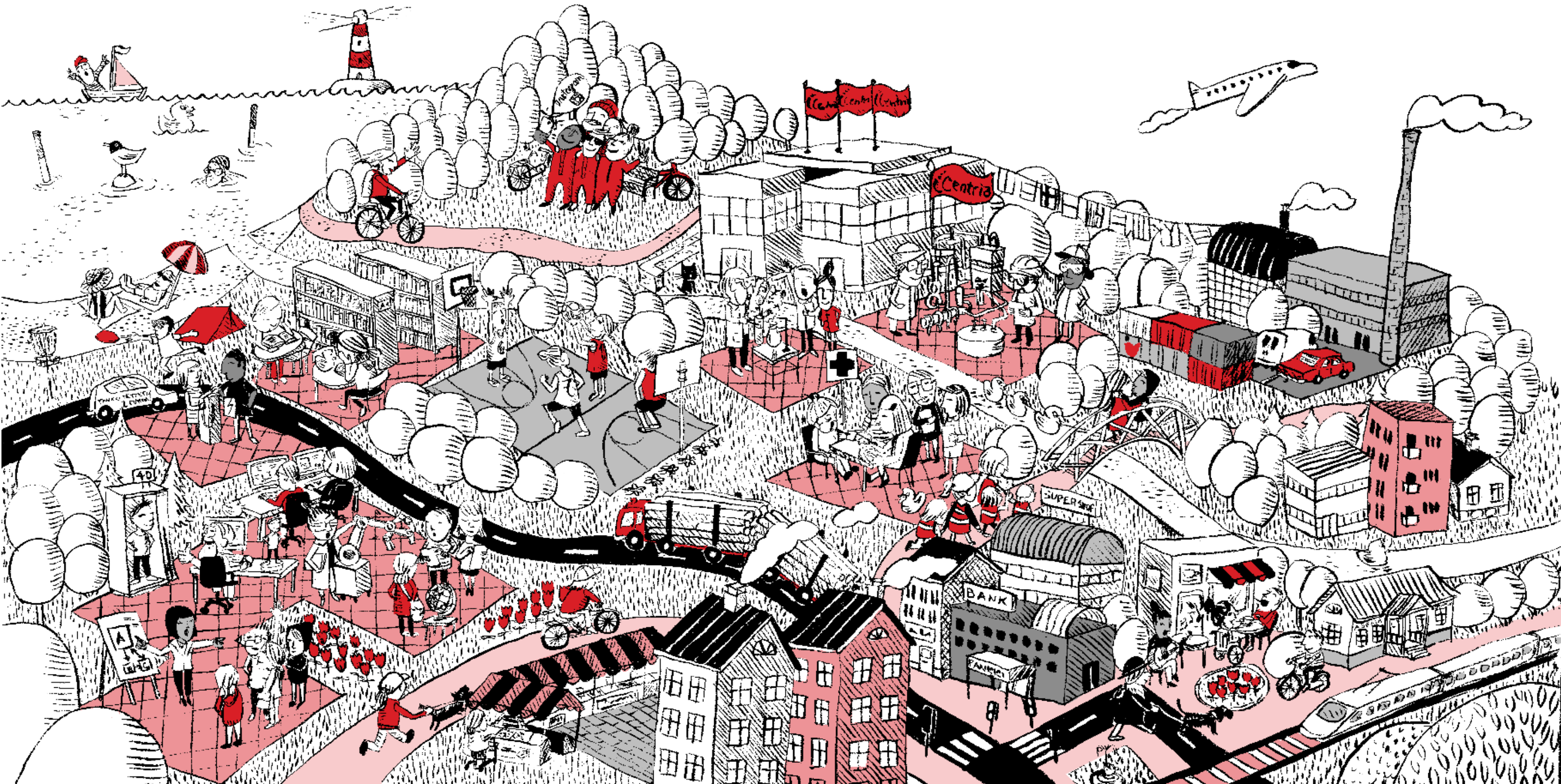
Tianyang Yuan

BIOMASS FERMENTATION FOR GAS PRODUCTION AND THE DEVELOPMENT OF BIOGAS IN CHINA

Thesis
CENTRIA UNIVERSITY OF APPLIED SCIENCES
Degree Programme
May 2022



ABSTRACT

Centria University of Applied Sciences	**Date** May 2022	**Author** Tianyang Yuan
Degree programme Bachelor of Engineering, Environmental Chemistry and Technology		
Name of thesis BIOMASS FERMENTATION FOR GAS PRODUCTION AND THE DEVELOPMENT OF BIOGAS IN CHINA		
Centria supervisor Yue Dong		**Pages** 24

At present, the international community is facing a series of energy problems such as the increasing shortage of fossil fuel resources and the deteriorating ecological environment. The development and application of renewable energy sources has become an important part of the world's development.

Methane and hydrogen are two new renewable and clean energy sources that can be a good substitute for fossil fuels, and their development status and prospects have received wide attention. With the development of biorefinery technology, the production of methane and hydrogen from biomass fermentation is becoming increasingly mature. The selection of microbial flora, the choice of reactor type and the efficiency of biomass utilisation are issues that still remain. Therefore, exploring various better biorefinery technologies should be a key research direction.

Methane production from biomass fermentation obtains a much higher energy conversion rate than hydrogen production from biomass fermentation, but the rate of hydrogen production is much faster than that of methane production. Biomass fermentation for methane production is much older and the technology is more mature. More than two hundred species of bacteria have been identified that can be used to produce methane, far more than those found in the production of hydrogen. The main method of fermenting methane and hydrogen is currently anaerobic fermentation, with anaerobic sludge bed reactors and CSTR reactors being the most widely used. The selection of suitable bacterial species and the synergistic fermentation of multiple bacterial groups are the main research directions in anaerobic fermentation for gas production at present.

