UTILIZATION OF STRAW PELLETS AND BRIQUETTES AS CO-SUBSTRATES AT BIOGAS PLANTS

Interreg
Öresund-Kattegat-Skagerrak
European Regional Development Fund

EUROPEAN UNION

BioGas2020

Energiforsk

Utilization of straw pellets and briquettes as co-substrates at biogas plants

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ISBN 978-91-7673-[XXX-X] | © 2015 ENERGIFORSK

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Foreword

This project evaluated co-digestion of food waste with straw pellets and straw briquettes for biogas production at mesophilic and thermophilic conditions. The hypothesis was that this approach would improve the biogas production and allow higher organic loads as compared to digestion of food waste alone. If successful this project can contribute with information to reach more efficient production of biogas as well as illustrate new possibilities to use straw as a substrate. Straw represents a huge resource for biogas production but the potential is presently not realized.

The report has been produced by the University of Borås, RISE and the Swedish University of Agricultural Sciences. All authors have participated actively in the work and contributed both to the development of the project and to the implementation and collection of data.

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The authors would like to acknowledge the Swedish Energy Agency, Energiforsk - Swedish Energy Research Center, for its financial contribution to the project within the scope of the program "Samverkansprogram Energigasteknik" – The cooperation research program Energy gas technology.

The authors would also like to thank their RISE colleagues Johnny Ascue, Leticia Pizzul and Xinmei Feng for their valuable technical help. Thanks to Li Sun, SLU, for assisting with microbial sequence data analysis.

They would also like to thank Per Wennerberg, Technofarm, for initiating and following up the project and for taking part in the discussions as well as our straw product suppliers, LagaBioenergy for straw pellets and C/F Nielsen for straw briquettes. We are grateful for Borås Energy and Environment for their support during the project and for providing the food waste slurry for the experiments and for Interreg, VGR as well as for Göteborg Energi's research foundation for contributing with financial support.

Summary

Co-digestion of straw, particularly in the form of straw pellets, with food waste, resulted in synergistic effects. The substrate mixture resulted in a higher methane production from food waste compared to when food waste was mono-digested. The effect was seen both at thermophilic and mesophilic conditions and at average and high ammonia levels. The addition of straw did not affect the retention time.

Straw is an abundant source of biomass that has a great potential to be used in the biogas industry, specifically in co-digestion with other substrates. Straw is poor in nitrogen and has a lignocellulosic structure giving a slow degradation. However, straw can be interesting as co–digestion material with substrates rich in easily degradable carbon and protein.

The digestion of substrates rich in easily degradable carbon and high nitrogen (proteins) can result in fast production and accumulation of organic acids (volatile fatty acids, VFA), a process accentuated by high ammonia/ammonium concentrations, causing inhibition of the methanogens, consequently giving low methane yields. These problems increase at high organic loadings and at digestion performed at higher temperatures (thermophilic conditions). Currently, low organic loadings and dilution with water are some of the strategies to try to overcome these problems. However, this results in the dilution of the digester content with water, increases the need for heating and results in large amount of digestate to handle.

By co-digesting, easily degradable carbon and nitrogen rich substrates together with a slow degradable carbon and nitrogen poor substrates, as straw, the biogas yield can potentially be improved. One disadvantage of using straw is that it requires some kind of pretreatment, as for example reduction of particle size, prior to its use in a biogas reactor. Straw pellets and briquettes here represent an interesting alternative. These are established, easily accessible and easy-to-use products, consisting of ground and pressed straw, which can be used directly in the biogas process.

The aim of this project was to evaluate the co-digestion of straw pellets (SP) or briquettes (SB) with food waste. The substrates were evaluated in the laboratory using both batch and semi-continuous digestion experiments. The anaerobic reactors were operated at two laboratories, at RISE in Uppsala and at the University of Borås. The purpose of the batch tests was to determine the biochemical methane potential (BMP) of each of the substrates used within the investigations, while the objectives of the continuous digestion experiments were to evaluate if a) addition of straw could give an improved utilization of the plant capacity and b) process stability could be improved when easily degradable carbon and nitrogen-rich substrates were co-digested with straw. To understand the effects of straw additional investigations regarding the microbial community structure as well as compositional and structural characterization of straw were performed. Different theoretical calculations were also performed to estimate the efficiency of the studied processes.

The results showed that the BMP for the straw products was 340 ± 19 NmL CH_4/g VS and no significant difference (t-test, $p<0.05$) was observed between SP and SB. The results confirmed that the briquetting and pelleting processes have a positive effect on the degradability of straw, as SP and SB showed a 9% (t-test $p>0.05$) higher BMP compared to virgin straw (313 ± 1 NmL CH_4/g VS). Equal results were obtained at the two laboratories. The BMP for food waste was however significantly higher (t-test $p<0.05$) when the test was performed at RISE, Uppsala (607 NmL CH_4/g VS) compared at UB, Borås (445 NmL CH_4/g VS). The difference was likely be explained by different experimental conditions in the different laboratories.

Results from continuous laboratory processes operated at thermophilic conditions showed that the presence of straw positively affected food waste digestion. An increased volumetric methane production (VMP) was observed compared to when food waste was mono-digested. The effect was clearer when the straw addition represented 20% compared with 10% of the organic loading rate (OLR), both at low (3.6 g VS/L day) and high OLR (8.4 g VS/L day). The effect was also mainly seen for SP and SB did not improve the productivity as much. Using data from both batch and continuous experiments for theoretical calculations of process performance suggested that addition of SP actually improved the degradation of the food waste. Assuming that the straw in the reactors resulted in yields similar to the obtained methane potential of 340 NmL CH_4/g VS, the remaining methane production in the continuous experiments should arise from the food waste. Comparing this calculated methane yield from the food waste with that obtained in the semi continuous reactor operation without the presence of straw, it was concluded that food waste produced 5 to 8% more methane in the co-digestion reactors than when it was mono-digested, indicating synergetic effects. Thus, addition of straw can be done without affecting the hydraulic retention time (HRT) and it appears to improve the degradation of the food waste. Conclusively addition of straw gives a better utilization of the reactor volume.

Comparing SP and SB and at the conditions tested in this study, the enhancement of methane production was observed mainly with SP as co-substrate and not with SB. The mixing was affected in the reactors when SB was used, especially at the higher OLR level. The smaller particle size of SP compared to that of SB likely explains this difference. Also, the analysis with Simon staining to determine the accessibility of the material for the cellulose degrading enzyme complex showed that SP particles had a much higher porosity than SB, which may allow a better attachment and activity of cellulose degrading microbes. In line with these results the microbial community analysis also illustrated only a clear effect when SP was added as a co-substrate and not with SB. Addition of SP at the highest load resulted in an increase of the order *Porphyromondaceae*. This group harbors genes encoding enzymes for degradation of complex carbohydrates, such as cellulose.

In line with the results described above, straw pellets also resulted in improved efficiency when co-digested with food waste in a high ammonia mesophilic process. Different to the thermophilic systems this process was in pseudo-stable conditions, *i.e.* high VFA and sensitive to OLR changes. In the presence of straw, and after subtracting the methane contribution from straw, the high nitrogen food

waste produced 3 318 NmL CH_4/d compared to the 2 430 NmL CH_4/d produced when mono-digested, *i.e.* 37% more methane. However, the specific methane production (SMP) of the SP amended process was still lower than that in a control reactor using the same substrate but amended with trace elements and iron.

In summary, straw is suitable for biogas production in co-digestion with food waste. The addition of straw, specifically straw pellets, gave positive effects resulting in higher volumetric methane production and synergistic effects without affecting the hydraulic retention time.

Sammanfattning

Samrötning av matavfall med halm, särskilt i form av halmpellets, resulterade i synergistiska effekter. Substratblandningen resulterade i en högre metanproduktion från matavfall jämfört med när matavfall användes som enda substrat. Effekten sågs både vid termofila och mesofila förhållanden och vid medelhög och hög ammoniaknivå. Tillsatsen av halm påverkade inte retentionstiden.

Halm är en riklig källa till biomassa som har stor potential att användas inom biogasindustrin, speciellt vid samrötning med andra substrat. Halm innehåller låga halter kväve och har en komplex struktur av lignocellulosa som ger en långsam nedbrytning. Halm kan emellertid ändå vara intressant som samrötningsmaterial till andra material som har hög andel lättnedbrytbart kol och protein och kan här potentiellt ha en stabiliserande effekt.

Substrat med hög andel lättnedbrytbart kol och protein kan resultera i snabb produktion och ackumulering av organiska syror (flyktiga fettsyror, VFA), en process som accentueras av höga ammoniak/ammoniumkoncentrationer från proteinnedbrytningen, vilket orsakar inhibering av metanogenerna, vilket följaktligen ger låga metanutbyten. Dessa problem ökar vid höga organiska belastningar och vid rötning vid högre temperaturer (termofil process). För närvarande är låg organisk belastning och utspädning med vatten vanliga strategier för att överkomma dessa problem. Detta resulterar emellertid i utspädning, ökar behovet av uppvärmning och resulterar i en stor mängd av rötrest att hantera.

Genom samrötning av substrat med hög andel lättnedbrytbart kol och kväve tillsammans med ett material som halm, med lågt kväveinnehåll och hög andel av kol som bryts ner mer långsamt, kan biogasutbytet eventuellt förbättras. En nackdel med att använda halm är emellertid att det kräver någon form av förbehandling som minskar partikelstorlek innan det kan användas i en biogasreaktor. Halmpellets och briketter representerar här ett intressant alternativ. Pellets och briketter är etablerade, lättillgängliga och lättanvända produkter, som består av finfördelat och pressat halm, och dessa kan användas direkt i biogasprocessen.

Syftet med detta projekt var att utvärdera samrötning av halmpellets eller briketter med matavfall. Substraten utvärderades i laboratoriet i satsvisa och semi-kontinuerliga biogasreaktorer. Reaktorerna drevs i två laboratorier, hos RISE i Uppsala och vid Högskolan i Borås. Syftet med testerna i de satsvisa reaktorerna var att bestämma den biokemiska metanpotentialen (BMP) för varje substrat som användes inom projektet. Målet med de semi-kontinuerliga försöken var att utvärdera om samrötning av matavfall med halm kunde ge a) ett förbättrat utnyttjande av reaktorvolymen och b) en högre processtabilitet/effektivitet jämfört med när matavfall används som enda substrat. För att förstå effekterna av halmtillförsel utfördes ytterligare undersökningar avseende den mikrobiella samhällsstrukturen samt kompositions- och strukturkarakterisering av halm. Olika

teoretiska beräkningar utfördes också för att uppskatta effektiviteten hos de studerade processerna.

Resultaten visade att BMP för halmprodukterna var 340 ± 19 NmL CH_4/g VS och ingen signifikant skillnad (t-test, p <0,05) observerades mellan halmpellets och briketter. Resultaten bekräftade att briketterings- och pelleteringsprocessen har en positiv effekt på halmens nedbrytbarhet, eftersom pellets och briketter visade en 9% (t-test p> 0,05) högre BMP jämfört med rå halm (313 ± 1 NmL CH_4/g VS). Lika resultat erhölls för halm vid de två laboratorierna. BMP för matavfall var dock signifikant högre (t-test p <0,05) när testet utfördes vid RISE, Uppsala (607 NmL CH_4/g VS) jämfört med den som utfördes vid Högskolan i Borås (445 NmL CH_4/g VS). Skillnaden berodde troligen på olika experimentella förhållanden i de olika laboratorierna.

Resultat från de semi-kontinuerliga laboratorieprocesser som kördes vid termofila förhållanden visade att närvaron av halm påverkade rötningen av matavfall positivt. En ökad volymetrisk metanproduktion (VMP) observerades jämfört med matavfall som enda substrat. Effekten var tydligare när halmen representerade 20% jämfört med 10% av den organiska belastningen (OLR), både vid både lågt (3,6 g VS / L dag) och hög OLR (8,4 g VS / L dag). Effekten sågs också främst för halmpellets, och för briketter erhölls inte en lika tydlig ökning av gasproduktionen. Teoretiska beräkningar baserade på data från både satsvisa och semi-kontinuerliga försök visade att tillsats av halmpellets faktiskt förbättrade nedbrytningen av matavfallet. Om man antar att halmen i reaktorerna resulterade i utbyten som liknar den erhållna metanpotentialen av 340 NmL CH_4/g VS, skulle den återstående metanproduktionen i det kontinuerliga experimentet uppstå ur matavfallet. Genom att jämföra detta beräknade metanutbyte från matavfallet med det som erhölls i den semi-kontinuerliga processen som drevs utan halmtillsats kunde slutsatsen dras att matavfallet producerade 5 till 8% mer metan under samrötning med halm än när det användes som enda substrat. Halm kan alltså tillsättas utan att påverka HRTn nämnvärd och också medge en förbättrad nedbrytning av matavfall. Konklusionen blev därför att tillsats av halm ger ett bättre utnyttjande av reaktorvolymen.

Vid jämförelse av halmpellets och briketter vid de betingelser som testades i denna studie observerades förbättringen av metanproduktionen huvudsakligen med halmpellets som samrötningssubstrat och inte med briketter. Störst effekt erhölls också vid en högre OLR-nivå. Den mindre partikelstorleken hos halmpellets jämfört med briketter förklarar sannolikt denna skillnad. En metod, ”Simon-staining”, som används för bedömning av materialets tillgänglighet visade också att halmpellets hade en mycket högre porositet än briketter, vilket kan möjliggöra en bättre fastsättning och aktivitet för cellulosanedbrytande mikrober. I linje med dessa resultat visade den mikrobiella analysen endast en klar effekt när halmpellets tillsattes som ett samrötningssubstrat och inte briketter. Tillsats av halmpellets vid den högsta belastningen resulterade i en ökning av bakterier inom ordningen *Porphyromondaceae*. Denna grupp har gener som kodar enzymer för nedbrytning av komplexa kolhydrater, såsom cellulosa.

I linje med resultaten som beskrivits ovan resulterade halmpellets också i förbättrad effektivitet när de samrötades med matavfall i en mesofil process med hög ammoniakhalt. Till skillnad från de termofila systemen var denna process en pseudo-stabil process (hög VFA och känslig för OLR-förändringar). I närvaro av halm och efter att ha subtraherat metanproduktionen från halm, producerade detta matavfall med hög kvävehalt 3 318 NmL CH_4/dygn jämfört med 2 430 NmL CH_4/dygn vid rötning av endast matavfallet, dvs. 37% mer metan. Emellertid var den specifika metanproduktionen i den processen med halm fortfarande lägre än den i en kontrollreaktor som använde samma substrat men med en tillsats av spårelement och järn.

Sammanfattningsvis är halm lämplig för biogasproduktion och samrötning med matavfall. Tillsats av halm, speciellt halmpellets, gav positiva effekter som resulterade i högre volymetrisk metanproduktion och synergistiska effekter på nedbrytningen, utan att påverka den hydrauliska retentionstiden.

