. In ACS Symposium Series. https://doi.org/10.1021/bk-2004-0889.ch001
- Diez-Simon, C., Mumm, R., & Hall, R. D. (2019). Mass spectrometry-based metabolomics of volatiles as a new tool for understanding aroma and flavour chemistry in processed food products. In Metabolomics (Vol. 15, Issue 3). Springer New York LLC. https://doi.org/10.1007/s11306-019-1493-6

Ding, L., Cheng, J., Qiao, D., Yue, L., Li, Y. Y., Zhou, J., & Cen, K. (2017). Investigating

hydrothermal pretreatment of food waste for two-stage fermentative hydrogen and methane co-production. *Bioresource Technology*, *241*, 491–499. https://doi.org/10.1016/j.biortech.2017.05.114

- Duangmanee, T., Padmasiri, S. I., Simmons, J. J., Raskin, L., & Sung, S. (2007). Hydrogen Production by Anaerobic Microbial Communities Exposed to Repeated Heat Treatments. *Water Environment Research*, 79(9), 975–983. https://doi.org/10.2175/106143007x175762
- Dwyer, J., Starrenburg, D., Tait, S., Barr, K., Batstone, D. J., & Lant, P. (2008). Decreasing activated sludge thermal hydrolysis temperature reduces product colour, without decreasing degradability. Water Research, 42(18), 4699–4709. https://doi.org/10.1016/j.watres.2008.08.019
- Echavarrla, A. P., Paglin, J., & Ibarz, A. (2012). Melanoidins Formed by Maillard Reaction in Food and Their Biological Activity. In *Food Engineering Reviews* (Vol. 4, Issue 4, pp. 203–223). Springer Science and Business Media, LLC. https://doi.org/10.1007/s12393–012–9057–9
- Elaigwu, S. E., & Greenway, G. M. (2016). *Microwave–assisted and conventional hydrothermal carbonization of lignocellulosic waste material: Comparison of the chemical and structural properties of the hydrochars.*
- Elbeshbishy, E., Hafez, H., & Nakhla, G. (2010). Enhancement of biohydrogen producing using ultrasonication. *Renewable Energy*, *35*(12), 6184–6193. https://doi.org/10.1016/j.ijhydene.2010.03.119
- Ferrer, I., PonsD, S., VDzquez, F., & Font, X. (2008). Increasing biogas production by thermal (70° C) sludge pre-treatment prior to thermophilic anaerobic digestion. Biochemical Engineering Journal, 42(2), 186–192.

https://doi.org/10.1016/j.bej.2008.06.020

- Galiwango, E., Abdel Rahman, N. S., Al–Marzouqi, A. H., Abu–Omar, M. M., & Khaleel, A. A. (2019). Isolation and characterization of cellulose and *a* –cellulose from date palm biomass waste. *Heliyon*, 5(12). https://doi.org/10.1016/j.heliyon.2019.e02937
- Gandhi, P., Paritosh, K., Pareek, N., Mathur, S., Lizasoain, J., Gronauer, A., Bauer, A., & Vivekanand, V. (2018). Multicriteria decision model and thermal pretreatment of hotel food waste for robust output to biogas: Case study from city of Jaipur, India. *BioMed Research International, 2018.* https://doi.org/10.1155/2018/9416249
- Ghimire, A. (2015). Dark fermentative biohydrogen production from organic waste and application of by-products in a biorefinery concept. https://tel.archives-ouvertes.fr/tel-01404689
- Goletz, C., Wagner, M., Grlbel, A., Schmidt, W., Korf, N., & Werner, P. (2011). Standardization of fluorescence excitation-emission-matrices in aquatic milieu. *Talanta*, 85(1), 650–656. https://doi.org/10.1016/j.talanta.2011.04.045
- Herbert H.P. Fang, Hong Liu, Effect of pH on hydrogen production from glucose by a mixed culture, Bioresource Technology, Volume 82, Issue 1, 2002, Pages 87–93, ISSN 0960-8524, https://doi.org/10.1016/S0960-8524(01)00110-9
- He, L., Huang, H., Zhang, Z., Lei, Z., & Lin, B. Le. (2017). Energy Recovery from Rice Straw through Hydrothermal Pretreatment and Subsequent Biomethane Production. *Energy and Fuels*, *31*(10), 10850–10857. https://doi.org/10.1021/acs.energyfuels.7b01392
- Hu, B., & Chen, S. (2007). Pretreatment of methanogenic granules for immobilized hydrogen fermentation. *International Journal of Hydrogen Energy*, 32(15 SPEC. ISS.), 3266–3273. https://doi.org/10.1016/j.ijhydene.2007.03.005

