



LUND UNIVERSITY

Factors that Influence the Biochemical Methane Potential (BMP) Test

Steps towards the Standardisation of BMP Test

Wang, Bing

2016

Document Version:

Other version

[Link to publication](#)

Citation for published version (APA):

Wang, B. (2016). *Factors that Influence the Biochemical Methane Potential (BMP) Test: Steps towards the Standardisation of BMP Test*. [Doctoral Thesis (compilation), Lund University]. Biotechnology, Lund University.

Total number of authors:

1

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Factors that Influence the Biochemical Methane Potential (BMP) Test

Steps towards the Standardisation of BMP Test

Bing Wang



LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Engineering, Lund University, Sweden.

To be defended at the Center for Chemistry and Chemical Engineering.

Date 20th of May, 2016 at 13:15 in Lecture Hall B.

Faculty opponent

Prof. Christof Holliger, Laboratory for Environmental Biotechnology
School of Architecture, Civil and Environmental Engineering (ENAC), EPFL
Lausanne, Switzerland

Organization LUND UNIVERSITY Division of Biotechnology P.O. Box 124 SE 22100 Lund, Sweden Author(s) Bing Wang	Document name Doctoral dissertation	
	Date of issue 2016-05-20	
	Sponsoring organization China Scholarship Council, Swedish International Development Agency and Swedish Energy Agency	
Title and subtitle Factors that Influence the Biochemical Methane Potential (BMP) Test: Steps towards the Standardisation of BMP Test		
Abstract <p>Anaerobic digestion (AD) has gained increasing attention nowadays as an approach for both waste treatment and renewable energy generation. Currently, many different types of materials can be used as feedstock for biogas production via AD process, but their biodegradability (based on methane yield, BD_{CH_4}) and potential to produce biogas might vary significantly, and these properties are key parameters that should be taken into consideration for economy, design and operation of a full-scale biogas plant during the selection of potential feedstock.</p> <p>The BD_{CH_4} and methane potential of a material are commonly determined using the Biochemical Methane Potential (BMP) test. However, a number of factors, e.g., temperature, pH, inoculum preparation, inoculum to substrate ratio (ISR), substrate concentration, mixing, etc. can affect the BMP test results. Moreover, the experimental setups, data analysis and presentation vary in different laboratories, and therefore, the results from different studies are difficult to compare. To improve the reliability and reproducibility of the BMP test and ensure that the results are more comparable, this PhD study evaluated the influences of various factors on the methane potential and degradation kinetics of a standard substrate (i.e., cellulose) and certain other types of materials. For example, mixing plays an important role in the BMP test because it aids in the distribution of microorganisms, substrates and nutrients; release of produced gases; and equalisation of the temperature in the digester, thereby enhancing the digestion process. In Paper IV, different mixing strategies were applied to evaluate the influences of mixing on the BMP test. The results showed that the methane potential of blank (inoculum only) was increased approximately 77% and 220% by automated continuous mixing at low intensity (10 rpm) and high intensity (160 rpm), respectively, compared with the methane production obtained from the manually shake system. For the most viscous substrate investigated, i.e., dewatered sludge (DWS), automated continuous mixing significantly improved the methane production even at low mixing intensity. However, for cellulose (fine-powdered and easily degraded) and much diluted substrate 8*DWS (i.e., DWS diluted by a factor of 8), mixing is not necessary or the manual shaking once per day is sufficient during the BMP test. Furthermore, certain other important factors, such as experimental setup, inoculum preparation and substrate concentration, were also evaluated and displayed a significant impact on the BMP test.</p> <p>Finally, as an application of the BMP test, a case study was performed to evaluate the effects of different pre-treatments on lignocellulosic biomass (<i>Miscanthus</i>) for improved methane production. <i>Miscanthus</i> has been proven as one of the highest energy biomasses in recent years; however, its conversion to biogas/methane is limited due to its recalcitrant structure. The study showed that methane production of <i>Miscanthus</i> was significantly improved after size reduction, steam explosion (SE) and alkali pre-treatment.</p>		
Key words Anaerobic Digestion; Biochemical Methane Potential; Degradation Kinetics; Lignocellulosic Biomass; Mixing; Pre-treatment; Standardisation		
Classification system and/or index terms (if any)		
Supplementary bibliographical information		Language English
ISSN and key title		ISBN 978-91-7422-436-8
Recipient's notes	Number of pages 112	Price
	Security classification	

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature _____ Date _____

Factors that Influence the Biochemical Methane Potential (BMP) Test

Steps towards the Standardisation of BMP Test

Bing Wang



LUND
UNIVERSITY

Cover: The opening moment of flow cell of Automatic Methane Potential Test System II (AMPTS II), with real-time information registered.

Photo by Kairang Chen

© Bing Wang

Division of Biotechnology
Faculty of Engineering
Lund University

ISBN 978-91-7422-436-8
ISRN LUTKDH/TKBT-16/1166-SE

Printed in Sweden by Media-Tryck, Lund University
Lund 2016



To my parents and beloved family

Content

Popular Summary	1
Abstract	3
List of Papers	5
My Contributions to the Papers	6
Abbreviations	7
1. Introduction	9
1.1 Aims of the study	10
2. Anaerobic Digestion	11
2.1 Degradation pathway during anaerobic digestion	11
2.1.1 Hydrolysis	11
2.1.2 Acidogenesis	13
2.1.3 Acetogenesis	13
2.1.4 Methanogenesis	13
2.2 Factors that influence the AD process	13
2.2.1 Temperature	14
2.2.2 pH	14
2.2.3 Alkalinity	15
2.2.4 Mixing	15
2.2.5 Inhibitory compounds	15
2.3 Feedstock analysis	16
3. Biochemical Methane Potential Test	19
3.1 Factors that influence the BMP test	20
3.1.1 Experimental conditions	20
3.1.2 Experimental setups and potential errors induced	21
3.1.3 Data analysis and presentation	22
3.2 Degradation kinetics	23
3.3 Own contributions to optimise and standardise the BMP test	24
3.3.1 Experimental setups for the BMP test	24
3.3.2 Inoculum preparation	26
3.3.3 Substrate concentration	27

3.3.4 Mixing strategies	28
4. Application of the BMP Test: A Case Study	31
4.1 Physical pre-treatment: size reduction	31
4.2 Steam explosion	32
4.3 Alkali pre-treatment	33
4.4 Acid pre-treatment	33
5. Concluding Remarks and Future Perspectives	35
Acknowledgement	37
References	39

Popular Summary

The amount of waste currently produced is increasing rapidly together with a growing population and rising living standard. More people are aware of the importance of sustainable development of our society, because of global warming, finite fossil fuel resources (i.e., coal, oil and natural gas), and the increasing price of these fuels. Therefore, an urgent need exists for the waste management and alternative energy sources. Is there an approach which can be applied for both waste treatment and energy generation, converting the waste to valuable resource? The answer is yes.

Anaerobic digestion (AD) is such a process which can convert waste to renewable energy biogas (a gas mixture of consisting primarily of methane and carbon dioxide) in the absence of oxygen. The produced biogas/methane can be used as a vehicle fuel, for generation of electricity and heat. In addition, the residuals from this process are rich in nutrients and can be used as fertilizer. Different types of waste materials that can be used as feedstock to produce biogas via the AD process, but the percentage of biodegradable content (i.e., the biodegradability based on methane yield, BD_{CH_4}) and methane potential of each material can differ significantly. BD_{CH_4} and methane potential are two important parameters for design, operation and economy of a full-scale AD process. Therefore, it is necessary to fully understand these two parameters of the feedstock prior to the AD process.

A Biochemical Methane Potential (BMP) test is commonly performed to understand the BD_{CH_4} and methane potential of the investigated material. However, the results from the BMP tests reported in different studies can vary and are difficult to compare due to the existence of different protocols, the tests might differ in terms of experimental setups, experimental conditions, and data analysis, among others.

The goal of this study is to gain the knowledge on optimisation and standardisation of the BMP test, and thereby increase the reliability and comparability of the test results. Paper I studied the experimental setups used for the BMP test, Papers II-IV examined the influences of different experimental conditions on the BMP test and Paper V presented a case study of BMP applications.

The work presented throughout this thesis consists of steps towards standardisation of the BMP assay, which is an effort to convert waste to valuable resource.

Abstract

Anaerobic digestion (AD) has gained increasing attention nowadays as an approach for both waste treatment and renewable energy generation. Currently, many different types of materials can be used as feedstock for biogas production via AD process, but their biodegradability (based on methane yield, BD_{CH_4}) and potential to produce biogas might vary significantly, and these properties are key parameters that should be taken into consideration for economy, design and operation of a full-scale biogas plant during the selection of potential feedstock.

The BD_{CH_4} and methane potential of a material are commonly determined using the Biochemical Methane Potential (BMP) test. However, a number of factors, e.g., temperature, pH, inoculum preparation, inoculum to substrate ratio (ISR), substrate concentration, mixing, etc. can affect the BMP test results. Moreover, the experimental setups, data analysis and presentation vary in different laboratories, and therefore, the results from different studies are difficult to compare. To improve the reliability and reproducibility of the BMP test and ensure that the results are more comparable, this PhD study evaluated the influences of various factors on the methane potential and degradation kinetics of a standard substrate (i.e., cellulose) and certain other types of materials. For example, mixing plays an important role in the BMP test because it aids in the distribution of microorganisms, substrates and nutrients; release of produced gases; and equalisation of the temperature in the digester, thereby enhancing the digestion process. In Paper IV, different mixing strategies were applied to evaluate the influences of mixing on the BMP test. The results showed that the methane potential of blank (inoculum only) was increased approximately 77% and 220% by automated continuous mixing at low intensity (10 rpm) and high intensity (160 rpm), respectively, compared with the methane production obtained from the manually shake system. For the most viscous substrate investigated, i.e., dewatered sludge (DWS), automated continuous mixing significantly improved the methane production even at low mixing intensity. However, for cellulose (fine-powdered and easily degraded) and much diluted substrate 8*DWS (i.e., DWS diluted by a factor of 8), mixing is not necessary or the manual shaking once per day is sufficient during the BMP test. Furthermore, certain other important factors, such as experimental setup,

inoculum preparation and substrate concentration, were also evaluated and displayed a significant impact on the BMP test.

Finally, as an application of the BMP test, a case study was performed to evaluate the effects of different pre-treatments on lignocellulosic biomass (*Miscanthus*) for improved methane production. *Miscanthus* has been proven as one of the highest energy biomasses in recent years; however, its conversion to biogas/methane is limited due to its recalcitrant structure. The study showed that methane production of *Miscanthus* was significantly improved after size reduction, steam explosion (SE) and alkali pre-treatment.

List of Papers

This thesis is based on Papers I-V, which are attached as appendices at the end of the thesis. Papers I-III and V are reproduced by the permission of the respective journals.

- I. **Bing Wang**, Ivo Achu Nges, Mihaela Nistor, and Jing Liu. Determination of Methane Yield of Cellulose Using Different Experimental Setups. *Water Science & Technology*, 70 (2014), 599-604.
- II. **Bing Wang**, Sten Strömberg, Ivo Achu Nges, Mihaela Nistor, and Jing Liu. Impacts of Inoculum Pre-treatments on Enzyme Activity and Biochemical Methane Potential. *Journal of Bioscience and Bioengineering*, 121 (2016), 557-560.
- III. **Bing Wang**, Sten Strömberg, Chao Li, Ivo Achu Nges, Mihaela Nistor, Liangwei Deng, and Jing Liu. Effects of Substrate Concentration on Methane Potential and Degradation Kinetics in Batch Anaerobic Digestion. *Bioresource Technology*, 194 (2015), 240-246.
- IV. **Bing Wang**, Annika Björn, Sten Strömberg, Ivo Achu Nges, Mihaela Nistor and Jing Liu. Evaluating the Influences of Mixing Strategies on the Biochemical Methane Potential (BMP) Test. *Manuscript*
- V. Ivo Achu Nges, Chao Li, **Bing Wang**, Liang Xiao, Zili Yi, and Jing Liu. Physio-Chemical Pretreatments for Improved Methane Potential of *Miscanthus Lutarioriparius*. *Fuel*, 166 (2016), 29-35.