Key words
Bacteria, biomass, fermentation, hydrogen, methane, microorganisms

CONCEPT DEFINITION

List of abbreviation

BTAC

Biomass Technology Advisory Committee

DOE

U.S. Department of Energy

FAO

Food and Agriculture Organization of the United Nations

NADH

Reduced Nicotinamide Adenine Dinucleotide

OPEC

Organization of Petroleum Exporting Countries

TS

Total feed solids concentration

TSr

Total solids concentration in the reactor

CSTR

Continuous Stirred-Tank Reactor

VFA

Volatile fatty acids

ABSTRACT
CONCEPT DEFINITIONS
CONTENT

1 INTRODUCTION

Fossil resources are the material basis of modern industry and human civilization. As the demand for energy increases, non-renewable energy sources such as oil are gradually being depleted and by the end of the 20th century the world energy crisis was becoming increasingly serious. According to the Organization of the Petroleum Exporting Countries (OPEC) monthly oil market report, total global oil demand reached an average of 15.4 billion litre by December 2021, and the volume will continue to grow year by year . Researchers predict that world oil consumption will increase to an average of 18.8 billion litre per day by 2030, and at this rate, global crude oil reserves will be depleted by 2060. (OPEC 2021.) At the same time, large-scale climate change like global warming is more often than ever a major concern for the international community due to the rapid accumulation of greenhouse gases such as carbon dioxide as a result of human activities (e.g. burning fossil fuels to meet energy demand). As a result of global warming, sea ice melting and sea level rise are occurring at a rapid rate in the Earth's ecology. (IPCC 2018.)

Bioenergy has an important role in the current and future energy landscape, and many EU Member States have recognised the role of bioenergy in increasing the share of renewable energy and reducing greenhouse gas emissions, helping countries to reduce their dependence on fossil fuels and making a significant contribution to the bioeconomy (Moulin, Fritsche & Junginger 2019). Therefore, the production of gas from biomass fermentation is widely valued in countries around the world as an important way to produce bioenergy.

As a relatively young field of research, systematic research and development in biorefineries has taken place mainly in Europe; the industrial sector has been developed mainly in the USA. The US Biomass Technology Advisory Council (BTAC) has developed a long-term plan and target for bioenergy, biofuels and bioproducts, with biomass-based transportation fuels growing from 0.5 % of US fuel consumption in 2001 to 20 % by 2030 (BTAC 2002). The production of hydrogen and methane from biomass has good industrial prospects as hydrogen and methane are more easily separated as gases than solid and liquid fuels. (Claassen 1999.)

This thesis describes the technology of biomass fermentation for the production of methane and hydrogen and describes the development of biogas technology in China. The paper is divided into four parts. The first part describes the pre-treatment process before the refining of biomass gas. The second part describes the process of biomass fermentation for methane production in detail. The third part introduces the biomass fermentation to hydrogen technology. The fourth part introduces the development of biogas technology in China.

2 BIOMASS PRETREATMENT

For this process, pretreatment is a very critical step as it has a significant impact on the overall efficiency of the biotransformation. The aim of the pretreatment is to break down the recalcitrant structure of the macromolecular biomass and make it more accessible to the enzymes that convert carbohydrate polymers into fermentable sugars. Among the various types of biomass used today, agricultural waste, organic household waste, animal manure and others. contain a large number of carbohydrates that are relatively simple in structure and easy to handle. However, woody fibre biomass such as crop straw and wood waste are difficult to process. Industry therefore needs to find suitable treatment methods to break down these complex wood fibres into simple molecules so that microorganisms can be quickly and efficiently absorbed by micro-organisms and reacted with, therefore, pretreatment became an essential process. Pretreatment can be divided into biological pretreatment, physical pretreatment and chemical pretreatment. (Mosier 2005.)

2.1 Physical and chemical pretreatment

Physical pre-treatment is considered a prerequisite before further application of lignocellulosic biomass. The basic aim of the physical treatment prior to treatment is to reduce the particle size of the biomass. A significant increase in specific surface area is achieved by reducing particle size, crystallinity and breaking polymeric chains. The fine particle size of the biomass can easily be used in the digestion process. This type of pretreatment is environmentally friendly as there is no exposure to toxic chemicals. Process conditions such as pressure, temperature, holding time and type of feedstock have a significant effect on the effectiveness of physical pretreatment. Physical treatments can also be referred to as mechanical treatments and include milling, grinding and chipping. The particle size of biomass can be reduced by up to 0.2 millimeter through the milling process. Pretreatment by milling can increase the yield of bioethanol and biogas. However, physical pretreatment can significantly reduce energy consumption when used in combination with chemical pretreatment. (Shah, Seehar ,Sharma & Toor 2022.)

In physical pretreatment, the biomass is reduced to smaller particle size by mechanical comminution or the surface area of the biomass is increased without reducing the particle size. The surface area can often be increased by mechanical refining, during which the biomass is subjected to shear-induced fibrillation. Physical pretreatment increases the hydrolysis rate by increasing the surface area, thus making it easier for cellulase to access the biomass surface. However, information on the mode of action, such as physical pretreatment modifying the chemical composition or how it affects the structure of the cell wall, is limited, although physical pretreatment appears to cause changes in the cell wall similar to changes produced by mechanical pulping techniques on wood, such as thermal pulping, milling, and pressurised grinding. Some forms of physical pretreatment have been observed to alter the structure of lignin. A disadvantage of physical pretreatment is that it lacks the ability to remove lignin, which limits the contact of enzymes with cellulose. Other disadvantages of this pretreatment method are the high energy consumption, the high cost of large-scale implementation, and the significant environmental and safety issues associated with it. Studies have shown that feedstock delignification may be responsible for the high energy consumption of physical pretreatment methods, and therefore, physical pretreatment ultimately affects the overall energy efficiency of biorefining. (Aslanzadeh, Ishalo, Richards & Taherzadeh 2014.)