- Hussy, I., Hawkes, F. R., Dinsdale, R., & Hawkes, D. L. (2003). Continuous Fermentative
  Hydrogen Production from a Wheat Starch Co-Product by Mixed Microflora. *Biotechnology and Bioengineering*, 84(6), 619–626.
  https://doi.org/10.1002/bit.10785
- Im, W. T., Kim, D. H., Kim, K. H., & Kim, M. S. (2012). Bacterial community analyses by pyrosequencing in dark fermentative H 2-producing reactor using organic wastes as a feedstock. *International Journal of Hydrogen Energy*, 37(10), 8330–8337. https://doi.org/10.1016/j.ijhydene.2012.02.167
- Jaenicke, S., Ander, C., Bekel, T., Bisdorf, R., Drlge, M., Gartemann, K. H., Jllnemann, S., Kaiser, O., Krause, L., Tille, F., Zakrzewski, M., Plhler, A., Schllter, A., & Goesmann, A. (2011). Comparative and joint analysis of two metagenomic datasets from a biogas fermenter obtained by 454–pyrosequencing. *PLoS ONE*, 6(1). https://doi.org/10.1371/journal.pone.0014519
- Khan, M. U., Usman, M., Ashraf, M. A., Dutta, N., Luo, G., & Zhang, S. (2022). A review of recent advancements in pretreatment techniques of lignocellulosic materials for biogas production: Opportunities and Limitations. In *Chemical Engineering Journal Advances* (Vol. 10). Elsevier B.V. https://doi.org/10.1016/j.ceja.2022.100263
- Kim, M. S., Cha, J., & Kim, D. H. (2013a). Fermentative Biohydrogen Production from Solid Wastes. Biohydrogen, 259–283. https://doi.org/10.1016/B978–0–444– 59555–3.00011–8
- Kim, S., Choi, K., Kim, J. O., & Chung, J. (2013b). Biological hydrogen production by anaerobic digestion of food waste and sewage sludge treated using various pretreatment technologies. *Biodegradation*, 24(6), 753–764. https://doi.org/10.1007/s10532-013-9623-8

- Kim, S., Lee, C., & Young Kim, J. (2023). Effects of alkaline thermal hydrolysis on the formation of refractory compounds and energy balance of anaerobic digestion of cattle manure. *Applied Energy*, 342, 121097. https://doi.org/10.1016/j.apenergy.2023.121097
- Krishna, D., & Kalamdhad, A. S. (2014). Pre-treatment and anaerobic digestion of food waste for high rate methane production – A review. In *Journal of Environmental Chemical Engineering* (Vol. 2, Issue 3, pp. 1821–1830). https://doi.org/10.1016/j.jece.2014.07.024
- Kroh, L. W., Fiedler, T., & Wagner, J. (2008). α Dicarbonyl compounds Key intermediates for the formation of carbohydrate–based melanoidins. *Annals of the New York Academy of Sciences, 1126*, 210–215. https://doi.org/10.1196/annals.1433.058
- Kubovský, I., Kačíková, D., & Kačík, F. (2020). Structural changes of oak wood main components caused by thermal modification. *Polymers*, 12(2). https://doi.org/10.3390/polym12020485
- L. Segal, J.J. Creely and A.E. Martin et al. An Empirical Method for Estimating the Degree of Crystallinity of Native Cellulose Using the X-Ray Diffractometer. *Textile Research Journal*. 1959. Vol. 29(10):786-794. DOI: 10.1177/004051755902901003
- Lee, Z. K., Li, S. L., Lin, J. S., Wang, Y. H., Kuo, P. C., & Cheng, S. S. (2008). Effect of pH in fermentation of vegetable kitchen wastes on hydrogen production under a thermophilic condition. *International Journal of Hydrogen Energy*, 33(19), 5234– 5241. https://doi.org/10.1016/j.ijhydene.2008.05.006

