# Biogas from agricultural waste

## Turning unavailable residues into accessible resources

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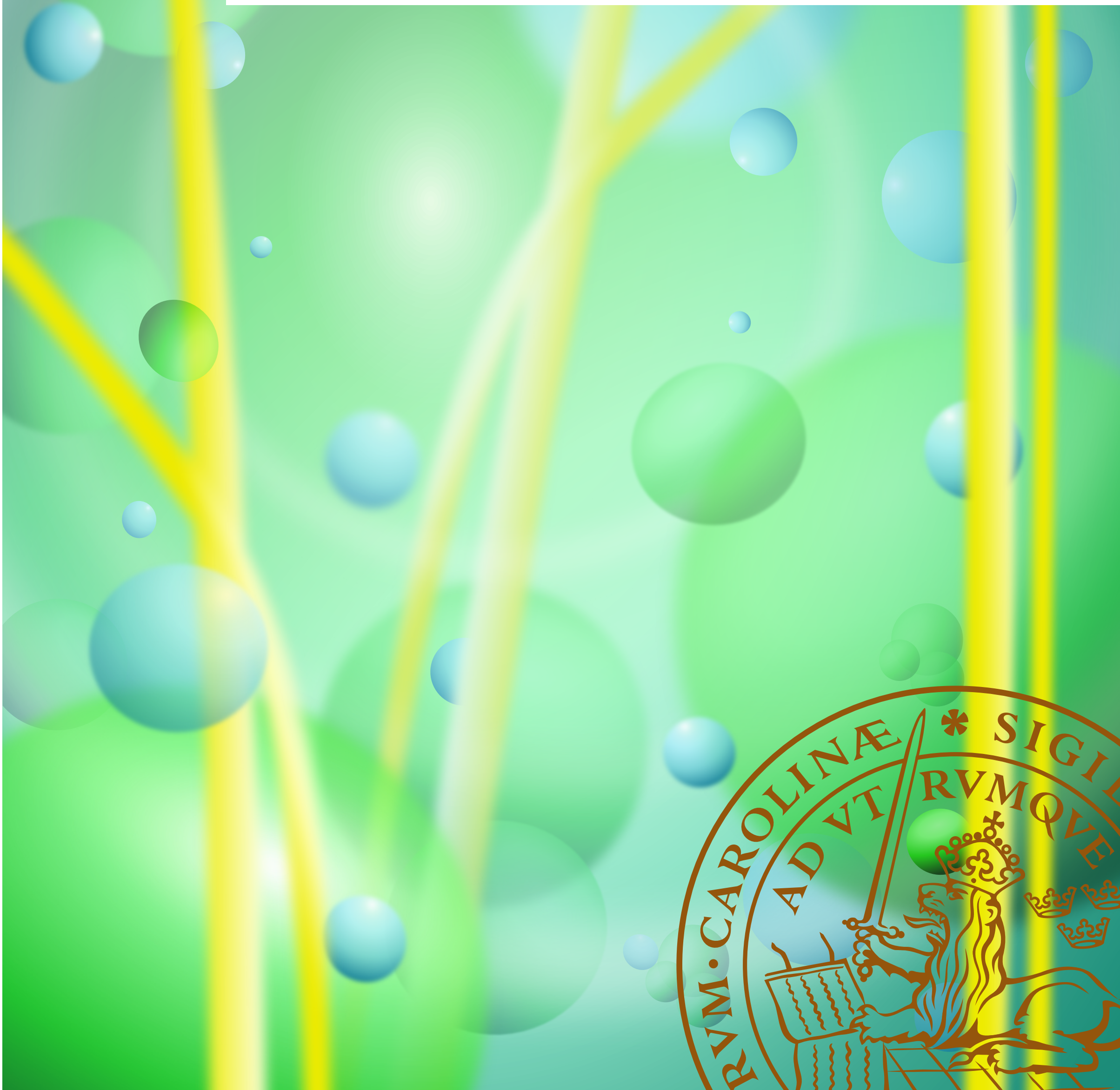

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Mirjam Victorin

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**Abstract:**
Moving from a fossil dependent to a fossil free economy requires increased energy production from renewable resources. This thesis discusses the utilization of agricultural waste streams, such as straw and manure, for biogas production. The first part of the research presented focuses on pretreatment of straw with the aim to reduce the handling issues concerning straw and improve the degradation of the material during anaerobic digestion. The second part concerns process design of agro-based biogas production plants with the aim to find process configurations and feedstocks that lead to high bioenergy yields. The effect of co-digesting a manure-rich stream with a carbohydrate-rich stream and the role of the degree of carbohydrate accessibility on the methane production is also discussed.

Wheat straw is a problematic material to digest due to its high porosity which causes it to float and makes it hard to pump/feed. Mechanical pretreatments that applied higher shearing to the straw, such as pelletization and extrusion, led to reduced floating layers. Particle size reduction of wheat straw impacted the methane production rate below 3 mm but did not have an impact on the methane yield. The particle size was, however, not the only factor affecting the methane production rate. Hammer milled straw and extruded straw had a similar particle size but the degradation of extruded straw was faster. To increase the methane production rate, a shearing effect of the pretreatment may be more important. To solve only the handling issues of the straw, it may not be worth the high energy demand of those pretreatments.

