Alkali-Microwave Pre-treatment of Wheat Straw for Production of Biogas by Anaerobic Digestion

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Declaration

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نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة عمادة البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحثة دنيا محمد زكريا حسن أبو حميد لنيل درجة الماجستير في كلية العلوم قسم التكنولوجيا الحيوية وموضوعها:

معالجة قش القمح بالقاعدة وأشعة الميكروويوف لإنتاج الغاز الحيوي

Alkali-Microwave Pre-treatment of Wheat Straw for Production of Biogas by Anaerobic Digestion

وبعد المناقشة العلنية التي تمت اليوم الأربعاء 11 ربيع أول 1439 هـ الموافق 29/11/2017م، الساعة الواحدة مساءً بقاعة مؤتمرات مبنى اللحيدان، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

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ويعد المداولة أوصت اللجنة بمنح الباحثة درجة الماجستير في كلية العلوم/ قسم التكنولوجيا الحيوية.
واللجنة إذ تمنحها هذه الدرجة فإنها توصيها بتقوى الله تعالى ولزوم طاعته وإن تسخير علماً في خدمة دينها ووطنها.

وallah واله تعالى

عميد البحث العلمي والدراسات العليا
أ.د. مازن اسماعيل هنية
Abstract

Background: the concern related to fossil energy sources is increased because of the energy crisis that facing the world, therefore, it is necessary to find renewable and environmentally friendly energy sources alternative to fossil energy. Lignocellulosic materials such as wheat straw can be considered for biogas production, however, its recalcitrant structure restrained its utility. Therefore, applying a convenient pre-treatment method is necessary to increase sugar yield and biogas production. Carbon to nitrogen ratio of wheat straw is high, so it is necessary to mix it with proper amounts of low carbon to nitrogen ratio material such as a chicken feather to get suitable carbon to nitrogen ratio for anaerobic digestion which is 20-30. Alkali-microwave pre-treatment assisted to break down the hard structure of wheat straw to improve degradation of cellulose and to release reducing sugars and biogas. This study aims to examine the effect of alkali-microwave pre-treatment of wheat straw on biogas production with or without pre-treated chicken feather.

Objective: evaluating pre-treatment of wheat straw using microwave irradiation and sodium hydroxide on biogas production from wheat straw.

Methods: in this study, the effectiveness of alkali-microwave pre-treatment on biogas production was investigated, wheat straw was ground and pre-treated with NaOH and microwave irradiation at 2450 MHz and 450 W for (5, 10, 15 and 20 min). For anaerobic digestion, pre-treated wheat straw was inoculated with an anaerobic consortium, and biogas produced was detected at mesophilic conditions.

Results: it was found that when wheat straw was pre-treated by 2% NaOH at a microwave power of 450 W with 40 g of wheat straw for 10 min in the microwave and mixed with pre-treated chicken feather, the cumulative biogas produced was 1870 ml.

Conclusions: alkali-microwave pre-treatment can enhance reducing sugar yield for anaerobic digestion of wheat straw, while the long time of irradiation lead on the decrease in reducing sugar yield. Adding pre-treated chicken feather for adjusting carbon to nitrogen ratio gave positive results of biogas production.

Key Words: Wheat Straw, Anaerobic Digestion, Fermentation, Biogas, Alkali Pre-treatment, Microwave Irradiation.
ملخص الدراسة
المقدمة: يواجه العالم أزمة في الطاقة نتيجة اعتماده بشكل أساسي على الوقود الأحفوري، مما يتسبب في تغير المناخ وأضرار البيئة. لذلك كان من الضروري إيجاد مصادر طاقة بديلة لمجاعة وصديقة للبيئة.

ويمكن استخدام المواد الغنية بالسليزول واللجنين مثل قش القمح، وما أن من الصعب استخدامه مباشرة في عملية التخمير اللاهواني لإنتاج الغاز الحيوي، إلا أن استخدامه يزيد عمليات التخمير في نسبة السكر فيه وبالتالي زيادة إنتاج الغاز الحيوي. نسبة الكربون إلى النتروجين في القمح عامل، لذلك من الضروري إضافة مواد أقل في نسبة الكربون إلى النتروجين مثل ريش الدجاج وذلك للحصول على نسبة مناسبة لحدود التخمير اللاهواني وهي 20-30.

معالجة القش باستخدام مواد قاعدية وأشعة الميكروويف تؤدي إلى تحضير التركيب الصلب للقش وبالتالي تحسين عملية هضم السليزول لإنتاج السكر والغاز الحيوي. تحتوي هذه الدراسة إلى دراسة تأثير معالجة قش القمح بالقاعدة وأشعة الميكروويف على إنتاج الغاز الحيوي سواء مع أو بدون استخدام ريش المعالج.

الهدف: تقييم عملية إنتاج الغاز الحيوي باستخدام قش القمح المعالج بـ هيدروكسيد الصوديوم وأشعة الميكروويف.

الطرق: في هذه الدراسة تم دراسة أثر معالجة قش القمح باستخدام القاعدة وأشعة الميكروويف على إنتاج الغاز الحيوي. تم طحن القش ومعالجه بـ هيدروكسيد الصوديوم وأشعة الميكروويف على 2400 ميغاهرتز و450 وات لمدة (5، 10، 15 و 20 دقيقة).

تمت عملية البقاء اللاهواني من خلال إضافة للفتش إلى القش والماء وملعقي البقية والقش المعالج المخلوط بالريش المعالج تحت ظروف معتدلة الحرارة.

النتائج: أفضل إنتاج للغاز الحيوي كان باستخدام 40 بالمئة من القش المخلوط بـ ريش الدجاج والماء بـ 2% هيدروكسيد الصوديوم ولمدة 10 دقائق في الميكروويف على 450 واط حيث تم إنتاج 1870 مل من الغاز الحيوي.

الاستنتاج: معالجة قش القمح باستخدام القاعدة وأشعة الميكروويف بإمكانها زيادة إنتاج السكر وتحسين عملية البقاء اللاهواني.

استخدام فترات طويلة للمعالجة بأشرعة الميكروويف تؤدي إلى التقليل من كمية السكر الناتج.

إضافة ريش الدجاج المعالج لضبط نسبة الكربون إلى النتروجين في القش أدت إلى تحسين إنتاج الغاز الحيوي.

الكلمات المفتاحية: قش القمح، البقاء اللاهواني، التخمير، الغاز الحيوي، المعالجة بالقاعدة، أشعة الميكروويف.
DEDICATION

To those who promised to raise me while I was young and lightened my way with advice and guidance while I'm older, mother and father, may Allah protect you.
To those who sympathized with me, supported me and motivated me for more progress, brothers and sisters, may Allah save you.
To everyone who taught me a letter and helped me in gaining knowledge.
To those who defended this nation and instilled the high spirit inside of us, and I particularly single out my beloved brother and martyr Ahmed, may Allah rest his soul in peace and make paradise his abode.
To everyone who participate in accomplishing this humble work, they deserve all my recognition and gratitude.
I dedicate them all the results of my painstaking effort and my humble research.

Dunia Abu Hmead
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My thanks and gratitude to my dear mother who devoted her life for us to be.

To my father, the reason of my existence.

To my brothers Hasan, Sayaf, Humam, and Yahia and my sisters, Maysoon, Maryam, and Islam.

And finally to the pure soul my beloved brother and martyr Ahmed.

Those I'm mentioned. I've thanked and those I've forgotten, they're the first people to thank and appreciate.

Finally, I can just say thanks for everything and may Allah give all who stood beside me, all the best in return.
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<td>Anaerobic Digestion</td>
</tr>
<tr>
<td>AFEX</td>
<td>Ammonia Fiber Expansion</td>
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<tr>
<td>C/N</td>
<td>Carbon To Nitrogen Ratio</td>
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<tr>
<td>CF</td>
<td>Chicken Feather</td>
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<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
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<tr>
<td>EFB</td>
<td>Empty Fruit Bunch</td>
</tr>
<tr>
<td>GHG</td>
<td>Greenhouse Gases</td>
</tr>
<tr>
<td>HMF</td>
<td>Hydroxymethyl Furfural</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic Retention Time</td>
</tr>
<tr>
<td>LCFA</td>
<td>Long Chain Fatty Acid</td>
</tr>
<tr>
<td>LHW</td>
<td>Liquid Hot Water</td>
</tr>
<tr>
<td>MSW</td>
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</tr>
<tr>
<td>MW</td>
<td>Microwave</td>
</tr>
<tr>
<td>OFMSW</td>
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<tr>
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<td>Organic Loading Rate</td>
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Chapter I
Introduction
Chapter I
Introduction

1.1 Overview

Nowadays, the main fears ascended considered by human being are air pollution and global warming. This issue caused by the massive liberation of greenhouse gases (GHG) including methane (CH$_4$) carbon dioxide (CO$_2$), and nitrous oxide (N$_2$O) that released as a result of fossil fuels combustion accompanied with the increasing of population density in the world (Hosseini, Wahid, & Aghili, 2013; Bansal, Sreekrishnan, & Singh, 2013; Abdeshahian, Dashti, Kalil, & Yusoff, 2010). GHGs acting as a determinate cause in global warming, because it reflects the heat from earth surface. Such GHG effect resulted mainly from CO$_2$ (60%) and while CH$_4$ contributes with CH$_4$ (15%) (Rahimnejad, Adhami, Darvari, Zirepour, & Oh, 2015; Hosseini & Wahid, 2014; Hosseini et al., 2013; Williams, Shobrak, Wilms, Arif, & Khan, 2012). So that, it is important to replace renewable energy sources such as biogas instead of fossil fuels (Horváth, Tabatabaei, Karimi, & Kumar, 2016).

Different types of wastes could be used to produce biogas, such as sewage sludge, source separated food waste and food industry wastes (Energimyndigheten, 2015). Production of biogas anaerobically is an environmentally friendly process utilizing the increasing amounts of organic waste produced worldwide, also, it offers another advantage, such as the prevention of odor release and pathogens removal (Horváth et al., 2016).

Anaerobic treatment or digestion (AD) is an assembly of compound processes by which microorganisms breakdown the biodegradable materials in the absence of oxygen (Klimiuk, Gusiatin, Bułkowska, Pokój, & Rynkowska, 2015).

During the AD process, cellulose and hemicellulose are degraded quite easily and then converted into soluble compounds such as volatile fatty acids (VFAs). While the presence of lignin limits the accessibility of microbial enzymes and thus prevents the degradation of the cellulosic material and reduce the expected yield of the produced biogas. High VFAs concentration could increase the acidity of the medium and then reduce the biogas yields (Yu, Bule, Ma, Zhao, Frear et al., 2014).
The crop/manure volatile solids ratio is important, so that, the biogas plant must be loaded properly to avoid process failure, and to avoid that, many studies are investigated to avoid process breakdown by monitoring program parameters such as propionic acid, organic loading rates (OLRs), etc. (Kampmann, Ratering, Geißler-Plaum, Schmidt, Zerr et al., 2014; Linke, Muha, Wittum, & Plogsties, 2013; Comino, Rosso, & Riggio, 2010). Another important way to avoid AD process breakdown, pre-treatment of lignocellulosic substrate is important which resulted in increasing the biogas yield also. Pre-treatment process is important because it makes the cellulose accessible to hydrolysis process and thereby improve the final methane conversion and reduce the hydraulic retention time (HRT) (Lv, Schanbacher, & Yu, 2010).

Development of suitable pre-treatment process of lignocelluloses is recommended for biogas production. Various technologies are there for pre-treatment of lignocellulosic substrates, these technologies include chemical, mechanical, biological, and thermal techniques as well as various combinations of those (Behera, Arora, Nandhagopal, & Kumar, 2014).

Production of biofuels from lignocelluloses such as wheat straw seems as an important possibility for increasing the material efficiency and energy production, and to decrease the coasts of biomass options and then mitigate GHG emissions, that’s because its price is low and it has a wide distribution. Lignocellulosic feedstocks can be used instead of fossil fuels to produce renewable energy, since these feedstocks do not compete with feed crops of food.

1.2 Objectives

The main objective of this work was to evaluate the alkali-microwave pre-treatment of wheat-straw for biogas production by anaerobic digestion.

The following specific objectives were achieved:

1. Evaluate the biogas production potential of untreated and pretreated wheat-straw (WS) through anaerobic batch digestion assays.
2. Enhancement of biogas production by:
a) Estimate physical microwave (MW) treatment on biogas production potential.

b) Estimate chemical pre-treatment by Alkali on biogas production potential.

c) Evaluation the MW-assisted alkali pre-treatment method on biogas production potential.

1.3 Significance

Production of biogas is one of the most interesting areas in respect to environmental and renewable energy issues. Palestine suffers from a shortage of energy supply and sustains development resources. The use of straw for the production of biogas represents a sustainable option since it does not compete with human food resources. The study focused on the evaluation of production of biogas from WS by the aid of MW and alkali treatment.
Chapter II

Literature review
Chapter II
Literature review

2.1 Energy challenge:

The Palestinian territories are facing a critical situation with regard to the accomplishment of sustainable development. Many problems participate in the continuous decline of the political conditions, economic, social and environmental situations and handicapped the development creativities. The lack of a Palestinian infrastructure for about four decades has hindered any realistic progress on the energy front. Deficiency of conventional energy resources has created many problems such as shortage of energy, unrealistic price control, and energy crisis in the future. The Israeli military occupation, fragile and weakness of institutional framework and the imperfect structure of a Palestinian state offered an unclear picture about the national and comprehensive energy policy. The political stability in the region, rising demand for energy, the fragile economic system and the absence indigenous resources, all of these reasons are strongly affect the renewable energy market.

Despite the challenges that facing Palestine such as The environment of political risk, it is trying to exploit its natural resources for rehabilitation and construction (Yaseen, 2009).

One of the main pollution problems around the world is air pollution which is considered as a threat to national health. Most countries enforce strict regulations to reduce this problem as much as possible (Karaeen, 2012). The main sources of air pollution in Palestine are transportation, heavy dust from quarries, dust from seasonal dust storms and smoking factories (Khader & Hasan, 2005). As we know, burning of fossil fuels generates gases that are pollutant and harmful and cause environmental problems (Bose, 2013). As pollution is a troubling problem, it does not impair human health directly, but the GHGs trap the earth's heat and participate in climate change and global warming. The major sources of air pollution are diesel-powered vehicles, quarrying, melting lead and burning solid wastes (Karaeen, 2012).

