IMPROVING METHANE PRODUCTIVITY OF FOODWASTE BY ENZYMATIC PRETREATMENT AND ELECTRODE MODIFICATION IN MEC-AD HYBRID SYSTEM

BY

ASEEL ABDELRAHIM ABDALLAH ALDAAS

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Kulliyyah of Engineering
International Islamic University Malaysia

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ABSTRACT

Hydrolysis has been identified as a rate-limiting stage in anaerobic-digestion. While it's been widely used in biomethane production, biomethane only accounts for 50-60%. A Therefore, an integrated AD-MEC system was developed to increase the biomethane content using food waste. However, high electrode's cost in the hybrid system poses an economical challenge to the market. Moreover, the microbial community plays a crucial role in the system, yet, minimal studies address the enhancement of microbial community and diversity. Hence, the characterization of food waste was performed in terms of carbohydrates, lipids, proteins, chemical oxygen demand, moisture content, solids, and volatile solids. The enzymatic hydrolysis of food waste was conducted to obtain the hydrolysate by one-factor-at-a-time (OFAT) through various factors including reaction time, temperature, enzyme loading, substrate concentration, and pH. The results showed that the optimum pH of 7, substrate concentration of 6%TS, the temperature of 50°C, and time of 16h gave the best release of reducing sugars. followed by the statistical optimization using faced centred central composite design (FCCCD) of selected factors, namely enzyme loading and substrate concentration,. The optimum conditions were enzyme loading of 6% (w/v) and a substrate concentration of 10% as the total solids (TS). Another pre-treatment, the acidic-enzymatic treatment using different concentrations of acids were performed. An acid concentration of 0.5% (v/v) showed the best hydrolysis effect achieving a value of 20 g/L reducing sugar,34.2% solids reduction, and 90 g/L soluble chemical oxygen demand (SCOD). However, the biogas production and free amino nitrogen release from acidic-enzymatic treated samples were lesser than only enzymatically treated samples. For MEC system, the effect of electrode modification using multiwall carbon nanotubes (MWCNT) and microbial growth into the electrodes was monitored using scanning electron microscope (SEM) images. The MWCNT growth was in-between the carbon felt fibres and the stainless steel mesh strands. The effectiveness of the electrodes was tested by inserting them into the hybrid system with glucose as the main substrate. Stainless steel mesh-modified cathode showed the highest biogas and methane production with a value of 14.4 ml CH_4/g glucose. In addition, carbon-felt modified electrodes showed a maximum substrate degradation value of 93% and a current density of 4.5 mA/m^2. The SEM imaging of the microbial growth on the electrodes showed that the microbes followed a different growth behaviour in modified and unmodified electrodes. In addition, MWCNT-modified Stainless steel mesh(SSTM) showed a potential hydrogenotrophic growth selectivity, unlike unmodified SSTM, which had a more syntrophic microbial community. Hybrid systems showed a higher hydrolysis efficiency especially modified systems, with a percentage of 39.4% by the 48th hour, followed by unmodified systems. The acidogenesis efficiency results showed that the hybrid systems were dominated by the acetic acid pathway, which is favourable in the hybrid system, unlike the conventional digester, which was dominated by a different pathway. Mixing the original inoculum obtained from a previous AD with cow manure has enhanced and increased the competitiveness of the microbial community. Thus, it was positively reflected on the biomethane production potential and rate, with a value of 38ml/g COD and 1.2 ml/h, respectively. In this study, we successfully enhanced the hydrolysis rate, improved the selectivity of microbes in the system, and introduced a set of commercially available electrodes. Our findings also provided compelling evidence that increasing microbial diversity significantly enhances the overall performance of the system.

