

THE INVESTIGATION OF METHANE PRODUCTION FROM AN
AGRICULTURAL WASTE, CORNCOB, AND ITS ENHANCEMENT VIA CO-
DIGESTION AND PRETREATMENT

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ABSTRACT

THE INVESTIGATION OF METHANE PRODUCTION FROM AN AGRICULTURAL WASTE, CORNCOB, AND ITS ENHANCEMENT VIA CO-DIGESTION AND PRETREATMENT

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In recent years, the renewable energy requirement is increased with the population increase and reduction of fossil fuel resources. In terms of biomass energy, corn waste constitutes the highest portion (33%) among crop wastes in the world. Thus, corncob, which is a corn waste and also non-food waste, has a great potential as a renewable energy source in the country. In this thesis study, the biochemical methane potential (BMP) of corncob (raw CC) was investigated initially and assessed by adopting co-digestion with feed (chicken manure (83%) and poppy (17%)), digestate or post-digestate and a pretreatment method, alkaline hydrothermal pretreatment at 240°C (AHP_240). BMP of corncob was 103 mL CH₄/g VS_{added}, and the highest synergistic effect was observed in Raw CC+Digestate reactors with 172 mL CH₄/g VS_{added}. After AHP_240 method was applied, although sCOD of AHP_240 CC increased 17 times compared to Raw CC, methane yield of both AHP_240 CC and its co-digestion reactors did not significantly improve. After that, hydrothermal, alkaline and alkaline hydrothermal pretreatment methods were applied on Raw CC by using various temperatures, namely 150°C, 180°C and 210°C for their optimization. The hydrothermal pretreated corncob (HP CC) had 55-61% higher

methane yield than Raw CC and there was no statistically significant difference among methane yields obtained for different temperatures studied. Thus, HP_150 method was selected as the optimum pretreatment method. Lastly, raw CC or hydrothermal pretreated corncob at 150 °C (HP_150 CC) were used as substrates with digestate in semi-continuous co-digestion reactors to investigate the optimum operational conditions, i.e., hydraulic retention time (HRT) and organic loading rate (OLR), leading to the maximum methane production. The highest methane yield of HP_150 CC and Digestate reactor was 392 mL CH₄/g VS_{added} at an HRT of 10 days and OLR of 4.5 g VS/L.d. Raw CC apparently requires more than 15 days of HRT for higher solubilization. But, when HP_150 method is applied, HRT of 10 days seems to be profitable. This means smaller volume and lower capital cost for biogas plant. The use of HP_150 CC with Digestate in semi continuous reactors seems to be promising and feasible for methane production in large-scale biogas plant.

Keywords: Corncob, Anaerobic co-digestion, Alkaline Hydrothermal Pretreatment, Biochemical methane potential (BMP), Semi-continuous reactor

ÖZ

TARIMSAL ATIK OLAN MISIR SÖMEĞİNDEN METAN ÜRETİMİNİN ORTAK ÇÜRÜTME VE ÖN İŞLEM İLE GELİŞTİRİLMESİNİN İNCELENMESİ

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Son yıllarda nüfus artışı ve fosil yakıt kaynaklarının azalması ile yenilenebilir enerji ihtiyacı artmaktadır. Biyokütle enerjisi açısından mısır atığı, dünyadaki tahıl atıkları içinde en yüksek payı (%33) oluşturmaktadır. Böylelikle mısır atığı ve ayrıca gıda dışı atık olan mısır sömeği, ülkemizde yenilenebilir enerji kaynağı olarak da büyük bir potansiyele sahiptir. Bu tez çalışmasında, ilk olarak mısır sömeğinin (Ham MS) biyokimyasal metan potansiyeli (BMP) araştırılmış ve Ham MS'nin BMP değeri Feed (tavuk dışkısı ve haşhaş (%17)), Digestate ve Post-Digestate ile birlikte çürütme uygulanarak ve ön işlem yöntemi olan alkali hidrotermal (AHÖ_240) yöntemi, kullanılarak değerlendirilmiştir. Mısır sömeğinin BMP'si 103 mL CH₄/g UKM idi, ve en yüksek sinerjistik etki Ham MS + Digestate reaktörlerinde gözlemlendi ve 172 mL CH₄ / g UKM 'ye ulaştı. AHÖ_240 yöntemi uygulandıktan sonra, AHÖ_240 MS'nin çKOİ'si, Ham MS'ye kıyasla 17 kat artmasına rağmen, hem AHÖ_240 MS hem de bu MS ile yapılan birlikte çürütme reaktörlerinin metan verimi önemli ölçüde iyileşmedi. Daha sonra ön işlem optimizasyonu için 150 °C, 180 °C ve 210 °C gibi sıcaklık değerleri kullanılarak Ham MS üzerinde hidrotermal, alkali ve alkali hidrotermal ön işlem yöntemleri uygulanmıştır. Hidrotermal ön işlem görmüş mısır sömeği (HÖ MS), Ham MS'ye göre %55-61 daha yüksek metan

verimine ulaşmıştır ve çalışılan farklı sıcaklıklarda saptanan metan verimi arasında istatistiksel olarak anlamlı bir fark yoktu. Böylece HÖ_150 yöntemi optimum ön işlem yöntemi olarak seçildi. Son olarak, maksimum metan üretimine yol açan hidrolik bekleme süresi (HBS) ve organik yükleme hızı (OYH) gibi optimum çalışma koşullarını araştırmak için yarı-sürekli birlikte çürütme reaktörlerinde sübstrat olarak Ham MS veya HÖ_150 MS ile Digestate kullanıldı. HÖ_150 MS ve Digestate reaktörünün en yüksek metan verimi 10 günlük HBS ve 4.5 g UKM/L.g OYH'de 392 mL CH₄/g UKM_{eklenen} olarak saptandı. Ham MS'nin daha fazla çözünürlük için 15 günlük HBS'den daha fazlasına ihtiyacı vardır. Ancak HÖ_150 yöntemi uygulandığında 10 günlük HBS karlı görünmektedir. Bu, biyogaz tesisi için daha küçük hacim ve daha düşük sermaye maliyeti anlamına gelir. HÖ_150 MS'nin Digestate ile kullanımının umut verici ve büyük ölçekli biyogaz tesisinde metan üretimi için uygulanabilirdi olduğu görülmektedir.

Anahtar Kelimeler: Mısır sömeği, Anaerobik birlikte çürütme, Alkali hidrotermal ön işlem metotları, Biyokimyasal metan potansiyeli (BMP), Yarı-sürekli reaktör

To freedom...

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TABLE OF CONTENTS

| | |
|--|------|
| ABSTRACT..... | v |
| ÖZ | vii |
| ACKNOWLEDGMENTS | x |
| TABLE OF CONTENTS..... | xi |
| LIST OF TABLES | xv |
| LIST OF FIGURES | xvii |
| LIST OF ABBREVIATIONS..... | xix |
| CHAPTERS | |
| 1 INTRODUCTION | 1 |
| 2 LITERATURE REVIEW | 5 |
| 2.1 Sources of Biomass Energy..... | 5 |
| 2.2 Structure and Composition of Lignocellulosic Biomass | 8 |
| 2.2.1 Agricultural biomass sources | 11 |
| 2.2.2 Properties and generation of corncob..... | 15 |
| 2.3 Anaerobic Digestion Process..... | 16 |
| 2.4 Process Parameters Affecting Anaerobic Digestion | 20 |
| 2.4.1 The Substrate to Inoculum Ratio (SIR)..... | 20 |
| 2.4.2 Organic Loading Rate (OLR) | 20 |
| 2.4.3 Retention Time..... | 21 |
| 2.4.4 pH and Alkalinity..... | 21 |

| | | |
|-------|---|----|
| 2.4.5 | Temperature..... | 22 |
| 2.4.6 | Carbon/Nitrogen (C/N) ratio | 23 |
| 2.4.7 | Nutrients Requirement..... | 24 |
| 2.4.8 | Toxicity..... | 25 |
| 2.5 | Anaerobic Digestion of Lignocellulosic Materials and Its Enhancement | 26 |
| 2.5.1 | Co-digestion | 27 |
| 2.5.2 | Pretreatment application..... | 28 |
| 2.6 | Anaerobic Digestion of Corncob | 32 |
| 2.6.1 | Enhancement of anaerobic digestion of corncob..... | 34 |
| 3 | BIOCHEMICAL METHANE POTENTIAL OF CORNCOB AND THE EFFECT OF CO-DIGESTION AND ALKALINE HYDROTHERMAL PRETREATMENT..... | 35 |
| 3.1 | Introduction..... | 35 |
| 3.2 | Materials and Methods..... | 38 |
| 3.2.1 | Characteristic of corncob..... | 38 |
| 3.2.2 | Pretreatment of corncob | 39 |
| 3.2.3 | Characteristic of co-substrates..... | 41 |
| 3.2.4 | Inoculum (seed sludge)..... | 42 |
| 3.2.5 | Basal medium | 43 |
| 3.2.6 | Experimental procedure..... | 43 |
| 3.2.7 | Analytical methods | 48 |
| 3.3 | Results and Discussion | 52 |
| 3.3.1 | Results of BMP_Set-1: Raw CC | 52 |
| 3.3.2 | Results of BMP_Set-2: AHP_240 CC..... | 66 |

| | | |
|-------|--|-----|
| 3.4 | Conclusions | 82 |
| 4 | EFFECT OF HYDROTHERMAL, ALKALINE AND ALKALINE HYDROTHERMAL PRETREATMENT ON BIOCHEMICAL METHANE POTENTIAL OF CORNCOB | 84 |
| 4.1 | Introduction | 84 |
| 4.2 | Materials and Methods | 87 |
| 4.2.1 | Characteristic of the pretreated corncob | 88 |
| 4.2.2 | Inoculum (seed sludge) | 89 |
| 4.2.3 | Basal medium (BM)..... | 89 |
| 4.2.4 | Experimental procedure | 90 |
| 4.2.5 | Analytical methods | 91 |
| 4.2.6 | Statistical analysis | 93 |
| 4.3 | Results and Discussion..... | 93 |
| 4.3.1 | The results on pretreatment studies of corncob | 93 |
| 4.3.2 | BMP test results of the used different pretreatment method on corncob 99 | |
| 4.3.3 | Characterization results of BMP reactors | 107 |
| 4.4 | Conclusions | 109 |
| 5 | SEMI-CONTINUOUS CO-DIGESTION OF CORNCOB WITH DIGESTATE: INFLUENCE OF HYDROTHERMALLY PRETREATED CORNCOB, HYDRAULIC RETENTION TIME AND ORGANIC LOADING RATE ON METHANE YIELD..... | 111 |
| 5.1 | Introduction | 111 |
| 5.2 | Materials and Methods | 114 |
| 5.2.1 | Characteristic of Raw CC and HP_150 CC | 114 |

| | | |
|-------|---|-----|
| 5.2.2 | Characteristic of the co-substrates | 115 |
| 5.2.3 | Inoculum (seed sludge)..... | 116 |
| 5.2.4 | Experimental procedure..... | 116 |
| 5.2.5 | Analytical methods | 119 |
| 5.3 | Results and Discussion | 121 |
| 5.3.1 | Methane Potential of Reactor-1 | 121 |
| 5.3.2 | Methane Potential of Reactor-2 | 129 |
| 5.3.3 | Mini Energy Analysis of Reactor-2..... | 139 |
| 5.4 | Conclusions..... | 140 |
| 6 | CONCLUSIONS AND RECOMMENDATIONS..... | 141 |
| | REFERENCES | 144 |
| | APPENDICES..... | 167 |
| A. | Preliminary Study to Find Optimum Parameter for Pretreatment Application | 167 |
| B. | Reactor Configuration of BMP_Set-1 and BMP_Set-2..... | 168 |
| C. | Calibration Curves of Gas Measurement in GC | 170 |
| D. | Reactors Characterization Results (Raw CC with/without co-substrate. | 172 |
| E. | Reactors Characterization Results (AHP_240 CC with/without co substrate)..... | 174 |
| F. | Characterization Results of Pretreated Corncob Reactors..... | 176 |
| G. | The Results of ANOVA for HP method at different temperature | 178 |
| H. | The Semi-Continuous Reactor Setup Condition at time zero | 179 |
| I. | Calibration Curve of Nessler Method..... | 180 |

LIST OF TABLES

TABLES

| | |
|--|----|
| Table 2.1 Composition of lignocellulosic materials | 11 |
| Table 2.2 Total annual production of field crops and waste quantities in Turkey in 2003 (Başçetinçelik et al., 2005)..... | 14 |
| Table 2.3 Amount of corn waste in Turkey (TÜİK, 2018; Başçetinçelik et al., 2005) | 16 |
| Table 2.4 C/N ratio of biomass | 24 |
| Table 2.5 Type of pretreatment method and advantages and challenges in lignocellulosic biomass (Kumari and Singh, 2018; Taherzadeh and Karimi, 2008; Kainthola et al., 2019; Chandra et al., 2012; Paul et al., 2018; Kucharska et al., 2018; Shah et al., 2015) | 29 |
| Table 2.6 Effect of pretreatment methods on biogas production (Paul et al., 2018) | 32 |
| Table 2.7 Studies of corncob in anaerobic digestion up to date | 33 |
| Table 3.1 Characteristics of corncob..... | 39 |
| Table 3.2 Characterization results of AHP_240 CC | 41 |
| Table 3.3 Characterization results of co-substrates | 42 |
| Table 3.4 Characterization results of inoculum | 43 |
| Table 3.5 Content of the BMP_Set-1 | 45 |
| Table 3.6 Content of BMP_Set-2 Reactors..... | 47 |
| Table 3.7 Exp _{CH₄} Yield, Theo _{CH₄} Yield and Exp-Estimated _{CH₄} Yield of BMP_Set-1 | 57 |
| Table 3.8 BMP of Raw CC studies in the literature up to date..... | 62 |
| Table 3.9 Average values of TAN, TKN and pH in BMP_Set-1 | 65 |
| Table 3.10 The change of sCOD and VS concentrations in BMP_Set-1 reactors.. | 66 |
| Table 3.11 Differences between characterization of Raw CC and AHP_240 CC.. | 67 |

| | |
|---|-----|
| Table 3.12 Exp _{CH₄} Yield, Theo _{CH₄} Yield and Exp-Estimated _{CH₄} Yield of BMP_Set-2 | 76 |
| Table 3.13 The production time required for 90% of the total methane produced in test reactors | 78 |
| Table 3.14 The change of TAN and pH in BMP_Set-2 | 81 |
| Table 3.15 The change of sCOD and VS concentrations in BMP_Set-2 reactors .. | 82 |
| Table 4.1 Characterisation of Pretreatment Studies on Raw CC..... | 95 |
| Table 4.2 Severity factor of the HP methods applied at different temperatures | 99 |
| Table 4.3 Exp _{CH₄} Yield, Theo _{CH₄} Yield, BD _{CH₄} of Raw CC, HP CCs, | 103 |
| Table 4.4 The change of TAN and pH values in the test reactors | 108 |
| Table 4.5 The change in average VS, tCOD and sCOD concentrations in the test reactors | 109 |
| Table 5.1 Characterisation of Raw CC and HP_150 CC..... | 115 |
| Table 5.2 Characterisation of Digestate and Inoculum | 116 |
| Table 5.3 Operation parameters of the reactors..... | 118 |
| Table 5.4 Some researches with lignocellulosic biomass and various types of manures in semi-continuous co-digestion reactors..... | 124 |
| Table 5.5 Comparison of the average methane yield of the Reactors | 138 |
| Table 5.6 Energy balance between using and production of energy | 139 |

LIST OF FIGURES

FIGURES

| | |
|---|----|
| Figure 2.1 Category of biomass type (Sánchez et. al., 2019) | 6 |
| Figure 2.2 a) Total primary energy supply in the world (IEA, 2019) b) Utilization rate of renewable energy supply according to OECD data in 2018..... | 7 |
| Figure 2.3 Categorization of biofuels (Kumari and Singh, 2018) | 8 |
| Figure 2.4 Structure of lignocellulosic biomass (Isikgor et al., 2015)..... | 10 |
| Figure 2.5 Worldwide crop production in 2018-2019 (FAO, 2018-2019) | 12 |
| Figure 2.6 Crop production in Turkey (TUIK, 2018)..... | 13 |
| Figure 2.7 The biological conversion process of anaerobic digestion (Mes et al., 2003) | 17 |
| Figure 2.8 Operating temperature of methanogenic bacteria concerning HRT (Gerardi, 2003)..... | 23 |
| Figure 3.1 Raw CC a) before grinding b) after grinding and screening | 38 |
| Figure 3.2 Average cumulative methane production of a) Raw CC, b) Feed, c) Digestate and d) Post-Digestate mono-digestion reactors | 54 |
| Figure 3.3 Average cumulative methane production of a) Raw CC+Feed, b) Raw CC+Digestate and c) Raw CC+Post-Digestate co-digestion reactors | 55 |
| Figure 3.4 Average methane yields of Raw CC mono- and co-digestion reactors. | 61 |
| Figure 3.5 Average cumulative methane production of the a) AHP_240 CC, b) Feed, c) Digestate and d) Post-Digestate mono-digestion reactors of BMP_Set-2 | 71 |
| Figure 3.6 Average cumulative methane production of the a) AHP_240 CC+Feed, b) AHP_240 CC+Digestate and c) AHP_240 CC+Post-Digestate co-digestion reactors of BMP_Set-1 | 72 |
| Figure 3.7 The comparison between methane yields of BMP_Set-1 and BMP_Set-2 test reactors | 79 |
| Figure 4.1 Schematic representation of pretreatment methods applied | 88 |
| Figure 4.2 Removal efficiency of cellulose, hemicellulose and lignin at different pretreatment methods..... | 97 |

| | |
|---|-----|
| Figure 4.3 Average cumulative methane production of a) HP_150 CC,..... | 100 |
| Figure 4.4 Average cumulative methane of production a) AHP_150 CC, b) AHP_180 CC, c) AHP_210 CC reactors..... | 101 |
| Figure 4.5 Average cumulative methane production of AP CC reactors | 102 |
| Figure 4.6 Daily methane production of HP_150 CC, HP_180 CC and HP_210 CC reactors | 106 |
| Figure 5.1 The photograph of the reactors with water displacement device..... | 119 |
| Figure 5.2 The a) operational conditions applied, b) cumulative methane production, c) cumulative methane yield of Reactor-1 | 122 |
| Figure 5.3 The a) operational conditions applied, b) pH, c) daily methane production, d) daily methane yield of Reactor-1 | 123 |
| Figure 5.4 The average influent and effluent concentrations of a) VS, b) sCOD, c) TAN of Reactor-1 | 127 |
| Figure 5.5 Methane and carbon dioxide content of biogas for Reactor-1 | 129 |
| Figure 5.6 The a) operational conditions applied, b) cumulative methane production, c) cumulative methane yield of Reactor-2 | 132 |
| Figure 5.7 The a) operational conditions applied, b) pH, c) daily methane production, d) daily methane yield Reactor-2 | 133 |
| Figure 5.8 The average influent and effluent concentrations of a) VS, b) sCOD, c) TAN of Reactor-2..... | 135 |
| Figure 5.9 Methane and carbon dioxide content in the biogas for Reactor-2 | 137 |

LIST OF ABBREVIATIONS

ABBREVIATIONS

| | |
|-----------------|------------------------------------|
| AHP | Alkaline Hydrothermal Pretreatment |
| Ar | Argon Gas |
| BM | Basal Medium |
| CC | Corncob |
| CH ₄ | Methane |
| CO ₂ | Carbon Dioxide Gas |
| COD | Chemical Oxygen Demand |
| HP | Hydrothermal Pretreatment |
| HRT | Hydraulic Retention Time |
| N ₂ | Nitrogen Gas |
| OLR | Organic Loading Rate |
| S/I | Substrate to Inoculum Ratio |
| sCOD | Soluble Chemical Oxygen Demand |
| SMA | Specific Methanogenic Activity |
| TAN | Total Ammonia Nitrogen |
| TS | Total Solid |
| VS | Volatile Solid |

CHAPTER 1

INTRODUCTION

Energy is an essential requirement for the economic and social development of all countries. Energy needs are increasing in parallel with the enhancement of urbanization and population. Currently, fossil fuels account for 80% of the total primary energy supply in the world; on the other hand, fossil fuels are limited reservoirs and a running out source (IEA, 2019). This situation has revealed the need for renewable energy. Nowadays, biomass energy covers 53% of the renewable energy which corresponds to 11% of the total primary energy supply in the world, and only 6% of biomass source is used for energy production (IEA, 2019; Kumari et al., 2018). The number of biogas plants is correspondingly increasing with the requirement of biomass energy. According to the World Biogas Association report, there were 17783 biogas plants with 10.5 GW installed capacity in Europe in 2017 (WBA, 2017). On the other hand, it is reported by BEPA (2020) that Turkey has only 199 biogas plants with 1.238 MW installed capacity in 2020.

One of the biomass energy sources is lignocellulosic biomass including wood, yard waste, agricultural residues (Paul and Dutta, 2018). Moreover, the potential energy equivalence of total wastes is 395378 GWh in Turkey, also vegetal waste, which is a kind of biomass source, constitutes 75% of this energy (BEPA, 2020). In Turkey, most of the agricultural waste is crop wastes because crop production and livestock farming corresponds 90% of the agricultural production (Ozturk et al., 2017); thus, it plays a key role in biomass energy.

Corn waste constitutes 33% of the crop waste, which is the highest amount among crop waste (Başçetinçelik et al., 2005). The main types of corn waste are straw and

cob. The corn straw includes leaves and stalk used as animal food while corncob is in the center of the corn ear and it is a non-food part of corn waste, which is generally used for heating in small villages due to its high calorific value with 18.4 MJ/kg, and this situation causes the release of high amount of CO₂ emission (Başçetinçelik et al., 2005). Corncob is a type of lignocellulosic biomass, of which structural contents are in the range of 34% to 41% cellulose, 32% to 36% hemicellulose and 6% to 19% lignin (Kumari et al., 2018). Some of the advantages of corncob are its being potential resource for renewable energy production and source of valuable chemicals such as xylitol, furfural due to its hemicellulose content highest among crop waste (Li et al., 2015). Yet, most of the corncob produced are kept in solid waste landfills. Despite the abovementioned advantages, the studies about methane production from corncob via anaerobic digestion are limited (Seppa et al., 2012; Li et al., 2015; Pérez-Rodríguez et al., 2016; Blandino et al., 2016; García-bernet and Domínguez, 2017; Pan-In and Sukasem 2017; Huang et al., 2017; Ali et al., 2018; Surra et al., 2018; Shah and Tabassum, 2018).

This study focuses on the use of non-food corn waste, i.e., corncob; thus, both the sustainable waste management and energy production can be provided (Zahan et al., 2018). One of the waste management methods is the anaerobic digestion method, of which has benefits of waste reduction, lower operation cost and the support of renewable energy production (Demirel and Yenigün, 2002).

The biochemical methane potential (BMP) of corncob has been investigated and found to be in the range of 139 to 222 mL CH₄/ gVS according to Li et al. (2015), Blandino et al. (2016) and Ali et al. (2018). The reasons of lower methane yield of corncob compared to theoretical methane yield (395 mL CH₄/ g COD) can be explained mainly by two means which are the deficiency of nitrogen in corncob content and its low biodegradability due to the rigid structural properties and recalcitrant components (Chandra et al., 2012; Paul and Dutta, 2018). These disadvantages of using corncob in anaerobic digestion can be eliminated with some applications such as the use of co-substrates to supply enough nitrogen sources and

thus adjust COD/N ratio, (optimum range:50-143) and suitable pretreatment applications to enhance biodegradable compositions (Speece, 1996). There is one study with raw corncob (CC) and pig manure in co-digestion where methane yield reached 139 mL CH₄/g VS (Li et al., 2015). Moreover, two BMP studies applied some pretreatment applications on corncob. One of the studies used alkali, enzymatic hydrolysis and alkali with enzymatic hydrolysis together, and the highest methane yield was found as 264 mL CH₄/g VS (Pérez-Rodríguez et al., 2017). The other one applied ultrasound pretreatment, then methane yield reached 291 mL CH₄/g VS (Pérez-Rodríguez et al., 2016). According to the literature review, there is no study on co-digestion of corncob or pretreated corncob with co-substrate.

There are various types of pretreatment methods, namely, physical, chemical, thermophysical, thermochemical and biological, to enhance the anaerobic degradability of corncob (Kumari and Singh, 2018). The determination of pretreatment methods in this study was based on the structural properties of the corncob. Alkaline pretreatment and hydrothermal pretreatment are mainly used to destroy lignin and to improve solubility and accessibility of cellulose and hemicellulose, respectively (Chandra et al., 2012; Kumari et al., 2018). Therefore, in this study, alkaline, hydrothermal and alkaline hydrothermal pretreatments were applied, besides, different temperatures were investigated for hydrothermal and alkaline hydrothermal pretreatment.

This thesis study aims;

- To investigate the effect of co-digestion on BMP of both raw CC and alkaline hydrothermal pretreated corncob. As co-substrates, the use of an industrial-scale two stage anaerobic digester's influent and effluent, namely, Feed (chicken manure (83%) and poppy mixture (17%)), Digestate and Post-Digestate were investigated.
 - To investigate and compare the methane yield of mono-digestion (raw CC/alkaline hydrothermal pretreated CC or one of the co-

substrates) and co-digestion (raw CC/alkaline hydrothermal pretreated CC and one of the co-substrates together)

- To research the effect of different pretreatment methods, which are hydrothermal, alkaline and alkaline hydrothermal pretreatment, on BMP of corncob
 - To assess the optimum pretreatment and temperature conditions leading to the highest methane yield.
- To investigate the anaerobic digestion of corncob in semi-continuous co-digestion reactors.
 - To investigate optimum operational conditions, that is hydraulic retention time (HRT) and organic loading rate (OLR) leading to the highest methane production/yield
 - To investigate the co-digestion of both raw corncob and hydrothermal pretreated CC in semi-continuous reactors with Digestate as the co-substrate.

The results of this thesis study might be of significance for researching an alternative energy resource, which is an important waste produced in our country and for indicating the optimum conditions to improve its methane potential in semi-continuous reactors.

CHAPTER 2

LITERATURE REVIEW

2.1 Sources of Biomass Energy

Biomass can be generally defined as the “organic matter derived from living, or recently living organisms” (Al Seadi et al., 2013). Therefore, biomass is not a fossil energy source. Biomass energy source is a renewable energy source, so this energy comes from the sun (Sánchez et al., 2019). For vegetal biomass sources, solar energy is used by vegetables to fulfill the photosynthesis process. During this process, energy is captured via plants. The release of the energy can be done by burning to create heat, fermenting to produce ethanol, distillation to produce methanol, digestion to produce biogas (Kaygusuz and Türker, 2002). Biomass type can be separated into three categories: the first category is based on chemical composition, the second category is based on origin, the third category is based on biomass end-use (Sánchez et al., 2019). The classification of biomass type is summarized in Figure 2.1.

| Chemical Composition | Origin | Biomass End Use |
|--|---|---|
| <ul style="list-style-type: none"> •Lignocellulosic biomass (corn,straw,tree etc.) •Sugar-rich biomasses (sugar beet, sugar cane etc.) •Starch-rich biomasses (wheat,corn, patato etc.) •Oil-rich biomasses (micro-macro algae etc.) •Protein-rich biomasses (soyabean, sunflower etc.) | <ul style="list-style-type: none"> •Agricultural biomass (lignocellulosic, starch, oil crops, food and energy crop etc.) •Forest biomass •By-products, residues, and waste •Aquatic biomass (microalgae, seaweed, and aquatic plants) | <ul style="list-style-type: none"> •Transport biofuels (bioethanol, biobutanol, biodiesel, biogas, bio-hydrogen etc.) •Biomass for heat and power (biogas, bio-syngas, biodiesel, bio-crude oil, or vegetable oil etc.) •Biomass for biorefineries •Intermediates or energy carriers (roasted, ground, pelletized biomass etc.) |

Figure 2.1 Category of biomass type (Sánchez et. al., 2019)

One of the categories of biomass type is depended on the origin. The origin type is divided into four categories. One of the classes is by-products, residues, and waste. This type can be categorized as:

- By-products of agricultural or crop residues are agricultural products such as wheat straw, corn straw, corncob, animal manure, and so on.
- By-products/residues of the forest come from harvesting of trees such as the trimming of forest, branches, leaves, etc.
- By-products/residues of agro-industrial are produced generally agricultural processing industries such as sugar beet /sugar cane molasses, coconut shell, coconut husks, coconut fiber, corn leaves, corncob, corn straw, manure, etc.
- By-products/residues of the wood industry are produced mainly pulp and paper industries such as fiberboard by-products, grinding dust, etc.
- Waste biomass originates from a different type of biowaste, such as food waste from the house, cafeteria, restaurant; biodegradable municipal solid waste (MSW); anaerobic digester waste (digestate, post-digestate) and so on.

Figure 2.2 shows the total primary energy, the renewable energy sources and their utilization rate in the world. Renewable energy covers 10.5 % of the total primary energy supply in the world (IEA, 2019). The most important part of the renewable energy is biofuels and waste. Renewable energy consists of 53.3 % biofuels and waste (OECD, 2018).

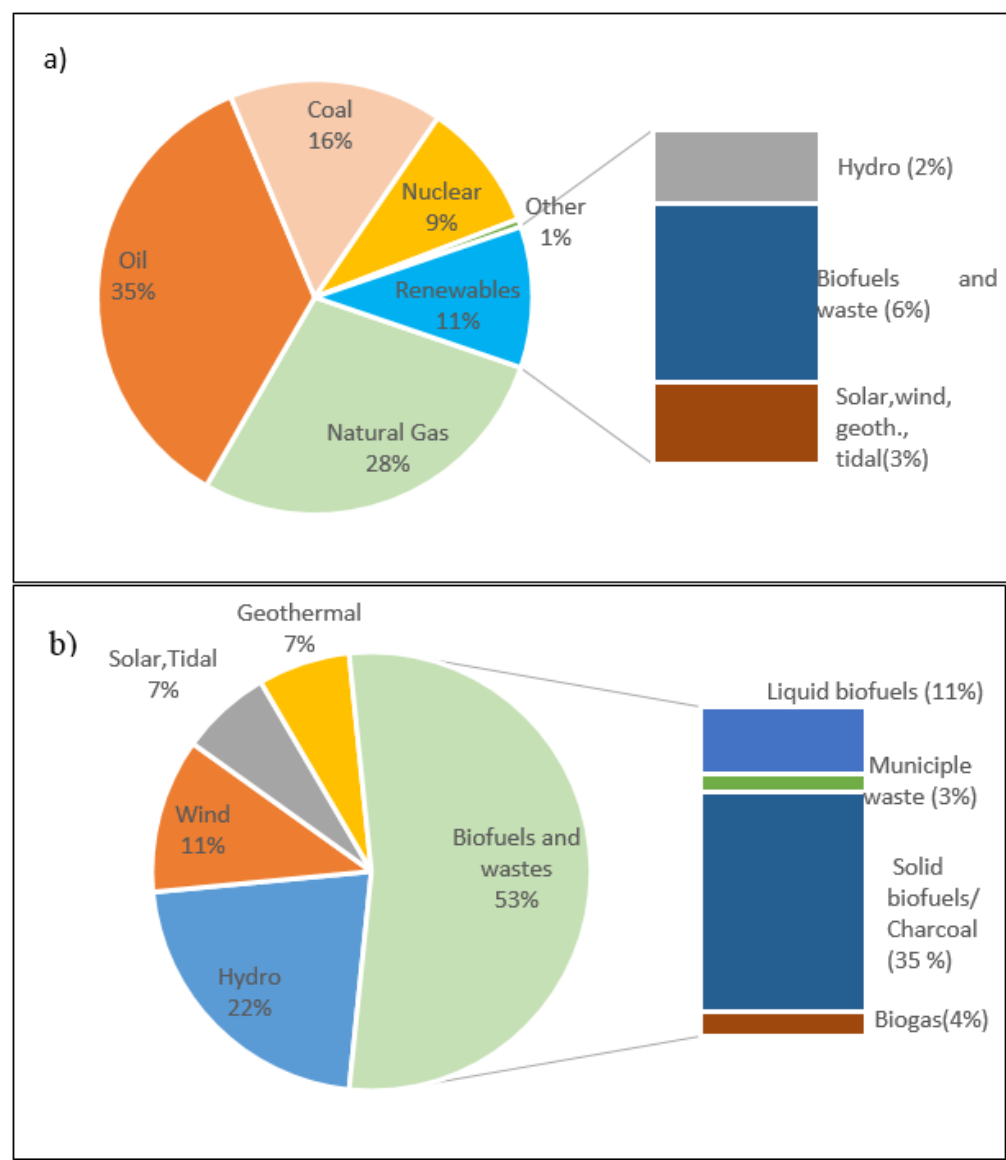


Figure 2.2 a) Total primary energy supply in the world (IEA, 2019) b) Utilization rate of renewable energy supply according to OECD data in 2018

If only fossil fuels are used to meet energy requirements, it will not be provided within the next 50 years due to the depletion of fossil fuels (Kumari and Singh, 2018). Thus, biofuels are the key solution to supply energy necessity. Biofuels can be divided into four categories that are first-generation, second-generation, third-generation and fourth-generation biofuels (Kumari and Singh, 2018). One of the second generation biofuels sources is lignocellulosic biomass.

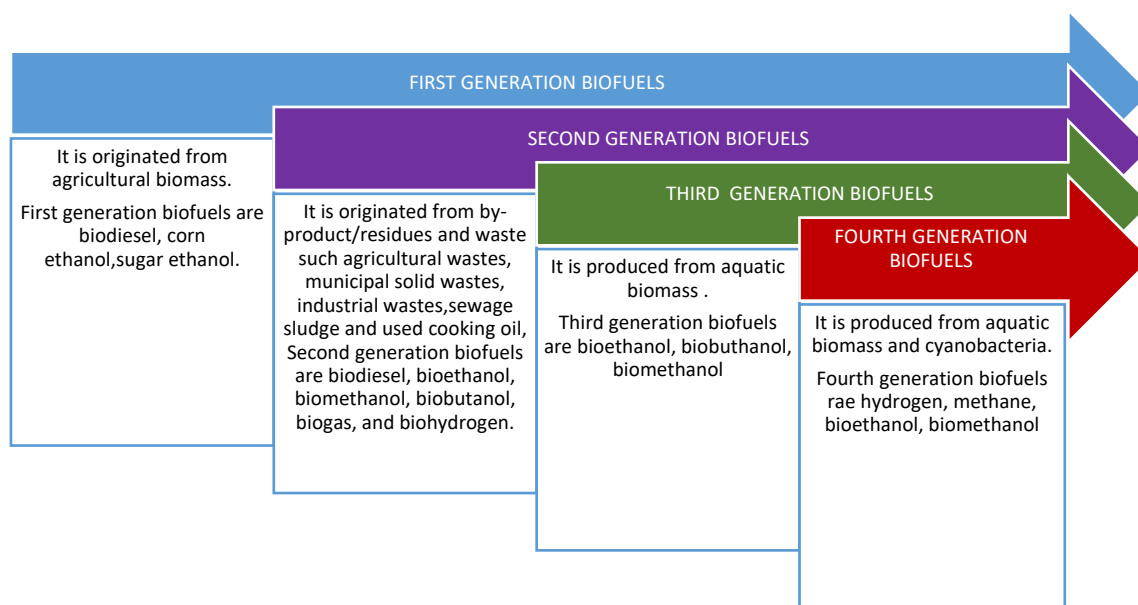


Figure 2.3 Categorization of biofuels (Kumari and Singh, 2018)

2.2 Structure and Composition of Lignocellulosic Biomass

One of the types of biomass resource is lignocellulosic biomass. Lignocellulosic biomass has become widespread to produce biofuels, recently. Lignocellulosic biomass is mainly wood, yard waste, energy crop, and agricultural residue (Paul and Dutta, 2018). It consists of chiefly cellulose, hemicellulose and lignin. In addition to this, there

are small amounts of inorganic mineral, pectin, protein and extractive (Chandra et al., 2012).

Cellulose is the main constituent structure of lignocellulosic biomass (Andrade and Rojas, 2012). Moreover, the amount of cellulose covers half of the organic carbon amount in the world. So, cellulose can be used to produce biofuels and valuable chemicals that are exalted importance. The structure of cellulose is not like glucose, so that it has a repetitive unit, which is a disaccharide (Isikgor et al., 2015).

Hemicellulose is the second widest biopolymer. Its structure differs from cellulose because hemicellulose contains heteropolymer forms, which are namely galactomannan, glucuronoxylan, xylan, arabinoxylan, xyloglucan, and glucomannan. Different types of lignocellulosic biomass have a different form of hemicellulose, such as hardwood biomass involve mainly xylan; however, softwood biomass includes mainly glucomannan (Isikgor et al., 2015). Moreover, arabinose and xylose have five-carbon monosaccharide (pentoses); glucose, galactose, and mannose have six-carbon monosaccharide (hexoses) (Isikgor et al., 2015). Hemicellulose has a hydrophilic structure, so the hydrolyze step occurs quickly compared to cellulose (Horn et al., 2012). The hemicelluloses are buried into the cell wall. Thus, hemicellulose has a complex network bond in order to ensure strong linkage with cellulose fiber into micro-fibrils and cross-linking with lignin (Isikgor et al., 2015).

Although there are lots of advantages of lignocellulosic biomass, lignin is one of the most critical challenges of using lignocellulosic biomass in the biological systems (Taherzadeh and Karimi, 2008). The structure of lignin is very complicated. This structure consists of phenylpropane units in a three-dimensional configuration. Also, lignin is not present in the biodegradable form. Lignin protects cellulose and hemicellulose from damage, like physical or chemical (Taherzadeh and Karimi, 2008). Besides, lignin is a recalcitrant component that is not easily broken down by microorganisms (Andrade and Rojas, 2012). Softwoods have more lignin in the structure

in comparison with hardwoods and the majority of agricultural waste (Taherzadeh and Karimi, 2008). All of the components in lignocellulosic biomass are shown in Figure 2.4.

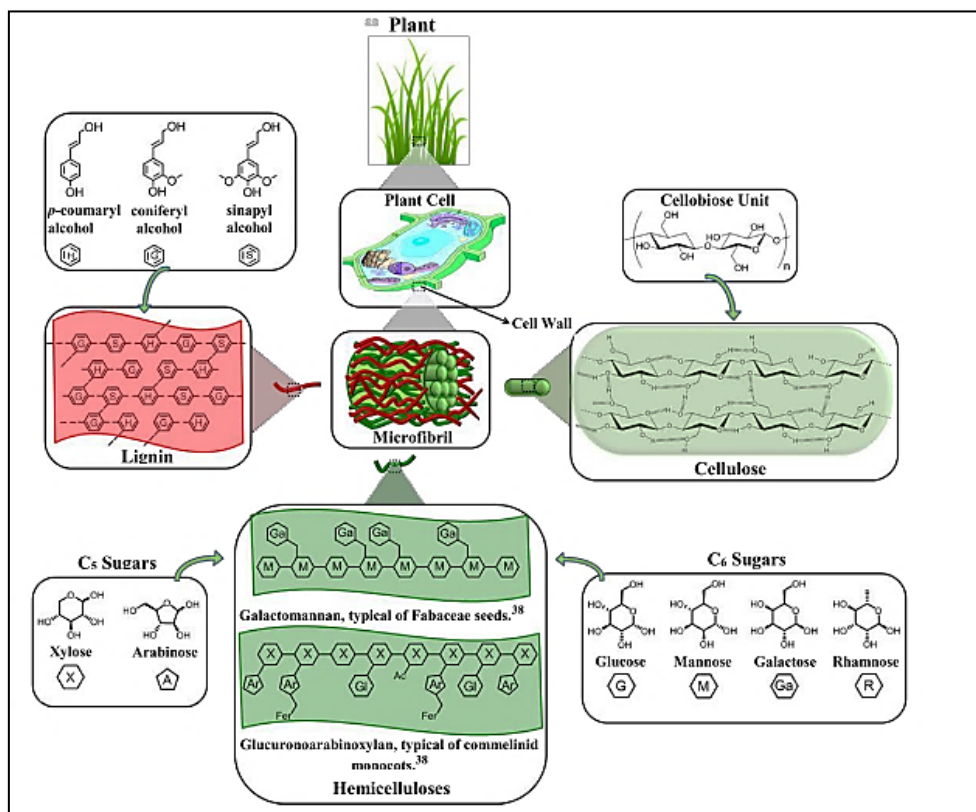


Figure 2.4 Structure of lignocellulosic biomass (Isikgor et al., 2015)

Fer and Gl mean ferulic acid and glucuronic acid, respectively.

Each type of cell structure of the plant has not a regular distribution of cellulose, hemicellulose and lignin component. Mostly, lignocellulosic materials compose of 35–50 % cellulose, 20–35 % hemicellulose, and 10–25 % lignin (Isikgor et al., 2015). The composition of lignocellulosic biomass is summarized in Table 2.1, below.

Table 2.1 Composition of lignocellulosic materials

| Lignocellulosic | Biomass | Cellulose(%) | Hemicellulose(%) | Lignin(%) | References |
|-----------------------|----------------------|--------------|------------------|-----------|---------------------------|
| Forest | Hardwood | 40.4-53.3 | 18.4-28.7 | 15.5-24.1 | Isikgor et. al. (2015) |
| | Softwood stems | 45-50 | 25-35 | 25-35 | Chandra et al. (2012a) |
| Agricultural Waste | Corn cob | 33.7-41.2 | 31.9-36.0 | 6.1-19.3 | Kumari et al. (2018) |
| | Corn stover | 40 | 25-31 | 14-17 | Paul and Dutta (2018) |
| | Wheat straw | 33-45 | 20-32 | 8-20 | Tye et al. (2016) |
| | Rice straw | 17 | 17 | 3 | Paul and Dutta (2018) |
| | Sugarcane bagasse | 40-45 | 20-24 | 25-30 | Paul and Dutta (2018) |
| | Cotton straw | 42 | 12 | 15 | Paul and Dutta (2018) |
| | Rice straw | 35-44 | 27-34 | 12-13 | Paul and Dutta (2018) |
| | Oat Straw | 31-35 | 20-26 | 10-15 | Isikgor et. al. (2015) |
| | Switchgrass | 36-45 | 28-30 | 12-26 | Paul and Dutta (2018) |
| | Grasses | 25-40 | 25-50 | 10-30 | Isikgor et. al. (2015) |

2.2.1 Agricultural biomass sources

Most of the countries attend an agricultural activity which is essential for the sustainable life cycle. The number of agricultural activities increases day by day due to the growing population. Thus, amount of agricultural residues increases in parallel with the production amount. Agricultural waste sources are food waste (sugarcane, fruit, corn)

and non-food waste(rice husk, corncob, corn straw, leaves) and animal wastes (Chandra et al., 2012). Agricultural wastes are produced from growing shape, harvesting, processing of the agricultural products. There are three types of agricultural waste. One of them is crop residues such as corncob, corn silage, wheat residue, nutshells; the other one is animal manure from poultry, cattle, swine, and the third one is forestry residues.