One of the effects of the unjust siege of The Gaza Strip on the environment is the distressing facts about air pollution. Many of toxic gases such as carbon
monoxide and sulphur dioxide, which affect human respiration, are released from running home generators to recompense electricity shortage caused by the acute shortage of fuel. About 100,000 of the used generators consume about 500,000 liters of fuel per day (Karaeen, 2012). Therefore, it is necessary to provide clean and less expensive fuel sources for the Gaza Strip.

Many benefits could reap from using renewable energies instead of fossil fuels, including increasing energy services in rural areas, as a result, improvement of the economic sector, environmental issues and social sector (Thiam, 2011).

### 2.2 Biogas:

Biogas is a workable, renewable and clean energy source offered itself as an alternative to conventional sources of energy which are causing many ecological issues and because of the GHGs that released from it, and eventually, they will run out (Yadvika, Santosh, Sreekrishnan, Kohli, & Rana, 2004).

In Europe, the annual potential biogas production that out of all anaerobic processes was estimated over 200 billion m$^3$.

Statistics issued obtained by the European Union, it was found that the highest rate of biogas obtained from sludge was produced from Germany and Denmark, proportional to their population. Though Italy, Spain, and France, is similar to other countries, have indicated good performance with respect to their population (Hanjie, 2010; De Baere, 2006).

The main components of biogas are methane (55-70%) and carbon dioxide (35-40%), and a mix of trace gases including hydrogen sulfide, nitrogen, oxygen, and hydrogen. If methane ratio exceeded 45%; the composition will be flammable. As methane ratio be more high the obtainable energy from biogas will increase (Deublein & Steinhauser, 2011).

Many types of wasted organic substrates are applicable for biogas production processes. Such types of materials included wastes resulted from agricultural and industrial processes or household. Since biogas production utilizes waste streams, it is not expected to exert negative effects on food production regimes and surely will not cause deforestation. The created biogas is applicable for heating, energy
generation, transport activities, power plants or producing fertilizers (Benjaminsson & Nilsson, 2009).

Methane is non-toxic, colorless, odorless, and flammable gas and widely distributed in nature. The molecular weight of methane is 16.04 including 74.87% carbon and 25.13% hydrogen. Additionally, it represents the main component of the outer planets of our solar system as well as it used as cooking fuel. Methane is also used in the manufacturing many of gases such as hydrogen, hydrogen cyanide, formaldehyde, ammonia and organic synthesis (Clemens, Trimborn, Weiland, & Amon, 2006).

**Figure (2.1):** The sustainability of biogas production from anaerobic digestion process (Al Seadi, 2001)

AD is a biological process for renewable energy production in which biodegradable material such as an animal or food waste is broken down and converted into biogas by microorganisms in the absence of oxygen Fig 2.1 (Montgomery & Bochmann, 2014; Al Seadi, 2001).

The process of AD comprises four dissimilar phases: hydrolytic, acidogenic, acetogenic and methanogenic Fig 2.2 (Schön, 2010); and in each individual phase, different groups of facultative or obligatory anaerobic microorganisms are involved as shown in Fig 2.3 (Abdeshahian, Lim, Ho, Hashim, & Lee, 2016; Chasnyk, Sołowski, & Shkarupa, 2015; Christy, Gopinath, & Divya, 2014).
2.3 Benefits of biogas production

There are numerous advantages of biogas operations including production of valuable clean energy such as electricity and heat, decreasing the negative impressions of organic wastes on the environment (decreasing of GHG emissions and minoring bad effect on water resources and bodies) and augmenting the worth of remaining products (Das & Mondal, 2006).

Biogas is an appropriate energy source in many areas, that is because it is rich in energy. It is used for warming buildings (Alkhamis, El-Khazali, Kablan, & Alhusein, 2000), Electricity and heat production by a gas generator (Johansson & Burnham, 1993), Automobile fuel, gas network for cooking stoves, water heaters, stoves, etc. (Pöschl, Ward, & Owende, 2010), also it can be used in a lot of industries.
like sugar refineries, dairies, distilleries and paper mills and in processing produced wastes that can be digested directly on the site (Jiang, Sommer, & Christensen, 2011).

Using new energies have major problems, such as hard accessibility, discontinuity and the high cost of such energies in most countries across the world. While the generation of energy that produced from biogas emitted from wastewater treatment is considered as a new constant source of energy due to continuous production processes of sewage in cities (Bodík, Sedláček, Kubaská, & Hutňan, 2011; Malik & Bharti, 2009).

The key advantage of application AD systems emerged from the fact that biogas will be produced directly from anaerobic treatment of sludge. At present, some of the produced biogas is utilized for heating the AD reactors. Heating of the reactors will promote the efficiency of the process. (Coelho, Velázquez, Martins, & de Abreu, 2006).

The utilization of biogas produced from household could reduce rural energy demand and provide part of the electric energy for the rural consumption, and therefore reducing electricity costs imposed on villagers and farmers. Furthermore, residues remained after AD process can be applied as bio-fertilizer since it contains good ratios of nitrogen and other plant nutrients. (Li, Liao, Wen, Wang, & Yang, 2015; Dai, Chen, Hayat, Alsaedi, & Ahmad, 2015; Cheng, Li, Mang, Huba, Gao et al., 2014)

2.4 Biogas digesters:

Human and animal manure could be processed as a feedstock using biogas digesters to produce biogas, this method can reduce the bad grounds and cut disagreeable odors and diseases by excluding the pathogenic agents like viruses and worm eggs. This will, promote health quality and ecological life. (Li et al., 2015; Dai et al., 2015; Cheng et al., 2014).

The anaerobic reactor is a closed, heated tank deprived of oxygen which offers proper conditions for indigenous anaerobic bacteria to grow, multiply, and convert the organic wastes into biogas and a weak-odor effluent. Typical anaerobic digesters
have to be insulated, having short, silo-like arrangements. It can be in-ground rounded or rectangular concrete tanks having a stretchy or stiff covers. Organic wastes are supplied daily to the reactor, stays there for about 20 days and then it allowed to flow to a storage system. The same amount of material added daily to the anaerobic digester is also removed daily, that’s because there is no volume reduction with AD. During operation of the digester bacteria work to convert the added organic wastes to biogas and utilisable effluent. (Leggett, Graves, & Lanyon, 2006).

One of the advantages of digesters is that it can significantly reduce the emission of GHGs and provide useful products to society. Using biogas generated from agricultural wastes for electricity production could promote the affordability of the agricultural sectors involved in the operations of AD processes (Das & Mondal, 2006).

Introducing feedstock into the digester includes two methods: batch or continuous.

Batch systems involved the processing of bulk material of feedstock, where the digester loaded with the appropriate amount of fresh material, which is allowed to digest and then is completely removed at the end of the process. After that, the digester is fed again with a new portion and the process is repeated. Batch-type digesters are usually used for AD and also they are simple to build.

Compared to other systems, the advantage of batch digestion is the low operating costs and costs of the mechanical technology behind it, while it consumes energy (Al Seadi, Rutz, Prassl, Köttner, Finsterwalder et al., 2008).

The batch digestion system yields a cost-effective and economically viable means for the conversion of the ever-abundant municipal solid waste (MSW) to useful energy. It is therefore recommended that such AD systems be increasingly employed in order to (i) utilize this source of “waste” energy and (ii) therefore protect the environment (Igoni, Ayotamuno, Eze, Ogaji, & Probert, 2008).

In a continuous digester, the feedstock is constantly fed into the digester by two ways either mechanically or by the pressure of the new feed substrate, to push out the digested material. Continuous digesters differ from batch digesters, in the
continuous, there is no need to interrupt biogas production for lading new feedstock and unload the digested effluent, but the produced biogas predictable.

Continuous tank digesters can be in one of three forms horizontal, vertical, or multiple tank systems. It depends on the style of stirring of the substrate, continuous digesters can be completely mixed digesters and plug flow digesters. Completely mixed digesters are typically vertical digesters while plug-flow digesters are horizontal digesters (Al Seadi et al., 2008).

2.5 Challenges of the AD industry

Various challenges are facing the development of the AD industry include biological, economic, technical and environmental criteria (Table 2.1). To control AD process there is a need to maintaining delicate microbial ecosystems. Both the temperature and physical input into the digesters (e.g., inorganic and non-digestible waste) must be closely regulated to enable optimal gas production and to avoid the failure of process. The optimum ranges of parameters that encourage the constancy of anaerobic digestion system are exhibited in (Table 2.2) (Korres, O'Kiely, Benzie, & West, 2013).

**Table (2.1) challenges of the AD industry**

<table>
<thead>
<tr>
<th>Technical</th>
<th>Biological</th>
<th>Financial</th>
<th>Environmental and regulatory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactor design</td>
<td>Digestate stabilization</td>
<td>Capital cost and financing</td>
<td>Feedstock collection</td>
</tr>
<tr>
<td>Process control system</td>
<td>Inoculum stabilization</td>
<td>Incentives</td>
<td>Utility connections</td>
</tr>
<tr>
<td>Downstream products and processing</td>
<td>Feedstock stabilization</td>
<td>Co-products and markets</td>
<td>Permits and regulatory approvals</td>
</tr>
<tr>
<td>Digester operations</td>
<td>Feedstock Characteristics</td>
<td>On-farm versus centralized digesters</td>
<td>Nutrients</td>
</tr>
<tr>
<td>Temperature operating regime</td>
<td>Mono or co-digestion</td>
<td></td>
<td>management issues</td>
</tr>
<tr>
<td>Feedstock type</td>
<td>Inhibitory substances</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Process scale</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: (Nizami & Murphy, 2011; Saville, Duff, & Porter, 2008).
Table (2.2) Conditions of a stabilized anaerobic digester

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Optimal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkalinity</td>
<td>1,500-4,000 mg $\text{CaCO}_3 \text{ L}^{-1}$</td>
</tr>
<tr>
<td>pH</td>
<td>6.8-7.2</td>
</tr>
<tr>
<td>VS/TS (volatile solids/total solids)</td>
<td>&gt; 45%</td>
</tr>
<tr>
<td>$\text{NH}_4^+\text{N}$ (ammonium)</td>
<td>&lt; 1,500 mg $\text{L}^{-1}$</td>
</tr>
<tr>
<td>C/N/P (carbon to nitrogen to phosphorus proportion)</td>
<td>100-120:5:1</td>
</tr>
<tr>
<td>C/N (carbon to nitrogen ratio)</td>
<td>20-30:1</td>
</tr>
<tr>
<td>IA/TA (intermediate alkalinity to total alkalinity)</td>
<td>0.1-0.2 (&lt; 0.4)</td>
</tr>
</tbody>
</table>

Source: (Crolla, Duke, Kinsley, & Sauvé, 2012)

There is less work carried out to analyze lignocellulosic materials which aim to improve biogas yield. Thus, the research literature on best digester configurations for lignocellulosic materials is scarce (Nizami, Thamsiriroj, Singh, & Murphy, 2010). Digesters can be evaluated for optimal reactor configuration while at the same time using equal quantities of the same substrate loaded at the same rate. As the substrate is an important factor, the digester configuration requires optimization of different feedstocks (e.g., organic fraction of municipal solid waste (OFMSW), green waste), in order to determine the best configuration for a specific high solids substrate. Another aspect important to be considered in the feedstock are solid content and volatile content (Nizami, Korres, & Murphy, 2009).

A light-weight material such as hair or bedding animal can float to the top of the digester during the retention time, forming a crusty scum and insoluble or heavy material for instance dirt can deposit at the bottom which results in reducing the effective volume of the reactor and then incomplete digestion and odor problems, while crusting can keep gas from escaping the surface of the digesting manure. To control these problems, there are many ways to agitate the material inside the digester such as slurry pumps, strategic placement of the heating pipes and mechanical stirrer. Slurry pumps can keep the material in the digester effectively mixed. Strategic placement of the heating pipes will encourage thermal circulation and decrease the problems resulted from settling. Mechanical mixing can aid thermal uniformity, reduce settling, and break up crust formation. Mechanical mixing may be necessary for certain manure handling systems such as flush systems where solid and liquid portions can be separated easily into distinct layers within the digester. (Leggett et al., 2006).
2.6 Biogas production processes

Production of biogas from complex biomass materials attracted pronounced attention during the last years. Because of that many works have been conducted to realize AD process and identify the microbial consortia in charge of such bioconversion of organic matter to beneficial materials (biogas). In spite that studies in the field offered a great growing knowledge about biogas generation phases but still we need more studies to have adequate information about hydrolytic microbes within the process. (Azman, Khadem, Van Lier, Zeeman, & Plugge, 2015).

AD is a complex course includes actions of chemolithoautotrophic and chemoheterotrophic bacteria in addition to methanogens. The actions of microbes can be categorized into four diverse chronological interactions; hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During the whole process the complex biopolymers broke to shorter molecules and subsequently, the final product emerges as biogas (Azman et al., 2015). As occurred in any multi-step reaction courses, it is known that the slower reactions will control the whole rate of the transformation process. Such slow reactions frequently called as "rate limiting step". During the AD process situation is somewhat different as the nature of raw materials and working conditions decide the rate limiting step (Speece, 1983). As an example, if simple organic substrate is in use, like starch and simple sugar, degrading of those simple substances will be fast and easy converted to methane. In such case methanogenesis phase will be the rate limiting step (Noike, Endo, Chang, Yaguchi, & Matsumoto, 1985). This occurred because acidogenic reaction rates will be higher than methanogenic reaction rates. However, when the system is fed with complex biomass, hydrolysis phase will represent the rate limiting step. This attributed to hard and complex structure of plant materials (e.g. straw, wood and corn stover) which make hydrolysis step hard and slow. (Vidal, Dien, Ting, & Singh, 2011; Appels, Baeyens, Degrève, & Dewil, 2008; Palonen & Viikari, 2004; Lynd, Weimer, Van Zyl, & Pretorius, 2002). It is estimated that efficacies of methane production from agricultural wastes was around 50%. This is referred to slow incompetent hydrolysis of biomass within large-scale reactors (Weiland, 2010). However, theoretical yield calculations depending on the cellulose content of the biomass predict that about 90% of the biomass energy can be produced as methane (Azman et al., 2015).
2.6.1 Hydrolysis

Hydrolysis is a chemical reaction, in this step, insoluble complex organic polymers such as proteins, polysaccharides, and lipids are hydrolyzed into small sizes soluble products such as simple sugars, amino acids, and fatty acids, by extracellular enzymes, while another products of hydrolysis including hydrogen and acetate may be used later by methanogens. The appropriate small size allows the transportation of these soluble products across the bacterial cell membrane to be used generate energy and to synthesize cellular components.