Related publication that is not included in this thesis:

- VI. Ivo Achu Nges, **Bing Wang**, Zhifang Cui, and Jing Liu. Digestate Liquor Recycle in Minimal Nutrients-Supplemented Anaerobic Digestion of Wheat Straw. *Biochemical Engineering Journal*, 94 (2015), 106-114.

My Contributions to the Papers

All the studies presented in this thesis are under the supervision of Associate Professor Jing Liu, Dr. Mihaela Nistor and Dr. Ivo Achu Nges.

- I. I planned and performed the experimental work. I performed all the data analysis and wrote the first draft of the manuscript.
- II. I had major role in the planning of the study and did all the experimental work. I also had a major role in analysing the results and writing the manuscript.
- III. I planned and did all the experimental work. I also had a major role in analysing the results and writing the manuscript.
- IV. I made a major contribution to the design and planning of the study. I performed most part of the experimental work. I also had a significant contribution in the analysis of the results and writing the manuscript.
- V. I was involved in the planning of the study and assisted to perform the experimental work. I contributed to improve the manuscript.
- VI. I was involved in the planning of the study, I performed part of the experimental work and data analysis. I contributed to improve the manuscript.

Abbreviations

AD	Anaerobic Digestion
AMPTS	Automatic Methane Potential Test System
BD _{CH₄}	Biodegradability based on Methane Yield
BDM	Bidirectional Mixing
BMP	Biochemical Methane Potential
CMCaseA	Carboxymethyl Cellulase Activity
COD	Chemical Oxygen Demand
DW	Distilled Water
DWS	Dewatered Sludge
FA	Free Ammonia
GC	Gas Chromatography
IA	Intermediate Alkalinity
ISR	Inoculum to Substrate Ratio
LCFAs	Long Chain Fatty Acids
NBS	Nutrient/Buffer Solution
NM	No Mixing
PA	Partial Alkalinity
TA	Total Alkalinity
TS	Total Solids
SE	Steam Explosion
SD	Standard Deviation
SKM	Shake Manually
SKWB	Shaking in Water Bath

STP	Standard Temperature and Pressure
UDM	Unidirectional Mixing
VFAs	Volatile Fatty Acids
VS	Volatile Solids

1. Introduction

Together with continued population growth and improvement in living standards, the amount of waste produced from industry, agriculture, stockbreeding and daily life is rapidly increasing. The population of the European Union (EU-28) was estimated at 508.2 million in January of 2015, and the total waste generated by all economic activities and households amounted to 2514 million tons (Eurostat, 2015a; Eurostat, 2015b). Therefore, one considerable challenge is to find an efficient approach for waste treatment.

Furthermore, fossil fuels (i.e., coal, oil and natural gas) have made modern life possible since the industrial revolution. These sources of energy are used to generate steam, electricity and power transportation systems. The global energy demand is projected to increase by 37% by 2040, and 75% of the energy supply will be fossil fuels (IEA, 2014). However, fossil fuels are non-renewable resources because of the long period required for their formation, i.e., millions of years, and because the reserves are being consumed much faster than new ones are being formed. Moreover, the use of fossil fuels releases a notably large amount of carbon dioxide, which is a greenhouse gas that contributes to global warming. As a result, society is facing the grand challenge of finding alternative energy sources and reducing the dependency on fossil fuels.

Anaerobic digestion (AD) is a complex process in which biodegradable materials (e.g., household waste, manure, crop straws, etc.) are broken down in the absence of oxygen and the renewable energy biogas is produced (Gunnerson et al., 1986). Biogas can be used as a vehicle fuel and for generation of electricity and heat. In the past few decades, AD has been considered as a popular approach that offers environmentally friendly solutions simultaneously address the two looming challenges mentioned above, i.e., waste handling and renewable energy generation. In addition to these two advantages, anaerobic digestate contains rich nutrients and can be used as fertilizer (Alfa et al., 2014).

Most types of organic waste can be degraded and used as feedstock to produce biogas via AD process. However, the feedstock can differ significantly in characteristics such as, methane potential, biodegradability (based on methane yield, BD_{CH_4}) and nutrient content, and all of these properties play significant role in the design, economy and management of full-scale implementation of AD (Møller et al., 2004). The methane potential and BD_{CH_4} of a potential

feedstock are commonly analysed by the Biochemical Methane Potential (BMP) test (Fannin et al., 1980; Owen et al., 1979). To carry out a BMP test, the investigated material is mixed with an anaerobic inoculum, which is normally collected from an active biogas plant, then the mixture is incubated under mesophilic or thermophilic condition for a period of 30-60 days or even longer (Labatut et al., 2011; Owen et al., 1979). In recent years, studies that address the BMP test have been extensively published, and different procedures and instruments have been applied. Unfortunately, no qualified recommendation exists for the BMP assay. Consequently, it is difficult to evaluate and compare results from different studies due to possible differences in the experimental protocol as well as in data analysis and presentation (Raposo et al., 2011). To improve knowledge on BMP test and ensure the results more comparable, the influences of several important factors were evaluated in this PhD study. In Paper I, different experimental setups are used for the determination of the methane yield of a standard substrate (i.e., cellulose), and the differences among these setups are compared. Papers II and III evaluate the effects of inoculum preparation and substrate concentration on the BMP assays, respectively. Paper IV evaluates the impacts of mixing on the BMP test, and Paper V presents a case study that demonstrates one application of BMP assay.

This thesis includes five sections. Section 1 presents an introduction to the research field and the aims of the study. Section 2 offers the general information on AD. Section 3 describes the factors that affecting the BMP test and the contributions of this thesis to optimisation and standardisation of the BMP test; Section 4 presents a case study of BMP application and Section 5 includes the conclusions of this study and future perspectives.

1.1 Aims of the study

This study aims to improve the knowledge of optimisation and standardisation of the BMP test and thereby increase the reliability and comparability of the results reported by different studies. The aims are accomplished by: i) optimisation of experimental conditions (i.e., experimental setup, inoculum preparation, substrate concentration and mixing) for the BMP test (Papers I-IV) and ii) reduction of errors induced during the test and normalisation of the data analysis and presentation (Paper I).

2. Anaerobic Digestion

AD is a complex process in which organic material is broken down by several groups of microorganisms in the absence of oxygen and renewable energy biogas is generated (Chen et al., 2008). AD occurs spontaneously in anaerobic environments such as lake and oceanic basin sediments (Koyama, 1963; Pamatmat & Bhagwat, 1973). The biogas generated from the AD process is a renewable energy source consisting mostly of methane (CH_4) and carbon dioxide (CO_2), with a small amount of hydrogen (H_2) and traces of hydrogen sulphide (H_2S) and ammonia (NH_3) (Abatzoglou & Boivin, 2009).

AD is an attractive technique by which both waste treatment and renewable energy recovery can be achieved. In addition to these two benefits, AD also reduces the odour of waste material and the digestate is rich in nutrients that can be used as fertilizer (Pain et al., 1990; Welsh et al., 1977).

2.1 Degradation pathway during anaerobic digestion

AD is a sequential complex process that involves several groups of microorganisms (Amani et al., 2010). The degradation process can be simplified into four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Angelidaki et al., 1993; Gavala et al., 2003; Tiehm et al., 2001). A schematic overview of the degradation process is presented in Figure 1. The product of one step acts as a substrate for the subsequent step, and thus imbalance in any one of the steps might have a negative effect on the overall process (Gerardi, 2003).

2.1.1 Hydrolysis

Hydrolysis is the first step in AD process. Complex feedstocks such as carbohydrates, proteins and lipids are all broken down by extracellular hydrolytic enzymes into small molecules (e.g., sugars, amino acids and fatty acids) prior to absorption and further degradation by acidogenic microorganisms. Hydrolysis has been considered as the rate-limiting step in a

number of cases, as many materials consist of complex structures and thus results in a degradable component that is less accessible to microorganisms due to the physical barrier and possible chemical bonds (Tong et al., 1990). Therefore, hydrolysis is viewed as one of the most important steps under certain conditions in the AD process and needs to be improved to achieve more efficient digestion.

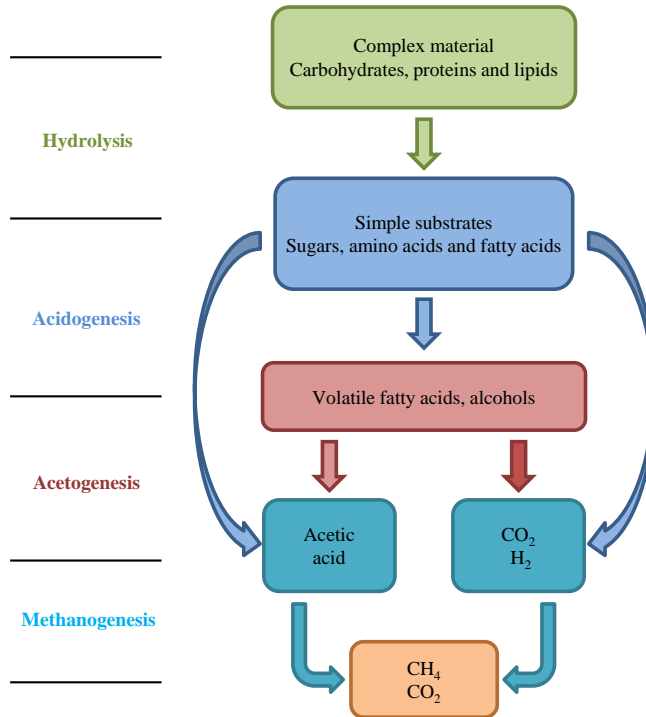


Figure 1. Schematic overview of the degradation pathway during anaerobic digestion of complex materials.

For instance, lignocellulosic biomass has gained greater attention in recent years as a resource for biogas production. However, the conversion of this material to biogas is limited by hydrolysis because of the refractory structure. Therefore, this type of biomass needs to be pre-treated for an efficient biogas production prior to the AD process. In Paper V, physio-chemical pre-treatments of *Miscanthus* (commonly known as Elephant Grass) were performed for improved biogas production. *Miscanthus* is a lignocellulosic energy crop and has been proven as one of the highest energy biomasses (Clifton-brown et al., 2004; Hastings et al., 2008; Sanchez et al., 2001) consisting mostly of cellulose and hemicellulose, but covered by a sheath of lignin (Nges et al., 2016; Shen et al., 2014). The effects of pre-treatments were evaluated using the BMP test.

2.1.2 Acidogenesis

Sugars, amino acids and fatty acids generated during the hydrolysis step are taken up by acidogenic microorganisms and converted to volatile fatty acids (VFAs), e.g., acetate, propionate, butyrate, etc., as well as alcohols, carbon dioxide and hydrogen. These products are the precursors for methane production. Acidogenesis is usually the quickest step in the AD process (Gerardi, 2003; Vavilin et al., 2008).

2.1.3 Acetogenesis

In the acetogenesis stage, conversion of VFAs and alcohols to substrates such as acetic acid, carbon dioxide and hydrogen is performed by acetogenic microorganisms. These microorganisms only thrive when the hydrogen partial pressure is very low, and therefore, they have a syntrophic relationship with hydrogen consuming methanogens (Schink, 1997).

2.1.4 Methanogenesis

Methane is produced in the final step by methanogens which belong to the *Archaea* domain and are obligate anaerobes. Methanogens are considered to be the most sensitive microorganisms in AD, and factors such as pH and high concentrations of ammonia (NH_3) might significantly inhibit their activity and can be fatal to the entire process (Koster & Koomen, 1988). In a normal AD process, approximately 70% of the methane is produced via the acetoclastic pathway (degradation of acetate), and the remaining 30% is generated from the hydrogenotrophic pathway (reaction between carbon dioxide and hydrogen) (Jetten et al., 1992). The acetogenesis and methanogenesis processes take place at similar optimal conditions (Gerardi, 2003; Weiland, 2010).

2.2 Factors that influence the AD process

The AD process is affected by several parameters that might slow or inhibit the process if they are not maintained within a certain range (Angelidaki et al., 2003; Espinoza-Escalante et al., 2009). A few of the most important factors are briefly presented.