Lewis, M. J. (2009). Physical properties of foods and food processing systems.

- Liu, C., Li, H., Zhang, Y., Si, D., & Chen, Q. (2016). Evolution of microbial community along with increasing solid concentration during high—solids anaerobic digestion of sewage sludge. *Bioresource Technology, 216*, 87–94. https://doi.org/10.1016/j.biortech.2016.05.048
- Liu, J., Yin, J., He, X., Chen, T., & Shen, D. (2018a). Three–Dimensional Excitation and Emission Fluorescence–Based Method for Evaluation of Maillard Reaction Products in Food Waste Treatment. *Journal of Chemistry, 2018.* https://doi.org/10.1155/2018/6758794
- Liu, N., Jiang, J., Yan, F., Gao, Y., Meng, Y., Aihemaiti, A., & Ju, T. (2018b). Enhancement of volatile fatty acid production and biogas yield from food waste following sonication pretreatment. *Journal of Environmental Management, 217*, 797–804. https://doi.org/10.1016/j.jenvman.2018.03.135
- Liu, X., Lee, C., & Kim, J. Y. (2020). Thermal hydrolysis pre-treatment combined with anaerobic digestion for energy recovery from organic wastes. *Journal of Material Cycles and Waste Management, 22*(5), 1370–1381. https://doi.org/10.1007/s10163-020-01025-2
- Li, C., & Fang, H. H. P. (2007). Fermentative hydrogen production from wastewater and solid wastes by mixed cultures. In *Critical Reviews in Environmental Science and Technology* (Vol. 37, Issue 1, pp. 1–39). https://doi.org/10.1080/10643380600729071
- Li, S. L., Lin, J. S., Wang, Y. H., Lee, Z. K., Kuo, S. C., Tseng, I. C., & Cheng, S. S. (2011). Strategy of controlling the volumetric loading rate to promote hydrogen-production performance in a mesophilic-kitchen-waste fermentor and the microbial ecology analyses. *Bioresource Technology*, 102(18), 8682–8687.

https://doi.org/10.1016/j.biortech.2011.02.067

- Li, Y., Jin, Y., Li, J., Li, H., & Yu, Z. (2016). Effects of thermal pretreatment on the biomethane yield and hydrolysis rate of kitchen waste. *Applied Energy*, 172, 47–58. https://doi.org/10.1016/j.apenergy.2016.03.080
- Li, Y., Zhang, R., Liu, X., Chen, C., Xiao, X., Feng, L., He, Y., & Liu, G. (2013). Evaluating methane production from anaerobic mono- and co-digestion of kitchen waste, corn stover, and chicken manure. *Energy and Fuels*, 27(4), 2085–2091. https://doi.org/10.1021/ef400117f
- Lin, Y., Ge, Y., Xiao, H., He, Q., Wang, W., & Chen, B. (2020). Investigation of hydrothermal co-carbonization of waste textile with waste wood, waste paper and waste food from typical municipal solid wastes. *Energy*, 210. https://doi.org/10.1016/j.energy.2020.118606
- Łukajtis, R., Hołowacz, I., Kucharska, K., Glinka, M., Rybarczyk, P., Przyjazny, A., & Kamiński, M. (2018). Hydrogen production from biomass using dark fermentation. In *Renewable and Sustainable Energy Reviews* (Vol. 91, pp. 665–694). Elsevier Ltd. https://doi.org/10.1016/j.rser.2018.04.043
- Mohan, S. V., & Pandey, A. (2013). Biohydrogen Production: An Introduction.
   *Biohydrogen*, 1–24. https://doi.org/10.1016/B978-0-444-59555-3.00001-5
- Mohsin, G. F., Schmitt, F. J., Kanzler, C., Dirk Epping, J., Flemig, S., & Hornemann, A. (2018). Structural characterization of melanoidin formed from D–glucose and L– alanine at different temperatures applying FTIR, NMR, EPR, and MALDI–ToF–MS. *Food Chemistry*, 245, 761–767. https://doi.org/10.1016/j.foodchem.2017.11.115