ملخص البحث

يعد الهضم اللاهوائي نهج لتحويل النفايات العضوية ، مثل مخلفات الطعام ، والحمأة ، والنفايات الزراعية ، إلى منتجات قيمة مثل الغاز الحيوي. ولكن محتوى الميثان الحيوي من الهضم الحيوي يمثل فقط 50-60٪ ، والباقي (40-50٪) هو ثاني أكسيد الكربون. أدت هذه المشكلة إلى الحد من تطبيق (اللاهوائي لانتاج الطاقة. للتغلب على هذه المشكلة، يمكن انتاج الميثان الحيوي من ثاني أكسيد الكربون داخل النظام باستخدام تطبيق خلية التحليل الكهربائي الميكروبية (MEC) ؛بحيث يتم إدخال مجموعة واحدة أو أكثر من الأقطاب الكهربائية في الهاضمة ، مع توصيل مصدر للطاقة. أولاً، تحليل محتوى نفايات الطعام. ثم تم فحص العوامل التشغيلية لتحلل نفايات الطعام باستخدام الإنزيمات باستخدام طريقة معامل واحد لكل دورة. أظهر الفحص أن الرقم الهيدروجيني 7 وتركيز مخلفات الطعام 6 ودرجة الحرارة 50 والوقت 16 ساعة هم الأفضل لمعالجة بقايا الطعام. وبعد ذلك, تم دراسة تأثير عوامل محددة وهي تركيز الإنزيم وتركيز مخلفات الطعام باستخدام خبير تصميم FCCCD. كانت الظروف المثلى هي تركيز الإنزيم بنسبة 6 ٪ (وزن / حجم) وتركيز مخلفات الطعام بنسبة 10 ٪ من إجمالي المواد الصلبة. تليها المعالجة الحمضية الأنزيمية. أظهر تركيز حمض 0.5٪ (حجم / حجم) أفضل تأثير في معالجة مخلفات الطعام محققًا قيمة 20 جم / لتر سكر مختزل، 34.2٪ اختزال للمواد الصلبة ، 90 جم / لتر SCOD. ومع ذلك، فان إنتاج الغاز الحيوي وانتاج النيتروجين الأميني الحر من العينات المعالجة بالإنزيم الحمضي أقل من العينات المعالجة بالإنزيم فقط. بعد ذلك، تم استخدام المجهر الالكتروني لمراقبة تأثير طلي الأقطاب المستخدمة للخلية الحيوية باستخدام جزيئات الكربون النانوية والنمو الميكروبي في الأقطاب باستخدام. كان نمو الجزيئات النانوية بين الألياف والشبكة بشكل منتظم. بعد ذلك ، تم اختبار فعالية الأقطاب الكهربائية عن طريق إدخالها في النظام. أظهر الكاثود شبكة الفولاذ المقاوم للصدأ المعدل بجزيئات الكربون النانوية أعلى إنتاج للغاز الحيوي والميثان. بالإضافة إلى ذلك، أظهرت الأقطاب الكهربائية المصنوعة من اللباد الكربوني المعدلة بجزيئات الكربون النانوية قيمة قصوى لاختزال السكر بقيمة تبلغ (93 ٪) وكثافة تيار كهربائي بلغ 4.5 مللي أمبير / م 2. أظهر تصوير بالمجهر الالكتروني للنمو الميكروبي على الأقطاب الكهربائية أن الميكروبات اتبعت سلوك نمو مختلف في الأقطاب الكهربائية المعدلة وغير المعدلة. بالإضافة إلى ذلك، أظهر شبكة الفولاذ المقاوم للصدأ المعدل بجزيئات الكربون النانوية انتقائية محتملة للنمو ميكروبات المنتجة للميثان عن طريق اختزال الهيدروجين، على عكس شبكة الفولاذ المقاوم للصدأ غير المعدلة ، والتي كان لديها مجتمع ميكروبي أكثر تنوعا. أظهرت الأنظمة الهجينة كفاءة تحلل مادي أعلى خاصة الأنظمة المعدلة بنسبة 39.4٪ خلال 48 ساعة تليها الأنظمة غير المعدلة. أظهرت نتائج كفاءة التولد الحمضي أن الأنظمة الهجينة قد سادت مع مسار حمض لأسيتيك، وهو مفضل في النظام الهجين. على عكس الهاضم التقليدي، الذي سيطر عليه مسار مختلف. أدى خلط حصيلة الميكروب التي تم الحصول عليه من خلية هضم لاهوائي سابقة مع روث البقر إلى تعزيز وزيادة القدرة التنافسية بين المجتمع الميكروبي. وبالتالي، فقد انعكس إيجابًا على إمكانات ومعدل إنتاج الميثان الحيوي ، بقيمة 38 مل ، و 1.2 مل / ساعة على التوالي.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science in Engineering