2.2.1 Temperature

Temperature plays an important role in methane production in the AD process because it affects the activities of microorganisms and enzymes. The microorganisms are active at four different temperature ranges: psychrophilic (5-25°C), mesophilic (30-35°C), thermophilic (50-60°C) and hyperthermophilic (> 65°C). Variations in temperature affect all microorganisms in the AD process, especially the methane-forming microorganisms. Moreover, fluctuation in temperature will influence the activity of methane-forming microorganisms to a greater extent than the operating temperature, and therefore, fluctuation in temperature during the process should be minimised, i.e., < 1°C per day for thermophilic digestion and 2-3°C per day for mesophilic digestion (Gerardi, 2003).

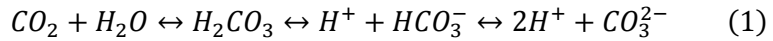
AD is mainly taking place at either mesophilic or thermophilic conditions (Pfeffer, 1974), and most methanogens are active in these two temperature ranges. The ultimate methane yield from substrate is not significantly changed in the temperature interval of 30-60°C (Hashimoto et al., 1981), but the activity of microorganisms is generally 25-50% higher under thermophilic temperatures leading to a higher digestion rate. In addition, thermophilic temperatures can improve solid settling and destruct pathogens more efficiently to satisfy regulations for disposal and reuse of the digestate (Hashimoto, 1983; Hashimoto, 1982; Varel et al., 1980). However, the free ammonia concentration increases with increased temperature, which has been considered to be an inhibitory compound and therefore has a negative effect on the process (Angelidaki & Ahring, 1994; Braun et al., 1981; Zeeman et al., 1985). Moreover, inhibition by volatile acids might occur if the temperature falls below 32°C, since the formation of volatile acids continues at depressed temperatures, but the conversion of volatile acids to methane slows (Gerardi, 2003).

2.2.2 pH

pH is one of the most important factors that affect enzymatic activity or digester performance. The microbial groups involved in the AD process have various optimal pH ranges, and to ensure a well-functioning process, the pH should be maintained at a level that can accommodate all microbial groups. Methanogens are sensitive to pH and thrive at an optimal pH interval between 6.5 and 7.2, whereas, the acidogenic microorganisms are less sensitive and functions well in a wider range of 4-8.5 (Hwang et al., 2004; Turovskiy & Mathai, 2006). The optimal pH range required to obtain the maximal biogas yield is 6.5-7.5 (Liu et al., 2008).

2.2.3 Alkalinity

Alkalinity reflects the buffering capacity and maintains the proper pH for the digester content. Alkalinity is presented primarily in the form of bicarbonates that exist in equilibrium with released carbon dioxide at a given pH. The released carbon dioxide results in production of carbonic acid, bicarbonate alkalinity and carbonate alkalinity (Equation 1). The produced ammonia results in the generation of ammonium ions (Equation 2) (Gerardi, 2003).



The total alkalinity (TA) is usually determined by titration with acid to an endpoint of pH 4.3, whereas, titration down to pH 5.75 denotes the partial alkalinity (PA). The difference between TA and PA is the intermediate alkalinity (IA), which approximates the VFAs (Jenkins et al., 1983; Ripley et al., 1986). The AD process functions well in a wide range of alkalinity between 2000 to 18000 mg CaCO₃/L (Cuetos et al., 2008; Gelegenis et al., 2007; Murto et al., 2004). The ratio of IA to PA is used to monitor the process and should be maintained at less than 0.3 (Ripley et al., 1986).

2.2.4 Mixing

Mixing is an important aspect in the AD process, because it can influence heat and mass transfer as well as the release of gas bubbles trapped in the digester liquid (Chae et al., 2008; Lindmark et al., 2014b; Sanchez et al., 2001; Sung & Dague, 1995) and therefore affects the efficiency of the process.

In an industrial AD process, different types of mixing are used, i.e., mechanical mixing, hydraulic mixing and pneumatic mixing (Dieter & Steinhauser, 2008). Mechanical mixing is most commonly used in Europe today. The influences of mixing in batch BMP tests are discussed in detail in Section 3.3.4.

2.2.5 Inhibitory compounds

The microorganisms in the anaerobic digester are quite sensitive to various compounds, especially acids- and methane-forming microorganisms. These compounds might originate from the substances or are intermediates generated during the degradation process. A substantial concentration of these compounds might upset the balance between the acids- and methane-forming microorganisms, and thereby inhibit or destroy the process (Demirel &

Yenigün, 2002; Pohland & Ghosh, 1971). Inhibition of the AD process is often indicated by a decrease in the steady-state rate of methane production and accumulation of volatile acids (Kroeker et al., 1979).

2.2.5.1 Ammonia

Ammonium ion (NH_4^+) and free ammonia (FA) are the two forms of inorganic ammonia nitrogen in the digester (Chen et al., 2008). FA has been suggested to play a major role in inhibition because it can freely pass through the membrane of the microorganisms and diffuse into the cell, leading to proton imbalance and/or potassium deficiency (Gallert et al., 1998; Sprott & Patel, 1986). Methanogens are most sensitive to ammonia (Kayhanian, 1994). Ammonia concentration of less than 200 mg/L is beneficial for the AD process (Liu & Sung, 2002). A decrease in pH and temperature within the appropriate range might increase the methane yield and reduce the ammonia inhibition (Angelidaki & Ahring, 1994; Zeeman et al., 1985).

2.2.5.2 Volatile fatty acids

Volatile fatty acids (VFAs) are important intermediate products in the AD process, and are the main substrates of methanogens used to produce methane. However, accumulation of VFAs is inhibitory to methanogens because it decreases the pH in the digester, which could lead to the loss of acid-sensitive glycolytic enzymes activity (Bouallagui et al., 2005; Misi & Forster, 2001). Moreover, unionized VFAs can penetrate the membranes of the microorganisms, and dissociate by releasing proton, thus leading to acidification of the cytoplasm (Cotter & Hill, 2003).

2.2.5.3 Long chain fatty acids

Long chain fatty acids (LCFAs) are generated by hydrolysis of lipids in anaerobic digestion. LCFAs are inhibitory to microorganisms by attachment to the membrane and disturb the transport or protective function (Rinzema et al., 1994). It has been reported that LCFAs exert a bactericidal effect and thereby result in irreversible inhibition (Angelidaki & Ahring, 1992).

2.3 Feedstock analysis

Most types of organic materials can be degraded under anaerobic conditions and can be used as feedstock to produce biogas in the AD process. The organic fraction of municipal solid waste (e.g., food waste), animal manure (in particular dairy and swine manure), energy crops, lipid-rich waste and sewage

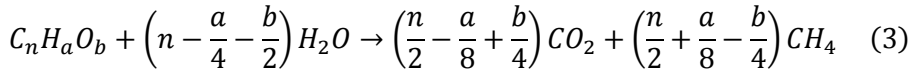
sludge generated from municipal wastewater treatment plants is the most commonly used feedstock.

However, organic materials might significantly differ in their degradation rate and methane potential and also might lack certain essential nutrients. All of these parameters play an important role in the economy, design and management of a biogas plant (Møller et al., 2004). For example, if one material lacks essential nutrients and others contain an excess of the same nutrients, then mixing of two or more such materials can balance the nutrients and will be beneficial to improve the methane yield. This approach is known as co-digestion, which is an important approach to improve the waste treatment and biogas production as well as to reduce the risk of inhibition (Álvarez et al., 2010). The main goal of co-digestion process is to balance several factors for an optimal co-substrate mixture, e.g., macro- and micro- nutrients, pH, inhibitors/toxic compounds, C/N ratio, biodegradable organic and dry matter (Hartmann et al., 2002). An optimal value of approximately 20 for the C/N ratio has been suggested to ensure the stability of the process (Burton & Turner, 2003; Chen et al., 2008). Among the above mentioned feedstock, animal manure contains high content of nitrogen, and therefore has been considered as a good co-substrate for many feedstock types with lower amounts of this nutrient, e.g., energy crops (Mata-Alvarez et al., 2014). Energy crops are slowly degraded, due to limited nutrient content and their complex structure. For such slowly degraded feedstock, longer retention times and/or large digester volume are required to generate the appropriate biogas yield, and this leads to an inefficient process and lower economic benefits (Carrère et al., 2010). Pre-treatment of such feedstock prior to AD might increase the degradation rate and/or BD_{CH_4} , and has been suggested as an important approach to improve the efficiency and economic margins of the process (Carlsson et al., 2012).

Therefore, feedstock analysis is of great importance with respect to know the methane potential, degradation rate and nutrient content. The BMP test is a batch trial used to analyse the BD_{CH_4} and methane potential of organic material and is discussed in detail in Chapter 3.

3. Biochemical Methane Potential Test

The characteristics of the feedstock are important in the design, economy and management of the AD process. BD_{CH_4} and methane potential are two of the most important characteristics and commonly analysed by the BMP test (Owen et al., 1979). Assuming the organic material (e.g., $C_nH_aO_b$) is completely converted to methane and carbon dioxide, the theoretical methane yield can be calculated according to the Buswell equation (Equation 3) (Symons & Buswell, 1933). The anaerobic BD_{CH_4} of an organic material is defined as the ratio between the experimental methane yield (BMP_{exp}) and theoretical methane potential (BMP_{th}) values (Equation 4).



$$BD_{CH_4}(\%) = \frac{BMP_{exp}}{BMP_{th}} \cdot 100 \quad (4)$$

In the BMP test, the investigated material is mixed with active anaerobic inoculum collected from a full-scale digester. The mixture is incubated under either thermophilic or mesophilic conditions and well mixed for optimal mass and heat transfer.

The test continues to run until the material is considered fully degraded or the daily gas production is less than 1% of the accumulated gas production as recommended by German standard VDI 4630 (2006), which depends on the physical and chemical properties of the material and the activity of the inoculum. During the test, the volume of produced gas is measured using either manometric or volumetric methods. At the end of the process, the BMP is calculated and adjusted to standard temperature and pressure under dry conditions (STP: 0°C, 101.325 kPa, zero moisture content) and plotted in the form of accumulated methane potential curves, as shown in Figure 2. The BMP is expressed as the volume of methane per gram of organic material added, which is often based on volatile solids (VS) or chemical oxygen demand (COD)

(Strömberg et al., 2014). However, the data analysis and presentation of methane yields in different studies are varying, and therefore might lead to incorrect and incomparable results.

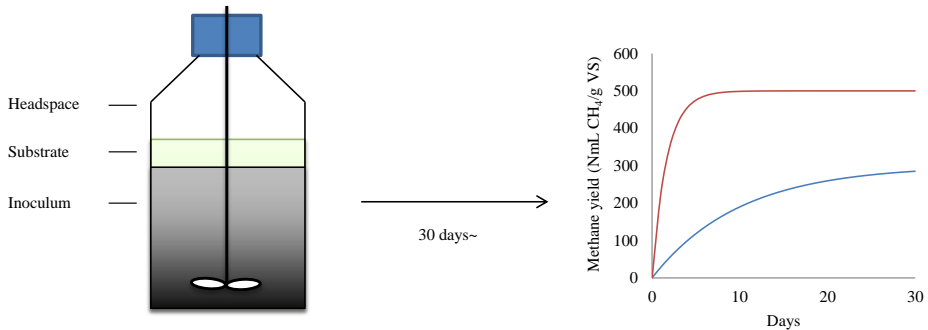


Figure 2. Overview of the Biochemical Methane Potential test.

3.1 Factors that influence the BMP test

A large number of factors can affect the BMP test and lead to incorrect and incomparable results from different studies. A number of guidelines and standards are available on how to determine the BD_{CH_4} of organic material, e.g., standards of the International Organisation of Standardisation (ISO-11734, 1995; ISO-14853, 2005), American Society for Testing and Materials (ASTM-D5210, 2007; ASTM-E2710, 2008), or German standard (VDI 4630, 2006), etc. Currently, the protocols used for the BMP test are derived from these guidelines and standards, but the protocols are quite different in terms of experimental conditions, experimental setups, data analysis and presentation. All of these aspects can impact the BMP test and lead to varying results. Moreover, even if the same instructions for experimental conditions are followed, the results might still differ. For example, one inter-laboratory study shows that the methane production of cellulose ranged from 175 ± 6 mL CH_4 /g VS to 412 ± 8 mL CH_4 /g VS (Raposo et al., 2011). Therefore, standardisation of the BMP test with respect to procedure, experimental setups, data analysis and presentation is required to obtain reliable and comparable results.