- Montgomery, L. F. R., & Bochmann, G. (2014). *Pretreatment of feedstock for enhanced biogas production Pretreatment of feedstock for enhanced biogas production (electronic version).*
- Nagarajan, S., Jones, R. J., Oram, L., Massanet-Nicolau, J., & Guwy, A. (2022). Intensification of Acidogenic Fermentation for the Production of Biohydrogen and Volatile Fatty Acids—A Perspective. In *Fermentation* (Vol. 8, Issue 7). MDPI. https://doi.org/10.3390/fermentation8070325
- Najafpour, G. D. (2015). Biological Treatment. *Biochemical Engineering and Biotechnology*, 557–595. https://doi.org/10.1016/B978–0–444–63357–6.00019–5
- Nguyen, T. T., Chu, C. Y., & Ou, C. M. (2021). Pre-treatment study on two-stage biohydrogen and biomethane productions in a continuous co-digestion process from a mixture of swine manure and pineapple waste. *International Journal of Hydrogen Energy*, 46(20), 11325–11336. https://doi.org/10.1016/j.jjhydene.2020.05.264
- Orlando, M. Q., & Borja, V. M. (2020). Pretreatment of animal manure biomass to improve biogas production: A review. In *Energies* (Vol. 13, Issue 14). MDPI AG. https://doi.org/10.3390/en13143573
- Pachapur, V. L., Kutty, P., Pachapur, P., Brar, S. K., Le Bihan, Y., Galvez-Cloutier, R., & Buelna, G. (2019). Seed pretreatment for increased hydrogen production using mixed-culture systems with advantages over pure-culture systems. In *Energies* (Vol. 12, Issue 3). MDPI AG. https://doi.org/10.3390/en12030530
- Park, M., & Snyder, S. A. (2018). Sample handling and data processing for fluorescent excitation-emission matrix (EEM) of dissolved organic matter (DOM). *Chemosphere*, 193, 530–537. https://doi.org/10.1016/j.chemosphere.2017.11.069

Parshetti, G. K., Chowdhury, S., & Balasubramanian, R. (2014). Hydrothermal conversion of urban food waste to chars for removal of textile dyes from contaminated waters.
 *Bioresource Technology*, 161, 310–319.
 https://doi.org/10.1016/j.biortech.2014.03.087