Hydrolysis process is slow and consumes energy and is normally considered as the rate-limiting step for the complete AD of complex polymers. This step involves the dissociation of water, so that it is known as liquefaction process (Gallert & Winter, 1999; Vavilin, Ryto, & Lokshina, 1996; McCarty & Mosey, 1991; Pavlostathis & Giraldo - Gomez, 1991)

Hydrolytic reactions which comprise two phases are propelled by extracellular enzymes secreted by bacteria which are obligate or facultative anaerobes. The facultative anaerobes take the dissolved oxygen and thus cause the low redox potential necessary for obligate anaerobes (Gerardi, 2003). In the first phase, a bacterial colonization takes place where the hydrolytic bacteria cover the surface of solids. Bacteria located on the particle surface release enzymes and produce the monomers which can be utilized by the hydrolytic bacteria themselves, as well as by other bacteria. In the second phase, the bacteria will degrade the particle surface at a constant depth per unit of time (Vavilin et al., 1996).

2.6.2 Acidogenesis

Acidogenesis or acidification is the second step of the AD process, which results in the conversion of the hydrolyzed products into simple molecules with a low molecular weight, such as VFAs (e.g. acetic-, propionic- and butyric acid), alcohols, aldehydes and gases like H₂, CO₂ and NH₃. However, oxidizing long chain fatty acids (LCFA) are required organisms to utilize an external electron acceptor such as hydrogen ions or CO₂ to produce H₂ or formate (CH₂O₂). The degradation of monosaccharides (e.g. glucose) can be observed in different pathways which lead to the production of different products (Table 2.3) such as VFAs, lactate, and ethanol.
with different yields of energy. Several factors determine the dominant pathway such as substrate concentration, pH and dissolved hydrogen concentrations (Batstone, Keller, Angelidaki, Kalyuzhnyi, Pavlostathis et al., 2002).

Acidogenesis is often the quickest step in the anaerobic conversion of complex organic matter in liquid phase digestions. So, various toxic or inhibitory components can lead to process-failure in the AD process and therefore, halt methane production and an accumulation of long- and short-chain fatty acids (Vavilin et al., 1996).

Table (2.3): examples of different products from glucose degradation

<table>
<thead>
<tr>
<th>Products</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>$\text{C}<em>6\text{H}</em>{12}\text{O}_6 + 2 \text{ H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH} + 2\text{CO}_2 + 4\text{H}_2$</td>
</tr>
<tr>
<td>Propionate + Acetate</td>
<td>$3\text{C}<em>6\text{H}</em>{12}\text{O}_6 \rightarrow 4\text{CH}_3\text{CH}_2\text{COOH} + 2\text{CH}_3\text{COOH} + 2\text{CO}_2 + 2\text{H}_2\text{O}$</td>
</tr>
<tr>
<td>Butyrate</td>
<td>$\text{C}<em>6\text{H}</em>{12}\text{O}_6 \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{CO}_2 + 2\text{H}_2$</td>
</tr>
<tr>
<td>Lactate</td>
<td>$\text{C}<em>6\text{H}</em>{12}\text{O}_6 \rightarrow 2\text{CH}_3\text{CHOHCOOH}$</td>
</tr>
<tr>
<td>Ethanol</td>
<td>$\text{C}<em>6\text{H}</em>{12}\text{O}_6 \rightarrow 2\text{CH}_3\text{CH}_2\text{OH} + 2\text{CO}_2$</td>
</tr>
</tbody>
</table>

Source: (Batstone et al., 2002)

2.6.3 Acetogenesis

As already mentioned before, the degradation of higher organic acids formed in acidogenesis is an oxidation step with no internal electron acceptor. Thus, the oxidizing organisms (normally bacteria) require an additional electron acceptor such as hydrogen ions or $\text{CO}_2$ for the conversion to acetate, carbon dioxide and hydrogen (Batstone et al., 2002). This intermediate conversion is crucial for the successful production of biogas, as these compounds cannot be utilized directly by methanogens. Since acetogens are obligate hydrogen producers and in the same time depend on a low partial pressure of hydrogen, they maintain a syntrophic (mutually beneficial) relationship with hydrogen-consuming methanogenic archaea. This interspecies hydrogen transfer where the methanogens serve as a hydrogen sink allows the fermentation reactions to proceed. Syntrophy means, literally, “eating together” and is a special case of symbiotic cooperation between two metabolically different types of microbial organisms which depend on each other for degradation of a certain substrate, typically for energetic reasons (Björnsson, 2000; Schink, 1997). In this stage, acetogens further break down the hydrogen and carbon dioxide to produce mainly acetic acid and organic acids and alcohols are converted into
acetate. The acetate serves as a substrate for the next step, methanogenesis (Chandra, Takeuchi, & Hasegawa, 2012).

2.6.4 Methanogenesis

Methanogens are members of the Archaea domain and inhabit certain anaerobic environments, such as freshwater sediments abundant in organic matter, swamps and waterlogged soils, sewage treatment plants as well as the rumen of ruminants (Woese, Magrum, & Fox, 1978). During hydrolysis, the amounts of produced biogas are relatively small. Whereas, biogas production reaches its peak during methanogenesis (Al Seadi et al., 2008).

During methanogenesis, the fermentation products such as acetate and H₂/CO₂ are converted into CH₄ and CO₂ by methanogenic archaea which are strict obligate anaerobes. Other methanogens are able to grow on one-carbon compounds such as formate, methanol, and methylamine. Generally, methanogenic bacteria are specialists in substrate utilization, as some of these methanogens can use only one substrate (Björnsson, 2000; Pavlostathis & Giraldo-Gomez, 1991; Klass, 1984).

Three groups of the archaea that are relevant for AD. These groups are 1) the hydrogenotrophic methanogens, 2) the acetotrophic methanogens, and 3) the methylotrophic methanogens. The term “trophic” (from *trophe*, “nourishment”) refers to the substrates used by the bacteria.

**Group 1: Hydrogenotrophic methanogens**

The hydrogenotrophic methanogens convert carbon dioxide to methane using hydrogen (Equation 2.1). During this reaction, these organisms help to maintain a low partial hydrogen pressure in an anaerobic digester that is desired for acetogenic bacteria.

\[
\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \quad \text{(Equation 2.1)}
\]

**Group 2: Acetotrophic methanogens**

The acetotrophic methanogens “split” acetate into methane and carbon dioxide (Equation 2.2). The carbon dioxide produced from acetate may be converted to
methane by hydrogenotrophic methanogens (Equation 2.1). Some hydrogenotrophic methanogens produce methane from carbon monoxide (Equation 2.3).

\[ 4\text{CH}_3\text{COOH} \rightarrow 4\text{CO}_2 + 2\text{H}_2 \quad \text{(Equation 2.2)} \]
\[ 4\text{CO} + 2\text{H}_2\text{O} \rightarrow \text{CH}_4 + 3\text{CO}_2 \quad \text{(Equation 2.3)} \]

The acetotrophic methanogens reproduce more slowly than the hydrogenotrophic methanogens as the accumulation of hydrogen has a negative effect on it. Therefore, the maintenance of a low partial hydrogen pressure in an anaerobic digester is favorable to guarantee the activity of not only acetate-forming bacteria but also acetotrophic methanogens. Under a relatively high hydrogen partial pressure, acetate and methane production are reduced.

**Group 3: methylotrophic methanogens**

The methylotrophic methanogens are microorganisms that grow on substrates that contain the methyl group (−CH₃) to produce methane. Examples of these substrates include methanol (CH₃OH) (Equation 2.4) and methylamines [(CH₃)₃−N] (Equation 2.5). Group 1 and 2 methanogens produce methane from CO₂ and H₂, while Group 3 methanogens produce methane directly from methyl groups not from CO₂.

\[ 3\text{CH}_3\text{OH} + 6\text{H} \rightarrow 3\text{CH}_4 + 3\text{H}_2\text{O} \quad \text{(Equation 2.4)} \]
\[ 4(CH_3)_3N + 6H_2O \rightarrow 9CH_4 + 3CO_2 + 4NH_3 \quad \text{(Equation 2.5)} \]

Methane-forming bacteria differ on energy gain according to the substrate used. For example, production of methane from hydrogen results in more energy gain for methane-forming bacteria than acetate degradation. Although using hydrogen for methane production is the more effective process of energy capture by methane-forming bacteria, this method produces less than 30% of the methane in an anaerobic digester.

Nearly all known methanogenic species are able to utilize H₂/CO₂ to produce methane, whereas only a few species of methanogens are capable of utilizing acetate as a substrate. However, from stoichiometric relations, it has been estimated that about 70% of the methane formed in anaerobic digesters is derived via the acetate
pathway (Björnsson, 2000; Pavlostathis & Giraldo-Gomez, 1991; Klass, 1984), this is because of the limited supply of hydrogen in an anaerobic digester. Most of methane obtained from acetate is produced by two genera of acetotrophic methanogens, *Methanosarcina* and *Methanothrix* (Gerardi, 2003). The hydrogen pathway is more energy yielding than the acetate pathway and is normally not rate limiting. It is, however, of fundamental importance due to its ability to keep the hydrogen pressure low in the system (Björnsson, 2000; Pavlostathis & Giraldo-Gomez, 1991; Klass, 1984).

The methanogenic community might be affected potentially by methanogenesis. Such practices include changing the process operating parameters, such as temperature, OLR, HRT and substrate composition, as well as process environmental conditions such as pH and level of ammonia and VFAs. The pretreatment strategy chosen for the substrate may also affect the methanogenic community, as some pre-treatment methods can result in the release of inhibitors or methanogenic substrates, such as acetate or furfurals. It is noted that many reactions in the anaerobic process are affected by each other and that the methanogenic community shaped by the operating parameters might influence the overall degradation of the substrate (Sun, 2015).

### 2.7 Substrates traditionally used

A huge amount of organic solid waste is generated through human activities, which can be used as feedstock for biogas production. Based on the origin, the different waste streams can be classified as MSW, agricultural residues, and wastes from industrial activities. According to world bank report issued in 2012, 1.3 billion tons of MSW was generated per year by 3 billion urban residents all over the world, which by 2025 will increase to 2.2 billion tons (Hoornweg & Bahada-Taha, 2012). MSW mainly consists of food waste, paper, and paperboard, yard trimmings, plastic, wood, glass, and metal. However, its composition differs depending on regions and countries from these were collected. To be able to utilize this fraction of wastes for biogas production, all the inert material, should be removed prior to AD such as glass, metal, and plastic. Moreover, around 15 billion tons of waste, such as crop residues and animal manure, is generated worldwide annually from the agricultural
sector (Donkin, Doane, & Cecava, 2013). Food processing industries also generate waste, however, it is excessively difficult to estimate its amount, since it greatly depends on the industry and technology applied. For example, in industries based on juice production up to 50% of the processed fruit will end up as waste. Moreover, 30% of the weight of a chicken is unsuitable for human consumption, and it is therefore removed as waste during slaughtering and other processing steps (Forgács, Pourbafrani, Niklasson, Taherzadeh, & Hováth, 2012; Salminen & Rintala, 2002). Although all these different waste fractions are suitable for the production of biogas, their biogas potential varies significantly, whereas the biogas yield mainly depends on the composition and the biodegradability (under anaerobic conditions) of the waste. Theoretically, the highest biogas yield can be achieved from lipids (1.01 Nm$^3$ CH$_4$/ kg VS), followed by proteins (0.50 Nm$^3$ CH$_4$/ kg VS), and carbohydrates (0.42 Nm$^3$ CH$_4$/ kg VS) (Møller, Sommer, & Ahring, 2004). On the other hand, biodegradability defines how much of a given material is actually consumed during the process. Some compounds such as sugars degrade fast and completely, while the degradation of some other materials are take longer times which degrades at very low rates as for example, lignocellulose rich biomass.

2.7.1 Lignocelluloses as possible feedstock for biogas production

Lignocellulosic material is the most remarkable plant material resource for obtaining energy; however, its recalcitrant structure restrains its utility. Therefore, it is necessary to apply a suitable pre-treatment method to increase the sugar yield (Inan, Turkay, & Akkiris, 2014).

Lignocelluloses can be turned into commodity chemicals and a variety of biological fuels (Xiao, Shen, Zhang, & Gu, 2009).

2.7.2 Wheat straw

Cereal straws can serve as a potential feedstock for the production of biofuel where it is an abundant resource of lignocelluloses (Sun, 2010).

Many lignocellulosic plants are currently subject of studies concerning their ability to serve as substrates for biofuel production, from these plants sugarcane, sweet sorghum, maize, wheat, and their byproducts. The advantage of using such
materials as biomass for biofuel production is that these plants are short-rotation crops (Ivanova, Rákhely, & Kovács, 2009).

As with other lignocellulosic materials, WS is rough and easily available. Hydrogen bonding plays a major role in the combination of cellulose molecules and hemicellulose molecules, or the coalescence of lignin molecules. There are another main bonds link the hemicellulose and lignin molecules like ether bonds, ester bonds, glycoside bonds and acetal bonding. There is no chemical chain between the cellulose, hemicellulose or lignin molecules at all. However, the chemical chain can join hemicellulose and lignin molecules with hydrogen to make the cellulose molecule difficult to be hydrolyzed (Mandels & Sternberg, 1976).

Agricultural residues like WS represent large renewable resources for lignocellulosic bioconversion. WS is a widely available substrate and it is an environmental problem so as disposal of it is an essential issue, therefore, the transformation of this byproduct is, therefore, desirable. Bioconversion of WS is favored because of its relatively low lignin content (< 20%, w/w) and high carbohydrate content (hemicellulose (50%) and cellulose (30%)) (Harper & Lynch, 1981). These sugars can potentially be used microbial fermentations to produce biofuels using microbial fermentation, such as biogas, bioethanol, and hydrogen (Pawar, Nkemka, Zeidan, Murto, & van Niel, 2013). The typical chemical composition of WS is shown in (Table 2.4) (Wang, Zhou, & Fu, 2014c).