Crop production and livestock farming constitute 90 % of the agricultural sector in Turkey (Ozturk et al., 2017). The percentage of produced crop types in the worldwide is shown in Figure 2.5. The percentage of produced crop type in Turkey is shown in Figure 2.6. According to these data, corn is produced more than other crops in the world which covers 43% of the crop production in 2018-2019 (FAO, 2018-2019). But in Turkey, wheat is the crop that is mostly produced (36%), which is followed by sugar beet (34%), barley (12%), and corn (10%) in 2018 (TUIK, 2018).

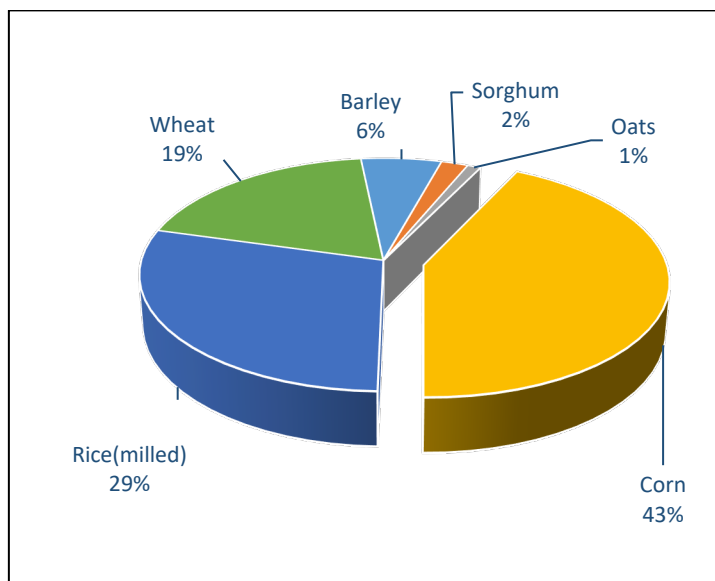


Figure 2.5 Worldwide crop production in 2018-2019 (FAO, 2018-2019)

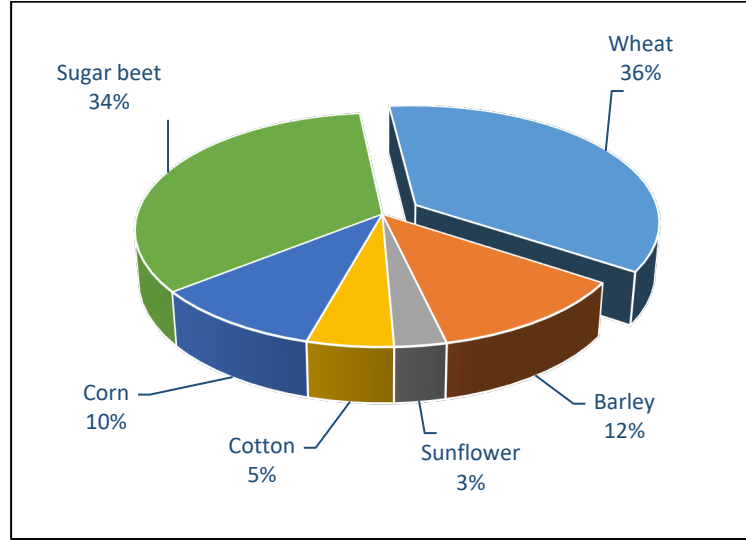


Figure 2.6 Crop production in Turkey (TUIK, 2018)

As mentioned above, crop production associates with waste generation. However, the amount of crop waste is not directly related to the amount of crop production. According to the TUIK in 2018, although wheat production was higher than other crop production, the amount of corn waste was the highest among the crop waste. The crucial issue is the amount of crop production how related with the amount of crop waste. Table 2.2 shows the relation between annual crop production and crop waste in Turkey 2003. The highest amount of crop wastes is produced corn, wheat and cotton production, respectively. The amounts of corn waste, wheat waste, and cotton waste constitute 33.4 %, 27.6 % and 18.1 % of the annual produced crop waste in Turkey in 2003, respectively (Başçetinçelik et al., 2005).

Table 2.2 Total annual production of field crops and waste quantities in Turkey in 2003 (Başçetinçelik et al., 2005)

| Products | Waste | Production (tone) | Area (hec.) | Teoritical | Real | Available Waste(tone) | Availability (%) | Calorific Value(MJ/kg) |
|-----------|------------|-------------------|-------------|------------|----------|-----------------------|------------------|------------------------|
| Wheat | Straw | 22439042 | 9424785 | 29170755 | 23429907 | 3514486 | 15 | 17.9 |
| Barley | Straw | 8327457 | 3732992 | 9992948 | 8963012 | 1344452 | 15 | 17.5 |
| Rye | Straw | 253243 | 145907 | 405188 | 358040 | 53706 | 15 | 17.5 |
| Oat | Straw | 322830 | 150459 | 419678 | 321236 | 48115 | 15 | 17.4 |
| Corn | Straw | 2209601 | 565109 | 5911902 | 4970259 | 2982155 | 60 | 18.5 |
| | Cob | | | 596592 | 1907307 | 1144384 | 60 | 18.4 |
| Rice | Straw | 331563 | 59879 | 582555 | 209532 | 125719 | 60 | 16.7 |
| | Shell | | | 88527 | 77747 | 62198 | 80 | 13 |
| Tobacco | Stalk | 181382 | 222691 | 362763 | 410778 | 246467 | 60 | 16.1 |
| Cotton | Stalk | 2292988 | 680177 | 6317181 | 2520281 | 1512169 | 60 | 18.2 |
| | Cotton gin | | | 481527 | 732220 | 585776 | 80 | 15.7 |
| Sunflower | Stalk | 836269 | 545963 | 2341554 | 2259121 | 1355472 | 60 | 14.2 |
| Peanut | Straw | 55241 | 25167 | 127054 | 28638 | 22910 | 80 | 20.7 |
| | Shell | | | 27621 | | | | |
| Soybean | Straw | 28795 | 15064 | 60468 | 21872 | 13123 | 60 | 19.4 |

2.2.2 Properties and generation of corncob

Corn waste mainly consists of two parts which are straw and cob. Corn straw that is also called corn stover includes leaves, hulks and stalks. Corn straw can generally be used as animal food. Corncob which is inside of the corn, and separated from straw and kernels, has a hard structure compared to corn stover. Corncob can be easily separated from straw and kernels by using a mechanical combine-harvester (Blandino et al., 2016). The general structural contents of corncob are in the range of 34% to 41% cellulose, 32% to 36% hemicellulose, and 6% to 19% lignin (Table 2.1), (Kumari et al., 2018). Corncob has the highest hemicellulose amount other crop wastes (Blandino et al., 2016). This situation contributes to using it for beneficial purposes that are: i) the production of some valuable chemicals such as xylitol, furfural, and so on ii) the production of biofuels such as ethanol, methanol, hydrogen and methane etc. (Li et al., 2015).

Besides, animals cannot eat corncob due to involving rigid structure. The calorific value of corncob is almost the highest among the others, as shown in Table 2.2. Therefore, corncob is generally used for heating purposes (Başçetinçelik et al., 2005). This situation causes the production of greenhouse gases, carbon dioxide, methane and nitrous oxide. These gases threaten human health and the environment (Levine, 1996).

As mentioned before, the generation of corn waste which is a second-generation biofuel source, is higher than other crop wastes in the world and Turkey (FAO, 2018-2019; TÜİK, 2018). Corn waste constitutes 33 % of the crop wastes (Table 2.2), (Başçetinçelik et al., 2005). Table 2.2 shows the amount of corn waste produced for only the year 2003. In order to calculate the amount of corn waste produced recently, TÜİK data and Table 2.2 were used. Corn production data of 2018 was taken from TÜİK, then waste to product ratio was used in Table 2.2. The amount of corn waste and corncob was calculated by using this correlation. These calculated data are given in Table 2.3. Corncob amount increased from 1.1 to 3.0 megatons from 2003 to 2018 years (Table

2.3). With the increasing corncob amount, some problems can be observed such as, the storage problem and air pollution problem due to burning of corncob. Thus, using corncob for the beneficial purpose should be increased and enhanced

Table 2.3 Amount of corn waste in Turkey (TUIK, 2018; Başçetinçelik et al., 2005)

| Year | Amount of product (megatons) | Amount of waste (megatons) | | Total waste (megatons) | Total waste /product | Corncob /product |
|------|---------------------------------|-------------------------------|------------|---------------------------|-------------------------|---------------------|
| | | Corn straw | Corncob | | | |
| 2003 | 2.2 | 3.0 | 1.1 | 4.1 | 1.8 | 0.5 |
| 2018 | 5.7 | 7.7 | 3.0 | 10.7 | | |

^a The calculated volumes are shown in bold

2.3 Anaerobic Digestion Process

During anaerobic digestion, the degradation of organic materials occurs via a consortium of microorganisms without any oxygen to produce valuable products and bioenergy, which is methane. Organic materials are animal manure, industrial residuals, solid residual substrates, which are crop residuals, agricultural waste, food residuals (Raposo et al., 2012). Biogas is a mixture of methane (55-75%) and carbon dioxide (25-45 %) (Mes et al., 2003). The biological conversion process can be subdivided into the following four steps, which are hydrolysis, acidogenesis, acetogenesis, methanogenesis. Figure 2.7 shows the biological conversion process of anaerobic digestion.

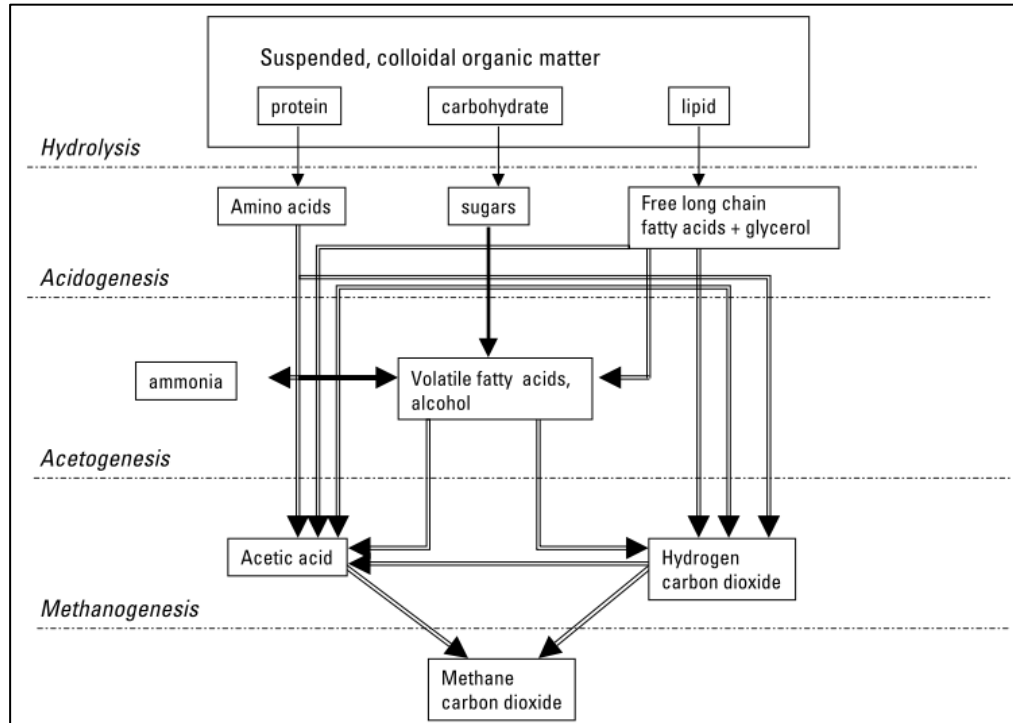
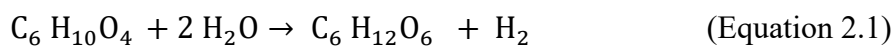


Figure 2.7 The biological conversion process of anaerobic digestion (Mes et al., 2003)

Hydrolysis Step:

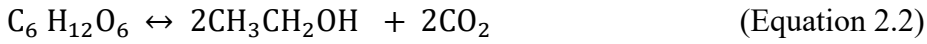
In the first step, large complex organic molecules which have higher molecular weight are converted into smaller molecular weight components in a soluble form (Bajpai, 2017a). Proteins are converted to amino acid, carbohydrates are hydrolyzed to soluble sugars and lipids are transformed into long-chain fatty acids and glycerin. Fermentative bacteria excretes exo-enzymes to accomplish the hydrolysis step. Moreover, the chemical band of complex organic molecules can be broken down by using water. Thus, organic molecules solubilize in water in order to degrade microorganisms. When solid wastes are used in anaerobic digestion, hydrolysis step is generally the rate-limiting step (Monge et al., 2013). An example for hydrolysis is given in Equation 2.1 (Bajpai, 2017a).



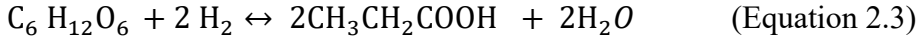
Acidegonesis Step:

Acidegonesis step comes after the hydrolysis step. Soluble components are converted to higher organic acids via acidogenesis. The end products are mainly intermediate volatile fatty acids (VFAs), propionic acid, butyric acid, acetic acid, ethanol, hydrogen and carbon dioxides. Example equations of acidegonesis are given in Equation 2.2, Equation 2.3, Equation 2.4 (Bajpai, 2017a).

Ethanol formation:



Propionic acid formation:

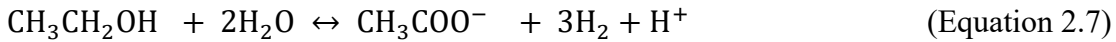
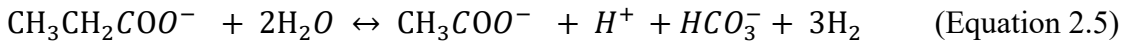


Acetic acid formation:



Acetogenesis Step:

Intermediate VFAs are converted into acetate, hydrogen, and carbon dioxides (Tchobanoglous et al., 2014). Example equations of acetogenesis step are given in Equation 2.5, Equation 2.6, Equation 2.7 (Bajpai, 2017a).



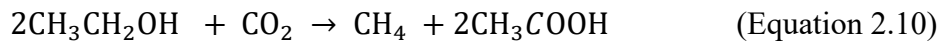
Methanogenesis Step:

Products of acetogenesis step are used to produce methane by methanogens (Bajpai, 2017b). Methanogens are divided into two groups that are acetotrophic and hydrogenotrophic methanogens (Teodorita Al Seadi et al., 2008). Acetotrophic methanogens convert acetate to methane production. Also, hydrogenotrophic methanogens use carbon dioxides as electron acceptor and hydrogen as electron donor (Enzmann et al., 2018). Acetotrophic methanogens contribute 70 % of the methane generation, and the rest of the methane is occurred by hydrogenotrophic (Teodorita Al Seadi et al., 2008). Equations of methanogenesis step are given in Equation 2.8, Equation 2.9, Equation 2.10 (Bajpai, 2017a).

Acetotrophic methanogenesis



Hydrogenotrophic methanogenesis:



The methanogenesis step is crucial in the entire conversion process of anaerobic digestion. The growing rate of methanogens is slower than other microorganism, so this step is the rate-limiting step in anaerobic digestion. Among the methanogens, hydrogenotrophic methanogens are generally the limiting step in methane production. Additionally, methanogens are more sensitive than other microorganism in the anaerobic digestion (Bajpai, 2017a). So, methanogens affect easily environmental conditions such as temperature changes, overloads, the presence of oxygen, substrate types, pH changes (Teodorita Al Seadi et al., 2008).

2.4 Process Parameters Affecting Anaerobic Digestion

Several parameters effect the performance of anaerobic digestion. Thus, the determination of process parameters is critical for the optimum biogas production (Teodorita Al Seadi et al., 2008). These process parameters are temperature, pH, alkalinity, the substrate to inoculum ratio, hydraulic retention time (HRT), organic loading rate (OLR), C/N ratio, inhibition, and toxicity factors.

2.4.1 The Substrate to Inoculum Ratio (SIR)

Biochemical Methane Production (BMP) tests are crucial to measuring the performance of anaerobic digestion. The optimum SIR is such an essential parameter to enhance the anaerobic digestion (Pellera and Gidarakos, 2016). Nevertheless, SIR is a complicated issue, so that there is a wide range of SIR in the literature. Volume of inoculum can change depending on the substrate type/characterization. The optimum range of SIR was found 0.25-0.5 (expressed as VS content) for using solid agro-industrial waste (Pellera and Gidarakos, 2016). However, the range of SIR was determined 0.8 to 3 (g VS /g VS) to produce maximum methane for sunflower oil cakes as a substrate (Raposo et al., 2009). The optimum range of SIR is 0.5 to 1 (expressed as VS content) for some substrates that are freshwater, marine, herbaceous, and woody feedstocks and municipal wastes (Chynoweth et al., 1993).

2.4.2 Organic Loading Rate (OLR)

This term is generally used for the process design of continuous anaerobic digester (Mao et al., 2015; Meegoda et al., 2018). OLR (g VS/L.d) is a vital parameter to balance in the anaerobic system between production and consumption components. If huge amount of volatile solid adds in the anaerobic digester, itor may cause inhibition in the anaerobic digester. After overloading, hydrolysis and acidogenesis steps occur more rapidly than the methanogenesis step. Moreover, VFA production increases due to irreversible

acidification and methanogens are inhibited due to decreasing pH value (above 6.8). Thus, methanogens cannot convert VFA to methane (Mao et al., 2015; Meegoda et al., 2018; Teodorita Al Seadi et al., 2008).

2.4.3 Retention Time

Retention time is a necessary time to degrade organic material via bacteria. The growing rate of bacteria is related to retention time, which depends on the characteristics of the substrate, operational temperature, and OLR. There are mainly two types of retention time, namely hydraulic retention time (HRT) and solid retention time (SRT). Concentration of bacteria (solid) in the anaerobic digestion is checked by using SRT term. HRT is explained by Equation 2.11 (Henze et al., 2008).

$$\text{HRT: } \frac{V}{Q} \quad (\text{Equation 2.11})$$

V: reactor volume (m³)

Q: the influent flow rate in time (m³/day)

The range of optimum retention time is 15 to 30 days to degrade organic solid for mesophilic anaerobic digestion. The reduction of the HRT generally causes VFA accumulation, whereas, long retention time leads to inadequate consumption of digester components (Gerardi, 2003).

2.4.4 pH and Alkalinity

pH significantly influences the performance of the anaerobic digestion and also is a crucial parameter for the growth rate of microorganisms in the anaerobic digester. pH value in the anaerobic digestion depends on the generation of intermediate products. pH is below 6 at the beginning of the anaerobic digestion due to the production of volatile

acid and carbon dioxides. After these products are consumed to produce methane by methanogens and the pH range increases 7 to 8. When pH drops below 6.6, activities of methanogens are adversely affected. Additionally, pH drops below 6.2, which is toxic for methanogens. Acidogens can produce acid even at pH of 4.5 to 5. The optimum pH range is 6.8 to 7.2 due to the production of sufficient methane by methanogens (Gerardi, 2003; Richard, 1968). The amount of carbon dioxide in the biogas influences the pH range in the anaerobic digester (Chandra et al., 2012).

Alkalinity concentration is a vital parameter to maintain the neutral pH. Thus, the optimum alkalinity concentration range is 2000 to 4000 mg/L as CaCO_3 (Tchobanoglous et al., 2014).

2.4.5 Temperature

There are three temperature ranges, namely psychrophilic, mesophilic and thermophilic, for the anaerobic digester. The optimum temperature of the psychrophilic range is from 5 to 25 °C, the mesophilic range is from 30 to 35 °C and the thermophilic range is from 50 to 65 °C (Abbasi et al., 2012; Gerardi 2003). The growth of methane-forming bacteria influences negatively at the temperature between 40 to 50 °C due to the transition from mesophilic to thermophilic microorganisms (Chandra et al., 2012). Many temperature ranges are suitable for the growing of methanogens and also methane production. Mesophilic conditions suit for the majority of methanogens; however, a few methanogens can grow under thermophilic conditions. Lastly, as long as temperature ranges increase, hydraulic retention time decreases which is shown in Figure 2.8 (Gerardi, 2003).

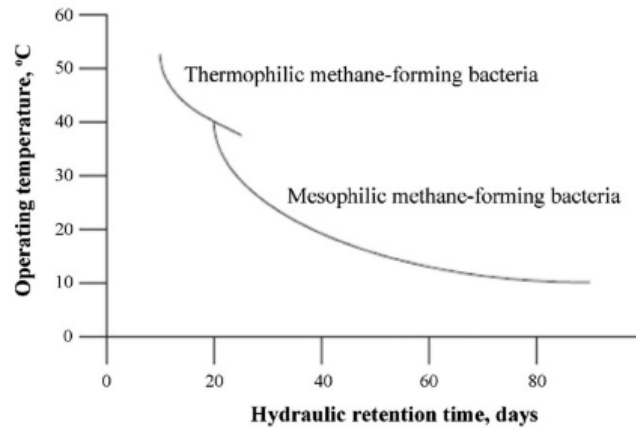


Figure 2.8 Operating temperature of methanogenic bacteria concerning HRT (Gerardi, 2003)

2.4.6 Carbon/Nitrogen (C/N) ratio

Organic materials mainly consist of a carbon source and a nitrogen source. The proportions of carbon and nitrogen are defined as a carbon/ nitrogen (C/N) ratio (Abbasi et al., 2012). The optimum range of the C/N ratio is 20-30 to enhance biogas production in the anaerobic digestion system. If the C/N ratio is lower than the optimum range of it, methanogens will quickly consume nitrogen sources to supply their requirement of protein; then, there will not be enough nitrogen sources in the organic material in order to use methane production. Otherwise, if the C/N ratio is higher than the optimum range of it, nitrogen will release ammonium ion (NH_4^+). Thus, there will be excess ammonium ion in the anaerobic digestion after that pH can increase 8.5, which is toxic for the methanogens in the anaerobic digester (Chandra et al., 2012). The C/N ratios of some biomass are given in Table 2.4.

Table 2.4 C/N ratio of biomass

| Raw Material | C/N | References |
|-------------------|-----------|------------------------|
| Corn cob | 123 | Kumari et al. (2018) |
| Corn stover | 50-63 | Paul and Dutta. (2018) |
| Wheat straw | 50-60 | Tye et al. (2016) |
| Softwood stems | 511 | Chandra et al. (2012a) |
| Rice straw | 47-67 | Chandra et al. (2012a) |
| Sugarcane bagasse | 118-150 | Chandra et al. (2012a) |
| Swine manure | 13.2-13.9 | Chen et al. (2003) |
| Poultry manure | 7.7-12 | Chen et al. (2003) |
| Cattle manure | 14.2-27.4 | Chen et al. (2003) |

The COD/N ratio is the same concept with the C/N ratio. The optimum range of COD/N ratio is 350/7 - 1000/7 to enhance methane production in anaerobic digestion (Speece, 1996).

2.4.7 Nutrients Requirement

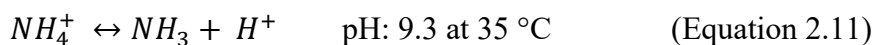
Nutrient requirements are categorized into two types that are macronutrients and micronutrients. Nitrogen and phosphorus are mainly macronutrients for the anaerobic digestion. Ammonical-nitrogen ($\text{NH}_4^+\text{-N}$) and orthophosphate phosphorus ($\text{HPO}_4\text{-P}$), which are suitable to use anaerobic microorganisms, are the soluble form of nitrogen and phosphorus, respectively. Especially methanogens need more micronutrients compared to macronutrients. These are basically cobalt, iron, nickel, zinc and sulfide. Moreover, trace elements which are tungsten, selenium, manganese, molybdenum and boron can be used by methanogens (Speece, 1996; Gerardi, 2003).

2.4.8 Toxicity

Anaerobic microorganisms are affected by toxic material types and toxic material concentration. Indicators of toxicities are the inhibition of hydrogen-producer and methane-producer, reduction of alkalinity and/or pH, and increase in amount of the volatile acid. Toxic material can be mainly classified into two types, namely ammonia toxicity and hydrogen sulfide toxicity (Gerardi, 2003).

Ammonia Toxicity:

Nitrogen is reduced to ammonium nitrogen ($\text{NH}_4^+\text{-N}$) or ammonium ions (NH_4^+) to degrade organic nitrogen source or generate amino acid and protein during the anaerobic digestion. Total ammonia nitrogen (TAN) involves ammonium nitrogen ($\text{NH}_4^+\text{-N}$) and NH_3 , which is free ammonia nitrogen (FAN). Anaerobic microorganisms can use ammonium ions (NH_4^+) for the nutrient requirements as a nitrogen source and ammonium ions supply buffering capacity in the anaerobic digester. However, the toxic compound is FAN for anaerobic microorganisms (Gerardi, 2003). The ionization of NH_3 can be adjusted by pH and temperature. Ammonium ions are rapidly converted to FAN at pH 9.3 and 35 °C (Speece, 1996). Equation 2.11 shows the conversion of ammonium ions to free ammonia. If the pH value increase, free ammonia concentration can also increase. Free ammonia concentration is 1 % of total reduced nitrogen at pH 7 (Speece, 1996; Gerardi, 2003).



The toxicity level of TAN concentration begins from 1500 mg /L at high pH in the anaerobic digester. If the TAN concentration increases up to 3000 mg/L, methanogens cannot tolerate this TAN value (Gerardi, 2003). On the other hand, methane is not produced due to ammonia inhibition, and the pH level decreases, volatile acid concentration increases. At this time, FAN can convert to ammonium ions due to decreasing pH, so this process can be called “self-correcting” (Gerardi, 2003; Yenigün

and Demirel, 2013). Methanogenic activity can be inhibited if FAN concentration reaches 150 mg/L (McCarty and McKinney, 1961).

Hydrogen Sulfide Toxicity

Anaerobic bacteria can sufficiently use soluble sulfide (HS^-) as a nutrient. But the excessive soluble sulfide amount or dissolved hydrogen sulfide gas inhibits the anaerobic digestion. The most toxic compound is hydrogen sulfide for the anaerobic bacteria. Hydrogenotrophic methanogens are more sensitive to hydrogen sulfide than acetotrophic methanogens. Additionally, acid-forming bacteria can be affected from the toxicity of hydrogen sulfide. The sulfide inhibition mechanism is not clear up to now. The toxicity level of sulfide concentration is 200 mg/L at 7 pH (Gerardi, 2003).

2.5 Anaerobic Digestion of Lignocellulosic Materials and Its Enhancement

Using lignocellulosic material as a substrate in the anaerobic digester is a crucial method due to the conversion of residual energy to renewable energy (Xu et al., 2019). Lignocellulosic material is one of the most abundant biomass energy sources; besides, this source is increasing by nearly 20 billion tons year by year (Patinvoh et al., 2017). To produce biomethane from lignocellulosic cellulose, anaerobic digester is one of the most cost-effective methods in the worldwide (Grosser, 2017). However, in the anaerobic digestion, using lignocellulosic biomass includes some challenges. Firstly, there are some complexities of lignocellulosic structure in the way of degraded from anaerobic microorganisms, which decreases conversion to biomethane from organic material (Paul and Dutta, 2018). The recalcitrance component is generally lignin, which covers the cellulose and hemicellulose to protect microorganisms. The lignocellulosic biomass can be pretreated to overcome the recalcitrance component. There are lots of pretreatment applications, which are categorized as lignocellulosic biomass type, composition, and why they are used (Xu et al., 2019). Another challenge is about deficiency in the content of lignocellulosic biomass. Compositions of lignocellulosic

biomass are different from each other such as carbohydrate-rich biomass, protein-rich biomass, and fat-rich biomass (Hagos et al., 2017). Lignocellulosic material can be arranged with using co-substrate to reach the optimum range of the C/N ratio (Babae et al., 2013).

2.5.1 Co-digestion

Anaerobic mono-digestion, in which only one substrate as a organic sources is used, includes some drawbacks such as high/low nitrogen or carbon source, heavy metal compound, including high amount of toxic component. Thus, the anaerobic co-digestion method was developed to overcome the drawbacks of the mono-digestion. Co-digestion is defined as two or more substrates are used in an anaerobic digester to enhance the methane production (Mata-Alvarez et al., 2014). Anaerobic co-digestion consists of the following advantages which are (Shah et al., 2015; Kainthola et al., 2019; Azbar et al., 2008):

- Dilution of the inhibitory component
- Providing stabilization of the process
- Improvement of the methane production rate
- Favorable effect on bacteria synergy
- Nutrients stability
- Cost-effective method due to providing biodegradable source and nutrients requirement
- Increasing the amount of biodegradable organic component

Co-substrates depend on the content of the main substrate in the anaerobic digestion. Meanwhile, the ratio of substrate to co-substrate is generally based on the optimization of the C/N ratio. But, different parameters such as pH, alkalinity, etc. also play an essential function in anaerobic co-digestion (Kainthola et al., 2019). Types of animal manure such as pig manure, chicken manure, cow manure, biodegradable industrial waste, municipal solid waste, algae, sewage sludge are commonly used as co-substrate

to enhance lignocellulosic biomass (Shah et al., 2015). Additionally, the effluent stream of the anaerobic digester that is called digestate is used as a co-substrate to improve performance of anaerobic digestion, recently (Thygesen et al., 2014).

2.5.2 Pretreatment application

The aim of the pretreatment applications is generally the conversion of the lignocellulosic biomass complexity to simple forms that are cellulose, hemicellulose and soluble lignin. After pretreatment application, changes in biomass structure are the destruction of the lignin component, conservation of hemicellulose, conservation of cellulose structure, decreasing the crystallinity of cellulose and increasing the size of biomass porosity (Kumari and Singh, 2018). There are lots of pretreatment applications of lignocellulosic biomass, but the selection of optimum pretreatment application is crucial to enhance methane production. Therefore, optimum pretreatment method should meet the following necessities (Kumari and Singh, 2018; Taherzadeh and Karimi, 2008):

- Preventing the generation of inhibitory by-product
- Enhancing sugar formation to degrade microorganisms
- Minimum energy requirement
- Suitable for biomass characterization
- Cost-effective method
- Generation of less waste

The pretreatment applications can be categorized into physical pretreatment, chemical pretreatment, thermophysical, thermochemical pretreatment, biological pretreatment and combined pretreatment (Kumari and Singh, 2018; Taherzadeh and Karimi, 2008; Kainthola et al., 2019; Chandra et al., 2012; Paul et al., 2018; Kucharska et al., 2018; Shah et al., 2015). These pretreatment applications are given in Table 2.6.

Table 2.5 Type of pretreatment method and advantages and challenges in lignocellulosic biomass (Kumari and Singh, 2018; Taherzadeh and Karimi, 2008; Kainthola et al., 2019; Chandra et al., 2012; Paul et al., 2018; Kucharska et al., 2018; Shah et al., 2015)

| Pretreatment method | Type of method | Expected positive change in biomass | Negative effect in anaerobic digestion |
|-----------------------|--|--|---|
| Physical Pretreatment | Fragmentation (milling, hacking, rolling, grinding) | Reduction of biomass size | |
| | Irritation (microwave, gamma-ray, electron-beam) | Increasing the pore size of biomass | |
| | Sonication | Reduction of cellulose crystallinity | |
| | Extrusion(Ext) | Breaking down of hydrogen band | |
| | Freezing (Frz) | Increasing degradation capacity via bacteria | Decreasing flowability |
| | Pyrolysis | Cellulose decomposition into the gaseous product at 300 °C | Generation of inhibitory by-product (furfural, guaiacols, fural etc.) |

| | | |
|---|--|--|
| Acid (H ₂ SO ₄ , HCl, H ₃ PO ₄) | Solubilization of especially hemicellulose into acid | Generation of inhibitory by-product(acetic acid, furfural, and 5-hydroxymethyl furfural) |
| Alkaline (Sodium hydroxide, calcium hydroxide, potassium hydroxide, and ammonia) | Disruption of the cell wall, and then solubilization of cellulose, hemicellulose and lignin Increasing porosity size of biomass | Excess using alkali solution can cause inhibition. |
| Oxidative (Hydrogen peroxide or peracetic acid) | Solubilized lignin by oxidative agent | |
| Ozonolysis | Disruption of lignin, and hemicellulose component and increasing cellulose degradation | Produced soluble aromatic compound due to lignin oxidation |
| Ionic liquid(IL) | Increasing solubilization of carbohydrates and lignin | Not a cost-effective method |
| Organosolv (Ethyl alcohol, methyl alcohol, acetone, ethylene glycol, and tetrahydrofurfuryl alcohol) | Breaking down of lignin and hemicellulose band | Costly method and not enough data about the toxicity |

| | | | |
|-------------------------------|--|---|--|
| Physico-chemical Pretreatment | Explosion (Steam, CO ₂ , SO ₂ Ammonia fiber(AFEX)) | Disruption of cellulose structure | Solubilization of inhibitory by-product(furfural and hydroxyl methyl furfural) |
| | Oxidation (Wet oxidation) | Hydrolyzation of hemicellulose and oxidation of lignin | Production of inhibitory component due to lignin oxidation |
| | Liquid Hot Water/Hydrothermal | Solubilization of cellulose, and removal of hemicellulose | Generation of inhibitory by-product |
| Biological Pretreatment | Fungal | Hemicellulose hydrolyzation | Costly |
| | Microbial consortium | Delignification | Slow pretreatment rate |
| | Enzymatic | Lignin decomposition | |
| Combine Pretreatment | Combined SO ₂ and steam explosion | Removal of lignin | |
| | Combined dilute acid and steam explosion | Increasing pore size and reactive surface | Generation of inhibitory by-product |
| | Combined alkali and hydrothermal | Decreasing cellulose crystallinity Solubilization of hemicellulose | Not a cost-effective method |

Combined pretreatment method can be constituted with using two or more pretreatment methods at time same time. Lignocellulosic biomass types, composition and characterization are vital parameters to determine suitable combine pretreatment application. Thus, Table 2.7 gives the information about the effect of pretreatment methods on biogas production (Paul et al., 2018).

Table 2.6 Effect of pretreatment methods on biogas production (Paul et al., 2018)

| Biomass Type | Level of pretreatment effect on biogas production | | | | |
|--------------|---|---------|--------------|--------------------|-------------------------|
| | Physical | Thermal | Hydrothermal | Alkaline - Thermal | Alkaline - Hydrothermal |
| Woody | Lowest | Low | Medium | High | High |
| Non-woody | Lowest | Low | Medium | High | Highest |

2.6 Anaerobic Digestion of Corncob

Amount of corn wastes are the highest among crop wastes in the world; however, 35 % of total corn waste was used as a substrate or co-substrate at laboratory scales research or industry scales to produce valuable component (Ramos-Suárez et al., 2017). Nevertheless, corn wastes, especially as an energy crop, are the most used agricultural waste in the industry scale (Achinas et al., 2017). Types of corn waste are straw and cob which have different characteristic properties. There are lots of research study about methane production with corn straw; moreover, corn straw can be also used animal food. Although corncob consists of the highest hemicellulose content compared to agricultural waste and it has limited usage area out of burning, there is not enough research study about using corncob in the anaerobic digester to produce methane. The reasons for this may be a structural challenge, nitrogen deficiency, and difficulty of separation from corn waste (Surra et al., 2018). The disadvantages of corncob can be easily overcome. For instance, structural challenges can be removed by pretreatment method and nitrogen

deficiency can be solved by adding a co-substrate (nitrogen-rich), and there is a new device, which is used for separation of corncob from corn waste during harvesting. So, out of corncob can use animal food (Surra et al., 2018; Pan-in and Sukasem, 2017; Blandino et al., 2016). Moreover, corncob is used for heating in the small villages thanks to its high calorific value which is 18.4 MJ/kg (Başçetinçelik et al., 2005). Thus, greenhouse gas emissions increase due to burning of the corncob. Corncob should be used as valuable products due to having a high amount, which is 30 % of corn waste (Başçetinçelik et al., 2005). The research studies of corncob in anaerobic digester are given in Table 2.8.

Table 2.7 Studies of corncob in anaerobic digestion up to date

| Type of pretreatment | Co-substrate | Range of max. methane (mL CH ₄ /g VS) | Ref. ^a |
|--|-----------------------|---|-------------------|
| Milling, Alkaline and/or Enzymatic hydrolyze | - | 269±1 - 309±5 | 1 |
| - | - | 139 | 2 ^c |
| Ultrasonic and/or Enzymatic hydrolyze | - | 243±2 - 297±3 | 3 |
| Peroxides Microwave and Chemical (Alkaline, Peroxides, Glycerol) | MSW | 124 - 331 | 4 ^b |
| - | - | 146±8 | 5 |
| Alkaline (Lime) | - | 104 | 6 |
| Alkaline (NaOH) | Pig, Cow, Goat manure | 27-106 | 7 ^c |
| - | - | 207-307 | 8 ^c |
| Acid hydrolyze | - | 267 | 9 ^{c,d} |
| - | - | 251-419 | 10 ^c |
| - | - | 222 | 11 |

^a References; ¹ García-bernet and Domínguez, (2017); ² Li et al., (2015), ³ Pérez-rodríguez et al. (2016), ⁴ Surra et al., (2018), ⁵ Blandino et al. (2016), ⁶ Shah and Tabassum (2018), ⁷ Pan-In and Sukasem (2017), ⁸ Menardo et al., (2014), ⁹ Huang et al.,(2017), ¹⁰ Seppa et al., (2012), ¹¹ Ali et al., (2018)

^b Temperature was generally at 35±1, and the only 4.ref is 50±2°C.

^c Corncob was mixed with different types of lignocellulosic; 2.ref: whole corn, 7.ref: cornhusks, 8.ref: the different ratio of corn waste mixtures, 9.ref: bagasse and rice straw, 10.ref: various types of corn waste.

^d Batch reactor was mainly used, only for 9. ref used up-flow anaerobic bioreactor.

The methane potential of corncob can be enhanced by the different types of pretreatment methods and co-digestion. The range of methane production yield is 104 to 309 mL CH₄/g VS, according to Table 2.8.

2.6.1 Enhancement of anaerobic digestion of corncob

As mentioned in Section 2.5, corncob, which is a lignocellulosic biomass, has a complex structure for biogas production in anaerobic digester (Surra et al., 2018). However, there are also enhancement methods to overcome the disadvantages of the corncob. Corncob generally contains 33.7-41.2 % cellulose, 31.9-36 % hemicellulose and 6.1-19.3 % lignin component (Kumari and Singh, 2018). Using a suitable pretreatment method can improve the degradation of corncob due to increasing cellulose and hemicellulose solubilization and the destruction of lignin (Kumari and Singh, 2018). However, by-products can occur during the pretreatment application. By-products contain both useful components and hazardous components for anaerobic microorganisms (Wang et al., 2018). Thus, the selection of a suitable pretreatment method is vital to enhance methane production. Corncob has high amount of lignin content; thus, in literature, alkaline pretreatment is one of the best methods to remove lignin (Paul et al., 2018). Also, corncob involves the highest amount of hemicellulose content compared to the agricultural residuals (Blandino et al., 2016). The hydrothermal pretreatment method is suitable for the solubilization of hemicellulose to ensure easy degradation of microorganisms (Paul et al., 2018). Another challenge of corncob is low-nitrogen content, as mentioned in Section 2.5. Addition of rich-nitrogen as a co-substrate can overcome this problem. When the co-substrate (nitrogen-rich) is mixed with corncob, COD/N ratio of the mixture should be controlled to ensure whether it is in the optimum range of it (Surra et al., 2018 and Pan-In and Sukasem, 2017).

CHAPTER 3

BIOCHEMICAL METHANE POTENTIAL OF CORNCOB AND THE EFFECT OF CO-DIGESTION AND ALKALINE HYDROTHERMAL PRETREATMENT

3.1 Introduction

With the increasing population in the world, the energy requirement is correspondingly increasing. Currently, biomass energy covers 53% of the renewable energy which corresponds to 11% of the total primary energy supply in the world (IEA, 2019). The amount of biomass sources is nearly 220 thousand tons; however, only 6% of these sources are used to produce energy (Kumari et al., 2018).

The crucial biomass energy sources as lignocellulosic biomass are mainly wood, yard waste, energy crop and agricultural residues (Paul and Dutta, 2018). One of the significant agricultural residues is crop waste which is the second-generation biofuel source. Moreover, one of the types of crop waste is corn waste which constitutes the highest portion (33%) among crop wastes in the world (Başçetinçelik et al., 2005). Corn waste can be separated into two parts that are straw and cob. Corn straw contains leaves and stalk used for animal food (Başçetinçelik et al., 2005). Corncob, of which shape is long rounded, is in the center of the corn ear. The general structural contents of corncob are in the range of 34% to 41% cellulose, 32% to 36% hemicellulose and 6% to 19% lignin (Kumari et al., 2018).

Corncob can be used to produce valuable chemicals and biofuels such as xylitol, furfural, hydrogen, ethanol, biodiesel, biogas, biomethane; unfortunately, most of the corncob wastes are kept in solid waste landfills (Li et al., 2015). There are lots of advantages of corncob. For instance, i) corncob has the highest hemicellulose content among the lignocellulosic biomass; therefore, it is the potential resource for renewable energy

production (Li et al., 2015) ii) if the corncob is not used to produce biofuels and valuable chemicals, it is generally used for heating purpose in the small villages due to its high calorific value (18.4 MJ/kg). However, it should be noted that the latter results in a high amount of CO₂ emission (Başçetinçelik et al., 2005). Despite the abovementioned advantages, the studies about methane production from corncob with anaerobic digestion are limited, and also these studies have been conducted for the last ten years (Seppa et al., 2012; Li et al., 2015; Pérez-Rodríguez et al., 2016; Blandino et al., 2016; García-bernet and Domínguez, 2017; Pan-In and Sukasem 2017; Huang et al., 2017; Ali et al., 2018; Surra et al., 2018; Shah and Tabassum, 2018).