3.1.1 Experimental conditions

The results of BMP tests are dependent on the experimental conditions, e.g., environmental conditions (i.e., ambient pressure and temperature), inoculum,

inoculum to substrate ratio (ISR), substrate concentration, the composition of flush gas, mixing etc. For instance, the ISR reflects the relationship between the microbial biomass and substrate. A high ISR commonly leads to more rapid and more stable degradation but less conclusive results, i.e., the risk of less gas produced from the substrate compared with that of the inoculum. In contrast, a low ISR will generate distinct data but might result in inhibition due to the accumulated intermediate products (Maya-Altamira et al., 2008). It has been reported that a higher ISR leads to a higher methane yield in an ISR range of 0.1-3 (Maya-Altamira et al., 2008; Raposo et al., 2009; Raposo et al., 2008). However, no general rule applies with respect to the setting of ISR, although a ratio ≥ 2 is recommended by the German standard (VDI 4630, 2006) for a stable digestion process. Additional experimental factors are discussed in Section 3.3.

3.1.2 Experimental setups and potential errors induced

Various experimental setups are used to perform the BMP test in different laboratories. Each setup might differ in terms of incubation unit, total and active volume of reactors/digesters, gas sampling, gas collection, mixing regimes, gas measuring techniques, etc.

For instance, the produced gas volume can be measured using both manometric and volumetric methods. Measuring the gas volume by the manometric method requires a good gas-tight system (e.g., reactor and all the connections), a manometer, and gas chromatography (GC) for determination of gas composition. However, underestimation of the gas volume might occur because of gas leakage, and due to the solubility of gases in liquid under overpressure, the dissolved gases could influence the pH in the reactor and further inhibit the process (Rozzi & Remigi, 2004). Moreover, when taking the samples manually for gas component measurements, random errors can be induced because the injected gas volume might affect the gas composition determined by GC. Due to the pressure built up by the produced biogas, the gas in the reactor should be released and analysed regularly, which is highly time consuming and labour intensive and requires personal analysis skills. Gas volume measurements by volumetric methods are either based on the water displacement principle or collection of produced gas in a gas-tight bag. Afterwards, the gas volume is determined by measuring the amount of displaced liquid or measured with the aid of a graduated syringe by sucking the gas collected in gas bag (Liu et al., 2004; Wang et al., 2014). However, different sources of errors are induced when the gas volume measurement follows the water displacement principle, e.g., the adsorption and diffusion of gas components into the barrier solution, and carbon dioxide loss occurs more easily. It is therefore recommended to

remove the carbon dioxide before measuring the produced gas (Walker et al., 2009).

Whether manometric or volumetric methods are used, both random and human errors can't be avoided and result in unsatisfactory results. In addition to methane yield, the degradation kinetics of potential feedstock is also important. To well know the degradation profile of the feedstock, gas production should be monitored in real-time instead of by periodic measurement. Therefore, a robust experimental setup is required for the BMP test and should be automated to minimise human errors and workload. An automatic system can record the real-time gas volume, pressure and temperature to describe the entire degradation profile of the feedstock and includes the function of data normalisation (Paper I).

3.1.3 Data analysis and presentation

Environmental conditions need to be taken into consideration when calculating the data obtained from BMP test, e.g., ambient temperature and pressure, because the gases are compressible, and the volume is highly dependent on these two parameters. However, the data analyses are poorly reported in scientific papers and could potentially induce errors. A literature study, found that only one of 24 papers addressing BMP test of dairy manure reported the data analysis and correction for temperature, pressure and water vapour content, and eleven of these studies didn't report information on whether these parameters were considered (Strömberg et al., 2014). Therefore, the data obtained from the BMP test should be calculated and presented in a normalised manner to ensure correct and comparable results.

It is generally suggested that the BMP of substrate should be normalised and expressed in terms of NmL CH₄/g VS or NmL CH₄/g COD at STP conditions (0°C, 101.325 kPa, zero moisture content) using the ideal gas law as presented in Equation 5 (Nasr et al., 2012; Strömberg et al., 2014).

$$V_{STP} = \frac{p_{gas}}{p_{STP}} \cdot \frac{T_{STP}}{T_{gas}} \cdot V_{gas} \quad (5)$$

In Equation 5, V_{STP} is the volume adjusted to STP conditions, p_{gas} is the pressure of the measured gas, p_{STP} is the standard pressure, T_{STP} is the standard temperature in Kelvin (K), T_{gas} is the temperature of measured gas in K, and V_{gas} is the measured gas volume.

However, several different STP conditions are currently used, e.g., STP of 0°C and 101.325 kPa defined by the former International Union of Pure and Applied

Chemistry and STP of 25°C, 100 kPa defined by the National Bureau of Standards. Calculation of the gas volume under these two different STP conditions results in a volume difference of more than 10% for the same mass of gas (Walker et al., 2009). Therefore, the STP conditions should be clearly stated when reporting the results.

In addition to the data adjustment to STP, the water vapour content is also necessary to consider (VDI 4630, 2006; Walker et al., 2009). For volumetric gas measurement, it is worth noting that the ambient pressure and temperature should be recorded at each gas volume measurement point and used for the analysis, not the values in the reactor. Strömberg et al. (2014) reported that water vapour induced an overestimation of 2-8% for gas volume in the normal ambient temperature range (10-40°C). Moreover, the overestimation induced by flush gas characteristics should be eliminated if only the volume of methane is measured. Strömberg et al. (2014) demonstrated that the overestimation increased greatly with low methane content in the flush gas, and when a small amount of gas was produced relative to the headspace volume. Up to 50% or even greater error might be induced.

Therefore, standardisation of the BMP test is required to ensure that the results from different laboratories are reliable and comparable.

3.2 Degradation kinetics

The BMP test analyses the BD_{CH_4} and methane potential of the investigated material, and also collects information on the degradation kinetics (Jensen et al., 2011). All of these parameters offer key information on substrate selection and AD process optimisation. The degradation kinetics of the substrate is sensitive to experimental conditions, such as the pre-incubation/storage conditions of inoculum (Paper II), substrate concentration (Paper III), mixing (Paper IV), etc. As an example, size reduction has been shown to increase the degradation rate of the substrate (Vavilin et al., 2008), which is validated in Paper V.

Mathematical models are commonly used to better understand the degradation kinetics of the investigated substrate. The degradation kinetics provides valuable information on how rapidly the material is degraded for selection of a potential substrate and/or evaluation of the effects of different pre-treatment methods. The hydrolysis of substrate has been generally assumed to follow the first-order model (Equation 6), and therefore it is the model most commonly used to evaluate the degradation kinetics of a BMP test (Myint & Nirmalakhandan, 2006; Shahriari et al., 2012; Vavilin et al., 2008).

$$B(t) = B_0 \cdot (1 - \exp(-k \cdot (t - \theta))) \quad (6)$$

In Equation 6, $B(t)$ is the methane yield (NmL CH₄/g VS) at a given time t (day), B_0 is the value of the ultimate methane yield (NmL CH₄/g VS) or maximum value at infinite digestion time, k is the rate or hydrolysis constant (day⁻¹) and θ is the lag time constant (day).

3.3 Own contributions to optimise and standardise the BMP test

This PhD study focuses on evaluation of the influences of various factors on the BMP test, and the specific contributions are presented below.

3.3.1 Experimental setups for the BMP test

The experimental setups used to perform the BMP test vary among laboratories and each experimental setup has advantages and disadvantages.

Traditionally, BMP tests have been performed with in-house developed lab setups, e.g., pressure-based gas measurement system aided by manometer (Ferrer et al., 2008), water-column-based gas measurement system (Mallik et al., 1990), gas-bag-based measurement system (Mshandete et al., 2006), etc. These systems are manually operated and therefore have a high risk of inducing random and human errors. Due to the large number of vials commonly employed and the long period required (30-60 days or even longer) (Owen et al., 1979), use of traditional systems for BMP test is highly time-consuming and labour-intensive. Therefore, the need exists for a robust system for the BMP test, which could reduce workload, minimize human errors, and collect high quality data, among other improvements.

One such lab system is the Automatic Methane Potential Test System (AMPTS) II (Bioprocess Control, Sweden AB), which contains three components (Figure 3). Unit A consists of a temperature controlled water bath and 15 reactors of 500 mL volume, and each reactor is equipped with a mixer to ensure good mass and heat transfer, and aid in gas release. Unit B is CO₂-fixing unit with an alkaline solution. Unit C is a gas volume measuring device with 15 flow cell arrangements, where the normalised (STP: 0°C, 101.325 kPa, dry conditions) gas volume is measured according to the principle of liquid displacement and buoyancy. The real-time normalised gas volume, temperature and pressure are registered at each measurement point. A report is generated in Excel format

with normalised accumulated and daily methane volume. This system can significantly reduce workload and human errors.

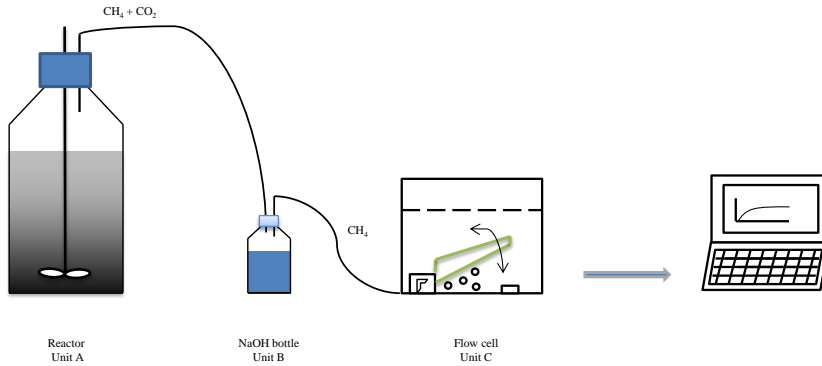


Figure 3. Schematic overview of the Automatic Methane Potential Test System (AMPTS) II.

In Paper I, the methane yield of a standard substrate (i.e., cellulose) was determined using three in-house developed experimental setups (i.e., manometer-, water-column- and gas-bag-based systems) and AMPTS II. The results (Table 1) show that the methane yield of cellulose obtained from the pressure-based system is slightly lower compared with the other systems, perhaps due to the increasing amount of gases in the liquid phase due to the high pressure built up by the produced biogas. In contrast, the methane yield of cellulose obtained from AMPTS II shows the lowest standard deviation (SD), thus representing a higher precision and less random errors.

Table 1. Methane yields obtained and workload demanded of different experimental setups.

Experimental setups	Methane yields (Nml CH ₄ /g VS)	Workload (min/sample)
Manometer	340 ± 18	540
Water-column	354 ± 13	220
Gas-bag	345 ± 15	220
AMPTS II	366 ± 5	40

Workload includes inoculum and substrate addition, gas volume measurement and gas composition analysis as well as the data management and analysis. Reproduced from Paper I.

In summary, the methane yields obtained from all systems are comparable, but the workloads differ significantly. The AMPTS II was used hereafter to perform all studies in this thesis because it has been demonstrated as a time- and labour-saving system, and more importantly, it delivers reliability with high precision and reproducibility.

3.3.2 Inoculum preparation

Inoculum plays a vital role in the BMP test and is the most complex factor that affects the results of the test, and is also the most difficult for standardisation due to the diversities of microorganisms included and their metabolic activities. Different approaches exist for inoculum preparation or storage prior to the BMP test, e.g., pre-incubate the inoculum at $35 \pm 2^\circ\text{C}$ for up to 7 days to reduce background gas production and decrease the influence of the blanks (ISO-11734, 1995), filtrate the inoculum with a 2 mm sieve to remove large particles or grit (Browne & Murphy, 2013), and store the inoculum at 4°C (Cabbai et al., 2013), etc. The inoculum storage conditions and preparation (i.e., pre-incubation, filtration) influence the metabolic activities of the microorganisms, secretion of the extracellular enzymes, and consequently, hydrolysis of the substrate (Sambusiti et al., 2014). However, to date, no qualified recommendation exists for inoculum preparation prior to a BMP test.