Dilute acid pretreatment has been widely used due to its low cost, its effectiveness in converting hemicellulose to monomeric sugars and inducing structural transformations in other biomass components, thus increasing enzyme accessibility and improving cellulose conversion (Antonopoulou, Dimitrellos, Beobide, Vayenas & Lyberatos 2015). To date, different organic and inorganic acids have been used at different residence times, temperatures, pressures and different concentrations. The acid has no differential effect on the polymerised sugar chains of cellulose, hemicellulose and lignin. Dilute sulphuric acid was used to successfully hydrolyse hemicellulose to sugar and to increase the specific surface area of cellulose. Sulphuric acid is the most widely used acid, but other acids such as hydrochloric acid, phosphoric acid, nitric acid and acetic acid have also been used for different lignocellulosic biomasses. The only disadvantage is that during acid pretreatment the hemicellulose fraction of the biomass is hydrolysed not only to soluble sugars but also to compounds such as furfural and furfural and aliphatic acids (such as formic and acetic acids). These compounds may have inhibitory or toxic effects on microorganisms used in the subsequent biotransformation process. (Antonopoulou et al. 2015) Pretreatment of sunflower straw with dilute sulphuric acid revealed the greatest release of sucrose at a temperature of 167.2 °C and an acid concentration of 1.3 %. Under these conditions, the pretreated solid retained 87 % of the glucose in the feedstock, enzymatic digestion released 56 % of the glucose and approximately half of the hemicellulose sugars (xylose) were recovered in the liquid phase. (Ruiz , Romero , Moya , Cara , Vidal & Castro 2013.)

The ester bond in the lignin/phenolic-carbohydrate complex was broken upon alkali pretreatment, which served to remove the lignin. Sodium hydroxide and calcium hydroxide are suitable chemicals for this type of pretreatment and are usually used at lower temperatures than acid pretreatment methods. However, the time required is on the order of hours or days, rather than the few minutes required for acid pretreatment. (Antonopoulou et al. 2015) Sunflower straw was subjected to different alkaline pretreatments to increase its methane potential and it was found that treatment with 4 g sodium hydroxide per 100 g solid at 55 °C for 24 hours was the best pretreatment condition for effective lignin removal (Monlau, Aemig, Barakat, Steyer & Carrere 2013).

2.2 Biological pretreatment

Biological pretreatment methods include fungal pretreatment, enzymatic digestion and aeration. They are used to break down the cross-linked structures in lignocellulosic waste. These enzymes are mainly produced by fungi, such as white rot, soft rot and brown rot. Specific extracellular enzymes secreted by fungi increase the rate of hydrolysis by degrading cellulosic compounds into readily fermentable sugars. Fungal pretreatment can increase cumulative biohydrogen production from maize stover fermentation by 209 %. In addition to fungal pretreatment, several industrial enzymes such as α-amylase, hemicellulase, arabinase, and xylanase can be used directly for hydrolysis purposes. Biological pretreatment methods are considered environmentally friendly as they are carried out at low temperatures and do not require any chemicals; however, this treatment is often used in combination with other methods as it has been reported that increased incubation time is required for successful enzyme saccharification. (Keskin, Abubackar, Arslan & Azbar 2019.)

Aeration is another biological pretreatment as it has been shown that the catalytic activity of extracellular enzymes secreted by hydrolytic microorganisms during aeration can enhance the solubilisation of composite granular materials. Aeration also promotes the hydrolysis of carbohydrates and proteins in the waste, as well as the activity of cellulase and protease hydrolases. Pre-aeration has also recently been applied to food waste in a semi-pilot-scale biological system in order to produce bio-based products (biohydrogen, volatile fatty acids and bioethane); the presence of molecular oxygen in the air not only inhibits methanogenic bacteria, but also helps to break down complex waste into simpler molecules. It is reported that aeration increased biohydrogen conversion efficiency by 97 % and volatile fatty acids (VFA) production by 10 % compared to the control group. (Keskin et al. 2019.)

3 BIOMASS MICROBIAL FERMENTATION FOR METHANE PRODUCTION

In the methane-producing microbial fermentation state, organic matter is eventually broken down into carbon dioxide and methane through a series of biochemical processes. The ecosystem of biomass fermentation for methane production consists of three stages: hydrolytic liquefaction, acid production and methane production. (FAO 2021) All methanogenic bacteria are archaea and are highly sensitive to oxygen, making them one of the most difficult bacteria to study. In actual methane fermentation systems, mixed bacterial systems such as anaerobic granular sludge are more commonly used. In micro-ecological pellets of anaerobic sludge, methanogenic bacteria are easily protected by low redox potential environments in the core of the pellet. The methane fermentation process is a microbial process that is carried out in concert by a variety of bacteria and essentially involves the flow of electrons. (Turick, Peck, Chynoweth, Jerger, White, Zsuffa & Kenney 1991) At neutral pH conditions, approximately 70 % of methane is formed from the decomposition of acetic acid produced by hydrolysis, while 30 % is derived from hydrogen and carbon dioxide or formic acid produced by hydrolysis. At higher temperatures (60 to 70 °C), most of the methane is derived from hydrogen and carbon dioxide, as anaerobic decomposition of acetic acid to hydrogen and carbon dioxide occurs at higher temperatures. At lower temperatures (lower than 15 °C), the contribution of acetic acid decomposition to methane is higher than 70 % (Claassen 1999).

Polymeric materials such as lipids, proteins and carbohydrates are mainly hydrolysed by extracellular hydrolytic enzymes which are secreted by the microorganisms present in the first phase. The hydrolytic enzymes (lipases, proteases, cellulases, amylases) hydrolyse their respective polymers into smaller molecules, mainly monomeric units, which are then consumed by the microorganisms. In methane fermentation of wastewater containing high concentrations of organic polymers, the hydrolytic activity associated with each polymer is critical, as polymer hydrolysis may become a rate limiting step for the production of simpler bacterial substrates in subsequent degradation steps. (FAO 2021.)

Lipase converts lipids to long-chain fatty acids. There are 104 to 105 lipolytic bacteria per millilitres of digest. Clostridium difficile and Micrococcus appear to be the majority of extracellular lipase producers.