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1 Background

An increased utilization of biomass-derived biofuels will result in environmental benefits such as reduction of fossil fuel consumption and greenhouse gas emissions. To produce biofuel from biomass based biogas therefore has a great potential. Furthermore, anaerobic digestion has additional values as the process in addition to produce renewable energy can be used for treatment of organic waste and production of a digestion residue possible to use as a fertilizing agent (Ward et al. 2008).

In the agricultural sector there are several biomass residue streams available for biogas production, such as manure and residues generated during crop cultivation. In Sweden the total biogas potential from animal manure and agricultural crop residues is calculated to 4.2 TWh/year and 6.6 TWh/year, respectively (Linné et al. 2008). The total production of biogas was in 2015 around 2 TWh/year in Sweden (Table 1) and if taking into the account the utilization of these agricultural residues it would be possible to increase the biogas production by several times.

Table 1. Swedish biogas production at different plant types (data from 2015) (EnergiGas Sverige 2016)

Plant type	**Number of plants**	**Biogas production[a] (GWh/year)**
Biowaste	35	854
Sewage sludge	140	697
Landfills	60	187[b]
Industrial	6	121
Agriculture	40	50
Gasification	1	38
Total	**282**	**1947**

[a]The biogas production is expressed as the energy content in the biogas

[b]Based on collected biogas not the actual biogas produced in from the landfills.

One abundant source of biomass is straw, which has a great potential for the production of biogas. During grain production approximately fifty present of the produced biomass will remain as straw. In Sweden about 2.9 million tons of straw is produced each year, of this roughly 1.6 million tons can be collected with an estimated biogas potential of 5.8 TWh/year (Linné et al. 2008). Today about 0.9 million tons straw are used as bedding material for domestic animal (Nilsson et al. 2009) and about 0.1 million tons are utilized for energy production by combustion. It is predicted that the utilization of straw for energy production will increase in line with higher energy prices and demand. Straw is particularly interesting as energy source as it does not compete with food production. Despite the comparably higher energy efficiency of combustion, the utilization of straw for biogas production is still interesting. Combusting of straw cannot be used for

biofuel production neither does it represent a possibility to reintroduce the nutrients back to the farmland. Other practical problems connected to straw combustion are e.g. fly ashes and boiler corrosion due the high content of calcium chloride in the straw (Kaparaju et al. 2009).

However, the chemical structure of straw, with its high portion of lignocellulose, represents a limitation and results in a slow degradation with low biogas yield (Risberg et al. 2013). An option to increase the biogas yield from straw is to use a suitable pretreatment method, *i.e*. either mechanical, thermal and chemical methods, or the combination of those, aiming to open up the complex and recalcitrant structure and making it more accessible for microbial degradation. Nevertheless, most of these methods are expensive as they have a high energy or chemical demand, or require the use of expensive equipment (Taherzadeh et al. 2008, Kim et al. 2002).

Pelleting or briquetting of straw works as a mechanical pretreatment, as the straw is highly compressed to form either larger briquettes or smaller pellets. These processes have the potential to increase the microbial accessibility by reducing the particle size of the straw. It can also improve the biogas yield since the material structure is ruptured and the particle size is reduced (Moset et al. 2015). Straw pellets are an established, easily accessible and easy-to-use product, consisting of finely ground and pressed straw. Straw briquettes have similar properties and function as straw pellets, but they are larger and have a slightly coarser structure and need therefore to be crushed before being used as animal feed. The simpler production process, however, makes them a cheaper alternative to straw pellets. Furthermore, briquettes and pellets have a much higher density compared with the virgin straw. Thus, this treatment gives a product which has a reduced bulk volume giving reduced storage, handling and transportation costs, which to some extent compensates for the cost of the briquetting or pelleting process itself. Furthermore, when compressed straw is used in a biogas process the briquetting and pelleting prevents the material from forming a floating layer of straw inside the bioreactor. This is otherwise a typical problem for virgin straw. Utilizing straw pellets or briquettes instead of virgin straw hence can be a more feasible option for a biogas plant.

During anaerobic degradation of organic material to biogas several groups of microorganisms with different metabolic activity need to cooperate (Schnürer 2016). In order to achieve a stable and robust biogas process all conversion steps during biogas production, hydrolysis, acidogenesis, acetogenesis and methanogenesis, and the microorganisms involved in each step, need to be synchronized. In the process different degradation steps can be rate limiting. When lignocellulosic material is converted to biogas the first degradation step, hydrolysis, is typically the slowest and rate determining step (Neyens et al. 2003).

In case of more easily accessible materials the last step performed by the methanogens is instead the slowest step (Schnürer and Jarvis 2017). A prerequisite for an efficient degradation and biogas production is that the degradation steps are in balance. For example, if the first step goes too fast in relation to the methanogenic step this can result in accumulation of degradation intermediates such as VFA. Today the overall biogas production routes are fairly known, but still there are many unanswered questions when it comes to the microbiology and specific groups of microorganisms. It is clear that more knowledge is needed to fully understand the complexity of this process and to optimize the process. For degradation of lignocellulosic materials such as straw it is important that the process harbors microorganisms with the ability to depolymerize these compounds. It is well known that the activity of the cellulose degrading microorganisms depends on a high surface area as these microorganisms bind to the material during the degradation process. When the microorganisms colonize the material surface, the production of cellulose degrading enzymes is stimulated (Azman et al. 2015). Some studies have been performed to specifically investigate the cellulose degrading microorganisms in biogas processes but still the knowledge level is rather low (Sun 2015, Azman et al. 2015). Most knowledge about these bacteria has been produced in studies from rumen or soil samples (Lynd et al. 2002; Morrison et al. 2009; Ransom-Jones et al. 2012).

Straw is rich in cellulose but low in nutrients and therefore utilizing straw for biogas production requires co-digestion with some other complementary substrates. By mixing substrates with different character in a co-digestion process the biogas yield can in many cases be improved because of synergistic effects (Mata-Alvarez et al. 2000). Optimally straw should be co-digested with a substrate having comparably higher levels of nitrogen and micro nutrients. Previous research on biogas production from straw has mainly focused on co-digestion with manure (Xavier, et al. 2015). Manure is rich in nutrient and also supports the process with alkalinity. However, manure also contains a large fraction of lignocellulose and thus the overall degradation efficiency is already low. More interesting would be to use co-digestion with a somewhat more easily available material, such as for example food waste. Using straw combined with food waste could potentially results in a more efficient utilization of the straw and also a stabilizing effect regarding the overall process. Food waste is generally a good substrate for biogas production as it already represents a mixture of different compounds. However, problems can arise if the level of easily digested carbohydrates and proteins are too high (Schnürer and Jarvis 2017). The carbohydrates are converted rapidly to organic acids (volatile fatty acids, VFA), which might accumulate. High levels of organic acids have a negative effect on the methane production, both as they represent a carbon fraction not converted to methane but also as high levels can be inhibitory for the process (Schnürer et al.

2016b). Proteins are converted to ammonium/ammonia, which also can cause additional problems as these compounds can inhibit the methanogens (Chen et al 2008). These problems increase at high organic loadings and at digestion performed at thermophilic conditions. Using straw in co-digestion with food waste might thus represent a possible way to reduce the risk for instability. The straw contains high levels of slowly degradable carbon and low levels of nitrogen, complementary to the food waste

The aim of this project was thus to evaluate the potential of using straw pellets or briquettes in co-digestion with food waste. The hypothesis was that the straw would give a stabilizing effect at high organic loads and as a consequence have positive effect on the methane yield and productivity and in turn a better utilization of plant capacity. An additional aim was to investigate the effect of straw addition on the microbial community and to reveal further information about the important cellulose degraders.

2 Introduction

Straw is an abundant source of biomass generated during harvesting of crops. In Sweden about 2.9 million tons of straw are produced each year, of this roughly 1.6 million tons can be collected with an estimated biogas potential of 5.8 TWh/year (Linné et al. 2008). However, due to its recalcitrant nature and low energy content only a small amount is used today for biogas production. To reach the potential of this material it is important to find methods by which straw can be utilized for biogas production with profitability. The main goal for this project was to investigate the potential of using wheat straw in the form of pellets or briquettes to generate biogas. The purpose was to evaluate if the addition of straw could allow better utilization of plant capacity by enhancing methane yield, as well as give improved stability and robustness of the process.

More specifically, the aim of the project was to evaluate the effects of straw pellets or briquettes on biogas production and process stability when co-digested with food waste. The effect of straw addition was investigated in laboratory scale digesters, both batch and continuous digesters. One experiment was performed at thermophilic temperature at average ammonia level and one experiment was performed at mesophilic condition and high ammonia levels. The processes were evaluated with both chemical and microbiological analyses. In addition, the structural and chemical composition of the substrates was also investigated. The experiments were performed in collaboration between University of Borås (UB), RISE-Research Institutes of Sweden, Uppsala and SLU, Uppsala.

The results of the project are relevant for several industries:

- Biogas plants (stabilizing the co-digestion process)
- Agricultural- and animal feed industries (improve the quality and value of bedding material)
- Industries which process straw (utilize straw for biofuel production)

The long term goal was to contribute with information of importance for an improved utilization of agricultural residues for biogas production.

In order to evaluate the potential of using straw in the form of pellets or briquettes during co-digestion with food waste the project was divided into five sub-projects.

- Sub-project 1 – Biochemical methane potential (BMP) of the substrates

The methane potential of straw briquettes, pellets and food waste was determined using batch cultures. Raw virgin straw was included as a reference. The purpose was to evaluate the effect of the treatment on the methane potential of the straw as well as generate data to be used for the evaluation of the laboratory scale processes

- Sub-project 2 – Semi-continuous laboratory processes - Co-digestion of food waste and straw products (briquettes and pellets) during thermophilic conditions

The co-digestion of food waste and straw pellets was investigated by UB and the co-digestion of food waste and straw briquettes was investigated by RISE, Uppsala. The continuous digestion experiments were performed in three digesters at each laboratory. One reactor was fed with food waste and the other two with a mixture of food waste and straw pellets or briquettes. The organic load was increased in all reactors over time. Both laboratories used the same inoculum, taken from the full-scale biogas plant of Borås Energy and Environment in Borås, to start up the digestion processes. The purpose was to evaluate the effect of straw on the biogas yield and overall performance and stability of the biogas process.

- Sub-project 3 – Semi-continuous laboratory processes - Co-digestion of food waste and straw pellets in a mesophilic process at high ammonia levels

The purpose was to evaluate the effect of straw pellets on the stability and biogas yield using a process with a comparably higher ammonia level that investigated in Sub-project 2. Continuous laboratory experiments in CSTR reactors were run at RISE, Uppsala.

- Sub-project 4 – Structural characterization of digested and raw straw

The purpose of this sub-project was to study potential structural changes in straw/straw fraction before and after the briquetting/pelleting processes and the biogas process. To assess changes in crystallinity and accessible surface area, FTIR and Simon staining analyses methods were performed at UB.

- Sub-project 5 – Evaluation of the microbial community structure

The microbial community structure developed in the reactors operated in Sub-project 2, was investigated by Illumina sequencing using 16 S rDNA as a target gene. The purpose was to reveal information of microbial population developed in response to the addition of straw. The analyses were performed by SLU.

- Sub-project 6 – Process efficiency calculations

Different calculations were performed to determine how the addition of different straw products, pellets or briquettes, influenced the biogas yield and productivity of the process

3 Material and methods

3.1 ANALYTICAL METHODS

Unless otherwise stated, the following analytical methods have been used to monitor digestion processes and to characterize the substrates, inocula and digestate:

- Chemical characterization of substrates, inocula and digestate residues was carried out through standard analyzes by the laboratory AgriLab AB (TS, VS, TKN, org-N, NH_4-N, C, P, K, Mg, Ca, Na, S).
- TS, VS, total nitrogen and NH_4-N digestion processes have been analyzed according to APHA 1995.
- Extractives, both hot water and ethanol, ash, total lignin (soluble and Klason lignin), and other sugars were analyzed according to NREL/TP-510-42618.
- pH was measured with pH meter Jenway 3510.
- Analysis of biogas methane content:
    - at RISE, Uppsala – gas chromatograph (GC) (Perkin Elmer Arnel, Clarus 500; column: 7 'HayeSep N 60/80, 1/8 "SF; Detector, FID 250 ° C, Carrier Gas: He, Flow 31 mL/min, Injection Temperature: 60 ° C, (Westerholm et al. 2012). For injection, a TurboMatrix 110 sampler was used.
    - at UB, Borås – GC (Auto System, Perkin Elmer, U.S.A.) equipped with a packed column (Column 8000 PKD, Perkin Elmer, U.S.A.) and a thermal conductivity detector (Perkin Elmer, U.S.A.), with an injector temperature of 150 °C. Nitrogen served as the carrier gas with a flow rate of 20 mL/min at 60 °C was used.
- Volatile fatty acids (VFA) in the digestates and substrates were analyzed:
    - at RISE, Uppsala: according to Westerholm et al. 2012.
    - at UB, Borås: high performance liquid chromatograph (HPLC) (Waters 2695, Millipore, Milford, U.S.A.), equipped with a refractive index (RI) detector (Waters 2414) and an ion-exchange column (Aminex HPX-87H column, Bio-Rad, USA) operating at 60 °C. Sulfuric acid (5 mM) was used as eluent with a flow rate of 0.6 mL/min.
- Biochemical Methane Potential (BMP) was performed at UB, Borås according to Teghammar et al. 2010 and at RISE, Uppsala according to Westerholm et al. 2012.

- The structure of virgin straw as well as straw briquettes and pellets prior to and after digestion was analyzed using Simon staining and Fourier transform infrared (FTIR) spectrometry according to the methods described in Teghammar et al (2012).

3.2 SUBSTRATES

The substrates used in this study were wheat straw pellets (SP), wheat straw briquettes (SB) and food waste (FW) (Fig. 1).

3.2.1 Wheat straw briquettes and pellets

Pelleting and briquetting are mechanical processes in which the raw straw, with a low initial density, is first shredded, milled and then subjected to high pressure promoting its agglomeration and densification.

The wheat straw briquettes (Fig. 1a) were provided by CF Nielsen, Denmark. Briefly, the briquetting process proceeded as follows: a BP 6500 briquetting unit linked to a hammer mill with 20 mm sieve producing cylindrical briquettes with 68 mm diameter was used for briquetting farmland wheat straw. No external binding agent was added and the pressures applied ranged between 150 to 200 MPa above atmospheric pressure (Xavier et al, 2015). Prior to its use in the laboratory trials the briquettes were disaggregated by hand liberating straw particles with sizes ranging mostly between 10-15 mm (Fig. 1a).

Wheat straw pellets (Fig. 1a) were provided by Laga BioEnergy, Laholm, Sweden. The pelleting process consisted of milling of farmland wheat straw to a particle size of around 2 mm using a hammer mill. These particles were pressed under high pressure and at a temperature of around 70-80 °C, forming straw pellets with 5 mm of diameter and 8-10 mm of length (https://www.lagabioenergi.se/Halmpellets-Videos).

SB and SP were analyzed chemically and structurally and their methane potential was determined in BMP tests. Raw wheat straw (RS) cut by hand (20-30 mm), was included as a control.

3.2.2 Food waste

Two different food wastes were used in this study.