Inoculum pre-incubation (37°C , 5 days), filtration (2 mm mesh) and storage (4°C , 5 days) were performed prior to the tests to evaluate the influences of inoculum preparation on the BMP test, also to examine the effects of enzyme activities (Carboxymethyl Cellulase Activity, CMC_{CaseA}) of the inoculum on methane production and kinetic degradation of the substrates (cellulose and wheat straw). The enzyme activity can be used to assess the hydrolytic potential of inocula.

The results show that the CMC_{CaseA} in the inoculum decreased slightly after filtration (Figure 4: A), this is probably caused by the loss of microorganisms bound to the removed particles (Vavilin et al., 2008). Moreover, the CMC_{CaseA} had a positive correlation with the methane yields of the substrates (Figure 4: B). With respect to the kinetic degradation of the substrate, fresh inocula led to considerably shorter lag time θ , which corresponds well to higher CMC_{CaseA}, as presented in Figure 4: A. This result implies that fresh inoculum has a higher hydrolytic activity from the start. Therefore, fresh inoculum is recommended for the BMP test because it has demonstrated the highest enzyme activity and methane yield, whereas, filtration of inoculum should be avoided unless large particles are contained. Additional details can be found in Paper II.

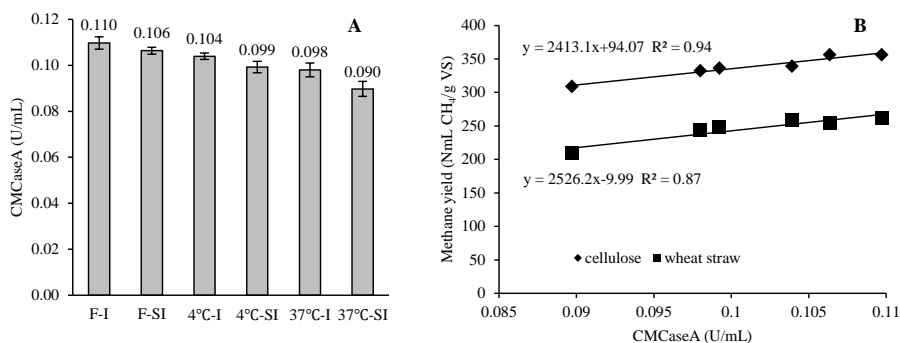


Figure 4. Enzyme activity (CMAaseA) of inocula (A) and the correlation between CMCCaseA and methane yields of substrates (B). F-I: Fresh inoculum; F-SI: sieved fresh inoculum; 4°C-I: inoculum stored at 4°C; 4°C-SI: sieved inoculum stored at 4°C; 37°C-I: inoculum pre-incubated at 37°C; 37°C-SI: sieved inoculum pre-incubated at 37°C. Reproduced from Paper II.

3.3.3 Substrate concentration

Substrate concentration has been considered as an important parameter that influences the efficiency of the AD process. At low substrate concentration, the microorganisms might exhibit low metabolic activity due to the low availability of substrate. The substrate concentration couldn't be too high also, as the overload situation might occur leading to inhibition caused by the accumulation of intermediate products (Tanimu et al., 2014; Zhang et al., 2014). With respect to methane yield, certain results in the literatures show that a higher substrate concentration leads to a higher methane yield (Maamri & Amrani, 2014; Tanimu et al., 2014), whereas, other studies present the opposite results (Zhang et al., 2014). Moreover, it is quite common to dilute the substrate to ensure that the methane potential of the substrate is not underestimated due to overload or possible inhibition (Angelidaki et al., 2009). The types of dilution media used to adjust substrate concentration are numerous, such as a medium consisting of nutrients, trace elements and vitamins (Angelidaki & Sanders, 2004; Raposo et al., 2012) or only water (Angelidaki et al., 2009). However, only few studies have investigated a fixed ISR for both diluted and undiluted substrate and it is therefore difficult to determine whether the methane yield is affected by the substrate concentration or ISR.

Thus far, the results in the literatures are inconclusive and lack of recommendations for a substrate concentration that should be used in a BMP test. The German standard VDI 4630 (2006) suggests to use an inoculum with a VS of 1.5-2.0% and an $ISR \geq 2$, which translates to a substrate concentration of ≤ 10 g VS/L.

In Paper III, five different substrate concentrations of cellulose were evaluated at a fixed ISR of 2 with or without dilutions by nutrient buffer solution (NBS) or distilled water (DW) to evaluate whether the substrate concentration has effect on methane potential and the degradation kinetics. The results show that the methane potential increases with higher substrate concentration for the two dilution series (Figure 5). To verify the results, additional BMP tests were performed. In these experiments, the substrate concentration was adjusted by dilution at a fixed ISR and by varying the ISR instead of dilution. These results offer additional evidence that substrate concentration influence the methane potential and a higher substrate concentration leads to a higher methane potential. Furthermore, these results show that this trend also occurs when the substrate concentration is adjusted by varying the ISR instead of dilution. However, these results are too limited to draw firm conclusions, and additional studies are needed to verify these observations on a wide-scale with more types of substrates, inocula and experimental conditions.

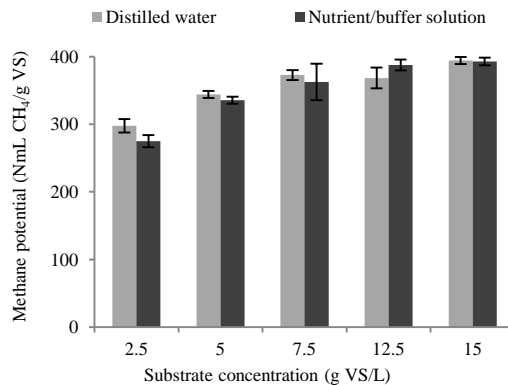


Figure 5. Methane potential of cellulose under different concentrations at ISR of 2. Reproduced from Paper III.

3.3.4 Mixing strategies

In a batch BMP test, different mixing types are applied, e.g., mixing by manually shake (Kafle & Kim, 2013) or with the aid of a magnetic bar (Raposo et al., 2011), shakers (Guendouz et al., 2010) and stirrers driven by geared motors (Raposo et al., 2006). The BMP test has also been reported under static conditions (Raposo et al., 2011). In addition to mixing types, the mixing mode (i.e., continuous or intermittent) and intensity at different frequencies and speeds can further influence the test. However, the results related to mixing are conflicting. One inter-laboratory study showed that methane yields are comparable independent of mixing, but the biogas production rate are

inconsistent (Raposo et al., 2011), whereas, other study showed that a lower mixing intensity leads to both higher biogas production rate and higher total biogas production (Lindmark et al., 2014a).

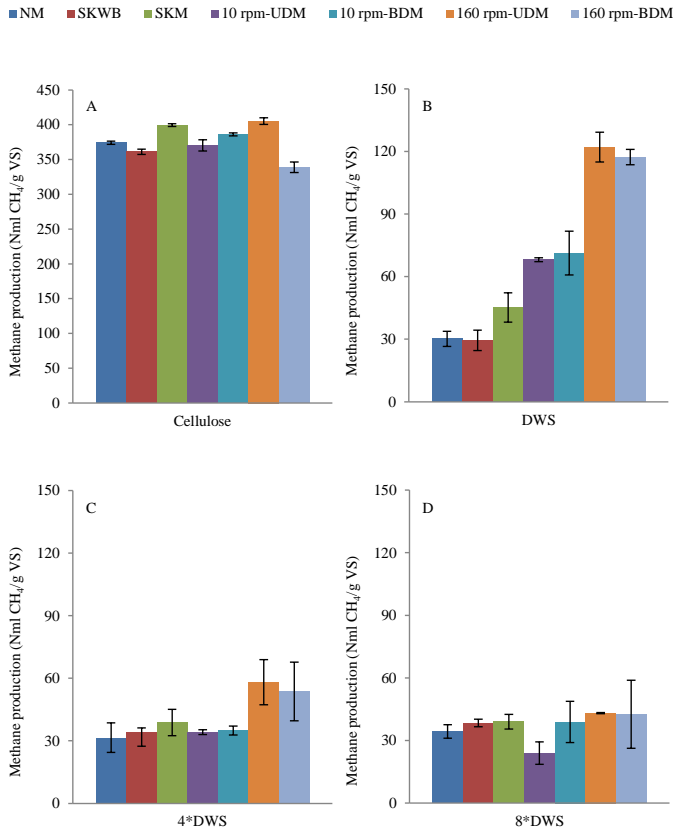


Figure 6. Methane potentials of substrates under different mixing strategies. NM: no mixing; SKWB: shaking in water bath; SKM:shake manually once per day; UDM: unidirectional mixing; BDM: bidirectional mixing; DWS: dewatered sludge. Paper IV.

Moreover, evaluation of mixing is complicated by differences in waste characteristics, organic loading, mixing system, active volume, etc. (Ganidi et al., 2009). For instance, higher gas production of palm oil mill effluents is obtained from a continuous mixing digester compared with an unmixed digester (Ho & Tan, 1985), but changing from continuous mixing to intermittent mixing leads to significantly higher gas production from a liquid municipal waste stream (Dague et al., 1970). Nevertheless, the influences with respect to high mixing intensity are in agreement, showing that high mixing intensity leads to increased shear stress, which has a negative effect on flock formation and gas

production (Kim et al., 2002; McMahon et al., 2001; Stroot et al., 2001; Whitmore et al., 1987).

In Paper IV, the influences of mixing strategies, i.e., no mixing (NM), shaking in water bath (SKWB), shake manually once per day (SKM), automated unidirectional and bidirectional mixing (UDM and BDM) with respect to mixing types, modes (continuous unidirectional and bidirectional mixing) and intensities on methane production from the BMP test are evaluated. As expected, the effects of mixing strategies are prominent for the most viscous substrate, i.e., DWS, both the highest methane potential (Figure 6: B) and highest maximal daily specific methane production were obtained at the highest mixing intensity. However, the organic removal efficiencies among all test samples are not affected by mixing, which could offer evidence that mixing aids in the release of gas bubbles trapped in the viscous liquid. Mixing is required for an efficient process when the digester content is viscous. However, mixing is not necessary or the shake manually once per day might be sufficient during the BMP test if the digester content is quite dilute or the substrate is easily degraded (Figure 6: D).

4. Application of the BMP Test: A Case Study

The degradation of a large number of organic materials used as substrates to produce biogas is difficult and slow because of their recalcitrant structures. In a full-scale biogas plant, such substrate often leads to an inefficient process with low economic benefits. Therefore, the biodegradability of such material needs to be improved by pre-treatment, and the performance of pre-treatment can be evaluated using the BMP test. Many different pre-treatment technologies have been suggested during the last few decades and can be classified into four types, i.e., biological, physical, chemical and physio-chemical pre-treatments (Alvira et al., 2010).

Lignocellulosic biomass has attracted much interest in recent years as a resource for biogas production because the raw material is abundant, cheap and with no conflict with food (Chandra et al., 2007; Mosier et al., 2005; Taherzadeh & Karimi, 2008). *Miscanthus* is a high yielding lignocellulosic biomass that is cultivable in various climate zones, soils and regions, and has been proven as one of the highest energy biomass sources (Clifton-brown et al., 2004; Hastings et al., 2008; Heaton et al., 2008). However, its conversion to biogas is limited by hydrolysis because the digestible contents are covered by a sheath of recalcitrant lignin (Eliana et al., 2014). Several pre-treatment methods were applied in Paper V for improved methane production of *Miscanthus* and the effects of these pre-treatments were evaluated by BMP tests.

4.1 Physical pre-treatment: size reduction

Size reduction is one of the physical pre-treatment, and it can improve the enzymatic hydrolysis or biodegradability of lignocellulosic biomass by increasing the accessible surface area and reducing the crystallinity and degrees of polymerization (Taherzadeh & Karimi, 2008). In Paper V, four different sizes were evaluated, i.e., 0.5, 5, 10 and 20 mm. The results presented in Figure 7: A showed that the particle sizes from 10 mm down to 0.5 mm didn't lead to a

significant difference in methane yield, whereas the methane yield at particle size of 20 mm was significantly lower compared to the rest. In addition, size reduction improved the methane production rate as evidenced by the increased hydrolysis constant (k).