Wheat straw cannot be digested without the addition of nutrients. Co-digestion with manure or animal bedding is thereby a promising solution. By washing the animal bedding, it was possible to separate out the fibers and subject them to pretreatment with similar yields as pretreatment of wheat straw. Such a process design opened up for parallel production of biogas from the manure-fraction and fiber hydrolysate, and bioethanol from the steam pretreated fibers. Co-digestion of manure and readily available hydrolysate led to an increased initial lag phase and additional studies presented in this thesis showed that a too high degree of carbohydrate accessibility will increase the risk of process instability due to volatile fatty acids accumulation.

Further, because part of the carbohydrates was diverted for yeast fermentation, the C/N ratio in the anaerobic digestion step became low. To solve this, cow manure and additional wheat straw were added to the production process. Energy balances and estimated energy demands over the process, in comparisons with other designs, showed that biofuel production was more energy efficient without co-production of ethanol. However, because of the recovery of lignin, there is a great potential of covering most of the energy demand by on-site steam production. Like so, the energy efficiency would much improve.

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*Man levde nära skiten. Den lockade till skratt.*
*(Annie Ernaux)*

# Abstract

Moving from a fossil dependent to a fossil free economy requires increased energy production from renewable resources. This thesis discusses the utilization of agricultural waste streams, such as straw and manure, for biogas production. The first part of the research presented focuses on pretreatment of straw with the aim to reduce the handling issues concerning straw and improve the degradation of the material during anaerobic digestion. The second part concerns process design of agro-based biogas production plants with the aim to find process configurations and feedstocks that lead to high bioenergy yields. The effect of co-digesting a manure-rich stream with a carbohydrate-rich stream and the role of the degree of carbohydrate accessibility on the methane production is also discussed.

Wheat straw is a problematic material to digest due to its high porosity which causes it to float and makes it hard to pump/feed. Mechanical pretreatments that applied higher shearing to the straw, such as pelletization and extrusion, led to reduced floating layers. Particle size reduction of wheat straw impacted the methane production rate below 3 mm but did not have an impact on the methane yield. The particle size was, however, not the only factor affecting the methane production rate. Hammer milled straw and extruded straw had a similar particle size but the degradation of extruded straw was faster. To increase the methane production rate, a shearing effect of the pretreatment may be more important. To solve only the handling issues of the straw, it may not be worth the high energy demand of those pretreatments.

Wheat straw cannot be digested without the addition of nutrients. Co-digestion with manure or animal bedding is thereby a promising solution. By washing the animal bedding, it was possible to separate out the fibers and subject them to pretreatment with similar yields as pretreatment of wheat straw. Such a process design opened up for parallel production of biogas from the manure-fraction and fiber hydrolysate, and bioethanol from the steam pretreated fibers. Co-digestion of manure and readily available hydrolysate led to an increased initial lag phase and additional studies presented in this thesis showed that a too high degree of carbohydrate accessibility will increase the risk of process instability due to volatile fatty acids accumulation.

Further, because part of the carbohydrates was diverted for yeast fermentation, the C/N ratio in the anaerobic digestion step became low. To solve this, cow manure and additional wheat straw were added to the production process. Energy balances and estimated energy demands over the process, in comparisons with other designs, showed that biofuel production was more energy efficient without co-production of ethanol. However, because of the recovery of lignin, there is a great potential of covering most of the energy demand by on-site steam production. Like so, the energy efficiency would much improve.

# Populärvetenskaplig sammanfattning

## Explosion av halm! För mer gas i tanken

**Vi blir fler och fler som äter (och slänger) mer och mer. Med en ökad matproduktion kommer också en ökad mängd avfall from jordbruket. Detta avfall måste tas om hand. Vi blir också fler och fler som vill köra mer och mer. Med klimatmål i sikte kan detta behov tillgodoses via ökad biogasproduktion från jordbruksrester. Då måste vi också ta om hand de krångligare avfallen – halm och gödsel.**

Inrikes transporter står för 31% av Sveriges totala utsläpp av växthusgaser (15 miljoner ton CO2-ekv., år 2022). För att minska detta klimatavtryck och nå Sveriges klimatmål om ett nettonollutsläpp år 2045 måste användningen av bensin och diesel minska. Energimyndigheten och Naturvårdsverket beskriver därför ett framtidsscenario där vägtrafiken förlitar sig på förnybara biobränslen, dvs. bränslen som härstammar från en naturresurs där det organiska materialet redan är en del av kretsloppet och ingen ny koldioxid tillförs atmosfären. Det minst klimatbelastande förnybara biobränslet är biogas. Trots detta så är den svenska produktionen och användningen av biogas fortfarande väldigt låg.