In Sub-project 2 the food waste (FW1) consisted of source-sorted food waste collected from households, milled and tank-stored food waste from restaurants and sludge from restaurants' fat separators, all obtained in Borås. This mixture was then processed into a slurry at the biogas plant of Borås Energi & Miljö at Sobacken, Borås Sweden (Figure 1b).

In Sub-project 3 the food waste (FW2) consisted of source-sorted organic fraction of municipal solid waste from Uppsala, supplemented with egg albumin powder (Westerholm et al. 2016).

All food wastes were chemically characterized.

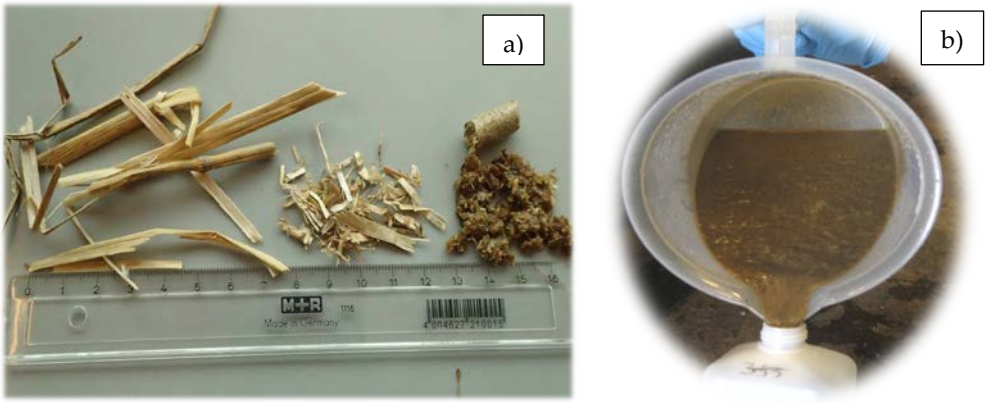


Figure 1. Substrates used in the study: a) virgin straw, straw-briquettes, -pellets; b) food waste.

3.3 SUB-PROJECT 1 - DETERMINATION OF BIOCHEMICAL METHANE POTENTIAL OF THE SUBSTRATES

The biochemical methane potential (BMP) of each substrate was determined in parallel at RISE, Uppsala and UB, Borås, using in house protocols. Each laboratory used digestate from their respective laboratory reactors as inoculum for the batch trials. At RISE, Uppsala, the digestate from three reactors was collected during the period 1 to 8 February, 2016 (Sub-project 2). At UB, Borås, the digestate was collected during the period 20 to 27 May, 2016 (Sub-project 2). In both cases the digestates corresponded to the process where only food waste was used as substrate and at OLR of 3.0 g VS/L/d.

The experiments were run in three replicates according to the experimental set up presented in Table 2. The study included virgin straw as non-pretreated straw control and cellulose as control to measure the inoculum activity. Inoculum without any substrate was also run to determine the methane contribution from endogenous material.

Gas production was determined by:

a) RISE, Uppsala – measuring the pressure in the bottle with a pressure gauge (GMH 3111 with pressure sensor GMSD 2BR). The pressure was then recalculated via the general gas law and normalized (1 atm and 0 ° C). Gas samples were taken (1 mL) and the methane content was analyzed by a gas chromatograph (an external standard curve used for calibration) (Westerholm 2012).

b) UB, Borås – sampling regularly the gas from the headspace of each bottle, using a 0.25 mL pressure-tight syringe (VICI, Precision Sampling Inc., Baton Rouge, LA, USA), and the samples were then immediately analyzed by gas chromatography (Teghammar et al. 2010). The results were normalized (1 atm and 0 ° C).

The cumulative methane production at the end of the tests divided by the total g VS added gives the biochemical methane potential (BMP) or ultimate methane yield of the substrates expressed as NmL CH_4/g VS.

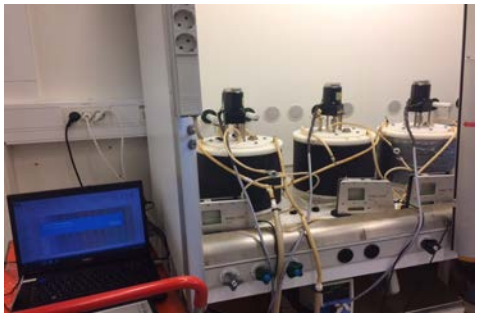
Table 2. Determination of the Biochemical Methane Potential – Substrates and operational parameters at each laboratory. The experiments were run in three replicates.

Substrates/Parameters	**BMP at RISE**	**BMP at UB**
Food waste (FW1)	Sobacken, Borås	Sobacken, Borås
Straw briquettes (SB)	C/F Nielsen	C/F Nielsen
Straw pellets (SP)	Laga bioenergi	Laga bioenergi
Raw straw (RS)	Farmland	Farmland
Cellulose (control)	Sigma-Aldrich C6288 (fibers, medium)	Sigma-Aldrich C6663 (fibers, long)
Inoculum/substrate ratio in VS	3:1	2:1
Substrate loading (g VS/L)	3	8
Reactor size (mL)	500	110
Wet volume (mL)	300	60
Temperature (°C)	53	53
Incubation time (d)	60	42

3.4 SUB-PROJECT 2 - SEMI-CONTINOUS LABORATORY PROCESSES - CO-DIGESTION OF FOOD WASTE AND STRAW PRODUCTS (BRIQUETTES AND PELLETS)

The co-digestion of food waste and straw (pellets or briquettes) was studied in semi-continuous laboratory scale reactors to evaluate the effect of straw addition at different OLRs. The co-digestion of food waste and straw briquettes was performed at RISE, Uppsala, while the co-digestion of food waste and straw pellets was studied at the University of Borås.

Three reactors (R1, R2, and R3) were run at each location. The reactor system at RISE, Uppsala (Dolly, Belach Bioteknik) had a total volume of 10 L and a working volume of 5 L. The reactor system at UB, Borås consisted of self-made reactors with a total volume of 5 L and a working volume of 3 L (Figure 3).



UB, Borås

RISE, Uppsala

Figure 3. Semi-continuous laboratory reactors used in the study.

Digestate collected in September 2015 from the large-scale biogas plant at Sobacken, Borås was used for the start-up of the reactors at RISE, Uppsala and UB, Borås (Inoculum I). However, due to technical problems the reactors at UB, Borås collapsed and were started again in January 2016. Digestate from the Uppsala reactors collected during the period of 30 November-17 December 2015 was then used as inoculum (Inoculum II) to re-start the reactors. Both inocula were chemically characterized.

Table 3 shows the general experimental set-up used at each laboratory. Reactor R1 was run as control with only food waste as substrate. The food waste in R2 and R3 was complemented with straw (10% and 20% of the OLR, respectively). The processes were operated at thermophilic (53 °C) conditions. The OLR was gradually increased in all reactors causing the respective changes in the HRT (Table 3).

Table 3. Experimental set-up in the processes studied in Sub-project 2

Reactor	**Substrate**	**Temp °C**	**OLR g VS/L/d** initial→target	**HRT days** initial→target
R1	Food waste	53	3.0→7.0	35->15
R2	Food waste+ 10% straw (briquettes or pellets)*	53	3.3→7.7	35->15
R3	Food waste + 20% straw (briquettes or pellets)*	53	3.6→8.4	35->15

*The percentage of added straw was in relation to the OLR in VS basis. The mixture food waste (FW1) and straw briquettes (SB) was studied at RISE, Uppsala. The mixture food waste (FW1) and straw pellets (SP) was studied at UB, Borås.

Three steady-state periods were evaluated (Table 4). At the start-up (Period 1), the three reactors at each location (Uppsala and Borås) were run at the same conditions using only food waste as substrate and at an OLR of 3.0 g VS/L/d and an HRT of 30 and 35 days at UB and RISE, respectively. This was to ensure that all three

processes were equal in regard to chemical parameters measured. Period 1 lasted almost 3 HRT.

During Period 2, R1 stayed as control with only FW1 as substrate while R2 and R3 were fed with a mixture of FW1 and straw, resulting in an increased OLR by 10 and 20%, respectively. More specifically, the OLR was 3.0 g VS/L/d in the control reactor R1 and to 3.3 and 3.6, respectively in R2 and R3. The HRT was kept at 35 days in all reactors at RISE, Uppsala and at 30 days at UB, Borås. Period 2 lasted 1.4 HRT (RISE, Uppsala) and 4.0 HRT (UB, Borås) (Table 4).

After Period 2 the OLR was increased gradually at a rate of 0.5 g VS/L per week until it reached an OLR of 7.0 g VS/L/d in the control reactor R1 and 7.7 and 8.4 g VS/L/d in R2 and R3, respectively. The HRT decreased to 14-15 days in line with the increase in OLR. Period 3 represented continuous operation at this OLR for another 2.8 HRT (RISE, Uppsala) and 1.5 HRT (UB, Borås) (Table 4).

The reactors were monitored with conventional process parameters. The parameters analyzed were biogas production and composition, methane production, fatty acids concentration, nitrogen levels, pH and alkalinity.

The produced gas was collected in a bag and the volume was measured with: a) Ritter Model TG05 / 5 wet gas meter (RISE, Uppsala) and b) μFlow volumetric gas flow meter from Bioprocess Control, Sweden (UB, Borås). Gas production was normalized (0 ° C, 1 atm) and the volume was expressed as N followed by a volume measurement. In addition to methane determination by GC, a portable instrument BIOGAS 5000 from Geotech was also used at RISE, Uppsala to determine the biogas composition (methane, carbon dioxide, oxygen and hydrogen sulphide). The daily monitoring of carbon dioxide content in the biogas was measured in an Einhorn fermentation saccharometer (Schnürer and Jarvis 2017) at both laboratories.

Table 4. Subproject 2 - Operational parameters in the semi-continuous processes at both locations, RISE (Uppsala) and UB (Borås).

Reactor	Lab.	Inoc.[a]	Temp °C	Period 1				Period 2				Period 3			
				Substr.	OLR g VS/L/d	HRT[b] days	# of HRT[b]	Substr	OLR g VS/L/d	HRT[b] days	# of HRT[b]	Substr	OLR g VS/L/d	HRT[b] days	# of HRT[b]
R1	Upp	I	53	FW1	3.0	35	2.8	FW1	3.0	35	1.4	FW1	7.0	15	2.8
R2	Upp	I	53	FW1	3.0	35	2.8	FW1+10%SB[c]	3.3	35	1.4	FW1+10%SB[c]	7.7	15	2.8
R3	Upp	I	53	FW1	3.0	35	2.8	FW1+20%SB[c]	3.6	35	1.4	FW1+20%SB[c]	8.4	15	2.8
R1	Borås	II	53	FW1	3.0	30	2.6	FW1	3.0	30	4.0	FW1	7.0	14	1.5
R2	Borås	II	53	FW1	3.0	30	2.6	FW1+10%SP[c]	3.3	30	4.0	FW1+10%SP[c]	7.7	14	1.5
R3	Borås	II	53	FW1	3.0	30	2.6	FW1+20%SP[c]	3.6	30	4.0	FW1+20%SP[c]	8.4	14	1.5

[a]Inoculum

[b]Hydraulic retention time (HRT). #HRT, number of retention times. The number of HRT (# HRT) in Period 2 and Period 3 at UB and RISE was different due to practical reasons at the different laboratories and not due to stability problems.

[c]The percentage of added straw was in relation to the OLR in VS basis.

3.5 SUB-PROJECT 3 - SEMI-CONTINOUS LABORATORY PROCESSES - CO-DIGESTION OF FOOD WASTE AND STRAW PELLETS IN HIGH AMMONIA PROCESSES

The effect of straw pellets during co-digestion of high nitrogen food waste (FW2) was studied in three (R4, R5 and R6) semi-continuous laboratory scale reactors at RISE, Uppsala. Before addition of the straw pellets these reactors had been run and under investigation for several years by SLU (Westerholm, et al. 2015). Previous to the start of the actual study a chemical characterization of the content of each reactor as well as the substrates was performed. The reactors were run with nitrogen enriched food waste. The high-ammonia level in the food waste was achieved by supplementing egg albumin (Alb) to the original substrate. The control reactor (R4) was run with albumin enriched food waste and in addition amended with trace elements (TE) and iron (Table 5). Reactors R5 and R6 were run only with albumin enriched food waste and without addition of the trace elements mixture and were considered as duplicates during Period 1 (Table 5). The earlier studies (Westerholm, et al 2015) showed that Reactor R4 (amended with TE and iron) had a higher methane production than reactors R5 and R6 and lower VFA levels. During Period 2, reactor R6 was amended with straw pellets corresponding to 10 % of the OLR of food waste and R5 stayed as a control reactor without any addition of either TE/iron or SP. The OLR was kept at 3.0 g VS/L/d in relation to food waste. The OLR in R6 was 3.3 g VS/L/d when amended with SP. The HRT was 35 days (Table 5). All the processes operated at mesophilic (37 °C) conditions.

Table 5. Operational parameters in the semi-continuous processes at high ammonia levels

R	Temp	Period 1				Period 2			
	°C	Substrate	OLR g VS/L/d	HRT[a] days	# HRT[a]	Substrate	OLR g VS/L/d	HRT[a] days	# HRT[a]
R4	37	FW2+Alb[b]+ TE[c]	3.0	35	1.2	FW2+Alb[b]+ TE[c]	3.0	35	4.0
R5	37	FW2+Alb[b]	3.0	35	1.2	FW2+Alb[b]	3.0	35	4.0
R6	37	FW2+Alb[b]	3.0	35	1.2	FW2+Alb[b]+ 10% SP[d]	3.3	35	4.0

[a]Hydraulic retention time (HRT). #HRT, number of retention times

[b]Egg albumin (Alb) was added to increase the nitrogen content according to Westerholm, et al 2015.

[c]Trace elements (TE): Kemira, BDP-868 (0.009 L/kg digester sludge) according to Westerholm, et al 2015.

[d]The percentage of added straw was in relation to the OLR.

The reactors were monitored with conventional process parameters. The parameters analyzed were biogas production and composition, methane production, volatile fatty acids (VFA) concentration, nitrogen levels, pH and alkalinity.

The reactor system used for this study (Dolly, Belach Bioteknik) had a total volume of 10 L and a working volume of 5 L (Figure 3). The produced gas was collected in a bag, the volume was measured with a Ritter Model TG05 / 5 wet gas meter and the gas production normalized (0 ° C, 1 atm), the portable instrument BIOGAS 5000 from Geotech and the Einhorn fermentation saccharometer were also used for gas composition measurements, as described before.

3.6 SUB-PROJECT 4 - STRUCTURAL AND COMPOSITIONAL CHARACTERIZATION OF DIGESTED AND RAW STRAW

Structural characterization of virgin straw, straw product as well as digested straw obtained from the reactors at the end of the continuous digestion experiments were performed using a Fourier transform infrared (FTIR) spectrometer (Impact 410 iS10, Nicolet Instrument Corp., Madison, WI, USA). The spectral data were generated by Nicolet OMNIC 4.1 software (Nicolet Instrument Corp) and analyzed by eFTIR (Essential FTIR, USA). FTIR stands for Fourier Transform InfraRed. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. The crystallinity index is calculated as the ratio of the absorbance at wavelengths of 1420 cm^{-1} and 898 cm^{-1} (Nelson and O'Connor, 1964).