Literature studies indicate that biochemical methane potential (BMP) of corncob is in the range of 139 to 222 mL CH₄/g VS (Li et al., 2015; Blandino et al., 2016; Ali et al., 2018). This value is lower than the theoretical methane yield (395 mL/g COD), assuming that 1 gram VS of corncob has 1 gram COD equivalence. The causes of lower methane production from corncob can be explained mainly by two ways: i) composition of corncob has not enough nitrogen content for methane production, ii) corncob is not readily biodegradable for microorganisms due to the structural properties of corncob (Chandra et al., 2012).

Co-substrates were used in the reactors to supply enough nitrogen sources so that the optimum COD/N range of 50 to 143 can be obtained and the methane production might be enhanced (Speece, 1996). In literature, there are some studies about using corn waste in the co-digestion method. Surra et al. (2018) studied pretreated corncob with municipal solid waste and biogas yield increased by 46%. The methane yield of corn stalk increased from 217 to 259 mL CH₄/g VS when the vermicompost was added (G. Chen et al., 2010). Nevertheless, there is one study with Raw CC and pig manure in co-digestion of which methane yield reached 139 mL CH₄/g VS (Li et al., 2015).

The structural problem of corncob mainly results from lignin, which covers cellulose and hemicellulose to prevent the microbial attack. Thus, the cellulose and hemicellulose cannot be easily accessed and thus degraded by the microorganisms. To remove structural difficulties, pretreatment methods such as alkaline pretreatment, hydrothermal, enzymatic, microwave and ultrasound pretreatment can be used (Kumari et al., 2018). The aim of the alkaline pretreatment is especially the lignin destruction (Chandra et al., 2012). Hydrothermal pretreatment focuses on the increase of solubility and accessibility of hemicellulose and cellulose (Kumari et al., 2018). For hydrothermal pretreatment, the important point is the arrangement of the temperature. When the temperature is higher than 250 °C, inhibitory by-products that can damage the methanogens might be formed (Paul et al., 2018). The alkaline hydrothermal can be a potential pretreatment method for the lignocellulosic biomass to overcome the whole structural problem. Paul et al. (2018) studied an alkaline hydrothermal pretreatment method on the corn residue and found methane yield as 278 mL/g VS. Surra et al. (2018) studied the microwave pretreatment method that was catalysed by H₂O₂ at the pH value of 9.2. According to this study, solubility of lignin, cellulose, and hemicellulose was increased by 69%, 63%, and 62%, respectively. Moreover, the methane yield was increased by 36% with the co-digestion effect. In Pérez-Rodríguez et al. (2016)'s study the BMP of pretreated corncob with enzymatic hydrolysis was increased by 15 %.

This study aims to investigate the BMP of corncob and its enhancement by using co-digestion and pretreatment approaches. This is the first study for corncob to use the alkaline hydrothermal pretreatment method at 240°C (AHP_240) with the aim to remove the structural difficulties of the corncob. In addition, three co-substrates, which are chicken manure, digestate, and post-digestate, are used for the first time to adjust the nitrogen content. So far, there has been little discussion about both the effects of different co-substrates and combined effect of pretreatment and co-digestion on methane production from corncob. Thus, insufficient information about corncob to improve methane production in the literature might be increased. To this end, BMP experiments

were carried out to investigate and compare the methane yields of anaerobic mono-digestion of both raw corncob and pretreated corncob and co-digestion of each substrate with three abovementioned different co-substrates.

3.2 Materials and Methods

This section informs about the characteristics of raw corncob (Raw CC), co-substrates, inoculum and alkaline hydrothermal pretreated corncob at 240 °C (AHP_240 CC). The experimental procedures are explained and analytical methods are given.

3.2.1 Characteristic of corncob

Corn cob was collected from a corn field within the city limits of Zonguldak. This raw material was dried at room temperature and grinded in an electric shredder. Then it was screened from 2.36 mm sieve (Endecotts Ltd, London, England). After these steps, corncob was sealed in a plastic bag and stored at -20 °C (Surra et al., 2018). Picture of the screened Raw CC is given in Figure 3.1. The properties of corncob are shown in Table 3.1.

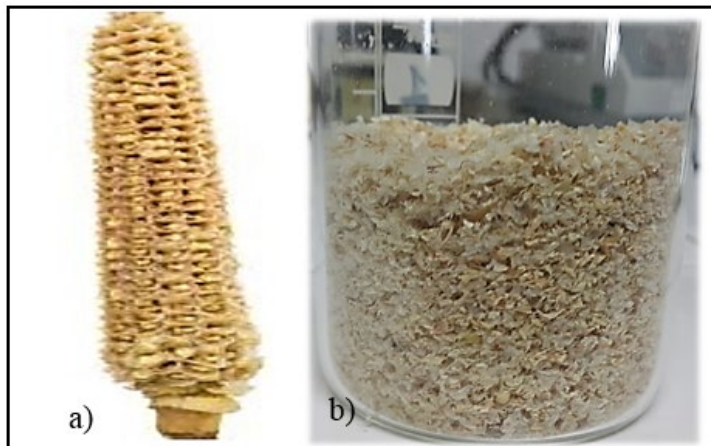


Figure 3.1 Raw CC a) before grinding b) after grinding and screening

Table 3.1 Characteristics of corncob

| Parameter | Value |
|-------------------|-------------|
| pH | 5.26 |
| TS (g/g) | 0.89±0.00 |
| TS (%) | 89.70±0.14 |
| VS (g/g) | 0.88±0.00 |
| VS % (% of TS) | 98.42±0.15 |
| tCOD (mg/g) | 1340±12 |
| tCOD/VS(g/g) | 1.52 |
| sCOD (mg/L) | 385±13 |
| sCOD (mg/g) | 30.90±0.47 |
| sCOD/tCOD(g/g) | 0.02 |
| TKN (mg /g) | 4.03±0.38 |
| TKN (%) | 0.40±0.04 |
| TAN (mg/g) | 0.0047±0.00 |
| TP (mg/g) | 0.260±0.00 |
| Cellulose (%) | 31.50±1.84 |
| Hemicellulose (%) | 35.54±0.65 |
| Lignin (%) | 21.27±0.31 |

3.2.2 Pretreatment of corncob

There are various pretreatment applications in the literature. When Table 2.7 is seen (Section 2.5.1), the most efficient pretreatment method seems to be the alkaline hydrothermal pretreatment method to overcome structural challenges of corncob as a lignocellulosic biomass. There are three parameters to consider in alkaline hydrothermal pretreatment method, which are NaOH concentration, liquid to solid ratio, and oven temperature. The oven was used for heat without pressure application. Paul et al. (2018)'s study mentioned that the degradation temperatures of cellulose is in the range

of 220 to 240°C, at these temperatures cellulose is converted to glucose. Moreover, hemicellulose is converted to xylose at 230°C in 30 minutes. However, 250°C and above might end up with inhibitory by-products. Therefore, in this study a temperature that would not cause a toxicity but improve the accessibility of cellulose and hemicellulose was selected as 240°C. Then, a preliminary study was done to select a suitable concentration of NaOH and liquid to solid ratio (Appendix A). Different NaOH concentrations, namely, 3%, 4%, 5%, 10%, 15% and 20% NaOH solution were used to investigate the suitable NaOH concentration. Liquid to solid ratios (mL/g) studied were 5, 10, 15 and 20. According to the increase in sCOD concentration and sCOD/tCOD ratio, 5% NaOH solution and liquid to solid ratio of 20 mL/g were decided to use during pretreatment application (Appendix A).

Alkaline Hydrothermal Pretreatment at 240°C (AHP_240): For alkaline hydrothermal pretreatment, 1 gram of corncob, which was dried and screened from 2.36 mm, was soaked into the 20 mL of 5% (w/v) NaOH solution for 3 hours. After the soaking period, this mixture was heated in the oven at 240°C for 30 minutes. Lastly, the pH value was adjusted at a neutral level by using HCl. The properties of AHP_240 CC are shown in Table 3.2.

Table 3.2 Characterization results of AHP_240 CC

| Parameter | Value |
|-------------------|-------------------|
| pH | 13.2 ^a |
| TS (g/g) | 0.95±0.00 |
| TS (%) | 95.40±0.08 |
| VS (g/g) | 0.70 ±0.06 |
| VS % (% of TS) | 73.01± 5.33 |
| tCOD (mg/g) | 1163 ± 14 |
| tCOD/VS | 1.66 |
| sCOD (mg/L) | 26350±250 |
| sCOD (mg/g) | 527±5 |
| sCOD/tCOD | 0.45 |
| TKN (mg /g) | 4.2±0.38 |
| TKN (%) | 0.40±0.40 |
| TAN (mg/g) | 0.28 ±0.00 |
| TP (mg/g) | 0.12± 0.07 |
| Cellulose (%) | 4.74± 1.99 |
| Hemicellulose (%) | 17.06± 1.26 |
| Lignin (%) | 12.15±0.38 |

^a Before adjustment of pH to neutral level

3.2.3 Characteristic of co-substrates

Co-substrates were used in the reactors to supply enough nitrogen sources so that the optimum COD/N range of 50 to 143 can be obtained and the methane production might be enhanced (Speece, 1996). Co-substrates were taken from Afyon Energy Biogas Plant. The anaerobic digester in this Plant is a two-stage process; the first digester is fed with chicken manure (83%) and poppy (17%) mixture. The first anaerobic digester's effluent is fed to the second anaerobic digester. For this study, three different co-substrates,

namely, Feed, Digestate and Post-digestate, were used. The first co-substrate, i.e., Feed, is the influent of the first digester in the Plant, which is chicken manure and poppy (17%) mixture. The second co-substrate is the effluent of the first digester, which is defined as Digestate in this study. The third co-substrate, namely Post-digestate, is the effluent of the second digester in the Plant. These co-substrates were put in plastic bottles and stored at -20°C. Bottles were thawed at 4°C before use. Table 3.3 shows the characterization results of these three co-substrates.

Table 3.3 Characterization results of co-substrates

| Parameter | Feed | Digestate | Post-digestate |
|----------------|--------------|------------|----------------|
| pH | 7.93 | 9.31 | 8.78 |
| TS (g/L) | 171.36±14.92 | 74.60±8.41 | 72.23±9.63 |
| VS (g/L) | 97.88±8.86 | 37.37±3.18 | 38.68±3.45 |
| VS % (% of TS) | 57.10±1.00 | 50.2±2.32 | 53.8±2.45 |
| tCOD (g/L) | 233.30±18.54 | 95.68±4.01 | 90.29±1.78 |
| tCOD/VS | 2.38 | 2.56 | 2.33 |
| sCOD (g/L) | 37.35±3.60 | 12.37±0.06 | 10.56±0.212 |
| sCOD/tCOD | 0.16 | 0.13 | 0.11 |
| TKN (g /L) | 8.40±0.48 | 7.16±0.56 | 6.87±0.10 |
| TAN (g/L) | 2.61±0.08 | 3.73±0.16 | 3.22±0.00 |
| TP (mg/L) | 30.61±1.87 | 50.45±0.94 | 61.36±2.34 |

3.2.4 Inoculum (seed sludge)

Inoculum is the seed sludge used in the experiments. Inoculum was obtained from the effluent of the anaerobic digesters in the Ankara Central Municipal Wastewater Treatment Plant. The seed sludge was stored at room temperature in a plastic container. Inoculum characteristics are shown Table 3.4.

Table 3.4 Characterization results of inoculum

| Parameter | Value |
|----------------|------------|
| pH | 7.60 |
| TS (g/L) | 6.82 ±0.1 |
| VS (g/L) | 3.41 ±0.07 |
| VS % (% of TS) | 50.1 ±0.92 |
| tCOD (g/L) | 6690±140 |
| sCOD (g/L) | 310 ±2.10 |
| TKN (mg/L) | 528±2.1 |
| TAN (mg/L) | 245±2.1 |
| TP (mg/L) | 65±1.1 |

3.2.5 Basal medium

Basal medium provides optimum growth conditions for microorganisms. Basal medium was applied to the reactors which includes only corncob. Basal medium ingredients and concentrations obtained in the reactors (shown in parentheses) are as follows: NH_4Cl (1200 mg/L), NaHCO_3 (6000 mg/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (400 mg/L), KCl (400 mg/L), $(\text{NH}_4)_2\text{HPO}_4$ (80 mg/L), $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (300 mg/L), $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (40 mg/L), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (50 mg/L), KI (10 mg/L), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (10 mg/L), $(\text{NaPO}_3)_6$ (10 mg/L), NH_4VO_3 (0.5 mg/L), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (0.5 mg/L), ZnCl_2 (0.5 mg/L), $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (0.5 mg/L), $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.5 mg/L), $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (0.5 mg/L), $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (0.5 mg/L), H_3BO_3 (0.5 mg/L), $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ (0.5 mg/L), Na_2SeO_3 (0.5 mg/L) and Cysteine (10 mg/L) (Speece, 1996).

3.2.6 Experimental procedure

Raw CC and AHP_240 CC were used as substrates. Co-substrates, namely, Feed, Digestate, or Post-digestate, were also used in the experiments.

3.2.6.1 BMP experiments

Experiments were conducted in batch reactors with 110 mL total volume and 60 mL effective volume. Two different reactor set-ups, namely BMP_Set-1, BMP_Set-2, were carried out for this study. Raw CC with and without co-substrates were used for the first set-up (BMP_Set-1). AHP_240 CC with and without co-substrates were used for the second set-up (BMP_Set-2). Both Sets were performed with mono-digestion and co-digestion aspects. In other words, when corncob or only one of the co-substrates were used as organic source, the reactor was also categorized as mono-digestion. If both corncob (either raw or pretreated) and one of the co-substrates were used as organic sources, the reactor was categorized as co-digestion reactor.

BMP_Set-1 contained three different reactor types, namely, control (C), blank (B), and test (T) (Table 3.5). Mono-digestion blank reactors contained Raw CC or one of the co-substrates but no seed sludge; while co-digestion blank reactors involved Raw CC and one of the co-substrates together but no seed sludge. If only Raw CC was used in the blank reactors, BM was added to supply the nutrients required. Mono-digestion test reactors contain Raw CC or one of the co-substrates with seed sludge; while co-digestion test reactors involved Raw CC and one of the co-substrates together with seed sludge. BM was added only to T-Raw CC (test) reactors. There were two types of control reactors. The first type of control reactors involved seed sludge and BM as the seed control of the T-Raw CC reactors. The other type of control reactor contained only seed sludge but no BM as the seed control of the other test reactors containing no BM. All control and test reactors contained an equal amount of seed sludge (10 g/L VS). S/I (substrate to inoculum, g COD/ gVS) was set in the range of 0.5-1.5. This study focused on the set of optimum COD/N ratio in reactors. Thus, while achieving a constant seed sludge concentration (Inoculum) and predetermined COD/N ratio in the reactors, S/I ratios ranged within 0.5-1.5. These S/I ratios are in the range given for agricultural and woody wastes' digestion such as 0.5-3.0 (Chynoweth et al., 1993; Raposo et al., 2009).

Table 3.5 Content of the BMP_Set-1

| | Reactor-Name ^a | Seed Sludge | BM | Raw CC | Co-substrates | | |
|----------------|---------------------------|-------------|-----|--------|---------------|-----------|----------------|
| | | | | | Feed | Digestate | Post-Digestate |
| | Control | + | +/- | - | - | - | - |
| Mono-digestion | B-Raw CC | - | + | + | - | - | - |
| | B-Feed | - | - | - | + | - | - |
| | B-Digestate | - | - | - | - | + | - |
| | B-Post-Digestate | - | - | - | - | - | + |
| Co-digestion | B-Raw CC+Feed | - | - | + | + | - | - |
| | B-Raw CC+Digestate | - | - | + | - | + | - |
| | B-Raw CC+Post-Digestate | - | - | + | - | - | + |
| Mono-digestion | T-Raw CC | + | + | + | - | - | - |
| | T-Feed | + | - | - | + | - | - |
| | T-Digestate | + | - | - | - | + | - |
| | T-Post-Digestate | + | - | - | - | - | + |
| Co-digestion | T-Raw CC+Feed | + | - | + | + | - | - |
| | T-Raw CC+Digestate | + | - | + | - | + | - |
| | T-Raw CC+Post-Digestate | + | | + | - | - | + |

^a B and T represent blank reactors and test reactors, respectively.

BMP_Set-2 also contained three different reactor types, namely, control, blank, and test (Table 3.6). Mono-digestion blank reactors contained AHP_240 CC or one of the co-substrates but no seed sludge; while co-digestion blank reactors involved AHP_240 CC and one of the co-substrates together but no seed sludge. If only AHP_240 CC was used in the blank reactors, BM was added in the reactor to supply the nutrients required. Mono-digestion test reactors contained AHP_240 CC or one of the co-substrates with seed sludge while co-digestion test reactors involved AHP_240 CC and one of the co-substrates together with seed sludge. BM was added only to T-AHP_240 CC reactors. There were again two types of control reactors. The first type of control reactors involved seed sludge and BM as the seed control of T-AHP_240 CC reactors. The other type of control reactor contained only seed sludge but no BM as the seed control of the other test reactors containing no BM. All control and test reactors contained an equal amount of seed sludge (10 g/L VS). S/I (substrate to inoculum, g COD/ gVS) was set in the range of 0.5-1.5.

The difference between the BMP_Set-1 and BMP_Set-2 was the corncob used which are Raw CC for the BMP_Set-1 and AHP_240 CC for the BMP_Set-2. The corncob and co-substrates were added in equal amounts in BMP_Set-1 and BMP_Set-2. Thus, the range of TS amount was from 0.3% to 2% in the test reactors. For all co-digestion reactors, substrate to co-substrate ratio was calculated by using mass balance to keep it in the optimum COD/N range. The optimum COD/N ratio ranges from 50 to 143 (Speece, 1996). Accordingly, the COD/N ratio for co-digestion reactors was set as 85 (Syaichurrozi et al., 2013).

Table 3.6 Content of BMP_Set-2 Reactors

| | Reactor-Name ^a | Seed Sludge | BM | AHP_240 CC | Co-substrates | | |
|----------------|------------------------------|-------------|-----|------------|---------------|-----------|----------------|
| | | | | | Feed | Digestate | Post-Digestate |
| | Control | + | +/- | - | - | - | - |
| Mono-digestion | B- AHP_240 CC | - | + | + | - | - | - |
| | B-Feed | - | - | - | + | - | - |
| | B-Digestate | - | - | - | - | + | - |
| | B-Post-Digestate | - | - | - | - | - | + |
| Co-digestion | B- AHP_240 CC+Feed | - | - | + | + | - | - |
| | B- AHP_240 CC+Digestate | - | - | + | - | + | - |
| | B- AHP_240 CC+Post-Digestate | - | - | + | - | - | + |
| Mono-digestion | T- AHP_240 CC | + | + | + | - | - | - |
| | T-Feed | + | - | - | + | - | - |
| | T-Digestate | + | - | - | - | + | - |
| | T-Post-Digestate | + | - | - | - | - | + |
| Co-digestion | T-AHP_240 CC+Feed | + | - | + | + | - | - |
| | T-AHP_240 CC+Digestate | + | - | + | - | + | - |
| | T-AHP_240 CC+Post-Digestate | + | | + | - | - | + |

^aB and T represent blank reactors and test reactors, respectively.

The contents of each reactor type are given in Appendix B. pH value was set between 7 to 7.5, which is the optimum pH value to produce methane (Gerardi, 2003). After suitable conditions were obtained, all reactors were sealed with rubber stoppers. Then, all reactors were purged with 100 % N₂ gas for 6 minutes to create anaerobic conditions. Each reactor type was carried out in duplicate. All of the reactors were operated under anaerobic batch conditions at 35±2 °C in a temperature-controlled incubator at 120 rpm (ZHWY-2008, Incubator Shaker). Biogas production was measured periodically, and also gas composition was analysed to calculate the methane production potential of the reactors.

3.2.7 Analytical methods

Total Solids and Volatile Solids

Standard methods were used for the determination of total solids (TS) and volatile solids (VS). For TS analyses, 2540 B Total Solid Dried at 103-105 °C method was used (APHA, 2005). For the VS analyses, 2540 E Fixed and Volatile Solids Ignited at 550°C method was used (APHA, 2005).

Total Chemical Oxygen Demand and Soluble Chemical Oxygen Demand

Determination of Total Chemical Oxygen Demand (tCOD) was performed by EPA approved digestion method (for COD range of 0-1500 mg/L) and spectrophotometric detection was carried out by using a spectrophotometer (SN 05827, PC Multidirect).

For the determination of Soluble COD (sCOD) of solid samples such as corncob and digestate, 1 gram corncob was added into 20 mL deionized water, then this mixture was stirred for an hour. Lastly, mixtures were filtered through 0.45 µm pore sized filters (Millipore). For sCOD analyses of liquid samples, similar filtration procedure was applied. Filtered samples were then analysed for their COD contents.

Total Kjeldahl Nitrogen

Determination of Total Kjeldahl Nitrogen (TKN) was done with Standard Methods 4500-Norg B. Macro Kjeldahl Nitrogen method (APHA, 2005).

Total Ammonia Nitrogen

Determination of Total Ammonia Nitrogen ($TAN = NH_4^+ - N + NH_3 - N$) was done with Standard Methods 4500 NH₃ C Titrimetric Method (APHA, 2005).

Total Phosphorus

Determination of Total Phosphorus (TP) was done with Standard Methods 4500-P B.5 Persulfate Digestion Method and 4500-P E. Ascorbic Acid Method.

Cellulose, Hemicellulose, and Lignin

The determination of lignin value was done with the Klason method, which contains two parts. One of them is sulfuric acid hydrolysis, and the other part is the determination of acid-soluble lignin (Biorefinery Test Methods, 2016).

For cellulose and hemicellulose analyses, Van Soest Method was used. This method has two different procedures (and reagents), namely Neutral Detergent Fiber (NDF) and Acidic Detergent Fiber (ADF) (Van Soest, 1991). When NDF procedure is applied, NDF content is analyzed which gives the summation volume of cellulose, hemicellulose, and lignin. When ADF procedure is applied, ADF content, which is the summation of cellulose and lignin, is obtained. The value of lignin is subtracted from ADF content to calculate cellulose content of the sample. Lastly, the value of ADF content is subtracted from the NDF content to calculate hemicellulose content of the sample.

pH

One gram corncob sample was put in the 20 mL distilled water, then enclosed and stirred for 5 minutes. This mixture was kept for 1 hour to settle down suspended solids (EPA-

Method 9045D). pH values of the feed, digestate, post-digestate and inoculum were directly measured. For pH measurement, pH meter (Mettler Toledo 33111) was used. pH meter was calibrated with the buffer solutions which has 4, 7 and 10 pH values.

Biogas Measurement Device

Water displacement device was used for daily biogas production measurements. This equipment comprises of a burette that has a volume as 50 mL joined to a water reservoir that has a volume as 500 mL. The burette was attached to a needle by using latex tubing. In order to measure biogas volume in the headspace, the needle was inserted to the rubber stopper of the reactor. The volume displacement of water in the burette was recorded as the volume of biogas produced.

Composition of Biogas

Gas chromatograph (Thermo Electron Co.) was used for the determination of biogas composition. Gas chromatograph (GC) consists of two detectors that are thermal conductivity detector (TCD) and a flame ionization detector (FID). Biogas composition was measured with TCD. The columns in series (CP- Moliseve 5A and CP-Porabond Q) were used to separate as CH₄, O₂, CO₂, H₂ from injected biogas at 45 °C oven temperature. Helium is the carrier gas at 100 kPa constant pressure. The detector, inlet and oven temperatures were arranged to 80°C, 50°C and 35°C respectively. The calibration curves prepared for the gas content analysis are given in Appendix C.

Methane Yield Calculation

Experimental methane yield (Exp_{CH₄} Yield) was calculated by using Equation 3.1. The calculation of theoretical methane yield (Theo_{CH₄} Yield) is grounded on the fact that removal of 1 gram COD produces 395 mL methane at 35 °C and 1 atm. To calculate Theo_{CH₄} Yield, tCOD (or sCOD) equivalents of 1 gram VS of the solid samples was used as shown in Equation 3.2 (Raposo et al., 2011).

sCOD value was used for the calculation of minimum Theo_{CH₄} Yield (Theo_{CH₄}-sCOD Yield). tCOD value was used for the calculation of maximum Theo_{CH₄} Yield (Theo_{CH₄}-tCOD Yield).

$$Exp_{CH_4} Yield \left(\frac{mL CH_4}{g VS_{added}} \right) = \frac{mL CH_4 (experimental)}{g VS_{added}} \quad (\text{Equation 3.1})$$

$$Theo_{CH_4} Yield \left(\frac{mL CH_4}{g VS_{added}} \right) = a \left(\frac{g COD}{g VS} \right) .395 \left(\frac{mL CH_4}{g COD} \right) \quad (\text{Equation 3.2})$$

a: tCOD (or sCOD) equivalents of 1 gram VS of the solid sample studied

e.g. a is 1.52 g sCOD/g VS of corncob (as shown in Table 3.1, 3.2 and 3.3)

For the co-digestion reactors, Experimentally Estimated Methane Yield (Exp-Estimated_{CH₄} Yield) was calculated using Equation 3.3. Exp-Estimated_{CH₄} Yield was calculated by summation of Exp_{CH₄} Yield of corncob and one of the co-substrates. The aim of using this equation shows obviously potential synergetic effect of co-digestion.

$$(Exp - Estimated_{CH_4} Yield) \left(\frac{mL CH_4}{g VS_{added}} \right) = CH_4 yield_{corncob} + CH_4 yield_{co-substrate} \quad (\text{Equation 3.3})$$

The value of biodegradability based on methane yield (BD_{CH₄}) was calculated by using Equation 3.4.

$$BD_{CH_4} (\%) = (Exp_{CH_4} Yield / Theo_{CH_4} Yield) .100 \quad (\text{Equation 3.4})$$

Exp_{CH₄} Yield and Theo_{CH₄} Yield represent experimental methane yield and theoretical methane yield, respectively. For the value of Theo_{CH₄}, either sCOD or tCOD can be used.

3.3 Results and Discussion

This section is structured in two main parts, namely, results of BMP_Set-1 (unpretreated corncob, ie. Raw CC with and without co-substrate) and results of BMP_Set-2 (alkaline hydrothermal pretreated corncob (AHP_240 CC) with and without co-substrates).

3.3.1 Results of BMP_Set-1: Raw CC

The main aim of BMP_Set-1 was to investigate the methane potential of Raw CC and the effect of co-digestion on methane potential. Each reactor type was conducted in duplicate and the average values of the results are presented in tables and figures.

3.3.1.1 Methane production potential of BMP_Set-1

These BMP tests were conducted with two different aspects: mono-digestion and co-digestion. Average cumulative methane production results of mono-digestion and co-digestion reactors are given in Figure 3.2 and Figure 3.3, respectively. Control, B-Digestate (Blank Digestate) and B-Post-Digestate (Blank Post-Digestate) reactors did not produce methane during 60 days of operation period. B-Raw CC (Blank Raw CC) and B-Feed (Blank Feed) reactors, on the other hand, produced 25 and 34 mL methane, respectively (Figure 3.2).

B-Raw CC+Feed, one of the co-digestion reactors, produced nearly 100 mL methane, which was the highest amount of methane among blank reactors (Figure 3.2 and Figure 3.3). Although B-Raw CC+Feed reactor did not involve seed sludge, microbial community of Feed, which is mainly (83%) chicken manure, could contribute to methane production during digestion period. Dominant bacterial community of Feed, namely, Firmicutes, Bacteroidetes and Proteobacteria are commonly identified in anaerobic digesters (Paranhos et al., 2020). Carbohydrate, protein and complex organic matters can be hydrolysed through dominant microorganisms in the Feed. Moreover,

Proteobacteria and Firmicutes contain cross-feeding, and acetogenic microorganisms can degrade VFA (Bassani et al., 2015). Feed might have contributed by two ways, i.e. by increasing the amount of the microbial community in the anaerobic digestion and by raising the extent of hydrolysis in the reactor, which might accelerate the Raw CC degradation. Other blank co-digestion reactors, B-Raw CC+Digestate and B-Raw CC+Post-Digestate reactors, produced 43 and 52 mL methane, respectively. Microbial community of Digestate and Post-Digestate were similar to that of the Feed. The main difference of the microbial community in Digestate and/or Post-Digestate is their being already acclimated to another lignocellulosic material, poppy, which is 17% of the Feed by mass. Despite of no gas production in mono-digestion of these co-substrates, their co-digestion with Raw CC resulted methane production which is even higher than that of sole Raw CC. This might be attributed to the synergistic effects of Digestate and Post-Digestate.

In mono-digestion test reactors, T-Raw CC and T-Feed reactors produced 73 and 61 mL methane; however, T-Digestate and T-Post-Digestate reactors did not produce significant methane (Figure 3.2). On the other hand, in co-digestion reactors, the cumulative methane production of T-Raw CC+Feed, T-Raw CC+Digestate and T-Raw CC+Post-Digestate, were 182 mL, 138 mL and 130 mL, respectively (Figure 3.4). When the results of mono-digestion and co-digestion are compared, co-digestion seems to create a positive synergistic effect on anaerobic digestion. Balancing of COD/N ratio and increasing the diversity of anaerobic microorganisms may have resulted in positive synergistic effects. It should be noted that test co-digestion reactors contain microorganisms of both seed sludge and the co-substrates.

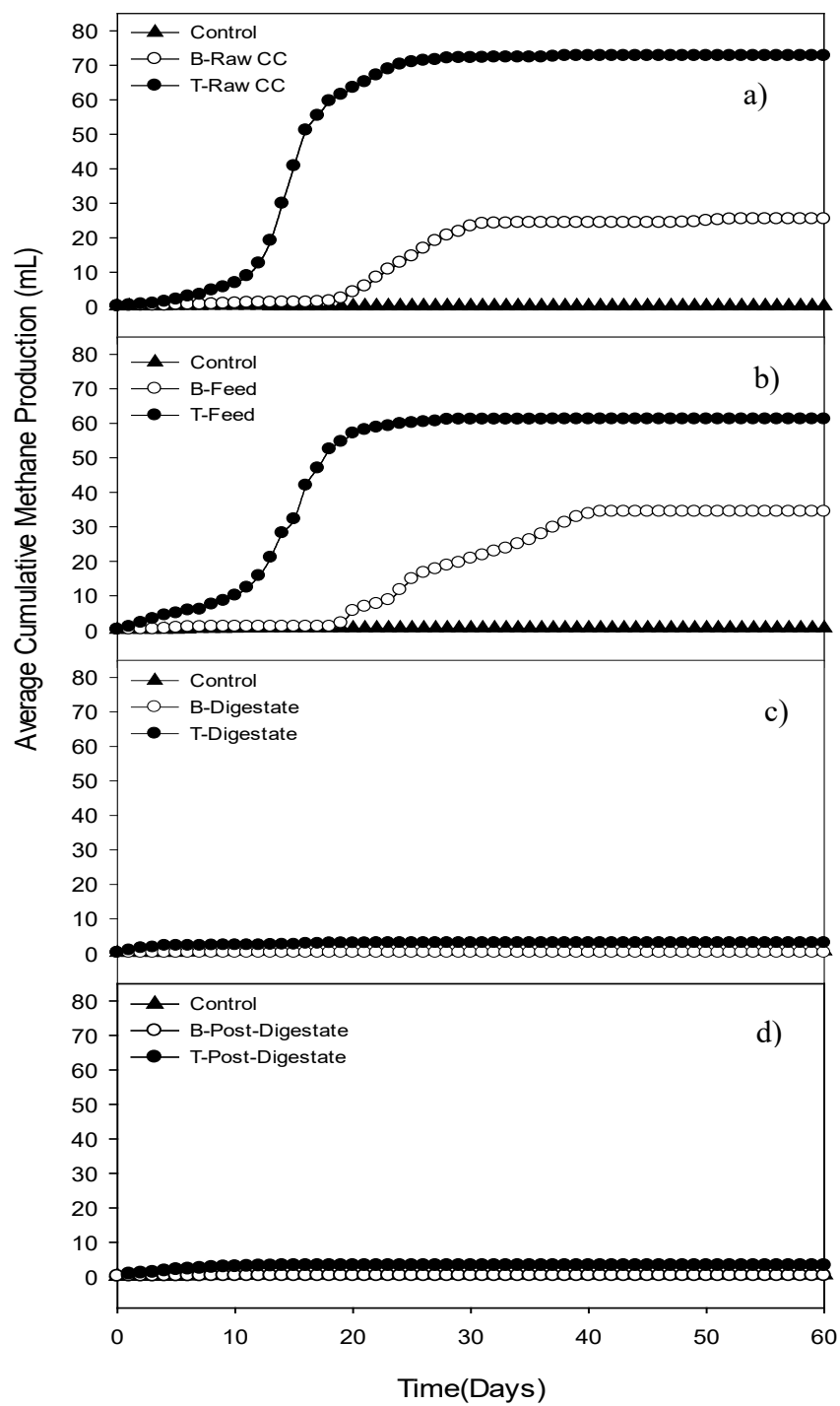


Figure 3.2 Average cumulative methane production of a) Raw CC, b) Feed, c) Digestate and d) Post-Digestate mono-digestion reactors

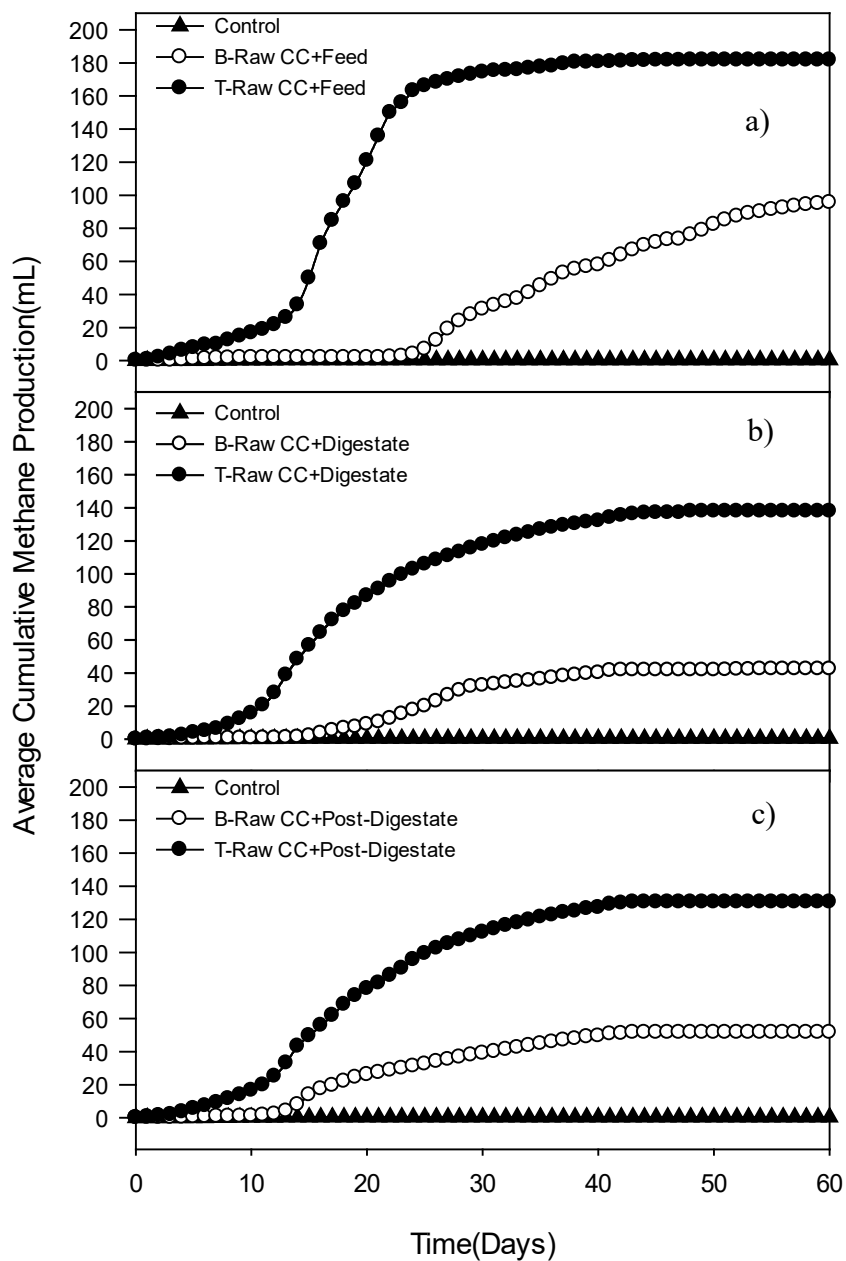


Figure 3.3 Average cumulative methane production of a) Raw CC+Feed, b) Raw CC+Digestate and c) Raw CC+Post-Digestate co-digestion reactors

Exp_{CH₄} Yield, the ratio of mL produced methane to gram added VS in the reactor, is a fundamental unit for observation of performance of the anaerobic digestion. Therefore, methane yield values are calculated and shown in Table 3.7. Theo_{CH₄} Yield for the lignocellulosic biomass was calculated for both sCOD and tCOD; therefore, methane yield of soluble and insoluble components could be compared. sCOD value generally represents the concentration of readily biodegradable material to be used by anaerobic microorganisms. In anaerobic digestion, first, soluble organic material is degraded then, the form of insoluble organic matter can be converted to a soluble form by some microbial community. For co-digestion reactors, the Exp-Estimated_{CH₄} Yield values were calculated to observe the synergistic effect of co-digestion, if any.

One of the mono-digestion reactors is Raw CC, whose Exp_{CH₄} Yield was 792% higher than the Theo_{CH₄}-sCOD Yield. However, it constituted only 18% of the Theo_{CH₄}-tCOD Yield, as Raw CC is not an easily degradable biomass. This result is comparable to the literature data (Li et al., 2015; Blandino et al., 2016). A higher experimental methane yield compared to Theo_{CH₄}-sCOD Yield, indicates the increase in solubilisation of substrate during digestion.

Table 3.7 Exp_{CH4} Yield, Theo_{CH4} Yield and Exp-Estimated_{CH4} Yield of BMP_Set-1

| Reactors | Exp _{CH4} Yield ^a | | Theo _{CH4} Yield ^a | | BD _{CH4} ^c | | Exp-Estimated _{CH4} Yield ^{a,b,d} | | Exp _{CH4} Yield / Exp-Estimated _{CH4} Yield ^{c,d} | |
|-----------------------|---------------------------------------|------------|--|-----------------|--------------------------------|-----------------|---|------------|--|------|
| | Blank | Test | Min. | Max. | Min. | Max. | Blank | Test | Blank | Test |
| | | | (based on sCOD) | (based on tCOD) | (based on sCOD) | (based on tCOD) | | | | |
| Raw CC | 36 | 103 | 13 | 584 | 7.92 | 0.18 | NA | NA | NA | NA |
| Feed | 109 | 195 | 151 | 941 | 1.29 | 0.21 | NA | NA | NA | NA |
| Digestate | 0 | 29 | 131 | 1011 | 0.22 | 0.03 | NA | NA | NA | NA |
| Post-Digestate | 0 | 30 | 108 | 921 | 0.28 | 0.03 | NA | NA | NA | NA |
| Raw CC+Feed | 94 | 179 | 56 | 694 | 3.20 | 0.26 | 145 | 298 | 0.65 | 0.60 |
| Raw CC+Digestate | 54 | 172 | 28 | 635 | 6.14 | 0.27 | 36 | 132 | 1.50 | 1.30 |
| Raw CC+Post-Digestate | 64 | 162 | 25 | 626 | 6.48 | 0.26 | 36 | 133 | 1.78 | 1.22 |

^a Unit of all values is mL CH₄/g VS_{added}

^b It was calculated by summation of methane yields of corncob and one of the co-substrates

^c BD_{CH4} represents biodegradability based on methane yield (BD_{CH4}). BD_{CH4}= Exp_{CH4} Yield/ Theo_{CH4} Yield

^d N.A: Not applicable

Both B-Feed and T-Feed reactors had the highest methane yields, among all blank and test reactors, respectively (Table 3.7). BD_{CH_4} of T-Feed reactors was 129% calculated by using $Theo_{CH_4-sCOD}$ Yield but it was 21% by using the $Theo_{CH_4-tCOD}$ Yield, because soluble organic portion of Feed was low. T-Digestate and T-Post-Digestate reactors achieved low methane yields, which are 29 and 30 mL CH_4/g VS, respectively (Table 3.7). Although their $Theo_{CH_4-sCOD}$ Yield values were high, Exp_{CH_4} Yield was the lowest among test reactors. A possible explanation for this might be that VS% of Digestate and Post-Digestate was lower than that of Feed; thus, there may not enough VS concentration to the use of microbial community. It should be noted that the amount of co-substrates to be added were determined based on the optimum COD/N ratio arrangement for the co-digestion reactors. Then, Digestate and Post-Digestate were added in the same amount in their both mono digestion and co-digestion reactors.

In co-digestion reactors, methane yield of B- Raw CC+Feed and T-Raw CC+Feed reactors were 94 and 179 mL CH_4/g VS, respectively. When methane yield of Raw CC (103 mL CH_4/g VS) and T-Raw CC+Feed are compared, co-digestion resulted in a higher methane yield than that of sole Raw CC, as expected. However, methane yields of B-Feed and T-Feed reactors were higher than that of Raw CC+Feed reactors. This can be explained with microbial community of Feed, which was negatively affected from Raw CC. The comparison of Exp_{CH_4} Yield and $Exp-Estimated_{CH_4}$ Yield is important for the observation of synergistic effect of co-digestion. Exp_{CH_4} Yield of B-Raw CC+Feed and T-Raw CC+Feed reactors constituted 65 and 60% of the $Exp-Estimated_{CH_4}$ Yield. These results showed that dominant microorganisms in Feed may need to adapt to Raw CC. It was also calculated that Exp_{CH_4} Yield of T-Raw CC+Feed reactors was 326% times higher than the $Theo_{CH_4-sCOD}$ Yield, as seen in BD_{CH_4} calculation, this indicates that the soluble portion of organic content can be enhanced by digestion, and anaerobic digestion was achieved effectively. Also, BD_{CH_4} of T-Raw CC+Feed reactors was determined as 26%. This result is expected because all organic

content of lignocellulosic biomass cannot be degraded by microorganisms due to its structural challenges.