Ett stort problem för svenska biogasproducenter har länge varit det låga priset på konkurrerande fossilgas. Det har inneburit att produktionen av biogas måste vara billig för att investeringskostnaderna ska kunna täckas av biogasintäkterna. Halm, kogödsel och djupströbädd (halm blandat med gödsel) är tre restprodukter som skulle kunna fungera som råvara för biogasproduktion. De stora utmaningarna ligger dels i hanteringen av halm och gödsel. Halm och gödsel går inte att pumpa. Dessutom har fibrerna i materialen en styv struktur som gör det svårt för mikroorganismerna att bryta ned dem till biogas. För att göra materialet pumpbart och öka dess nedbrytbarhet krävs en lämplig förbehandling. Jag har i min forskning studerat tre olika metoder; tvättning av djupströbädd, sönderdelning av halm samt ångexplosion av halm. Vidare måste halm också rötas tillsammans med ett näringsrikt material för mikroorganismernas överlevnad. Gödsel innehållet mycket näring, varför jag har studerat effekterna av att blanda obehandlad och förbehandlad halm med just gödsel.

Genom att tvätta djupströbädd med vatten kunde halmstråna separeras ut och förbehandlas ytterligare. Förutom att stora fibrer är svåra att pumpa så innebär de också problem vid omrörningen av materialet i biogasreaktorn. Är fibrerna dessutom väldigt porösa, som halmstrån tenderar att vara, är det inte ovanligt att de flyter upp och bildar tjocka täcken. I min forskning har jag visat att sönderdelning av halm inte alltid räcker till för att motverka dess flytkraft. Även själva metoden för sönderdelning – hacka, krossa eller mala – har en inverkan. Förutom att minska halmens flytkraft visade mina försök att en minskad partikelstorlek också leder till en snabbare nedbrytningsprocess och därigenom effektivare biogasproduktion.

I ett försök att förbättra nedbrytningsprocessen av halmen ytterligare så undersöktes även en mer aggressiv förbehandlingsmetod; ångexplosion. Ångexplosion fungerar så att het ånga pressas in i halmen under högt tryck och höga temperaturer i en försluten reaktor. När reaktorn sedan öppnas så exploderar den fuktiga halmen och delar av den förvätskas. Ångexplosionen gjorde det möjligt att producera bioetanol parallellt med biogas. Bioetanol är en dyrbarare produkt än biogas och kan därför bära en del av produktionskostnaderna och öka processens lönsamhet. Men, den förvätskade halmen från ångexplosionen visade sig höja risken för försurning under rötningen. Det ledde till att mikroorganismerna slutade producera biogas. Genom att tillsätta ett mer svårnedbrytbart material, som exempelvis obehandlad halm, kunde däremot stabiliteten förbättras och försurningen förhindras.

Från mina experiment drar jag slutsatsen att optimering av förbehandlings-metoderna av halm inte bara bör göras med avseende på halmen. Det är även viktigt att ta hänsyn till vad halmen sedan ska blandas med för annat material under själva rötningsprocessen. Via tillgängliggörandet av halm för biogasproduktion kan halmen bidra med en trefaldig ökning av den nuvarande svenska fossilfria fordonsgasen.

Forskningen som presenteras i denna avhandling berör ett område som har stor inverkan på Sveriges klimatmål. Enligt FN: s globala mål för hållbar utveckling, Agenda 2030, har Sverige som mål att minska den fossila energianvändningen inom transportsektorn med 80% jämfört med utsläppsnivåerna år 2010. För att vara i fas med målen borde vi ha nått en dubbelt så stor minskning än vad vi gjort. Sverige är helt enkelt inte i fas. Men, i och med den rådande diskursen i samhället kring energisäkerhet, global uppvärmning och uppbyggandet av en cirkulär ekonomi så är min förhoppning att satsningarna på förnybara bränslen och biogas tar fart.

# List of Papers

**Paper I**

Victorin, M., Davidsson, Å., Wallberg, O. (2020). Characterization of Mechanically Pretreated Wheat Straw for Biogas Production. BioEnergy Res. 13, 833-844.

**Paper II**

Victorin, M., Sanchis-Sebastiá, M., Davidsson, Å., Wallberg, O. (2019). Production of Biofuels from Animal Bedding: Biogas or Bioethanol? Influence of Feedstock Composition on the Process Layout. Ind. Eng. Chem. Res. 58, 21927-21935.

**Paper III**

Victorin, M., Davidsson, Å., Wallberg, O. (2023). Carbohydrate accessibility in anaerobic co-digestion – increased productivity with decreased accessibility. *(Manuscript submitted).*

**Paper IV**

Victorin, M., Davidsson, Å., Wallberg, O. (2023). Process design of manure-based biogas solutions for increased material and energy efficiency. *(Manuscript).*

# My contributions to the studies

**Paper I**

I planned and performed the experiments. I also evaluated the results. I wrote the paper with input from the co-authors.

**Paper II**

I planned and performed the experiments with M. Sanchis-Sebastiá. I evaluated the results and wrote the paper with M. Sanchis-Sebastiá with input from the co-authors.