In addition, structural characterization of the samples was performed using a modified version of the Simons' Staining procedure, developed previously by Chandra et al. (2008). Simon staining is used to determine the accessibility of the material for the cellulose degrading enzyme complex. Two different dyes were used, an orange dye which exclusively penetrates into the larger pores, large enough for the enzyme to be able to attach on the surface, and a blue dye, which has a lower affinity to the cellulose and that penetrate into the smaller pores (Chandra et al. 2008). The measurements were performed as described in detail by Teghammar et al. (2012).

Regarding chemical composition, the cellulose, hemicellulose, and lignin contents of the materials were determined according to NREL procedures (Sluiter et al. 2008). In this method, a two-step acid hydrolysis with concentrated and diluted sulfuric acid was performed to liberate the sugars from the cellulose and the hemicellulose. The formed sugars were then quantified by HPLC. The acid-soluble lignin was measured using UV spectroscopy at 280 nm, and acid-insoluble lignin was determined after drying followed by ignition at 575 °C. All lignin and carbohydrate analyses were performed in duplicate.

The total carbohydrate (cellulose and hemicelluloses) was analyzed using HPLC

(Waters 2695, Millipore, Milford, U.S.A.) equipped with a refractive index (RI) detector (Waters 2414, Millipore, Milford, U.S.A.) and an ion-exchange column (Aminex HPX- 87P, Bio-Rad, U.S.A.) at 85 °C using ultra-pure water as the eluent with a flow rate of 0.6 mL/min.

3.7 SUB-PROJECT 5 - EVALUATION OF THE MICROBIAL COMMUNITY STRUCTURE

The composition of the microbial community was analyzed using a DNA-based technique. DNA was extracted from different reactor samples over time (at the end of phase1, 2 and 3) and subjected to so-called Illumina sequencing using 16S rRNA as a target gene. The analysis was conducted with the aim of gaining a picture of the general development of the microbiological community and to generate knowledge of changes caused by the addition of the straw. During the analysis DNA sequences was generated from different microorganisms in the sample and these sequences were later on compared with information available in data bases for the identification. The databases comprise sequences found earlier in different environments, both from previously know and characterized microorganisms but also from unknown. A more accurate description of the method can be found in some recently published scientific articles (Sun et al. 2016, Müller et al. 2016)

To compare the results obtained from the sequencing of samples from the different reactors, the following analyses were made:

• Principal Component Analysis (PCA): This is a statistical method to compare the whole microbial community between different samples. The analysis shows correlations between the community in different sampling points and from different treatment, such as with or without straw.

• Diversity analysis: Diversity is a measure of diversity and can be divided into two parts, number of different microorganisms and distribution. Each identified DNA sequence is unique and called an OTU. This OTU can represent a known or unknown organism. The number of different OTUs in a sample shows how many different organisms that are present in a particular sample, *i.e.* is a measure of number. Distribution is a measure of the abundance of each identified OTU, *i.e.* if there are many different OTUs at the same abundance or if some OTUs that exist in higher or lower number. Diversity can be calculated with different indices, and in this report two different diversity indices have been used, Shannon and Simpson.

• Rarefaction: By comparing the number of different OTUs with the total number of analyzed sequences, a so-called rarefaction curve can be build. This curve shows the number of different OTUs found in relation to the number of sequences generated for a particular sample. Using this curve it is possible to estimate how many different species that potentially exist in the sample but are not captured (covered) in the analysis. If all possible OTUs are identified, this curve levels out to form a plateau and then the coverage is 100%. This, however, rarely happens as the microbial communities in a biogas reactor are very complex. Many organisms are at a very low level and are difficult to "find" among those that are at a higher level. Common numbers for this parameter are between 50-90%.

3.8 SUB-PROJECT 6 – PROCESS EFFICIENCY CALCULATIONS

The efficiency of the investigated processes was determined by calculating the specific methane production (SMP) (Eq. 1), volumetric methane production (VMP) (Eq. 2) and by calculating the degree of degradation (DD) (Eq. 3).

$$SMP = \frac{\text{Total biogas production (NmL) x methane content (\%)}}{\text{VS (g)}} \quad \text{(Eq. 1)}$$

$$VMP = \frac{\text{Total biogas production (NL) x methane content (\%)}}{\text{Reactor active volume (L)}} \quad \text{(Eq. 2)}$$

The degree of degradation, based on TS and VS, was determined by using a simplified calculation that is usually used in Swedish full scale installations where substrate and digestate volumes are assumed to be equal (Eq. 3).

$$DD = \left(\frac{(TS_{subs} \times VS_{subs}) - (TS_{digestate} \times VS_{digestate})}{(TS_{subs} \times VS_{subs})}\right) * 100 \quad \text{(Eq. 3)}$$

Where TS is expressed as % of wet weight and VS as % of TS.

These data were furthermore used for detailed calculations regarding the contribution of biogas from the straw and synergistic effects caused by the co-digestion (see chapter 4.6).

4 Results

4.1 SUB-PROJECT 1 - DETERMINATION OF THE BIOCHEMICAL METHANE POTENTIAL OF THE SUBSTRATES

The biochemical methane potential of the substrates: food waste (FW1), straw briquettes, straw pellets and raw straw as well as cellulose was determined at both laboratories (Table 6).

Table 6. Cumulative methane production and methane production rate from food waste (FW1), straw pellets (SP), straw briquettes (SB) and raw straw (RS).

	NmL CH_4/g VS			**Time (days) to reach the percent of the final methane potential**		
	RISE, Uppsala	UB, Borås	Average from both laboratories	70%	60%	50%
FW1	607±14	445±5**	607±14	14	13	12
SP	332±6	340±4	336±6	20	18	17
SB	366±39	323±7	344±31	21	19	17
RS	313±11	312±6	313±1	22	20	19
Cellulose*	417±15	383±10	400±24	-	-	-

** Control for the evaluation of the inoculum activity.*

***This result was not used in the determination of the average BMP for food waste as discussed later in chapter 5.*

The results showed no significant differences (t-test, $p<0.05$) in the BMP values for SP, SB, RS and cellulose between laboratories. Therefore, the average results of these substrates from both laboratories were calculated and used for comparison with the results for the semi-continuous reactors. Using the average values both SP and SB showed a significant higher BMP value (t-test $p<0.05$) than raw straw. However, no significant differences were found in the methane production rate for SP and SB compared to raw straw (Table 6). In contradiction to the result for the straw, the BMP value for food waste obtained at RISE, Uppsala (607 NmL CH_4/g VS) was significantly higher (t-test $p<0.05$) than that obtained at UB, Borås (445 NmL CH_4/g VS).

4.2 SUB-PROJECT 2 - SEMI-CONTINOUS LABORATORY PROCESSES - CO-DIGESTION OF FOOD WASTE AND STRAW PRODUCTS (BRIQUETTES AND PELLETS)

The purpose was to evaluate whether the addition of straw would give a better utilization of the plant capacity, since the organic load in the reactors can be increased by addition of straw without effecting the hydraulic retention time. Hence, the goal with the semi-continuous laboratory processes within this sub-project was to evaluate how the biogas production and digestion parameters were influenced when wheat straw, either in the form of briquettes or pellets, was added at two different VS loads as co-substrate to the digestion of food waste and with increasing organic loads.

4.2.1 Substrate and inocula characterization

The inocula and the substrates used in these continuous laboratory experiments were subjected to detailed chemical characterization (Table 7). The dry matter and volatile solids content for SP was respectively 91% and 86%. For SB, the dry matter and volatile solids content was 89% and 86%, respectively. These differences were not significant (t-test $p<0.05$). Comparing the two different inocula used, a higher total nutrient content was observed in Inoculum II compared to Inoculum I. This difference may be explained by the operational parameters (*i.e.* large-scale operation vs laboratory experiment) and the homogeneity of substrate used in the process from which the inocula were taken.

4.2.2 Results from Period 2 – lower organic loads

When steady state had been achieved at conditions 3gVS/L/d organic load of food waste in all reactors (Period 1), the addition of straw started. The process operated under these conditions until reaching a new steady state and results obtained are shown in Table 8. Because no changes were applied to the control reactor R1 the average of both period 1 and 2 is reported. The TS and VS in the substrate increased with the presence of straw (reactors R2 and R3). In line with this the TS and VS in the effluents from these reactors were also higher compared to the reference reactor R1, with only FW1. Low levels of VFA (0.2 – 0.4 g/L) and a pH around 8 were observed in all reactors. Only small differences were seen between the reactors R1-R3, however some differences were observed between the processes run at RISE, Uppsala compared to those at UB, Borås, independent on straw addition or not (Table 8). The alkalinity was higher (12 775 – 15 138 mg/L $CaCO_3$) in the reactors operated at RISE, Uppsala compared to the reactors operated at UB, Borås (8 584 – 9 156 mg/L $CaCO_3$). The level of total nitrogen was approximately 4.0 g/L in the reactors at RISE, Uppsala, while at UB, Borås the values fluctuated between 4.6 and 4.9 g/L. The ammonium nitrogen level (1.8 g/L) determined in the reactors at UB, Borås was lower than the level in the reactors at RISE, Uppsala, where ammonium nitrogen concentrations of between 2.6 and 2.8 g/L were obtained. The methane content in the UB reactors fluctuated between 71-72 % CH_4 while it was between 64-65 % CH_4 in the RISE reactors (Table 8). A trend of higher levels of H_2S was observed with higher levels of SB in the substrate (987

and 1 040 ppm for reactors R2 and R3, respectively) compared to reactor R1 (859 ppm) with only FW1. No H_2S measurements were made in the SP reactors.

The specific methane production (SMP) showed the same trends at both laboratories (Table 8). The presence of straw (SP or SB) had no effect on the specific methane production (SMP, NmL CH_4/g VS) and no statistical differences (t-test $p>0.05$) were observed in reactors R2 (FW1+10% straw) and R3 (FW1+20% straw) compared to R1 (control process with only FW1).

The volumetric methane production (VMP, NL/L/d) in RISE, Uppsala in reactor R3 (FW1+20%SB) was significantly (t-test $p>0.05$) higher compared to the control reactor R1 (only FW1) and to R2 (FW1+10%SB), but no difference was observed between R2 (FW1+10% SB) and R1 (only FW1). At UB, Borås a significant (t-test $p>0.05$) difference in VMP was seen between all three reactors R1 (FW1), R2 (FW1+10%SP) and R3 (FW1+20%SP). The VMP order was R3>R2>R1 (Table 8).

The degree of degradation (DD) was significantly higher in the processes with only FW1 as substrate as compared to those containing SB (Table 8). However, the degree of degradation in the processes supplemented with SP showed no significant differences compared to the reference reactor with only FW1.

Table 7. Chemical characterization of the inocula, food waste, straw briquettes and straw pellets used in the laboratory scale reactors (Sub-project 2).

		Inoc.-I*	Inoc.-II**	Food waste (FW1)	Straw briquettes (SB)	Straw pellets (SP)
TS	%	3.3	2.9	12.7	89.4	90.8
VS	%	1.9	1.7	10.9	85.9	85.6
VS	% of TS	58	59	86	96	94
Tot-N	kg/ton	4.1	4.1	4.9	5.5	5.3
Org-N	kg/ton	0.7	1.1	4.4	-	-
NH_4-N	kg/ton	3.5	3.0	0.6	-	-
Tot-C	kg/ton	4.4	9.9	60.3	436	437
C/N		1.1	2.4	12.3	79.3	82.5
Tot-P	kg/ton	0.2	0.4	0.5	0.8	0.5
Tot-K	kg/ton	1.1	1.4	1.5	11.8	8.8
Tot-Mg	kg/ton	0.1	0.2	0.2	0.8	0.6
Tot-Ca	kg/ton	0.3	1.4	2.4	1.9	2.5
Tot-Na	kg/ton	1.8	1.3	1.4	0.2	0.4
Tot-S	kg/ton	0.1	0.2	0.3	1.2	1.2
Tot-Fe	mg/kg TS	2 090	3 962	1 454	57	78
Tot-Cu	mg/kg TS	12	49	13	2	2
Tot-Mn	mg/kg TS	143	197	84	20	39
Tot-Zn	mg/kg TS	141	238	46	27	17
As	mg/kg TS	8.4	4.6	1.3	< 0.08	< 0.08
Cd	mg/kg TS	0.3	0.4	0.1	0.1	0.1
Co	mg/kg TS	3.9	3.4	2.6	0.02	0.03
Cr	mg/kg TS	6.7	36.4	18.1	0.1	0.2
Hg	mg/kg TS	0.1	0.1	0.03	< 0.01	< 0.01
Ni	mg/kg TS	11.9	17.9	8.7	0.02	0.03
Pb	mg/kg TS	2.1	5.7	2.0	0.1	0.1
Se	mg/kg TS	2.2	0.8	0.2	< 0.02	< 0.02

*Inoculum collected in September 15, 2015 from the large-scale biogas plant at Sobacken, Borås.

**Inoculum collected at RISE, Uppsala from the laboratory reactors running with food waste during the period between 30 November to 17 December 2015.