Exp_{CH₄} Yields of Raw CC+Digestate reactors, namely blank and test reactors, reached 54 and 172 mL CH₄/g VS, respectively. Exp_{CH₄} Yields of blank reactors, which was 150% higher than Exp-Estimated_{CH₄} Yield, showed that microbial community of Digestate created a significant positive effect on digestion of Raw CC. These results can be explained by two ways as the microbial content of Digestate and the balanced COD/N ratio via co-digestion. Some of the microbial content of the Digestate such as Flavobacteriaceae, Xanthomonadaceae, Carnobacteriaceae, Bacillaceae and Alcaligenaceae are capable of converting lignocellulosic material to fermentable sugar (Paranhos et al., 2020). Furthermore, Flavobacteriaceae excretes α -L-arabinofuranosidase enzyme, which contributes to the degradation of hemicellulose content (Paranhos et al., 2020). Exp_{CH₄} Yield of T-Raw CC+Digestate reactor was 130% and 614% times higher than Exp-Estimated_{CH₄} Yield and Theo_{CH₄}-sCOD Yield, respectively. A possible explanation for these results might be similarly that conversion of fermented sugar from cellulose and hemicellulose in Raw CC could be easily done by those specific microbial content in the Digestate. Moreover, as it was previously mentioned, the microorganisms in the Digestate obtained from digester of Afyon Energy Plant, which is operated with Feed (chicken manure+poppy(17%)), has been already acclimated to a lignocellulosic biomass like poppy. Similarly, Bassani et al. (2015) mentioned in their study that some of the microorganisms of chicken manure are able to degrade lignocellulosic component.

Exp_{CH₄} Yields of B- Raw CC+Post-Digestate and T-Raw CC+Post-Digestate reactors were 64 and 162 mL CH₄ /g VS, respectively (Table 3.7). Exp_{CH₄} Yields of B-Raw CC+Post-Digestate and T-Raw CC+Post-Digestate reactors were 178% and 122% higher than Exp-Estimated_{CH₄} Yield, respectively. Both blank and test results showed that Raw CC and Post-Digestate have a significant synergistic effect on co-digestion.

These results were related to the microbial content of Post-Digestate and the balanced COD/N ratio via co-digestion. Microbial community of Post-Digestate was expected to be similar with the Digestate; however, the difference between those was the adaptation period with lignocellulosic biomass, like poppy. Digestate was obtained from a digester with 20 days of SRT while Post-Digestate was obtained from the following digester, thus have a longer SRT of 30 days in total. Therefore, Exp_{CH_4} Yields of B-Raw CC+Post-Digestate reactors was higher than that of B-Raw CC+ Digestate reactors.

Figure 3.4 shows the cumulative methane yields of Raw CC and co-digestion test reactors. Methane yield of Raw CC increased by 74%, 67% and 57% via co-digestion with Feed, Digestate and Post-Digestate, respectively.

The studies on corncob and corn waste uses various S/I ratios, which are in the range of 0.5 to 2.5 (Amon et al., 2007; Seppälä, et al., 2012; Li, et al., 2015; Menardo et al., 2015; Pérez-Rodríguez, et al., 2016). The arrangement of COD/N ratio was focused on in this study. Thus while achieving that COD/N ratio as well as same inoculum concentration in the reactors, S/I ratio was used in the range of 0.5 to 1.5. The observation of different lag phase in Figure 3.2, 3.3 and 3.4 can be related to the use of various S/I ratio in each reactor. This result was expected because different S/I ratios affect the kinetics of methane production. Moreover, methane yield do not relate to S/I ratio in BMP test (Raposo, et al., 2012).

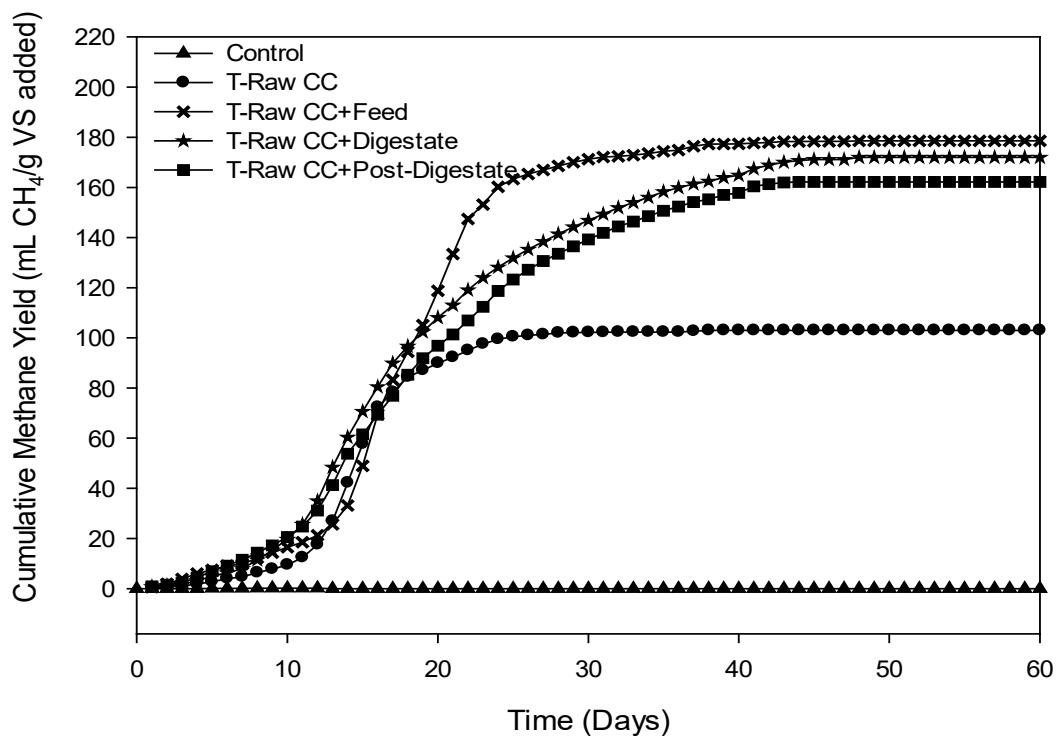


Figure 3.4 Average methane yields of Raw CC mono- and co-digestion reactors

Literature studies of Raw CC and various corn wastes are given in Table 3.8. In research studies, Raw CC generally was mixed with different types of corn waste in mono-digestion and co-digestion. There is one study with Raw CC and pig manure in co-digestion whose methane yield reached 139 mL CH₄/g VS (Li et al., 2015). As seen in Table 3.8, the methane yields obtained in this study are higher than obtained by Li et al. (2015). Menardo et al. (2014) inoculated Raw CC was inoculated with seed sludge taken from an agricultural biogas plant at 40 °C; thus, methane yield of their study reached higher than this study due to both the use of inoculum acclimated with agricultural waste and higher temperature application compared to this study. The aim of Seppa et al. (2012)'s study was to determine the best harvesting time and types of corn waste to achieve high methane yield. Therefore, corns were grown in a specific area by using different harvesting periods.

Table 3.8 BMP of Raw CC studies in the literature up to date

| Substrate | Co-substrate | Range of max. methane yield (mL CH ₄ /g VS) ^b | Ref ^c |
|------------------------------|-------------------|---|------------------|
| Raw CC | Pig manure | 139 | 1 |
| Raw CC w/ whole corn(50-50%) | - | 146±8 | 2 |
| Raw CC | - | 206 ^c | 3 |
| Various types of corn waste | - | 251-419 ^d | 4 |
| Raw CC | - | 222 | 5 |
| Raw CC | - | 103 | This study |
| Raw CC | Feed ^a | 179 | This study |
| Raw CC | Digestate | 172 | This study |
| Raw CC | Post-Digestate | 162 | This study |

^a Feed is mixture of chicken manure and poppy (17%)

^b Most of the studies were conducted in batch reactors at 35±2 °C

^c This study was performed at 40 °C

^d This study was conducted with different types waste of corn.

^e References; ¹ Li et al. (2015), ² Blandino et al. (2016), ³ Menardo et al. (2014), ⁴ Seppa et al. (2012), ⁵ Ali et al. (2018)

3.3.1.2 Characterization study of BMP_Set-1

The reactors were sampled at the initial and final days of the incubation period for TS, VS, TKN, TAN, TP and sCOD analyses. The detailed results of these analyses are given in Appendix D.

The initial and final pH value of the reactors were in the range of 7.1 to 8.1, (Table 3.9) which is in the optimum pH range (6.5-8.2) (Speece, 1996). The final pH value was higher than the initial pH value because alkalinity value increased due to degradation of protein. These results show that anaerobic reactors worked efficiently (Labatut and Gooch, 2014).

Total Kjeldahl Nitrogen (TKN) contains organic nitrogen and TAN (NH_4+NH_3). Organic nitrogen is converted to TAN during anaerobic digestion. Then, TAN is used by microorganisms to supply nitrogen requirements. The use of TAN content is observed as reduction of TKN value (Ghyselbrecht et al., 2017). TKN removal values were calculated as 35-55 % in the anaerobic reactors. These results are slightly higher than the data found in the literature as 10-50% (Surra et al., 2018).

TAN concentration gives information about the methanogenic activity in the reactors. There is an equilibrium between ammonium and ammonia. When the pH increases above 9.3, dominant component is determined as ammonia in this equilibrium (Speece, 1996). The removal efficiency of TAN and the initial and final concentrations of TAN are given in Table 3.9. TAN concentration increased at the end of anaerobic digestion because of the fact that decomposition of organic nitrogen leads to the release of ammonium during anaerobic digestion. There are different limits for ammonia inhibition, which are 150 mg/L (McCarty and McKenney, 1961), 345 mg/L (Ripley et al., 1985) and 1500 mg/L (Gerardi, 2003; Speece, 1996). If there is an ammonia inhibition in the anaerobic reactors, reduction in methane production can be observed also due to pH increase. The highest TAN concentration, 856 mg/L TAN, was observed in the T-Feed reactor at the end of the digestion (Table 3.9). In addition, the test reactors containing Raw CC had final TAN concentrations less than 512 mg/L and pH levels were below 8, usually in the range of 7.3-7.7. These values are above the inhibition levels with respect to McCarty and McKenney (1961) and Ripley et al. (1985) but much lower than the inhibition limits set by Gerardi (2003) and Speece (1996). As mentioned previously, the pH value in the reactors is a significant parameter for the indication of ammonia formation (Speece, 1996). pH value was measured below 8.1 and usually in the range of 7.3-7.7 in Raw CC containing test reactors; therefore, ammonia is not expected to be the dominant one and no inhibition due to TAN accumulation was expected.

The VS removal associates with the methane yield in anaerobic digestion (Nasir et al., 2012). The VS removal efficiencies of mono-digestion blank and test reactors are in the ranges of 2-21% and 36-50%, respectively (Table 3.10). VS removal efficiencies of co-digestion blank and test reactors are in the range of 12-38% and 36-59%, respectively. These results are consistent with those of Li et al. (2013), who found the VS removal efficiencies of corn stover and chicken manure mixture as $59 \pm 5\%$. Another result that is found in the literature was that Raw CC and Organic Fraction of Municipal Solid Waste (OFMSW) in co-digestion reactor achieved 65.5% VS removal (Surra et al., 2018). The removal of VS was in the range of 13-46% for various types of lignocellulosic biomass (Zhu et al., 2010; Romano et al., 2009). The VS removal efficiency was found as 17 to 41 % by using the anaerobic digestion effluent of cattle slurry (Hawkes et al., 1984; Rico et al., 2011).

Table 3.9 Average values of TAN, TKN and pH in BMP_Set-1

| Reactors | TAN change in the test reactors | | | TKN change in the test reactors | | | pH change in the test reactors | |
|-----------------------|---------------------------------|-----------------|----------------|---------------------------------|-----------------|----------------|--------------------------------|---------|
| | Initial (mg/L) | Final (mg/L) | Removal (%) | Initial (mg/L) | Final (mg/L) | Removal (%) | Initial | Final |
| Raw CC | 362 | 419 | -16 | 580 | 375 | 35 | 7.1 | 7.9-8.0 |
| Feed | 439 | 856 | -95 | 840 | 386 | 54 | 7.2-7.3 | 7.9 |
| Digestate | 484 | 544 | -12 | 702 | 431 | 39 | 7.3-7.4 | 8.0-8.1 |
| Post-Digestate | 321 | 352 | -10 | 690 | 328 | 52 | 7.1-7.2 | 8.0-8.1 |
| Raw CC+Feed | 397 | 512 | -29 | 848 | 536 | 37 | 7.1 | 7.6-7.7 |
| Raw CC+Digestate | 472 | 419 | 11 | 711 | 323 | 55 | 7.3 | 7.3-7.3 |
| Raw CC+Post-Digestate | 403 | 472 | -17 | 698 | 433 | 38 | 7.3 | 7.4-7.5 |

Table 3.10 The change of sCOD and VS concentrations in BMP_Set-1 reactors

| Reactors | Average VS removal in the reactors (%) | | Average sCOD removal in the reactors (%) | |
|-----------------------|--|------|--|------|
| | Blank | Test | Blank | Test |
| Raw CC | 9 | 50 | -154 | -157 |
| Feed | 10 | 52 | 44 | 60 |
| Digestate | 2 | 36 | -9 | -69 |
| Post-Digestate | 21 | 36 | 12 | -25 |
| Raw CC+Feed | 23 | 53 | -53 | 58 |
| Raw CC+Digestate | 38 | 59 | -117 | -12 |
| Raw CC+Post-Digestate | 12 | 51 | -306 | -15 |

Another critical parameter is sCOD concentration in the reactors after incubation period. sCOD values generally increase at the end of digestion because of increased solubilization of organic content during digestion. As seen in Table 3.10, effluent sCOD values increased in most of the reactors which is indicated by negative removal values. On the contrary, effluent sCOD values decreased in the T-Feed and T-Raw CC+Feed reactors. This reveals the consumption of the produced soluble organic matter more compared to other reactors, as also observed from higher CH₄ yields obtained (Table 3.7).

3.3.2 Results of BMP_Set-2: AHP_240 CC

To enhance the methane production of lignocellulosic biomass, pretreatment methods are mostly used. In this study, in order to improve the BMP of corncob, alkaline hydrothermal pretreatment at 240 °C (AHP_240) method was applied. The produced pretreated corncob (AHP_240 CC) was analyzed for its content and compared to the analyses results of Raw CC. Characterization results of Raw CC and AHP_240 CC are given in Table 3.11.

Table 3.11 Differences between characterization of Raw CC and AHP_240 CC

| Parameter | Raw CC | AHP_240 CC |
|-------------------|--------------|-------------------|
| pH | 5.26 | 13.5 ^a |
| TS (g/g) | 0.89 ±0.00 | 0.95±0.00 |
| TS (%) | 89.70±0.14 | 95.40±0.08 |
| VS (g/g) | 0.88 ±0.00 | 0.70 ±0.06 |
| VS % (% of TS) | 98.42± 0.15 | 73.01± 5.33 |
| tCOD (mg/g) | 1340 ±12 | 1163 ± 14 |
| sCOD (mg/L) | 385±13 | 26350±250 |
| sCOD(mg/g) | 31±0.5 | 527±5 |
| sCOD/tCOD | 0.02 | 0.45 |
| TKN (mg /g) | 4.2±0.38 | 4.03±0.38 |
| TKN (%) | 0.40±0.04 | 0.40±0.40 |
| TAN (mg/g) | 0.0047 ±0.00 | 0.28 ±0.00 |
| TAN/TKN(%) | 0.11 | 6.95 |
| TP (mg/g) | 0.260± 0.00 | 0.12± 0.07 |
| Cellulose (%) | 31.50±1.84 | 4.74± 1.99 |
| Hemicellulose (%) | 35.54 ±0.65 | 17.06± 1.26 |
| Lignin (%) | 21.27±0.31 | 12.15±0.38 |

^a Before adjustment of pH to neutral level

TS value of AHP_240 CC (0.95 g/g) was higher than Raw CC (0.89 g/g). The usage of alkali solution during pretreatment might contribute to the increase in solid portion of AHP_240 CC. On the contrary, VS content of the AHP_240 CC was 0.70 g/g, which was lower than that of Raw CC (0.88 g/g). Organic solid content of Raw CC, that is its VS content, might be disrupted by pretreatment effect due to application of alkali solution and high temperature leading to VS reduction. sCOD and sCOD/tCOD ratio are important parameters to observe the efficiency of pretreatment methods. After pretreatment, the sCOD value increased 17 times compared to Raw CC. sCOD/tCOD

increased by 95% after pretreatment application. This result shows that alkaline hydrothermal pretreatment increased the solubility of Raw CC. These results support the main aim of pretreatment methods, which is to increase the solubility of the organic content for easy degradation of microorganism (Kumari and Singh, 2018).

The change in cellulose, hemicellulose, and lignin is also vital to observe efficiency of pretreatment application on Raw CC. When the amount of these components decreases after pretreatment with the increase in sCOD, the main aim of pretreatment and increased solubility of biomass, can be fulfilled (Surra et al., 2018). The removal of cellulose, hemicellulose and lignin were calculated as 85%, 52% and 43%, respectively, and these results are comparable to the data found in literature. Surra et al. (2018) found that removal of cellulose, hemicellulose and lignin was 63.4%, 61.9%, 68.6% by using peroxide at pH 9.8 for 3 days, respectively. After alkali pretreatment method, the amount of hemicellulose and cellulose in Raw CC decreased from 42.97 to 12.74% and from 33.7 to 24.95%, respectively in another study (Pan-in and Sukasem, 2017). Shah and Taassubum (2018) measured only lignin removal from Raw CC, which was 57.8%, by using $\text{Ca}(\text{OH})_2$ for 30 days. However, the use of Raw CC with alkaline hydrothermal pretreatment has not been investigated, so there is not exactly comparable data found in literature. Alkaline hydrothermal pretreatment is applied in two steps which are alkali and hydrothermal pretreatment. The basic aim of alkali pretreatment is the disruption of lignin component to improve accessibility of cellulose and hemicellulose (Paul et al., 2018). Hydrothermal pretreatment contributes by increasing solubility and accessibility of cellulose and hemicellulose (Blandino et al., 2016). The soluble organic content is directly related to soluble form of cellulose, hemicellulose and lignin (Bayard et al., 2016). Identification of soluble component is a crucial point to observe negative or positive effect on microbial degradation (Paranhos et al., 2020). The soluble portion is categorized into two parts, which are monomeric sugar and phenolic compounds. The increase in monomeric sugar is useful for the anaerobic microorganisms; on the contrary,

the increase in phenolic compounds can damage microbial community during digestion (Wang et al., 2018; Surra et al., 2018).

As seen in Table 3.11, TAN value increased in the AHP_240 CC compared to Raw CC. This also indicates the pretreatment affect because Raw CC was destructed during pretreatment. Thus, the ammonium was released from organic nitrogen easily, and TAN value increased, as expected.

3.3.2.1 Methane production potential of BMP_Set-2

The aim of BMP_Set-2 was to investigate the effect of pretreatment on BMP of Raw CC. Thus, the same amounts of corncob and co-substrates were used as in the BMP_Set-1 to compare these two sets.

Methane production results of mono-digestion and co-digestion reactors in BMP_Set-2 are given in Figure 3.5 and Figure 3.6, respectively. Control, B-AHP_240 CC, B-Digestate and B-Post-Digestate reactors did not produce methane during 60 days of the incubation period. Yet, B-Feed reactors produced 40 mL methane (Figure 3.5). One of the co-digestion reactors, that is B-AHP_240 CC+Feed reactors, produced nearly 112 mL methane, which was the highest amount of the methane produced among the blank reactors (Figure 3.5 and Figure 3.6). Although B-AHP_240 CC+Feed reactors contained higher soluble content compared to B-Raw CC+Feed reactors (Table 3.11), methane production of B-AHP_240 CC+Feed was slightly higher than B-Raw CC+Feed (Figure 3.3; Figure 3.6). The soluble component of B-AHP_240 CC+Feed reactors might not have been used by microorganisms due to its potential inhibitory composition. It should be noted that B-AHP_240 CC+Feed and B-Raw CC+Feed reactors contain equal and same microbial community originated from Feed. Microbial communities of Feed, such as Firmicutes, Bacteroidetes and Proteobacteria, contribute to the hydrolyse carbohydrate, protein and complicated organic matters (Paranhos et al., 2020). Thus,

hydrolysis step, which is rate-limiting step, may have been accelerated. Other co-digestion reactors were B-AHP_240 CC+Digestate and B-AHP_240 CC+Post-Digestate reactors, which produced 65 and 55 mL methane, respectively. As mentioned previously, microbial community of Digestate and Post-Digestate were similar to that of the Feed, yet acclimated to poppy fed to the digesters of Afyon Energy Plant. Thus, despite being blank reactors, these reactors also produced methane.

In mono-digestion test reactors, methane production of T-AHP_240 CC and T-Feed reactors were 64 and 76 mL methane, but T-Digestate and T-Post-Digestate reactors produced 4 and 8 mL of methane, respectively (Figure 3.5). The acclimation period in T-AHP_240 CC reactors lasted 15 days till the cumulative methane amount reached 10 mL. Yet, T-Raw CC reactors had an acclimation period of only 11 days (Figure 3.2). This was unexpected because hydrolysis rate of pretreated corncob was expected to increase due to higher soluble content, so start time of methane production should decrease. This unexpected result may partly be explained by the effect of formed inhibitory by-product such as furfuran, after pretreatment and excess sodium ion coming from alkali solution (Singh et al., 2017). In the co-digestion reactors, the methane production of T-AHP_240 CC+Feed, T-AHP_240 CC+Digestate and T-AHP_240 CC+Post-Digestate reactors were 188 mL, 94 mL and 114 mL, respectively (Figure 3.6).

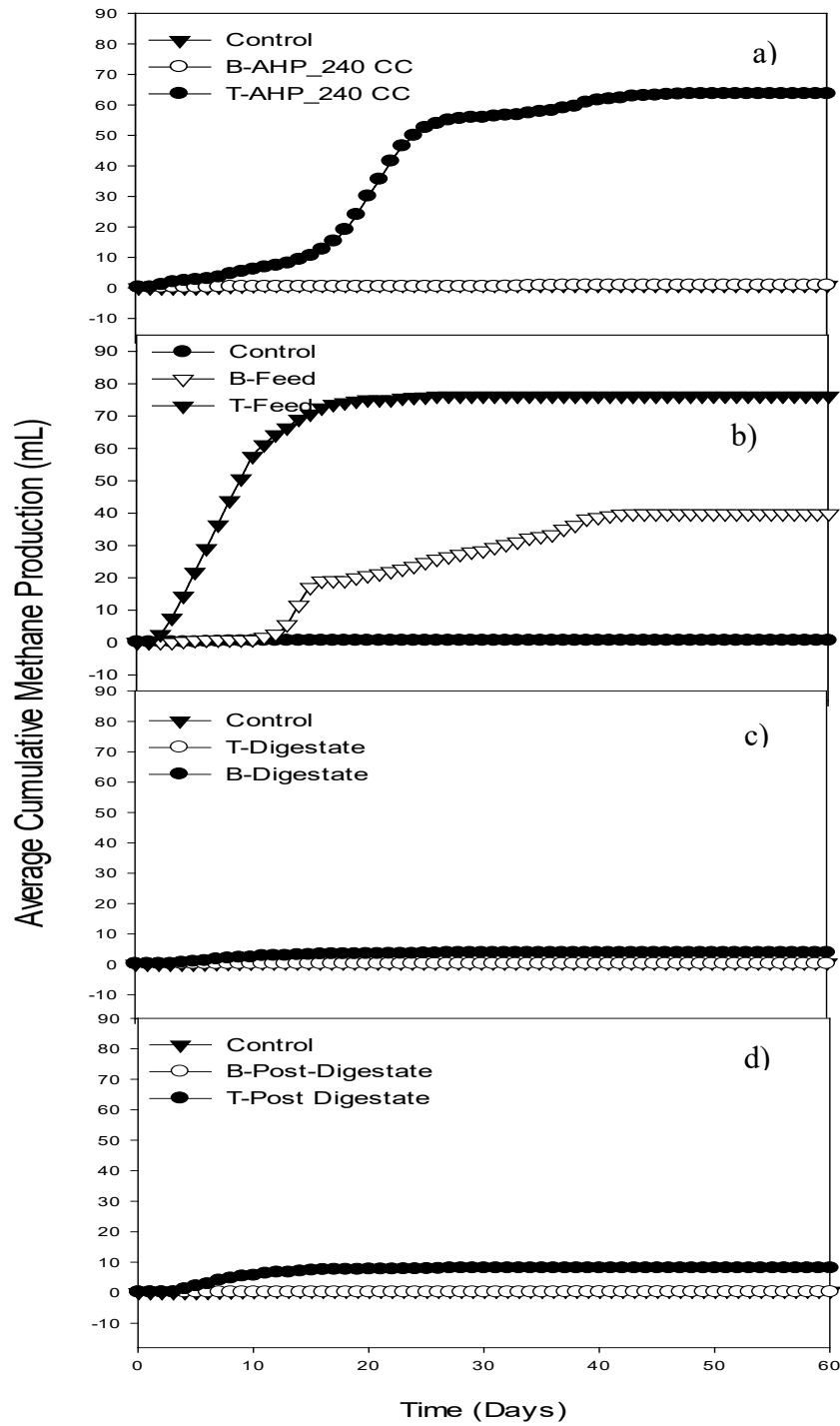


Figure 3.5 Average cumulative methane production of the a) AHP_240 CC, b) Feed, c) Digestate and d) Post-Digestate mono-digestion reactors of BMP_Set-2

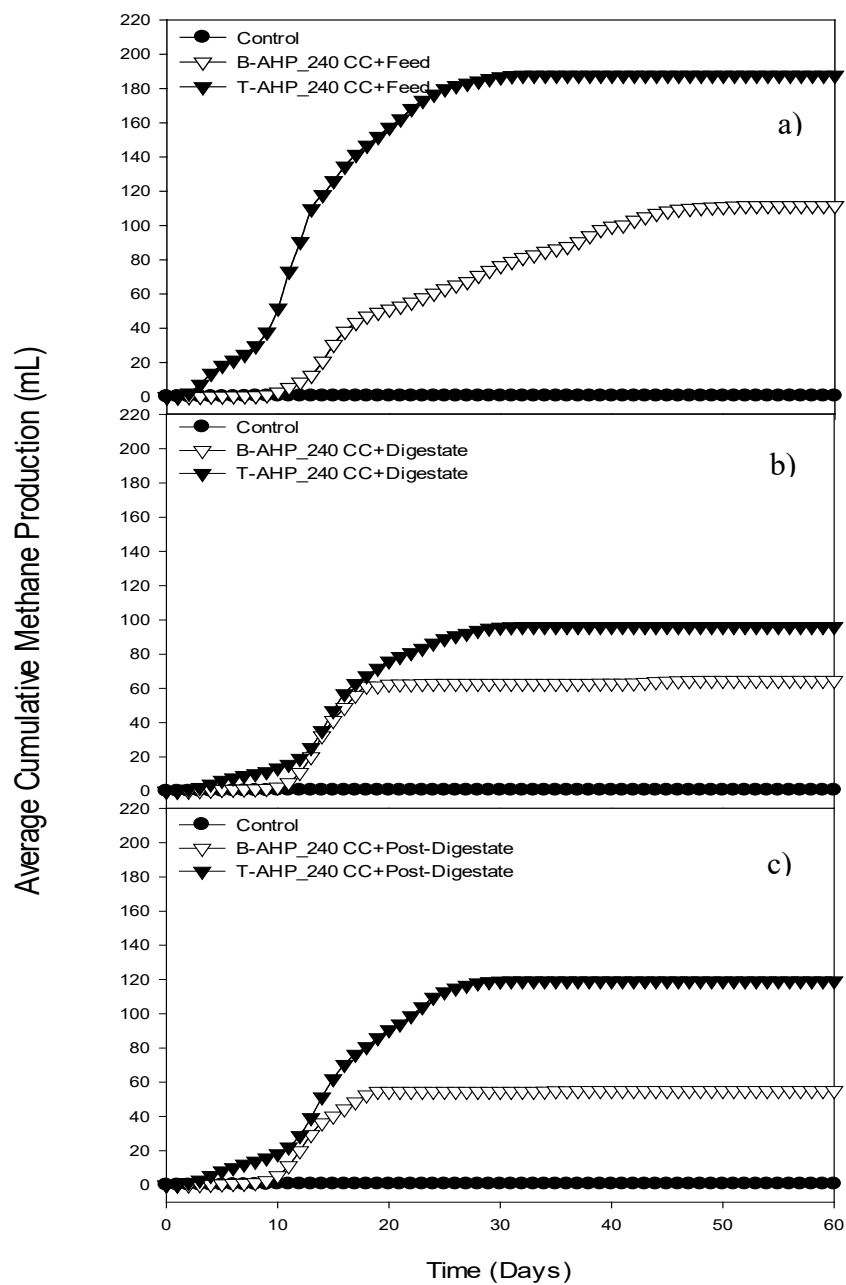


Figure 3.6 Average cumulative methane production of the a) AHP_240 CC+Feed, b) AHP_240 CC+Digestate and c) AHP_240 CC+Post-Digestate co-digestion reactors of BMP_Set-2

When the results of mono-digestion and co-digestion are compared, it is seen that co-digestion created positive synergistic effects on anaerobic digestion. As mentioned previously in BMP_Set-1, co-digestion had a positive synergistic effect due to the balanced COD/N ratio and increased microbial community with addition of chicken manure co-substrates, especially with Digestate and Post-Digestate. Although hydrolysis rate of Raw CC seems to decrease after alkaline hydrothermal pretreatment, co-substrate affected the digestion of AHP_240 CC positively. Thus, methane production of AHP_240 CC was increased in the range of 49%-198%, thanks to the co-substrate (Figure 3.5a; Figure 3.6). Methane production of co-digestion test reactors followed low rate until 10th day; however, methane production rate increased between 10th day and 28th day of the incubation. On the other hand, methane production of T-AHP_240 CC reactors was low until 15th day and slightly increased between 15th day and 30th day of the incubation. These different results between AHP_240 CC and co-digestion test reactors obviously show that co-substrate contributed by increasing the hydrolysis rate and decreasing the potential effects of inhibitory by-products produced during pretreatment which is discussed below.

Methane yield values are given in Table 3.12. The Exp_{CH₄} Yield of AHP_240 CC and co-digestion reactors were determined in the range of 114-216 mL CH₄ /g VS in BMP_Set-2. This is close to the methane yield of alkaline hydrothermal pretreated miscanthus at (175°C) found as 135 mL CH₄ /g VS where 8% NaOH was used for pretreatment (Xue et al., 2020).

One of the mono-digestion reactors is AHP_240 CC, of which Exp_{CH₄} Yield was only 38% of the Theo_{CH₄}-sCOD Yield. Even though AHP_240 CC included more soluble organic content than Raw CC, AHP_240 method did not significantly improve the BMP of Raw CC. As also mentioned for methane production comparison, this was a surprising result because soluble portion is a readily biodegradable compound which is used in methane production, but apparently not all soluble portion increased after AHP_240

method was used by microbial community. The soluble portion is generally classified into two types: monomeric sugars, which are glucose and glucan coming from cellulose, and phenolic compounds, which are furfural, arabinose, xylose, xylooligosaccharides, formic acid etc coming from lignin and hemicellulose (Chen et al, 2014). Less sCOD consumption may be explained by the fact that phenolic compounds, which could be formed during pretreatment, may be inhibitory to microbial community producing methane (Wang et al., 2018; Surra et al., 2018). One of the inhibitory by-products is formic acid which is rapidly released due to the increase in pretreatment temperature. In this regard, methanogens activity could be repressed, and resulting in decreased methane yield (Wang et al., 2018). Hereby, AHP_240 method could have not made a significant positive effect on methane yield of Raw CC despite the increased soluble organic content after pretreatment

Both B- Feed and T-Feed reactors produced the highest methane yields, among all blank and test reactors, respectively (Table 3.12). Exp_{CH_4} Yield of T-Feed reactor was 161% higher than the $\text{Theo}_{\text{CH}_4\text{-sCOD}}$ Yield, while it constituted 26 % of the $\text{Theo}_{\text{CH}_4\text{-tCOD}}$ Yield. Although Feed was used in same amounts for both BMP_Set-1 and BMP_Set-2, the Exp_{CH_4} Yield of Feed was found to be different for each set as 195 mL CH_4 /g VS and 243 mL CH_4 /g VS, respectively. This was first attributed to the different methanogenic activities of the seed sludge which was obtained from Ankara Municipal Wastewater Treatment Plant at different times of the year. Thus, Specific Methanogenic Activity (SMA) test was done to test the methanogenic activity of seed sludge by using acetic acid, which can be degraded by microbial community easily. The results of SMA test of seed sludges used in BMP_Set-1 and BMP_Set-2 revealed that BMPs obtained were almost 40% and 60% of the theoretical methane production, respectively, which was not that different considering that the seed sludge of BMP_Set-1 was stored more than 90 days in the laboratory at room temperature before SMA test. Therefore, the difference in T-Feed and B-Feed reactors of BMP_Set-1 and BMP_Set-2 was attributed

to the different, maybe more biodegradable, organic content of the Feed used in BMP_Set-2.

T-Digestate and T-Post-Digestate test reactors achieved low methane yields, which is 38 and 79 mL CH₄/g VS, respectively (Table 3.12). Different methane yields were also unexpected, because characteristic results of Digestate and Post-Digestate were similar with each other (Table 3.3).

In co-digestion reactors, B-AHP_240 CC+Feed and T-AHP_240 CC+Feed reactors achieved methane yield of 128 and 216 mL CH₄/g VS, respectively. T-AHP_240 CC+Feed reactors achieved 20% more methane yield compared to the T-Raw CC+Feed reactors (Table 3.7 and Table 3.13). The observed increase in methane yield could be attributed higher soluble content of AHP_240 CC and the resistance of microbial community of Feed against the potential inhibitory by-products produced during pretreatment. Due to the fact that the gastrointestinal system of chicken has some enzymes to utilize carbon rich content nutrition, microbial content of Feed (consist of 83% chicken manure) might adapt to some soluble inhibitory by-products (Paranhos et al., 2020). It should be noted that although the Exp_{CH₄} Yield of B-AHP_240 CC+Feed reactors was the same as Exp-Estimated_{CH₄} Yield which might also verify that the microbial content of Feed is well adapted and has tolerance to the AHP_240 CC content. On the other hand, Exp_{CH₄} Yield of T- AHP_240 CC+Feed constituted 61% of Exp-Estimated_{CH₄} Yield. Apparently, seed sludge used was not positively affected by the co-digestion of AHP_240 CC and Feed.

Table 3.12 Exp_{CH₄} Yield, Theo_{CH₄} Yield and Exp-Estimated_{CH₄} Yield of BMP_Set-2

| Reactors | Exp _{CH₄} Yield ^a | | Theo _{CH₄} Yield ^a | | BD _{CH₄} ^c | | Exp-Estimated _{CH₄} Yield ^{a,b,d} | | Exp _{CH₄} Yield / Exp-Estimated _{CH₄} Yield ^{c,d} | |
|----------------------------|--|------------|---|-----------------|---|-----------------|--|------------|--|------|
| | Blank | Test | Min. | Max. | Min. | Max. | Blank | Test | Blank | Test |
| | | | (based on sCOD) | (based on tCOD) | (based on sCOD) | (based on tCOD) | | | | |
| AHP_240 CC | 1 | 114 | 297 | 584 | 0.38 | 0.20 | N.A | N.A | N.A | N.A |
| Feed | 127 | 243 | 151 | 941 | 1.61 | 0.26 | N.A | N.A | N.A | N.A |
| Digestate | 0 | 38 | 131 | 1011 | 0.29 | 0.04 | N.A | N.A | N.A | N.A |
| Post-Digestate | 0 | 79 | 108 | 921 | 0.73 | 0.09 | N.A | N.A | N.A | N.A |
| AHP_240 CC +Feed | 128 | 216 | 245 | 694 | 0.88 | 0.31 | 128 | 357 | 1.00 | 0.61 |
| AHP_240 CC +Digestate | 99 | 147 | 273 | 635 | 0.54 | 0.23 | 1 | 152 | 99.0 | 0.97 |
| AHP_240 CC +Post-Digestate | 84 | 181 | 269 | 626 | 0.67 | 0.29 | 1 | 193 | 84.0 | 0.94 |

^a Unit of all values is mL CH₄/g VS_{added}^b It was calculated by summation of methane yields of corncob and one of the co-substrates^c BD_{CH₄} represents biodegradability based on methane yield (BD_{CH₄}). BD_{CH₄}= Exp_{CH₄} Yield/ Theo_{CH₄} Yield^d N.A: Not applicable

Exp_{CH₄} Yield of B-AHP_240 CC+Digestate and Exp_{CH₄} Yield of T-AHP_240 CC+Digestate reactors reached 99 and 147 mL CH₄/g VS, respectively. The result of blank reactors, which was 99 times higher than Exp-Estimated_{CH₄} Yield, showed that microbial community of Digestate created positive effect on digestion of AHP_240 CC. A possible explanation for this might be that some of the microbial community in Digestate could be adapted to the inhibitory by-products, produced during pretreatment such as arabinose, xylose etc due to the fact that some of the community can convert lignocellulosic material to soluble components including phenolic compounds and monomeric sugar (Paranhos et al., 2020). This could be verified by the ratio of Exp_{CH₄} Yield to Exp-Estimated_{CH₄} Yield for B-AHP_240 CC+Digestate and B-Raw CC+Digestate reactors determined as 99 and 1.5, respectively.

Exp_{CH₄} Yield of B-AHP_240 CC+Post-Digestate and T-AHP_240 CC+Post-Digestate were 84 and 181 mL CH₄/g VS, respectively (Table 3.12). Exp_{CH₄} Yield of B-AHP_240 CC+Post-Digestate reactors was 84 times higher than the Exp-Estimated_{CH₄} Yield. Also, Exp_{CH₄} Yield of T-AHP_240 CC+Post-Digestate reactors constituted 94% of the Exp-Estimated_{CH₄} Yield. AHP_240 CC and Post-Digestate mixture have a positive synergistic effect on co-digestion which was also observed for AHP_240 CC+Digestate.

The primary aim of pretreatment was to increase soluble portion of raw corncob, so digestion period can decrease due to the increase in hydrolysis rate, which is rate-limiting step for lignocellulosic biomass (Chen et al., 2014). If the alkaline hydrothermal pretreatment method increase the hydrolysis rate and methane production, the time required for 90% of the total methane produced is expected to decrease when compared to results of BMP_Set-1. However, the production time of 90% of the total methane in T-AHP_240 CC reactors was calculated as 33 days; this value was higher than that of T-Raw CC reactors (Table 3.13). As mentioned previously, this result might be related to the inhibitory by-products produced during AHP_240 CC application such as furfural,

arabinose, xylose, xylooligosaccharides, formic acid etc. In mono-digestion, AHP_240 CC reactors could not overcome their inhibition effect. Yet, the production time of 90% of the total methane in co-digestion reactors of BMP_Set-2 is much lower than that of BMP_Set-1 reactors. The co-digestion reactors in BMP_Set-2 produced 90% of the cumulative methane production in 23-24 days (Table 3.13). This reveals that co-digestion with Feed, Digestate and Post-Digestate decreases the incubation period for AHP_240 CC, most probably due to the tolerance and adaptation of co-substrates to the products produced during pretreatment.

Table 3.13 The production time required for 90% of the total methane produced in test reactors

| Reactors ^a | The time of 90% of the total methane production (day) | |
|-----------------------|---|-----------|
| | BMP_Set-1 | BMP_Set-2 |
| T-CC | 22 | 33 |
| T-CC+Feed | 28 | 23 |
| T-CC+Digestate | 37 | 24 |
| T-CC+Post-Digestate | 37 | 24 |

^a CC refers to Raw CC for BMP_Set-1 while AHP_240 CC for BMP_Set-2

Lastly, both co-digestion and AHP_240 method applications were investigated to observe their effects on corncob in BMP_Set-1 and BMP_Set-2. The methane yields of BMP_Set-1 test reactors were divided by the Exp_{CH_4} Yield of T-Raw CC to determine the co-digestion effect on Raw CC and to observe which reactors achieved higher methane yields than Raw CC. The same calculation was applied on BMP_Set-2 but this time by dividing with the Exp_{CH_4} Yield of T-AHP_240 CC. Although T-Feed reactors reached almost 2 times higher methane yields of Raw CC or AHP_240 CC in both sets, T-Raw CC+Feed and T-AHP_240 CC+Feed reactors did not achieve double methane

yield of Raw CC or AHP_240 CC (Figure 3.7). This revealed that Feed and corncob, either raw or pretreated do not have a significant synergistic effect and Feed should be used in the mono-digestion reactor. Although T-Digestate and T-Post-Digestate reactors did not reach adequate methane yield for both BMP_Set-1 and BMP_Set-2, their co-digestion reactors achieved almost 1.5 times higher methane yield of Raw CC or AHP_240 CC. Therefore, Digestate and Post-Digestate can be used as a co-substrates. Furthermore, no significant difference was observed between co-digestion reactors of BMP_Set-1 and BMP_Set-2 (Figure 3.7). Thus, these results show that AHP_240 method does not seem to be an effective pretreatment application for corncob to improve its methane production potential.