- Perera, K. R. J., Ketheesan, B., Gadhamshetty, V., & Nirmalakhandan, N. (2010). Fermentative biohydrogen production: Evaluation of net energy gain. International Journal of Hydrogen Energy, 35(22), 12224–12233. https://doi.org/10.1016/j.ijhydene.2010.08.037
- Podgorbunskikh, E. M., Bychkov, A. L., & Lomovsky, O. I. (2019). Determination of surface accessibility of the cellulose substrate according to enzyme sorption. *Polymers*, 11(7). https://doi.org/10.3390/polym11071201
- Rajesh Banu, J., Merrylin, J., Mohamed Usman, T. M., Yukesh Kannah, R., Gunasekaran, M., Kim, S. H., & Kumar, G. (2020). Impact of pretreatment on food waste for biohydrogen production: A review. *International Journal of Hydrogen Energy*, 45(36), 18211–18225. https://doi.org/10.1016/j.ijhydene.2019.09.176
- Ryu, D., Lee, J., Kim, D., Jang, K., Lee, J., & Kim, D. (2022). Enhancement of the Biofuel Characteristics of Empty Fruit Bunches through Hydrothermal Carbonization by Decreasing the Inorganic Matters. *Energies*, 15(21). https://doi.org/10.3390/en15218154
- Setlow, P. (2003). Spore germination. In *Current Opinion in Microbiology* (Vol. 6, Issue 6, pp. 550–556). Elsevier Ltd. https://doi.org/10.1016/j.mib.2003.10.001
- Singh, D., & Yadav, S. (2021). Evaluation of the physico-chemical development of kitchen food wastes through torrefaction – a biodiversity case study. https://doi.org/10.1007/s13399-019-00526-x/Published

- Sivagurunathan, P., Kumar, G., Park, J. H., Park, J. H., Park, H. D., Yoon, J. J., & Kim, S. H. (2016). Feasibility of enriched mixed cultures obtained by repeated batch transfer in continuous hydrogen fermentation. *International Journal of Hydrogen Energy*, 41(7), 4393–4403. https://doi.org/10.1016/j.ijhydene.2015.06.133
- Song, C., Zheng, H., Shan, S., Wu, S., Wang, H., & Christie, P. (2019). Low-Temperature Hydrothermal Carbonization of Fresh Pig Manure: Effects of Temperature on Characteristics of Hydrochars. *Journal of Environmental Engineering*, 145(6). https://doi.org/10.1061/(asce)ee.1943-7870.0001475
- Tang, G. L., Huang, J., Sun, Z. J., Tang, Q. Q., Yan, C. H., & Liu, G. Q. (2008). Biohydrogen production from cattle wastewater by enriched anaerobic mixed consortia: Influence of fermentation temperature and pH. *Journal of Bioscience and Bioengineering*, *106*(1), 80–87. https://doi.org/10.1263/jbb.106.80
- Tang, J. Y., Xiong, Y. S., Li, M. X., Jia, R., Zhou, L. S., Fan, B. H., Li, K., Li, W., Li, H., & Lu, H. Q. (2023). Hyperbranched polyethyleneimine—functionalised chitosan aerogel for highly efficient removal of melanoidins from wastewater. *Journal of Hazardous Materials*, 447. https://doi.org/10.1016/j.jhazmat.2023.130731
- Valdez-Vazquez, I., & Poggi-Varaldo, H. M. (2009). Hydrogen production by fermentative consortia. In *Renewable and Sustainable Energy Reviews* (Vol. 13, Issue 5, pp. 1000–1013). https://doi.org/10.1016/j.rser.2008.03.003
- Val del Rio, A., Morales, N., Isanta, E., Mosquera–Corral, A., Campos, J. L., Steyer, J. P., & Carrère, H. (2011). Thermal pre–treatment of aerobic granular sludge: Impact on anaerobic biodegradability. *Water Research*, 45(18), 6011–6020. https://doi.org/10.1016/j.watres.2011.08.050

Veluchamy, C., & Kalamdhad, A. S. (2017). Enhancement of hydrolysis of lignocellulose

waste pulp and paper mill sludge through different heating processes on thermal pretreatment. *Journal of Cleaner Production, 168,* 219–226. https://doi.org/10.1016/j.jclepro.2017.09.040