The long-chain fatty acids produced are further degraded by oxidation to produce acetyl coenzyme. Proteins are generally hydrolysed to amino acids by proteases secreted by Bacillus spp, Vibrio butyricus, Clostridium spp, Clostridium spp, Selenomonas spp and Streptococcus spp. The resulting amino acids are then degraded into fatty acids such as acetic, propionic and butyric acids, and ammonia found in Clostridium, Gastrococcus, Aeromonas, Campylobacter and Synechococcus. (FAO 2021.)

Hexoses and pentoses are usually converted to C_2 and C_3 intermediates and reducing electron carriers via a common pathway. Most anaerobic bacteria metabolise hexose via the Emden-Meyerhof-Arnas Pathway (EMP), which together with reduced Nicotinamide Adenine Dinucleotide (NADH) produces pyruvate as an intermediate. The resulting pyruvate and NADH are converted to endofermentative producers such as lactic acid, propionic acid, acetic acid and ethanol by other enzymatic activities, which vary according to the microbial species. During the acid production phase of hydrolysis, the sugars, amino acids and fatty acids produced by the microbial degradation of the biopolymer are metabolised in turn by the bacterial population and fermented mainly into acetic acid, propionic acid, butyric acid, lactic acid, ethanol, carbon dioxide and hydrogen. (FAO 2021.)

During anaerobic digestion, methanogenic bacteria are physiologically uniform as methane producers; although acetate and hydrogen/carbon dioxide are the main substrates available in the natural environment, formate, methanol, methylamine and carbon monoxide are also converted to methane. As methanogenic bacteria, being exclusively anaerobic, require a redox potential below 300 mV to grow, their isolation and culture is somewhat elusive due to the technical difficulties encountered in handling them under completely anaerobic conditions. Over 40 strains of pure methanogenic bacteria have been isolated. Methanogenic bacteria can be divided into two groups: those that consume hydrogen/carbon dioxide and those that consume acetate. While some hydrogen and carbon dioxide consumers are able to use formate, acetate can only be consumed by a limited number of strains, such as the methanogenic and methanogenic genera that are unable to use formate. Since large quantities of acetate are produced in the natural environment, methanogenic saccharomyces and methanogenic filamentous bacteria play an important role in completing anaerobic digestion and accumulating hydrogen, inhibiting acetate and methanogenic bacteria. Methanogenic bacteria that consume hydrogen and carbon dioxide eventually produce methane by binding to unusual coenzymes that reduce carbon dioxide in the form of electron acceptors through the formyl, methyl and methyl levels. As a small proportion of carbon dioxide is also formed from carbon derived from methyl groups, it can be suspected that the reduction potential generated by methyl groups may reduce carbon dioxide to methane. (FAO 2021.)

Pretreatment of cedar methane fermentation to improve methane production. Another advantage of using fungi is their high tolerance to salt and metal ions. Pretreatment of Japanese cedar chips by different methods such as refining, steam treatment, biological treatment with Streptomyces fungi, steam blasting and biogas conversion are examples that show that steam blasting are very effective pretreatment methods. The results showed that steam blasting is a very effective pretreatment method for methane gas production. Steam blasting was the most effective pretreatment method at a steam pressure of 4.51 MPa and a cooking time of 5 minutes. (Amirta 2006.) Biological pretreatment of straw using white rot fungi destroys the straw structure, reduces the lignin content, greatly shortens the anaerobic fermentation cycle and increases the efficiency of methane conversion. However, the fungus was used for biomass pretreatment at a slower rate, affecting the overall duration of the fermentation process. (Yang 2007.)

To date, several methanogenic genomes have been sequenced. The genomic information has led to a better understanding of the cellular structure, evolution, metabolism and environmental adaptations of methanogenic bacteria, and has better guided the genetic modification of methanogenic bacteria to obtain "super" methanogenic bacteria to reduce methanogenic process time and increase methanogenic conversion rates. In general, a methanogenic bacterium has only one methanogenic pathway, but the multicellular methanogenic Bacillus Octococcus has three methanogenic pathways and can use at least nine methanogenic substrates. During methane biosynthesis, the formation of methane is accompanied by the formation of a chemical gradient inside and outside the cell membrane. Based on the metabolic pathways of methane synthesis, metabolic fluxes can be analysed, taking into account external factors (pH, temperature) and the manipulation of metabolic pathways (enhancement, deletion or addition of new pathways) to promote methane production from a material and energetic perspective. (Alexei 2002.)

The design of a methane fermentation reactor must take into account, from a system perspective, the policy basis, the general layout, the process design (determining the best applicable process: including pretreatment, biogas fermentation, post-treatment), the selection and design of the plant, the design of the gas delivery system, the design of the storage tank, the desulphurisation of the biogas and the safety and fire protection (Rao & Singh 2004). In developing countries, batch and semi-continuous anaerobic fermentation systems are two widely used technologies for the bioenergetic conversion of organic fractions of waste. Batch fermentation systems are the simplest, easy to operate and have low equipment and associated maintenance costs. In order to achieve rapid and efficient anaerobic digestion, several processes have been developed, such as up-flow anaerobic filtration processes, up-flow anaerobic sludge bed reactors, anaerobic fluidised bed methods, and anaerobic fluidised bed reactors, to increase cell retention, and a two-stage digestion process to optimise acid production and methanogenesis. (Naomichi & Nakashimada 2004.)

A solid-liquid coupled bioreactor, which is a combination of a solid waste reactor for waste acidification and a Upflow Anaerobic Sludge Blanket reactor (UASB) for methane production, enhances the two-stage fermentation process (Xu, Wang & Zhang 2002). The two-stage anaerobic intermittent reactor process can be used for the anaerobic treatment of food and vegetable waste. The greatest advantage of this two-stage process is that the organic loading rate is buffered in the first stage, creating a more stable feed rate for methanogenesis. In addition, the inherent operational flexibility of the reactor is characterised by a high degree of process flexibility in terms of cycle time and sequence, eliminating the need for a separation clarifier and retaining a higher concentration of low growth anaerobic bacteria in the reactor. High solids waste degradation and suspended solids removal rates of 93.5 % were achieved. (Bouallagui 2004.)