Table 2.4: Main components of wheat straw

<table>
<thead>
<tr>
<th>Component</th>
<th>Cellulose</th>
<th>Hemicellulose</th>
<th>Lignin</th>
<th>Ash content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Percentage (%)</td>
<td>51.16</td>
<td>23.69</td>
<td>12.12</td>
</tr>
</tbody>
</table>

Today, straw is often burned in fields to get rid of it without any energy recovery. In the future, straw could make a sustainable contribution to the power supply if it is fermented in a biogas plant (Deublein & Steinhauser, 2011).

2.8 Pre-treatment

Pretreatment is a necessary step to increase the accessibility of cellulose to the enzymatic attack for biogas production, therefore, alter some structural
characteristics of cellulosic materials (Beszédes, Tachon, Lemmer, Ábel, Szabó et al., 2012). The main goal of pre-treatment is to overcome the recalcitrance of lignocelluloses and therefore, increase the digestibility of maximum available sugars.

Many technologies used for pretreatment of lignocelluloses, physical (mechanical), physicochemical, chemical and biological. (Taherzadeh & Karimi, 2007). Each pre-treatment process has a specific effect on cellulosic components which are cellulose, hemicellulose, and lignin fraction, thus different pre-treatment methods and conditions must be chosen according to the process configuration selected for the subsequent hydrolysis and fermentation steps (Maurya, Singla, & Negi, 2015).

The pre-treatment process can change the compact structure of lignocelluloses; destroy the chemical and physical chains so as to decrease the cellulose crystallinity. It can also remove lignin and increase osteoporosity of the material to increase the effective contact with the cellulose system (Wang et al., 2014c). Therefore, an appropriate pre-treatment method should be selected to increase the efficiency of cellulose hydrolysis (Han, Feng, Zhang, Ma, Wang et al., 2012).

2.9 Pre-treatment methods

2.9.1 Physical pre-treatment:

2.9.1.1 Milling

Milling is a mechanical pre-treatment that breaks down the structure of lignocellulosic materials and decrease crystallinity of the cellulose (Sun & Cheng, 2002). Reducing the particle size of the material up to 0.2-2 mm is performed, which is advantageous in that it is helpful in increasing the surface/volume (Taherzadeh & Karimi, 2008) and a reduction of the degree of polymerization of cellulose ratio and consequently increase the exposure to enzymatic hydrolysis (Palmowski & Müller, 2000), which improves the efficiency of subsequent processing by reducing transportation costs, improving flow properties, and overcome heat and mass transfer limitations (Bitra, Womac, Chevanan, Miu, Igathinathane et al., 2009). Milling can be achieved by chopping or grinding. There are different types of milling such as ball
milling, hammer milling, disk milling, and vibratory milling, all these types are in use for improving enzymatic hydrolysis and saccharification (Taherzadeh & Karimi, 2008).

A disadvantage of milling is that the machine requires high power and consequently high energy costs (Hideno, Inoue, Tsukahara, Fujimoto, Minowa et al., 2009), it is likely that this process is not economically feasible (Hendriks & Zeeman, 2009).

### 2.9.1.2 Irradiation

Irradiation pre-treatment can be conducted by microwaves, electron beaming or gamma rays. It aids in improving the degradation process of lignocellulososes to some extent. The success of using this technique seems to be correlated to the amount of lignin as well as the degree of crystallinity characterizing the lignocellulososes (Taherzadeh & Karimi, 2008). MW irradiation has been increasingly used in the recent years because the heating efficiency is high and its operation is easy (Maurya et al., 2015).

Compared with conduction/convection heating, MW heating enables an electromagnetic field to directly interact with the heated object and generate heat. Therefore, it is more direct, rapid and uniform (Macquarrie, Clark, & Fitzpatrick, 2012) with no heat loss (Bordeleau & Droste, 2011). An internal heating of MW irradiation process is preferable because of the direct absorption of energy occurs by polar molecules, which differs from conventional heating that is based on transfer of heat (Ninomiya, Yamauchi, Ogino, Shimizu, & Takahashi, 2014; Wang, Maxim, Gurau, & Rogers, 2013; Hu, Han, Zhang, Ho, & Chen, 2012; Wang, Li, Cao, & Tang, 2011). Microwave irradiation affects the material by two ways, thermally and non-thermally. Thermally microwaves achieved by interacting between the molecules of the material and electromagnetic field of the MW energy to generate heat, non-thermal microwaves achieved by the effect of polar molecules and ions in the material which resulted in direct chemical, physical, and biological reactions (Yu, Chan, Liao, & Lo, 2010; Hong, Park, & Lee, 2004; Thostenson & Chou, 1999; Sridar, 1998).
MW can selectively heat the more polar parts in lignocellulosic material and creates “hot spots” in heterogeneous materials, this unique heating mechanism could result in an ‘explosion’ effect, which further enhances the disruption of the recalcitrant structure of lignocelluloses (Hu & Wen, 2008). Many researches has been done in the field of MW pre-treatment, with different feedstocks, such as sugarcane bagasse (Chen, Tu, & Sheen, 2011), rapeseed straw (Lu, Xi, Zhang, & Angelidaki, 2011), switchgrass (Keshwani & Cheng, 2010), and WS (Zhu, Wu, Yu, Wang, Yu et al., 2006b). The MW processing efficiency strongly depends on the physical properties of the subject, such as structural arrangements, conductivity and dielectric properties (Kappe, 2004).

Generation of a rapid volumetric heating of the material during MW irradiation, led to alternation of physicochemical characteristics of the material, which resulting in breaking down of the hemicellulose and lignin and increase the surface area of cellulose to cellulases (Jackowiak, Frigon, Ribeiro, Pauss, & Guiot, 2011; Ma, Liu, Chen, Wu, & Yu, 2009). It found that the MW pre-treatment affects the AD process and biogas production depending on various parameters (Rani, Kumar, Kaliappan, Yeom, & Banu, 2013) such as power and irradiation time, it was observed the sugar yield increased with the increase of irradiation time and power (Pang, Xue, Yu, Zhang, Li et al., 2012; Ma et al., 2009).

The exposure time of the material to MW irradiation ranges from 5 to 20 min. During this period, lignin and hemicelluloses are degraded, and with the aid of enzymes the ultra-structure of cellulose will change (Maurya, Vats, Rai, & Negi, 2013). The effect of MW irradiation on biogas production from wastewater was investigated by Passos et al., using various parameters, operating power, time, and temperature. They observed that the microalgae solubilization were enhanced by using MW pre-treatment and the production of methane also improved (Passos, Solé, García, & Ferrer, 2013). Jin et al., compared the effect of conventional heating and MW heating on AD process. The results obtained indicates that the solubilization of lignocellulose using MW heating were more than that of conventional heating, and the anaerobic digestibility also enhanced using MW heating (Jin, Hu, & Wen, 2009).
Wanitwattanarumlug et al., treat corn crops using MW-alkali, they observed structural changes occur by removing hemicellulose and lignin and thus increasing the total sugar yield (Wanitwattanarumlug, Luengnaruemitchai, & Wongkasemjit, 2012). Another study showed that there was a loss of total reducing sugars of 41% from oil palm empty fruit bunch fiber pretreated with MW in the presence of alkaline conditions. They also observed a loss of lignin and holocellulose of about 74% and 24.5% respectively (Nomanbhay, Hussain, & Palanisamy, 2013). Gabhane et al., reported that there was an improvement of about 10% in sugar yield from MW pre-treated garden biomass compared to autoclave and hot plate treatment techniques. (Gabhane, William, Vaidya, Mahapatra, & Chakrabarti, 2011). Kuglarz et al., study the effect of MW irradiation on filter paper with no lignin, they observed that the enzymatic hydrolysis does not improved, while treatment of newspaper with small amounts of lignin was slightly improved the enzymatic hydrolysis (Kuglarz, Karakashev, & Angelidaki, 2013), which means that the MW irradiation affects directly on lignin content, and consequently, estimation of lignin content is an indicator about MW irradiation effect (Kumakura & Kaetsu, 1983; Kumakura, Kojima, & Kaetsu, 1982; Kamakura & Kaetsu, 1982; Kumakura & Kaetsu, 1978). However, irradiation methods have disadvantages because the high cost and the difficulties in industrial application (Taherzadeh & Karimi, 2008).

Combination of MW irradiation and chemicals could be good choice to improve substrate digestibility and increase sugar yield.

MW pre-treatment could be further combined with the addition of chemicals to improve the sugar yield from the substrate (Maurya et al., 2015).

Microwave-assisted pre-treatment of lignocellulosic biomass have many advantages, it could save time, energy, and decrease inhibitors production. It is a promising technique for pre-treatment because it could change the native structure of lignocellulosic material by degradation of lignin, cellulose, and hemicellulose, and thus increasing the susceptibility of the substrate for hydrolysis enzymes (Lu et al., 2011).
2.9.2 Chemical pre-treatment

2.9.2.1 Alkaline pre-treatment

Alkali pre-treatments conducted using either mineral catalyst such as lime and NaOH or organic catalyst such as ammonia. Chemical catalysts can solubilize both lignin and hemicellulose molecules. Alkali pre-treatment is distinguish from acid pre-treatment in that the alkali-pretreatment conditions are mild and the degradation of sugars is minimal, and there is no inhibitory compounds formed.

Many researchers used alkali-pretreatment, several types of alkali pre-treatment are used, some of them are lime, ammonia percolation and ammonia fiber expansion (AFEX) (Volynets & Dahman, 2011). Alkali pre-treatment is most effective with the biomass with low lignin content such as agricultural residues but its effectively decrease with the increase of lignin content (Agbor, Cicek, Sparling, Berlin, & Levin, 2011). It is considered as thermochemical hydrolysis process leads to increase the transformation of non-soluble cellulosic compounds to soluble simple compounds (Hosseini, Aziz, Syafalni, & Kiamahalleh, 2011). It also reduces the inhibitory compounds during methane fermentation and the production cost is lower compared to other pre-treatment methods (Kumar, Barrett, Delwiche, & Stroeve, 2009; Pang, Liu, Li, Wang, & Yuan, 2008).

Compared to conventional heating NaOH/H₂SO₄ pre-treatment of Miscanthus, sugar yield using MW assisted pre-treatment was 12 times higher than the conventional heating. MW irradiation remove lignin and hemicellulose effectively as so as for makes the cellulose fibers more accessible to cellulases and consequently enhance the digestibility of the residue significantly (Zhu, Simister, Bird, McQueen-Mason, Gomez et al., 2015).

2.9.2.2 Acid pre-treatment

Many chemical bonds hold the lignocellulosic biomass, such as hydrogen bonds, van der Waals forces, and covalent bonds, these bonds could disrupt using acid pretreatment (Li, Knierim, Manisseri, Arora, Scheller et al., 2010). This consequently, resulted in solubilization of hemicelluloses and destroy the crystallinity of cellulose in order to loosen the structure of raw material, which can
improve the fermentability of cellulose. It is one of the most effective technique for lignocellulosic biomass treatment (Harmsen, Huijgen, Bermudez, & Bakker, 2010).

Many acids are used for wheat straw pre-treatment such as dilute or concentrated sulfuric acid, phosphoric acid, and propionic acid (Wang et al., 2014c). There are many advantages of dilute acid pre-treatment for industrial applications and it could be applied on different types of biomass. This method could solubilize hemicellulose and convert it into fermentable sugars. Using dilute acids in pre-treatment biomass produce fewer degradation products of sugar than the pre-treatment with concentrated acids (Alvira, Tomás-Pejó, Ballesteros, & Negro, 2010).

Treating wheat straw with sulfuric acid was a better-established and simpler method; however; it had an obvious shortfall in more cost because difficulties incurred for recycling the materials. Furthermore, sulfuric acid can corrode the reaction vessel and damage the instrument (Wang et al., 2014c). Moreover, acid pre-treatment or other pre-treatments performed at low pH may result in the production of fermentation inhibitors, like furfural and hydroxymethyl furfural (HMF). These inhibitors usually inhibit the microbial growth and consequently result in less yield and productivity of biogas or ethanol (Volynets & Dahman, 2011; Taherzadeh & Karimi, 2008).

2.9.3 Physico-chemical

2.9.3.1 Steam explosion

Steam explosion is the most widely demonstrated physicochemical method for treatment of lignocellulosic biomass. It is a hydrothermal pre-treatment process in which the biomass undergoes to pressurized steam for a period of time ranging from seconds to several minutes, and then suddenly depressurized (Pan, Xie, Gilkes, Gregg, & Saddler, 2005).

Steam explosion is done in the presence of steam along with some alkali or acid to increase the pre-treatment effectiveness of biomass under high pressure, 0.69 to 4.83 MPa and temperature (160–260°C) up to 20–30 min, and as a result the high extending of lignocellulosic biomass occurs, which facilitates hydrolysis of cellulose
(Soccol, de Souza Vandenberghhe, Medeiros, Karp, Buckeridge et al., 2010; Öhgren, Vehmaanperä, Siika-Aho, Galbe, Viikari et al., 2007).

This pre-treatment method has some weakness, which makes it less efficient for biomass with higher lignin content (e.g. softwood newspaper) as well as solubilization of very small fraction of the lignocellulosic material, mostly hemicellulose (Sun & Cheng, 2002).

2.9.3.2 Liquid hot water pre-treatment (LHW)

LHW is a hydrothermal pre-treatment, where use the pressure to maintain water in the liquid state, at a particular temperature. LHW pre-treatment can remove up to 80% of the hemicellulose and it can also increase the enzymatic digestibility of pretreated material (Ingram, Rogalinski, Bockemühl, Antranikian, & Brunner, 2009; Pérez, Ballesteros, Ballesteros, Sáez, Negro et al., 2008). LHW pre-treatment has the advantage of that it is conducted without adding chemicals and also minimizes hemicellulose degradation (Kristensen, Thygesen, Felby, Jørgensen, & Elder, 2008). The major challenge facing this pre-treatment method is to reduce the usage of water to make the process feasible economically for industrial scale operation (Volynets & Dahman, 2011).