**Paper III**

I planned and performed the experiments. I evaluated the results with input from Å. Davidsson and O. Wallberg. I wrote the paper with input from the co-authors.

**Paper IV**

I planned and performed the experiments with input from the co-authors. I also evaluated the results and wrote the paper.

# Acknowledgements

If I would not have been surrounded by so many great and supportive people during my time as a PhD student. I feel overjoyed (and sentimental) when I think about all the wonderful people that I have met during my time at Lund University. I have so many people to thank and I will try to cover it all before the music starts playing.

I would first and foremost like to thank my main supervisor – Ola – for your open-mindedness. It was your enthusiasm for the topic of process design and biorefineries that first inspired me to seek my way to the Department of Chemical Engineering.

I would also like to thank my co-supervisor, Åsa, for always cheerfully making sure that I come back to reality once in a while and focus on what an engineer should be doing – to engineer.

To my co-author Miguel: for being such a great colleague and friend. Anyone who gets to work with you can consider themselves lucky. To Bori: the first day in the lab, we bonded over how the heck we ended up pipetting pig manure when we were both obviously born to dance. You have always helped when I have asked, and I don't think we've had a single interaction where I didn't end up laughing. Thank you. To Gertrud: for helping whenever I have needed it and for the chair under the tree in your office.

To all my friends and colleagues at the department: Thank you for contributing to such a welcoming and friendly work environment. I will for sure miss all the fika conversations, all the office interruptions at 4 pm on Friday afternoons, all the laboratory meetings about everything that has nothing to do with lab work, and so much more.

To my parents: thank you for your love and support and thank you for teaching me to find laughter in fiascos and failures. Doing a PhD is a smorgasbord of problems and thereby potential utter joy! To my partner and my daughter: I love you.

Finally, to all the new PhD students that might open this thesis I would like to pass on some words of wisdom that I received when my imposter syndrome was at its peak: *Don't doubt yourself, doubt the world.*

# Abbreviations

| | |
|---|---|
| AD | Anaerobic digestion |
| BMP | Biochemical methane production |
| Co-AD | Anaerobic co-digestion |
| C/N | Carbon-to-nitrogen ratio |
| DM | Dry matter |
| DMP | Daily methane production rate |
| GHG | Greenhouse gas |
| HRT | Hydraulic retention time |
| I/S | Inoculum-to-substrate ratio |
| OLR | Organic loading rate |
| TAN | Total ammonium nitrogen |
| TC | Total carbon |
| TN | Total nitrogen |
| TS | Total solids |
| VFA | Volatile fatty acid |
| VS | Volatile solids |

# Table of Contents

# Chapter 1. Introduction

Biogas—or 'flammable air,' as it was first described—has been used by humans for over 2000 years (World Biogas Association, 2023). In China and India, people used such gas for heating and cooking (Negi et al., 2019). In the 17th century, scientists began to document and realize that flammable gas originated from organic matter (Helmont, 1662). Later, it was understood that methane was produced in the absence of oxygen at natural anaerobic sites (Negi et al., 2019).

At the start of the 19th century, methane was discovered to exist in gases that were produced from the anaerobic digestion of manure. Subsequently, the first anaerobic digester was built in 1859 in Bombay (now Mumbai), and awareness of biogas then reached Europe at the end of the 19th century, where it was collected from sewer systems for use as city gas in streetlamps.

Sweden began performing anaerobic digestion in 1934 as a treatment step for sludge that was produced at wastewater treatment plants (Energigas, 2015). However, it was during and after World War II that the production of biogas as an energy source gained interest, culminating in the introduction of biogas to the local city gas grid in 1948. Several decades later, following the oil crisis in the 1970s, industries began generating biogas from their wastewater treatment facilities for internal production of heat and electricity. Natural gas appeared on the Swedish market in 1976, and several years later, Sweden started harvesting biogas from landfills, in parallel with the construction of the Swedish natural gas grid.

In the late 1980s, the first gas-powered vehicles were developed to reduce pollution in urban environments, which led to the upgrade of biogas technologies. In 1994, the first fill-in station for private gas-powered cars was installed in Gothenburg, and in 2014, biogas was introduced to the transmission grid. Since then, national production and consumption of biogas have accelerated, and Sweden is now one of Europe's main producers of upgraded biogas.

Anaerobic digestion of organic matter to produce biogas has many important functions. Today, biogas solutions can be described as i) waste handling facilities; ii) energy production plants for heat, electricity, and fuel; and iii) resource recycling plants. The main driver behind the modern development of biogas production has been the ongoing transition from an oil-dependent to fossil-free society, as part of climate change mitigation efforts.