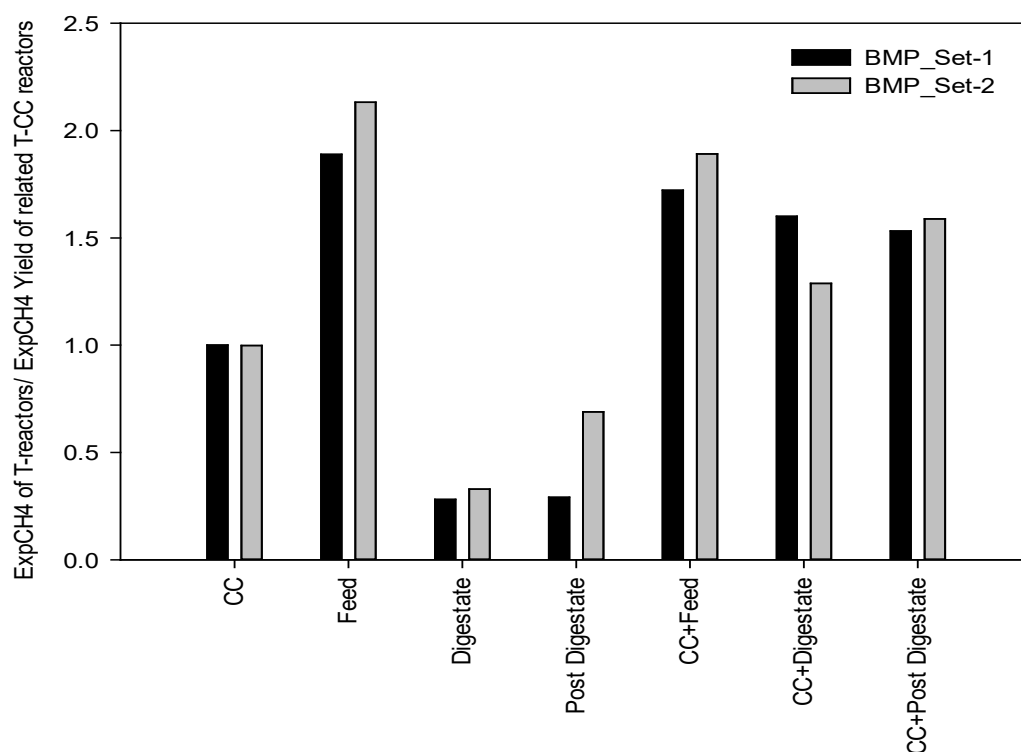


Figure 3.7 The comparison between methane yields of BMP_Set-1 and BMP_Set-2 test reactors

3.3.2.2 Characterization study of BMP_Set-2

The initial and final values of TS, VS, TAN, tCOD and sCOD were measured. The detailed results of these analyses are given in Appendix E.

The initial and final pH value was observed between 7.0 to 8.2 (Table 3.14). TAN initial value, final value, and removal efficiency are also given in Table 3.14. At the end of the anaerobic digestion, TAN value increased due to the release of ammonium during organic nitrogen decomposition via digestion. Although the range of effluent TAN values in BMP_Set-1 were 352-856 mg/L (Table 3.9), the range of effluent TAN values in BMP_Set-2 was 632-899 mg/L (Table 3.14). Higher solubilization was achieved due to alkaline hydrothermal pretreatment in BMP_Set-2. Therefore, influent TAN concentration was already higher in BMP_Set-2. The highest TAN concentration of 899 mg/L was in T-AHP_240 CC+Feed reactors (Table 3.14). This value is above the ammonia inhibition limit of 150 mg/L according to McCarty and McKenney (1961) and the inhibition limit of 345 mg/L Ripley et al. (1985), yet, it is below the ammonia inhibition level of 1500 mg/L respect to Gerardi (2003) and Speece (1996). As mentioned previously, the pH value in the reactors is a crucial point for the indication of ammonia formation (Speece, 1996). pH value was measured below 8.2; thus, ammonia inhibition was not expected in these reactors.

Table 3.14 The change of TAN and pH in BMP_Set-2

| Reactors | TAN change in the test reactors | | | pH change in the test reactors | |
|---------------------------|---------------------------------|--------------|-------------|--------------------------------|-------|
| | Initial (mg/L) | Final (mg/L) | Removal (%) | Initial | Final |
| AHP_240 CC | 496±2 | 632±16 | -21 | 7.4 | 8.1 |
| Feed | 387±1 | 856±14 | -121 | 7.1 | 8.1 |
| Digestate | 196±1 | 861±17 | -339 | 7.0 | 8.1 |
| Post-Digestate | 547±3 | 803±12 | -47 | 7.3 | 8.2 |
| AHP_240 CC+Feed | 418±1 | 899±32 | -115 | 7.3 | 7.9 |
| AHP_240 CC+ Digestate | 444±1 | 856±14 | 93 | 7.2 | 7.9 |
| AHP_240 CC+Post Digestate | 573±2 | 725±9 | -27 | 7.3 | 7.8 |

The VS removal efficiencies of mono-digestion blank and test reactors were in the range of 11-41% and 23-31%, respectively. Besides, the VS removal efficiencies of co-digestion blank and test reactors were in the range of 19-60%, 32-49%, respectively (Table 3.15). In the literature, corncob was pretreated with H₂O₂ at pH 9.8 for 3 days and co-digestion reactor, which was conducted with pretreated corncob and (OFMSW,) achieved removal of VS in the range of 58-63% (Surra et al., 2018). Surra et al. (2018) is the only that determined the removal values of VS of pretreated corncob in digester. Thus, the removal values of VS were comparable with the study of Surra et al. (2018).

When BMP_Set-1 and BMP_Set-2 were compared, the significant differences were found for T-AHP_240 CC and co-digestion reactors. The sCOD removal values of T-AHP_240 CC reactors was determined as (-32)% (Table 3.15) while the sCOD removal value of T-Raw CC reactors reached (-154)% Table(3.10). The removal of sCOD in blank and test reactors of BMP_Set-2 was observed in the range of 22 to (-72) % and (-20) to (-152)% , respectively (Table 3.15).

Table 3.15 The change of sCOD and VS concentrations in BMP_Set-2 reactors

| Reactors | VS removal in the reactor (%) | | sCOD removal in the reactor (%) | |
|----------------------------|-------------------------------|------|---------------------------------|------|
| | Blank | Test | Blank | Test |
| AHP_240 CC | 11 | 23 | -72 | -32 |
| Feed | 38 | 27 | 22 | -93 |
| Digestate | 30 | 31 | -68 | -156 |
| Post-Digestate | 41 | 31 | -15 | -142 |
| AHP_240 CC +Feed | 60 | 49 | -58 | -20 |
| AHP_240 CC +Digestate | 32 | 32 | -1 | -46 |
| AHP_240 CC +Post-Digestate | 19 | 45 | -31 | -31 |

3.4 Conclusions

This study aims to investigate the BMP of corncob and its enhancement by using co-digestion and pretreatment approaches. The results of this study were summarized as follows;

In BMP_Set-1:

- Exp_{CH₄} Yield of T-Raw CC reached 103 mL CH₄/g VS, and it constituted only 18% of the Theo_{CH₄}-tCOD. These results show that Raw CC was not easily degraded via digestion.
- Exp_{CH₄} Yield of T-Raw CC+Digestate and T-Raw CC+Post-Digestate were 172 mL CH₄/g VS and 162 mL CH₄/g VS, respectively. Yet, Exp_{CH₄} Yield of T-Raw CC, Exp_{CH₄} Yield of T-Digestate and Exp_{CH₄} Yield of T-Post-Digestate were 103 mL CH₄/g VS, 29 mL CH₄/g VS and 30 mL CH₄/g VS, respectively. It should be noted that co-digestion with Digestate and Post-Digestate seems to create a positive synergistic effect on anaerobic digestion. The reason of this synergistic effect may be attributed to the balanced COD/N ratio and increased the diversity of anaerobic microorganisms.

In BMP_Set-2:

- Although sCOD of AHP_240 CC increased 17 times compared to Raw CC, Exp_{CH_4} Yield of T-AHP_240 CC reactors were low being only 10% greater than T-Raw CC reactors (114 mL $\text{CH}_4/\text{g VS}$). This can be explained by the fact that soluble portion may include inhibitory by-products produced during pretreatment, and activity of methanogens may be repressed by inhibitory by-product, resulting in reduced methane yield. Moreover, non-biodegradable sCOD can cause low methane yield.
- Exp_{CH_4} Yield of T-AHP_240 CC+Digestate reactor was 147 mL $\text{CH}_4/\text{g VS}$, while Exp_{CH_4} Yield of T-AHP_240 CC and T-Digestate reactors was 114 mL $\text{CH}_4/\text{g VS}$ and 38 mL $\text{CH}_4/\text{g VS}$, respectively. Exp_{CH_4} Yield of T-AHP_240 CC reactor increased almost 30% with co-digestion (Digestate).
- The ratios of Exp_{CH_4} Yield / $\text{Exp-Estimated}_{\text{CH}_4}$ Yield of T-AHP_240 CC+Digestate and T-Raw CC+Digestate were 0.97 and 1.3, respectively. Pretreatment seems to have slightly negative effect on methane yield, which was also observed for the reactor with Post-Digestate as the co-substrate.

Digestate used in this study was obtained from a digester in Afyon Energy Plant with 20 days SRT while Post-Digestate was obtained from the following digester, thus have a longer SRT of 30 days in total. Yet, this study meanwhile revealed that B-Digestate and B-Post-Digestate did not produce significant amount of methane. Thus, this result showed that the second digester, which is fed with Digestate, did not produce methane and thus is not meaningful to use in Afyon Energy Plant. Digestate or Post-Digestate should be mixed with a lignocellulosic biomass such as corncob in the second digester (of the Plant) in order to both effectively produce residual methane and use bacterial consortiums of Digestate or Post-Digestate.

CHAPTER 4

EFFECT OF HYDROTHERMAL, ALKALINE AND ALKALINE HYDROTHERMAL PRETREATMENT ON BIOCHEMICAL METHANE POTENTIAL OF CORNCOB

4.1 Introduction

The number of biogas plants in the world is increasing day by day. According to World Biogas Association report, there were 17783 biogas plants with 10.5 GW installed capacity in Europe in 2017. Germany, which is the leader among the European Union countries, has 10971 biogas plants. Moreover, China has numerous large-scale anaerobic digester plants, which were 6972 in 2015. There were 2200 biogas plants with 977 MW installed capacity in the USA in 2017 (World Biogas Association, 2019). On the other hand, it is reported by BEPA (2020) that Turkey has only 199 biogas plants with 1.238 MW installed capacity in 2020.

In Turkey, energy potential of total wastes equals 395.378 GWh, and 75% of this energy is constituted by vegetal waste in 2020 (“BEPA,” 2020). Agricultural residual is an important component in the vegetal waste, and it plays a key role in biomass energy. Most of this agricultural waste is constituted by crop wastes. However, these wastes are mostly used for burning or mulching on the field (Dell’Omo and Spena, 2020). Corn waste accounts for 33% of the crop waste in Turkey, which is a type of agricultural residue as biomass resources in Turkey (Başçetinçelik et al., 2005).

This study focuses on the use of non-food corn waste to reduce storage problem and to increase the use of corn waste as energy source. Non-food corn waste is the corncob, which is the centre of the corn ear, and used for heating in small villages

due to its high calorific value of 18.4 MJ/kg (Başçetinçelik et al., 2005). Corncob is also a lignocellulosic biomass, whose content is in the range of 34% to 41% cellulose, 32% to 36% hemicellulose and 6% to 19% lignin (Kumari et al., 2018). Advantages of corncob is that it contains the highest hemicellulose content among crop wastes, and this component plays an important role in the biomass energy (Blandino et al., 2016).

Despite having such a desired property, corncob is used in limited number of studies to produce methane, owing to its low biodegradability. The soluble component of corncob is poor. This means that easily biodegradable fraction of corncob is low. The reason of low biodegradability is mainly recalcitrant component in lignocellulosic structure (Paul and Dutta, 2018). The recalcitrance component generally refers to lignin, which covers the cellulose and hemicellulose for protection against microorganisms (Xu et al., 2019). Limited studies on corncob to produce methane still shows an important degradable portion for anaerobic digestion (Wang et al., 2018).

Pretreatment method can overcome the disadvantages of using corncob by increasing its biodegradability in order to produce methane effectively. There are various types of pretreatment methods, namely, physical, chemical, thermophysical, thermochemical and biological, to enhance the anaerobic degradability of corncob (Kumari and Singh, 2018). The selection of pretreatment method is a critical point for effective production of methane. Thus, to determine the components of substrate is crucial, and suitable pretreatment methods should be selected with respect to the substrate structure. For instance, alkaline pretreatment and hydrothermal pretreatment are mainly used to destroy lignin and to increase solubility and accessibility of cellulose and hemicellulose, respectively (Chandra et al., 2012; Kumari et al., 2018). Corncob has high lignin and hemicellulose content, so alkaline, hydrothermal and alkaline hydrothermal pretreatment methods can be used and optimized to determine an efficient method.

Alkaline pretreatment is a widely used method due to disruption of lignin in order to access cellulose and hemicellulose via anaerobic microorganisms easily (Chandra et al., 2012). Besides, cellulose and hemicellulose degradation can be observed. It is reported in a study (Monlau et al., 2015) that, the cellulose, hemicellulose and lignin in sunflower stalks were removed by 4%, 24 %, and 22 %, respectively, at optimum pretreatment condition (4 gram NaOH/100 g TS, 55 °C). The soluble portion increases after alkaline pretreatment. However, methane yield may not decrease only due to formation of undesirable by-products, which are mainly furfural, 5-hydroxymethyl furfural (5-HMF) and lignin derivatives, but also non-biodegradable sCOD. Besides, other soluble components might be organic acid, acetic acid, formic acid, monomeric and oligomeric sugar, which can be detected after pretreatment application (van der Pol et al., 2016; Wang et al., 2018).

Another significant pretreatment method is hydrothermal pretreatment, which contributes to the solubilization of cellulose and hemicellulose (Kumari and Singh, 2018). Different from other pretreatment methods, hydrothermal pretreatment is accepted as a green technology due to the use of only water for pretreatment (Wang et al., 2018). There is production of monomeric sugar such as glucose and glucan, as well as inhibitory by-products such as furfural, arabinose, xylose, xylooligosaccharides, formic acid etc in soluble portion after hydrothermal pretreatment (Kabel et al., 2007). Monomeric sugar can be degraded by microbial community. It is reported by Chandra et al. (2012a) that, when hydrothermal pretreatment was applied on rice straw at 200 °C for 10 minutes, methane production increased 222 % compared to raw rice straw. On the other hand, soluble portion might include inhibitory by-product can damage methanogenetic microorganisms. Wang et al. (2018)'s study was investigated that biogas yield was increased by 3 % after hydrothermal pretreatment (at 180 °C for 15 minutes) compared to raw rice straw.

Alkaline hydrothermal pretreatment is expected to be a more effective method than both alkaline and hydrothermal methods due to its success in both the disruption of

lignin and the increased solubilization of cellulose and hemicellulose. Yet, as mentioned previously, inhibitory-by products can still be formed in soluble content; thus, methane production may decrease. In previous study of this thesis work (Chapter-3), it was concluded that alkaline hydrothermal pretreatment at 240 °C did not improve the BMP of corncob effectively (only 10% increase) despite the 1605% increase in soluble portion potentially due to the formation of inhibitory by-products and non-biodegradable sCOD. The temperature and alkaline application might be the factors, leading to the inhibitory effects. Therefore, optimization of the temperature and sole application of hydrothermal and alkaline pretreatment, together with their combined effect at lower temperatures is still worth investigating.

There are several studies on the use of alkaline pretreatment method (Paul et al., 2018; Monlau et al., 2015); however, there is no study on hydrothermal and alkaline hydrothermal treatment with corncob in the literature. Considering the individual advantages of hydrothermal and alkaline pretreatment methods, its combination, that is alkaline hydrothermal pretreatment, is expected to be a more effective method than both alkaline and hydrothermal methods. This study, therefore, focuses on the comparison of different pretreatment methods and their optimization by using various temperature values, namely 150, 180 and 210 °C. In this context, the aim of this study is to investigate the effect of hydrothermal, alkaline and alkaline hydrothermal pretreatment on methane potential of corncob and to determine optimum pretreatment and temperature conditions leading to the highest BMP. Moreover, the relation of the increase in solubility and the change in methane yield were observed. In addition, the change of structural component of corncob, mainly cellulose, hemicellulose and lignin, was observed and the effects of structural change on methane production were assessed.

4.2 Materials and Methods

This section informs about characteristics of hydrothermal pretreated corncob at 150 °C (HP_150 CC), hydrothermal pretreated corncob at 180 °C (HP_180 CC) and

hydrothermal pretreated corncob at 210 °C (HP_210 CC); alkaline hydrothermal pretreated corncob at 150 °C (AHP_150 CC), alkaline hydrothermal pretreated corncob at 180 °C (AHP_180 CC) and alkaline hydrothermal pretreated corncob at 210 °C (AHP_210 CC); alkaline pretreated corncob (AP CC). The experimental procedures are explained and analytical methods are given.

4.2.1 Characteristic of the pretreated corncob

In this study, three different pretreatment methods were used, namely alkaline pretreatment (AP), hydrothermal pretreatment (HP) and alkaline hydrothermal pretreatment (AHP), which is the combination of alkali and hydrothermal pretreatment. Then, three different temperatures, namely 150, 180 and 210 °C, were used for hydrothermal and alkaline hydrothermal pretreatment methods. Figure 4.1 presents an overview of pretreatment methods. As mentioned in Chapter-3, the AHP_240 method could not improve to methane yield in the reactors. This was attributed to the inhibitory by-products which may inhibit the methane producers mostly (Wang et al., 2018). Therefore, in this part of the study, in Chapter-4, temperatures lower than 240 °C and known to have not resulted in inhibition in hydrothermal pretreatment were used (Paul et al., 2018).

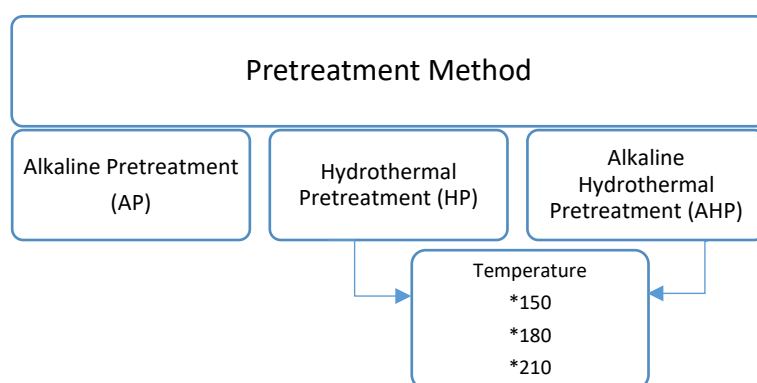


Figure 4.1 Schematic representation of pretreatment methods applied

Alkaline Hydrothermal Pretreatment (AHP): For alkaline hydrothermal pretreatment, 1 gram of corncob, which was dried and screened from 2.34 mm, was solubilized in 20 mL 5% (w/v) NaOH solution for 3 hours. After the soaking period, sample of this mixture was heated in the oven at 150 °C, 180 °C or 210 °C for 30 minutes. Lastly, the pH value was adjusted at a neutral level by using HCl. These samples were further dried at nearly 65 °C in the oven.

Hydrothermal Pretreatment (HP): 1 gram corncob, with particle size smaller than 2.34 mm, was mixed with 20 mL distilled water to arrange the liquid to solid ratio as 20 mL/g. The same liquid to solid ratio was used in all pretreatment method, which was assessed in a previous preliminary study (Appendix A). Then, the samples of this mixture were heated at 150 °C, 180 °C or 210°C by using the oven for 30 minutes. After these applications, the pH value was adjusted to neutral level. These samples were later dried at 65 °C in the oven.

Alkaline Pretreatment (AP): 1 gram corncob, with particle size smaller than 2.34 mm, was soaked in 20 mL NaOH solution (5%) for 3 hours. After that, pH value was adjusted to almost 7 by using HCl solution. This sample was further dried at 65 °C in the oven.

4.2.2 Inoculum (seed sludge)

Seed sludge was taken from anaerobic digester in the Ankara Central Municipal Wastewater Treatment Plant. The seed sludge was stored at room temperature in a plastic container. The characterisation of inoculum was given in Table 3.4 (Chapter-3, Section 3.2.4).

4.2.3 Basal medium (BM)

Basal medium ingredients and concentrations obtained in the reactors (shown in parentheses) are as follows: NH₄Cl (1200 mg/L), NaHCO₃ (6000 mg/L),

MgSO₄·7H₂O (400 mg/L), KCl (400 mg/L), (NH₄)₂HPO₄ (80 mg/L), Na₂S·9H₂O (300 mg/L), FeCl₂·4H₂O (40 mg/L), CaCl₂·2H₂O (50 mg/L), KI (10 mg/L), CoCl₂·6H₂O (10 mg/L), (NaPO₃)₆ (10 mg/L), NH₄VO₃ (0.5 mg/L), MnCl₂·4H₂O (0.5 mg/L), ZnCl₂ (0.5 mg/L), CuCl₂ ·2H₂O (0.5 mg/L), Na₂MoO₄·2H₂O (0.5 mg/L), AlCl₃·6H₂O (0.5 mg/L), NiCl₂·6H₂O (0.5 mg/L), H₃BO₃ (0.5 mg/L), Na₂WO₄·2H₂O (0.5 mg/L), Na₂SeO₃ (0.5 mg/L) and Cysteine (10 mg/L) (Speece, 1996).

4.2.4 Experimental procedure

BMP experiments were conducted in batch reactors with total volume of 60 mL and effective volume of 30 mL. There were three different reactor types, namely, control (C), blank (B), and test (T) reactors. Blank and test reactors were conducted with BM and one of the seven pretreated corncob types, which were AHP_150 CC, AHP_180 CC, AHP_210 CC, HP_150 CC, HP_180 CC, HP_210 CC and AP CC. S/I (substrate to inoculum, g COD/ gVS) was set as 1 (Chynoweth et al., 1993). The control reactors involved seed sludge and BM as the seed control of the test reactors. All control and test reactors contained an equal amount of seed sludge (22.8 g/L VS). Blank reactors did not contain any seed sludge. The substrates, namely HP CCs, AP CC and AHP CCs, were added in equal amounts in all test reactors, and TS amount of test reactors was almost 2.8%.

pH value of the reactor contents was set between 7 and 7.5, the optimum pH range value to produce methane (Gerardi, 2003). After suitable conditions were obtained, all reactors were sealed with rubber stoppers. Then, all reactors were purged with 100 % N₂ gas for 6 minutes to create anaerobic conditions. Each reactor type was carried out in duplicate. All of the reactors were operated under anaerobic batch conditions at 35±2 °C in a temperature-controlled incubator (ZHWY-2008, Incubator Shaker) at 120 rpm. Biogas production was measured periodically, and also gas composition was analysed to calculate the methane production potential of the reactors.

4.2.5 Analytical methods

Standard Methods (APHA, 2005) were used for determination of TKN, TP, TS, VS, TAN. tCOD was determined by EPA approved digestion method (for COD range of 0-1500 mg/L), heat was applied by Aqualytic AL 38 heater for 2 hours, then spectrophotometric detection was carried out by using the spectrophotometer (SN 05827, PC Multidirect). For sCOD measurement, firstly the sample was filtered with 0.45 μm pore-sized filters (Millipore); then the steps for tCOD were followed. For pH measurement, pH meter (Mettler Toledo 33111) was used. pH meter was calibrated with the buffer solutions which has 4, 7 and 10 pH amounts.

Lignin value was measured by using the Klason method (Biorefinery Test Methods, 2016). Neutral Detergent Fiber (NDF) and Acidic Detergent Fiber (ADF) methods were used for determination of the amounts of cellulose and hemicellulose (Van Soest, 1991). All measurements were performed in triplicate.

Water displacement device was used to measure the volume of biogas produced in each reactor daily. The measurement of the gas composition was done by Gas chromatograph (GC) device to specify methane amount in the biogas. Thus, whenever there is gas production, gas composition was also measured by GC at the same day. GC consists of two detectors that are thermal conductivity detector (TCD) and a flame ionization detector (FID). Biogas composition was measured with TCD. The columns in series (CP- Moliseve 5A and CP-Porabond Q) were used to separate as CH_4 , O_2 , CO_2 , H_2 from injected biogas at 45 °C oven temperature. Helium was the carrier gas at 100 kPa constant pressure. The detector, inlet and oven temperatures were arranged to 80°C, 50°C and 35°C respectively. The calibration curves prepared for the gas content analysis are given in Appendix C.

Severity factor

Empirical equation defines severity factor, which includes time and temperature, most important parameter of the hydrothermal pretreatment (Equation 4.1) (Hosseini and Shah, 2009). The severity factor is the impact force of the hydrothermal pretreatment method and is represented as $\log R_0$. The severity factor can be calculated by Equation 4.1. The optimum severity factor is in the range of 3 to 4.5 (Tingyue Gu, 2013). If the severity factor of the hydrothermal pretreatment is below the optimum range, solubilization efficiency can be lower than the expected value. However, if the severity factor is higher than the optimum range, inhibitory by-products can form during the solvation of component (Zakaria et al., 2014). Thus, severity factor calculation is important for hydrothermal pretreatment.

$$\log R_0 = \log \left(t \cdot \exp\left(\frac{T-100}{14.75}\right) \right) \quad \text{Equation 4.1}$$

In equation 4.1, t is the reaction time (min), and T is the hydrolysis temperature ($^{\circ}\text{C}$).

Theoretical methane yield ($\text{Theo}_{\text{CH}_4}$ Yield)

The calculation of theoretical methane yield ($\text{Theo}_{\text{CH}_4}$ Yield) is done based on the fact that removal of 1 gram COD produces 395 mL methane at 35 $^{\circ}\text{C}$ and 1 atm (Equation 4.2). In Equation 4.2, tCOD equivalents of 1 gram VS of the solid samples was used for the determination of $\text{Theo}_{\text{CH}_4}$ Yield.

$$\text{Theo}_{\text{CH}_4} \text{ Yield} \left(\frac{\text{mL CH}_4}{\text{g VS}_{\text{added}}} \right) = a \left(\frac{\text{g COD}}{\text{g VS}} \right) \cdot 395 \left(\frac{\text{mL CH}_4}{\text{g COD}} \right) \quad (\text{Equation 4.2})$$

a : tCOD (or sCOD) equivalents of 1 gram VS of the solid sample studied (as shown in Table 4.1)

Biodegradability based on methane yield (BD_{CH_4})

Experimental methane yield (Exp_{CH_4} Yield) and $Theo_{CH_4}$ Yield can be compared to determine the anaerobic biodegradability level of the substrate under BMP test conditions by using Equation 4.3 (Raposo et al., 2011).

$$BD_{CH_4} (\%) = (Exp_{CH_4} \text{ Yield} / Theo_{CH_4} \text{ Yield}) \cdot 100 \quad (\text{Equation 4.3})$$

Exp_{CH_4} Yield and $Theo_{CH_4}$ Yield represent experimental methane yield and theoretical methane yield, respectively.

4.2.6 Statistical analysis

To determine optimum pretreatment method for the corncob, one-way analysis of variance (ANOVA) program was used. The significance level (α) was taken as 0.05. If p-value is greater than α , it is accepted that there is no significant difference between values statistically (Seppälä et al., 2012).

4.3 Results and Discussion

This section is discussed in three main parts; pretreatment studies of corncob, BMP test results of pretreated corncob and characterisation results of BMP reactors.

4.3.1 The results on pretreatment studies of corncob

In pretreatment study, three different methods were applied to raw corncob, namely alkaline pretreatment (AP), hydrothermal pretreatment (HP) and alkaline hydrothermal pretreatment (AHP), which is the combination of alkali and hydrothermal pretreatment. Besides, three different temperatures, namely 150, 180 and 210 °C, were used to optimize hydrothermal and alkaline hydrothermal pretreatment. The change in the properties of raw corncob after pretreatment applications is presented in Table 4.1.

The pH values of hydrothermal pretreated corncob at different temperatures were 5.22-5.5, which was similar to that of Raw CC because no chemical solution was used during pretreatment. On the other hand, for AP CC and AHP CC samples, pH values were 13-14.4 due to the use of NaOH solution during both pretreatment.

TS values of AHP CC samples at different temperatures were 0.96-0.97 g/g, which was higher than that of Raw CC (0.89 g/g) as seen in Table 4.1. The usage of alkali solution during pretreatment might have contributed to the increase in the solid portion in AHP CC. The similar situation was seen for AP CC as well, as expected. However, TS values of HP at different temperatures were 0.91-0.92 g/g, which was close to the TS value of Raw CC (0.89 g/g).

One of the characterisation results is VS content, which represents organic content in the corncob. It is seen in Table 4.1 that, the differences in VS content of AHP CC and Raw CC is almost 32%. It is reported that the VS reduction was in the range of 54.3% to 72.1% and it is related to the decrease in cellulose, hemicellulose and lignin content of Raw CC after pretreatment (Chen et al., 2014). Yet, it seems that HP method did not affect the VS content of Raw CC substantially. Song et al. (2019) found that VS reduction was 0.06-6.3% after hydrothermal pretreatment at 100, 150, 200°C, which is close to values obtained in this study (1.1-5.7%).

The amount of soluble portion is an important parameter for its potentially easily degradation by bacterial community. After the application of AHP and HP methods, sCOD values increased in the range of 12- to 26-fold and 1.1- to 1.4-fold, respectively; moreover, sCOD increased by 9-fold after AP method. The results of sCOD/tCOD ratio also reveal that AHP method contributed more to the increase in solubility of Raw CC compared to AP and then HP methods. It seems that as the temperature increases, the solubilization increases in AHP method.

Table 4.1 Characterisation of Pretreatment Studies on Raw CC

| | Raw CC ^b | AHP_150 CC | AHP_180 CC | AHP_210 CC | HP_150 CC | HP_180 CC | HP_210 CC | AP |
|--------------------|---------------------|--------------------|--------------------|--------------------|------------------|------------------|-------------------|-------------------|
| pH | 5.26 ^a | 13.18 ^a | 13.53 ^a | 14.34 ^a | 5.5 ^a | 5.4 ^a | 5.22 ^a | 13.8 ^a |
| TS (g/g) | 0.89±0.00 | 0.97±0.00 | 0.97±0.00 | 0.96±0.00 | 0.92±0.00 | 0.912±0.00 | 0.92±0.00 | 0.96±0.00 |
| TS (%) | 89.70±0.14 | 96.90±0.04 | 96.90±0.00 | 96.30±0.06 | 91.50±0.08 | 91.50±0.02 | 91.60±0.05 | 95.80±0.07 |
| VS (g/g) | 0.88±0.00 | 0.28±0.02 | 0.32±0.00 | 0.24±0.00 | 0.83±0.00 | 0.89±0.00 | 0.85±0.00 | 0.34±0.00 |
| VS % (% of TS) | 98.42±0.15 | 28.80±2.41 | 32.70±00 | 25.80±0.4 | 90.2±0.35 | 97.6±0.06 | 92.7±0.24 | 35.8±0.35 |
| tCOD (mg/g) | 1340±12 | 1053±38 | 1073±45 | 1068±1 | 1255±46 | 1267±62 | 1433±100 | 1017±4 |
| tCOD/VS | 1.52 | 5.46 | 3.35 | 4.45 | 1.51 | 1.42 | 1.68 | 2.99 |
| sCOD (mg/L) | 385.33±12.58 | 17820±1570 | 19107±95 | 40559±625 | 1278±74 | 1713±38 | 2074±74 | 14010±339 |
| sCOD(mg/g) | 30.90±0.47 | 356±31 | 382±2 | 811±12 | 34±1 | 34±1 | 42±0 | 280±7 |
| sCOD/tCOD | 0.02 | 0.33 | 0.35 | 0.75 | 0.03 | 0.03 | 0.03 | 0.28 |
| TKN (mg /g) | 4.03±0.38 | 0.21±0.02 | 0.23±0.02 | 0.29±0.02 | 1.04±0.11 | 1.40±0.75 | 0.51±0.04 | 0.21±0.02 |
| TAN (mg/g) | 0.0047±0.00 | 0.06±0.01 | 0.14±0.01 | 0.20±0.01 | 0.04±0.00 | 0.05±0.00 | 0.08±0.01 | 0.12±0.01 |
| TP (mg/g) | 0.260±0.00 | 0.11±0.00 | 0.10±0.00 | 0.12±0.00 | 0.23±0.00 | 0.25±0.01 | 0.23±0.00 | 0.11±0.00 |
| Cellulose (%) | 31.50±1.84 | 7.1 ±1.9 | 10.4±1.6 | 4.6±1.6 | 25.8±0.3 | 27.3±1.2 | 25.6±0.6 | 10.3±0.8 |
| Hemicellulose (%) | 35.5±0.7 | 11.2±2 | 13.7±2.6 | 12.8±1.7 | 30.5±0.9 | 33±1.5 | 30.8±1.6 | 9.7±0.9 |
| Soluble Lignin (%) | 3.4±0.4 | 4.0±0.0 | 4.2±0.0 | 4.2±0.0 | 2.8±0.3 | 2.8±0.0 | 2.7±0.0 | 2.7±0.0 |
| Lignin (%) | 21.27±0.31 | 11.8±0.9 | 10.4±0.1 | 10±1.1 | 25.2±0.4 | 27.5±0.5 | 26.1±0.4 | 10±0.7 |

^a Before adjustment of pH to neutral level^b The characterization results of Raw CC was taken from Chapter-3

Yet, temperatures of 150°C and 180°C have close sCOD/tCOD ratios, while a temperature of 210°C in AHP method almost doubled the solubilization compared to 150-180°C. On the other hand, temperatures of 150-180°C did not lead to any increase in solubilization during HP method. Interestingly, all three HP methods had similar solubilization effect. The difference in solubilization effect of alkaline and hydrothermal pretreatments was also observed for different lignocellulosic wastes.

sCOD concentration of asparagus stems increased in the range of 1.8- to 5.7- times after alkaline pretreatment (Chen et al., 2014); while sCOD concentration of rice straw increased only 0.68% after hydrothermal pretreatment (Du et al., 2019).

TAN value dramatically increased after the usage of all pretreatment methods due to the contribution of temperature change, which is higher in AHP and AP methods compared to HP, as expected (Table 4.1).

In AHP_150, AHP_180 and AHP_210 methods, cellulose, hemicellulose and lignin content of solid corncob dramatically reduced compared to Raw CC value. The cellulose, hemicellulose, lignin contents in the solid form might have either transferred to the soluble form or disrupted due to degradation of cell wall, as expected from AHP method. The content of cellulose, hemicellulose and lignin also decreased after AP method. The decrease in cellulose and hemicellulose contents of corncob was seen in HP_150, HP_180 and HP_210 methods; however, the lignin solid content increased after HP methods. The increase in lignin content is an unexpected result. The main purpose of HP method is to increase the solubility of cellulose and hemicellulose while lignin content is not change during HP method (Kumari and Singh, 2018). The increase in lignin content could be attributed the reaction between lignin and by-products produced after HP pretreatment (Garrote et al., 2007). During HP method, lignin is crosslinking with arabinoxylans, which is hemicellulose component, and these formations are measured as lignin (Jönsson and Martín, 2016). Thus, the increase of lignin content might be observed after HP method. Besides, monomeric sugar can be

converted to the pseudo-lignin component after pretreatment (Wang et al., 2018). This might be the explanation of why lignin content increased.

The removal efficiencies of cellulose, hemicellulose and lignin after pretreatment are presented in Figure 4.2. The highest removal in cellulose, hemicellulose and lignin contents was achieved in AHP method. The removal efficiencies of cellulose, hemicellulose and lignin were in the range of 67-85%, 61-68% and 45-53%, respectively. There was a direct relationship between the removal efficiency of lignin and the increase in pretreatment temperature in this study. However, this relationship was not observed for cellulose and hemicellulose. This situation could be explained by the fact that lignin components could be affected by alkaline solution and temperature used during AHP firstly because lignin covers the cellulose and hemicellulose. Thus, lignin might have been disrupted via AHP method, then cellulose and hemicellulose components might have been affected from pretreatment.

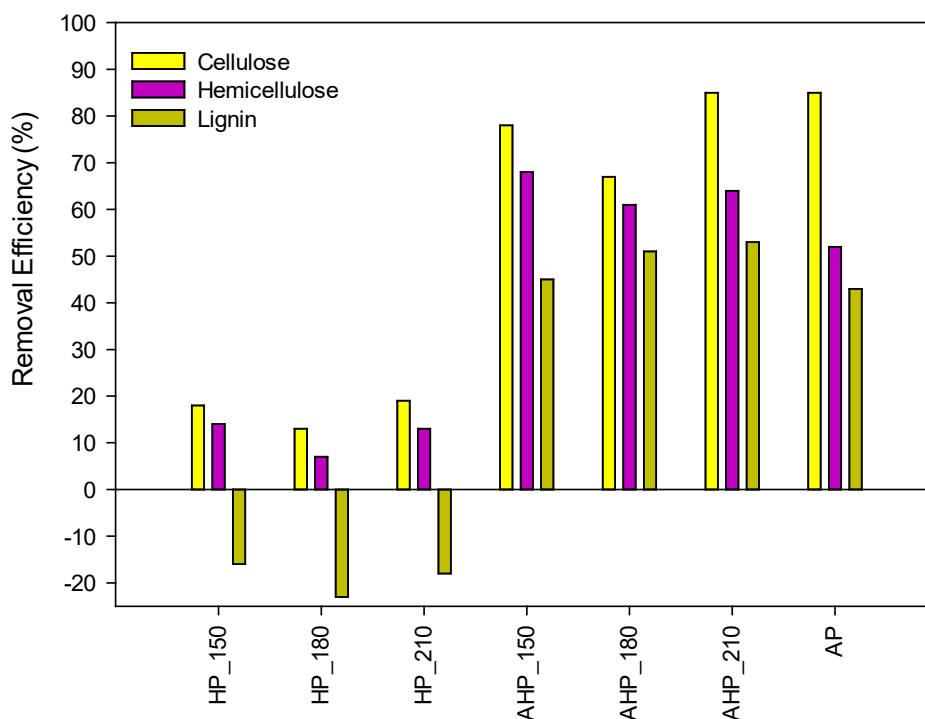


Figure 4.2 Removal efficiency of cellulose, hemicellulose and lignin at different pretreatment methods

For HP method, the removal efficiencies of cellulose and hemicellulose were in the range of 13-19 % and 7-14 %, respectively. Song et al. (2019) observed higher hemicellulose and cellulose removal from corn stover as 22-31% and 35-42 % (at 50-70 °C for 24 and 72 hours), respectively. In another study, the removal efficiencies of cellulose, hemicellulose and lignin content were in the range of (-5) to (-40)%, (-6) to 98% and (-6) to (-52)% , respectively, during HP application (90, 150, 180, 210 °C for 30 minutes), indicating a production, even for cellulose and hemicellulose (Wang et al., 2018). The removal efficiencies of cellulose, hemicellulose and lignin content during AP were obtained as 85%, 52%, 43% in this study (Figure 4.2). The cellulose, hemicellulose and lignin of sunflower stalks were removed by 4%, 24 %, and 22%, respectively at optimum pretreatment condition, which was found as 4 g NaOH/100 g TS at 55 °C (Monlau et al., 2015). These values are much lower than the ones obtained in this study. These results indicate that the removal efficiency of composition in lignocellulosic biomass depends on the substrate type, the alkali solution concentration and duration times.

As abovementioned, solubility of Raw CC was increased the most in AHP (the highest being at the highest temperature of 210°C), then AP and finally in HP. Yet, it should be noted that the highest solubility is not necessarily related to the highest BMP values. During pretreatment, some inhibitory by-products and non-biodegradable sCOD that might decrease the methane yield might be produced such as furfural, arabinose, xylose, xylooligosaccharides, formic acid etc (Kabel et al., 2007; Wang et al., 2018). In order to understand the potential effects of pretreatment, one of the methods used in HP is severity factor model. This model uses the temperature and time factors during the pretreatment application (Equation 4.1, Section 4.2.5). The optimum severity factor is in the range of 3 to 4.5 (Tingyue Gu, 2013). When the severity factor of the HP method is below the optimum range, solubilization efficiency can be lower than the expected value. However, when the severity factor is higher than the optimum range, inhibitory by-products can form during the solvation of component (Zakaria et al., 2014). This

situation creates negative effects on methanogens (Nitsos et al., 2012). The severity factors of HP_150, HP_180 and HP_210 applied in this study are given in Table 4.2. These results show that HP method used for this study is almost in the range of optimum value. Therefore, despite the lowest sCOD/tCOD ratios obtained and thus lower solubility effect compared to the AHP and AP methods, the solubilization obtained in HP methods seems to be as expected, and might not lead to inhibition.

Table 4.2 Severity factor of the HP methods applied at different temperatures

| Temperature (°C) | Time (min.) | Severity Factor (log Ro) |
|------------------|-------------|--------------------------|
| 150 | 30 | 2.9 |
| 180 | 30 | 3.8 |
| 210 | 30 | 4.7 |

4.3.2 BMP test results of the used different pretreatment method on corncob

Methane production results of HP, AHP and AP applications are given in Figure 4.3, Figure 4.4 and Figure 4.5 respectively. Control, B-HP_150 CC and B- HP_210 CC reactors did not produce methane, while B- HP_180 CC reactors produced 20 mL methane during incubation period (Figure 4.3). Methane production in B-HP_180 reactors were not observed during the first 23 days, then reactors start to produce methane suddenly. Therefore, adequate biodegradable component may have accumulated for methane production during this period and intrinsic microorganism somehow survived despite the HP_180 application and produced methane. Methane production of T-HP_150 CC, T-HP_180 CC, T-HP_210 CC were measured as 77, 85 and 83 mL CH₄, respectively. All of T-HP CC reactors almost started to produce methane on Day 3, while Raw CC reactors started to produce methane on Day 8 (Figure 3.2, Section 3.3.1 and Figure 4.3). These results showed that soluble portions of cellulose and hemicellulose were increased by HP method; thus, hydrolysis step, which is the rate-limiting step for lignocellulosic biomass, may be accelerated via HP method.