- Wang, J., & Wan, W. (2008). Comparison of different pretreatment methods for enriching hydrogen–producing bacteria from digested sludge. *International Journal of Hydrogen Energy*, 33(12), 2934–2941. https://doi.org/10.1016/j.ijhydene.2008.03.048
- Wang, J., & Yin, Y. (2017). Principle and application of different pretreatment methods for enriching hydrogen—producing bacteria from mixed cultures. In *International Journal* of Hydrogen Energy (Vol. 42, Issue 8, pp. 4804–4823). Elsevier Ltd. https://doi.org/10.1016/j.ijhydene.2017.01.135
- Wang, N., Chen, Q., Zhang, C., Dong, Z., & Xu, Q. (2022). Improvement in the physicochemical characteristics of biochar derived from solid digestate of food waste with different moisture contents. *Science of the Total Environment*, 819. https://doi.org/10.1016/j.scitotenv.2022.153100
- Wang, X., Duan, X., Chen, J., Fang, K., Feng, L., Yan, Y., & Zhou, Q. (2016). Enhancing anaerobic digestion of waste activated sludge by pretreatment: Effect of volatile to total solids. *Environmental Technology (United Kingdom)*, 37(12), 1520–1529. https://doi.org/10.1080/09593330.2015.1120783
- Wei, Y., Gao, J., Shi, Z., Li, X., Ma, W., & Yuan, H. (2022). Effect of hydrothermal pretreatment on two-stage anaerobic digestion of food waste and Enteromorpha: Digestion performance, bioenergy efficiency, and microbial community dynamics. *Fuel, 318.* https://doi.org/10.1016/j.fuel.2022.123639

Wirth, R., Kovlics, E., Marlti, G., Bagi, Z., Rikhely, G., & Kovlics, K. L. (2012).

Characterization of a biogas-producing microbial community by short-read next generation DNA sequencing. *Biotechnology for Biofuels, 5.* https://doi.org/10.1186/1754-6834-5-41

- Wong, Y. M., Wu, T. Y., & Juan, J. C. (2014). A review of sustainable hydrogen production using seed sludge via dark fermentation. In *Renewable and Sustainable Energy Reviews* (Vol. 34, pp. 471–482). Elsevier Ltd. https://doi.org/10.1016/j.rser.2014.03.008
- Xia, A., Cheng, J., & Murphy, J. D. (2016). Innovation in biological production and upgrading of methane and hydrogen for use as gaseous transport biofuel. In *Biotechnology Advances* (Vol. 34, Issue 5, pp. 451–472). Elsevier Inc. https://doi.org/10.1016/j.biotechadv.2015.12.009
- Xu, D., Han, X., Chen, H., Yuan, R., Wang, F., & Zhou, B. (2020). New insights into impact of thermal hydrolysis pretreatment temperature and time on sewage sludge: Structure and composition of sewage sludge from sewage treatment plant. *Environmental Research*, 191. https://doi.org/10.1016/j.envres.2020.110122
- Xu, Z. (2007). Biological Production of Hydrogen from Renewable Resources. Bioprocessing for Value-Added Products from Renewable Resources: New Technologies and Applications, 527–557. https://doi.org/10.1016/B978– 044452114-9/50022-0
- Xu, Z. X., Song, H., Deng, X. Q., Zhang, Y. Y., Xue-Qin, M., Tong, S. Q., He, Z. X., Wang, Q., Shao, Y. W., & Hu, X. (2019). Dewatering of sewage sludge via thermal hydrolysis with ammonia-treated Fenton iron sludge as skeleton material. *Journal of Hazardous Materials*, 379. https://doi.org/10.1016/j.jhazmat.2019.120810

Yanga, G., & Wang, J. (2017). Fermentative hydrogen production from sewage sludge.

*Critical Reviews in Environmental Science and Technology*, *47*(14), 1219–1281. https://doi.org/10.1080/10643389.2017.1348107