2.9.3.3 Carbon dioxide Explosion (CO₂ Explosion)

CO₂ Explosion depends on the utilization of CO₂ as a supercritical fluid, which refers to a fluid that is in a gaseous form but is compressed at temperatures above its critical point to a liquid. Supercritical pre-treatment conditions can effectively remove lignin increasing substrate digestibility. Addition of co-solvents such ethanol can improve removal of lignin. Supercritical carbon dioxide (SC-CO₂) has been used mostly as an extraction solvent but it is being considered for non-extractive purposes due to its many advantages (Schacht, Zetzl, & Brunner, 2008). CO₂ penetrates the biomass at high pressure and once it dissolved in water, CO₂ will form carbonic acid, which assists in the hydrolysis of hemicellulose. The release of the pressurized gas results in the disruption of the biomass native structure and increasing the accessible surface area (Zheng, Lin, Wen, Cao, Yu et al., 1995).
2.9.4 Microwave alkaline pre-treatment

MW pre-treatments are now being used to enhance other pretreatments by using the combination of MW with other pretreatments. MW assisted chemical pre-treatments are by far the most common and successful technique in this regard. In this technique, the chemical pretreatment is enhanced by the assistance of MW so it is termed as MW assisted chemical pre-treatments (Laghari, Isa, Abdullah, Laghari, & Saleem, 2015).

The combination of MW and alkaline treatments techniques could improve the hydrolysis process by accelerating the main reaction of alkaline-pretreatment-delignification (Ding, Hii, & Ong, 2012). Preliminary experiments identified alkalis as suitable chemical reagents for MW-based pre-treatment (Zhu et al., 2006b). Keshwani and Cheng reported that the effect of MW treatment increased when it combined with the alkaline pre-treatment, decreases in the overall pre-treatment time and thus an increase in delignification efficiency. (Keshwani & Cheng, 2009), therefore the increased production of reducing sugars is predominantly attributed to the greater exposure of cellulose surface area of the lignocellulosic biomass treated with MW-assisted alkaline (Kumar et al., 2009).

Sodium hydroxide identified as the most effective alkali reagent for MW-based pretreatment (Alvira et al., 2010). Zhu et al., reported that when using MW-assisted alkali pre-treatment of WS, they observed a lower sugars losses and higher hydrolysis rates than the conventional alkali pre-treatment methods were observed (Zhu, Wu, Yu, Chen, Wu et al., 2006a).

Another study on MW-based alkali pretreatment of switchgrass observed that there was low energy requirement for extended pretreatment time and obtained 70-90% sugar yields (Hu & Wen, 2008).

The combination of MW irradiation with chemical pre-treatments as well as the MW irradiation at temperatures higher than 145 °C resulted in a larger component of refractory material per gCOD, causing a decrease in the biogas generation. Similar results were observed with pig manure pretreated with lime and heated at temperatures higher than 110 °C (Rafique, Poulsen, Nizami, Murphy, & Kiely, 2010; Carrère, Sialve, & Bernet, 2009).
2.9.5 Biological

Biological pre-treatment methods are used for the delignification of lignocellulosic biomass (Chan, Rudravaram, Narasu, Rao, & Ravindra, 2007); it basically uses microorganisms such as brown-, white- and soft-rot fungi, which are capable of producing various enzymes have an ability to degrade lignin and hemicellulose (Balat, 2011), this strategy of pre-treatment requires low energy input, low capital cost, and mild environmental conditions. Otherwise, it is not preferred at industrial scale because of slow degradation rates (AITA & KIM, 2010; Hahn-Hägerdal, Galbe, Gorwa-Grauslund, Lidén, & Zacchi, 2006).

2.10 Operational Conditions of anaerobic digestion:

Methanogens are strict anaerobes and are very sensitive to changes in alkalinity, pH, and temperature. Therefore, operational conditions in the bioreactor should be periodically monitored and maintained within optimum conditions. Any drastic change in these parameters can adversely affect the production of biogas. In addition to alkalinity, pH, and temperature, several other operational conditions must be monitored and maintained within optimum ranges for the acceptable activity of methane-forming bacteria. These conditions are gas HRT, composition, oxidation-reduction potential (ORP), and volatile acid concentration.

2.10.1 pH

pH is an important environmental factor that affects the stability of biogas plants. Low pH can inhibit acidogenesis and pH below 6.4 can be toxic for methane forming bacteria. The preferred pH within the AD process is generally within the range 6.0-8.5. The methanogenic bacteria grow optimally at around neutral pH and a pH value outside this range can inhibit the bacterial growth, thus resulting in unstable digester performance and sometimes even process failure (Chandra et al., 2012).

2.10.2 Temperature

Most methane-forming bacteria are active in two temperature ranges, the mesophilic range 30–35°C and the thermophilic range 50–60°C. Methane-forming bacteria are inhibited at temperatures between 40 and 50°C (Deublein & Steinhauser,
Digester performance falters somewhere near 42 °C, as this temperature represents the transition from mesophilic to thermophilic bacteria (Gerardi, 2003).

Any fluctuating or changing of temperatures within stable bioreactors may cause lower biogas production or temporarily disturbance in methanogenic activity (Cioabla, Ionel, Dumitrel, & Popescu, 2012; Chae, Jang, Yim, & Kim, 2008), whereas, with raising the temperature the toxicity of H₂S increase (Deublein & Steinhauser, 2011). The optimum temperature of digestion may vary depending on feedstock composition and type of digester, but in most AD processes, it must be maintained relatively constant to sustain the gas production rate.

Thermophilic digesters are more efficient in terms of loading rate, retention time, and nominally gas production, but they need a higher heat input and have a greater sensitivity to operating and environmental variables, which make the process more problematic than mesophilic digestion (Verma, 2002).

### Table (2.5): Comparison of mesophilic and thermophilic AD

<table>
<thead>
<tr>
<th>Process operation</th>
<th>Mesophilic (35°C)</th>
<th>Thermophilic (55°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degradation rate</td>
<td>Lower</td>
<td>Higher</td>
</tr>
<tr>
<td>Methane yield</td>
<td>Lower</td>
<td>Higher</td>
</tr>
<tr>
<td>Hydraulic retention time</td>
<td>Longer, or the same</td>
<td>Shorter, or the same</td>
</tr>
<tr>
<td>Sanitation</td>
<td>No</td>
<td>Possible</td>
</tr>
<tr>
<td>Energy demand</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Temperature sensitivity</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Process stability</td>
<td>Higher lower</td>
<td></td>
</tr>
</tbody>
</table>

Source: (Forgács, 2012)

However, higher temperatures require more energy, and the process is more sensitive to changes in the operational conditions. For example, at high temperatures, thermophilic methanogens are more sensitive to the accumulation of VFAs, and the increasing ammonium pKa at elevated temperature leads to increasing of free ammonia, which is more toxic. Table 2.5 summarizes the differences in AD under mesophilic and thermophilic conditions (Forgács, 2012).
2.10.3 Carbon to nitrogen ratio (C/N ratio)

C/N ratio represents the relationship between the amount of carbon and nitrogen present in an organic material.

A too high C/N ratio indicates a lack of nitrogen, which then results in lower gas production (Verma, 2002). Nitrogen deficiency has severe consequences for protein formation and thus the energy and structural material metabolism of the microorganisms result (Deublein & Steinhauser, 2011). On the other hand, a low C/N ratio causes accumulation of ammonia and then pH values may override 8.5. Such conditions can be toxic to methanogenic bacteria (Verma, 2002). To achieve optimum C/N ratios of the digester materials, different substrates of high and low C/N ratios can be mixed together, such as organic solid waste mixed with sewage or animal manure (Vögeli, Lohri, Gallardo, Diener, & Zurbrügg, 2014).

A balanced composition is necessary; e.g. the mixture of WS and chicken feather.

2.10.4 Retention (residence) time:

In anaerobic digesters, there are two important retention times which are solids retention time (SRT) and HRT. The SRT is a significant design and operating parameter, it is the average time that bacteria (activated-sludge solids) are in the anaerobic digester. While the HRT means the average length of time that the sludge or wastewater remains in the anaerobic digester (Gerardi, 2003).

The nature of feedstock and process temperature applied could affect and vary the retention time within the reactor. The digesters that operate in the thermophilic range are require lower retention time (Verma, 2002).

High retention time value have many advantages, it increases required digester volume, maximizes volatile mass removal capacity, and provide buffering capacity for protection against the effects of shock loadings and toxic compounds in wastewaters and sludge of the reactor. (Gerardi, 2003) High retention time values also help to permit biological acclimation to toxic compounds, on the other hand, a disadvantage of high retention time is that a larger volume of the reactor at a particular OLR is required (Yadvika et al., 2004).
Methanogens have a long retention time generally, and it must be at least 10-15 days with reactor systems to avoid washing out from the reactor which do not have means for returning and retaining the biomass (Deublein & Steinhauser, 2011).

2.10.5 Particle size

It has been repeatedly shown that smaller particle sizes result in higher yields (Teghammar, Karimi, Horváth, & Taherzadeh, 2012; Lennartsson, Niklasson, & Taherzadeh, 2011; Monavari, Galbe, & Zacchi, 2009; Jin & Chen, 2006). The effect of particle size on biomethane production from food waste was studied by Izumi et al. They obtained about 40% higher chemical oxygen demand (COD) solubilization when the size of the substrate was reduced through a beads mill. While more reduction of the particle size to smaller than 0.7 mm caused an accumulation of VFAs (Izumi, Okishio, Nagao, Niwa, Yamamoto et al., 2010).

It is recommended to reduce the particle size to produce higher amounts of biogas, because this process increases the efficiency of hydrolysis process and consequently, improving the overall digestion process (Deublein & Steinhauser, 2011; Yadvika et al., 2004; Gunaseelan, 1997).

2.10.6 Volatile fatty acids (VFAs):

VFAs represent fatty acids from C2 to C5 (acetic, propionic, butyric, etc.) (Fontanille, Kumar, Christophe, Nouaille, & Larroche, 2012). It can be obtained from agro-industrial lignocellulosic sludge, wastes, and a variety of biodegradable organic wastes (Chang, Kim, Kang, & Jeong, 2010). Recently, VFAs are derived from AD of biomass as a cost-effective and environmentally friendly alternative for the production of VFAs (Wang, Yin, Shen, & Li, 2014a).

VFAs are important mid-products in the production of methane, and the efficiency of fermentation affected by their concentration (Wang, Zhang, Wang, & Meng, 2009). An accumulation of VFAs will result in lowering pH value, under such conditions methanogenesis cannot occur anymore, and consequently more decreasing of pH value. Too high hydrogen pressure will increase the VFAs which will decrease pH value again.
Accumulation of VFAs has been regarded as a sign of process failure in AD process (Vanwonterghem, Jensen, Rabaey, & Tyson, 2015) which inhibit the methanogenic activity and then decrease the methane production rate during the AD processes (Xu, Zhao, Miao, Huang, Gao et al., 2014), and results in formation of toxic compounds in the reactor (Murto, Björnsson, & Mattiasson, 2004; De Mes, Stams, Reith, & Zeeman, 2003). So, VFAs concentrations must be used as an important operational parameter to control and manage the AD process (Lee, Lee, Bae, Kang, Kim et al., 2015).

2.10.7 Sulphur compounds:

Sulfate represents a problem, because it helps in production of H₂S in a step before methane formation, and then, the process can be inhibited.

\[
\begin{align*}
\text{SO}_4^{2-} + 4\text{H}_2 & \rightarrow \text{H}_2\text{S} + 2\text{OH}^- \\
\text{SO}_4^{2-} + \text{CH}_3\text{COOH} & \rightarrow \text{H}_2\text{S} + 2\text{HCO}_3^-
\end{align*}
\]

Sulphate compounds can also inhibit production of methane, because sulphate-degrading microorganisms are dominant and they need less energy and/or do not a symbiosis partner (Deoblein & Steinhauser, 2011).

2.11 Previous studies

Pre-treatment of WS with NaOH could change its composition and structure, and therefore, exhibits good performance on enhancing enzymatic hydrolysis (Jiang, Zeng, Nie, Li, Ding et al., 2016).

For methane production, an efficient pre-treatment process of wheatgrass by MW is obtained by increasing the temperature up to 150°C, which increased sample solubilization corresponding to the maximum volume of generated methane (Jackowiak, Bassard, Pauss, & Ribeiro, 2011).

Farm animal waste treatment by using the AD process could potentially contribute in production of large amounts of biogas as a renewable energy source. Also, treating organic matter anaerobically could be used as fertilizer and biochemicals for crop cultivation in the agriculture land. Furthermore, treating
animal waste anaerobically could enhance the public health by reducing the detrimental impacts (Abdeshahian et al., 2016).

Alkaline pre-treatment of WS was optimized to maximize the fermentable sugar yield and to remove as much lignin as possible, that’s for improving rheological attributes of the media. Enzymatic hydrolysis of WS achieved 93.1% conversion of cellulose to glucose and 80.3% yield of monosaccharides (Jaisamut, Paulová, Patáková, Rychtera, & Melzoch, 2013).

The biomethane potential of Ca(OH)$_2$-pre-treated WS was 36% higher than untreated WS (Sträuber, Bühligen, Kleinsteuber, Nikolausz, & Porsch, 2015).

The highest yield of fermentable sugars were obtained from MW assisted-alkaline pre-treated coconut husk after enzymatic hydrolysis. There were a significant and obvious disruption of the structure of coconut husks after pre-treatment process (Ding et al., 2012).

A sequential dilute acid/alkaline process was developed for efficient pre-treatment of rice straw, resulting in high enzymatic digestibility and high sugar recovery from the solid residues (Weerasai, Suriyachai, Poonsrisawat, Arnthong, Unrean et al., 2014).

The MW assisted alkali pre-treatment of empty fruit bunch (EFB) using NaOH, improved the enzymatic saccharification of EFB, this pre-treatment process remove lignin and hemicellulose and therefore increase the accessibility of EFB to hydrolytic enzymes (Nomanbhay et al., 2013).