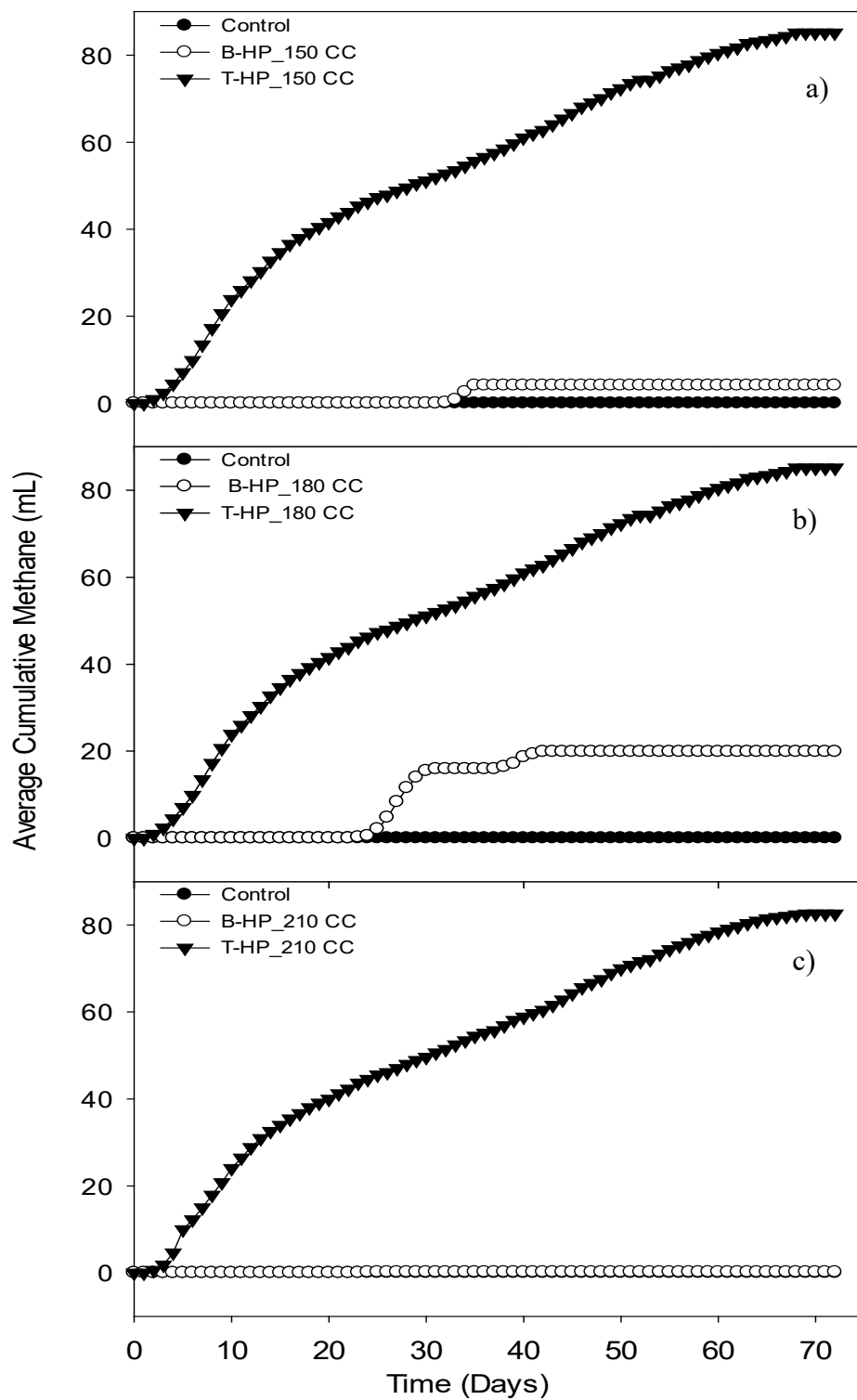


Figure 4.3 Average cumulative methane production of a) HP_150 CC,

b) HP_180 CC, c) HP_210 CC reactors

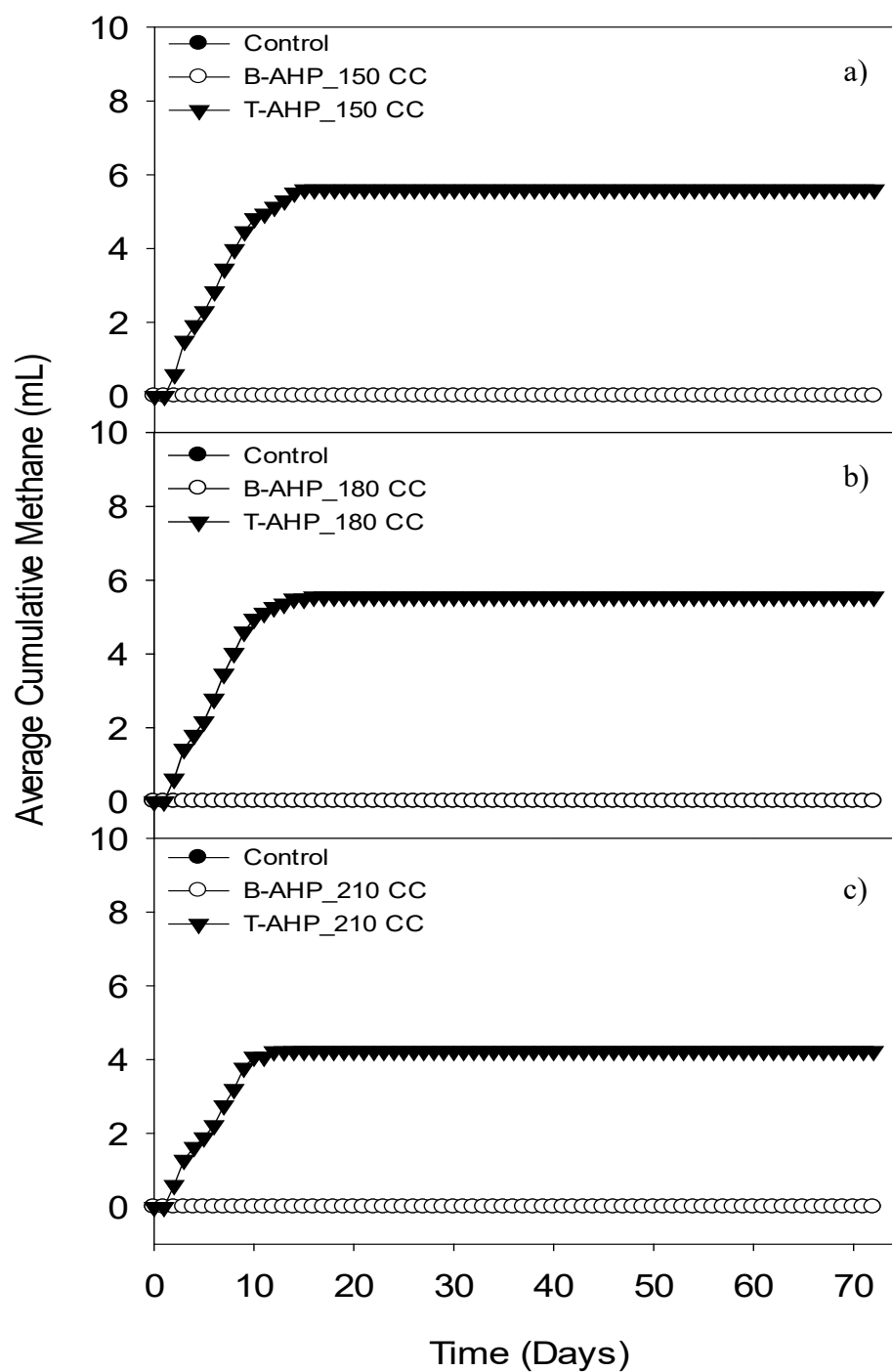


Figure 4.4 Average cumulative methane of production a) AHP_150 CC, b) AHP_180 CC, c) AHP_210 CC reactors

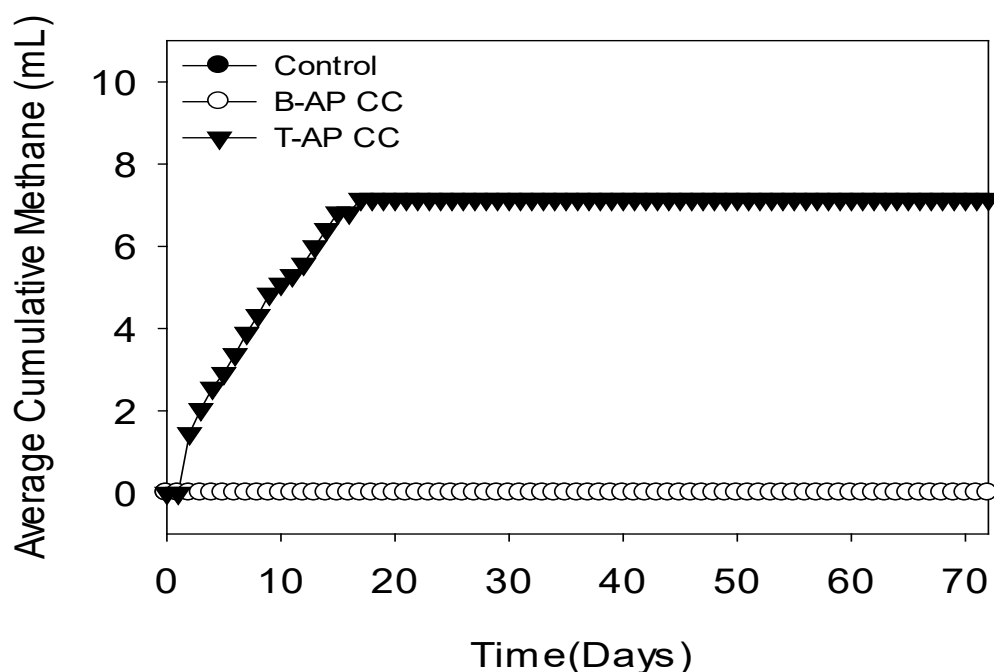


Figure 4.5 Average cumulative methane production of AP CC reactors

B-AHP CC and B-AP CC reactors did not produce methane during incubation period. T-AHP_150, T-AHP_180, T-AHP_210 and T-AP CC reactors produced in the range of 4 to 6 mL methane (Figure 4.4 and Figure 4.5). These are disappointing results because AHP and AP methods lead to both reduction of lignin in the corncob and the increase in solubility of hemicellulose and cellulose (Table 4.1). As mentioned previously, the whole soluble portion may not be a useful biodegradable component for the microbial community, it may be even inhibitory (Chen et al, 2014). Cumulative methane production of AHP graph (Figure 4.4) showed that the methane production ceased in all three types of T-AHP reactors almost 10-15 days, independent of the temperature applied during pretreatment. Methane yield results of HP, AHP and AP methods are presented in Table 4.4. B-HP_150 CC and B-HP_210 CC blank reactors hardly produced no methane, as expected. However, HP_180 CC blank reactors achieved 20 mL CH₄/gVS. This result was unexpected compared to the results in the literature because the increase in temperature generally damages intrinsic microorganisms in the lignocellulosic biomass.

T-HP_150 CC, T-HP_180 CC and T-HP_210 CC reactors achieved 158, 160, 162 mL CH₄/g VS, respectively (Table 4.3). These results can be explained by adopting two approaches, which are biodegradability and the increase in methane potential of Raw CC. Firstly, the values of biodegradability, which is the ratio of Exp_{CH₄} Yield to Theo_{CH₄} Yield, for T-HP_150 CC, T-HP_180 CC and T-HP_210 CC were 27%, 28%, 27%, respectively. These results were slightly low when the literature is considered; however, biodegradability value of Raw CC increased by 47%-53% after HP application at different temperatures. These showed that the solubility and accessibility of the cellulose and hemicellulose were improved by HP method at different temperatures. Yet, as mentioned previously, it seems that applied temperatures had similar effects and resulted in same biodegradability.

Table 4.3 Exp_{CH₄} Yield, Theo_{CH₄} Yield, BD_{CH₄} of Raw CC, HP CCs, AHP CCs and AP CC

| Reactors | Exp _{CH₄} Yield ^a (mL CH ₄ /g VS) | | Theo _{CH₄} Yield ^b (mL CH ₄ /g VS) | BD _{CH₄} ^c (%) |
|---------------------|--|------|---|--|
| | Blank | Test | | |
| Raw CC ^d | 36 | 103 | 584 | 18 |
| HP_150 CC | 8 | 156 | 571 | 27 |
| HP_180 CC | 20 | 159 | 577 | 28 |
| HP_210 CC | 0 | 162 | 604 | 27 |
| AHP_150 CC | 0 | 33 | 1552 | 2 |
| AHP_180 CC | 0 | 25 | 1358 | 2 |
| AHP_210 CC | 0 | 29 | 1810 | 2 |
| AP CC | 0 | 35 | 1175 | 3 |

^a Exp_{CH₄} Yield represents experimental methane yield

^b Theo_{CH₄} Yield represents theoretical methane yield based on tCOD

^c BD_{CH₄} represent biodegradability based on methane yield, and the calculation was done for test reactors.

^d The results of Raw CC was taken from Chapter-3

During HP method, water can diffuse into the lignocellulosic biomass increasing the solubility of the cellulose and hemicellulose and destructing lignin barely (Zheng et al., 2014). The amounts of cellulose and hemicellulose contents in Raw CC (in solid form) decreased after the HP method due to their conversion to soluble form. These soluble components are identified as monomeric and oligomeric sugars (Wang et al., 2018). Glucose and glucan, which are formed due to cellulose conversion from solid to liquid form, can be measured in soluble portion. Hemicellulose includes mainly combination of pentoses and hexoses; thus, its structure consists of complex components compared to cellulose (Hu and Ragauskas, 2012). The xylose converted to xylan and xylooligosaccharides components come from hemicellulose during the conversion of solid to liquid form after pretreatment application (Chen et al, 2014). These soluble components of cellulose and hemicellulose contribute to improved methane production. On the other hand, inhibitory by-products, namely furans and organic acid, e.g., formic acid, can form during HP. Furans, which are furoic acid, 5-hydroxymethylfurfural (HMF) and furfural, can also form because both monomeric sugars, glucose and glucan, might be converted to these components by the effect of high temperature and xylose is partly converted to furfural (Kabel et al., 2007; Wang et al., 2018; Hu and Ragauskas, 2012). One of the inhibitory by-products is that organic acid, formic acid, can form owing to the depolymerization of hemicellulose (van der Pol et al., 2016). Considering the severity factors calculated for HP (Table 4.2), it was expected that inhibitory by-products were not produced or produced in low amount. Low BD_{CH_4} (27-28%) obtained in this study compared to the ones obtained in literature such as 43-63% (Lee and Park, 2020) might be due to the low amounts of inhibitory by-products produced and/or structural properties of substrate. Yet, still, HP method was found to be the most effective method among the studied ones in improving the BMP of corncob (47-53%).

B-AHP_150 CC, B-AHP_180 CC, B-AHP_210 CC reactors did not produce methane, as expected. AHP_150 CC, AHP_180 CC and AHP_210 CC test reactors achieved 33, 25, 29 mL CH_4 /gVS, respectively (Table 4.4). These results were extremely low compared to both literature data and methane yield of Raw CC (103

mL CH₄ /gVS). Although solubility of the Raw CC was increased in the range of 11.5- to 26-fold after AHP method, methane yield of AHP decreased in the range of 67-75 %. Moreover, biodegradability value of AHP (2%) was also lower than that of Raw CC (18%). The cause of this reduction might be the corncob structure destructed by alkali solution. Thus, inhibitory by- products could have formed during pretreatment methods. The basic mechanism of AHP is de-esterification of the molecules, which are hemicellulose and lignin (Kim et al., 2013). This situation causes the change of physical properties of the substrate (Kim et al., 2016). Saccharinic acids, lactic acid, dicarboxylic acids come from polysaccharides degradation during AHP as by-products. Phenolic acid occurs under alkaline conditions, and it damages methanogens (Jönsson and Martín, 2016). The relationship between inhibitory by-products and reduction amount of hemicellulose and lignin can be considered as the reason of low methane yield.

B-AP reactors did not produce methane. As seen in Table 4.6, T-AP reactors achieved 35 mL CH₄/gVS. These results were also extremely low compared to both literature data and methane yield of Raw CC (103 mL CH₄ /gVS). The reason of reduction in methane yield can be explained by the formation of inhibitory by-products during AP method, which is similar to AHP method, and non-biodegradable sCOD.

In Figure 4.6, a few peaks in daily methane yield production in T-HP reactors were observed during incubation period due to the fact that corncob included different digestible portions. For example, glucan, soluble and readily biodegradable form, and lignin, insoluble form, existed in the same digestion period (Wang et al., 2018). The first methane yield peak was observed on Days 5-6 where it was 11 mL in HP_210 CC reactors. The first methane yield peak of HP_180 CC appeared on Days 7-8, and it was 7 mL in HP_180 CC reactors. Lastly, the first peak of the HP_150 CC reactors was observed on Days 8-9, and the methane yield was 5 mL. As seen in Figure 4.6, different temperatures affect the time of the first peak of methane yield. HP_150 CC and HP_180 CC reactors methane yield gradually dropped until Day 12;

however, HP_210 CC reactors dropped suddenly on Day 6. Higher temperature conditions may lead to higher acidification rate and extent in the reactor, the latter being also verified by increased sCOD values with temperature increase. After Day 20, all HP CC reactors reached nearly the same values in methane yield.

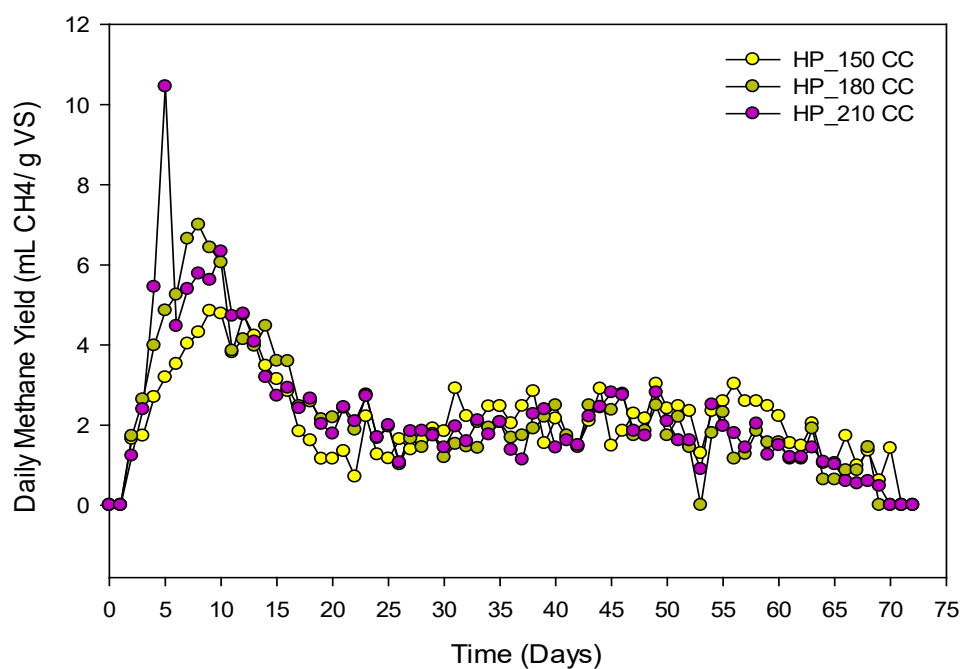


Figure 4.6 Daily methane production of HP_150 CC, HP_180 CC and HP_210 CC reactors

The methane yields of the T-HP CC reactors were very close (158-162 mL CH₄/g VS). Therefore, one-way ANOVA was used to determine if there is any significant effect of temperature on HP application, in terms of methane yield. These analyses were used just for HP applications due to the fact that HP applications reached the highest methane yield among used pretreatment methods. The detailed results of one-way ANOVA are given in Appendix G. Results revealed that there is no statistically significant difference between each methane yield values. This means that, T-HP_150 CC, T-HP_180 CC and T-HP_210 CC are not different in terms of their BMPs so the applied temperatures of 150, 180, 210 °C did not result in a different yield. In other words, HP methods applied for three different temperatures had the

same effect on the methane yield from corncob. The similar composition and soluble portion amount might be formed during hydrothermal pretreatment at different temperatures (150, 180 and 210°C). Yet, in Table 4.1 it is seen that sCOD concentration (mg/L) increases by almost 400-700 mg/L as temperature increases. Therefore, potential inhibitory by-products might be also formed. This finding was also reported by Lee and Park (2020).

4.3.3 Characterization results of BMP reactors

The reactors were sampled at the initial and final days of the incubation periods for TS, VS, TAN, sCOD, tCOD analysis. The detailed results of these analyses are given in Appendix D.

pH values of T-HP CC reactors were in the range of 7.75 and 7.81 at the final day of incubation (Table 4.4). These results were in the optimum pH range of 6.5 to 8.2 (Speece, 1996). The final pH values were in the range of 8.45-8.68 for T-AHP CC reactors (Table 4.4). These results were slightly higher than the optimum pH value.

TAN ($\text{NH}_4 + \text{NH}_3$) concentrations were in the range of 1110 to 1158 mg/L for T-HP CC reactors (Table 4.4). These values were below the ammonia inhibition limit, 1500 mg/L, as mentioned previously. The range of the TAN removal were (-13) % to (-42) % (negative signs represent accumulation of TAN). AHP CC reactors reached 1185-1330 mg/L TAN value at the final of digestion period (Table 4.4). The equilibrium between ammonium (NH_4) and ammonia (NH_3) depends on pH value in the environment, and if the pH increases above 9.3, ammonia becomes dominant component (Speece, 1996). In T-AHP reactors, both pH value was in the range of 8.45-8.68 (below 9.3) and the range of effluent TAN concentration was 1185-1330 mg/L (below 1500 mg/L); thus, no inhibition from ammonia accumulated was observed.

Table 4.4 The change of TAN and pH values in the test reactors

| Reactors | TAN value | | | The change of pH value | |
|---------------------|-------------------|-----------------|----------------|------------------------|---------|
| | Initial (mg/L) | Final (mg/L) | Removal (%) | Initial | Final |
| Raw CC ^a | 362±5 | 419±1 | -16 | 7.1 | 7.9-8.0 |
| HP_150 CC | 807±7 | 1010±4 | -25 | 7.28 | 7.81 |
| HP_180 CC | 981±12 | 1105±16 | -13 | 7.25 | 7.75 |
| HP_210 CC | 813±67 | 1158±67 | -42 | 7.25 | 7.8 |
| AHP_150 CC | 866±9 | 1185±20 | -37 | 7.3 | 8.6 |
| AHP_180 CC | 883±29 | 1330±4 | -51 | 7.25 | 8.68 |
| AHP_210 CC | 867±12 | 1290±8 | -49 | 7.32 | 8.45 |
| AP | 862±13 | 1350±8 | -57 | 7.25 | 8.35 |

^a The results of Raw CC was taken from Chapter-3

The VS removals of the T-HP CC reactors were in the range of 23-34 % in the test reactors (Table 4.5). The VS removal in T-AHP CC reactors were 14-33 % while VS removal in T-AP CC was 29 % (Table 4.5). In the literature, the VS removal of hydrothermal pretreated lignocellulosic biomass was in the range of 24% to 46 % (Romano et al., 2009), which is consistent with the findings of this study. Despite the lower methane yields of T-AHP and T-AP reactors compared to HP counterparts, they obtained close VS removals. This indicates that hydrolysis steps of anaerobic digestion were still active in AHP and AP reactors. This outcome was also verified by increased sCOD production and TAN concentrations as obtained by the end of the incubation period (Table 4.4; Table 4.5).

The tCOD removal in T-HP_150 CC, T-HP_180 CC and T-HP_210 CC reactors were in the range of 43 to 58% (Table 4.7). Yet, the removal of tCOD values in T-AHP_150 CC, T-AHP_180 CC and T-AHP_210 CC were only of 5 to 13%, while that of T-AP CC reactors were 12% (Table 4.6), which was related to the low methane yields.

sCOD concentration generally increased at the end of incubation period due to the increased solubilization of organic content during digestion. Effluent sCOD concentration increased in all of the test reactors independent of the pretreatment type. However, the increase was highest in T-AHP CC reactors (Table 4.6). This situation may be related to the still-active-hydrolysis-step in these reactors.

Table 4.5 The change in average VS, tCOD and sCOD concentrations in the test reactors

| Reactors | VS removal (%) ^a | tCOD removal (%) ^a | sCOD removal (%) ^a |
|---------------------|-----------------------------|-------------------------------|-------------------------------|
| Raw CC ^b | 50 | 34 | -157 |
| HP_150 CC | 31 | 43 | -206 |
| HP_180 CC | 23 | 58 | -181 |
| HP_210 CC | 24 | 48 | -201 |
| AHP_150 CC | 33 | 13 | -291 |
| AHP_180 CC | 14 | 5 | -245 |
| AHP_210 CC | 29 | 5 | -266 |
| AP CC | 29 | 12 | -187 |

^a These analyses was done triplicate.

^b The results of Raw CC was taken from Chapter-3

4.4 Conclusions

The main aim of this study is to investigate the effect of different pretreatment methods on methane yield of corncob.

Although the solubility of the Raw CC increased in the range of 11.5- to 26-fold after AHP method, methane yield of AHP CC reactors decreased in the range of 67-75 % compared to Raw CC. It should be noted that the highest solubility is not necessarily related to highest BMP values. During pretreatment, some inhibitory by-products

that might decrease the methane yield might be produced such as furfural, arabinose, xylose, xylooligosaccharides, formic acid etc because methanogens activity could be repressed by inhibitory by-products.

Despite the lowest sCOD/tCOD ratios obtained and thus lower solubility effect compared to the AHP and AP methods, the highest methane yields were obtained in HP CC reactors. The methane yields of T-HP CC reactors were 55-61% higher than that of T-Raw CC. The formation of inhibitory by-products might be minimized due to the fact that HP method was applied in the optimum range of severity factor. On the other hand, 22-23% biodegradability and similar yields obtained at three different temperature applications might indicate a low amount of inhibitory by-product formation as well as low biodegradability due to the rigid structural properties.

This study showed that the content of the soluble composition after pretreatment application was much more important than how much solubility was increased. Therefore, pretreatment methods should be compared with BMP tests. In this study, HP₁₅₀ method was selected as the optimum pretreatment method among the studied ones. For leading to similar methane yield, instead of HP applications at 180°C and 210°C, HP at 150°C was the optimum one; thus, unnecessary energy use can be avoided.

CHAPTER 5

SEMI-CONTINUOUS CO-DIGESTION OF CORNCOB WITH DIGESTATE: INFLUENCE OF HYDROTHERMALLY PRETREATED CORNCOB, HYDRAULIC RETENTION TIME AND ORGANIC LOADING RATE ON METHANE YIELD

5.1 Introduction

Sustainable waste management is a vital challenge due to the increasing amount of crop and animal wastes which are organic biomasses (Zahan et al., 2018). There is a variety of waste management methods one of which is the anaerobic digestion with some benefits such as the use of organic content to produce methane, and with some advantages such as less amount of waste generation after digestion, lower operation cost and the support of renewable energy production (Demirel and Yenigün, 2002). Thus, anaerobic digestion plays a critical role in terms of both waste management and meeting energy requirement (Li et al., 2015).

Although large scale anaerobic digester plants use mono-substrates generally which are types of manures as a feedstock, the mono-digestion has some disadvantages two of which are hardships in maintaining feedstock supply and ammonia inhibition (Li et al., 2018). Some researches show that the challenges of mono-digestion can be eliminated by adopting co-substrates, which are rich carbon sources such as lignocellulosic biomass (Aboudi et al., 2015; Zahan et al., 2018). Thus, disadvantages of mono-digestion can be removed by using co-digestion and in turn providing optimum COD/N ratio which is in the range of 350/7 - 1000/7 (Li et al., 2018; Speece, 1996). However, the usage of lignocellulosic biomass as a co-substrate may lead to some problems which are mainly about its degradation due to the inclusion of variable structural components of lignocellulosic biomass (Hagos et al., 2017).

Many pretreatment methods, such as alkaline, hydrothermal, enzymatic, microwave and ultrasound pretreatments, can be used in order to get rid of this lignocellulosic biomass drawback (Kumari et al., 2018). Different from other methods, hydrothermal pretreatment is accepted as a green technology due to the use of only water for pretreatment (Wang et al., 2018). The aim of using each pretreatment method is different owing to the application method and used materials. Hydrothermal pretreatment contributes to the solubilization of cellulose and hemicellulose (Kumari and Singh, 2018).

This study focuses on a non-food waste as the substrate and digestate, which is an effluent of anaerobic digester, as the co-substrate rather than manure. Corn waste, defined (used) as substrate in this study, accounts for 33% of the crop waste in Turkey, being the first most produced one. (FAO, 2019). Besides, agricultural residual is an important component in the vegetal waste, and plays a key role in biomass energy (Dell'Omo and Spena, 2020). On the other hand, digestate is used as a co-substrate to underline its feature as a valuable organic waste in this study, since its usage area has been limited just as a fertilizer. Moreover, digestate includes lots of nutrients and bacterial consortiums to enhance methane production in digester (Gioelli et al., 2011). Yet, some of the researches showed that it may bring about global warming for containing dissolved greenhouse gases. For that reason, they were opposed to its usage as fertilizer (Gioelli et al., 2011; Nkoa, 2013). Therefore, the use of digestate as a co-substrate might be a second alternative for their usage which might also indicate a residual BMP (Rico et al., 2011) worth to investigate.

Anaerobic digestion can be mainly categorised as batch, semi-continuous and continuous in terms of the feeding style (Forster-Carneiro et al., 2008). Mostly, methane production potential and biodegradability of feedstock can be determined by batch reactor studies (BMP tests). There are semi-continuous and continuous studies focusing on anaerobic digestion, which can indicate the stability and productivity of the digester. These studies are significant for industrial scale anaerobic digesters (Li et al., 2014). Many studies have discussed the effects of

hydraulic retention time (HRT) and organic loading rate (OLR) on digestion of different feedstocks. Y. Li et al. (2014) studied different OLR values, which are in the range of 1-4 g VS/L.d with chicken manure and corn stover mixed as C/N ratio of 25, in semi-continuous reactor. The highest methane yield was found to be 223 mL CH₄ /g VS at OLR of 4 g VS/L.d. K. Li et al. (2018) used manure with apple pulp or corn stover (as C/N ratio of 25) in semi-continuous reactor by using various HRT and OLR values to determine the highest methane production. The highest methane yield was found to be 340 mL CH₄/g VS at an HRT of 25 days and an OLR of 4 g VS/L.d. In another study, chicken litter with agricultural waste and/or food wastes were mixed at a C/N ratio of 20, and two different OLR values (2 and 3 g VS/L.d) were studied at an HRT 20 days. The highest methane yield was determined to be 237 mL CH₄/g VS at an OLR 2 g VS/L.d with an amount of chicken litter (60%), food waste (20%) and wheat straw (20%) (Zahan et al., 2018).

In the literature, lignocellulosic biomass and various types of manure were generally used in semi-continuous co-digestion studies, as some of them were already abovementioned (Comino et al., 2010; Y. Li et al., 2014; D. Li et al., 2015; Aboudi et al., 2016; Hassan et al., 2017; K. Li et al., 2018; Zahan et al., 2018). Although extensive co-digestion research has been carried out on various types of lignocellulosic biomass together with manure, to our knowledge, there is no study on co-digestion of lignocellulosic biomass with digestate. In addition, there is a limited number of studies about corncob co-digestated with different co-substrates in batch reactors (Pérez-Rodríguez et al., 2016; Surra et al., 2018). Yet, no study was found to investigate both the effect of pretreatment and the arrangement of optimum COD/N in continuous reactors.

In this study, raw corncob and hydrothermal pretreated corncob at 150 °C (HP_150 CC) were used as substrates with digestate (as the co-substrate), the latter for the adjustment of COD/N ratio. It was aimed to investigate the optimum operational conditions, i.e., HRT and OLR, leading to the maximum methane production during the co-digestion of these substrates in semi-continuous reactors. To this end, two

semi-continuous co-digestion reactors, one fed with raw corncob, the other with HP_150 CC as substrate, were operated to compare the methane yields and in turn effects of the pretreatment and optimum operational conditions on methane production.

5.2 Materials and Methods

5.2.1 Characteristic of Raw CC and HP_150 CC

Raw CC and HP_150 CC were previously used as substrates in batch reactors (Chapter-3 and Chapter-4, respectively). The characterisation results of these substrates previously given in Table 3.1 (Chapter-3) and Table 4.2 (Chapter-4), respectively, are shown together in Table 5.1 below. As the pretreated corncob, HP_150 CC was selected, because the results in Chapter-4 revealed that hydrothermal pretreatment led to the highest yield among others and the effect of temperatures applied on yield was not significantly different.

Table 5.1 Characterisation of Raw CC and HP_150 CC

| | Raw CC ^b | HP_150 CC ^c |
|--------------------|---------------------|------------------------|
| pH | 5.26 ^a | 5.5 ^a |
| TS (g/g) | 0.89±0.00 | 0.92±0.00 |
| TS (%) | 89.70±0.14 | 91.50±0.08 |
| VS (g/g) | 0.88±0.00 | 0.83±0.00 |
| VS % (% of TS) | 98.42±0.15 | 90.2±0.35 |
| tCOD (mg/g) | 1340±12 | 1255±46 |
| tCOD/VS(g/g) | 1.52 | 1.51 |
| sCOD (mg/L) | 385.33±12.58 | 1278±74 |
| sCOD(mg/g) | 30.90±0.47 | 34±1 |
| sCOD/tCOD | 0.02 | 0.03 |
| TKN (mg /g) | 4.03±0.38 | 1.04±0.11 |
| TAN (mg/g) | 0.0047±0.00 | 0.04±0.00 |
| TP (mg/g) | 0.260±0.00 | 0.23±0.00 |
| Cellulose (%) | 31.50±1.84 | 25.8±0.3 |
| Hemicellulose (%) | 35.5±0.7 | 30.5±0.9 |
| Soluble Lignin (%) | 3.4±0.4 | 2.8±0.3 |
| Lignin (%) | 21.27±0.31 | 25.2±0.4 |

^a Before adjustment of pH to neutral level

^b The characterization results of Raw CC were taken from Chapter-3

^c The characterization results of HP_150 CC were taken from Chapter-4

5.2.2 Characteristic of the co-substrates

Digestate taken from Afyon Energy Biogas Plant was used as the co-substrate. This plant includes a two-stages process. The first digester was fed with Feed (chicken manure and poppy (17%)), and the first digester's effluent is called as Digestate, which is fed with second anaerobic digester. The effluent of second anaerobic digester is Post-Digestate. Feed, Digestate and Post-Digestate were used as co-substrates with Raw CC in anaerobic co-digestion (Chapter-3). Digestate was

selected to use in semi-continuous digestion since it created more positive synergistic effect than Feed and Post-Digestate on anaerobic digestion. Digestate was put in a plastic bottle and stored at -20°C. It was thawed at 4°C before use. Table 5.2 presents the characterization results of Digestate.

Table 5.2 Characterisation of Digestate and Inoculum

| Parameter | Digestate | Inoculum |
|----------------|------------|------------|
| pH | 9.31 | 7.60 |
| TS (g/L) | 74.60±8.41 | 6.82 ±0.1 |
| VS (g/L) | 37.37±3.18 | 3.41 ±0.07 |
| VS % (% of TS) | 50.2±2.32 | 50.1 ±0.92 |
| tCOD (g/L) | 95.68±4.01 | 6690±140 |
| sCOD (g/L) | 12.37±0.06 | 310 ±2.10 |
| TKN (g /L) | 7.16±0.56 | 528±2.1 |
| TAN (g/L) | 3.73±0.16 | 245±2.1 |
| TP (mg/L) | 50.45±0.94 | 65±1.1 |

5.2.3 Inoculum (seed sludge)

Seed sludge was taken from the effluent of the anaerobic digesters in the Ankara Central Municipal Wastewater Treatment Plant. The inoculum was stored at room temperature in a plastic bottle. The characterisation results of inoculum are given in Table 5.2.

5.2.4 Experimental procedure

Experiments were performed in semi-continuous reactors with 3 L total volume and 2 L effective volume. Two different reactor set-ups, namely Reactor-1 and Reactor-2, were conducted for this research study. Raw CC with Digestate and HP_150 CC with Digestate were used for Reactor-1 and Reactor-2, respectively. Initially, the ratio of substrate to co-substrate was calculated depending on optimum COD/N ratio,

which was determined as 85 (Syaichurrozi et al., 2013). The adjustment of COD/N ratio was done for both feed and the reactors content at the very beginning of the operation. The reactors were inoculated with seed sludge at a concentration of 7.5 g/L and S/I value of 1. The content analysis of the inoculated reactors at the beginning of the operation are presented in Appendix H. Both reactors were operated at various operational conditions of HRTS (10, 15 days) and OLR (2, 3 and 4.5 g VS/L.d) in order to assess the optimum conditions and substrate type in terms of methane yields.

Reactor-1 was fed daily for the first 14 days, and Reactor-2 was fed daily the first 29 days. However, feeding regime was changed from daily to once-in-every-two-days, yet the VS concentration of the feed was doubled; therefore, the two times concentrated feed (as VS) was applied in once-in-every-two-days. The reason of changing feeding regime was the restriction of Covid-19 because daily feeding of the reactors was not possible during the lockdown. The feedstocks were prepared freshly before the feeding. The content of feedstock was adjusted with COD/N ratio of 85 by using mass balance calculation as mentioned previously. Basal medium, BM, was not used in the reactors due to the fact that nutrient requirements of the microbial consortium were supplied from Digestate. Reactor-1 and Reactor-2 were operated for 139 days and 154 days, respectively by adopting different operational parameters (Table 5.3). Duration time for each HRT application was not fixed, as the time to reach steady state condition was different. This means that, the HRT was changed when the methane production reached the steady state conditions. Generally, each reactor was operated at a specific operational condition for at least 4 HRTs.

Table 5.3 Operation parameters of the reactors

| Reactor-1 operation time (days) | Reactor-2 operation time (days) | HRT (days) | OLR (g VS/L.d) |
|------------------------------------|------------------------------------|------------|-------------------|
| 0-61 | 0-60 | 15 | 3 |
| 61-97 | 60-112 | 10 | 2 |
| 97-139 | 112-154 | 10 | 4.5 |

pH value was set between 7 to 7.2, which is the optimum pH value for methanogens (Gerardi, 2003). The purge process was applied in two steps. In the first step, the liquid parts of the reactors were purged with Argon gas (100%) for 5 minutes, and the reactors were sealed with rubber stopper. In the second step, the head spaces of the reactors were purged with Argon gas (100%) for 5 minutes to create anaerobic conditions. Accumulation of Argon gas in the headspace due to purging was prevented by using water displacement device; thus, the headspace pressure of the reactors was obtained at 1 atm. Reactor-1 and Reactor-2 were operated in a hot room at 35 ± 2 °C and mixed with magnetic stirrers at 200 rpm. The illustration of the reactors is given in Figure 5.1.

Volume of the biogas produced was daily measured with water displacement device. The solution of water displacement device was 270 g salt/L and the pH value of 2; therefore, the solubilisation of CO₂ in water was prevented with this solution (WRAP, 2010).



Figure 5.1 The photograph of the reactors with water displacement device

5.2.5 Analytical methods

Standard Methods (APHA, 2005) were used for determination of total solid (TS) and volatile solid (VS). Total Chemical Oxygen Demand (tCOD) and soluble Chemical Oxygen Demand (sCOD) were determined by EPA approved digestion method (for COD range of 0-1500 mg/L), heat was applied by Aqualytic AL 38 heater for 2 hours, then spectrophotometric detection was carried out by using the spectrophotometer (SN 05827, PC Multidirect). For the determination of sCOD of solid samples, i.e., corncob and digestate, 1 gram corncob was added into 20 mL deionized water, then this mixture was stirred for an hour. Lastly, mixtures were filtered through 0.45 μm pore sized filters (Millipore). sCOD value of this sample was measured with spectrophotometer (SN 05827, PC Multidirect). TAN analysis was measured with Nessler method (Crosby, 1968). The calibration curve is given in Appendix I. All measurements were performed triplicate. For pH measurement, pH meter (Mettler Toledo 33111) was used. pH meter was calibrated with the buffer solutions which has 4, 7 and 10 pH values. Water displacement device was used to measure the amount of biogas production in the reactor daily. The measurement of

the gas composition was done by a gas chromatograph (GC) to specify methane content of the biogas. GC consists of two detectors that are thermal conductivity detector (TCD) and a flame ionization detector (FID). Biogas composition was measured with TCD. The columns in series (CP- Moliseve 5A and CP-Porabond Q) were used to separate as CH₄, O₂, CO₂, H₂ from injected biogas at 45 °C oven temperature. Helium is the carrier gas at 100 kPa constant pressure. The detector, inlet and oven temperatures were arranged to 80°C, 50°C and 35°C respectively. The calibration curves prepared for the gas content analysis are given in Appendix C. Whenever the water displacement device was used to observe the biogas production, gas composition was also analyzed by GC at the same day.

Energy input (Ei) calculation was done to determine the energy used during pretreatment application (Equation 5.1). Energy output (Eo) calculation was done to find the energy equivalent of methane produced in each reactor (Equation 5.2). Equation 5.3 was used to calculate net energy. Ei and Eo calculations were done by using Equation 5.1 and Equation 5.2 (Xiong et al., 2020).

$$Ei \left(\frac{MJ}{kg} VS \right) = \frac{W(kWh).(3.6.10^6)}{m_{sample}(g VS).10^3} \quad \text{Equation 5.1}$$

$$Eo \left(\frac{MJ}{kg} VS \right) = \frac{P_{CH_4} \left(\frac{mL}{g VS} \right) . c \left(\frac{kJ}{m^3} \right)}{10^6} \quad \text{Equation 5.2}$$

$$E_{net} = Eo - Ei \quad \text{Equation 5.3}$$

Ei and Eo were input and output energy values, respectively. W is the consumption of the electricity during pretreatment, and power consumption of oven is 0.7 kWh (FN 055 Dry Heat Sterilizers/Ovens, 2021). P_{CH₄} is the methane yield of the reactors and the c represents lower methane heating value, 35800 kJ/m³ CH₄ (at STP).

5.3 Results and Discussion

5.3.1 Methane Potential of Reactor-1

The various HRT and OLR values were applied to investigate and determine the operational conditions leading to the highest methane yield. OLR value applied was 3 g VS/L.d at an HRT of 15 days and 2 g VS/L. d and 4.5 g VS/L.d at HRT of 10 days (Figure 5.2).

Initially, Reactor-1 was operated at 15-day HRT and OLR of 3 g VS/L.d, and it reached 270 L CH₄/kg VS_{added} stable daily methane yield (Figure 5.3c). The methane yield of Reactor-1 was comparable with the literature as given in Table 5.4. During this operational condition, the feeding regime was daily for the first 14 days. Then, the feeding regime was changed due to pandemic period. Thus, Reactor-1 was fed once-in-every-two-days yet with 2 times concentrated feed (i.e., 2C/ 2day). This situation sharply affected the daily methane production and yield in Reactor-1(Figure 5.3a, 5.3c). When Reactor-1 was fed daily, the maximum daily methane production and methane yield were obtained as 300 mL and 100 L CH₄/kg VS_{added} at 14th day, respectively. The methane production and yield reached 900 mL and 157 L CH₄/kg VS_{added} on the 16th day immediately after the feeding regime was converted to once-in-every-two-days (with 2 times concentrated VS feed). These results showed that the methane yield value increased 1.5-fold compared to daily feeding regime. Manser et al. (2015) investigated the relationship of feeding regimes and methane yield. The methane yield improved by 27 % with long feeding regime compared to the daily feeding regime in their study. This might be attributed to improvement in methanogenic activities due to the feeding regime. On Day 45, daily methane yield sharply doubled, and methane yield of 270 L CH₄/kg VS_{added} was observed during the following 15 days (Days 45-60). The reason of this unexpected increase in daily methane yield may be the solubilisation of the accumulated biodegradable components.