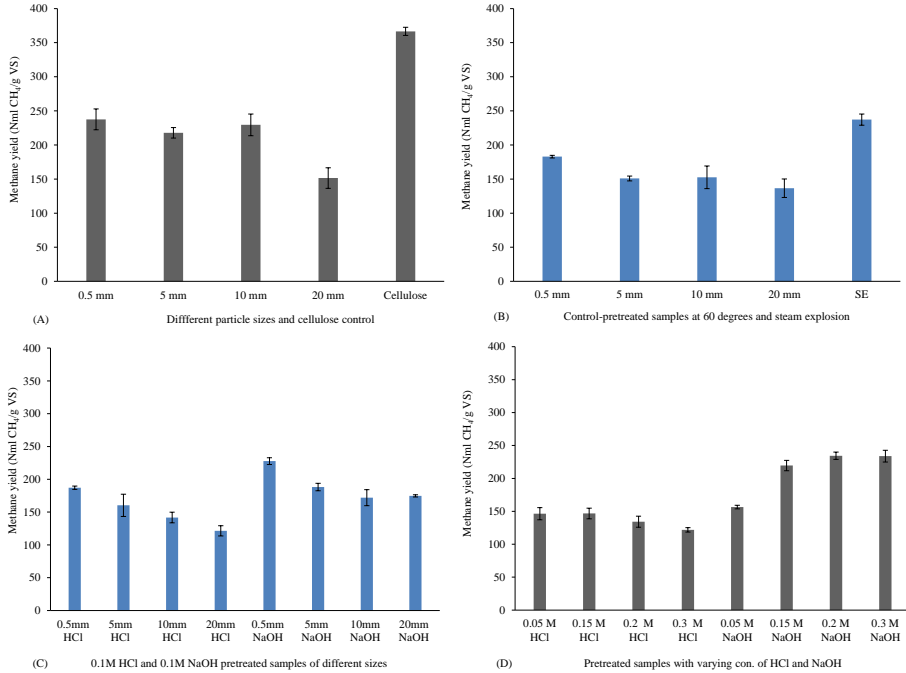


Figure 7. Methane yields of *M.luteivoriparius* after the various treatments. (A) shows the methane yields of the different particle sizes. (B) shows the methane yields of control and steam explosion pre-treated samples. (C) shows the methane yields of different particle sizes after acid and alkaline pre-treatments while (D) shows the methane yields of sample 20 mm after pre-treatment with increasing concentration of alkaline and acid. SE stands for steam explosion. Reproduced from Paper V.

4.2 Steam explosion

Steam explosion (SE) is the most commonly used physio-chemical pre-treatment for conversion of lignocellulosic biomass to biogas. During SE, the biomass is exposed to a pressurised steam and high temperature (typically between 160-260°C) for a period ranging from seconds to few minutes, and is subsequently subjected to a suddenly reduced pressure (Alvira et al., 2010; Sun et al., 2004; Varga et al., 2004). During this pre-treatment, the acetyl groups in hemicellulose are hydrolysed at high temperature and generate acetic acid and it is therefore a physio-chemical pre-treatment. In addition to partial hydrolysis of hemicellulose and solubilisation, the lignin is redistributed and removed in

certain ways (Pan et al., 2005). The methane yield was improved by SE as shown in Figure 7: A-B, and this result could be explained by better accessibility of cellulose for enzymatic hydrolysis caused by the defibrillation of cellulose bundles when the pressure was suddenly released (Menardo et al., 2013).

4.3 Alkali pre-treatment

The accessibility of enzymes to cellulose can be efficiently improved by alkali pre-treatment using sodium, potassium, calcium and ammonium hydroxide solutions, because lignin and a portion of hemicellulose can be removed during this pre-treatment (Tahezadeh & Karimi, 2008). Sodium hydroxide (NaOH) solution was applied for alkali pre-treatment of *Miscanthus* in Paper V. The results showed that the methane yields of different particle sizes pre-treated by 0.1 M NaOH were significantly increased, and pre-treatment of 20 mm particle by increasing NaOH concentrations higher than 0.05 M also lead to significant improvement of the methane yield as shown in Figure 7: A, C and D. Higher methane yields were achieved after alkali pre-treatment compared with that of acid pre-treatment (Figure 7: C-D). It is possible that the alkali solution is more effective in breaking the ester bonds among lignin, hemicellulose and cellulose, thereby exposing cellulose and hemicellulose for enzyme attack (Gáspár et al., 2007; He et al., 2008).

4.4 Acid pre-treatment

Almost all of the hemicellulose can be removed and the lignin disrupted by acid pre-treatment, and thus the susceptibility of cellulose to enzyme hydrolysis is increased (Yang & Wyman, 2004). However, in Paper V, the results showed that acid pre-treatment (0.1 M HCl) of different particle sizes resulted in significantly lower methane yields (Figure 7: A and C). Moreover, pre-treatment of 20 mm particles by increasing the concentration of acid led to decreased methane yield, and a significantly lower methane yield was obtained when the highest acid concentration was applied (Figure 7: A and D). This result is likely due to the formation of inhibitors, such as furfural, hydroxymethylfurfural, carboxylic acids, etc. at lower pH (Tahezadeh, 1999; Tahezadeh & Karimi, 2007). These inhibitors affect the metabolism of microorganisms and thereby decrease the methane yield (Saha et al., 2005).

5. Concluding Remarks and Future Perspectives

As an attractive technology for both waste treatment and renewable energy recovery, AD plays an important role in the sustainable development of society. Most organic materials can be degraded under anaerobic conditions to produce biogas. However, these materials can significantly differ in their degradation rates and methane potentials also might be lack of certain nutrients. All of these factors are key parameters for the economy, design and management of a biogas plant. Therefore, prior to the AD process, the materials should be fully analysed. The BMP test is commonly used to analyse the biodegradability and methane potential of organic materials. However, a number of factors can affect the BMP test and lead to unreliable and incomparable results. Therefore, better knowledge of the optimisation of the experimental conditions is required for comparable results from different studies related to the BMP test.

This PhD study focused on the evaluation of different aspects related to standardisation of the BMP test. As an application, the effects of different pre-treatments for lignocellulosic biomass were evaluated by the BMP test. The major findings from this study are summarised below.

- Manually operated/in-house developed experimental setups are low cost and easy to operate, but they are time- and labour-intensive, with a high risk of inducing random and human errors. During the BMP test, the real-time temperature and pressure at each gas volume measurement need to be recorded for data normalisation, and the gas volume should be measured continuously to better understand the degradation profile of the investigated substrate (Paper I).
- Inoculum preparation, such as pre-incubation, filtration and storage conditions affect the enzyme activity of the inoculum, and thereby influence the methane production and degradation kinetics of the investigated material. Filtration of inoculum should be avoided to prevent the loss of microorganisms. Fresh inoculum is recommended to use in BMP test as it shows the highest enzyme activity and methane production (Paper II).

- Substrate concentration is a key parameter which can influence the BMP test results. For the investigated substrate concentrations (2.5-15 g VS/L), the methane yield increases with the increasing substrate concentration. The dilution liquid used to adjust the substrate concentration influences the degradation rate of the substrate (Paper III).
- Mixing has strong effects on BMP test and is required for an efficient process if the digester content is viscous. However, mixing is not necessary or shake manually once per day might be sufficient during the BMP test if the digester content is quite diluted or the substrate is easily degraded (Paper IV).
- Size reduction improves both the methane yield and the degradation rate of the investigated lignocellulosic biomass *Miscanthus*. Acid pre-treatments lead to lower methane yields, whereas, alkali pre-treatment significantly improves the methane yield, and the higher alkaline concentration the higher methane yields (Paper V). The effects of pre-treatment might be varying for different types of feedstock.

In addition to these findings, many aspects still require further study to optimise the conditions for the BMP test. In particular, the influence of the inoculum needs to be studied in more depth, and additional research is needed to evaluate the influence of substrate concentration on a wider-scale with more types of substrates, inocula and experimental conditions.

In summary, these outcomes should be considered before performing a BMP test to ensure reliable and comparable results.

Acknowledgement

I started my PhD study at Division of Biotechnology in Lund University since March of 2012. It is quite a long, tough, challenging and rewarding journey, which I will remember forever. I have tried my best to turn out this thesis in the current form. I am so proud of myself, but it's not solely my own achievement, I would like to express my sincere gratitude to the following people, without whom I cannot come to this end.

First of all, my deep gratitude goes to my supervisor Associate Professor Jing Liu, for accepting and guiding me as a PhD student. You always steer me in the right direction.

My co-supervisor Dr. Mihaela Nistor, thank you for all your support and advice both in research and life. You have spent a lot of time to correct and improve my manuscripts in very details. I sincerely appreciate for this.

My co-supervisor Dr. Ivo Achu Nges, thank you for all your support and help. You are always patient to answer all my questions, even the stupid ones. You are such a funny person, I always feel happy and relaxing when talking to you.

Thanks to all the co-authors, Sten, Annika, Chao and Liangwei for smooth collaborations.

Emma Poaches, thank you for being so helpful with administrative issues.

Frans-Peder Nilson, thank you for solving all the problems related to my computer, and all the nice talking.

Thank you Paula for your help and input with the layout of my thesis.

Thanks to my current and previous office mates, Ying, Roxana, Linda K., Marisa, Maryam, Hugo, Solmaz, Ivo and Valentine for the social-academic environment.

Emma K., Linda Ö., Marisa, Lu, Hong and Roya, thank you for always being so kind and helpful whenever needed, and thanks to all the other members in environmental group and in Division of Biotechnology.

Nicklas, Susanne, Alberto, Kristina, Sofie, Sten and Mihaela, you have always made me feel welcome when I have been in Bioprocess Control. Thank you for the Easter gifts, Christmas gifts, cakes, fruits, candies and chocolates...

I would like to thank all my Chinese friends in Lund, you enrich my life. Thank you for all your help whenever I needed it, and our wonderful moments together, parties and delicious food.

Thanks to Hua and Qiao for keeping in touch. Thank you for always being there talking and listening to me.

Last but not least, I would like to thank my family for making the person I am today. Thanks to my father and mother, you give me lots of loves, support and encouragements and always believe in me. Thanks to my brother Kai for being a wonderful sibling, giving advice in both work and life.

Kairang, thanks for your tolerance and endless love. Thanks for the handsome you create. Nuoyi, my seven-month-old son, you make me happy and full of energy every day, thanks for being in my life.

References

- Abatzoglou, N., Boivin, S. 2009. A review of biogas purification processes. *Biofuels, Bioproducts and Biorefining*, **3** (1), 42-71.
- Alfa, M.I., Adie, D.B., Igboro, S.B., Oranusi, U.S., Dahunsi, S.O., Akali, D.M. 2014. Assessment of biofertilizer quality and health implications of anaerobic digestion effluent of cow dung and chicken droppings. *Renewable Energy*, **63**, 681-686.
- Álvarez, J.A., Otero, L., Lema, J.M. 2010. A methodology for optimising feed composition for anaerobic co-digestion of agro-industrial wastes. *Bioresource Technology*, **101** (4), 1153-1158.
- Alvira, P., Tomás-Pejó, E., Ballesteros, M., Negro, M. 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Bioresource Technology*, **101** (13), 4851-4861.
- Amani, T., Nosrati, M., Sreerkrishnan, T. 2010. Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects-a review. *Environmental Reviews*, **18**, 255-278.
- Angelidaki, I., Ahring, B. 1994. Anaerobic thermophilic digestion of manure at different ammonia loads: effect of temperature. *Water research*, **28** (3), 727-731.
- Angelidaki, I., Ahring, B.K. 1992. Effects of free long-chain fatty acids on thermophilic anaerobic digestion. *Applied Microbiology and Biotechnology*, **37** (6), 808-812.
- Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J., Guwy, A., Kalyuzhnyi, S., Jenicek, P., Van Lier, J. 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Science & Technology*, **59** (5), 927-934.
- Angelidaki, I., Ellegaard, L., Ahring, B.K. 2003. Applications of the anaerobic digestion process. *Advances in Biochemical Engineering/Biotechnology*, **82**, 1-33.
- Angelidaki, I., Ellegaard, L., Ahring, B.K. 1993. A mathematical model for dynamic simulation of anaerobic digestion of complex substrates: focusing on ammonia inhibition. *Biotechnology and bioengineering*, **42** (2), 159-166.