MW assisted KOH pre-treatment of bamboo significantly improved the enzymatic hydrolysis by removing the recalcitrance of bamboo structure, and increased the glucose yield by 8.7 folds and xylose by 20.5 folds, at 48 hr compared with untreated bamboo. Compared to acid pre-treatment, the fermentation inhibitors such as furfural, formic acid, levulinic acid and HMF were lower (Li, Jiang, Fei, Yu, & Cai, 2012).
Chapter III
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3.1 Materials:

3.1.1 Chemicals

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Manufacture</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrochloric acid</td>
<td>Himedia</td>
<td>India</td>
</tr>
<tr>
<td>Sodium Hydroxide</td>
<td>Oxford lab. Chem.</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>Sigma</td>
<td>USA</td>
</tr>
<tr>
<td>Calcium Hydroxide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose assay kit</td>
<td>Chemelex</td>
<td>Spain</td>
</tr>
</tbody>
</table>

3.1.2 Equipment and apparatus:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Manufacture</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shaking water bath</td>
<td>N-Biotech</td>
<td>Korea</td>
</tr>
<tr>
<td>Centrifuge combi 514r</td>
<td>Hanil science industrial</td>
<td></td>
</tr>
<tr>
<td>Microwave oven</td>
<td>JAC</td>
<td>China</td>
</tr>
<tr>
<td>Analytical balance (max 150g-d 0.005g)</td>
<td>Ae-ADAM equipment</td>
<td>U.S.A</td>
</tr>
<tr>
<td>PH/mV Meter</td>
<td>Azzota</td>
<td></td>
</tr>
<tr>
<td>Refrigerator 0 C up To 15</td>
<td>Boeco</td>
<td>Germany</td>
</tr>
<tr>
<td>Spectrophotometer (Ct-220)</td>
<td>ChromTeck</td>
<td>Taiwan</td>
</tr>
</tbody>
</table>

All the experiments performed in biology and biotechnology laboratories in Islamic university-Gaza.

3.1.3 Inoculum:

In this work, a consortium of anaerobic microorganisms was obtained from anaerobic digester of Sheikh Ijleen wastewater treatment plant.
3.1.4 Collection of wheat straw:

The WS used as a substrate were obtained from local farms of Gaza in Palestine.

3.1.5 Collection of chicken feather:

The chicken feather (CF) used to adjust C/N ratio for anaerobic digestion. CF was obtained from a local poultry slaughterhouse.

3.1.6 Reactor design:

There were six Erlenmeyer flasks capped with rubber stoppers. The reactors were placed into a water bath maintained at 38°C. There was a hole in each rubber cap attached to a tube; the tube then went from the bottle to a graduated cylinder containing water at a specified level. All graduated cylinders were calibrated before the tests were started and the biogas fermentation reactors were checked for any leakage. Biogas volume was periodically measured using water displacement method (Fig.3.1). A records date, time, temperature, and the volume of gas were achieved.
3.2 Methods:

3.2.1 Preparation of wheat straw:

Firstly, WS has been cut into pieces of size 1-2 cm. Now the prepared samples of WS are cleaned thoroughly using tap water until the washings became colorless and clear. Before any pre-treatment samples have been air dried (Singh, Tiwari, Srivastava, & Shukla, 2014) and ground to powder. Dried straw was stored in sealed glass containers at room temperature.

Part of the dried sample was used to estimate the C/N ratio and conducted in Environment & Earth Science department at the Islamic University of Gaza

3.2.2 Preparation of chicken feather:

Chicken feather was washed several times with tap water and detergents until the washings became colourless and clear and then air-dried at room temperature. Then, the dried feathers were ground into 2–3 mm particle and stored at 5 °C until use.

Part of the dried sample was used to estimate the C/N ratio and conducted in Environment & Earth Science department at the Islamic University of Gaza

3.2.3 Adjusting C/N ratio:

Optimal C/N ratio of biomass is an essential parametric requirement for efficient methane fermentation process. Since the optimal C/N ratio for AD is considered of about 20-30, the C/N ratio of WS were adjusted to 20:1 by adding an appropriate amount of pre-treated chicken feathers.

According to C/N ratio analysis, it was found that the C/N ratio of WS was about 83:1, while CF has a C/N ratio of about 6 (Table 3.1).

Table 3.1: C/N ratio of wheat straw and chicken feather.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total carbon (g/Kg)</th>
<th>Total nitrogen (g/Kg)</th>
<th>C/N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat straw</td>
<td>128.0</td>
<td>0.9</td>
<td>83:1</td>
</tr>
<tr>
<td>Chicken feather</td>
<td>10.8</td>
<td>0.1</td>
<td>6:1</td>
</tr>
</tbody>
</table>
The C/N ratio was determined by dividing the total organic carbon content by total nitrogen content according to the following equation (Eq. 3.1) (Wang, Lu, Li, & Yang, 2014b):

\[
C/N = \frac{W_1 \times C_1 + W_2 \times C_2}{W_1 \times N_1 + W_2 \times N_2}
\]

Eq. 3.1

Where W1 weigh of WS (Kg), W2 weigh of CF (Kg), C1 organic carbon content of WS (g Kg \(^{-1}\) VS), C2 organic carbon content of CF (g Kg \(^{-1}\) VS), N1 organic carbon content of WS (g Kg \(^{-1}\) VS), N2 organic carbon content of C (g Kg \(^{-1}\) VS).

According to the previous calculations, each 1Kg of WS needs 0.375 Kg of CF.

C/N ratio was also adjusted using urea according to (Eq. 3.1), each 1Kg of WS needs 0.108 Kg of urea. The results were compared with that from using CF.

### 3.2.4 Microwave-alkaline treatment of wheat straw

MW treatment was carried out using a domestic MW instrument (JAC, China). The MW instrument was operated at 2450 MHz and 450 W. WS samples of 10, 20, 30 and 40g were immersed in 300 ml solution of 1% and 2% NaOH in 500 ml Erlenmeyer flasks for 5 min and shaking well. The flasks subjected to MW irradiation in MW oven. The exposure time was varied at 5, 10, 15 and 20 min. The resulted mixture was passed to shaking water bath at 90°C temperature for 1, 2, 3, 4 and 5 hr. At the end, the reaction mixture was neutralized with 1N HCl solution to pH 7 (Binod, Satyanagalakshmi, Sindhu, Janu, Sukumaran et al., 2012).

Liquid supernatants were decanted into tubes, centrifuged, and evaluated for reducing sugar concentration. All experiments were done at least in duplicate, and the given numbers are the mean values

### 3.2.5 Chicken feather pre-treatment:

Feather samples were pre-treated with 0.1 g/g Ca(OH)\(_2\) for 30 min in an autoclave. (Forgács, Niklasson, Horváth, & Taherzadeh, 2014). After pre-treatment process, pH was neutralized to 7.0 with 1N HCl.
3.2.6 Fermentation:

Batch cultures of dark fermentation were performed in 500 mL reactors containing untreated WS, pre-treated WS or pre-treated WS mixed with pre-treated CF. All of the batch digestion experiments are conducting in duplicates. An inoculum of 10% was added to each flask containing either untreated or pre-treated sample.

3.2.7 Bioreactors parameters monitoring:

The batch experiments were carried out for 6-13 days until most of the gas production had stopped. To evaluate the biogas yield, the amount of biogas in each reactor was monitored during the experimental period.

3.2.8 Estimation of reducing sugars

Estimation of reducing sugars conducted by using glucose assay kit (BioSystems S. A., Spain).

3.2.8.1 Method

"GOD-POD": Enzymatic photometric test.

3.2.8.2 Principle

Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid. The formed hydrogen peroxide (H₂O₂) is detected by a chromogenic oxygen acceptor, phenol, 4 – aminophenazone (4- AP) in the presence of peroxidase (POD):

\[
\text{Glucose} + \frac{1}{2} \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{GOD}} \text{Gluconate} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4 - \text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4\text{H}_2\text{O}
\]

3.2.8.3 Assay procedure

- Wavelength: 500 nm.
- Cuvette: 1 ml
- Temperature: 16-25°C/ 37°C
1. Bring the reagent to room temperature.
2. Pipette into labeled test tubes

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Standard (S)</td>
<td>___</td>
<td>10 µL</td>
<td>___</td>
</tr>
<tr>
<td>Sample</td>
<td>___</td>
<td>___</td>
<td>10 µL</td>
</tr>
<tr>
<td>Reagent (A)</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
</tr>
</tbody>
</table>

3. Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25°C) or for 5 minutes at 37°C.
4. Measure the absorbance (A) of the standard and the sample at 500 nm against the blank. The color is stable for at least 2 hours.

### 3.2.8.4 Calculations

The glucose concentration in the sample is calculated using the following general formula:

$$C_{\text{sample}} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times C_{\text{standard}}$$

If the glucose standard provided has been used to calibrate:

$$\frac{A_{\text{sample}}}{A_{\text{standard}}} \times 100 = \text{mg/dL glucose}$$

$$\frac{A_{\text{sample}}}{A_{\text{standard}}} \times 5.55 \text{ mmol/L glucose}$$

### 3.2.9 Biomethane assay:

Biomethane estimation analysis was performed by volumetric method. Sixty ml of the collected gas was taken with a syringe from the top of the gas collecting cylinder that has rubber cover and the gas is injected through a series of scotch bottles tightly closed: The first contains 10% NaOH to precipitate CO₂ gas and the other contains 5% NH₄OH precipitate H₂S. The third bottle contains H₂O which is collected in measuring cylinder upon displacement and accounted for CH₄ gas (Figure 3.2).
Figure (3.2) Experimental setup of the biochemical methane potential assay used for the anaerobic digestion of substrates used in this study.
Chapter IV

Results
Chapter IV

Results

This study was conducted to determine the produced methane from untreated, NaOH -microwave pre-treated WS and NaOH -microwave pretreated WS mixed with pre-treated chicken feather through anaerobic batch digestion assays.

AD was attained for 13 days with untreated and pre-treated (with 1% and 2% NaOH at 90°C) WS to study the effect of different pre-treatment conditions on the biogas production.

The work started with the determination of C/N ratio of WS which was 83, thus, the C/N ratio of WS biomass was adjusted to 25 by adding the appropriate amount of pre-treated chicken feathers.

All experiments were carried out in a water bath in the range of 38°C-40°C. Glucose concentration was measured before biogas production and post-production of biogas in order to determine the glucose consumption in the process of AD in biogas production and to investigate the value of the remaining organic matter consumed during the AD process.

4.1 Effect of microwave irradiation time on alkaline-pretreated wheat straw:

In this part of work, four different amounts (10, 20, 30 & 40 g) of WS were subjected separately for NaOH-microwave treatment to enhance the digestibility of WS prior the biogas production.

Two concentrations of NaOH (1% and 2%) investigated with different time intervals between 5-20 min in microwave instrument to determine the best pre-treatment method. The results of the amount of glucose produced after alkali-microwave pre-treatment are summarized in (Figure 4.1 and 4.2).

In this study, the microwave-assisted NaOH pre-treatment positively affected the glucose concentration. It was found as a result of the alkali-microwave pre-treatment of 40 g WS, the glucose concentration was 31.6 and 45.3 mg/dl at 10 min irradiation time at 1% and 2% NaOH respectively. It can be noticed, that significant
difference was not observed in the quantity of produced sugar after MW pre-treatments for 10 min.

**Figure (4.1):** Effect of microwave irradiation time at 1% NaOH pre-treated wheat straw on glucose production.

**Figure (4.2):** Effect of microwave irradiation time at 2% NaOH pre-treated wheat straw on glucose production.
Because there was an increase of glucose concentration with an increase of WS weight for 1% and 2% NaOH, and to get an optimum concentration of NaOH, 40 g wheat straw were pre-treated with 3% and 4% NaOH, the results showed a decrease of glucose concentration to 40.4 and 29.2 mg/dl for 3% and 4% NaOH respectively (Figure 4.3) under MW treatment for 10 min.

**Figure (4.3):** Effect of microwave irradiation time at 3% and 4% NaOH of 40 g pre-treated wheat straw on glucose production.

### 4.2 Effect of heating time on alkali-microwave pre-treated straw

Applying thermal pre-treatment on alkali-microwave pre-treated WS at different time intervals (1-4 hr.) at 90 °C resulting in an increase of glucose concentration of about 58.6 mg/dl was observed at 2% NaOH concentration and 3 hr heating time, (Figure 4.4, 4.5 and 4.6).
Figure (4.4): Total sugar concentrations at different time intervals at 90°C in shaking water bath, 1% NaOH and 10 min irradiation time.

Figure (4.5): Total sugar concentrations at different time intervals at 90°C in shaking water bath, 2% NaOH and 10 min irradiation time.
Figure (4.6): Total sugar concentrations at different time intervals at 90°C in shaking water bath, 3% and 4% NaOH, 40 g wheat straw and 10 min irradiation time.

Based on the glucose concentration results, the following pre-treatments were selected for further investigations regarding the following gas production: 1%, 2%, 3% and 4% NaOH for 10 min in MW followed by incubating at 90 °C for 3hr in shaking water. The same procedure of pre-treatment was conducted and the pre-treated straw was used for biogas production with or without mixing with a pre-treated chicken feather to attain the C/N ratio of 25.

4.3 Effect of alkali-microwave treatment on biogas production:

Methane production of the selected WS samples was carried out in the bath of AD, the methane production of pre-treated WS with or without pre-treated CF and the methane production of untreated WS were compared.

Each treatment was duplicated, and the cumulative methane produced was calculated during the 13-day long incubation period. All treatments were operated for biogas production in parallel with a control bottles without any treatment.
4.3.1 Effect of alkali-microwave treatment on biogas production when total wheat straw was 10 g:

Table 4.1 and Figure 4.7 present the cumulative biogas production obtained from 10 g of NaOH-microwave pretreated WS (1% and 2% NaOH) with or without adding pre-treated chicken feather comparing with control bottles during the 13-day long incubation period.

For 1% and 2%, NaOH-microwave pretreated straw with or without mixing with a pretreated chicken feather, there was an increase in biogas production comparing to control samples, whereas the maximum biogas production was 700 ml for 2% NaOH-microwave pretreated straw.

1% and 2% NaOH-microwave pretreated straw gave a higher amount of biogas 450 ml and 700 ml respectively comparing to 1% and 2% NaOH-microwave pretreated straw mixed with pretreated chicken feather 300 and 480 ml respectively. While untreated WS gave 200 ml of biogas.

Table (4.1): Cumulative biogas production for samples pre-treated with NaOH 1%, 2% at 38 °C and total solid material of 10 g.

<table>
<thead>
<tr>
<th>Days</th>
<th>Cumulative biogas (ml) from 10 g wheat straw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
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<td>4</td>
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<td>6</td>
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</tr>
<tr>
<td>12</td>
<td>200</td>
</tr>
<tr>
<td>13</td>
<td>200</td>
</tr>
</tbody>
</table>
4.3.2 Effect of alkali-microwave treatment on biogas production when total wheat straw was 20 g:

The results of using 20 g WS are illustrated in (Table 4.2 and Figure 4.8). The maximum biogas production was 830 ml when WS was pre-treated with 2% NaOH while adding pre-treated feather to 2% NaOH pre-treated WS decrease the total biogas production to 640 ml.