- Yang, G., Yin, Y., & Wang, J. (2019). Microbial community diversity during fermentative hydrogen production inoculating various pretreated cultures. *International Journal of Hydrogen Energy*, 44(26), 13147–13156. https://doi.org/10.1016/j.ijhydene.2019.03.216
- Yang, G., & Wang, J. (2019). Changes in microbial community structure during dark fermentative hydrogen production. *International Journal of Hydrogen Energy*, 44(47), 25542–25550. https://doi.org/10.1016/j.ijhydene.2019.08.039
- Yang, S., Li, J., Zheng, Z., & Meng, Z. (2009). Lignocellulosic structural changes of Spartina alterniflora after anaerobic mono- and co-digestion. *International Biodeterioration* and Biodegradation, 63(5), 569–575. https://doi.org/10.1016/j.ibiod.2009.02.007
- Yang, S., McDonald, J., Hai, F. I., Price, W. E., Khan, S. J., & Nghiem, L. D. (2017). Effects of thermal pre-treatment and recuperative thickening on the fate of trace organic contaminants during anaerobic digestion of sewage sludge. *International Biodeterioration and Biodegradation*, 124, 146–154. https://doi.org/10.1016/j.ibiod.2017.06.002
- Yeshanew, M. M., Frunzo, L., Lens, P. N. L., Pirozzi, F., & Esposito, G. (2016). Mass loss controlled thermal pretreatment system to assess the effects of pretreatment temperature on organic matter solubilization and methane yield from food waste. *Frontiers in Environmental Science*, 4(SEP). https://doi.org/10.3389/fenvs.2016.00062
- Yu, S., Yang, X., Li, Q., Zhang, Y., & Zhou, H. (2023). Breaking the temperature limit of hydrothermal carbonization of lignocellulosic biomass by decoupling temperature

and pressure. Green Energy and Environment. https://doi.org/10.1016/j.gee.2023.01.001

- Zappi, A., Hernandez, R., & Holmes, W. E. (2021). A review of hydrogen production from anaerobic digestion. In *International Journal of Environmental Science and Technology* (Vol. 18, Issue 12, pp. 4075–4090). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s13762-020-03117-w
- Zeng, M., Ge, Z., Ma, Y., Zha, Z., Wu, Y., Hou, Z., & Zhang, H. (2023). Hydrothermal carbonization coupled with gasification for collaborative disposal of kitchen waste and yard waste. *Energy Conversion and Management, 283*, 116864. https://doi.org/10.1016/j.enconman.2023.116864
- Zhan, H., Zhang, S., Song, Y., Chang, G., Wang, X., & Zeng, Z. (2022). Hydrothermal cocarbonization of industrial biowastes with lignite toward modified hydrochar production: Synergistic effects and structural characteristics. *Journal of Environmental Chemical Engineering*, 10(3). https://doi.org/10.1016/j.jece.2022.107540

초록

수소는 깨끗하고, 재생 가능하며, 오염을 일으키지 않은 특성으로 인해 화석 연료를 대체할 수 있는 자원으로 각광받고 있다. 수소는 휘발유와 경유에 비해 높은 에너지를 가지고 있어, 에너지 효율성이 높다는 장점이 있으며, 교통, 산업 및 주거 분야 등 다양한 분야에 사용할 수 있다. 유기성 폐기물 또한 매년 증가하는 경향을 보여 유기성 폐기물로부터 수소를 생산하는 연구가 증가하고 있다. 다양한 수소 생산 방법 중 수소 발효는 수소 생산과 폐기물 처리를 한번에 할 수 있고, 다양한 분야에서 사용되는 휘발성 지방산 (VFAs)와 온실가스(CH<sub>4</sub>, CO<sub>2</sub>)를 적게 배출한다는 장점이 있다.

음식물 쓰레기와 가축분뇨와 같은 유기성폐기물은 건조 중량 기준으로 40-50 %의 리그노셀룰로오스를 포함한다. 리그노셀룰로오스는 생분해도를 감소시키고 가수분해 단계를 방해하여 바이오가스 생산 효율을 감소시키는 효과를 가져온다. 이러한 문제를 해결하기 위해 수소 발효 이전에 생물학적, 기계적, 화학적, 열가수분해 (Thermal hydrolysis process, THP) 등 다양한 전처리 방법을 적용함으로써, 리그노셀룰로오스를 분해하고 생분해도를 향상시키는 연구가 진행되고 있다. 이러한 다양한 전처리 중에서도 고온 및 고압의 증기를 이용하여 유기성 폐기물을 분해하는 열가수분해 공정(THP)이 성능을 가장 크게 향상시키는 것으로 알려져 있어, 널리 적용되고 있다.