The annual average global temperature is increasing. Thus, to avoid exceeding a rise in temperature of 2.0°C (per the Paris Agreement, 2015), greenhouse gas (GHG) emissions must be significantly reduced. However, the International Energy Agency

(IEA) has opined that neither the current global efforts nor ambitions are projected to reach this goal, based on energy-related sectors (Figure 1). The Stated Policies Scenario (STEPS) is an estimation of future global emissions, based on ongoing activities (Fig. 1, Stated Policies Scenario), and the Announced Pledges Scenario (APS) is the projected emission level, based on current climate commitments. These scenarios are predicted to lead to rises in global temperature of 2.6°C and 2.1°C, respectively, by 2100, compared with the Net Zero Scenario, which anticipates such a rise being limited to 1.5°C.

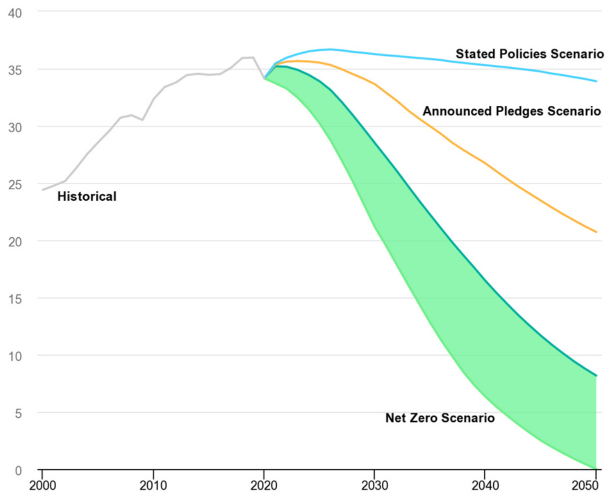


**Figure 1** Historical and projected global greenhouse gas emissions (Gt CO2-eq.) according to three scenarios (IEA, 2021). The Net Zero Scenario represents the goal of limiting the increase in global temperature to 1.5ºC, the Announced Pledges Scenario reflects the effect of current climate commitments, and the Stated Policies Scenario comprises emission levels based on what actions have been put in place.

In Sweden, the domestic transport sector is one of the largest sources of GHG emissions, constituting approximately 31% of total emissions, or 48 Mton $CO_2$-eq (Swedish Environmental Protection Agency, 2021), most of which is generated by private vehicles. According to Agenda 2030, a set of Swedish climate policy goals, these emissions should be reduced to approximately 20 Mton $CO_2$-eq by 2030. Börjesson et al. (2016) reported that replacing gasoline and diesel with biogas as a vehicle fuel can lower GHG emissions by up to 80% (as $CO_2$-eq), regardless of production system, distribution system, or type of vehicle. Despite the benefits of biogas as a vehicle fuel, annual national biogas production has stagnated at approximately 2.0 TWh for the past several years.

In contrast, total biogas imports to Sweden increased from 0 TWh in 2015 to 2.5 TWh in 2021 (Swedish Energy Agency, 2021). Two-thirds of the imported biogas was produced in Denmark, and the remainder generated in other parts of Europe.

Clearly, the greater national demand for fossil-free methane has not been met by increased national production. One of the underlying reasons is that Danish biogas actors have been awarded subsidies to produce biogas, whereas Sweden has implemented a system of tax exemptions that have been directed toward the consumer (Gustafsson et al., 2022), rendering imported gas cheaper than locally produced gas.

In addition, the lack of long-term policies for establishing a stable economic environment that encourages new investment has led to hesitation among biogas stakeholders (Swedish Environmental Protection Agency, 2011). But in 2022, the Swedish government enacted new legislation that ensures support for biogas producers of 0.3 SEK/kWh for upgraded biogas (CBG) and 0.45 SEK/kWh for liquified biogas (LBG).

Sweden has no specific national biogas production targets. Based on Agenda 2030, a national target of 15 TWh biogas was proposed by the biogas industry (National Biogas Strategy 2.0, 2018). More recently, a government-sponsored public investigation of the Swedish biogas market (Westlund et al., 2019) proposed a national biomethane production target of 10 TWh, of which 7 TWh should be obtained via anaerobic digestion, by 2030. This less ambitious goal corresponds to approximately 20% of the estimated national biogas potential for 2030 (Börjesson, 2021), of which the agricultural sector constitutes 20% to 30%. Based on its significant potential for biogas production, the agricultural sector has been identified as a key sector in reaching the proposed reduction targets.

Anaerobic digestion of manure serves many functions with regard to international sustainability goals (IPCC). Manure is used primarily as fertilizer, and its storage is a chief source of emission by the agricultural sector (Scarlat et al., 2018). In Sweden, the agricultural sector is responsible for 14% of total GHG emissions. Further, a by-product of the anaerobic digestion of manure is biofertilizer—a less odorous material than fresh manure with higher nutrient availability (Risberg et al., 2013; Orzi et al., 2015).

According to the Swedish Energy Agency, 64 of 280 biogas plants utilize manure as a substrate for their biogas production, with a total annual use of 1 million tons (wet basis) (Energigas Sverige, 2019). Most of this manure (68%, wet basis) is processed in centralized co-digestion plants, with the remainder being handled by farm-based anaerobic digestion plants. However, compared with other European countries, manure makes up a low share of the total feedstock that is used for biogas production in Sweden (Gustafsson et al., 2022).