Table 8. Summary of the operational parameters and chemical composition of the substrates, digestates and biogas in the processes operated during Period 2 at RISE, Uppsala and UB, Borås. (nd = not determined)

Laboratory		**Uppsala**			**Borås**		
Reactor		R1	R2	R3	R1	R2	R3
Temperature	°C	53	53	53	53	53	53
OLR – FW1	g VS/L/d	3.0	3.0	3.0	3.0	3.0	3.0
OLR - straw	g VS/L/d	0	0.3	0.6	0	0.3	0.6
OLR –	g VS/L/d	3.0	3.3	3.6	3.0	3.3	3.6
HRT	d	35	35	35	30	30	30
Number of HRT		4.2	1.4	1.4	6.6	4.0	4.0
Substrate		FW1	FW1 + 10% SB	FW1 + 20% SB	FW1	FW1 + 10% SP	FW1 +20% SP
TS_{in}	% ww	12.2±0.02	13.3±0.21	14.4±0.24	11.1±0.78	12.5±0.77	13.7±0.77
VS_{in}	% ww	10.6±0.01	11.6±0.18	12.6±0.18	9.3±0.73	10.6±0.72	11.6±0.72
VS_{in}	% of TS	86	87	88	84	85	85
Biogas quality							
CH_4 (GC)	%	64±2.0	65±1.5	64±2.3	72±2.4	71±2.1	71±2.2
CH_4 (B-5000)	%	66±2.0	65±1.2	64±1.0	nd	nd	nd
CO_2 (B-5000)	%	33±1.0	34±1.1	35±1.0	nd	nd	nd
H_2S (B-5000)	ppm	859±9	987±189	1 040±242	nd	nd	nd
O_2 (B-5000)	%	0.4±0.04	0.36±0.10	0.43±0.08	nd	nd	nd
Digestate							
pH		8.0±0.01	8.0±0.03	8.0±0.12	8.3±0.13	8.3±0.15	8.4±0.10
Alkalinity	$CaCO_3$ g/L	12 755±656	15 138±290	14 655±277	8 584±689	8 647±746	9 156±528
VFA (tot)	g/L	0.3±0.07	0.4±0.12	0.4±0.12	0.3±0.13	0.2±0.07	0.4±0.12
TS_{ut}	% ww	3.4±0.1	4.5±0.1	4.6±0.3	3.5±0.5	4.7±0.6	4.5±0.5
VS_{ut}	% ww	2.1±0.1	2.9±0.2	3.0±0.2	2.2±0.3	2.9±0.4	2.9±0.4
VS_{ut}	% of TS	62	64	65	60	63	65
Tot-N	kg/ton	4.0±0.3	4.1±0.0	4.1±0.0	4.9±0.2	4.6±0.6	4.8±0.1
NH_4-N	g/L	2.8±0.1	2.6±0.0	2.6±0.0	1.7±0.1	1.8±0.1	1.8±0.2
Org-N	kg/ton	1.2	1.5	1.5	3.2	2.8	3.0
SMP	NmL CH_4/g VS	500 ± 15	497 ± 34	473 ± 32	478 ± 23	469 ± 33	488 ± 38
VMP	NL CH_4/L_{RK}/d	1.53 ± 0.11	1.64 ± 0.12	1.71 ± 0.12	1.4 ± 0.07	1.5 ± 0.11	1.8 ± 0.14
DD	%	80 ± 0.8	75 ± 1	76 ± 2	76 ± 3	73 ± 6	75 ± 7

4.2.3 Results from Period 3 – higher organic loads

During Period 3, even though the OLR for the food waste increased to 7 g VS/L/d (*i.e.* the OLR was 7.7 and 8.4 g VS/L/d in the co-digestion reactors) the processes were still stable (Table 9). There was still no significant difference in the biogas composition observed among reactors with and without the presence of straw (Table 9). However, VFA levels (1.8 – 3.9 g/L) were higher compared with the levels at 3 g VS/L/d and regardless of the presence or absence of straw (Table 8).

The SMP (NmL CH_4/g VS) in the Uppsala reactor R1 (FW1) was significantly (t-test $p>0.05$) higher than R3 (FW1+20%SB), while no significant difference was found between R1 (only FW1) and R2 (FW1+10% SB). The SMP in the Borås reactors showed no significant differences between the processes with SP (reactors R2 and R3) compared to reactor R1 (only FW1). The VMP (NL CH_4/L/d) in the Uppsala reactors showed no statistical differences (t-test $p>0.05$) among the processes. On the contrary, a trend of higher volumetric methane production with higher levels of SP could be observed in the Borås reactors. However, the differences were not significant (Table 9). The degree of degradation was significantly higher in the processes with only FW1 as substrate as compared to those containing SB (Table 9). As for the lower OLR, the processes with SP showed similar degradation degrees as when only FW1 was digested (Table 9).

The straw particles in the reactors run with SB were not efficiently mixed and floating layers were observed (Figure 5). At the end of the experiment, when the reactors were opened, it was estimated that approximately 5% of the reactor active volume was occupied by the floating layer in reactor R2 (FW1+10%SB). In reactor R3 (FW1+20% SB) this figure was estimated to 35%. This influenced the mixing rate that had to be increased in reactor R3 to enable a better mixing. No such problems were observed using SP.

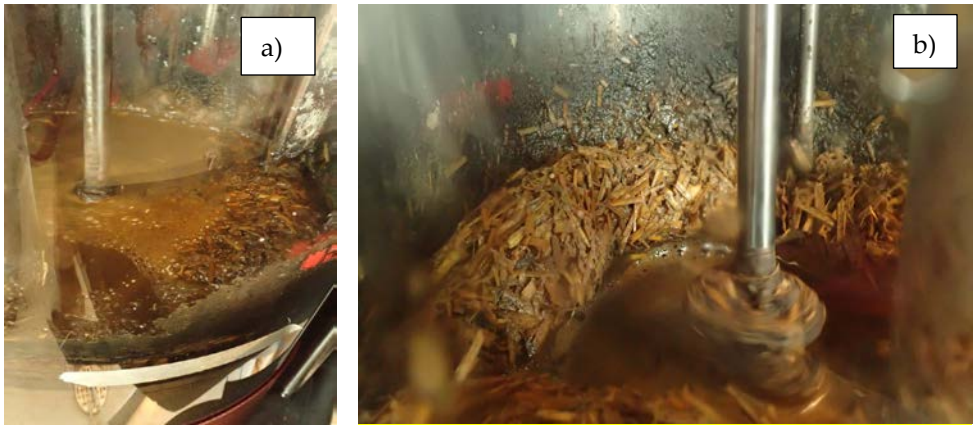


Figure 5. Straw layer formed in: a) reactor R2 (FW1+10 % SB) and b) R3 (FW1 + 20% SB) at an OLR of 7.7 and 8.4 g VS/L/d, respectively.

Table 9. Summary of the operational parameters and chemical composition of the substrates, digestates and biogas in the processes operated during Period 3 at RISE, Uppsala and UB, Borås. (nd = not determined)

Laboratory		**Uppsala**			**Borås**		
Reactor		R1	R2	R3	R1	R2	R3
Temperature	°C	53	53	53	53	53	53
OLR – FW1	g VS/L/d	7.0	7.0	7.0	7.0	7.0	7.0
OLR - SB	g VS/L/d	0	0.7	1.4	0	0.7	1.4
OLR – FW1+SB	g VS/L/d	**7.0**	**7.7**	**8.4**	**7.0**	**7.7**	**8.4**
HRT	d	**15**	**15**	**15**	**14**	**14**	**14**
Number of HRT		2.7	2.7	2.7	1.5	1.5	1.5
Substrate		FW1	FW1 + 10% SB	FW1 + 20% SB	FW1	FW1 + 10% SP	FW1 + 20% SP
TS_{in}	% ww	12.8±0.37	13.7±0.47	14.6±0.48	13.2±0.07	14.5±0.08	15.8±0.09
VS_{in}	% ww	11.0±0.30	11.9±0.38	12.9±0.39	11.4±0.02	12.6±0.03	13.9±0.04
VS_{in}	% of TS	86	87	88	86	87	88
Biogas quality							
CH_4 (GC)	%	68±1.0	68±1.5	65±1.6	75±1.0	74±1.8	71±2.1
CH_4 (B-5000)	%	69±0.6	68±2.7	67±1.2	nd	nd	nd
CO_2 (B-5000)	%	29±0.7	30±2.2	31±0.5	nd	nd	nd
H_2S (B-5000)	ppm	732±54	670±82	734±56	nd	nd	nd
O_2 (B-5000)	%	1.4±2.2	0.50±0.11	0.43±0.19	nd	nd	nd
Digestate							
pH		7.9±0.04	7.9±0.07	7.8±0.10	8.4±0.11	8.4±0.11	8.4±0.11
Alkalinity	$CaCO_3$ g/L	12 919±621	11 567±0	12 952±900	18 383±777	19 017±459	18 383±777
VFA (tot)	g/L	2.0±0.83	3.0±0.81	3.9±1.79	3.1±0.82	2.3±0.71	3.9±0.84
TS_{ut}	% ww	4.7±0.1	5.7±0.2	6.5±0.7	5.3±0.5	5.9±0.6	5.8±0.7
VS_{ut}	% ww	3.2±0.1	4.1±0.2	4.8±0.7	3.5±0.1	4.2±0.2	4.2±0.4
VS_{ut}	% of TS	68	72	74	66	71	72
Tot-N	kg/ton	4.7±0.0	4.6±0.0	4.6±0.0	4.9±0.0	5.1±0.0	4.9±0.0
NH_4-N	g/L	2.2±0.0	2.3±0.0	2.3±0.0	2.4±0.0	2.5±0.0	2.5±0.0
Org-N	kg/ton	2.5	2.3	2.3	2.5	2.6	2.4
SMP	NmL CH_4/g VS	494 ± 54	449 ± 30	377 ± 71	529 ± 45	495 ± 32	519± 54
VMP	NL CH_4/L_{RK}/d	3.47 ± 0.39	3.45 ± 0.23	3.17 ± 0.59	3.7 ± 0.3	3.8 ± 0.2	4.4 ± 0.5
DD	%	71 ± 2	65 ± 2	62 ± 7	69 ± 1	67 ± 2	70 ± 3

4.3 SUB-PROJECT 3 - SEMI-CONTINUOUS LABORATORY PROCESSES - CO-DIGESTION OF FOOD WASTE AND STRAW PELLETS IN HIGH AMMONIA PROCESSES

Biogas processes treating easily degradable carbon and nitrogen-rich substrates, such as food waste, might have instability and low efficiency. These processes typically show high levels of ammonia nitrogen and VFA levels, and hence are normally run at rather low OLR, are sensitive to small loading changes and consequently show low methane production. Our hypothesis was that in such cases stability could be reached when easily degradable carbon and nitrogen-rich substrates were co-digested with substrates with high levels of slowly degradable carbon, as in the case of straw.

Three reactors (R4, R5 and R6) used in this investigation had been run for a long time (Westerholm et al 2015) at high ammonia nitrogen levels. The three processes were run with a food waste substrate (FW2, C/N 14.2) amended with egg albumin to decrease the C/N ratio to 6.4. Reactor R4 was a reference reactor, which was running at stable conditions using a substrate (FW2+alb) supplemented with trace elements and iron. Reactors R5 and R6, not receiving this additive were at pseudo stable conditions (*i.e.* stable but at high VFA content and sensitive to changes). During Period 1, R5 and R6 were run at the same conditions (with only albumin-amended food waste). Later, during Period 2 (Table 5), the substrate in reactor R6 was further amended with SP (10% of the OLR). SP was chosen as this material performed better than SB during sub-project 2. Reactor R5 stayed as a control reactor without addition of straw. Before the initiation of the study, representative samples were collected from the content of each reactor and chemically characterized. The food waste with and without the amendments was also analyzed (Table 10).

Table 11 shows the results obtained during Period 1 and 2 of this sub-project. Because no changes were applied to the reference reactor R4 the average of both periods is reported. In general, it was observed that R4, receiving nitrogen rich food waste supplemented with trace elements and iron, showed very stable conditions during the whole study, with low levels of VFA, pH around 8 and low levels of H_2S in the biogas. R5 showed slightly higher specific and volumetric methane productions compared to R6, however the differences were not significant (t-test $p<0.05$) (data not shown). During Period 2, the OLR was increased by 10% through addition of SP to the substrate in reactor R6 (FW2 + Alb), while R5 stayed as control and was continuously run with only FW2+Alb. The reactors were operated under these conditions under four HRTs. At HRT2, R5 had a higher (t-test $p<0.05$) SMP than R6 (Table 11). However, at HRT4 the SMP and VMP in the straw amended reactor, R6, were significant higher (232 ± 21 NmL CH_4/g VS and 0.7 ± 0.1 NL CH_4/L_{RK}/d, respectively) than those in the control reactor, R5 (162 ± 21 NmL CH_4/g VS and 0.5 ± 0.02 NL CH_4/L_{RK}/d, respectively). However, the specific and volumetric methane production in the reference reactor, R4, was clearly much higher with 388 ± 14 NmL CH_4/g VS and 1.2 ± 0.05 NL CH_4/L_{RK}/d, respectively. This means that none of the other processes (R5 and R6) could reach the levels of the reference process amended with trace elements and iron (Table 11).

Table 10. Chemical characterization of the food waste and the initial conditions in reactors R4, R5 and R6 of Sub-project 3. Food waste 2 (FW2), Albumine (Alb), Trace elements (TE).

		Initial conditions at			FW2	FW2+Alb	FW2+Alb+ TE
		R4	R5	R6			
TS	%	4.5	5.5	5.7	19.3	15.2	15.2
VS	%	3.2	4.0	4.3	14.4	12.6	12.5
VS	% of TS	69.6	72.9	76.4	74.4	82.9	82.3
Tot-N	kg/ton	7.8	8.2	8.1	5.5	10.8	10.8
Org-N	kg/ton	2.2	2.2	2.3	5.0	10.5	10.5
NH_4-N	kg/ton	5.5	5.9	5.8	0.5	0.3	0.3
Tot-C	kg/ton	17.2	22.9	24.4	78.9	68.5	67.8
C/N		2.2	2.8	3.0	14.2	6.4	6.3
Tot-P	kg/ton	0.3	0.4	0.3	1.1	0.5	0.5
Tot-K	kg/ton	1.1	1.2	1.1	1.6	1.6	1.6
Tot-Mg	kg/ton	0.1	0.2	0.2	0.5	0.3	0.3
Tot-Ca	kg/ton	2.0	2.2	2.0	5.2	2.5	2.4
Tot-Na	kg/ton	0.9	1.0	0.9	0.9	1.3	1.3
Tot-S	kg/ton	0.5	0.4	0.4	0.4	1.0	1.0
Tot-Cu	mg/kg TS	49	48	46	64	37	35
Tot-Fe	mg/kg TS	14 564	3 941	2 944	4 532	2 752	8 016
Tot-Mn	mg/kg TS	128	134	124	140	80	85
Tot-Zn	mg/kg TS	76	82	76	71	44	40
As	mg/kg TS	5.0	5.6	3.6	1.5	0.9	0.8
Cd	mg/kg TS	0.2	0.2	0.2	0.3	0.2	0.2
Co	mg/kg TS	8.6	2.4	1.9	2.3	1.4	4.6
Cr	mg/kg TS	18.1	32.2	19.7	9.5	5.7	4.5
Hg	mg/kg TS	0.1	0.1	0.0	0.0	0.0	0.0
Ni	mg/kg TS	17.4	21.7	16.5	8.8	5.4	6.8
Pb	mg/kg TS	67.6	77.3	47.7	19.4	40.5	11.3
Se	mg/kg TS	2.0	0.7	0.6	0.2	0.3	1.1

Table 11. Summary of the operational parameters and chemical composition of the substrates, digestates and biogas in the high-ammonia processes. Period 2 lasted 4 HRT and the results reported here are from the second and the fourth HRT. (nd = not determined)