하지만, THP는 높은 열과 전기를 필요로 하며, 높은 온도에서는 마이야르 상과 탄화 현상으로 인해 난분해성 물질을 발생시킬 수 있다는 단점이 존재한다. 이전 수소 발효에 THP를 적용하는 연구는 대부분 수소 발생량 증가에 집중해왔으며, 공정의 에너지 수지 및 마이야르 현상과 탄화 현상의 메커니즘을 찾는 연구는 부족한 상황이다.

따라서, 본 연구의 주요 목적은 열가수분해 온도가 음식물 쓰레기의 수소 생산 및 순에너지에 미치는 영향을 조사하는 것이다, 이 연구는 생화학적 수소 발생량 실험(BHP test)을 통해 THP가 바이오가스 생산에 미치는 영향을 평가하였다. 또한, THP가 수소 발효에 어떠한 영향을 미치는지에 대한 이해를 높이기 위해 리그노셀룰로오스의 구조 별화에 대한 분석이 수행된다. 마지막으로 연속식 반응 실험(CSTR test)를 통해 음식물 쓰레기의 THP에 따른 수소 발효 공정의 순에너지량을 평가하기 위한 에너지 분석을 수행하였다.

THP를 이용하여 120, 140, 160, 180 ℃의 온도에서 음식물 쓰레기의 전처리를 수행하였다. BMP 실험 결과, 140 ℃에서 전처리한 경우, 전처리하지 않은 음식물 쓰레기에 비해 수소 발생량이 3배 증가하여 가장 높은 수소 발생량을 보였다. 또한, THP에 의해 음식물 쓰레기 내 리그노셀룰로오스의 형태학적 변화와 셀룰로오스의 결정화도 감소는 미생물의 이용성을 항상시켰으며, 이를 통해 수소 발생량이 증가하였다. 하지만. 160 ℃와 180 ℃의 경우, 작용기의 변화와 휴믹산 계열 물질의 형성이 관찰되어 Maillard 현상화 탄화 현상으로 인해 수소 발생량이 감소하였음을 시사하였다.

CSTR 실험에서 140 ℃에서 전처리한 음식물(THP HF)과 전처리하지 않을 음식물 쓰레기(Control HF)를 기질로 사용하였다. THP의 적용은 THP HF에서 유기물 제거율 향상 (1.1배) 및 부티르산과 아세트산의 비율(Bu/AC) 향상에 기여하였고, 이를 통해 수소 발생량이 1.6배 증가하였다. 또한, THP의 적용은 박테리아 군집의 변화로 이어졌고, 공정 안정도 향상에 기여하였다. 하지만, THP 전처리 시 사용된 에너지로 인해 THP HF의 순에너지는 Control HF에 비해 낮은 것으로 나타났다.

결론적으로, THP는 음식물 쓰레기의 생분해도를 향상시키고 공정의 안정도를 증가시켜 수소 발생량 향상에 효과적인 방법으로 입증되었다. 하지만

고온에서는 Maillard 현상과 탄화현상으로 인해 수소 발생량이 감소하였다. 이러한 THP의 수소 생산 향상에 대한 긍정적인 영향에도 불구하고, 전처리 단계에 소비되는 많은 에너지로 이해 순에너지가 감소하였다. THP 적용 공젖의 에너지 효율은 THP 공정의 열 회수율의 증가를 통해 향상시킬 수 있으며, 열 회수율이 95 %일 때, 양(+)의 순에너지 값을 보였다.

**주요어:** 수소 발효; 음식물 쓰레기; 열가수분해; 리그노셀룰로오스; 순에너지 **학 번:** 2021-26328