Due to its high concentration of nutrients, manure is suitable for co-digestion with less nutrient-dense materials, such as cereal straw. In Sweden, straw is primarily left in the fields or recovered for other purposes. Wheat straw, in particular, is recovered from fields in 37% to 44% of cases (total areal). Of this fraction, only 1% is used for biogas production; whereas the majority (73% to 74%) is sold as material for animal bedding; 6% to 11% is used as animal feed, and 6% to 15% is burned for heating purposes (Statistics Sweden, 2012). The low utilization rate of straw in biogas production is attributed to the low theoretical potential production of biogas and the poor handling properties of bulky, fibrous straw.

## Aims and outline of this thesis

The overarching aim of the work in this thesis was to examine the impact of the bioaccessibility of lignocellulosic biomass on anaerobic digestion processes. Finding technical solutions that increase the availability of feedstock for biogas production has been the overall incentive of my research. Specifically, I have focused on the function of agricultural residues in anaerobic digestion systems and have studied how pretreatment technologies affect lignocellulosic materials and their biological degradation. With this thesis, I aim to present my findings and discuss their relevance for academic research and Swedish society at large. The main research questions that have shaped my approach in addressing this aim are as follows:

1. How does mechanical pretreatment affect the bioavailability and bioaccessibility of wheat straw in biogas production? (**Paper I**)
2. How does the composition of animal bedding affect the process configuration during co-production of bioethanol and biogas? (**Paper II**)
3. How does the accessibility of carbohydrates impact biogas production and the risk of instability during co-digestion with manure? (**Paper III**)
4. How can a biogas production process on straw, cow manure, and animal bedding be designed for a high biofuel production efficiency? (**Paper IV**)

The agricultural residues that were examined in this research were wheat straw, animal bedding from cows, and cow manure. Chapter 2 provides the background on anaerobic digestion, and Chapter 3 introduces the relevant methods. Moreover, Chapter 4 discusses the impact of pretreatment methods on the digestion of straw (**Papers I, IV**). Chapter 5 delves into impact of co-digestion on the energy efficiencies of various proposed biogas solutions (**Paper IV**) and their relevance to Swedish regional energy production. The dynamics of co-digestion between carbohydrate-rich process streams and manure (**Papers II-IV**) are also discussed. The findings of my research are summarized in the Conclusions, whereby I make suggestions for future studies to be performed as an extension of my work. Lastly, I share my outlook on the future of climate change mitigation.

# Chapter 2. Anaerobic digestion of lignocellulosic biomass

## Composition of lignocellulosic biomass

Lignocellulose is the main constituent of the cell wall in all plants (Figure 2). It has evolved to strengthen the integrity of a plant and its resistance to microbial degradation.

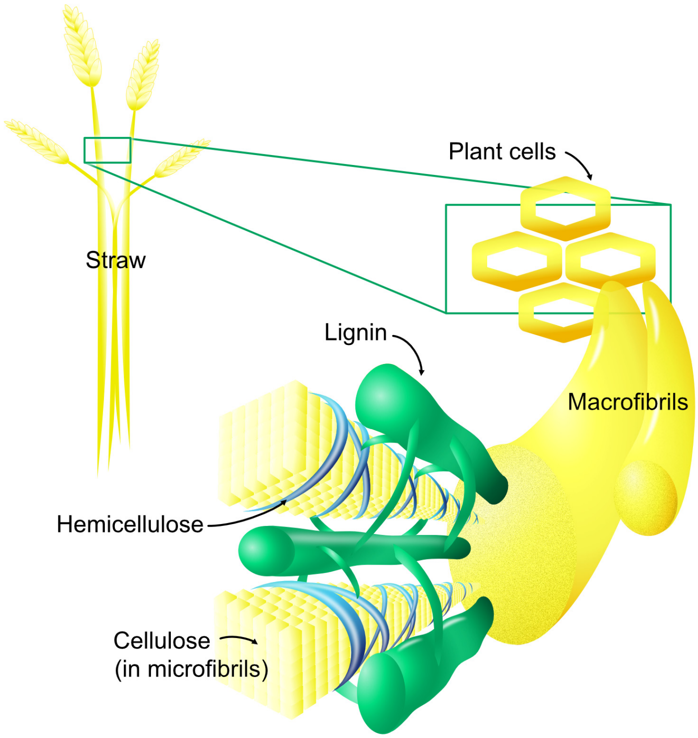


**Figure 2** The structure of lignocellulose and the main components; cellulose, hemicellulose, and lignin. The cellulose chains are structured in microfibrils and stabilized by a hemicellulose-lignin matrix, forming macrofibrils that are the core structure of plant cell walls.

Lignocellulose is composed primarily of cellulose, hemicellulose, and lignin. Cellulose comprises D-glucose units that are linked via $\beta$-1,4 glycosidic bonds, forming linear water-insoluble chains. The length of such chains varies and is measured in terms of degree of polymerization. These chains can organize themselves in parallel and, through hydrogen bonds, form a rigid and crystalline structure (O'Sullivan, 1997).