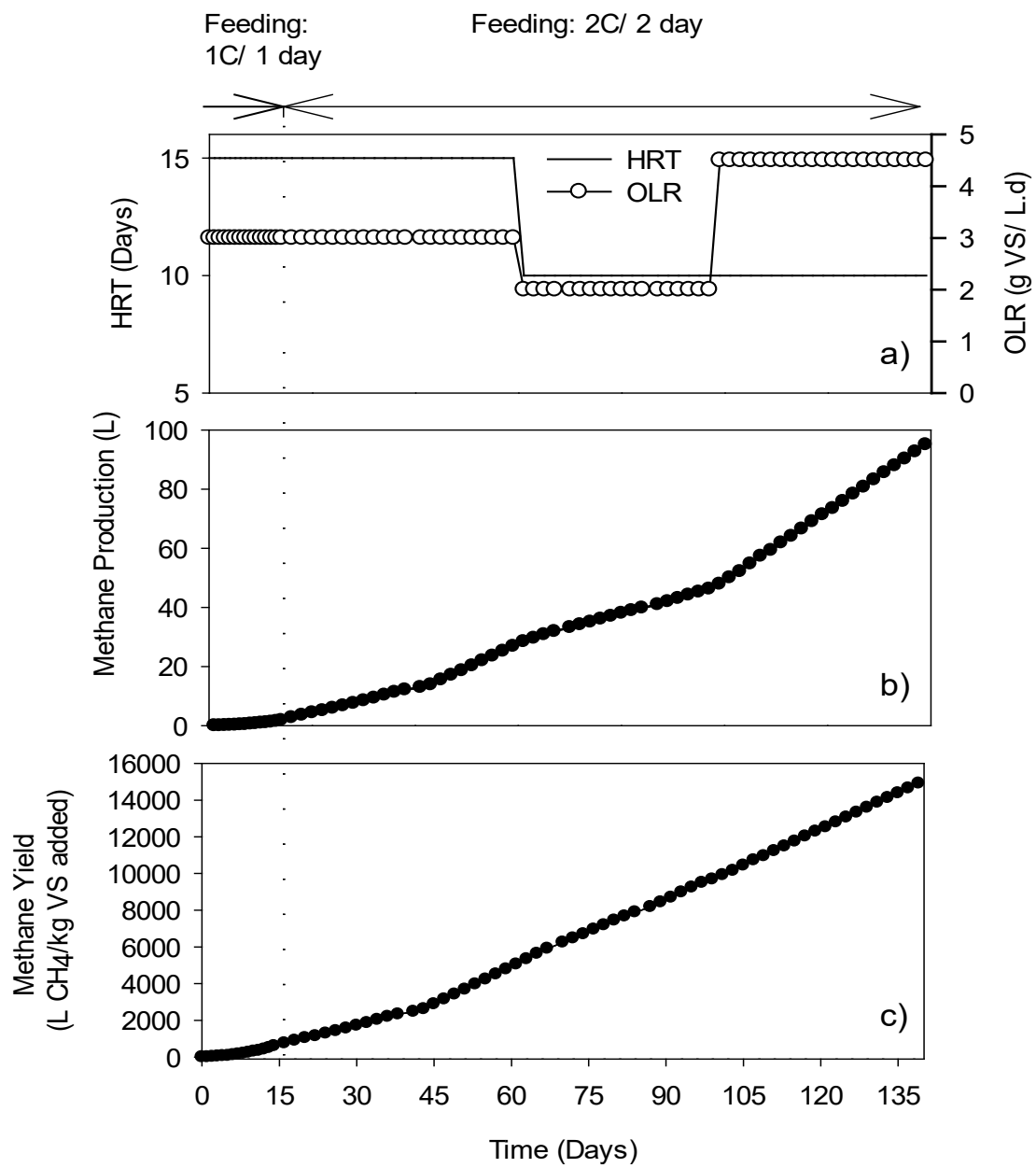


Figure 5.2 The a) operational conditions applied, b) cumulative methane production, c) cumulative methane yield of Reactor-1

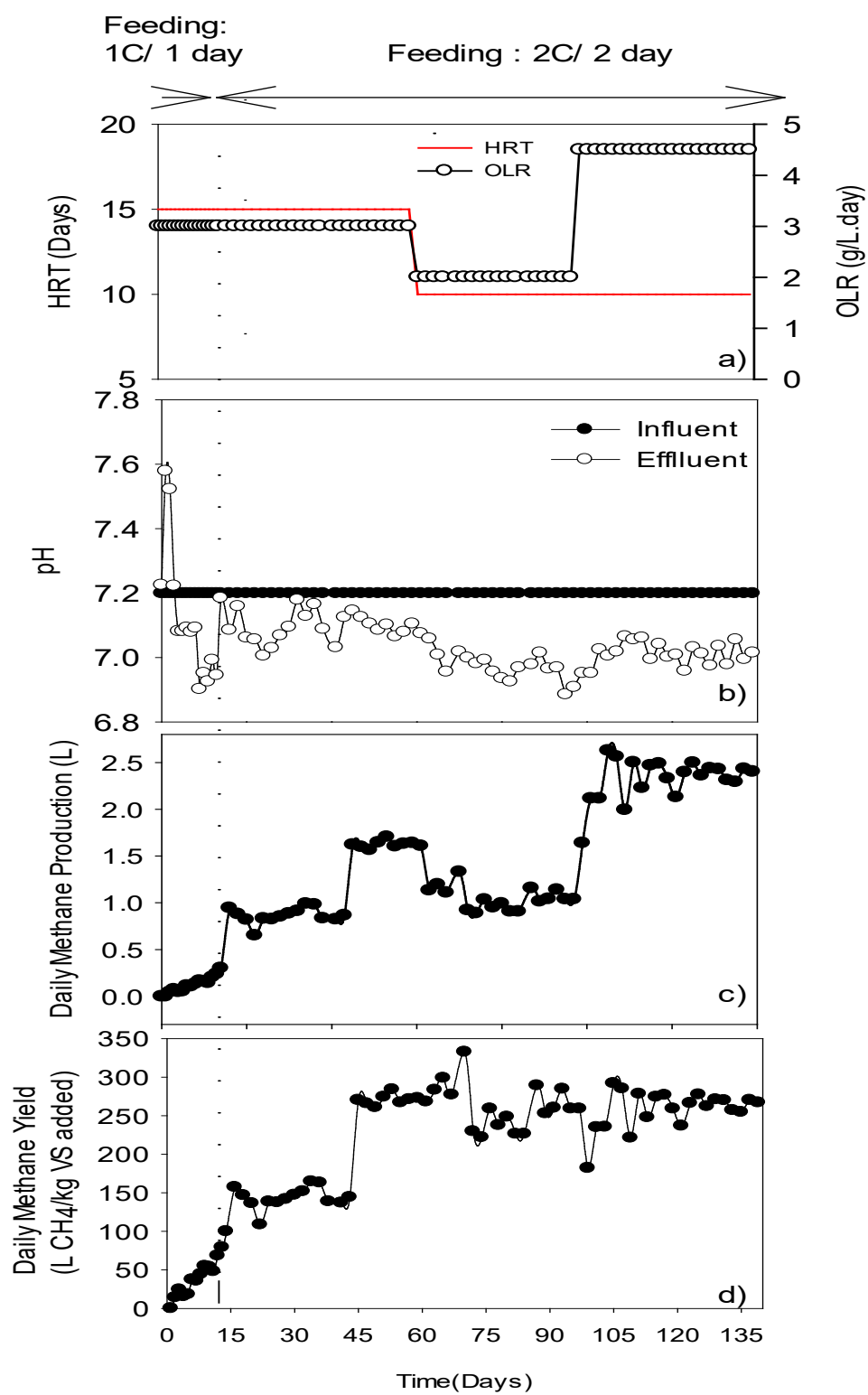


Figure 5.3 The a) operational conditions applied, b) pH, c) daily methane production, d) daily methane yield of Reactor-1

Table 5.4 Some researches with lignocellulosic biomass and various types of manures in semi-continuous co-digestion reactors

| Feedstocks | OLR (g VS/L. d) | HRT (days) | Highest Methane Yield (L/kg VS _{added}) | C/N ratio | References |
|--|------------------|--|---|-----------|---------------------|
| Chicken manure and corn stover | 1, 2, 3, 4 | 22-25 | 223 (OLR:4) | 25 | Y. Li et al., 2014 |
| Sugar beet and pig manure (PM) | 4.2-12.8 | 20-5 | 362 (OLR:7.4, HRT:12) | | Aboudi et al., 2015 |
| Rice straw (RS) and pig manure (PM) | 3-12 | 19+1 | 227 (OLR:3-8) | | D. Li et al., 2015 |
| Cow manure and sugar beet | 2.9-6.2 | 20(mono digestion) 15(co-digestion) | 313 (OLR:4.97, HRT:15) | | Aboudi et al., 2016 |
| Horse manure and grass | 1.25, 1.88, 2.50 | 15(start-up period:7 days) | 382 (OLR:1.88) | | Wangliang Li, 2016 |
| Goose manure and wheat straw | 1.5, 3, 4.5 | 10 | 254 (OLR:3, C/N:25) | 20-25 | Hassan et al., 2017 |
| Chicken manure and corn stover or apple pulp | 2.4,4.8,7.2,9,6 | 50, 25, 16.7, 12.5 | 340 (OLR:4.8, HRT:25) | 25 | K. Li et al., 2018 |

| Feedstocks | OLR (g VS/L. d) | HRT (days) | Highest Methane Yield (L/kg VS _{added}) | C/N ratio | References |
|---|------------------|------------|---|-----------------|--------------------|
| Chicken litter, food waste and wheat straw or hay grass | 2-3 ^a | 20 | 238 (OLR:2) | 20 | Zahan et al., 2018 |
| Raw CC and Digestate | 3, 2, 4.5 | 15, 10 | 270 (OLR:3, HRT:15) | 85 ^b | This study |
| HP_150 CC and Digestate | 3, 2, 4.5 | 15, 10 | 392 (OLR:4.5, HRT:10) | 85 ^b | This study |

^a OLR unit is g TS/L. d

^b This value is COD/N ratio, and the optimum COD/N ratio ranges from 50 to 143 (Speece, 1996)

There is a sequence in the degradation of organic substances during anaerobic digestion, this order is carbohydrate, protein and lipid (Morales-Polo et al., 2018). The sudden increase in daily methane yield despite no changes made in operational conditions might be due to the increased solubilization of the accumulated carbohydrate. It should be noted that effluent sCOD concentration increased from 2843 to 3707 mg/L between Days 35-43 in Reactor-1 (Figure 5.4b). Methane production might have improved since the concentration of soluble organic content increased during Days 6-45 (HRT: 15 days, OLR:3 g VS/L.d), indicating higher solubilization. Influent pH value was fixed at 7.2, and effluent pH value was measured in the range of 6.9 to 7.2, which were in the optimum pH range for methanogens (Figure 5.3a). Influent TAN concentration was determined as 112 mg/L and effluent TAN concentration range was 363- 648 mg/L in Reactor-1 during first 60 days, which were in the range of optimum TAN for anaerobic digestion (Figure 5.4c). The removal of VS was in the range of 58-70 % for 60 days.

At an HRT of 10 days and OLR of 2 g VS/L.d, daily methane yield of Reactor-1 was recorded as 260 L CH₄/kg VS_{added} at steady state conditions (Days 61-97) (Figure 5.3c). As Table 5.4 shows, this result was comparable with the literature. However, fluctuations were observed in methane yield. When a high daily methane yield (of 333 L CH₄/kg VS_{added}) was observed on Day 70, it is seen that average sCOD concentration decreased from 4050 to 3097 mg/L between Days 63-70. This result showed that the soluble biodegradable component were used resulting in high methane production on Day 70. During these operational conditions (HRT:10 days, OLR:2 g VS/L.d), average influent pH value was 7.2, and average effluent pH value was measured in the range of 6.9 to 7.1 (Figure 5.3a). Average influent TAN concentration was 196 mg/L and average effluent TAN concentrations were in the range of 180 - 648 mg/L (Figure 5.4c). The VS removal range was determined as 13-58 % between Days 61 to 97.

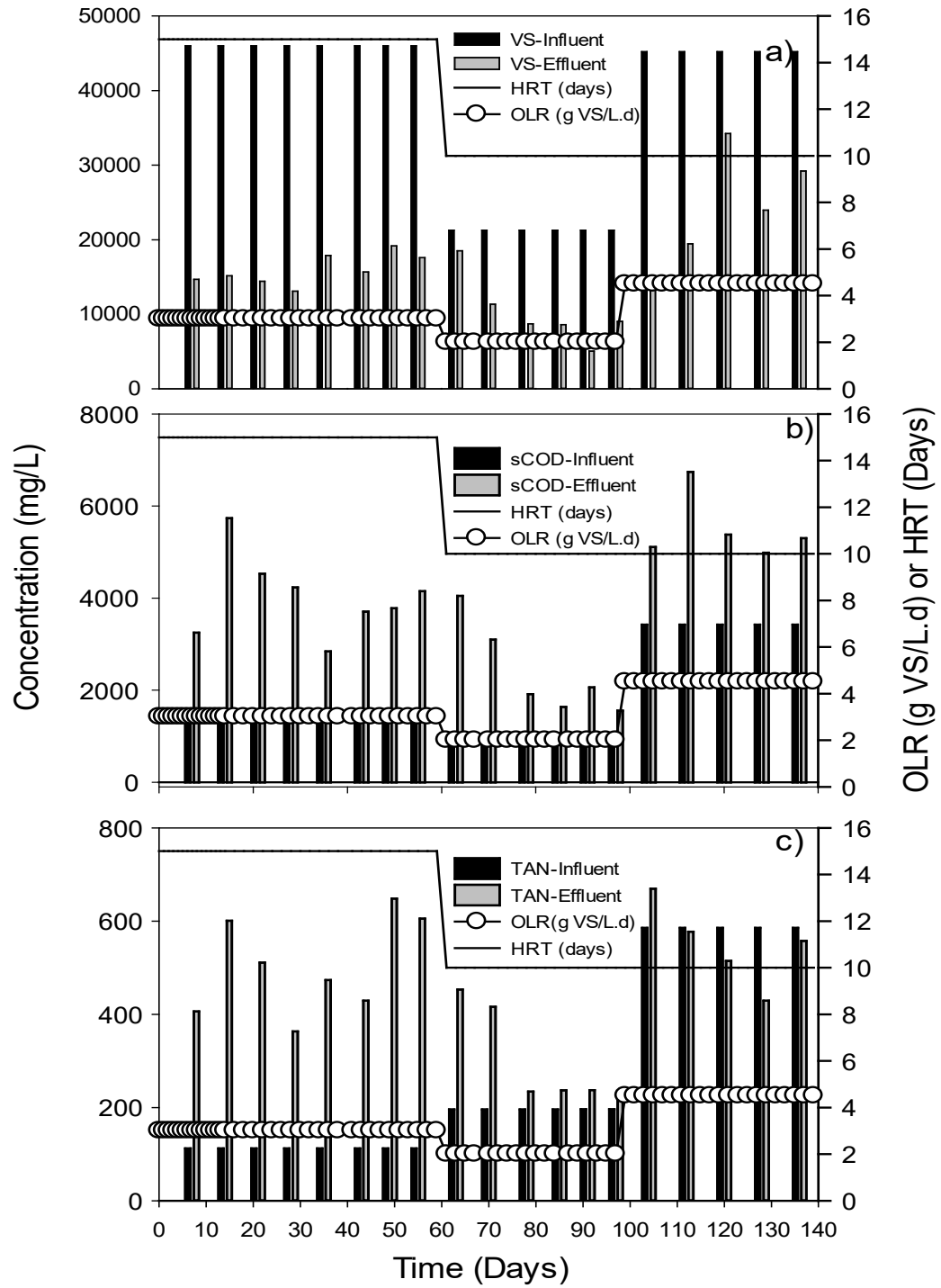


Figure 5.4 The average influent and effluent concentrations of a) VS, b) sCOD, c) TAN of Reactor-1

The VS removal was very low as 13 % on Day 63 compared to other VS removal values at the very beginning of these operational conditions. The reason for low VS removal could be that the system was not yet acclimated to new operational parameters of 10 day-HRT.

When OLR was increased from 2 to 4.5 g VS/L.d (on Day 97), the methane yield of Reactor-1 reached 265 L CH₄/kg VS_{added} at steady state conditions at an HRT of 10 days and OLR of 4.5 g VS/L.d (Days 97-139) (Figure 5.3c). The fluctuations in the methane yield were observed at the beginning of these operational conditions, which were attributed to the acclimation of the system to new OLR. After a while, Reactor-1 adapted as seen in Figure 5.3c, and daily methane production reached an average of 2.3 L. During Days 97-139, effluent pH value was in the range of 6.9 to 7.1 (Figure 5.3b). Average influent TAN concentration was 551 mg/L and effluent TAN concentrations were in the range of 266 - 624 mg/L (Figure 5.4c). The VS removal was determined in the range of 24 % to 70 % between Days 97-139.

For three different operational conditions studies, VS removal of Reactor-1 was comparable with literature (Table 5.4). The VS removal of sugar beet and pig manure in a semi-continuous co-digestion reactor was found as 68% at an HRT of 12 days and OLR of 4.5 g VS/L.d (Aboudi, et al., 2015). Zahan et al. (2018) studied with chicken litter (60%) and food waste (40%), and the VS removal was determined as 45.8%. Y. Li et al. (2014) found the VS removal of chicken manure and corn stover mixture in a semi-continuous reactor in the range of 55% to 79 % at OLR of 1-4 days.

The average methane content of biogas was measured in the range of 54% to 58 % after Days 20 (Figure 5.5). The fluctuations were observed almost for the first 20 days, after that, acclimation increased and the methane yield and methane content reached almost steady state conditions (Figure 5.3c and Figure 5.5). It should be noted that changing the feeding regime might have positively affected the system, and led to the achievement of steady state conditions sooner. D. Li et al. (2015) found that the methane content was 40-

60%, and in this study was in the range of 54-58 %, similar to that study. Thus, it was observed that methane content in this study was comparable with the literature.

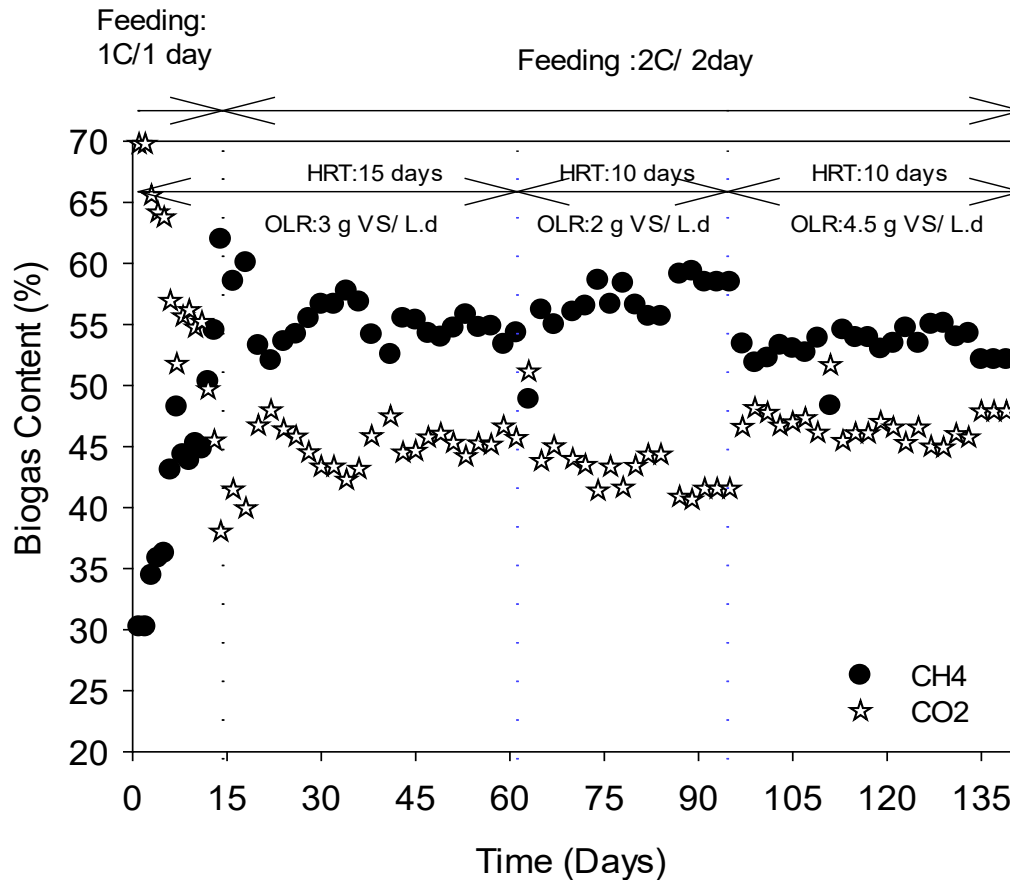


Figure 5.5 Methane and carbon dioxide content of biogas for Reactor-1

5.3.2 Methane Potential of Reactor-2

Among pretreatment applications, the optimum one was selected as HP_150 in Chapter-4. Therefore, in this study, HP_150 CC was used. Yet, in Chapter-4 co-digestion was not studied. But, HP_150 CC and Digestate batch test reactors were operated individually (Chapter-4 and Chapter-3, respectively). T-HP-150 CC reactors achieved 158 mL CH₄/gVS methane yield, T-Digestate reactors did not produce significant amount of methane. As abovementioned, the methane yield of HP_150 CC+Digestate

in batch reactor is not known. Therefore, Reactor-2 results would give the BMP yield of corncob, possible to have the optimum co-digestion and pretreatment conditions.

Reactor-2 was operated at operational conditions similar to that of Reactor-1. The HRT of 15 days was applied at an OLR of 3 g VS/L.d. Then HRT was decreased as 10 days, and OLR of 2 g VS/L. d and 4.5 g VS/L.d was applied at this operational condition(Figure 5.6a).

As seen in Figure 5.6a, the feeding regime was daily for the first 29 days. After the 29th day, Reactor-2 was fed once-in-every-two-days, yet with 2 times concentrated feed, owing to pandemic period. Reactor-2 achieved 136 L CH₄/kg VS_{added} average daily methane yield at an HRT of 15 and an OLR of 3 g VS/L.d at steady stated conditions (Figure 5.7d). The daily methane yield of Reactor-2 gradually increased and reached 220 L CH₄/kg VS_{added} on Day 29. Yet, after feeding regime was changed from daily to once-in-every-two-days, methane yield decreased by 180 L CH₄/kg VS_{added} on Day 31, suddenly. The changing feeding regime, once-in-every-two-days, meant that the VS content of feed doubled. Yet, HP_150 CC might include some by-products, i.e., xylose, acetic acid, trace amount furfural, glucose, oligomer, glucan based-sugar, etc., produced during HP_150 method applied. Some by-products can improve methane production such as glucose and oligomer while some of them, namely xylose and furfural, might affect the methane production negatively, and these components can be called inhibitory by-products (Wang et al., 2018). These by-products were increased due to 2 times concentrated feed. The methane yield decreased and averaged at 136 L CH₄/kg VS_{added} between Days 31-60 (Figure 5.8b). This could be attributed to microorganisms that might have not acclimated to some inhibitory by-products produced during HP_150 application. It should be noted that in Chapter-4, HP application at 150-210°C was thought to be not producing inhibitory by-products. Yet, having similar yields despite different temperatures applied might have indicated potential inhibitory by-products. When methane yields of Reactor-1 and Reactor-2 are compared, it is seen that methane

yield of Reactor-1 was 50% higher than methane yield of Reactor-2. The reason of 50% lower methane yield in Reactor-2 could be attributed to the accumulation of some inhibitory by-products due to the changing feeding regime (2 times concentrated feed), and microorganisms that might have not been acclimated to these products yet.

The stability of reactors can be observed with pH values, which is used as an indicator of the reactors (D. Li et al., 2015). The average influent pH value was arranged as 7.2, and average effluent pH was in the range of 7 to 7.5 during the first 60 days (Figure 5.7b). Average effluent TAN concentration was in the range of 822 mg/L - 598 mg/L for 60 days at an HRT of 15 and an OLR of 3 g VS/L.d (Figure 5.8c).

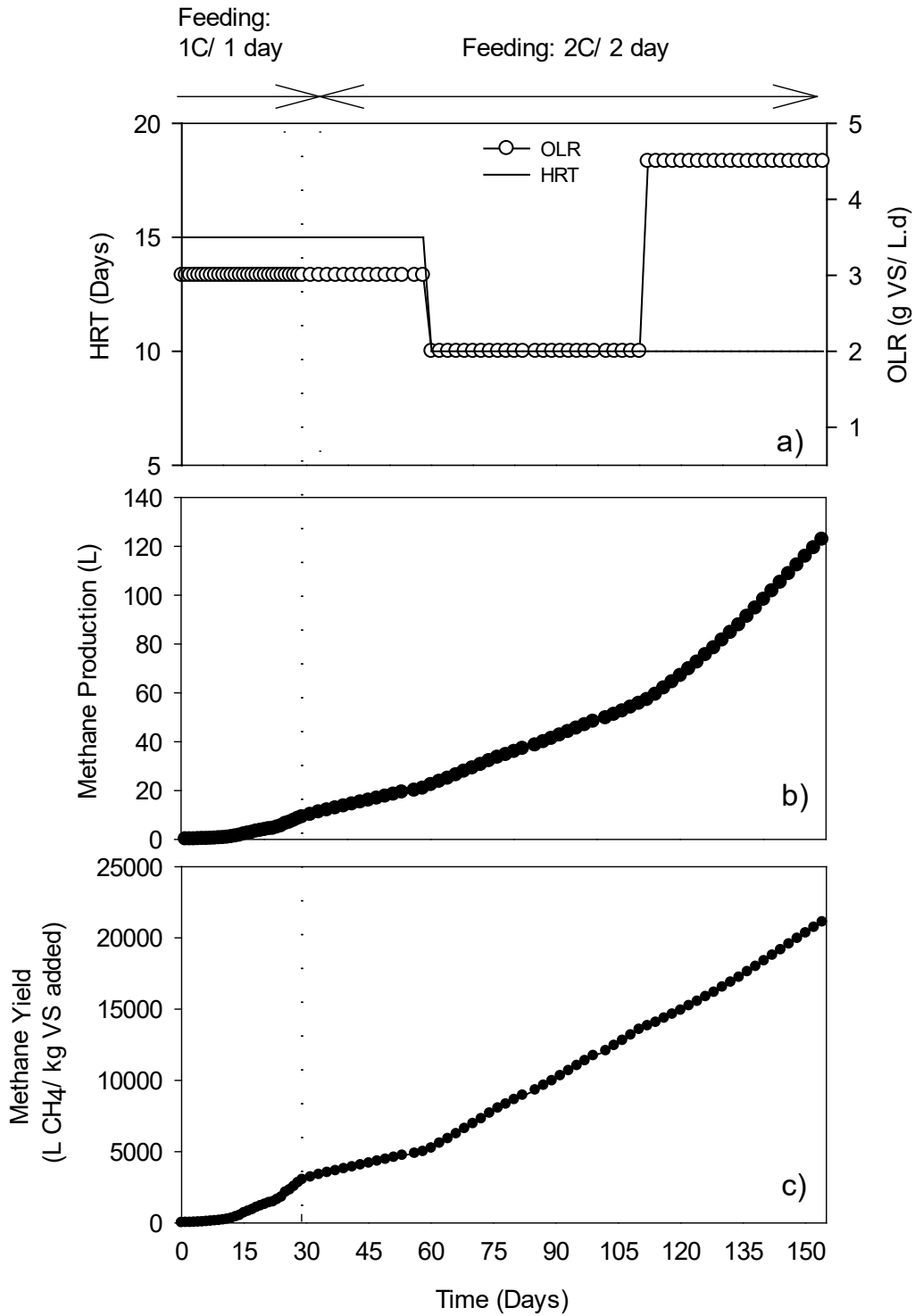


Figure 5.6 The a) operational conditions applied, b) cumulative methane production, c) cumulative methane yield of Reactor-2

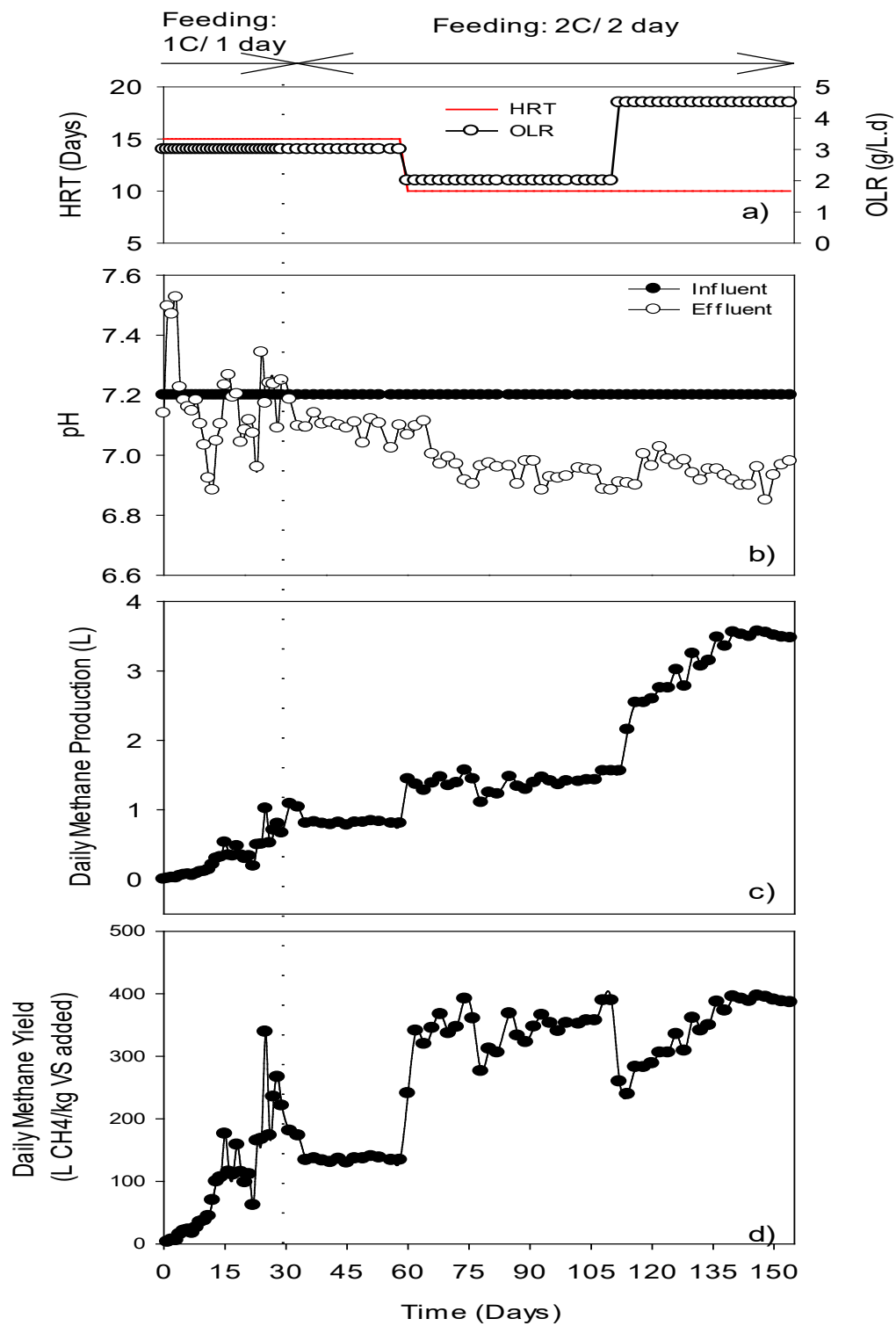


Figure 5.7 The a) operational conditions applied, b) pH, c) daily methane production, d) daily methane yield Reactor-2

The operational conditions of 10-day HRT and 2 g VS/L.d OLR were applied for 52 days (Days 60-112). At steady-state condition, the average daily methane yield reached 362 L CH₄/kg VS_{added} between Days 91-112 (Figure 5.7d). The methane yield of Reactor-2 was positively affected after the change in the operational condition. As mentioned previously, one of the feedstocks was HP_150 CC in Reactor-2, and HP_150 method contributed by improving the accessibility and solubility of cellulose and hemicellulose (Wang et al., 2018). Thus, Reactor-2 included more soluble component than Reactor-1 in order to produce more methane. Moreover, methane yield of Reactor-2 was 40% higher than methane yield of Reactor-1 during this operational condition in steady state conditions. Apparently, at this period, Reactor-2 seemed to acclimated to the potential inhibitory by-products. pH was measured in the range of 6.9 to 7.1 during Days 60-112 at an HRT of 10 days and an OLR of 2 g VS/L.d. Average TAN concentration range of the effluent were found between 180 mg/L to 457 mg/L between Days 60-112. Lastly, the removal of VS was determined in the range of 46% to 85%, which is higher than that of Reactor-1 (13-58 %), as expected.

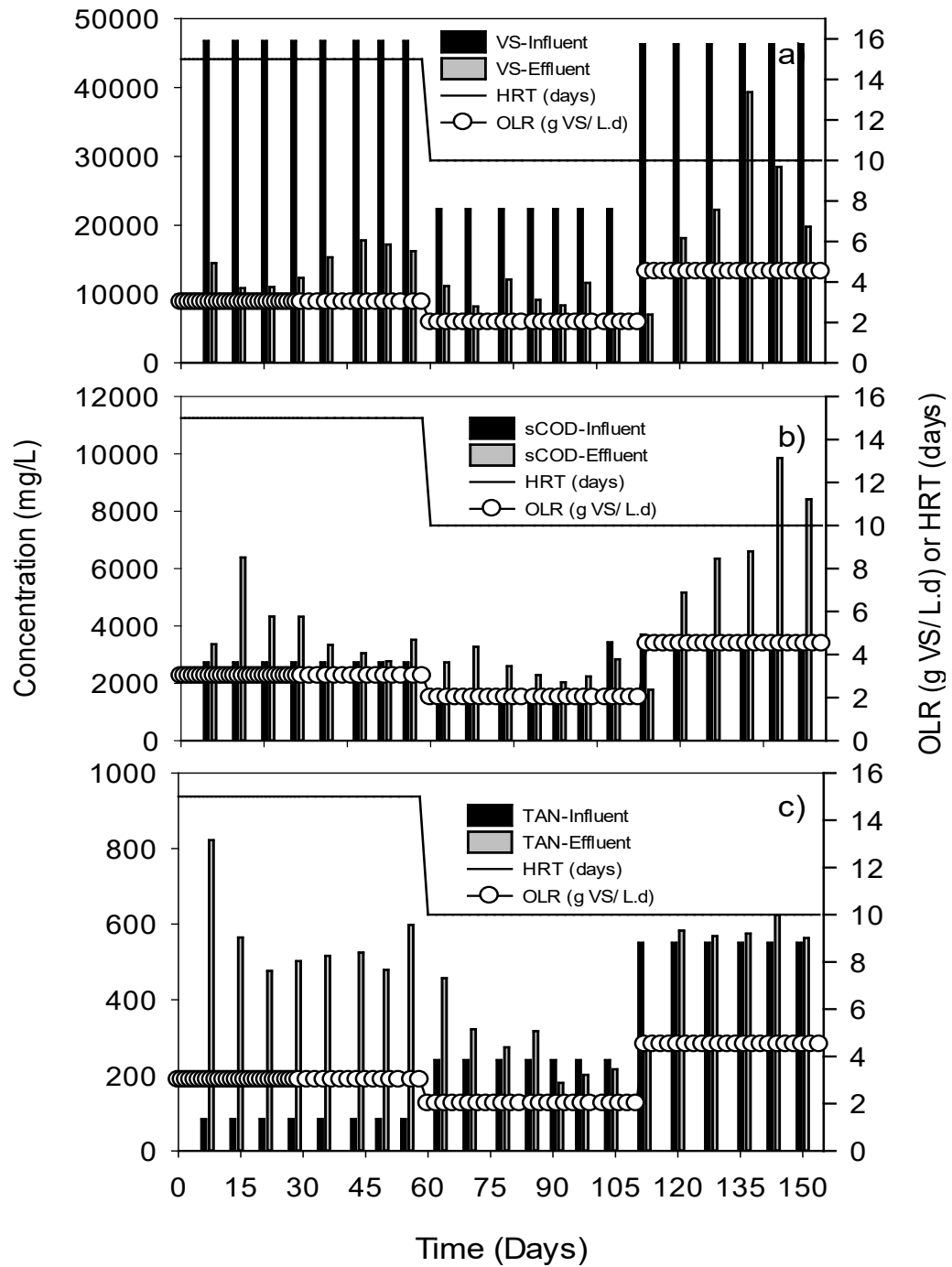


Figure 5.8 The average influent and effluent concentrations of a) VS, b) sCOD, c) TAN of Reactor-2

HRT of 10 days and OLR of 4.5 g VS/L.d was the last operation conditions applied during Days 112-154. Although fluctuations were noticed at the beginning of this period, steady-state conditions were reached after Day 136. The methane yield of Reactor-2 was determined as 392 L CH₄/kg VS_{added} at steady-state conditions (Days 136-154) (Figure 5.7d). In addition to fluctuation, a serious decrease in methane yield was observed at the beginning of this period. The reason of this situation might be the increase in OLR from 2 to 4.5 g VS/L.d. Although average effluent sCOD concentration was 1773 mg/L on Day 112, it increased and reached to a range of 5160 to 6597 mg/L between Days 120-136. This also indicates the acclimation of the system to new operational conditions because accordingly, the daily methane production and yield gradually increased and reached to 3.5 L and 392 L CH₄/kg VS_{added}, respectively. Daily methane yield averaged at 392 L CH₄/kg VS_{added} during Days 136-154. When Reactor-1 and Reactor-2 were compared, methane yield of Reactor-2 was found to be 48% higher than the methane yield of Reactor-1. It should be noted that the significant factor of obtaining almost 50% higher methane yield in Reactor-2 is that HP_150 method was used on corncob. Thus, HP_150 method seems suitable for corncob for this operational condition. During this period (Days 112-154) pH was measured in the range of 6.8 to 7.0, which is still suitable for methanogens. TAN concentration ranges of effluent were 266-624 mg/L. The VS removal was calculated in the range of 38% to 61%.

The methane concentration of biogas was measured in the range of 55% to 60% (Figure 5.9). After first 30 days, methane content reached steady-state conditions. Reactor-1 reached steady state conditions quicker than Reactor-2. After changing the feeding regime from daily to once-in-every-two-days, yet 2 times VS concentration of feed, Reactor-1 was positively affected the feeding regime (after Day 14) while the methane yield of Reactor-2 decreased after Day 29 (at feeding regime of once-in-every-two-days). Thus, although Reactor-1 reached steady-state conditions after Day 20, Reactor-2 reached steady-state conditions after Day 30.

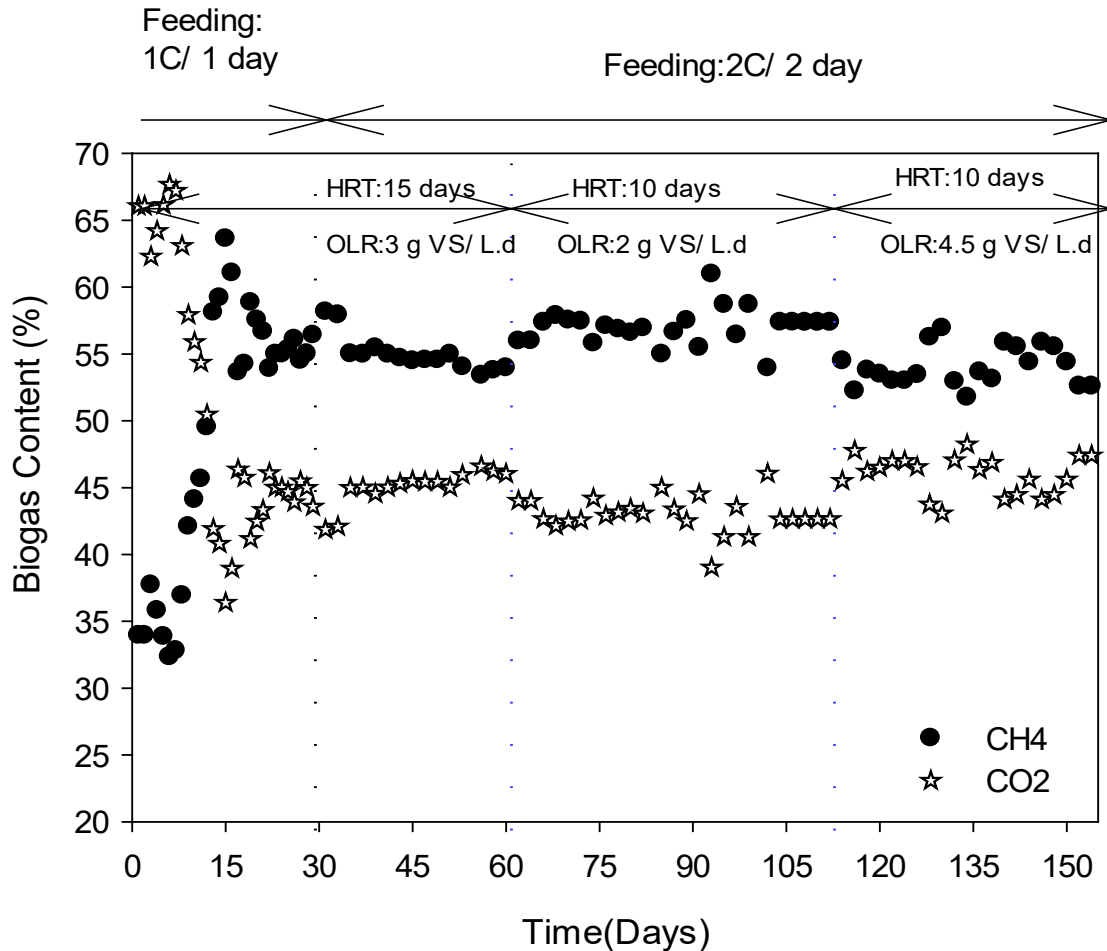


Figure 5.9 Methane and carbon dioxide content in the biogas for Reactor-2

The differences in Reactor-1 and Reactor-2 are presented in Table 5.5. At HRT of 15 days and OLR of 3 g VS/L.d, methane yield of Reactor-1 was higher than that of Reactor-2 at steady state conditions when feeding regime was once in every two days. However, before that, when daily feeding was applied, much higher yields were obtained in Reactor-2. Reactor-2 reached average methane yield of 240 mL CH₄/g VS_{added} between Days 25 to 29 in daily feeding regime but methane yield decreased by 104 mL CH₄/g VS_{added} after changing the feeding regime. Reactor-2 might have been negatively affected due to the change in feeding regime. As mentioned previously, this could be attributed to the accumulation of some inhibitory by-products due to the changing

feeding regime (2 times concentrated feed), and microorganisms might have not been acclimated to these.