…..…………………………..…………
Md Zahangir Alam
Supervisor

..………..... ……….
Azlin Azmi Suhaida
Co-Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science in Engineering

……….…..…………………..…………
Mohammed Saeid Jami
Internal Examiner

………………...………..…………
Ahmad Anas Nagoor
External Examiner

This thesis was submitted to the Kulliyyah of Engineering and is accepted as a fulfilment of the requirement for the degree of Master of Science in Engineering

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Sany Izan Ihsan
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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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LIST OF ABBREVIATIONS

AD	Anaerobic digestion/digester
MEC	Microbial electrolysis cell
OFAT	One factor at the time
FCCCD	Face-centred central composite design
TS	Total solids
TVS	Total volatile solids
MWCNT	Multi-wall carbon nanotubes
SEM	Scanning electron microscopy
SSTM	Stainless steel mesh
CF	Carbon felt
FW	Food waste
VFA	Volatile fatty acids
EPS	extracellular polymer substance
pH	Potential of hydrogen
FAN	Free amino nitrogen
COD	Chemical Oxygen demand
SCOD	Soluble chemical Oxygen demand
BSA	Bovine serum albumin
DNS	Dinitrosalicylic acid
HRT	Hydraulic retention time
DIET	Direct interspecies electron transfer
EAB	Electroactive bacteria
HER	Hydrogen evolution reaction
OLR	Organic loading rate
GAC	granular activated carbon
PAC	Powdered activated carbon
PBS	phosphate buffer saline

LIST OF SYMBOLS

CH_4	Methane
CO_2	Carbon dioxide
H_2	Hydrogen
H_2S	Hydrogen Sulphide
CH_3COOH	Acetic acid
H_2SO_4	Sulfuric Acid
NO_2-	Nitrite
NO_3-	Nitrate
Fe	Iron
Mn	Manganese
%	Percentage
V	voltage
W	weight
V	volume
mA	milli-Amber
m_2	Meter square
cm_2	Centimetre square
mL	Milli-litre
^oC	Celsius
mg	Milli-gram
Ω	Ohm

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The projection of food waste has been increasing the past 25 years, especially in Asian countries, Hodaifa et al., (2019)reported that there would be an increase from 278 to 416 million tonnes from 2005 to 2025. Food waste accounts for 23% of municipal waste, accounting for 30% of the total trash disposed into landfills and incinerators (Abdel-Shafy& Mansour, 2018). This problem has led to uncontrolled fermentation in landfills, emitting greenhouse gases, polluting groundwater, increasing the disposal cost, and damaging incinerators by high-temperature fluctuation due to high water content. On the contrary, food waste has a high content of fermentable substrates such as sugars, fats, starches, lipids, proteins, and cellulose (Moon et al., 2009), which makes it an excellent substrate for producing high-value products (e.g., biofuels and platform chemicals) (Uçkun Kiran et al., 2015).

Anaerobic digestion is an approach to converting organic waste, such as food waste, into valuable products like biogas. The digestion process involves four significant steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Wirth et al., 2012). During hydrolysis, complex organic matters like carbohydrates, protein, and fats are broken down into their monomers, reducing sugars, amino acids, and fatty acids, respectively. Next is acidogenesis, where acidogenesis microorganisms further break down the products of hydrolysis, producing ammonia, H_2, CO_2, H_2S, shorter volatile fatty acids, carbonic acids, alcohols, and trace amounts of other by-products (Kirk & Gould, 2020). Next is acetogenesis, where microbes produce acetate. Finally, acetogens utilize the products of acidogenesis to produce acetic acid, CO_2, and H_2. Methanogenesis is the last step of the pathway. Methanogens produce methane from the final products of acetogenesis as well as from some of the intermediate products from hydrolysis and acidogenesis, following two paths involving the utilization of acetic acid and CO_2 along with hydrogen as shown in the following equations below (Kumar et al., 2012; Salman et al., 2017):

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad (1.1)$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad (1.2)$$

Although anaerobic digestion is an attractive solution, biomethane production only accounts for 50-60%; the remaining is CO_2 (Xu et al., 2014; Zeppilli et al., 2019). To separate CO_2 from CH_4, conventional methods for biomethane purification includes the removal of CO_2 without the reduction of CH_4 mass; this includes pressure swing adsorption, membrane separation, or chemical CO_2- absorption (Cerrillo et al., 2017; Hassanein et al., 2017).