The disruption of hydrogen bonds along the cellulose creates an amorphous region locally. Such amorphous regions have a higher affinity to water, which facilitates the transport of enzymes and renders them more susceptible to hydrolysis (Fink et al. 1987). The ratio between crystalline to total cellulose in a material can be measured and is referred to as the crystallinity index (Park et al., 2010).

Hemicellulose is also a polysaccharide but consists of a variety of monomeric units, some of which act as branch chains that are bound to a backbone structure. The most common monomers in wheat straw are xylose, arabinose, mannose, and galactose; xylose monomers link together to form a xylan backbone. Cellulose and hemicellulose are biologically degradable in an anaerobic process. Hemicellulose, however, is more accessible to hydrolysis and is thus solubilized first (Li et al., 2018).

Lignin is a polyphenolic compound and an inert fraction of organic matter. By wrapping around cellulose and hemicellulose and forming lignin-carbohydrate bonds, lignin has a sheathing effect and gives its structure much of its rigidity (Tarasov et al., 2018). The exact chemical composition of lignin varies widely but is based on 3 primary monomers: *p*-coumaryl alchol, coniferyl alcohol, and sinapyl alcohol (Chundawat et al., 2011). Several chains of cellulose in parallel form microfibrils that, bound to lignin and hemicellulose, arrange into macrofibrils—the core structural components of the plant cell wall.

In addition to lignocellulose, vegetative biomass contains extractives (wax), proteins, and inorganic compounds. The precise composition varies significantly between species, growth conditions, and time of harvest (Kreuger et al., 2011).

## Manure-based biomass

Cows are herbivores and host specialized bacterial cultures in their *rumen* that hydrolyze structural carbohydrates and thus use the resulting derivatives as constituents of biomass and a source of energy. Consequently, the manure that is produced by these animals also contains a large fraction of recalcitrant lignocellulose (25% to 50% of DM); the remainder comprises proteins (15% to 30% of DM), fats (24% to 46%), and ash (10% to 16%) (Amon et al., 2007; **Paper II**, **IV**). In contrast to straw, cow manure also contains solubilized compounds, such as organic acids, urea, ammonium, and carbonates, contributing to its high alkalinity and buffering capacity.

Animal bedding, or spent animal bedding, naturally contains an even higher fraction of lignocellulosic fibers than pure manure, because excreted cow manure is mixed immediately with the bedding material, often straw. The composition depends in part on how frequently the bedding material is replaced, wherein the more recalcitrant fibers reside in the bottom layers of the bedding, which contains most of the soluble fraction and a higher proportion of lignin (Sanchis-Sebastiá et al., 2020). In this thesis, the composition of animal bedding and cow manure is regarded as the sum of two fractions that are obtained via washing: a fiber fraction and a non-fibrous manure fraction (also referred to as washing liquid in **Paper II** and filtered manure in **Paper III**).

**Table 1** Chemical composition of wheat straw, cow manure, and animal bedding by fractionation method per Sanchis-Sebastiá et al. (2019) and a standard method for determining structural carbohydrates per the NREL (National Renewable Energy Laboratory, Golden, Colorado, USA). DM=dry matter; TS=total solids, VS=volatile solids, n.d.=not determined.

| **Content** | **Wheat straw** | | **Cow manure** | **Animal bedding** | | |
|---|---|---|---|---|---|---|
| **(% of DM)** | Paper I | Paper II | Paper III | Paper II | Paper II | Paper II |
| **TS (% wet basis)** | | 92 | 17 | 39 | 25 | 25 |
| **Manure** | | - | 52 | 14 | 34 | 43 |
| **VS** | 95 | 95 | 86 | 90 | 87 | 84 |
| **Ash** | 5 | 5 | 14 | 10 | 13 | 16 |
| **Cellulose** | 35 | 41 | n.d. | 34 | 26 | 23 |
| **Hemicellulose** | 26 | 30 | n.d. | 20 | 16 | 14 |
| **Lignin** | 16 | 24 | n.d. | 19 | 15 | 13 |
| **Extractives** | 12 | 7 | n.d. | 5 | 4 | 3 |
| **Total carbon** | n.d. | 44 | 54 | 39 | 33 | 30 |
| **Total nitrogen** | n.d. | 0.3 | 2.3 | 0.9 | 1.2 | 1.3 |

# Metabolic pathway in anaerobic digestion

When substrate enters an anaerobic digester, it must undergo a series of conversion reactions to reach its final form, methane. All reactions occur in the absence of oxygen and are performed by anaerobes—thus collectively termed anaerobic digestion. These reactions are usually categorized into 4 steps: hydrolysis, fermentation/acidogenesis, acetogenesis, and methanogenesis (Figure 3) (Gujer and Zehnder, 1983; Pavlostathis and Giraldo-Gomez, 1991; Batstone et al., 2002).