- Angelidaki, I., Sanders, W. 2004. Assessment of the anaerobic biodegradability of macropollutants. *Reviews in Environmental Science & Bio/Technology*, **3** (2), 117-129.
- ASTM-D5210. 2007. Standard test method for determining the anaerobic biodegradation of plastic materials in the presence of municipal sewage sludge. *West Conshohocken, Pennsylvania*.
- ASTM-E2710. 2008. Standard test method for determining anaerobic biodegradation potential of organic chemicals under methanogenic conditions. *West Conshohocken, Pennsylvania*.
- Bouallagui, H., Touhami, Y., Ben Cheikh, R., Hamdi, M. 2005. Bioreactor performance in anaerobic digestion of fruit and vegetable wastes. *Process Biochemistry*, **40** (3-4), 989-995.
- Braun, R., Huber, P., Meyrath, J. 1981. Ammonia toxicity in liquid piggery manure digestion. *Biotechnology Letters*, **3** (4), 159-164.
- Browne, J.D., Murphy, J.D. 2013. Assessment of the resource associated with biomethane from food waste. *Applied Energy*, **104**, 170-177.
- Burton, C.H., Turner, C. 2003. Manure management: Treatment strategies for sustainable agriculture. *Silsoe Research Institute*.
- Cabbai, V., Ballico, M., Aneggi, E., Goi, D. 2013. BMP tests of source selected OFMSW to evaluate anaerobic codigestion with sewage sludge. *Waste Management*, **33** (7), 1626-1632.
- Carlsson, M., Lagerkvist, A., Morgan-Sagastume, F. 2012. The effects of substrate pretreatment on anaerobic digestion systems: A review. *Waste Management*, **32** (9), 1634-1650.
- Carrère, H., Dumas, C., Battimelli, A., Batstone, D.J., Delgenès, J.P., Steyer, J.P., Ferrer, I. 2010. Pretreatment methods to improve sludge anaerobic degradability: A review. *Journal of Hazardous Materials*, **183** (1-3), 1-15.
- Chae, K., Jang, A., Yim, S., Kim, I.S. 2008. The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure. *Bioresource Technology*, **99** (1), 1-6.
- Chandra, R.P., Bura, R., Mabee, W., Berlin, d.A., Pan, X., Saddler, J. 2007. Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics? *Advances in Biochemical Engineering/Biotechnology*, **108**, 67-93.
- Chen, Y., Cheng, J.J., Creamer, K.S. 2008. Inhibition of anaerobic digestion process: A review. *Bioresource Technology*, **99** (10), 4044-4064.
- Clifton-brown, J.C., Stampfl, P.F., Jones, M.B. 2004. Miscanthus biomass production for energy in Europe and its potential contribution to decreasing fossil fuel carbon emissions. *Global change biology*, **10** (4), 509-518.

- Cotter, P.D., Hill, C. 2003. Surviving the acid test: responses of gram-positive bacteria to low pH. *Microbiology and Molecular Biology Reviews*, **67** (3), 429-453.
- Cuetos, M.J., Gomez, X., Otero, M., Morán, A. 2008. Anaerobic digestion of solid slaughterhouse waste (SHW) at laboratory scale: influence of co-digestion with the organic fraction of municipal solid waste (OFMSW). *Biochemical Engineering Journal*, **40** (1), 99-106.
- Dague, R.R., McKinney, R.E., Pfeffer, J.T. 1970. Solids retention in anaerobic waste treatment systems. *Journal (Water Pollution Control Federation)*, R29-R46.
- Demirel, B., Yenigün, O. 2002. Two-phase anaerobic digestion processes: a review. *Journal of Chemical Technology and Biotechnology*, **77** (7), 743-755.
- Dieter, D., Steinhauser, A. 2008. Biogas from waste and renewable resources: an introduction. *John Wiley & Sons*.
- Eliana, C., Jorge, R., Juan, P., Luis, R. 2014. Effects of the pretreatment method on enzymatic hydrolysis and ethanol fermentability of the cellulosic fraction from elephant grass. *Fuel*, **118**, 41-47.
- Espinoza-Escalante, F.M., Pelayo-Ortíz, C., Navarro-Corona, J., González-García, Y., Bories, A., Gutiérrez-Pulido, H. 2009. Anaerobic digestion of the vinasses from the fermentation of Agave tequilana Weber to tequila: The effect of pH, temperature and hydraulic retention time on the production of hydrogen and methane. *Biomass and bioenergy*, **33** (1), 14-20.
- Eurostat. 2015a. Population and population change statistics.
- Eurostat. 2015b. Waste statistics.
- Fannin, K.F., Chynoweth, D.P., Ghosh, S., Srivastava, V.J. 1980. Anaerobic processes. *Journal (Water Pollution Control Federation)*, **52** (6), 1182-1195.
- Ferrer, I., Ponsá, S., Vázquez, F., Font, X. 2008. Increasing biogas production by thermal (70°C) sludge pre-treatment prior to thermophilic anaerobic digestion. *Biochemical Engineering Journal*, **42** (2), 186-192.
- Gallert, C., Bauer, S., Winter, J. 1998. Effect of ammonia on the anaerobic degradation of protein by a mesophilic and thermophilic biowaste population. *Applied Microbiology and Biotechnology*, **50** (4), 495-501.
- Ganidi, N., Tyrrel, S., Cartmell, E. 2009. Anaerobic digestion foaming causes—a review. *Bioresource technology*, **100** (23), 5546-5554.
- Gáspár, M., Kálmán, G., Réczey, K. 2007. Corn fiber as a raw material for hemicellulose and ethanol production. *Process Biochemistry*, **42** (7), 1135-1139.
- Gavala, H.N., Yenal, U., Skiadas, I.V., Westermann, P., Ahring, B.K. 2003. Mesophilic and thermophilic anaerobic digestion of primary and secondary sludge. Effect of pre-treatment at elevated temperature. *Water research*, **37** (19), 4561-4572.

- Gelegenis, J., Georgakakis, D., Angelidaki, I., Mavris, V. 2007. Optimization of biogas production by co-digesting whey with diluted poultry manure. *Renewable Energy*, **32** (13), 2147-2160.
- Gerardi, M.H. 2003. The microbiology of anaerobic digesters. *John Wiley & Sons*.
- Guendouz, J., Buffière, P., Cacho, J., Carrère, M., Delgenes, J.-P. 2010. Dry anaerobic digestion in batch mode: design and operation of a laboratory-scale, completely mixed reactor. *Waste Management*, **30** (10), 1768-1771.
- Gunnerson, C.G., Stuckey, D.C., Greeley, M., Skrinde, R. 1986. Anaerobic digestion: Principles and practices for biogas systems. *The World Bank, Washington, DC*.
- Hartmann, H., Angelidaki, I., Ahring, B.K. 2002. Co-digestion of the organic fraction of municipal waste with other waste types. *Biomethanization of the Organic Fraction of Municipal Solid Wastes*, 181-200.
- Hashimoto, A., Varel, V., Chen, Y. 1981. Ultimate methane yield from beef cattle manure: effect of temperature, ration constituents, antibiotics and manure age. *Agricultural Wastes*, **3** (4), 241-256.
- Hashimoto, A.G. 1983. Conversion of straw–manure mixtures to methane at mesophilic and thermophilic temperatures. *Biotechnology and bioengineering*, **25** (1), 185-200.
- Hashimoto, A.G. 1982. Methane from cattle waste: effects of temperature, hydraulic retention time, and influent substrate concentration on kinetic parameter (K). *Biotechnology and bioengineering*, **24** (9), 2039-2052.
- Hastings, A., Clifton-Brown, J., Wattenbach, M., Stampfl, P., Mitchell, C.P., Smith, P. 2008. Potential of Miscanthus grasses to provide energy and hence reduce greenhouse gas emissions. *Agronomy for sustainable development*, **28** (4), 465-472.
- He, Y., Pang, Y., Liu, Y., Li, X., Wang, K. 2008. Physicochemical characterization of rice straw pretreated with sodium hydroxide in the solid state for enhancing biogas production. *Energy & Fuels*, **22** (4), 2775-2781.
- Heaton, E.A., Dohleman, F.G., Long, S.P. 2008. Meeting US biofuel goals with less land: the potential of Miscanthus. *Global change biology*, **14** (9), 2000-2014.
- Ho, C., Tan, Y. 1985. Anaerobic treatment of palm oil mill effluent by tank digesters. *Journal of Chemical Technology and Biotechnology. Biotechnology*, **35** (2), 155-164.
- Hwang, M.H., Jang, N.J., Hyun, S.H., Kim, I.S. 2004. Anaerobic bio-hydrogen production from ethanol fermentation: the role of pH. *Journal of Biotechnology*, **111** (3), 297-309.
- IEA. 2014. World Energy Outlook 2014.

- ISO-11734. 1995. Water quality–Evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge–Method by measurement of the biogas production. *1 ed. Geneva.*
- ISO-14853. 2005. Plastics-Determination of the ultimate anaerobic biodegradation of plastic materials in an aqueous system-Method by measurement of biogas production. *British Standards Institute, London, UK.*
- Jenkins, S.R., Morgan, J.M., Sawyer, C.L. 1983. Measuring Anaerobic Sludge Digestion and Growth by a Simple Alkalimetric Titration. *Journal (Water Pollution Control Federation)*, **55** (5), 448-453.
- Jensen, P., Ge, H., Batstone, D.J. 2011. Assessing the role of biochemical methane potential tests in determining anaerobic degradability rate and extent. *Water Science and Technology*, **64** (4), 880.
- Jetten, M.S., Stams, A.J., Zehnder, A.J. 1992. Methanogenesis from acetate: a comparison of the acetate metabolism in *Methanotrix soehngenii* and *Methanosarcina* spp. *FEMS Microbiol. Rev.*, **88** (3-4), 181-197.
- Kafle, G.K., Kim, S.H. 2013. Anaerobic treatment of apple waste with swine manure for biogas production: batch and continuous operation. *Applied Energy*, **103**, 61-72.
- Kayhanian, M. 1994. Performance of a high-solids anaerobic digestion process under various ammonia concentrations. *Journal of Chemical Technology and Biotechnology*, **59** (4), 349-352.
- Kim, M., Ahn, Y.-H., Speece, R. 2002. Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. *Water research*, **36** (17), 4369-4385.
- Koster, I.W., Koomen, E. 1988. Ammonia inhibition of the maximum growth rate (μ_m) of hydrogenotrophic methanogens at various pH-levels and temperatures. *Applied Microbiology and Biotechnology*, **28** (4-5), 500-505.
- Koyama, T. 1963. Gaseous metabolism in lake sediments and paddy soils and the production of atmospheric methane and hydrogen. *Journal of Geophysical Research*, **68** (13), 3971-3973.
- Kroeker, E., Schulte, D., Sparling, A., Lapp, H. 1979. Anaerobic treatment process stability. *Journal (Water Pollution Control Federation)*, 718-727.
- Labatut, R.A., Angenent, L.T., Scott, N.R. 2011. Biochemical methane potential and biodegradability of complex organic substrates. *Bioresource Technology*, **102** (3), 2255-2264.
- Lindmark, J., Eriksson, P., Thorin, E. 2014a. The effects of different mixing intensities during anaerobic digestion of the organic fraction of municipal solid waste. *Waste Management*, **34** (8), 1391-1397.