Microbial electrolysis cell has been employed in the anaerobic digestion system to upgrade biomethane production. External energy is supplied to the system to drive a thermodynamic non-spontaneous reaction, like the conversion of CO_2 to CH_4 (Aryal et al., 2017; Cheng et al., 2009). In addition to conventional pathways of biomethane production, a unique pathway reaction occurs on the cathode by electro-methanogenesis; the electroactive microbes directly utilize electrons and organic compounds to produce methane(Zakaria et al., 2020). In addition, the enrichment of hydrogenotrophic methanogenesis on the cathode is a key factor in the hybrid system, decreasing the amount of CO_2 produced while increasing the biomethane yield in anaerobic digestion (Anukam et al., 2019; Eerten-Jansen et al., 2011).

The production of biomethane has two different extracellular electron transfer mechanisms, either indirectly by intermediate abiotic electrochemical and microbially catalysed hydrogen production in the cathodic compartment or directly by taking the electrons from the cathode reduction of CO_2 to methane.

1.2 STATEMENT OF THE PROBLEM

Food waste production is increasing with the increase in population. Conventional methods of FW disposal, like incineration and open landfills, are no longer feasible, due to the high operational cost, increased risk projected on the environment, and contribution to global warming (Gao et al., 2017). Although anaerobic digestors have been employed in the treatment of food-waste for biogas production, hydrolysis, which is the first stage, present a significant challenge in the effectiveness of the treatment, hence, limiting the capacity to handle large scales (Yin et al., 2016). Therefore, The pre-treatment of food-wate is crucial, as it helps speed up the hydrolysis stage, decrease hydraulic retention time, and improves the efficiency of the following stages, especially acidogenesis and methanogenesis (Moon & Song, 2011).

Moreover, Biomethane production through AD only accounts for 60% of the total biogas produced; the remaining 40% is CO_2 (Anukam et al., 2019; Enzmann et al., 2018). Hence, carbon dioxide absorption requires costly downstream processes that also limits the application of anaerobic digestion(Xu et al., 2014).

Microbial electrolysis cell is a new technology representing a new form of green energy. It has attracted considerable attention for the past few years as a promising technology for higher biogas production from organic matter (Huang et al., 2020a). microbe's cathodic reaction is responsible for reducing CO_2 into CH_4 (Kundu et al., 2013).

The high cost of electrodes, especially ones utilizing precious metals such as platinum and palladium have limited the implementation and economical viability of the system (Zakaria et al., 2020). Consequently, finding alternative electrode's material or replacing modifications using precious metal while maintaining the MEC-AD performance is crucial for more economically and sustainable energy production and food-waste treatment.

Lastly, microbial community is the driving force in the system, responsible for the fermentation process and biomethane production rate and volume (Yu et al., 2017). However, a gap exists in the research in terms of manipulating the microbial community, Although multiple studies have reported that inoculums rich in diverse types of microbes performed better than other systems seeded with conventional inoculums(Detman et al., 2021). In addition, there is a lack of study on the effect of mixing inoculum on biomethane production kinetics and enhancement, though mixing two inoculums rich in fermentative and methanogenic microbes could potentially enhance the overall system's performance.

1.3 SCOPE OF THE STUDY

The research focused on improving biomethane production from FW. AD was integrated with an MEC system to convert CO_2 into CH_4 through the cathodic reaction of hydrogenotrophic and electro methanogenic microbes through the following steps:

- Pre-treatment of the substrate: FW were pre-treated using two treatment methods. Acidic-enzymatic and enzymatic treatment only. Sulfuric acid was used in the acidic treatment, while a cocktail of hydrolytic enzymes produced from rice bran was used in the enzymatic treatment. Performing hydrolysis in a separate process(pre-treatment) from the methanogenesis process can minimize interspecific competition, thus increasing the reaction rate of methanogenesis in the MEC-AD system(Park et al., 2018). Hydrolytic enzymes broke down and solubilized complex organic matter into their monomers. Hence, it eased the substrate uptake by microorganisms and reduced the hydraulic retention time. Enzyme loading, TS concentration, pH, temperature, and reaction time were optimized to obtain maximum sugar and free amino acid recovery. Then the acidic-enzymatic treatment was tested; the acid solubilized the substrate, offering a higher area for the enzymes to attack. The effect of both treatments was monitored on the release of reducing sugar, free amino nitrogen, solubilization of substrate, total solids reduction, and biogas production.