In comparison with control, which produces 500 mL biogas; there was a significant increase to total biogas produced when WS pre-treated with 1% and 2% NaOH, 760 & 830 ml respectively. The lowest total biogas production was to 1% NaOH pre-treated WS mixed with pre-treated feather.
Table (4.2): Cumulative biogas production for samples pre-treated with NaOH 1%, 2% at 38 °C and total solid material of 20 g.

<table>
<thead>
<tr>
<th>Days</th>
<th>Cumulative biogas (ml) from 20 g wheat straw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>untreated</td>
</tr>
<tr>
<td>1</td>
<td>140</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
</tr>
<tr>
<td>3</td>
<td>310</td>
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<tr>
<td>4</td>
<td>420</td>
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<tr>
<td>5</td>
<td>470</td>
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<td>12</td>
<td>500</td>
</tr>
<tr>
<td>13</td>
<td>500</td>
</tr>
</tbody>
</table>

Figure (4.8): A Cumulative Biogas Production for Samples Pre-treated with NaOH 1% & 2% at 38°C Total Solid Material of 20g
4.3.3 Effect of alkali-microwave treatment on biogas production when total wheat straw was 30 g:

The results obtained when 30 g of WS used (Table 4.3 and Figure 4.9), are different in comparison to 10 and 20 g WS with respect to adding pre-treated feather.

The maximum total biogas production was 1000 ml obtained from 2\% NaOH-microwave pre-treated straw mixed with pre-treated feather, followed by 2\% NaOH-microwave pre-treated straw without mixing with a pre-treated feather which gave 900 mL total biogas. Whereas 1\% NaOH-microwave pre-treated straw and 1\% NaOH-microwave pre-treated straw mixed with pre-treated feather gave 870 and 630 mL total biogas respectively.

Table (4.3): Cumulative biogas production for samples pre-treated with NaOH 1\%, 2\% at 38 °C and total solid material of 30 g.

<table>
<thead>
<tr>
<th>Days</th>
<th>Cumulative biogas (ml) from 30 g wheat straw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>untreated</td>
</tr>
<tr>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>110</td>
</tr>
<tr>
<td>3</td>
<td>160</td>
</tr>
<tr>
<td>4</td>
<td>210</td>
</tr>
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<td>5</td>
<td>250</td>
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<td>6</td>
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<td>12</td>
<td>570</td>
</tr>
<tr>
<td>13</td>
<td>570</td>
</tr>
</tbody>
</table>
Figure (4.9): A Cumulative Biogas Production for Samples Pre-treated with NaOH 1% & 2% at 38°C Total Solid Material of 30g

4.3.4 Effect of alkali-microwave treatment on biogas production when total wheat straw was 40 g:

As shown in (Table 4.4 and Figure 4.10), the best result was to 2% NaOH-microwave pre-treated straw mixed with a pre-treated feather, followed by 2% NaOH-microwave pre-treated straw, 1870 and 1520 ml respectively. While 1% NaOH—microwave pre-treated straw and 1% NaOH-microwave pre-treated straw mixed with pre-treated feather gave low total biogas production of 1000 and 700 ml respectively. The control sample was the lowest amount of total biogas production, which was 670 ml
Table (4.4): Cumulative biogas production for samples pre-treated with NaOH 1%, 2% at 38 °C and total solid material of 40 g.

<table>
<thead>
<tr>
<th>Days</th>
<th>Cumulative biogas (ml) from 40 g wheat straw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>untreated</td>
</tr>
<tr>
<td>1</td>
<td>160</td>
</tr>
<tr>
<td>2</td>
<td>210</td>
</tr>
<tr>
<td>3</td>
<td>330</td>
</tr>
<tr>
<td>4</td>
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<tr>
<td>12</td>
<td>670</td>
</tr>
<tr>
<td>13</td>
<td>670</td>
</tr>
</tbody>
</table>

Figure (4.10): A Cumulative Biogas Production for Samples Pre-treated with NaOH 1% & 2% at 38°C Total Solid Material of 40g.
4.3.5 Effect of alkali-microwave treatment on biogas production when total wheat straw was 40 g treated with 3% and 4% NaOH

Pretreatment of 40g WS with 3% NaOH-microwave with or without adding pretreated chicken feather was conducted for biogas production.

Table (4.5) illustrates different results comparing previous results when 1% and 2% NaOH were used. The maximum total biogas production was 1430 ml obtained from 3% NaOH-microwave pre-treated straw only, while 3% NaOH-microwave pre-treated straw with pre-treated feather gave lower biogas amount which was about 880 ml.

Different results were obtained when 40g WS was pre-treated with 4% NaOH-microwave irradiation; there was a decrease in total biogas production. The maximum total biogas production was 725 ml obtained from 4% NaOH-microwave pre-treatment, while 4% NaOH-microwave pre-treated straw gave 580 ml biogas as shown in Figure 4.11.

Table (4.5): Cumulative biogas production for samples pre-treated with NaOH 3%, 4% at 38 °C and total solid material of 40 g.

<table>
<thead>
<tr>
<th>Days</th>
<th>Cumulative biogas (mL) from 40 g wheat straw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3% NaOH</td>
</tr>
<tr>
<td>1</td>
<td>125</td>
</tr>
<tr>
<td>2</td>
<td>275</td>
</tr>
<tr>
<td>3</td>
<td>855</td>
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<td>12</td>
<td>1430</td>
</tr>
<tr>
<td>13</td>
<td>1430</td>
</tr>
</tbody>
</table>
Figure (4.11): A Cumulative Biogas Production for Samples Pre-treated with NaOH 3% & 4% at 38°C Total Solid Material of 40g.

4.3.6 Cumulative gas production from pre-treated wheat straw mixed with urea:

10, 20, 30, and 40g of WS was pre-treated by microwave and 1% NaOH as mentioned previously, instead of a pre-treated feather, urea was added to adjust C/N ratio to 25. The results were unsatisfactory which were very low compared with results obtained from using a pre-treated feather. The maximum cumulative biogas was 350, 400, 490 and 530 for 10, 20, 30 and 40 g WS respectively as shown in (Table 4.6 and Figure 4.12).
Table (4.6): Cumulative biogas production for samples pre-treated with 1% NaOH mixed with urea at 38 °C.

<table>
<thead>
<tr>
<th>Days</th>
<th>Cumulative biogas (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 g WS</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
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<td>4</td>
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<td>13</td>
<td>350</td>
</tr>
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Figure (4.12): A Cumulative Biogas Production for Samples Pre-treated with 1% NaOH mixed with urea at 38°C.
4.4. Glucose concentration after biogas production:

Glucose concentration was estimated after fermentation process for 10, 20, 30 and 40 g WS as shown in (Figures 4.13, 4.14, 4.15 and 4.16) respectively glucose concentration remarkably went down after biogas production. As it noticed, for 10 g WS the lowest glucose concentration was to 2% NaOH pre-treated straw 2.3 mg/dl, while untreated WS gave the highest glucose concentration, 8.4 mg/dl. Similar results were obtained from 20 g WS, where glucose concentration was 4.1 and 9.3 mg/dl for 2% NaOH pre-treated straw and untreated WS respectively. With 30 g WS, the lowest concentration of glucose about 3.6 mg/dl for 2% NaOH pre-treated straw mixed with a pre-treated feather, while for 40 g WS, the lowest glucose concentration was 4.2 mg/dl for 2% NaOH pre-treated straw mixed with feather compared to 16.2 mg/dl for control.

Generally, there was a decrease in glucose concentration after AD process.

Figure (4.13): Glucose concentration after fermentation of 10 g wheat straw.
Figure (4.14): Glucose concentration after fermentation of 20 g wheat straw.

Figure (4.15): Glucose concentration after fermentation of 30 g wheat straw.
Glucose concentration after fermentation of 40 g wheat straw.

4.5 pH after biogas production:

Before fermentation, pH was adjusted to 7. pH was determined also after biogas production process, as illustrated in (Figure 4.17) the pH for untreated WS ranged from 6.4 to 6.8, while for pre-treated WS, its range was between 5.1 and 6.4.

Figure (4.16): pH after biogas production process for 10, 20, 30, and 40 g WS at 1%, 2%, 3% and 4% NaOH.
4.6 Effect of fermentation on total solid residue (TS):

The weight loss of WS is an important indicator of the effectiveness of the pre-treatment.

Figures 4.18, 4.19, 4.120 and 4.21, shows the weight loss of WS after AD for each treatment process (1%, 2%, 3% and 4% NaOH concentration) for 10, 20, 30 and 40 g WS. The 40g WS treated by 4% NaOH had the highest weight loss which was 42.7% for pretreated WS mixed with a pre-treated feather which was closed to 40 g pretreated straw which had a weight loss of about 40%. As shown in figures, pretreatment and AD could cause a loss in weight for all experiments. It was also observed that the increasing sodium hydroxide concentration increase the weight loss for all experiments.

![Figure (4.18): total solid residue after fermentation at a weight of 10g of untreated and pre-treated wheat straw.](image-url)
Figure (4.19): total solid residue after fermentation at a weight of 20g of untreated and pre-treated wheat straw.

Figure (4.20): total solid residue after fermentation at a weight of 30g of untreated and pre-treated wheat straw.
Figure (4.21): total solid residue after fermentation at a weight of 40g of untreated and pre-treated wheat straw.
Chapter V
Discussion
Chapter V

Discussion

The cellulosic biomass such as straw, cannot be fermentable in biogas plants without an appropriate pre-treatment process. So that, it must be disintegrated thermally and/or chemically (Deublein & Steinhauser, 2011).

The most commonly used mechanical pre-treatment methods are size reduction. Both chemical and thermochemical methods could yield up to 11.5-48% higher biogas yield depending on the pre-treatment conditions and substrate characteristics (Ariunbaatar, Panico, Esposito, Pirozzi, & Lens, 2014).

5.1 Effect of alkali-microwave irradiation on glucose production:

In this study, NaOH used for WS pre-treatment as it is the most alkaline and reducing agent commonly used for treatment (Costa, Barbosa, Alves, & Sousa, 2012). The effectiveness of alkali-microwave pre-treatment was evaluated by measuring the concentration of glucose. In comparison with sulphuric acid, Sodium hydroxide has a stronger performance on biomass surface leading to exposed biomass fibers (Zhu et al., 2015)

During alkali pre-treatment, the first reactions that occurs are solvation and saponification of intermolecular ester bonds, these bonds are cross-links xylan hemicelluloses and other components, which induce the recalcitrance structure of solids (Lagerkvist & Morgan–Sagastume, 2012). As a result, the specific surface area of the substrate will be increased and then the substrates will be easily accessible to digest anaerobically by microorganisms (Modenbach & Nokes, 2012; Hendriks & Zeeman, 2009; Torres & Lloréns, 2008).

The glucose concentration from hydrolysis of microwave pre-treatment combined with varying concentrations of NaOH are shown in (Figures 4.4 - 4.6) The results showed that the pre-treated WS with microwave assisted 2% NaOH at 3hr had significantly produced a higher concentration of reducing sugar yield, which means more degradation of lignin and hemicellulose. Results also showed that increasing NaOH concentration from 1% to 2% have a positive effect on glucose concentration.
which it increased from 51 to 58.6 mg/dl. This means that NaOH increased the digestibility of WS resulting in increasing of glucose concentration. Hardwood digestibility was increased from 14% to 55% when pre-treated by NaOH, and lignin content was decreased from 24-55% to 20% (Kumar et al., 2009). While increasing NaOH concentration over than 2% have a negative effect on glucose concentration. These results indicated that increasing NaOH concentration resulted in degradation of produced glucose. It is may be caused due to the conversion of NaOH into irrecoverable salts. As Zhang et al., reported the disadvantages of using alkali pre-treatment such as formation of irrecoverable salts, and/or incorporation of these salts into the biomass during pre-treatment of the substrate. Moreover, treatment of large amounts of these salts has become a challenging issue for alkaline pre-treatment (Zheng, Pan, & Zhang, 2009).

Using MW irradiation to generate heat is a good and promising choice to be applied for biomass fermentation and production of simple sugars. Jaisamut et al., are also reported that glucose yield is positively influenced mainly by increasing concentration of sodium hydroxide (Jaisamut et al., 2013).

Results obtained by (Nomanbhay et al., 2013) proved that the microwave assisted alkali pre-treatment of empty fruit bunch (EFB) using NaOH, significantly improved the enzymatic saccharification of EFB by removing more lignin and hemicellulose and increasing its accessibility to hydrolytic enzymes.

A review of other references agree with our results which indicate microwave pre-treatment in the presence of chemical reagents would be more effective, as reported by (Singh et al., 2014; Binod et al., 2012; Chang, Tyagi, & Lo, 2011)

Unlike acid pre-treatment, alkaline pre-treatment has been proven effective with a wide temperature range at various concentrations (Xu, Cheng, Sharma-Shivappa, & Burns, 2010).

(Hu & Wen, 2008) when conducted scanning electron microscope (SEM) analysis, they noticed that after alkali-treatment of the lignocellulosic material, its surface had become rough and loose. Also, they noticed that, the using of microwave irradiation after alkali pre-treatment make the lignocellulosic material more thinner
and striated. That’s because the degradation of lignin that took place, which resulted in increasing the exposure of the material components cellulose and hemicellulose.

(Jaisamut et al., 2013) reported that after alkali pre-treatment performed under different conditions, the percentage of lignin removal varied from 8% to 61%.

Interactive effects of time and temperature on the amount of glucose released were not as distinct as that of sodium hydroxide and temperature (Jaisamut et al., 2013).

(Torres & Lloréns, 2008) obtain an 11.5% increased methane production with alkaline pre-treatment of organic fraction of the municipal solid waste. The acidic and alkali pre-treatments are not suitable for substrates with a low lignin content.

Treatment of lignocelluloses using alkali pre-treatment is an effective method which reduce lignin content of the material, by a process called delignification. During this process, the ester bonds that cross-link lignin and xylan breakdown by sodium hydroxide, thus the porosity of the biomass is increased (Silverstein, Chen, Sharma-Shivappa, Boyette, & Osborne, 2007).