4 BIOMASS MICROBIAL FERMENTATION FOR HYDROGEN PRODUCTION

In a fermentation-based system, microorganisms, such as bacteria, break down organic matter to produce hydrogen. These organic materials can be biomass feedstocks such as sugar, corn stover or even waste water. These methods are sometimes referred to as "dark fermentation" methods because no light is required. In direct hydrogen fermentation, the microorganisms themselves produce hydrogen. These microbes can break down complex molecules through many different pathways, some of which have by-products that can be combined with enzymes to produce hydrogen. Researchers are investigating ways to make fermentation systems produce hydrogen faster (increase the rate of hydrogen production) and produce more hydrogen from the same amount of organic matter (increase production). Microbial electrolytic cell devices are devices that use the energy and protons produced by microorganisms breaking down organic matter, coupled with an additional small electric current, to produce hydrogen. (DOE 2022.)

Fermentative bacteria that produce molecular hydrogen during metabolism, including parthenogenic and specialized anaerobes, include Enterobacteriaceae, Clostridium, Megacoccus, Veronococcus, Mutococcus, Acinetobacter, Acinetomycetes, Vibrio, Bacillus, Fusobacterium, Rumenococcus, Eubacterium, and Faecococcus (Wang, Dong, Zhang, Fang, Chen, Zhao, Luo & Yang 2019). These bacteria are capable of decomposing the substrate to produce hydrogen gas. Hydrogen production mainly involves the formic acid dehydrogenation and NADH production pathways (Naomichi & Nakashimada 2002).

The most studied fermentation substrates for hydrogen production are still glucose, starch, molasses wastewater, sludge and crystalline cellulose. The microorganisms used are mainly pure cultures or anaerobic activated sludge, which involves the optimisation of culture conditions and the regulation of metabolic pathways. The corresponding reactors include fixed bed reactors, continuous stirred reactors, ascending flow anaerobic sludge bed reactors, fluidised bed reactors, which involve the use of activated carbon, algal sugar, lucerne, expanded clay. (Shiza & Bagley 2005.)

Using sweet sorghum as the fermentation substrate and a mixed microbial system as the fermentation flora, 10.4 L/kg of hydrogen was produced over a 12 hours reaction time and the hydrogen producing effluent could be used to produce methane. (Antonopoulou, Hariklia & Ioannis 2008.)

In addition to the necessary and effective pre-treatments for the production of hydrogen from biomass, there are three areas of technology that are being developed. The first is bacterial cell surface display technology, where different functional proteins are displayed on the cell surface to build multifunctional biocatalysts, using a single cell to perform tasks that previously required multiple cells. (Patrik, Elin & Nygren 2002) The cell surface engineering technology can be used to immobilise carboxymethylcellulase and β-glucosidase on the surface of microbial cells for the production of cellulose fermentation. Clostridium thermophilum is a thermophilic bacterium that contains a cellulosome structure, which is a unitary structure containing several enzymes such as endoglucanase, xylanase and gibberellinase bound to the cell surface, and can effectively degrade cellulose (Yuval, Raphael & Edward 1999). At the same time, Clostridium thermophilum can produce hydrogen. In addition, some studies have co-cultured cellulose-degrading bacteria with hydrogen-producing bacteria (Patrik et al. 2002). Meanwhile, Clostridium thermophilum can produce hydrogen (Levin, Rumana & Nazim 2006). In addition, some studies have co-cultured cellulose-degrading bacteria with hydrogen-producing bacteria (Wang, Ren & Shi 2002). Thirdly, the application and development of quantitative cytofluorimetric techniques in anaerobic hydrogen production systems can help to better understand anaerobic hydrogen production hybrid systems and guide the selection and process optimisation of fermentative hydrogen production strains (Zhang, Xing & Lou 2002).

5 BIOMASS FERMENTATION FOR CO-PRODUCTION OF METHANE AND HYDROGEN

Hydrogen and methane are two important energy carriers and are both very important energy and chemical feedstocks. A comparative study of hydrogen and methane production from biomass was carried out. The results show that greater energy recovery can be obtained from methane production. Although hydrogen has the largest calorific value per mass of any fuel (142 GJ/t), its low density (0.09 kg/m^3) results in a low volumetric calorific value (0.0127 GJ/m^3); methane has a lower calorific value per mass (55.6 GJ/t) than hydrogen, but a higher density (0.72 kg/m^3), resulting in a higher calorific value per volume (0.040 GJ/m^3). With a calorific value per unit volume ratio of 3.15 for methane to hydrogen, more energy will be available than for hydrogen. If methane is produced from biomass, the energy recovery rate can reach 13 % to 42 %, while for hydrogen, the energy recovery rate is 5 % to 10 %. This suggests that methane production from biomass is a more efficient conversion technology than hydrogen. However, hydrogen production is more rapid than methane, requiring only 6 to 10 hours to convert sugar to hydrogen, whereas conversion to methane takes 10 to 15 days. In addition, and very importantly, a high value and clean renewable source of hydrogen has the unique advantage of being linked to the renewable fuel group of fuel cells. In this case, there is a preference for hydrogen production over methane. (Levin 2007.)

A two-stage process for the co-production of hydrogen and methane from solid waste has been successful. In the first production stage, the hydrogen yield from volatile solids was 43 mL/g, and in the second stage methane production was 500 mL/g, with methane production 21 % higher than in the first stage process. The hydrogen reactor doubles the hydrogen production due to methane injection. pH is a key factor affecting the fermentation pathway in the hydrogen production stage. The optimum pH for hydrogen production in this system is 5.0 to 5.5. The results of this study are also directly evident in dynamic fermentation systems, showing that an increase in hydrogen reflects an increase in the acetate/butyrate ratio in the liquid phase. (Liu 2006.)