- Electrode modification and biofilm monitoring: Microbes, electrode interaction, and biofilm formation are crucial factors affecting the MEC-AD system. Thus, the set of electrodes was modified with MWCNT. The biofilm formation and interaction with surface-modified 3D electrodes were monitored to improve microbes formation, decrease adaptation time and increase biomethane production.

- The original inoculum obtained from a previous anaerobic digester was enhanced by adding cow manure to the hybrid system with modified electrodes. Cow manure is reported to have a high population of the methanogenic community. Mixing the original inoculum with cow manure gave the essential microbial community for treating high fermentable substrates like food waste. Testing the overall efficiency of the microbial stages was crucial to see the combined effect of food-waste pre-treatment, electrode modification, and inoculum mixing in enhancing the system.

1.4 RESEARCH OBJECTIVES

The study aimed to achieve the following objectives:

1. To identify and optimize the process parameters of enzymatic hydrolysis of food waste for maximum monomers in the hydrolysate.
2. To determine the effect of electrode surface modification on biomethane production and biofilm formation in the MEC-AD system.
3. To study the fermentation stages and the effect of mixed inoculum on biomethane kinetics production from food waste equipped with modified electrodes using modified gompertz model.

1.5 THESIS ORGANIZATION

This thesis consists of five chapters, including chapter one, which covers background information, problem statements, objectives, the scope of studies, and the overall flow of this study. Chapter two includes the literature on previous research on previous pre-treatment methods of FW, hybrid system of MEC-AD, and factors affecting the system like electrode choice, voltage, microbial community and altering it. Chapter three focuses on the detailed methodology of experiments applied in this study. Chapter four presents the results and discussion of each finding on substrate pre-treatment, electrode modification, and each microbial stage efficiency with mixing inoculum. Chapter five highlights this study's findings, conclusions, and recommendations for future studies..

CHAPTER TWO
LITERATURE REVIEW

2.1 INTRODUCTION

This chapter summarizes previous works in different treatment processes, anaerobic digestors, microbial electrolysis cells and a hybrid system of both. Followed by detailed comparisons of operational factors like the voltage, electrodes, electrode modification, and microbial community, in terms of culture, biofilm and suspension, and methods of enriching methane-producing microbes.

2.2 BIOGAS

Biogas is a renewable, environmentally friendly energy source. It's produced by the microbial breakdown of organic matter, such as food or animal waste, in an anaerobic digestion process (Scarlat et al., 2018). The production of biogas provides a versatile carrier of renewable energy, as methane can be used to replace fossil fuels in both heat and power generation and as a vehicle fuel (Weiland, 2010). Various process types are applied for biogas production, which can be classified into wet and dry fermentation systems. Most often applied are wet digester systems using vertical stirred tank digesters with different stirrer types dependent on the origin of the feedstock. Biogas is mainly utilized in engine-based combined heat and power plants. In contrast, micro-gas turbines and fuel cells are expensive alternatives that need further development work to reduce costs and increase reliability (Tian et al., 2020).