Table 5.5 Comparison of the average methane yield of the Reactors

| Methane Yield (mL CH ₄ /g VS _{added}) | | | | |
|--|------------------------------------|---|---|---|
| | HRT: 15 days OLR:3 g VS/L.d | | HRT: 10 days OLR: 2 g VS/L.d | HRT: 10 days OLR: 4.5 g VS/L.d |
| | Daily feeding regime (1C/ 1day) | Every two days feeding regime (2C/ 2 day) | Every two days feeding regime (2C/ 2 day) | Every two days feeding regime (2C/ 2 day) |
| Reactor-1 | 100 ^a | 270 ^b | 260 ^b | 265 ^b |
| Reactor-2 | 240 ^a | 136 ^b | 362 ^b | 392 ^b |

^a This is average value.

^b This value was obtained at steady-state condition

Despite the decrease in OLR from 3 to 2 g VS/L.d, the decrease in HRT from 15 to 10 days resulted in decrease in methane yield of Reactor-1 since there was not enough solubilization at an HRT of 10 days. However, the similar application led to the increase in methane yield of Reactor-2 because the substrate which pretreated (HP_150 CC) had already higher sCOD content. At an HRT of 10 days, when the OLR increased from 2 g VS/L.d to 4.5 g VS/L.d, methane yield of Reactor-2 increased from 362 mL CH₄/g VS_{added} to 392 mL CH₄/g VS_{added}, and methane yield of Reactor-1 almost did not change for not having enough solubilization. In the other words, anaerobic digestion of Raw CC requires more than 15 days of HRT. But, when HP_150 method is applied, HRT of 10 days seem to be profitable. This means smaller reactor volume and lower capital cost.

Results also clarified that the highest methane yield was achieved with Reactor-2 (392 mL CH₄/g VS_{added}) at HRT of 10 days and OLR of 4.5 g VS/L.d. Therefore, despite the

change in feeding regime, anaerobic digestion of HP_150 CC resulted in almost 50% increase in methane yield in semi-continuous reactors.

5.3.3 Mini Energy Analysis of Reactor-2

The important properties of the suitable pretreatment method are less energy requirement, cost-effectiveness and less waste generation (Kumari and Singh, 2018; Taherzadeh and Karimi, 2008). If the energy usage during pretreatment is higher than the produced energy during digestion, this is not an applicable method. Reactor-2 had 392 mL CH₄/g VS_{added}, which was the highest methane yield, at HRT of 10 days and OLR of 4.5 g VS/L.d. One of the feedstocks was HP_150 CC in Reactor-2. Energy analysis of HP_150 method was done by using Equation 5.1 and Equation 5.2 (Table 5.5). This analysis was done to observe whether used pretreatment method was suitable or not. According to Table 5.5, net energy (E_{net}) of Reactor-2 was determined slightly (almost 10%) higher than Reactor-1. Therefore, HP_150 method seems to be suitable application for corncob pretreatment.

Table 5.6 Energy balance between using and production of energy

| Operational Condition | Reactors | E _i ^{a,b} | E _o ^{a,c} | E _{net} ^{a,d} |
|-----------------------|-----------|-------------------------------|-------------------------------|---------------------------------|
| HRT:10 days | Reactor-1 | N.A ^e | 9.5 | 9.5 |
| and OLR:4.5 g VS/L.d | Reactor-2 | 3.5 | 14 | 10.5 |

^a Unit is MJ/kg VS

^b E_i means input energy value.

^c E_o means output energy value.

^d E_{net}=E_o-E_i

^e Not applicable

5.4 Conclusions

It was aimed to investigate the optimum operational conditions, i.e., HRT and OLR, leading to the maximum methane production during the co-digestion of Raw CC or HP_150 CC with Digestate in semi-continuous reactors.

At HRT of 15 days and OLR of 3 g VS/L.d, Reactor-1 reached higher methane yield (270 L CH₄/kg VS_{added}) than that of Reactor-2 (136 L CH₄/kg VS_{added}) at steady state conditions. The potential inhibitory by-products of HP_150 CC might create negative effect on methane yield of Reactor-2 because in this period, the daily feeding regime was changed once-in-every-two-days, yet with 2 times concentrated feed.

The highest methane yield of 392 mL CH₄/g VS_{added} was observed at HRT of 10 days and OLR of 4.5 g VS/L.d in Reactor-2. Moreover, methane yield of Reactor-2 was 48% higher than Reactor-1 in this operational period. Therefore, HP_150 method seems to be suitable for the pretreatment of corncob to enhance its anaerobic digestion and improve methane production.

The net energy value of Reactor-2 was found as 10.5 MJ/kg VS, which is almost 10% higher than the net energy value of Reactor-1. Thus, HP_150 method was also determined as feasible method at HRT of 10 days and OLR of 4.5 g VS/L.d.

Finally, anaerobic digestion of Raw CC requires more than 15 days of HRT. But, when HP_150 method is applied, HRT of 10 days seem to be profitable. This means smaller volume and lower capital cost in addition to the 10% higher net energy value.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

This thesis study aims to investigate the BMP of corncob and its enhancement by using co-digestion and pretreatment approaches. In addition, it was also aimed to investigate anaerobic co-digestion of corncob and the effect of different operational conditions on methane yield in semi-continuous reactors.

The main results are given as follows:

- This thesis study indicated that raw corncob (Raw CC), which is hardly biodegradable, can be an alternative energy source. BMP of corncob is found as 103 mL CH₄/g VS.
- Application of co-digestion has a synergistic effect if the co-substrate is Digestate or Post-Digestate. Digestate and Post-Digestate increased methane yield of corncob by 67% and 57%, respectively. This can be attributed to the presence of already acclimated microorganisms found in Digestate and Post-Digestate with a lignocellulosic biomass like poppy.
- All pretreatment applications used were not entirely useful methods to improve biodegradability.
 - The hydrothermal, alkaline and alkaline hydrothermal pretreatment were investigated as pretreatment applications.
 - The hydrothermal pretreatment application was selected as an optimum method. Methane yields of hydrothermal pretreated corncobs were in the range of 158-162 mL CH₄/g VS which were 55-61% higher than that of Raw CC. Three different temperatures, 150°C, 180°C and 210°C, were used for pretreatment application. Hydrothermal pretreatment at 150°C (HP_150) was selected because methane yields of hydrothermal

pretreated corncob at different temperatures were similar; thus, unnecessary energy use can be avoided.

- Although the solubility of the raw corncob was increased in the range of 9- to 26-fold after alkaline and alkaline hydrothermal pretreatment applications, methane yield of these reactors decreased in the range of 67-75% compared to Raw CC. It can be attributed to the formation of inhibitory by-products, furfural, arabinose, xylose, xylooligosaccharides, formic acid, during alkaline and alkaline hydrothermal pretreatment application and these could repress the methanogens.
- The optimum pretreatment application (HP_150) and co-substrate (Digestate) was used to investigate the highest methane yield in semi-continuous reactors. Methane yield of Raw CC+Digestate and HP_150 CC+Digestate was compared to observe the effect of pretreatment on optimum operational conditions.
 - HP_150 CC+Digestate reactor seems to be suitable in terms of having the highest methane yield.
 - Raw CC+Digestate reactor achieved the highest methane yield (270 L CH₄/kg VS_{added}) at an HRT of 15 days and OLR of 3 g VS/L.d among three operational conditions.
 - HP_150 CC+Digestate reached the highest methane yield (392 L CH₄/kg VS_{added}) at an HRT of 10 days and OLR of 4.5 g VS/L.d. The net energy value of HP_150 CC+Digestate was found as 10.5 MJ/kg VS, which is almost 10% higher than the net energy value of Raw CC+Digestate.
- Although anaerobic digestion of Raw CC requires more than 15 days of HRT, HP_150 CC method can be operated at an HRT of 10 days. In other words, HP_150 method provides smaller volume and lower capital cost in addition to the 10% higher net energy value.

Recommendations

- HP_150 CC+Digestate can be used in solid-state anaerobic digestion. Thus, the effects of both wet anaerobic digestion and solid-state anaerobic digestion can be compared.
- Enzymatic pretreatment method can be applied on Raw CC; thus, inhibitory by-products coming from lignin destruction might be prevented.
- The by-products produced during pretreatment application can be analyzed. Before anaerobic digestion, the optimum pretreatment method can be selected by using these results.
- Incineration is an alternative method to produce energy from corncob with adequate emission control systems. However, if incineration is to be applied, transportation should be also taken into consideration due to its cost which might be also the case for central anaerobic digesters. On the other hand, small-scale anaerobic digesters constructed in a farm or field would be eliminating the transport cost and be more economic than incineration while producing energy.

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APPENDICES

A. Preliminary Study to Find Optimum Parameter for Pretreatment Application

Table A.1: Effect of different liquid to solid ratio on sCOD value

| Liquid(mL) | Corncob (mg) | Liquid /Solid (mL/g) | sCOD(g/L) | sCOD(g/g) |
|------------|-----------------|-------------------------|-----------|-----------|
| 10 | 0.5 | 20 | 12.1 | 0.29 |
| 15 | 0.5 | 30 | 10.4 | 0.21 |
| 20 | 0.5 | 40 | 5.3 | 0.09 |

Table A.2: Effect of different NaOH concentration on sCOD value

| NaOH (%) | sCOD(g/L) | sCOD(mg/mg) | tCOD(mg/mg) | sCOD/tCOD (%) |
|-------------|-----------|-------------|-------------|------------------|
| 3 | 28.75 | 0.56 | 0.93 | 62.10 |
| 4 | 31.43 | 0.63 | 0.90 | 70.02 |
| 5 | 31.48 | 0.63 | 0.77 | 81.15 |
| 10 | 34.88 | 0.70 | 0.81 | 85.85 |
| 15 | 29.88 | 0.60 | 0.93 | 63.97 |
| 20 | 27.18 | 0.54 | 0.78 | 69.32 |

B. Reactor Configuration of BMP_Set-1 and BMP_Set-2

Table B.1 Reactor Configuration of BMP_Set-1

| | Reactors | Inoculum (mL) | Substrate (g) | Co-substrates (mL) | BM (mL) | Effective Volume(mL) |
|----------------|-------------------------|------------------|---------------|-----------------------|------------|-------------------------|
| | Control | 45 | - | - | 8.6 | 60 |
| | Control without BM | 45 | - | - | - | 60 |
| Mono-digestion | B-Raw CC | - | 0.8 | - | 8.6 | 60 |
| | B-Feed | - | - | 3.2 | - | 60 |
| | B-Digestate | - | - | 2.6 | - | 60 |
| | B-Post-Digestate | - | - | 2.6 | - | 60 |
| Co-digestion | B-Raw CC+Feed | - | 0.8 | 3.2 | - | 60 |
| | B-Raw CC+Digesate | - | 0.8 | 2.6 | - | 60 |
| | B-Raw CC+Post-Digestate | - | 0.8 | 2.6 | - | 60 |
| Mono-digestion | T-Raw CC | - | 0.8 | - | 8.6 | 60 |
| | T-Feed | 45 | - | 3.2 | - | 60 |
| | T-Digestate | 45 | - | 2.6 | - | 60 |
| | T-Post-Digestate | 45 | - | 2.6 | - | 60 |
| Co-digestion | T-Raw CC+Feed | 45 | 0.8 | 3.2 | - | 60 |
| | T-Raw CC+Digesate | 45 | 0.8 | 2.6 | - | 60 |
| | T-Raw CC+Post-Digestate | 45 | 0.8 | 2.6 | - | 60 |

B,T represents blank reactors and test reactors, respectively.

Table B.2 Reactor Configuration of BMP_Set-2

| | Reactors | Inoculum (mL) | Substrate (g) | Co-substrates (mL) | BM (mL) | Effective Volume(mL) |
|----------------|----------------------------|------------------|---------------|-----------------------|------------|----------------------|
| | Control | 45 | - | - | 8.6 | 60 |
| | Control without BM | 45 | - | - | - | 60 |
| Mono-digestion | B- AHP_240 CC | - | 0.8 | - | 8.6 | 60 |
| | B-Feed | - | - | 3.2 | - | 60 |
| | B-Digestate | - | - | 2.6 | - | 60 |
| | B-Post-Digestate | - | - | 2.6 | - | 60 |
| Co-digestion | B- AHP_240 CC+Feed | - | 0.8 | 3.2 | - | 60 |
| | B- AHP_240 CC+Digesate | - | 0.8 | 2.6 | - | 60 |
| | B-AHP_240CC+Post-Digestate | - | 0.8 | 2.6 | - | 60 |
| Mono-digestion | T- AHP_240 CC | - | 0.8 | - | 8.6 | 60 |
| | T-Feed | 45 | - | 3.2 | - | 60 |
| | T-Digestate | 45 | - | 2.6 | - | 60 |
| | T-Post-Digestate | 45 | - | 2.6 | - | 60 |
| Co-digestion | T-AHP_240 CC+Feed | 45 | 0.8 | 3.2 | - | 60 |
| | T-AHP_240 CC+Digesate | 45 | 0.8 | 2.6 | - | 60 |
| | T-AHP_240CC+Post-Digestate | 45 | 0.8 | 2.6 | - | 60 |

B,T represents blank reactors and test reactors, respectively.

C. Calibration Curves of Gas Measurement in GC

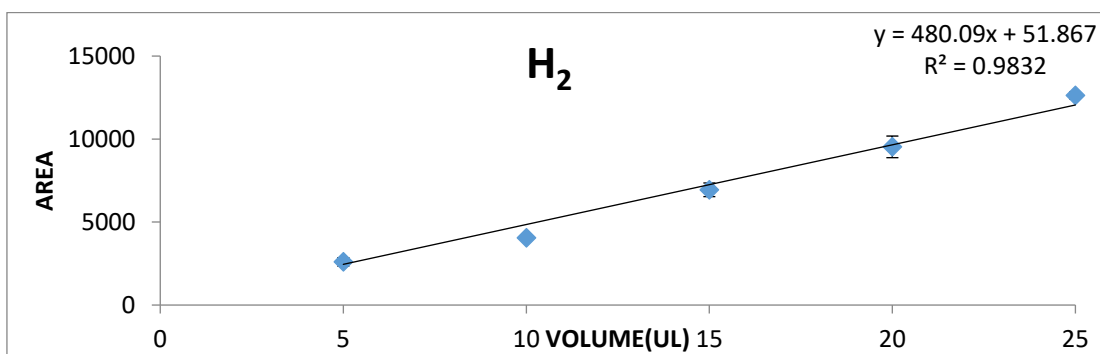


Figure C.1 Calibration curve for hydrogen

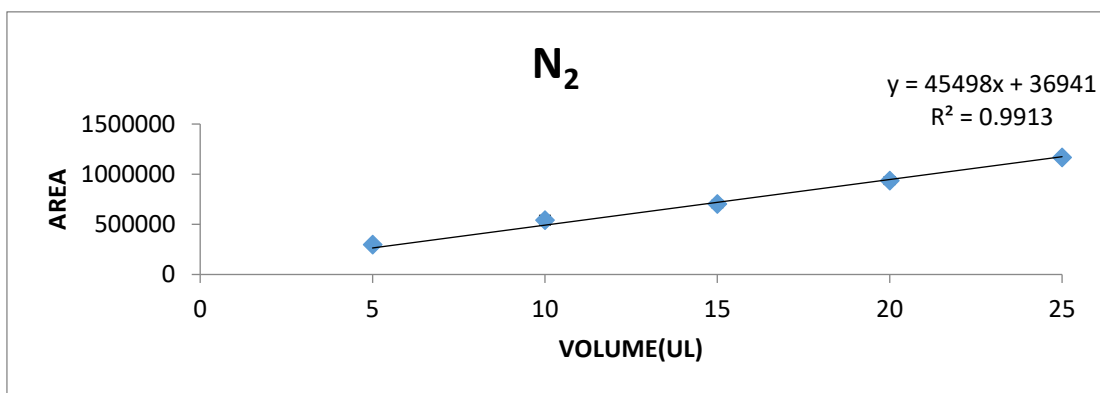


Figure C.2 Calibration curve for nitrogen

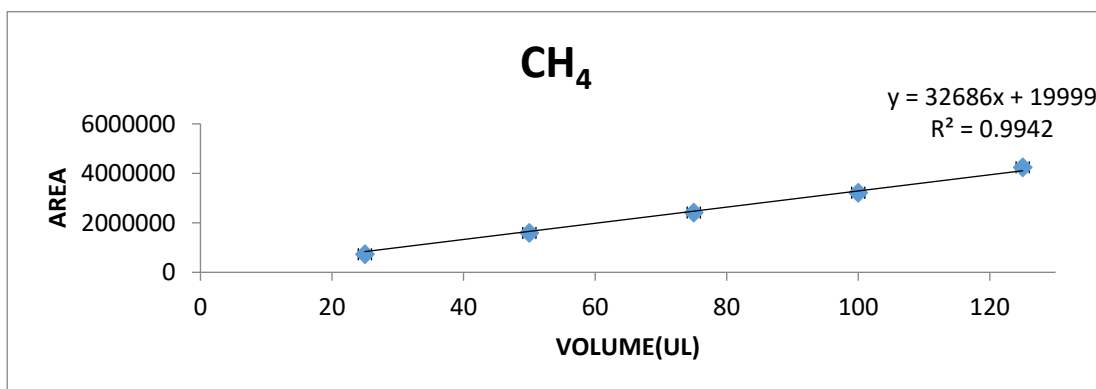


Figure C.3 Calibration curve for methane

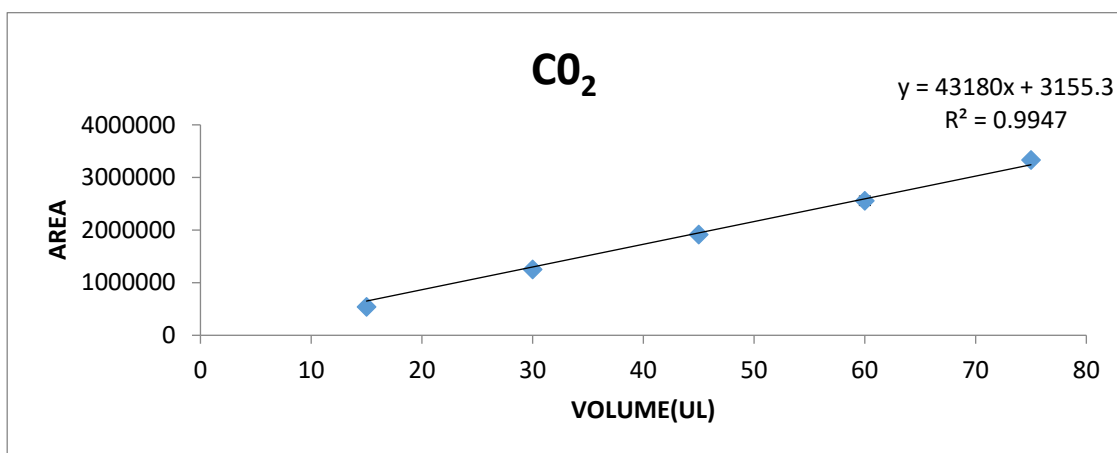


Figure C.4 Calibration curve for carbon dioxide

D. Reactors Characterization Results (Raw CC with/without co-substrate

Table D. Characterization Average Results of BMP_Set-1

| Reactors | TS (mg/L) | VS (mg/L) | sCOD (mg/L) | tCOD (mg/L) | TP (mg/L) | TAN (mg/L) | TKN (mg/L) | pH |
|-----------------------------|-----------|-----------|-------------|-------------|-----------|------------|------------|-----|
| Control-to | 29050 | 10700 | 1140 | 27050 | 22.7 | 525 | 465 | 7.0 |
| Control-tf | 25012 | 9987 | 1816 | 18750 | 13.0 | 611 | 615 | 8.2 |
| Control w/o BM-to | 29150 | 14975 | 819 | 27750 | 9.9 | 350 | 255 | 7.3 |
| Control w/o BM-tf | 21250 | 9875 | 840 | 13650 | 19.0 | 533 | 192 | 7.9 |
| B-Raw CC-to | 7450 | 3950 | 680 | 20450 | 9.7 | 220 | 154 | 7.1 |
| B- Raw CC -tf | 9383 | 3600 | 1724 | 13988 | 13.0 | 221 | 123 | 7.8 |
| B-Feed-to | 5825 | 3900 | 1861 | 50900 | 17.3 | 120 | 171 | 7.1 |
| B-Feed-tf | 4750 | 3500 | 1041 | 6400 | 3.0 | 387 | 182 | 7.6 |
| B-Digestate-to | 4775 | 3425 | 675 | 3750 | 7.9 | 198 | 434 | 7.3 |
| B-Digestate-tf | 4000 | 3350 | 738 | 1215 | 14.0 | 400 | 130 | 7.6 |
| B- Post-Digestate-to | 4250 | 2925 | 818 | 26700 | 8.3 | 121 | 95 | 7.2 |
| B-Post-Digestate-tf | 2537 | 2313 | 717 | 3088 | 10.0 | 261 | 123 | 7.7 |
| B- Raw CC+Feed-to | 8000 | 6729 | 1886 | 7800 | 5.2 | 145 | 182 | 7.1 |
| B- Raw CC+Feed-tf | 6533 | 5200 | 2886 | 5890 | 6.0 | 224 | 126 | 7.0 |
| B- Raw CC+Digestate-to | 8520 | 7780 | 564 | 12000 | 11.5 | 145 | 98 | 7.4 |
| B- Raw CC +Digestate-tf | 5743 | 4829 | 1225 | 6950 | 6.0 | 216 | 31 | 6.9 |
| B- Raw CC+Post-Digestate-to | 5575 | 4450 | 375 | 44900 | 14.6 | 85 | 244 | 7.2 |
| B- Raw CC+Post-Digestate-tf | 4332 | 3938 | 1524 | 13950 | 22.0 | 165 | 41 | 6.9 |
| T- Raw CC -to | 40975 | 19975 | 1823 | 44950 | 17.7 | 362 | 580 | 7.1 |
| T- Raw CC-tf | 29675 | 13425 | 1673 | 29838 | 19.0 | 419 | 375 | 7.9 |
| T-Feed-to | 24750 | 11250 | 2285 | 71350 | 12.5 | 439 | 294 | 7.2 |
| T-Feed-tf | 22612 | 9787 | 895 | 11363 | 12.0 | 856 | 386 | 7.9 |

| Reactors | TS (mg/L) | VS (mg/L) | sCOD (mg/L) | tCOD (mg/L) | TP (mg/L) | TAN (mg/L) | TKN (mg/L) | pH |
|-----------------------------|-----------|-----------|-------------|-------------|-----------|------------|------------|-----|
| T-Digestate-to | 39600 | 17125 | 795 | 63200 | 8.4 | 484 | 468 | 7.4 |
| T-Digestate-tf | 23512 | 10775 | 1346 | 19750 | 17.0 | 544 | 431 | 8.1 |
| T- Post-Digestate-to | 32175 | 14350 | 1038 | 115400 | 23.3 | 518 | 437 | 7.2 |
| T-Post-Digestate-tf | 24150 | 10700 | 1293 | 34450 | 15.0 | 352 | 328 | 8.1 |
| T- Raw CC+Feed-to | 28650 | 15525 | 2804 | 103550 | 30.1 | 397 | 566 | 7.1 |
| T- Raw CC+Feed-tf | 25637 | 15075 | 1107 | 21225 | 13.0 | 512 | 536 | 7.6 |
| T- Raw CC+Digestate-to | 41020 | 25300 | 1656 | 117100 | 16.2 | 472 | 423 | 7.3 |
| T- Raw CC+Digestate-tf | 23254 | 11672 | 1336 | 19000 | 29.0 | 419 | 323 | 7.4 |
| T- Raw CC+Post-Digestate-to | 41527 | 25143 | 1432 | 88000 | 34.7 | 403 | 454 | 7.3 |
| T- Raw CC+Post-Digestate-tf | 27775 | 13988 | 1279 | 21975 | 30.0 | 472 | 433 | 7.4 |

E. Reactors Characterization Results (AHP_240 CC with/without co substrate)

Table E. Characterization Results of BMP_Set-2

| | TS (mg/L) | TS Removal Eff. (%) | VS (mg/L) | VS Removal Eff. (%) | sCOD (mg/L) | sCOD Removal Eff. (%) | tCOD (mg/L) | tCOD Removal Eff. (%) | TAN (mg/L) | TAN Removal Eff. (%) | pH |
|-------------------------------|------------|---------------------------|------------|---------------------------|----------------|-----------------------------|----------------|-----------------------------|---------------|----------------------------|------|
| Control w BM-to | 17690±712 | 7 | 6412±664 | 15 | 1903±366 | -111 | 26533±3092 | 2 | 300±1 | -189 | 7.23 |
| Control w BM -tf | 16432±1310 | | 5459±731 | | 4017±592 | | 26100±2553 | | 867±9 | | 8.19 |
| Control w/o BM -to | 18415±1425 | 2 | 10385±1065 | 21 | 1160±66 | -162 | 36033±2843 | 36 | 437±2 | -76 | 7.38 |
| Control w/o BM-tf | 18073±882 | | 8241±418 | | 3037±254 | | 23033±1858 | | 771±9 | | 8.2 |
| B-AHP_240 CC -to | 10494±313 | 6 | 1830±157 | 11 | 3267±155 | -72 | 11467±1102 | 8 | 103±1 | -135 | 7.23 |
| B- AHP_240 CC -tf | 9878±475 | | 1633±136 | | 5627±428 | | 10533±231 | | 243±9 | | 7.3 |
| B-Feeding-to | 3680±174 | 15 | 2167±142 | 38 | 3727±273 | 22 | 35700±2606 | 36 | 203±1 | -37 | 7.15 |
| B-Feeding -tf | 3120±350 | | 1347±127 | | 2913±92 | | 23000±656 | | 277±5 | | 7.66 |
| B-Digestate-to | 2340±231 | 1 | 1153±83 | 30 | 1750±185 | -68 | 18233±1854 | 12 | 171±2 | -84 | 7.19 |
| B-Digestate -tf | 2319±109 | | 807±220 | | 2937±204 | | 16050±1344 | | 315±12 | | 7.86 |
| B-Post-Digestate-to | 2260±227 | 28 | 1187±101 | 41 | 2320±115 | -15 | 17067±1007 | 12 | 179±1 | -38 | 7.35 |
| B-Post-Digestate -tf | 1633±50 | | 700±35 | | 2660±151 | | 15067±1250 | | 248±14 | | 7.84 |
| B-AHP_240 CC +Feeding -to | 13733±643 | 12 | 8333±733 | 60 | 4450±491 | -58 | 59333±6417 | 46 | 202±1 | -55 | 7.16 |
| B -AHP_240 CC +Feeding -tf | 12087±810 | | 3333±306 | | 7020±677 | | 31767±2570 | | 312±16 | | 7.23 |

Table E. Characterization Results of BMP_Set-2 (Con't)

| | TS (mg/L) | TS Removal Eff. (%) | VS (mg/L) | VS Removal Eff. (%) | sCOD (mg/L) | sCOD Removal Eff. (%) | tCOD (mg/L) | tCOD Removal Eff. (%) | TAN (mg/L) | TAN Removal Eff. (%) | pH |
|---------------------------------|------------|---------------------------|------------|---------------------------|----------------|-----------------------------|--------------|-----------------------------|---------------|----------------------------|------|
| B-AHP_240 CC+Digestate-to | 10141±315 | 2 | 3344±429 | 32 | 4937±491 | -1 | 33433±1893 | 15 | 170±2 | -70 | 7.1 |
| B-AHP_240 CC+Digestate -tf | 9956±276 | | 2263±171 | | 4980±185 | | 28467±1762 | | 288±16 | | 7.54 |
| B-AHP_240 CC+Post-Digestate -to | 9660±381 | 28 | 2920±242 | 19 | 3433±329 | -31 | 24900±2170 | 28 | 200±2 | -11 | 7.19 |
| B-AHP_240 CC+Post-Digestate -tf | 6995±681 | | 2360±175 | | 4490±386 | | 17867±1858 | | 221±9 | | 7.49 |
| T-AHP_240 CC-to | 35539±1248 | 18 | 12117±810 | 23 | 3733±126 | 1 | 26600±404 | 12 | 496±2 | -27 | 7.36 |
| T-AHP_240 CC-tf | 29256±920 | | 9356±559 | | 3685±215 | | 23400±2987 | | 632±16 | | 8.1 |
| T-Feeding-to | 18113±1265 | 22 | 8400±787 | 27 | 3202±28 | -93 | 54300±4386 | 42 | 387±1 | -121 | 7.12 |
| T-Feeding -tf | 14167±854 | | 6139±269 | | 6180±403 | | 31550±3328 | | 856±14 | | 8.1 |
| T-Digestate-to | 18047±1457 | 24 | 8147±643 | 31 | 2058±113 | -156 | 50300±2514 | 30 | 196±1 | -339 | 7.04 |
| T-Digestate -tf | 13706±1192 | | 5655±251 | | 5260±623 | | 35400±2478 | | 861±17 | | 8.11 |
| T-Post-Digestate-to | 15775±926 | 25 | 7433±551 | 31 | 1915±22 | -142 | 45367±2548 | 28 | 547±3 | -47 | 7.32 |
| T-Post-Digestate -tf | 11906±1074 | | 5134±308 | | 4630±106 | | 32550±1690 | | 803±12 | | 8.17 |
| T-AHP_240 CC+Feeding -to | 37700±3421 | 34 | 18407±794 | 49 | 6057±105 | -20 | 114033±10155 | 53 | 418±1 | -115 | 7.27 |
| T -AHP_240 CC+Feeding -tf | 24864±1282 | | 9333±739 | | 7280±23 | | 53467±5829 | | 899±32 | | 7.91 |
| T-AHP_240 CC+Digestate-to | 31660±2545 | 34 | 11513±1187 | 32 | 4200±82 | -46 | 84600±2095 | 33 | 444±1 | -93 | 7.23 |
| T-AHP_240 CC+Digestate -tf | 20788±1989 | | 7867±882 | | 6145±668 | | 56467±7223 | | 856±14 | | 7.86 |
| T-AHP_240 CC+Post-Digestate -to | 36220±1840 | 36 | 16693±1295 | 45 | 4365±120 | -31 | 76567±2501 | 27 | 573±2 | -27 | 7.31 |
| T-AHP_240 CC+Post-Digestate -tf | 23329±1188 | | 9210±662 | | 5735±450 | | 55667±2948 | | 725±9 | | 7.8 |

F. Characterization Results of Pretreated Corncob Reactors

Table F. Characterization Results of Pretreated Corncob Reactors

| | TS (mg/L) | TS Removal Eff. (%) | VS (mg/L) | VS Removal Eff. (%) | sCOD (mg/L) | sCOD Removal Eff. (%) | tCOD (mg/L) | tCOD Removal Eff. (%) | TAN (mg/L) | TAN Removal Eff. (%) | pH |
|------------------|------------|---------------------------|--------------|---------------------------|----------------|-----------------------------|----------------|-----------------------------|---------------|----------------------------|------|
| Control -to | 17690±712 | 7 | 6412±664 | 16 | 1903±166 | -111 | 26533±3092 | 2 | 300±1 | -189 | 7.34 |
| Control -tf | 16432±1310 | | 5392±619 | | 4017±192 | | 26100±2553 | | 867±9 | | 8.27 |
| B-HP_150 CC-to | 9656±454 | 12 | 3578±217 | 8 | 922±90 | -457 | 8567±284 | 8 | 185±7 | -9 | 7.15 |
| B-HP_150 CC-tf | 8494±857 | | 3300±291 | | 5140±46 | | 7920±923 | | 202±1 | | 7.5 |
| B -HP_180 CC-to | 9368±1214 | 14 | 5459±512 | 48 | 1138±87 | -303 | 8900±606 | 16 | 219±5 | 19 | 7.12 |
| B-HP_180 CC-tf | 8033±504 | | 2833±379 | | 4587±57 | | 7500±721 | | 177±1 | | 7.79 |
| B-HP_210 CC-to | 10506±790 | 15 | 5430±536 | 38 | 1082±118 | -298 | 7333±1005 | 8 | 127±3 | -40 | 7.25 |
| B-HP_210 CC-tf | 8911±1162 | | 3344±532 | | 4307±194 | | 6770±723 | | 178±2 | | 7.75 |
| B-AHP_150 CC-to | 16706±487 | 4 | 3547±321 | 50 | 2166±124 | -111 | 11267±939 | 6 | 195±1 | -10 | 7.37 |
| B-AHP_150 CC-tf | 16111±1084 | | 1778±150 | | 4560±287 | | 10583±945 | | 214±2 | | 8.4 |
| B-AHP_180 CC-to | 14348±2226 | -10 | 2822±234 | 46 | 2797±156 | -86 | 9950±312 | 9 | 199±4 | -40 | 7.12 |
| B -AHP_180 CC-tf | 15844±828 | | 1511±51 | | 5190±469 | | 9100±321 | | 280±1 | | 8.32 |

Table F. Characterization Results of Pretreated Corncob Reactors (Con't)

| | TS (mg/L) | TS Removal Eff. (%) | VS (mg/L) | VS Removal Eff. (%) | sCOD (mg/L) | sCOD Removal Eff. (%) | tCOD (mg/L) | tCOD Removal Eff. (%) | TAN (mg/L) | TAN Removal Eff. (%) | pH |
|-----------------|------------|---------------------------|------------|---------------------------|----------------|-----------------------------|----------------|-----------------------------|---------------|----------------------------|------|
| B-AHP_210 CC-to | 17707±878 | 3 | 2942±15 | 38 | 2791±125 | -113 | 11683±1026 | 13 | 222±5 | 18 | 7.21 |
| B-AHP_210 CC-tf | 17156±693 | | 1822±255 | | 5943±143 | | 10220±938 | | 181±1 | | 7.66 |
| B-AP CC-to | 17104±1535 | 10 | 2633±231 | 46 | 2217±103 | -161 | 11733±980 | 21 | 226±2 | 1 | 7.27 |
| B-AP CC-tf | 15444±695 | | 1422±241 | | 5780±383 | | 9263±729 | | 223±2 | | 8.38 |
| T-HP_150 CC-to | 70233±5626 | 22 | 38100±4605 | 31 | 2004±229 | -206 | 74133±4987 | 42 | 807±7 | -25 | 7.28 |
| T-HP_150 CC-tf | 55122±5307 | | 26156±2811 | | 6125±632 | | 43000±1670 | | 1010±4 | | 7.81 |
| T-HP_180 CC-to | 64763±4400 | 3 | 40708±2894 | 23 | 1940±172 | -181 | 74133±4565 | 58 | 981±12 | -13 | 7.25 |
| T-HP_180 CC-tf | 63120±6548 | | 31221±3831 | | 5455±343 | | 30767±1270 | | 1105±16 | | 7.75 |
| T-HP_210 CC-to | 68444±5627 | 10 | 39832±3355 | 24 | 1992±127 | -201 | 69933±6145 | 48 | 813±67 | -42 | 7.25 |
| T-HP_210 CC-tf | 61567±6792 | | 30322±3227 | | 5995±359 | | 36053±920 | | 1158±67 | | 7.8 |
| T-AHP_150 CC-to | 72711±4088 | 24 | 31611±1641 | 33 | 2694±41 | -291 | 84700±6245 | 13 | 866±9 | -37 | 7.3 |
| T-AHP_150 CC-tf | 55133±2022 | | 21333±1242 | | 10545±403 | | 74067±3745 | | 1185±20 | | 8.6 |
| T-AHP_180 CC-to | 68556±2561 | 9 | 27200±2123 | 14 | 3220±133 | -245 | 87800±8052 | 5 | 883±29 | -51 | 7.25 |
| T-AHP_180 CC-tf | 62567±6325 | | 23333±1989 | | 11115±346 | | 83267±1721 | | 1330±4 | | 8.68 |
| T-AHP_210 CC-to | 67676±4359 | 20 | 25881±2582 | 29 | 3224±146 | -266 | 90767±8021 | 5 | 867±12 | -49 | 7.32 |
| T-AHP_210 CC-tf | 54297±6555 | | 18476±1242 | | 11800±179 | | 85900±8359 | | 1290±8 | | 8.45 |
| T-AP CC-to | 67767±4563 | 15 | 30456±2627 | 29 | 3733±205 | -187 | 78133±3700 | 12 | 867±13 | -57 | 7.25 |
| T-AP CC-tf | 57644±4171 | | 21678±1766 | | 10700±617 | | 69050±6023 | | 1350±8 | | 8.35 |

G. The Results of ANOVA for HP method at different temperature

Table G.1 Descriptive of HP method at different temperature by ANOVA

| Pretreatment Temperature | Number | Mean | Std. Dev. | Std. Error | Lower Bound ^a | Upper Bound ^a | Min. | Max. |
|--------------------------|--------|-------|-----------|------------|--------------------------|--------------------------|------|------|
| 150 | 2 | 155.5 | 0.7 | 0.5 | 149.1 | 161.9 | 155 | 156 |
| 180 | 2 | 158.5 | 0.7 | 0.5 | 152.1 | 164.9 | 158 | 159 |
| 210 | 2 | 162.0 | 9.9 | 7.0 | 73.1 | 250.9 | 155 | 169 |
| Total | 6 | 158.7 | 5.3 | 2.2 | 153.1 | 164.2 | 155 | 169 |

^a 95% Confidence Interval for Mean

Table G.2 The Results of ANOVA

| | Sum of Squares | df | Mean Squares | F-Value | Significant (P-Value) |
|----------------|----------------|----|--------------|---------|-----------------------|
| Between Groups | 42.333 | 2 | 21.167 | 0.641 | 0.586 |
| Within Groups | 99.000 | 3 | 33.000 | - | - |
| Total | 141.333 | 5 | - | - | - |

Table G.3 The Results of Post Hoc Tests

| (I)Var ^a | (J)Var ^a | Mean Diff.(I-J) | Std Error | Sig | Lower Bound | Upper Bound |
|---------------------|---------------------|-----------------|-----------|-------|-------------|-------------|
| 150 | 180 | -3 | 5.7 | 0.867 | -27 | 21 |
| | 210 | -6.5 | 5.7 | 0.562 | -30.5 | 17.5 |
| 180 | 150 | 3 | 5.7 | 0.867 | -21 | 27 |
| | 210 | -3.5 | 5.7 | 0.826 | -27.5 | 20.5 |
| 210 | 150 | 6.5 | 5.7 | 0.562 | -17.5 | 30.5 |
| | 180 | 3.5 | 5.7 | 0.862 | -20.5 | 27.5 |

^a Var is variation used pretreatment temperatures.

H. The Semi-Continuous Reactor Setup Condition at time zero

Table I.1 The Semi-Continuous Reactor Setup Condition at time zero

| | Effective Vol.(L) | CC(g) | Corncob VS(g/g) | Digester (mL) | Digester VS (g/L) | Total Subst. VS in reactor (g) | Total Subst. VS in reactor (g/L) |
|-----------|----------------------|-------|--------------------|------------------|----------------------|--------------------------------------|---|
| Raw CC | 2.05 | 16.1 | 0.84 | - | - | 15.4 | 7.5 |
| Pret.CC | - | 16.7 | 0.81 | | | | |
| Digestate | - | - | - | 49.4 | 37.4 | - | - |

Table I.2 The Semi-Continuous Reactor Setup Condition at time zero (Con't)

| | Effective Vol.(L) | Seed (L) | Total Seed VS in reactor (g) | Total Seed VS in reactor (g/L) |
|------|----------------------|-------------|---------------------------------|-----------------------------------|
| Seed | 2.05 | 1.88 | 15.375 | 7.5 |

I. Calibration Curve of Nessler Method

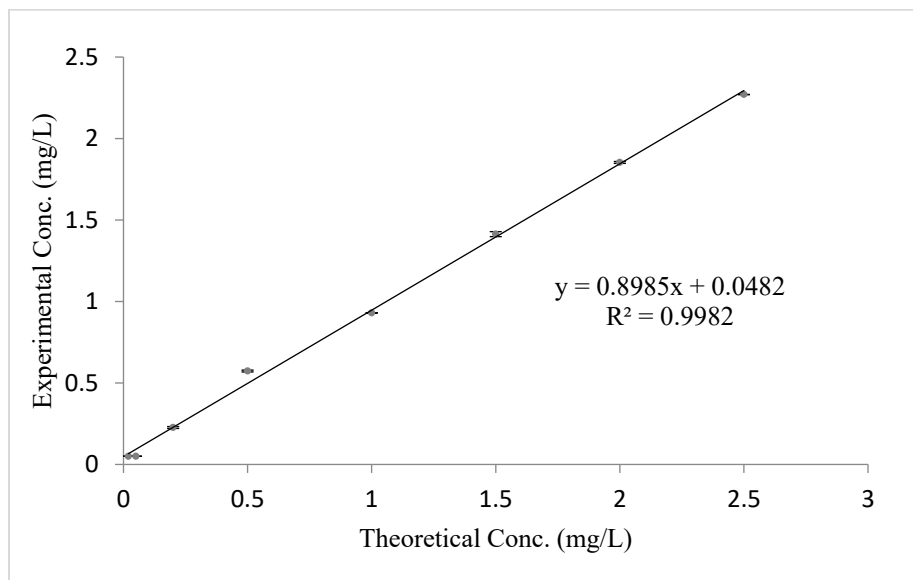


Figure I.1 Calibration curve of Nessler method for TAN analysis