A new two-stage process "Biocell Biofermentation" system was devised which is based on phase separation, reactor rotation mode and continuous batch technology to produce hydrogen and methane from food waste by applying different fermentation optimum conditions to the different components of the food waste. The biocell process consists of four leaching bed reactors for hydrogen recovery and post-treatment, and one ascending anaerobic sludge bed reactor for methane recovery. The leach bed reactors operate in rotary mode at two-day intervals during the degradation phase. The yield of hydrogen and methane from the added volatile solids was 0.31 m^3/kg and 0.21 m^3/kg respectively. In addition, the post-treatment output could be used as a soil amendment. (Kee & Sik 2004.)

6 DEVELOPMENT OF BIOGAS TECHNOLOGY IN CHINA

The straw used for anaerobic fermentation includes not only the traditional rice straw, maize straw, but also straw from crops with regional characteristics, such as banana straw, cassava straw, cotton straw, rape straw and peanut seedlings. There are currently large and medium-sized straw biogas projects in many areas such as Sichuan and Henan. There are currently four main processes for fermenting and producing biogas in China: high solids concentration, solid state, two-phase and mixed fermentation, all four of which are types of anaerobic fermentation. (Deng, Liu, Zheng, Wang, Pu, Song, Wang, Lei, Chen & Long 2017.)

Most of the early biogas projects in China operated under conditions where the total solids concentration of the feed or the total solids concentration in the reactor was less than 2 %, and the volumetric organic loading rate was usually less than 1.5 kg/m^{3}d, resulting in a low pool volume gas production rate, generally below 0.5 m^3/m^{3}d, thus causing the overall economy of the project to be poor. In 2010, with the introduction of fully mixed continuous stirred anaerobic reactor process digestion, China's large and medium-sized biogas projects began to commonly use high concentration medium temperature fermentation technology. The total feed solids concentration (TS) or total solids concentration in the reactor (TSr) of high solids concentration anaerobic digestion is generally higher than 8 % with a volumetric organic load of 3 to 6 kg/m^{3}d. (Li, Yuan & Sun 2010.)

The effect of solids concentration in the reactor on anaerobic digestion start-up and methane production showed that all three TSr (16.0 %, 13.5 % and 11 %) achieved normal start-up with no suppression of volatile organic acids and ammonia. Continuous fermentation was also carried out using food waste as feedstock, with anaerobic digestion operating at a maximum organic loading rate of 5.588 kg/m^{3}d, a tank capacity gas production rate of 4.41 m^3/m^{3}d and a feedstock methane production rate of 353 to 488 L/kg. The rapid start-up of medium temperature anaerobic fermentation of pig manure at high solids concentration (TS = 15 %) was achieved by inoculum domestication, and after 60 days of anaerobic fermentation, gas production rates of 320.5 to 488 L/kg of raw material were achieved. The gas production rate was 320.5 to 357.3 L/kg after 60 days of anaerobic fermentation. Focusing on the effect of ammonia concentration on continuous high-solids anaerobic digestion, the results show that long-term fermentation can significantly improve the tolerance of anaerobic microorganisms to high ammonia concentrations. Medium temperature batch fermentation was carried out with sludge at a TS concentration of 17 % and the whole fermentation process lasted 73 days. The average tank volume gas production rate was 0.498 m^3/m^3d and the organic matter removal rate was 67.3 %. (Li et al 2010.)

High-solids anaerobic digestion is usually carried out in a continuous stirred-tank reactor (CSTR), where the solids are pre-treated before feeding by shredding, mixing with digestate or other feedstocks, by screw pump feeding, piston feeding and mixed scour feeding. The high solids anaerobic digestion process is suitable for a variety of feedstocks such as livestock waste, light industrial wastewater sludge, domestic organic waste and sludge, but it is difficult to achieve results with straw. Due to the problems of stratification and crusting in anaerobic digestion of straw, the pool capacity gas yield of grasshopper biogas projects in China is low and straw biogas is suitable for development towards anaerobic dry fermentation technology. In addition to the CSTR, a horizontal high residual anaerobic reactor suitable for high concentration anaerobic digestion was designed by combining the CSTR with a plug flow reactor, using a hydrodynamic circulating mixing tube to mix the feedstock and inoculum and a screw thruster at the bottom of the reactor to remove high specific gravity impurities such as sand and gravel. (Weiland & Rozzi 1991.)

Solid-state anaerobic digestion, also known as dry fermentation, is suitable for treating solid feedstocks such as woody fibre feedstocks and domestic organic waste, with TSr generally in the range of 20 % to 50 %. Solid state anaerobic digestion generally does not require the addition of large amounts of water to dilute the feedstock. This saves water and avoids the problems of handling methane. Low particle size and impurity requirements and simple pre-treatment. High organic matter loading rates and reservoir volume gas production rates. Easy dewatering of the fermentation residue, which can be used directly without even dewatering. (Deng et al. 2017.)

At present, the main dry fermentation systems in China are percolation, membrane (air bag), garage and push-flow systems. In percolation dry fermentation systems, the lower part of the reactor is equipped with an orifice plate and the bottom is used to store leachate, which is circulated to spray the upper part of the feedstock (Deng et al. 2017). Similar to the Swiss Kompogas process, it is a flat push flow anaerobic reactor. It is a flat push anaerobic reactor characterised by a horizontal inlet and outlet, with the storage gas collected from the top. The material is incompletely mixed and pushed from the inlet side to the outlet side by slow agitation in a horizontal stirrer. The methane fraction at the outlet end returns to the inlet end where it is fully mixed with the new material. (Lissen, Vandevivere & De 2001.)

Two-phase anaerobic digestion is based on the two-stage theory of hydrolysis-acid production and methanogenesis in biogas fermentation, which aims to optimise the fermentation parameters for the different growth and metabolic characteristics of hydrolysing acid-producing and methanogenic microorganisms. For example, hydrolysing acid-producing bacteria are able to tolerate lower pH values and higher ammonia, and acidic conditions are also favourable for the hydrolysis of the feedstock; methanogenic bacteria can only grow and metabolise under neutral conditions and are more sensitive to high ammonia concentrations. When treating fluidised feedstock, two-phase anaerobic digestion can be used in various combinations of reactor types, with the hydrolysis acid production phase usually using CSTR. (Deng et al. 2017.)