2.3 FOOD-WASTE

Food waste is biodegradable waste discharged from a variety of sources. However, private households are the major source in food waste generation. The projection of food waste has been increasing in the current 25 years, mainly in Asian countries. It was reported by Paritosh et al., (2017) that there would be an increase from 278 to 416 million tonnes from 2005 to 2025. While food waste might not seem like a significant issue to environmental sustainability, almost 30-50% of the total food produced in the world goes to landfills, accounting for 30% of the complete waste disposed into landfills and burnt in incinerators. The uncontrolled fermentation of food waste in landfills causes groundwater contamination and greenhouse gas emissions, with an estimated 3.3 billion tons of CO_2 in the atmosphere annually (Paritosh et al.,2017). In addition, landfills have reached their capacity, incinerators require high capital costs and are insufficient in treating FW with high moisture content. On the contrary, FW is an excellent feedstock to produce high-value biofuels, owing to the high content of the fermentable substrate. Table 2.1 shows the characterization of food waste:

Table 2.1 Characteristics of food waste

Parameter	Content (w/w %)
pH	5.1
Total solid	22.1
Volatile solid	16.8
Moisture	76.6
Total sugar	65.1
Cellulose	3.7
protein	17.3
Lipid	13.9

Source: (Rueda et al., 2020)

2.4 FOOD-WASTE TREATMENT AND HYDROLYSIS

Food waste is readily biodegradable due to the high volatile fraction of total solids. However, the degradation of the substrate into soluble particles is a rate-limiting factor in anaerobic digestion. The pre-treatment of food waste is the process of reducing particle size to increase surface area accessibility by microbes or breaking down complex organic matters like carbohydrates, proteins and lipids to their monomers, reducing sugars, amino acids, and fatty acids, respectively. This process eases the substrate uptake by the microbes, improving biogas production. Hence, improve the hydrolysis kinetics.

2.4.1 Chemical Treatment

Chemical treatment is majorly involved in the solubilization of lignin and hydrolyzing cellulose in the agricultural and food-waste industries (Hodaifa et al., 2019). Chemical pre-treatment involves the usage of strong alkaline or acid to solubilize organic compounds. While alkaline treatment is used for the hydrolysis of proteins, lipids, and lignin, acidic pre-treatment is used for the hydrolysis of carbohydrates (Parthiba et al., 2018). Different acid treatments are performed, including concentrated and diluted acids. The principle of concentrated acid hydrolysis is that crystalline cellulose can be completely dissolved in 72% sulfuric acid or 42% hydrochloric acid, or 77–83% phosphoric acid at a lower temperature, resulting in the homogeneous hydrolysis of cellulose (Chen, 2015) .In the dilute process, acid pre-treatment, acid hydrolysis the hemicellulose portion of the biomass and causes structural changes, thereby improving the enzyme accessibility for hydrolysing cellulose (Achinas et al., 2021).

However, the chemical treatment has multiple drawbacks restricting the application in the food-waste pre-treatment, some of the drawbacks sited in previous literature are as follow:

- The usage of strong alkaline or acids can lead to the formation of toxic by-products such as hydrogen sulfide and ammonia (Carlsson et al., 2012)

- Chemical pre-treatment might disrupt the degradation efficiency of the microbial community in the subsequent treatment of anaerobic digestion, hindering the biomethane production(López et al., 2008).

2.4.2 Thermal Treatment

The process of thermal pre-treatment of food waste involves the disintegration of the cell membrane, which produces organic material solubilization, so it will make it easier for microorganisms to digest the feedstock within a shorter time, in other words, enhancing the solubilization of COD (Chemical Oxygen Demand), which will improve the efficiency of the anaerobic digestion process (Chua et al., 2019).

The process involves heating the food waste in different time intervals under different temperatures. There are two types of thermal pre-treatment:

- Low thermal pre-treatment: temperature range between 50-110 ^{o}C
- High thermal pre-treatment: temperature range between 110-250 ^{o}C

It was reported previously that treating FW pre-treated with low thermal pre-treatment did not show efficient COD solubilization in the temperature range between (50-90 ^{o}C) (Yingcun et al., 2016). Meanwhile, Parre et al., (2020) studied the effect of different temperatures ranging between 25-150^{o}C. The study showed that FW treated under 100 ^{o}C showed the highest solubilization of COD, while FW treated under the temperature range 130-150 ^{o}C showed the lowest solubilization efficiency.

2.4.3 Biological Treatment

Hydrolytic enzymes break down complex substrates into their monomers, allowing a higher surface area to be attacked by the microbes, thus improving the digestion of lignocellulosic biomass in the system, and reducing the hydraulic retention time (HRT) (Wang et al., 2020). Multiple hydrolytic enzymes, such as Protease, Lipase, and Carbohydrase enzymes,