Period		**Period 1 and 2**	**Period 2 (HRT2)**		**Period 2 (HRT4)**	
Reactor		**R4**	**R5**	**R6**	**R5**	**R6**
Temperature	°C	37	37	37	37	37
OLR – FW2	g VS/L/d	3.0	3.0	3.0	3.0	3.0
OLR - SB	g VS/L/d	0	0	0.3		0.3
OLR – FW+SB	g VS/L/d	3.0	3.0	3.3	3.0	3.3
HRT	d	35	35	35	35	35
Number of		5				4
Substrate		FW2+Alb+TE	FW2+Alb	FW2+Alb	FW2+Alb	FW2+Alb+SP
TS_{in}	% ww	12.9±0.3	11.7±1.3	12.6±1.3	12.9±0.1	14.0±0.1
VS_{in}	% ww	10.5±0.1	9.7±1.0	10.9±1.3	10.6±0.0	11.6±0.1
VS_{in}	% of TS	81	83	87	82	
Biogas quality						
CH_4 (GC)	%	64±2.0	49±7.0	46±0.9	51±1.4	58±2.2
CH_4 (B-5000)	%	63±0.6	48±6	45±1.2	49±0.7	56±0.8
CO_2 (B-5000)	%	35±0.6	45±5	50±1.2	44±0.5	39.2±0.8
H_2S (B-5000)	ppm	602±129	3 734±428	4 320±386	4 130±443	3 719±103
O_2 (B-5000)	%	0.4±0.1	0.4±0.1	0.4±0.1	0.4±0.0	0.5±0.1
Digestate						
pH		8.0±0.1	7.7±0.1	7.9±0.04	7.4±0.1	7.6±0.1
Alkalinity	$CaCO_3$ g/L	27 483±1 083	24 516±2471	17 810±1446	21 549±0	21 322±0
VFA (tot)	g/L	0.1±0.1	13.8±5	19.5±1.3	23.9±2.7	19.6±1.6
TS_{ut}	% ww	4.6±0.3	nd	nd	5.0±0.4	6.6±0.3
VS_{ut}	% ww	3.0±0.1	nd	nd	3.6±0.2	5.4±0.2
VS_{ut}	% of TS	65	nd	nd	72	82
NH_4-N	g/L	6.3±0.3	6.8±0.5	5.6±0.1	6.6±0.0	6.0±0.5
SMP	NmL CH_4/g VS	388 ± 14	164 ± 56	141 ± 10	162 ± 21	232 ± 21
VMP	NL CH_4/L_{RK}/d	1.2 ± 0.05	0.5 ± 0.2	0.3 ± 0.02	0.5 ± 0.02	0.7 ± 0.1
DD	%	71 ± 1	nd	nd	64 ± 3	53 ± 2

4.4 SUB-PROJECT 4 – COMPOSITIONAL AND STRUCTURAL CHARACTERIZATION OF RAW AND DIGESTED STRAW

The compositional analysis of raw straw, pellets and briquettes (Table 12), showed an almost similar composition of three raw materials suggesting that the pretreatment caused very little changes on the chemical composition. Furthermore, the FTIR analysis (Table 13) showed a comparable crystallinity index for the different straw products. This suggests that even though pretreatment reduces the particle size and makes it more amenable for microbes it does not considerably change the molecular fingerprints of straw.

Table 12. Compositional analysis of raw straw and the straw products, pellets and briquettes

Parameters		Raw straw	Pellets	Briquettes
Water soluble extractives	%	9.72	7.65	6.57
Ethanol soluble extractives	%	2.04	1.14	3.25
Total extractives	%	11.76	8.79	9.82
Ash	%	3.33	1.08	0.87
Lignin and carbohydrates after extractive and ash correction in whole biomass				
Klason lignin (Acid insoluble)	%	17.6	17,5	16,4
Acid soluble lignin	%	6.50	6.47	6.25
Total lignin	%	24.10	24.00	22.66
Holocellulose	%	60.81	66.13	66.65
Glucan	%	41.2	43.7	41.7
Mannan	%	0	0	0
Xylan	%	20.4	21.5	20.4
Arabinan	%	4.25	5.1	4.54

The values presented corresponds to % of TS basis

Table 13. FTIR analysis of straw pellets and briquettes

Straw type	Crystallinity index (CI)*
Raw straw	0.41
Pellets	0.66
Briquettes	0.57
Digested Pellets, 10% addition	0.76
Digested Pellets, 20% addition	0.89
Digested Briquettes, 10% addition	0.77
Digested Briquettes, 20% addition	1.00

**CI: ratio of absorbance at wavelength 1420 cm^{-1} and 898 cm^{-1}*

The straw particles remaining after the digestion process had a higher crystallinity index, indicating that straw particles with high crystallinity were less efficiently converted in the process as compared to particles with lower crystallinity (Table 13). Furthermore, Simon's staining on virgin, pretreated and digested straw showed that pretreated straw adsorbed more orange dye compared to virgin straw (Figure 6), indicating a more porous and open structure of the straw products. Moreover, the adsorption of the orange dye increased in both pellets and briquettes after digestion (Figure 6). These results indicate that the straw after digestion has larger pores as compared to before digestion.

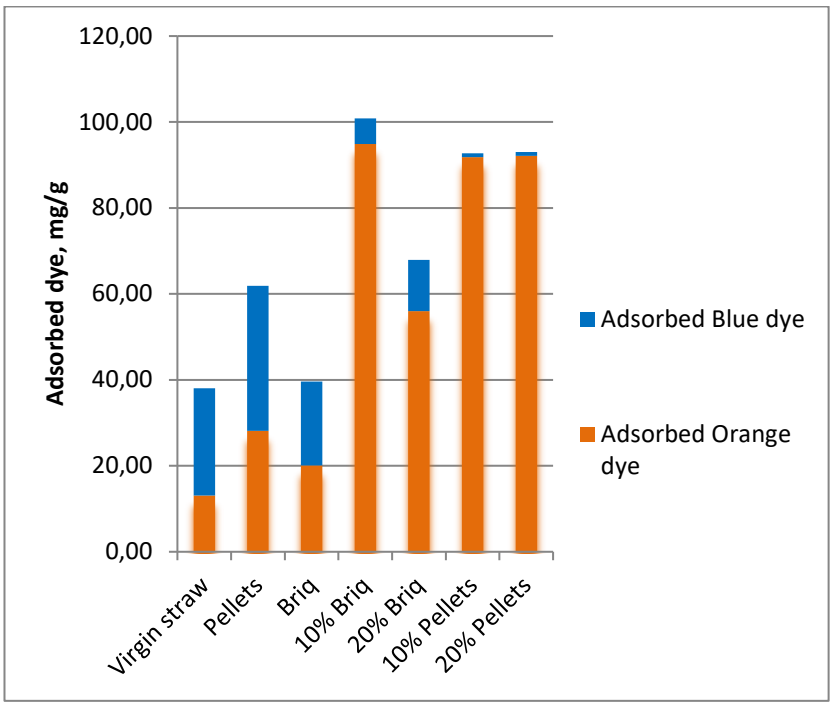


Figure 6. Simon's staining of virgin straw, undigested/digested pellets and briquettes

The effect was equally evident for both pellets and briquettes, with exception of straw briquettes at 20% loading which showed less adsorption of the orange dye after the digestion process. An explanation for this result could be the poor mixing conditions leading to a less efficient digestion (Figure 5). Pellets showed a very well digested and porous structure even at high loading of 20%.

4.5 SUB-PROJECT 5 - EVALUATION OF THE MICROBIAL COMMUNITY STRUCTURE

Samples were analyzed before addition of straw (sample 1), during OLR increase (sample 2 and 3) and after reaching full load (sample 4). The number of observed OTUs was similar in most samples, ranging from 1589 to 2002. One sample was different from the rest, having a slightly lower value ranging from 1916-1170 in the

triplicate samples (reactor at UB, Borås sampling 2). These samples also showed a comparably lower diversity in regard to evenness, while all other samples showed similar values. The coverage, *i.e.* the number of identified OTUs in relation to the actual number of organisms present ranged between 69 and 86%, with the lowest values for the sample showing the comparably lower diversity.

A statistical analysis of the whole community in the samples using PCA was performed using both weighted and unweighted analysis, *i.e.* the number of each identified OTU was counted for (weighted) or not (unweighted). The weighted analysis illustrated similar communities at start, but still with some separation between different reactors (Figure 7). Operation over time resulted in community changes, with most effect on the reactors operated by RISE, except for one sampling point of the Borås reactors (sampling 2). Still, at the end of the experiment, no clear trend could be seen in regard to the addition of straw and the communities formed clusters close to each other (Figure 7A). The unweighted analysis showed somewhat different results. Here, all sample from the RISE reactors formed a single cluster, clearly separated from the reactors operated by Borås (Figure 7B). The samples from Borås were furthermore separated from each other for each sampling occasion, *i.e.* an effect of time and/or increase in OLR was seen. However, no clear effect in response to SP addition could be seen.

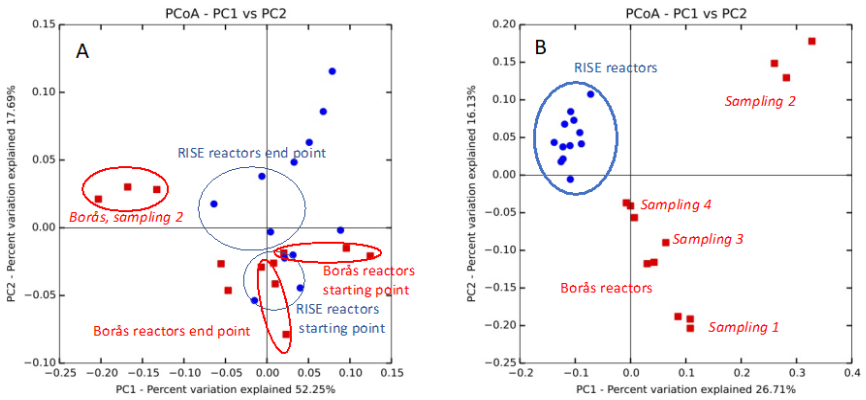


Figure 7. Weighted (A) and Unweighted (B) PCA analysis of the microbial communities in the reactors operated with food waste (FW1) alone or combined with straw pellets (SP; UB, Borås; red) or straw briquettes (SB; RISE, Uppsala, blue).

A more thorough taxonomic analysis was performed to generate knowledge of the overall community as well as reveal possible effects caused by the straw. Microorganisms can be divided into various so-called phylogenetic levels of which the top is domain; the next level is phyla, followed by class, order, family, genus and species. Bacteria and Archaea, including the methanogens, were the two dominating domains in the biogas community. In the samples analyzed, the bacteria represented approximately 98-99% of the whole community, some of which were unknown organisms (1.6-15.4%), and the Archaea were only 0.1-2%. The bacterial community in all reactors was dominated by members belonging to the phyla Firmicutes (29.5-57.4%), Thermotogae (31.4-67.1) and Synergistetes (0.8-

4.0%) (Figure 8). In addition, a low relative abundance of the phyla Proteobacteria, OP9 and Bacteroidetes (<1%) was also found in most samples. The general trend in the reactors was a slight increase and decrease in the relative abundance on the phylum Thermotogae and Firmicutes, respectively, in response to the increase in OLR. These changes were independent on the straw addition, *e.g.* the same trend was seen for the reactor R1 receiving food waste only. A response in the community related to the addition of straw was only found for the reactor operating with the highest level (20%) of straw pellets (R3, at UB, Borås). In this reactor, the abundance of Bacteroidetes increased over time to reach a final value of 3.2% (Figure 8). The phylum Euryarchaeota harboring the methanogenic groups represented only 0.1-2% of the community, with the highest level found at the first sampling occasion for the reactors operated at UB, Borås.

Looking at a lower taxonomic level, the Firmicutes were dominated by the class Clostridia, order *Clostridiales* (2.6-9.9%), MBA08 (8.3-30.9%), SHA-98 (2.7-9.8%) and Thermoanaerobacterales (0.6-7.7%), with no general trends for the different samples. Thermotogae and Synergistestes were represented only by the orders *Thermotogales* and *Synergestales*, respectively. Also for these groups no general trends could be seen for the different samples. Bacteroidetes, increasing over time in the reactor supplemented with 20% SP, was represented by the order *Porphyromondaceae*. Phylum Euryarchaeota was in all samples mainly represented by the genus *Methanothermobacter* (order *Methanobacteriales*).

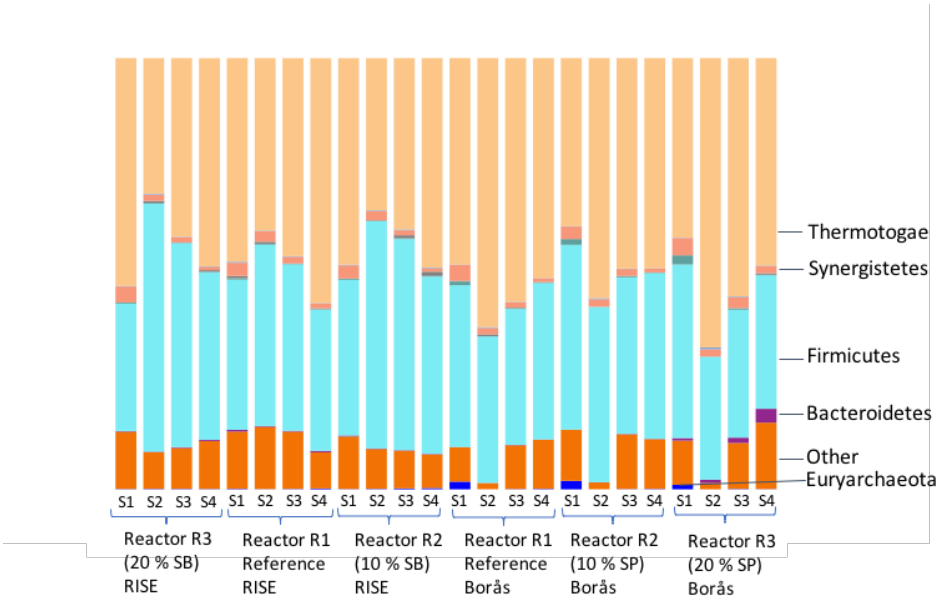


Figure 8. Distribution of Phyla in samples from reactors operated at RISE Uppsala and Borås University. Reactor R1 represented reference reactors operated with only food waste and R2 and R3 were experimental reactors supplemented with straw pellets (SP; UB, Borås) or straw briquettes (SB; RISE, Uppsala) in addition to food waste (FW1). Sample explanation: S1 (before straw addition), S2 and S3 (during increase of OLR); S4 (at the highest OLR). Sample explanation: S1(before straw addition, S2 and S3 (during increase of OLR); S4 (at the highest OLR)

4.6 SUB-PROJECT 6 – PROCESS EFFICIENCY CALCULATIONS

The process efficiency and the effect of the addition of straw on methane production were evaluated based on specific and volumetric methane production, determined at steady state conditions at two different organic loading (Tables 8 and 9), as well as the degree of degradation. Besides that, additional calculations were performed using data obtained in Sub-project 2, *i.e.* the theoretical maximum SMP in the reactors was calculated based on the BMP obtained for food waste (FW1, 607 NmL CH_4/g VS) and straw (340 NmL CH_4/gVS).

Comparing the experimental data with the maximum calculated SMP values, the results show that most of the processes produced between 67 to 87 % of the maximum calculated SMP (Table 14). The process that produced the highest value (92% of the maximum calculated SMP) was the one digesting the mixture of food waste with 20% of SP. The lowest values were obtained from processes with addition of SB at the higher OLR level.

Considering the VMP with and without the presence of straw at the lowest OLR level the co-digestion processes using 20% SB produced 13.5% more methane per day compared to the process with only FW1 (Table 15). When using SP as co-substrate at a level of 10 and 20% of the OLR, the daily methane production increased by 9.5 and 22.5%, respectively, compared to that obtained in the control process digesting only FW1. At the high OLR level the daily methane production was also higher as compared to the control reactor, but only when SP was added at a level of 20%. In that case 17.7% enhancement in the daily methane production was detected (Table 15).