A typical two-phase anaerobic digestion process for solid feedstocks is a combination of anaerobic percolation beds and ascending anaerobic sludge beds. This process is actually an upgrade of the leachate dry fermentation system, except that the leachate is transferred to a dedicated anaerobic reactor for methane production. In addition to the ascending anaerobic sludge bed, other anaerobic reactors suitable for organic wastewater treatment can be used for the methanogenic phase. For wood fibre feedstock, the process can compensate for the disadvantages of dry fermentation. For example by continuous gas production in a separate methanogenic stage, and for hydrolysis, which is the rate-limiting step in the entire biogas fermentation process. The hydrolysis of wood fibre feedstock can be enhanced by a separate hydrolysis acid production stage. The process has been used to treat straw, manure and water hyacinth with good results. For perishable feedstocks, where methanogenesis is the rate-limiting step, this process separates the acid and methanogenic processes, avoiding the inhibition of methanogenic bacteria by VFA. VFA inhibition under high organic loading conditions was successfully avoided by using this process to treat fruit and vegetable waste. (Nizami & Murphy 2010.)

A typical two-phase anaerobic digestion process for solid feedstocks is a combination of anaerobic percolation beds and ascending anaerobic sludge beds. This process is actually an upgrade of the leachate dry fermentation system, except that the leachate is transferred to a dedicated anaerobic reactor for methane production. In addition to the ascending anaerobic sludge bed, other anaerobic reactors suitable for organic wastewater treatment can be used for the methanogenic phase. The process has been used to treat straw, manure and water hyacinth with good results. For perishable feedstocks, where methanogenesis is the rate-limiting step, this process separates the acid and methanogenic processes, avoiding the inhibition of methanogenic bacteria by VFA. VFA inhibition under high organic loading conditions was successfully avoided by using this process to treat fruit and vegetable waste. (Nizami & Murphy 2010.)

In addition to feedstock pre-treatment, process and reactor design optimisation, gas production efficiency can also be improved by mixed anaerobic digestion. Mixed anaerobic digestion is the anaerobic digestion of two or more feedstocks with complementary characteristics. Mixed anaerobic digestion has the following advantages. Firstly, the carbon to nitrogen ratio of the fermentation feedstock is adjusted to balance the nutrient structure. Secondly, the moisture content of the fermentation feedstock is adjusted to meet the process requirements. Thirdly, mixing difficult to degrade and perishable feedstocks to avoid acidification and increase the tank capacity gas production rate. Fourth, improve the buffering capacity and stability of the system. (Ye, Li , Sun, Wang, Yuan, Zhen & Wang 2013.)

7 CONCLUSION

Global energy security issues provide good opportunities for the development of bioenergy and renewable energy. The new gas can relieve environmental pressure and the urgent need for oil energy shortage, and is also a promising industry for agriculture and rural areas. The development of bio-gas should rely on the synergistic development of rural areas, agriculture and agricultural engineering to promote the healthy, orderly and steady development of the bio-energy industry.

This thesis describes in detail the process of biomass fermentation for the production of methane and hydrogen. The two processes of biomass production of methane and hydrogen have much in common, for example both production processes are microbial fermentation processes and both require the use of some bacteria to aid production. However, the differences between the two are very significant, for example the choice of reactor and the type of microorganism or bacteria required. There are many different ways to produce the same gas, so choosing the right raw material, the right type of bacteria and the right reactor becomes an important factor for industry to calculate production costs.

At present, reactors for the production of methane from biomass are still mainly at the level of anaerobic wastewater treatment reactors. The next challenge for anaerobic biotechnology is to expand its scope of application, especially in the face of large amounts of solid resources such as lignocellulose. Therefore, innovative and more efficient microbial population building and reactor design are needed.

China is a large agricultural country and the deep integration of industry and agriculture is indispensable to achieve sustainable industrial and rural economic development and to solve the "three rural issues". In the process of biomass fermentation production, the whole process from raw material to gas and final use of waste should be considered, and the scale effect of new gas production should be considered, especially in rural areas, and the integrated use of production and products should be considered from a system engineering perspective. With the rapid development of biology, chemistry, engineering, mathematics and economics, the development of new gas production from biomass fermentation will be further promoted and the key issues involved will be gradually solved. China's new gas development has already taken a path of its own. Both the growth in Chinese methane production and China's unique methane industry model are worth studying and learning from for countries working on new natural gas technology development.There are many issues to be considered in the development of new gas, such as the acceptability of methane as a new energy source and the conflict between new gas development and the environment. These are a range of issues that need to be addressed.

REFERENCES

Alexei, S. 2002. The complete genome of hyper thermophile Methanopyrus kandleri AV19 and monophyly of archaeal methanogens. *PANS*, 99(7), 4644-4649.

Amirta, R. 2006. Methane fermentation of Japanese cedar wood pretreated with a white rot fungus, Ceriporiopsis subvermispora. *Journal of Biotechnology*, 123, 71-74.

Antonopoulou, G., Dimitrellos, G., Beobide, A.S., Vayenas, D. & Lyberatos, G. 2015. Chemical Pretreatment of Sunflower Straw Biomass: The Effect on Chemical Composition and Structural Changes. *Waste Biomass Valor*. 6, 733-746.

Antonopoulou, G., Hariklia, G. & Ioannis, S. 2008. Biofuels generation from sweet sorghum: Fermentative hydrogen production and anaerobic digestion of the remaining biomass. *Bioresource Technology*, 99, 110-119.

Aslanzadeh, S. ,Ishalo, M.M. , Richards, T. & Taherzadeh, M.J. 2014. An Overview of Existing Individual Unit Operations. *Biorefineries*, 3-36.

Bouallagui, H. 2004. Two-phases anaerobic digestion of fruit and vegetable wastes: bioreactors performance. *Biochemical Engineering Journal*, 21, 193-197.