- Lindmark, J., Thorin, E., Fdhila, R.B., Dahlquist, E. 2014b. Effects of mixing on the result of anaerobic digestion: Review. *Renewable and Sustainable Energy Reviews*, **40**, 1030-1047.
- Liu, C.-f., Yuan, X.-z., Zeng, G.-m., Li, W.-w., Li, J. 2008. Prediction of methane yield at optimum pH for anaerobic digestion of organic fraction of municipal solid waste. *Bioresource Technology*, **99** (4), 882-888.
- Liu, J., Olsson, G., Mattiasson, B. 2004. A volumetric meter for monitoring of low gas flow rate from laboratory-scale biogas reactors. *Sensors and Actuators B: Chemical*, **97** (2), 369-372.
- Liu, T., Sung, S. 2002. Ammonia inhibition on thermophilic aceticlastic methanogens. *Water Science and Technology*, **45** (10), 113-120.
- Maamri, S., Amrani, M. 2014. Biogas production from waste activated sludge using cattle dung inoculums: Effect of total solid contents and kinetics study. *Energy Procedia*, **50**, 352-359.
- Mallik, M.K., Singh, U.K., Ahmad, N. 1990. Batch digester studies on biogas production from Cannabis sativa, water hyacinth and crop wastes mixed with dung and poultry litter. *Biological Wastes*, **31** (4), 315-319.
- Mata-Alvarez, J., Dosta, J., Romero-Güiza, M., Fonoll, X., Peces, M., Astals, S. 2014. A critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renewable and Sustainable Energy Reviews*, **36**, 412-427.
- Maya-Altamira, L., Baun, A., Angelidaki, I., Schmidt, J.E. 2008. Influence of wastewater characteristics on methane potential in food-processing industry wastewaters. *Water research*, **42** (8), 2195-2203.
- McMahon, K.D., Stroot, P.G., Mackie, R.I., Raskin, L. 2001. Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions-II: microbial population dynamics. *Water research*, **35** (7), 1817-1827.
- Menardo, S., Bauer, A., Theuretzbacher, F., Piringer, G., Nilsen, P.J., Balsari, P., Pavliska, O., Amon, T. 2013. Biogas production from steam-exploded miscanthus and utilization of biogas energy and CO₂ in greenhouses. *BioEnergy Research*, **6** (2), 620-630.
- Misi, S.N., Forster, C.F. 2001. Batch co-digestion of multi-component agro-wastes. *Bioresource Technology*, **80** (1), 19-28.
- Møller, H.B., Sommer, S.G., Ahring, B.K. 2004. Methane productivity of manure, straw and solid fractions of manure. *Biomass and Bioenergy*, **26** (5), 485-495.
- Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y., Holtzapple, M., Ladisch, M. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, **96** (6), 673-686.
- Mshandete, A., Björnsson, L., Kivaisi, A.K., Rubindamayugi, M.S.T., Mattiasson, B. 2006. Effect of particle size on biogas yield from sisal fibre waste. *Renewable Energy*, **31** (14), 2385-2392.

- Murto, M., Björnsson, L., Mattiasson, B. 2004. Impact of food industrial waste on anaerobic co-digestion of sewage sludge and pig manure. *Journal of environmental management*, **70** (2), 101-107.
- Myint, M., Nirmalakhandan, N. 2006. Evaluation of first-order, second-order, and surface-limiting reactions in anaerobic hydrolysis of cattle manure. *Environmental engineering science*, **23** (6), 970-980.
- Nasr, N., Elbeshbishy, E., Hafez, H., Nakhla, G., El Naggar, M.H. 2012. Comparative assessment of single-stage and two-stage anaerobic digestion for the treatment of thin stillage. *Bioresource Technology*, **111**, 122-126.
- Nges, I.A., Li, C., Wang, B., Xiao, L., Yi, Z., Liu, J. 2016. Physio-chemical pretreatments for improved methane potential of *Miscanthus lutarioriparius*. *Fuel*, **166**, 29-35.
- Owen, W., Stuckey, D., Healy, J., Young, L., McCarty, P. 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water research*, **13** (6), 485-492.
- Pain, B.F., Misselbrook, T.H., Clarkson, C.R., Rees, Y.J. 1990. Odour and ammonia emissions following the spreading of anaerobically-digested pig slurry on grassland. *Biological Wastes*, **34** (3), 259-267.
- Pamatmat, M.M., Bhagwat, A.M. 1973. Anaerobic metabolism in Lake Washington sediments. *Limnology and Oceanography*, **18** (4), 611-627.
- Pan, X., Xie, D., Gilkes, N., Gregg, D.J., Saddler, J.N. 2005. Strategies to enhance the enzymatic hydrolysis of pretreated softwood with high residual lignin content. *Twenty-Sixth Symposium on Biotechnology for Fuels and Chemicals*, 1069-1079.
- Pfeffer, J.T. 1974. Temperature effects on anaerobic fermentation of domestic refuse. *Biotechnology and bioengineering*, **16** (6), 771-787.
- Pohland, F., Ghosh, S. 1971. Developments in anaerobic stabilization of organic wastes—the two-phase concept. *Environmental letters*, **1** (4), 255-266.
- Raposo, F., Banks, C., Siegert, I., Heaven, S., Borja, R. 2006. Influence of inoculum to substrate ratio on the biochemical methane potential of maize in batch tests. *Process Biochemistry*, **41** (6), 1444-1450.
- Raposo, F., Borja, R., Martín, M.A., Martín, A., de la Rubia, M.A., Rincón, B. 2009. Influence of inoculum–substrate ratio on the anaerobic digestion of sunflower oil cake in batch mode: Process stability and kinetic evaluation. *Chemical Engineering Journal*, **149** (1–3), 70-77.
- Raposo, F., Borja, R., Rincon, B., Jimenez, A.M. 2008. Assessment of process control parameters in the biochemical methane potential of sunflower oil cake. *Biomass and bioenergy*, **32** (12), 1235-1244.

- Raposo, F., De la Rubia, M., Fernández-Cegrí, V., Borja, R. 2012. Anaerobic digestion of solid organic substrates in batch mode: an overview relating to methane yields and experimental procedures. *Renewable and Sustainable Energy Reviews*, **16** (1), 861-877.
- Raposo, F., Fernández-Cegrí, V., De la Rubia, M.A., Borja, R., Béline, F., Cavinato, C., Demirer, G., Fernández, B., Fernández-Polanco, M., Frigon, J.C., Ganesh, R., Kaparaju, P., Koubova, J., Méndez, R., Menin, G., Peene, A., Scherer, P., Torrijos, M., Uellendahl, H., Wierinck, I., de Wilde, V. 2011. Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. *Journal of Chemical Technology & Biotechnology*, **86** (8), 1088-1098.
- Rinzema, A., Boone, M., van Knippenberg, K., Lettinga, G. 1994. Bactericidal effect of long chain fatty acids in anaerobic digestion. *Water Environment Research*, 40-49.
- Ripley, L.E., Boyle, W.C., Converse, J.C. 1986. Improved Alkalimetric Monitoring for Anaerobic Digestion of High-Strength Wastes. *Journal (Water Pollution Control Federation)*, **58** (5), 406-411.
- Rozzi, A., Remigi, E. 2004. Methods of assessing microbial activity and inhibition under anaerobic conditions: a literature review. *Reviews in Environmental Science & Bio/Technology*, **3** (2), 93-115.
- Saha, B.C., Iten, L.B., Cotta, M.A., Wu, Y.V. 2005. Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. *Process Biochemistry*, **40** (12), 3693-3700.
- Sambusiti, C., Rollini, M., Ficara, E., Musatti, A., Manzoni, M., Malpei, F. 2014. Enzymatic and metabolic activities of four anaerobic sludges and their impact on methane production from ensiled sorghum forage. *Bioresource Technology*, **155**, 122-128.
- Sanchez, E., Borja, R., Weiland, P., Travieso, L., Martín, A. 2001. Effect of substrate concentration and temperature on the anaerobic digestion of piggery waste in a tropical climate. *Process Biochemistry*, **37** (5), 483-489.
- Schink, B. 1997. Energetics of syntrophic cooperation in methanogenic degradation. *Microbiology and Molecular Biology Reviews*, **61** (2), 262-280.
- Shahriari, H., Warith, M., Hamoda, M., Kennedy, K.J. 2012. Anaerobic digestion of organic fraction of municipal solid waste combining two pretreatment modalities, high temperature microwave and hydrogen peroxide. *Waste Management*, **32** (1), 41-52.
- Shen, S., Nges, I.A., Yun, J., Liu, J. 2014. Pre-treatments for enhanced biochemical methane potential of bamboo waste. *Chemical Engineering Journal*, **240**, 253-259.
- Sprott, G.D., Patel, G.B. 1986. Ammonia toxicity in pure cultures of methanogenic bacteria. *Systematic and applied microbiology*, **7** (2), 358-363.

- Strömberg, S., Nistor, M., Liu, J. 2014. Towards eliminating systematic errors caused by the experimental conditions in Biochemical Methane Potential (BMP) tests. *Waste Management*, **34** (11), 1939-1948.
- Stroot, P.G., McMahon, K.D., Mackie, R.I., Raskin, L. 2001. Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions—I. Digester performance. *Water research*, **35** (7), 1804-1816.
- Sun, X., Xu, F., Sun, R., Wang, Y., Fowler, P., Baird, M. 2004. Characteristics of degraded lignins obtained from steam exploded wheat straw. *Polymer Degradation and Stability*, **86** (2), 245-256.
- Sung, S., Dague, R.R. 1995. Laboratory studies on the anaerobic sequencing batch reactor. *Water Environment Research*, **67** (3), 294-301.
- Symons, G., Buswell, A. 1933. The methane fermentation of carbohydrates^{1, 2}. *Journal of the American Chemical Society*, **55** (5), 2028-2036.
- Taherzadeh, M.J. 1999. *Ethanol from lignocellulose: physiological effects of inhibitors and fermentation strategies*. Chalmers University of Technology.
- Taherzadeh, M.J., Karimi, K. 2007. Enzymatic-based hydrolysis processes for ethanol from lignocellulosic materials: A review. *BioResources*, **2** (4), 707-738.
- Taherzadeh, M.J., Karimi, K. 2008. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. *International journal of molecular sciences*, **9** (9), 1621-1651.
- Tanimu, M.I., Ghazi, T.I.M., Harun, M.R., Idris, A. 2014. Effect of feed loading on biogas methane production in batch mesophilic anaerobic digesters treating food waste. *International Journal*, **5** (1).
- Tiehm, A., Nickel, K., Zellhorn, M., Neis, U. 2001. Ultrasonic waste activated sludge disintegration for improving anaerobic stabilization. *Water research*, **35** (8), 2003-2009.
- Tong, X., Smith, L.H., McCarty, P.L. 1990. Methane fermentation of selected lignocellulosic materials. *Biomass*, **21** (4), 239-255.
- Turovskiy, I.S., Mathai, P. 2006. Wastewater sludge processing. *John Wiley & Sons*.
- Varel, V., Hashimoto, A., Chen, Y. 1980. Effect of temperature and retention time on methane production from beef cattle waste. *Applied and environmental microbiology*, **40** (2), 217-222.
- Varga, E., Réczey, K., Zacchi, G. 2004. Optimization of steam pretreatment of corn stover to enhance enzymatic digestibility. *Applied biochemistry and biotechnology*, **114** (1-3), 509-523.
- Vavilin, V.A., Fernandez, B., Palatsi, J., Flotats, X. 2008. Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview. *Waste Management*, **28** (6), 939-951.

- VDI 4630. 2006. Fermentation of organic materials - Characterisation of the substrate, sampling, collection of material data, fermentation tests.
- Walker, M., Zhang, Y., Heaven, S., Banks, C. 2009. Potential errors in the quantitative evaluation of biogas production in anaerobic digestion processes. *Bioresource Technology*, **100** (24), 6339-6346.
- Wang, B., Nges, I.A., Nistor, M., Liu, J. 2014. Determination of methane yield of cellulose using different experimental setups. *Water science and technology*, **70** (4), 599-604.
- Weiland, P. 2010. Biogas production: current state and perspectives. *Applied Microbiology and Biotechnology*, **85** (4), 849-860.
- Welsh, F., Schulte, D., Kroeker, E., Lapp, H. 1977. The effect of anaerobic digestion upon swine manure odors. *Canadian Agricultural Engineering*, **19** (2), 122-126.
- Whitmore, T., Lloyd, D., Jones, G., Williams, T. 1987. Hydrogen-dependent control of the continuous anaerobic digestion process. *Applied Microbiology and Biotechnology*, **26** (4), 383-388.
- Yang, B., Wyman, C.E. 2004. Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn stover cellulose. *Biotechnology and bioengineering*, **86** (1), 88-98.
- Zeeman, G., Wiegant, W., Koster-Treffers, M., Lettinga, G. 1985. The influence of the total-ammonia concentration on the thermophilic digestion of cow manure. *Agricultural Wastes*, **14** (1), 19-35.
- Zhang, W., Lang, Q., Wu, S., Li, W., Bah, H., Dong, R. 2014. Anaerobic digestion characteristics of pig manures depending on various growth stages and initial substrate concentrations in a scaled pig farm in Southern China. *Bioresource Technology*, **156**, 63-69.