The contribution of straw to the methane production was also calculated by subtracting the methane volume (NmL/d) produced by food waste (R1) from the total daily methane volume produced in the co-digestion reactors (R2 and R3). According to these calculations, at the lower OLR level (*i.e.* 3 g VS/L/d of FW1) and in the case of addition of SB, a remaining methane volume of 701 NmL CH_4/d and 1 014 NmL CH_4/d in R2 (FW1+10% SB) and in R3 (FW1+20% SB), respectively, can be assumed to originate from the straw. While, in the case of addition of SP, the corresponding values were 341 NmL CH_4/d in R2 (FW1+10% SP) and 968 NmL CH_4/d in R3 (FW1+20% SP), respectively (Table 15). By dividing these values with the g VS/d of straw added, the respective specific methane production of straw can be estimated. This calculation gave values of 467 and 338 NmL CH_4/g VS_{straw} for addition of 10 and 20% SB, respectively, and 379 and 538 NmL CH_4/g VS_{straw}, respectively, when 10 and 20% of SP was added. A similar calculation at the higher OLR level (*i.e.* 7 g VS/L/d of FW1) showed an estimated methane production from SP of 155 and 469 NmL CH_4/g VS_{straw} calculated for R2 (FW1+10% SP) and R3 (FW1+20% SP), respectively (Table 15). The processes with SB showed a lower daily methane production compared to the reactor with only food waste indicating that the addition of SB had no or even a negative effect on the process (Table 15). These calculated methane production values (*i.e.* 467, 538 and 469 NmL CH_4/g

VS_{straw}) were all higher than the BMP values previously determined for straw in the batch digestion assays *i.e.* 340 NmL CH_4/g VS, (Table 6). This suggests that the straw either was more efficiently degraded in the semi continuous processes compared to the batch assay or alternatively that the extra methane was produced from the food waste, due to synergistic effects. Because the BMP value is the maximum that can be expected for a substrate (straw), it can be concluded that the extra methane volume must be coming from an enhanced degradation of food waste in the presence of straw.

Methane production from the food waste was also estimated by subtracting the methane contribution from straw (340 NmL CH_4/g VS) from the values obtained in the co-digestion reactors. At the lower level of OLR (*i.e.* 3 g VS/L/d of FW1) and in the presence of straw, FW1 produced between 1 to 8% more methane compared to the process where FW1 was digested alone (Table 16). The highest improvement of 8% was obtained from the process amended with 20% SP. Furthermore, according to these calculations at the higher OLR level (*i.e.* 7 g VS/L/d of FW1), 5% more methane was produced from the FW1 when co-digested with 20% SP. On contrary, the addition of SB (at the high OLR levels) gave a lower methane production from FW1, due to process disturbances observed at these high loads of straw briquettes (Figure 5).

This calculated increase in methane production (Table 15) due to the addition of straw, was used to estimate the excess energy produced in the co-digestion process. Using the energy content of 10 kWh/Nm^3 of methane and taking into account the energy consumption of 100 kWh/ton (Xavier et al (2015) for the briquetting process, 3 900 and 2 800 kWh/ton net energy production can be achieved using 10% and 20% SB, respectively, at the lower OLR of food waste. For SP these net energy values were calculated to about 3 000 kWh/ton and 4 350 kWh/ton when taking into account the energy consumption of 260 kWh/ton for the pelleting process (Lars-Erik Jansson, personal communication). In comparison, at higher loading rates only the addition of SP contributed to the daily increase in methane production in the co-digestion reactors, corresponding to 1 100 and 3 700 kWh/ton net energy production when 10 and 20% (VS basis in OLR) SP, respectively, was added to the digestion of FW1. In these calculations, the TS of 86% of straw (Table 7) was applied.

Similar process efficiency calculations were performed in Sub-project 3 to estimate the amount of additional methane produced in the presence of SP. In this case the comparison was made between reactors R5 and R6. Even though SMP and VMP in these reactors did not reach the SMP and VMP of the reference reactor R4 (with trace elements addition) the better performance of R6 in relation to reactor R5 due to the straw addition was clear. Correspondingly, the methane production from the food waste in the co-digestion reactor, R6, was estimated by subtracting the methane contribution from straw using the BMP value of 340 NmL CH_4/g VS (Table 17). After subtracting the methane contribution estimated for the straw, FW2 produced 3 318 NmL CH_4/d in the co-digestion with SP, compared to the 2 430 NmL CH_4/d produced when mono-digested, *i.e.* again a synergistic effect was indicated.

Table 14. Semi-continuous processes in Sub-project 2 – Comparison between the theoretical SMP calculated based on the BMP for each single substrate (FW= 607 NmL CH_4/g VS and straw = 340 NmL CH_4/g VS) and the experimental results obtained at RISE (with SB) and UB (with SP).

				SMP NmL CH_4/g $VS_{FW+Straw}$			**SMP experimental vs calculated (%)**	
		OLR_{FW} g VS/L/d	OLR_{straw} g VS/L/d	Calculated based on BMP	Experimental RISE reactors	Experimental UB reactors	RISE (SB)	UB (SP)
R1	FW1	3.0	0	**607**	500	478	82	79
R2	FW1+10% straw	3.0	0.3	**583**	497	469	85	80
R3	FW1+20% straw	3.0	0.6	**563**	473	488	84	87
R1	FW1	7.0	0	**607**	494	529	81	87
R2	FW1+10% straw	7.0	0.7	**583**	449	495	77	85
R3	FW1+20% straw	7.0	1.4	**563**	377	519	67	92

Table 15. Semi-continuous processes in Sub-project 2 – Calculation of straw specific methane (NmL CH_4/g VS) contribution by substracting the food waste methane production (NmL CH_4/d) in reference reactor R1 from the total methane production (NmL CH_4/d) in co-digestion reactors R2 and R3.

		OLR_{FW}	**OLR_{straw}**	**Total daily VS feed**	**SMP**	**Daily methane production**	**Increase in daily methane production**	**Calculated daily methane production from straw.**	**Calculated methane production from straw**
		g VS/L/d	g VS/L/d	g VS/d	NmL CH_4/g $VS_{FW+Straw}$	NmL CH_4/d	in % compared to that in respective R1	NmL CH_4/d	NmL CH_4/g VS_{Straw}
R1	FW1	3.0	0	15.0	500	7 500			
R2	FW1+10% SB	3.0	0.3	16.5	497	8 201	9.3	701	467
R3	FW1+20% SB	3.0	0.6	18.0	473	8 514	13.5	1 014	338
R1	FW1	3.0	0	9.0	478	4 302			
R2	FW1+10% SP	3.0	0.3	9.9	469	4 643	7.9	341	379
R3	FW1+20% SP	3.0	0.6	10.8	488	5 270	22.5	968	538
R1	FW1	7.0	0	35.0	494	17 290			
R2	FW1+10% SB	7.0	0.7	38.5	449	17 287	-	-	-
R3	FW1+20% SB	7.0	1.4	42.0	377	15 834	-	-	-
R1	FW1	7.0	0	21.0	529	11 109			
R2	FW1+10% SP	7.0	0.7	23.1	495	11 435	2.9	326	155
R3	FW1+20% SP	7.0	1.4	25.2	519	13 079	17.7	1 970	469

Table 16. Semi-continuous processes in Sub-project 2 – Calculation of the total methane production from FW (NmL CH_4/d) by substracting the straw gas contribution calculated on the value of 340 NmL CH_4/g VS obtained in the BMP test.

						Daily methane production NmL CH_4/d			**CH_4 production from FW**
		OLR_{FW}	OLR_{straw}	Total VS	SMP	Total	Straw*	FW	% of the volume in reference reactor R1
		g VS/L/d	g VS/L/d	g VS/d	NmL CH_4/g $VS_{FW+Straw}$				
R1	FW1	3.0	0	15.0	500	7 500		7 500	
R2	FW1+10% SB	3.0	0.3	16.5	497	8 201	510	7 691	103
R3	FW1+20% SB	3.0	0.6	18.0	473	8 514	1 020	7 494	100
R1	FW1	3.0	0	9.0	478	4 302		4 302	
R2	FW1+10% SP	3.0	0.3	9.9	469	4 643	306	4 337	101
R3	FW1+20% SP	3.0	0.6	10.8	488	5 270	612	4 658	108
R1	FW1	7.0	0	35.0	494	17 290		17 290	
R2	FW1+10% SB	7.0	0.7	38.5	449	17 287	1 190	16 097	93
R3	FW1+20% SB	7.0	1.4	42.0	377	15 834	2 380	13 454	78
R1	FW1	7.0	0	21.0	529	11 109		11 109	
R2	FW1+10% SP	7.0	0.7	23.1	495	11 435	714	10 721	97
R3	FW1+20% SP	7.0	1.4	25.2	519	13 079	1 428	11 651	105

**Calculated using the value of 340 NmL CH_4/g VS obtained in the BMP tests was used.*

Table 17. Semi-continuous processes in Sub-project 3 – Calculation of the total methane production from FW (NmL CH_4/d) by substracting the straw gas contribution calculated on the value of 340 NmL CH_4/g VS obtained in the BMP test.

		OLR_{FW}	**OLR_{straw}**	**Vol_{RK}**	**FW**	**Straw**	**Total**	**SMP calculated with BMP***	**SMP experm.**	**SMP**	**Daily methane prod.**	**Daily methane prod.***	**Daily methane prod.**	**Additional CH_4 prod. by FW**	**Additional CH_4 production from FW**
		g VS/L/d	g VS/L/d	L	g VS/d	g VS/d	g VS/d	NmL CH_4/g VS	NmL CH_4/g VS	% of calc.	NmL CH_4/d	NmL CH_4/d_{Straw}	NmL CH_4/d_{FW}	NmL CH_4/d_{FW}	%
R4	FW2+Alb+TE	3.0	0	5	15	0	15.0	**607**	388	64	5 820	0			
R5	FW2+Alb	3.0	0	5	15	0	15.0	**607**	162	27	2 430	0	2 430		
R6	FW2+Alb+SP	3.0	0.3	5	15	1.5	16.5	**583**	232	40	3 828	510	3 318	888	37

**Calculated using the values of 607 NmL/g VS (FW) and 340 NmL CH_4/g VS (straw) obtained in the BMP tests. This is an approximation because the BMP of the substrates was determined at another temperature and with another inoculum.*

5 Discussion

To achieve a stable and efficient biogas production process, the material added to digesters must have a good balance of both macro- and micronutrients (Angelidaki et al 2011, Schnürer et al. 2016). Some materials work well as a single substrate, whereas others can be only used in mixtures with other substrates. To overcome the drawbacks of a single material, simultaneous co-digestion using two or more substrates in a mixture is a feasible alternative to mono-digestion (Mata-Alvarez et al 2014). Moreover, the chemical composition of the material used as substrate will also affect the biogas yield and the methane content of the gas, as well as the biodegradability and degradation kinetics (Schnürer 2016). In this regard, straw has some clear disadvantages. It has a recalcitrant structure making it difficult to degrade and an unbalanced nutrient composition, with low levels of nitrogen and trace elements, in regard to microbial growth and activity. To use straw in a biogas process, a pretreatment method is needed as well as co-digestion with a complementary material. A pretreatment can reduce the particle size and porosity, making the material more accessible to microbial degradation, and in addition improve flow properties (Bitra et al 2011). In this project straw pellets and briquettes were evaluated for biogas production in co-digestion with food waste. Pretreated straw in the form of briquettes has been shown in a previous study to improve the methane production (Xavier 2015). Food waste was selected as a co-substrate based on the hypothesis that straw could contribute with complementary nutrient composition and thus give a stabilizing effect at high organic loads.

Methane potential of straw products and food waste

During batch digestion assays an average BMP value of 340 ± 19 NmL CH_4/g VS was determined for the straw products (SP and SB, Table 6). This result was in line with a previous study of Johansson et al. (2012) where the BMP for straw pellets (328 – 343 NmL CH_4/g VS) and other straw containing substrates was determined. The obtained value was significantly higher (9%, t-test $p>0.05$) than the value obtained for the virgin straw (313 NmL CH_4/g VS), supporting previous results that briquetting can be considered as a pretreatment with positive effect on the degradability. The improved accessibility of the straw after the processing to pellets or briquettes was also supported by the results of the structural characterization using Simon staining method (Figure 6), showing larger accessible area for the straw products, SP and SB, compared to that of virgin straw. This larger accessible area for the cellulolytic enzymes has previously been shown to accelerate the rate limiting hydrolysis steps during anaerobic degradation of lignocelluloses, achieving a faster degradation and a higher methane yield (Teghammar et al., 2012).

The BMP for food waste (FW1) was determined to 607 NmL CH_4/g V in the test performed at RISE, results in line with results previously reported in the literature for similar substrates (Schnürer and Jarvis 2017). However, here a significant difference was seen between the two laboratories (RISE and UB). Although several norms and guidelines for BMP tests exist, interlaboratory tests regularly still show high variability of BMPs for the same substrate. The ISR, *i.e.* the ratio of VS from the inoculum (partially due to actively degrading biomass) to VS from the

substrate, is a key parameter in the BMP tests. It is recommended that the portion of VS from the inoculum should be greater than that from the substrate, to minimize acidification or inhibition problems. Therefore, VS based ISRs should for most applications be between two and four (Holliger et al 2016). Here, the ISRs used were within this range, however they were different between the two laboratories and moreover, the organic loading in the RISE tests was of 3.0 g VS/L, while the load was much higher, *i.e.* 8 g VS/L, at UB, Borås (Table 2). Food waste is an easily degradable substrate compared to straw; hence a higher load of FW can lead to an overload resulting in high concentrations of VFAs and low pH in the system, which in turn will decrease the activity of methanogens lowering the methane yield. The value obtained at RISE, Uppsala was therefore chosen to be used in further calculations.

Co digestion of food waste and straw pellets/briquettes

In sub-project 2 two main questions were raised; 1) will addition of straw have a positive effect when co-digested with food waste, regarding stability of the process and 2) will the presence of straw (SP or SB) give a higher efficiency in regard to specific methane production (SMP) and/or the volumetric methane production (VMP)?

A comparison of the obtained yields in the semi-continuous processes (Tables 8 and 9) with the BMP values for the different substrates (Table 6) illustrated that the presence of straw positively affected the efficiency of the process. As expected, the addition of straw (SP and SB) did not increase the SMP when co-digested with FW1, because of the lower BMP from straw as compared to that of food waste. However, an increase in the VMP was observed in the presence of 20% of SB (at low OLR) and with 10 and 20% addition of SP (independent on OLR and HRT). By subtracting the methane produced by the straw (using the BMP value of 340 NmL CH_4/g VS) it appears that food waste produced between 3 to 8 % more methane (Table 16) in the presence of straw as compared to when digested alone, suggesting a synergetic effect. As the BMP for the straw represent the maximum value determined during an incubation period of more than 40 days, an even lower methane production can be expected at the shorter retention time applied in the co-digestion experiments. Thus consequently, the enhancement in methane production from food waste related to the synergetic effects of straw addition is likely even higher than calculated. We conclude that addition of straw leads to higher methane productivity without a considerable change in HRT, hence giving a better utilization of the reactor volume.