Using of microwave heating instead of conventional heating as means of pre-treatment was first reported by (Ooshima, Aso, Harano, & Yamamoto, 1984).

During microwave alkali pre-treatment of WS, fewer losses in sugars and higher rates of hydrolysis were observed than compared to alkali pre-treatment with conventional heating method (Zhu et al., 2006a).

Higher solubilization can be achieved with lower temperatures < 100 °C, (Mottet, Steyer, Déléris, Vedrenne, Chauzy et al., 2009) compared different thermal pre-treatment methods and found no significant difference between steam and electric heating, whereas microwave heating solubilized more biopolymers. The higher rate of solubilization with microwave pre-treatment can be caused by the polarization of macromolecules (Marin, Kennedy, & Eskicioglu, 2010; Toreci, Kennedy, & Droste, 2009). The released compounds are mostly phenolic compounds that are usually inhibitory to microbial populations (Hendriks & Zeeman, 2009).

Hence the advantage of using the microwave is that it reduces the time required for the delignification process significantly and uses less electrical energy.
Microwave-assisted alkali pre-treatment of WS removed more lignin and hemicellulose from biomass with shorter pre-treatment time than the alkali by conventional heating (Zhu et al., 2006a).

Here the glucose yield indicating that the microwave-assisted alkali pre-treatment enhanced the yields. With regard to energy saving, (Wang et al., 2014c) compared the energy requirement for microwave heating and conventional heating during biomass pre-treatment in Ionic liquids, they approximately estimated that the energy required in microwave pre-treatment in ionic liquids was much less than that required for conventional heating.

The effect of pre-treatment time was investigated at (450 W) for different durations (5–20 min). The results showed that the glucose concentration increased through the increasing the duration time of microwave from 5 min to 10 min, and there was no significant effect of pre-treatment after 10 minutes pre-treatment on glucose concentration. The results obtained by (Nomanbhay et al., 2013) showed that the optimum pre-treatment condition was 3% (w/v) NaOH at 180 W for 12 minutes.

The highest yield (51 mg/dl) for 40 g WS at 3 hr heating was obtained for pre-treatment with 2% NaOH for 10 min (Figure 4.5). For 1% 2%, 3% and 4% NaOH, peak yields were obtained at pre-treatment time of 10 min. The effect of alkali concentration on glucose yields also varied with pre-treatment time. For a pre-treatment time of 5 min, glucose yield significantly increased with increasing alkali concentration. At all other pre-treatment times (10 min and longer), glucose yield peaked at 2% NaOH with significantly lower yields at 1%, 3% and 4% NaOH. In a study using Coastal Bermuda grass, pre-treatment with higher NaOH concentrations (above 3%) and longer microwave exposure times, resulted in lower solid recovery, thus leading to less reducing sugars yield (Wang, Keshwani, Redding, & Cheng, 2010). The severity of the pre-treatment process is influenced by both the alkali concentration and pre-treatment time. Extended pre-treatment likely resulted in the loss of carbohydrates to the pre-treatment liquor, thereby will reduce the sugar yields during hydrolysis (Nomanbhay et al., 2013). High concentration of alkali or high temperature was necessary to solubilize or decompose the cellulose (Peng, Chen, Qu, Li, & Xu, 2014).
From this study, it was shown that the most efficient method for utilizing microwave irradiation as pre-treatment process for WS was 10 min in combination with dilute NaOH (2%).

5.2 Effect of alkali-microwave pre-treatment on biogas production:

For 10 and 20g WS the maximum biogas produced was 700ml and 830 ml respectively, for 2% NaOH, while using pre-treated feather give 480ml and 640ml respectively, these results indicated that using pre-treated feather did not increase biogas yield. It has been reported that according to the composition of the substrates, intermediate products of the decomposition can limit or inhibit the degradation. Thus, for example, the decomposition of proteins, methane fermentation can be restrained by the formation of ammonia and hydrogen sulfide (Deublein & Steinhauser, 2008), this may explain the lower biogas yield achieved. While for 30 and 40g WS, using pre-treated feather gave better results, which gave 1000ml and 1870ml respectively, this means that adjustment of C/N ratio using feather for low amounts of substrates not preferred.

High amounts of ammonia lead to a change in the pathway for acetate conversion to methane by applying mainly syntrophic acetate oxidation to CO₂ and H₂ coupled to hydrogenotrophic methane formation (Schnürer & Nordberg, 2008; Schnürer, Houwen, & Svensson, 1994).

Increasing the concentration of the substrate gave a positive effect on biogas production, and while all amounts of the substrate are pre-treated with the same concentration of NaOH, that means the inhibitory effect of alkali-microwave pre-treatment is decreased with an increase of substrate concentration which led to an increase in the volume of biogas produced.

Increasing the concentration of NaOH to 3% and 4% for 40g of WS decrease the cumulative biogas to 1430 and 580ml respectively, and to 580 and 725 ml respectively for the pre-treated WS mixed with pre-treated feather.

The reasons for the differences between the studies could be that different test system, inoculum slurries of different origins, the different substrate to inoculum ratios and different incubation temperatures were used.
We can conclude that alkali-microwave pre-treatment of WS prior to AD can significantly increase the cumulative biogas. The optimal cumulative biogas produced was for 2% alkali-microwave pre-treated straw mixed with pre-treated feather.

WS has a C/N ratio of 83:1, which is far from the 15-30 suggested as optimal for AD (Chandra et al., 2012). Adjusting the C/N ratio with pre-treated feather gave a more optimal substrate mix in this regard but, interestingly, a stable process was observed even with a C/N ratio as high as 83:1.

Presence of different phenolic compounds and furfurals, produced during the pre-treatment of the WS, which has been shown to cause inhibition in the biogas process (Hendriks & Zeeman, 2009).

The combination of microwaves with chemical pre-treatments as well as the microwave irradiation at temperatures higher than 145 °C resulted in a larger component of refractory material per gCOD, causing a decrease in the biogas production. A similar trend was observed with pig manure pretreated with lime and heated at temperatures higher than 110 °C (Rafique et al., 2010; Carrère et al., 2009).

The acidic pH also initiates further reactions of lignin. These reactions are not only degrading lignin, but the acidic conditions are also leading to a polymerization, which makes lignin less degradable (Hendriks & Zeeman, 2009).

Pre-treatment using NaOH 2% produced the highest volume of biogas of 1870ml. At NaOH 1% only about 700ml and the NaOH 4% has 725ml while biogas without pre-treatment has 570. Pre-treatment by using NaOH solution causes the volume of biogas produced is higher than the volume of biogas without pre-treatment. Research conducted by (Song, Yang, Han, Feng, & Ren, 2012) explained that with early treatment using NaOH alkaline solution will reduce lignin compounds in rice straw. The high concentrations of NaOH that used, then increasingly reduced lignin because NaOH will change microstructure of the cell walls of rice straw to facilitate microorganisms in the fermentation process for the production of biogas (Budiyono, Wicaksono, Rahmawan, Hawali Abdul Matin, Gumilang Kencana Wardani et al., 2017).
5.3 Effect of urea on biogas production

Using urea instead of a pre-treated chicken feather to adjust C/N ratio is not preferable, the cumulative biogas increased with increasing substrate concentration, however, the cumulative biogas from using feather was higher than that from using urea. Maybe there was an accumulation of ammonia rapidly comparing with using a feather.

5.4 pH after biogas production

pH is an important parameter affecting the growth of microbes during anaerobic fermentation. The initial pH values all patch fermentations were about 7.0. At the end of fermentation, pH values became acidic between 5.2 – 6.8 which were within the suitable range of 5.5–8.2 (Mao, Feng, Wang, & Ren, 2015). This result might be attributed to hydrolysis and acidogenesis. The desirable range for the methanogens reviewed by researchers (Sreekrishnan, Kohli, & Rana, 2004), which was 6.8–7.2. The activity of methanogens is usually inhibited due to a pH lowering from 7.2 to 5.3 because the substrate would rapidly degrade to VFA (Getachew, 2012).

The amount of carbon dioxide and VFAs produced during the anaerobic process affects the pH of the digester contents. For an anaerobic fermentation to proceed normally, the concentration of VFAs, acetic acid, in particular, should be below 2000 mg/l (Sreekrishnan et al., 2004). (Jain & Mattiasson, 1998) found that above pH 5.0, the efficiency of CH₄ production was more than 75%. Researchers have proved that inhibition caused by ammonia could lead to a decrease in pH, which would partially counteract the effect of ammonia by increasing the concentration of VFAs (Chen, Cheng, & Creamer, 2008), high VFAs concentrations are toxic to AD microorganisms (Veeken & Hamelers, 1999).

High concentration of VFAs leads to a pH drop and high levels of undissociated acids accumulation, both of which inhibit the methanogenic activity (Bouallagui, Touhami, Cheikh, & Hamdi, 2005; Misi & Forster, 2001). Results obtained by (Xu et al., 2014) demonstrated that acetic acid was the main factor in VFAs inhibition in anaerobic process (Xu et al., 2014).
Feathers make up approximately 5% of the chickens body mass. It consists mainly of the fibrous keratin and small amounts of lipids and water. Because of the complex, rigid and fibrous structure of keratin which is resistant to enzymatic digestion, feathers are poorly degradable under anaerobic conditions (Salminen, Einola, & Rintala, 2003). A pre-treatment method, where the tough structure is broken down is needed to be able to get a high biogas yield.

Previous studies have shown that lime treatment might be the only satisfying pre-treatment method for chicken feathers (Khorshidi Kashani, 2009; Salminen et al., 2003).

Lime pre-treatment of feathers with the lowest temperature, (100 °C), and shortest time (30 min) resulted in highest yield of biogas. This was because the degradation of protein and amino acids under lime conditions. Lime treatment resulted in ammonia production which at high levels would inhibit the AD digestion process (Deublein & Steinhauser, 2008). Lime treatment have many advantages, that lime is relatively inexpensive, recoverable, effective, and safe to use at low concentrations (Coward-Kelly, Agbogbo, & Holtzapple, 2006a).

When keratin is degraded ammonia and nitrates are formed (Coward-Kelly, Chang, Agbogbo, & Holtzapple, 2006b) which are inhibitors of AD at high concentrations (Deublein & Steinhauser, 2008). If more keratin is degraded thereby more ammonia and nitrate are formed this may explain the lower biogas yield achieved.

Pre-treatment of feathers with Ca(OH)\textsubscript{2} make it solubilize significantly, whereas, addition of lime even in very low concentration (0.1 g Ca(OH)\textsubscript{2}/g TS feather) increases the biodegradability of feather dramatically after treatments at temperature ranges of 100–120 °C (Forgács, Lundin, Taherzadeh, & Horváth, 2013). (Coward-Kelly et al., 2006b) also reported that solubilization of feather can significantly improved using low amounts of Ca(OH)\textsubscript{2}.

The chosen strategy for substrate pre-treatment may impact on community of the methanogens, as releasing some inhibitors or methanogenic substrates such as acetate or furfurals.
It is worth noting that many reactions in the anaerobic process are influenced by each other and that the methanogenic community shaped by the operating parameters might influence the overall degradation of the substrate (Sun, 2015).

5.5 Glucose concentration after biogas production:

Glucose concentration after AD process was also estimated in this study. Figures 4.12, 4.13, 4.14 and 4.15 show that there was a decrease of sugar concentration after anaerobic process compared to a control samples. This indicates that glucose was used by microorganisms for growth and biogas production.

Microwave irradiation can damage the structure of plant cells because of the sudden temperature rise and the internal pressure increase (Zhou & Liu, 2006).

As it was shown in Figures 4.17, 4.18, 4.19 and 4.20 there was a decrease in weight loss after AD process of WS for all experiments which means that microwave alkali pre-treatment increase degradation of WS components such as cellulose, hemicellulose and lignin.

(Hendriks & Zeeman, 2009; Taherzadeh & Karimi, 2008) agree that alkali pre-treatment could cause cellulose fibers to swell rather than directly degrading it, they conclude that this pre-treatment is the best method to avoid further fragmentation of hemicelluloses polymers as well as break up the ester bonds between lignin, hemicelluloses and cellulose (Gáspár, Kálmán, & Réczey, 2007) which make it more accessible to fermentable bacteria.

It was observed that increasing of sodium hydroxide concentration increase the weight loss after AD. These results indicate that microwave-alkali pre-treatment is an efficient WS technique.

5.6 Total solid residue after anaerobic digestion:

The highest weight loss was 40% for 40 g of WS was 42.7% for pretreated WS mixed with pretreated feather which was closed to results obtained by (Zhu et al., 2006b) which was 44.6%.

(Zhu, Wu, Yu, Liao, & Zhang, 2005) Indicate that microwave/alkali had a higher hydrolysis rate than alkali alone. These results demonstrate that microwave-
assisted alkaline pretreatment is an efficient way to improve the bioconversion of lignocellulosic biomass to biogas.
Chapter VI
Conclusion and recommendations
Chapter VI

Conclusion and recommendations

6.1 Conclusion:

The present study mainly focused on biogas production as a waste management tool for by-products from the food-processing industries, namely wheat straw. Wheat straw recalcitrant to the anaerobic digestion process in different ways. Pre-treatment by microwave irradiation and alkali increased the rate of degradation of wheat straw and showed possibilities of increasing the cumulative biogas. It made the substrate more available to anaerobic digestion because it increased the total accessible area for enzymes and bacteria. Microwave-assisted dilute sodium hydroxide pre-treatment could enhance the concentration of reducing sugars and biogas production from wheat straw when compared to untreated wheat straw.

Addition of pre-treated chicken feather to adjust C/N ratio appears to be a viable option to enhance biogas production compared to urea.

The highest cumulative biogas (1870ml) was obtained from 40 g wheat straw treated with 450w for 10 min in microwave, 2% NaOH and mixed with pre-treated chicken feather. While it was 1520 ml without addition of pre-treated chicken feather.

Wheat straw could be a potential lignocellulosic biomass resource for the biogas production

6.2 Recommendations:

In light of the above conclusions and based on the results of this study, the following recommendations are suggested:

1. Using of different lignocellulosic material to show its feasibility for biogas production.
2. Alternate processing methods to get higher release of reducing sugars could be tried to make the technology more efficient and profitable.
3. Applying the microwave-base pre-treatment techniques to other feedstocks, such as municipal solid wastes and other agricultural residues.
5. Evaluation of using the residue from fermentation process as a compost.
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