BTAC. 2002. Vision for bioenergy and biobased products in the United States. Academic Press, 9-11.

Claassen, P. A. M. 1999. Utilisation of biomass for the supply of energy carriers. *Applied microbiology and biotechnology*, 52, 741-755.

Deng L. W., Liu Y., Zheng D., Wang L., Pu X. D., Song L., Wang Z.Y., Lei Y. H. Chen Z. A. & Long Y. 2017. Application and development of biogas technology for the treatment of waste in China. *Renewable* *and Sustainable Energy Reviews*. 70, 845-851.

DOE. 2022. Hydrogen Production: Microbial Biomass Conversion. Hydrogen and Fuel Cell Technology Office. Available at: www.energy.gov/eere/fuelcells/hydrogen-production-microbial-biomass-conversion. Accessed: 26 March 2022.

FAO. Methane production. Renewable biological systems for alternative sustainable energy production. Available at: https://www.fao.org/3/W7241E/w7241e0f.htm

Kee, H. S. & Sik, S. H. 2004. Performance of an innovative Two- Stage Process Converting food waste to hydrogen and methane. *Journal of the Air & Waste Management Association*, 54, 242-249.

Keskin, K., Abubackar, H. N., Arslan, K. & Azbar, N. 2019. Biohydrogen Production From Solid Wastes. *Biohydrogen (scoend edition)*, 321-346.

Levin, D. B. 2007. Potential for hydrogen and methane production from biomass residues in Canada. *Bioresource Technology*, 98, 654-660.

Levin, D. B., Rumana, I. & Nazim, C. 2006. Hydrogen production by Clostridium thermocellum 27405 from cellulosic biomass substrates. *International Journal of Hydrogen Energy*, 31, 1496-1503.

Li D., Yuan Z. H. & Sun Y. M. 2010. Semi-dry mesophilic anaerobic digestion of water sorted organic fraction of municipal solid waste (WS-OFMSW). *Bioresource Technology*, 101(8), 2722-2728.

Lissen, S. G., Vandevivere, P & De, B.L. 2001. Solid waste digesters: Process performance and practice for municipal solid waste digestion. *Water Science Technology*, 44(8), 91-102.

Liu D. W. 2006. Hydrogen and methane production from household solid waste in the two- stage fermentation process. *Water Research*, 40, 2 230-2 236.

Monlau, F., Aemig, Q., Barakat, A., Steyer, J. P., Carrere, H. 2013. Application of optimized alkaline pretreatment for enhancing the anaerobic digestion of different sunflower stalks varieties.*Environ*, 34(13-14), 2155-2162.

Mosier, N. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, 96, 673-686.

Moulin, T. M., Fritsche, U. R. & Junginger, M. 2019. Charting global position and vision of stakeholders towards sustainable bioenergy. *Energy, Sustainability and Society*.9-48.

Naomichi, N. & Nakashimada, Y. 2004. High rate production of hydrogen/methane from various substrates and wastes. *Advances in Biochemical Engineering/Biotechnology*, 90, 63-87.

Nizami, A. S. & Murphy, J. D. 2010. What type of digester configurations should be employed to produce biomethane from grass silage. *Renewable and Sustainable Energy Reviews*, 14(6), 1558-1568.

OPEC. 2021. OPEC monthly oil market report-13 December 2021. Available at :https://www.opec.org/opec_web/en/publications/338.htm. Accessed:28 December 2021

Patrik, S., Elin, G. & Nygren, P. A. 2002. Display of proteins on bacteria. *Journal of Biotechnology*, 96, 129-154.

Rao, M. S. & Singh, S. P. 2004. Bioenergy conversion studies of organic fraction of MSW: kinetic studies and gas yield organic loading relationships for process optimization. *Bioresource Technology*, 95, 173-185.

Ruiz, E., Romero, I., Moya, M., Cara, C., Vidal, J. D., Castro, E. 2013. Dilute sulfuric acid pretreatment of sunflower stalks for sugar production. *Bioresour*, 140, 292-298.

Shah, A. A., Seehar, T. H., Sharma, K. & Toor, S. S. 2022. Biomass pretreatment technologies.

Hydrocarbon Biorefinery, 203-208

Turick, C. E., Peck, M. W., Chynoweth, D. P., Jerger, D. E., White, E. H., Zsuffa, L. & Kenney, W. A. 1991. Methane Fermentation of Woody Biomass. *Bioresource Technology*, 37, 141-147.

Wang A. J., Ren N. Q. & Shi Y. 2008. Bioaugmented hydrogen production from microcrystalline cellulose using co-culture-Clostridium acetobutylicum X9 and Ethanoigenens harbinense B49. *International Journal of Hydrogen Energy*. 33, 912-917.

Wang X. Q., Dong T. T., Zhang A. L., Fang Y.T., Chen D., Zhao C.X., Luo Q. & Yang H. H. 2019. Isolation of bacteria capable of hydrogen production in dark fermentation and intensification of anaerobic granular sludge activity. *International Journal of Hydrogen Energy*, 44(30), 15853-15862.

Weiland, P. & Rozzi, A. 1991. The Start-up Operation and Monitoring of High-Rate Anaerobic Treatment Systems: Discusser's Report. *Water Science and Technology*, 24(8) , 257-277.

Xu H. L., Wang J. Y. & Zhang H. 2002. A comparative study of anaerobic digestion of food waste in a single pass, a leachate recycle and coupled solid/liquid reactors. *Water Science and Technology*, 47(1), 319-324.

Yuval, S. Raphael, L. & Edward, B. A. 1999. The cellulosome concept as an efficient microbial strategy for the degradation of insoluble polysaccharides. *Trends in Microbiology*, 7 (7) ,275-281.

Zhang C., Xing X. H. & Lou K. 2005. The green fluorescent protein as a visible marker for quantifying the hydrogen - producing bacterium of Enterobacter aerogenes in an anaerobic complex system. *FEMS Microbiology Letters*. 249, 211-218.