Comparing SP and SB at the conditions tested in this study, the enhancement of methane production was observed clearly when using SP as co-substrate (independent on OLR and HRT). A positive effect of SB was only observed at the low OLR. The mixing efficiency was clearly affected in the reactors when SB was used, specifically at the higher OLR level. A good mixing allows an even distribution of the substrate in the reactor, avoiding foam formation and sedimentation and therefore a better use of the reactor volume. It is also important for a good contact of microorganisms with the nutrients and for an effective gas transfer (Schnürer et al. 2016). The poor mixing capacity in this study when using SB resulted in building up a surface layer of straw in the reactor (Figure 5). Likely,

this was one reason why no methane enhancement was observed in the presence of SB. One of the main differences between SP and SB is the size of the particles applied in the manufacturing process, with smaller particle size for SP. This smaller particle size likely explains the comparably higher methane yield in the co-digestion processes as well as the more efficient mixing. Smaller particles reduce risks of mechanical problems in mixing and will give a larger accessible area for the microorganisms to attach and degrade. Several studies have already shown a clear effect of particle size reduction causing an increase in methane production (Lindmark, et al. 2012; Motte et al. 2014).

The straw structure, before and after the digestion, was studied by FTIR analyses and Simon staining methods (Table 13 and Figure 6). The FTIR analyses showed that the remaining straw particles at the end of the digestion experiments had a higher crystallinity as compared to before the digestion, indicating that the amorphous parts of the straw particles were digested during the process (Table 13). Also, the straw pellets particles had a much higher porosity than straw briquettes after the digestion, suggesting a more effective degradation (Figure 6).

The results also indicate that the addition of straw had synergetic effects improving the degradation of the food waste. A possible explanation could be that the straw is used by the microorganisms as carrier material on which they can attach and grow. The formation of biofilms on the straw particles may allow a higher cell concentration compared with processes lacking carrier materials as when FW is digested alone.

The microbiological analysis illustrated a similar community in all investigated reactors, however still with some clear differences. The observed community was very typical for biogas digesters with dominance of phyla Firmicutes, Thermotogae and Synergistetes in all samples, with some additional group present at lower abundance. As typical for many biogas systems, the level of methanogens was low in comparison to the bacterial community and also a part of the community was represented by today unknown microorganisms (Schnürer 2016). In line with the results in this study the phyla Firmicutes and Thermotogae are typically present at high levels at thermophilic temperatures (Schnürer 2016). Firmicutes contain members with a wide metabolic capacity. Among the different detected orders *Clostridiales* was highly abundant. This group comprises many bacteria with the ability to degrade cellulose but also proteins and lipids. Some members within these orders are also acetogens, critical for the overall function of the biogas process (Schnürer 2016). In addition to *Clostridiales* the phylum Firmicutes was also comprised of the order MBA08 and SHA-98. The MBA08 cluster has been found previously in digesters operating at various condition such as at both mesophilic and thermophilic temperature, high and low ammonia and during degradation of manure and straw and municipal solid waste (Tanget al. 2004; Cheonet al. 2007; Sun et al 2016 Kouigas et al. 2017). The level of SHA-98 was recently shown to correlate with the ammonia level (Müller et al. 2016). At present the role of these groups in the biogas digesters is unclear. Thermotogales dominated the phylum Thermotoga and bacteria belonging to this order are involved in the fermentation of substrates such as glucose, acetate, methanol and starch (Balk et al., 2002; Feng et al., 2010). The phylum Synergistetes were composed only of the order

Synergistales, previously shown able to degrade amino acids and converts them to short-chain fatty acids (Vartoukian et al., 2007). In line with the increase/decrease in OLR/HRT some changes were seen in the community with a more pronounced effect of the community in the reactors operated by UB, Borås. This change (also seen in Fig 7B) was mainly caused by minor changes within the phylum Firmicutes, in the fraction of unknown bacteria, and the effect was in general independent of the straw addition. The difference between the effect of the OLR increase between the reactor operated at the different universities are likely caused by differences in reactor design and possibly by the fact that the reactors operated with different batches of the food waste. These differences resulted in slightly different level of alkalinity, pH and ammonium-nitrogen, all potentially influencing on the development of the microbial community. Still, the changes were small and likely not impacting on the overall degradation, a conclusion supported by the fact that the reactors overall showed very similar performance. Interestingly, the reactors showing the highest efficiency and also synergistic effects between the substrates, *e.g*. R3 (20% SP), showed an enrichment of members within the phylum Bacteroidetes, represented by the order *Porphyromonadaceae*. Bacteria within this order have previously been shown to have genes encoding for degradation of complex compounds such as carbohydrates, protein and peptides (Hahnke et al. 2014). In addition, in a recent publication this phylum was suggested to be involved in the improved degradation of VFAs connected to a recovery after a sudden increase in the levels of ammonia and LCFA (long chain fatty acids) (Reguiro et al. 2016). Thus, the increase of the relative abundance of this group could potentially relate both to an improved degradation of straw as well as of food waste. Anyway, the increase suggests a correlation with the improved efficiency.

Addition of straw pellets for stabilization at high ammonia conditions

Addition of straw pellets to the high ammonia processes in sub-project 3 indicated a positive effect by addition of straw, resulting in both significantly higher SMP and VMP, as compared to that obtained in the control reactor without straw addition and after four retention times (Figure 9). Still the gas production did not reach the same level as the reference reactor (R4), running with the same substrate but amended also with trace elements and iron (Table 11). Apparently, the addition of straw could not counteract the negative effects of high levels of hydrogen sulfide, as for rector R5 and R6. Hydrogen sulfide traps metal of critical importance for microbial activity and as a consequence accumulation of VFAs is typically seen (Thanh et al. 2016). Addition of iron result in precipitation of iron sulfide, which releases the important metals. Thus, addition of iron, alone or combined with extra trace metals, as for reactor R4, resulted in a higher microbial activity, which in turns improved the degradation of VFAs and resulted in a higher methane yield. A similar addition to reactor R6, receiving the straw, would likely have improved the overall degradation even further.

Energy efficiency

When it comes to the energy efficiency, the energy produced (as methane) due to the addition of the straw should be compared to the energy (electricity) needs of the pelleting or briquetting process. Our results show positive energy balances at those process conditions where the addition of straw did not lead to any

mechanical problems, namely at lower OLR in case of both straw pellets and briquettes, and additionally at high OLR in case of straw pellets (Table 18).

Table 18. Energy balance calculations - Net energy production as the result of straw addition. The energy requirements for SP and SB production are assumed to be 260 and 100 kWh/ton straw, respectively

Low OLR_{FW1}	**Calculated additional methane production due to the presence of straw** Nm^3/ton straw added	**Increased energy produced due to the addition of straw** kWh/ton straw added	**Net energy production** kWh/ton straw added
+ 10% SB	401	4 010	3 910
+ 20% SB	290	2 900	2 800
+ 10% SP	325	3 250	2 990
+ 20% SP	462	4 620	4 360
High OLR_{FW1}			
+ 10% SB	-	-	-
+ 20% SB	-	-	-
+ 10% SP	133	1 330	1 070
+ 20% SP	402	4 020	3 760

Since the briqetting compared to the pelleting process has lower energy requirements (100 and 260 kWh/ton straw respectively), at the lower organic load and with only 10% addition of straw products, a better energy efficiency can be achieved when straw briquettes are added. However, at all other cases the addition of straw pellets is more beneficial. Moreover, according to the results obtained in the laboratory scale reactors, when biogas processes are operated at high loading rates only the addition of straw pellets can be recommended due to mechanical problems which might arise in case of addition of straw briquettes.

6 Conclusions

Co-digestion of straw with food waste results in higher volumetric methane production compared to mono-digestion of food waste, and without affecting the hydraulic retention time. Hence the reactor volume in the digestion reactor could be utilized more effectively.

Theoretical calculations illustrated higher methane yields from food waste when it was co-digested with straw compared to that when digested alone, indicating synergistic effects during the co-digestion.

Positive co-digestion effects between straw and food waste were shown more clearly with straw pellets as compared to straw briquettes, especially at higher loading rates.

At the conditions tested in this work, the addition of straw briquettes at high loading rates caused mechanical problems for mixing, leading to building up of a straw layer at the surface.

The energy balance for the straw addition was found to be positive, since the excess energy produced in the co-digestion process due to the addition of straw was higher than the energy requirements for manufacturing the straw products, pellets or briquettes.

The BMP tests showed significantly higher methane potential values for the straw products, pellets or briquettes, compared to virgin straw.

The structural characterization of the straw products confirmed that the pelleting and briquetting process itself can be considered as a pretreatment process for the lignocellulosic biomass leading to smaller particle size of straw particles in the reactor and consequently providing larger accessible surface area for the degradation. Comparing the structural analyses results of pellets and briquettes it was found that pellets adsorbed more orange dye compared to briquettes, with molecular size comparable to the cellulose degrading enzyme complex, indicating a slightly more porous and open structure and hence a better digestibility.

Addition of straw during co-digestion with food waste had low effect on the microbial community. However, in line with the improve process efficiency when using straw pellets at a high organic load an increase in the abundance of the phylum Bacteroidetes, order *Porphyromonadaceae* was seen.

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8 Glossary

Acetate = CH_3COO^-, the anion of acetic acid (CH_3COOH).

Acetogen = acetate-producing microorganism.

Acetotroph = microorganism using acetate (acetic acid) as a substrate. One example is the acetic-acid-splitting microorganisms that form methane and carbon dioxide from acetate.

Alkalinity = measure of the amount of alkaline (basic) substances. Bicarbonate, carbonate and carbon dioxide are examples of substances that contribute to alkalinity in a biogas process.

Anaerobic = oxygen-free.

Anaerobic oxidation = degradation step between fermentation and methane formation. Intermediate products such as alcohols and fatty acids are broken down in this step to hydrogen, carbon dioxide, and acetate.

Archaea = a group of microorganisms with unique properties that have developed in parallel with bacteria and fungi. Methane-producing microorganisms belong to the group Archaea.

Batch digestion = material is digested without any material added or withdrawn during the process.

Biogas = the gas, consisting mostly of carbon dioxide and methane, which is produced when organic materials breaks down in an oxygen-free environment (anaerobic digestion).

Bio-manure = residue from biogas systems that digest relatively uncontaminated waste such as manure, source-separated food waste, waste from the food industry, agricultural crops, etc.

CSTR = Continuously Stirred Tank Reactor, that is, a biogas reactor in which the materials are mixed using an agitator.

Co-digestion = digestion of multiple substrates simultaneously. Often provides a higher methane yield than in the case where each material is digested separately.

Continuous digestion = new material (substrate) is pumped continuously into the digester with a steady flow during the day. This is feasible for liquid substrates (TS-content below 5%), while sludge-like substrates with higher TS levels are often pumped in portions over the day. This is known as semi-continuous digestion.

Degree of degradation (DD) = indicates, as a percentage, how much of the organic material has been broken down and converted into biogas during a certain period of time.

Digestion residue, digestate = liquid or sludge-like product that is formed after digestion and contains water, non-degraded material, nutrients and microorganisms (biomass).

Fermentation = the second degradation step of the biogas process in which sugars, amino acids etc. are broken down under oxygen-free conditions to various fermentation products, such as alcohols, fatty acids, carbon dioxide, and hydrogen.

Floating crust = may form when undegraded materials accumulate and float above the liquid surface in the digester or the residue storage tank.

Gas yield= Amount of biogas in Nm^3 produced per unit weight of organic material.

Hydrolysis = the first degradation step in the biogas process in which large organic molecules (proteins, sugars, fats) are broken down into smaller components.

Load = usually stated as organic load or organic loading rate (OLR). Describes how much organic material is introduced into the process per digester volume and day.

Mesophilic temperature = within the range of about 25° C - 40° C. Mesophilic biogas processes typically run at a temperature of about 35° to 37° C.

Methane = CH_4, the simplest hydrocarbon, an odorless gas of high energy value (9.81 kWh/Nm^3).

Methanogen = methane-producing microorganism.

Methane yield = amount of methane in Nm^3 formed per unit weight of organic matter load.

Nm^3 = normal cubic meter, volume at normal conditions, *i.e.* 0°C and atmospheric pressure (1.01325 bar).

Propionate = $CH_3CH_2COO^-$, anion of propionic acid (CH_3CH_2COOH).

Retention time = time that the substrate is in the digester. Frequently referred to as hydraulic retention time (HRT) and describes the time it takes to replace all the material in the digester. Sometimes the retention time is instead given as the residence time for the particulate material in the digester, solids retention time (SRT).

SB = Straw briquettes

SP = Straw pellets

Specific methane production (SMP) = the quantity of methane produced per quantity of organic matter input (m^3 CH_4 per kg VS per day).

Substrate = organic material suitable for digestion.

Support or carrier material = material, which can be added to the digester to retain microorganisms.

Syntrophy = collaboration between two organisms where both benefit from the cooperation. An example of syntrophy in the biogas process is the transfer of hydrogen (IHT) between microorganisms that carry out anaerobic oxidation and methane producers.

Syntrophic acetate oxidation = SAO, alternative methane formation pathway from acetate, where acetate is broken down first to hydrogen and carbon dioxide by non-methane-producing bacteria. These products are then used by another microorganism, a hydrogenotrophic methane producer, to produce biogas.

Thermophilic temperatures = temperatures above 40°C. Thermophilic biogas processes typically run at temperatures around 50° - 55°C.

TS = total solids or dry solids, what is left when a material is dried. Usually stated as a percentage of wet weight.

VFA = volatile fatty acids.

Volumetric methane production (VMP) = the quantity of methane produced per wet volume reactor (m^3 CH_4 per m^3 reactor per day).

VS = volatile solids, organic content, *i.e.* dry weight minus ash. Usually stated as a percentage of TS. Sometimes referred to as loss on combustion.

UTILIZATION OF STRAW PELLETS AND BRIQUETTES AS CO-SUBSTRATES AT BIOGAS PLANTS

Biogas reactors can be utilized more efficiently when straw and food waste are digested together instead of separately. In the present study, straw in the form of pellets and briquettes has been used in experiments and calculations. Co-digestion of different substrates can give a more optimal substrate composition and a more efficient utilization of available digester volume.

The pelleting and briquetting process has been shown to be an adequate pretreatment method of the straw. Digesting food waste and straw together showed synergistic effects with improved degradation of the food waste as well as a higher total volumetric methane production as compared to when food waste was used as the sole substrate. Energy produced through increased biogas production was higher than the energy needed for the pelleting and briquetting process.

The positive effect in regard to gas production was mainly seen for the straw pellets, results supported by both chemical and microbiological analysis. These effects were observed in both mesophilic and thermophilic conditions.

In conclusion, this study illustrates that straw is a suitable co-digestion substrate to food waste and can be used to improve gas yields as well as for more efficient utilization of the digester volume. These results show the biogas potential of straw, today not yet used as a substrate to a large extent.