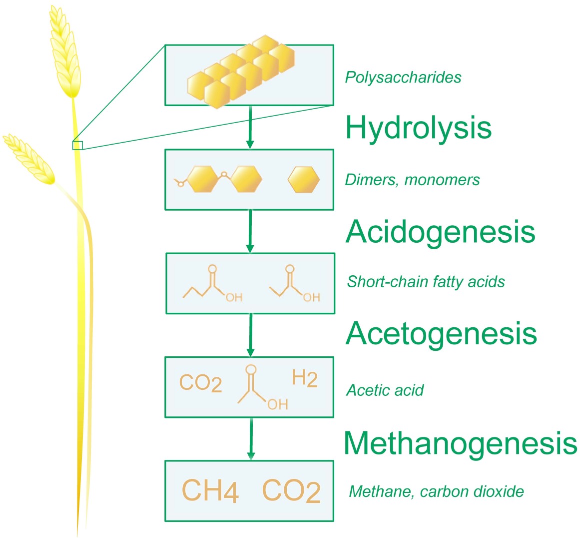


**Figure 3** The polysaccharides in lignocellulose are disintegrated and hydrolyzed to monomeric units through hydrolysis, whereby fermenting bacteria transform sugars into short-chain fatty acids (volatile fatty acids) via acidogenesis. The acids are then digested to acetic acid, carbon dioxide, and hydrogen through acetogenesis, constituting the main reactants for the final step, methanogenesis, which transforms them into methane and carbon dioxide.

Substrate particles are disintegrated and solubilized, wherein the polymers are hydrolyzed to monomeric units by hydrolytic enzymes. Hydrolytic and fermenting bacteria transform the monomers into volatile fatty acids via acidogenesis. The acids are then further digested into acetic acid, carbon dioxide, and hydrogen through acetogenesis, becoming the main reactants for the final step—methanogenesis—in which they are transformed into methane and carbon dioxide.

All conversion steps are performed by various microorganisms, the slowest of which becomes the rate-limiting step in methane production. The total degradability of a substrate is governed by its *bioavailability*—i.e., the sum of the soluble and hydrolysable fractions of volatile solids in the substrate. Alternatively, the degradation rate of a substrate depends on its *bioaccessibility*—i.e., how easily active degrading enzymes or bacteria can reach the volatile solids.

## Hydrolysis of lignocellulose

All bioaccessible polymers are degraded into dimers or monomers through hydrolysis, an enzymatically catalyzed reaction with water. Although *hydrolysis* per se refers specifically to the enzymatic reaction, it is frequently used to describe the cumulative effect of disintegration, solubilization, and hydrolysis (Eastman and Ferguson, 1981).

Cellulose and hemicellulose are converted into sugars by extracellular cellulytic and hemicellulytic enzymes. These enzymes are free or integrated components of larger enzyme complexes (cellulosomes) that are bound to the cell wall of hydrolytic bacteria (Vélez-Mercado et al., 2021). As such, hydrolytic bacteria can benefit directly from hydrolysis by digesting the resulting monomers. The enzymes adsorb onto the structural carbohydrates and then diffuse to specific regions on the cellulose and hemicellulose chains—i.e., their active sites, where the hydrolytic reaction starts.

In systems with excess levels of enzymes, the degree of accessibility of the structural carbohydrates to these enzymes thus determines the rate of hydrolysis (Walker and Wilson, 1991). When the hydrolysis is the rate-limiting step during anaerobic digestion, the degree of accessibility will also govern the rate of methane production (Pavlostathis and Giraldo-Gomez, 1991). Thus, in anaerobic digestion of lignocellulosic biomass, the recalcitrance of a substrate is critical.

Bioaccessibility to hydrolytic enzymes and bacteria

Due to the challenge in controlling and altering the individual physiochemical properties of lignocellulosic materials, the exact impact of various parameters on bioaccessibility remains *inconclusive*, rendering it difficult to assess the bioaccessibility of a material through physiochemical characterization. Because hydrolytic enzymes attach to specific active sites on a substrate, its surface area is sometimes used to approximate bioaccessibility.

Particle size is sometimes used as a proxy for estimates of surface area. However, such calculations fail to consider the internal surface area, which is the predominant one compared to the external surface area for straw particles. With more refined methods, the true accessible surface area can differ significantly from the measured value, depending on the analysis method (Palmowski and Müller, 2003). For example, one must consider whether the pore openings to the internal surface area are large enough for enzymes and the bacteria to which they are attached to enter—bacteria, cellulosomes, and free hydrolytic enzymes are approximately 1-10 µm, 18 nm, and 4-5 nm in diameter, respectively (Raven et al., 2005; M. Schülein, 1988; Cowling and Kirk, 1976; Lamed et al., 1983).

Moreover, the properties of the liquid with regard to conductivity and sugar concentration, for instance, affect its interaction with solid particles (Weiss et al., 2016). Also, the properties of the exposed surface area are determinants. For example, the presence of carboxylic groups in the cell wall (fibers) changes the charge density and thus induces the absorption of water by the cell wall (Scallan 1983).

Water is necessary as a transport medium and reactant for hydrolysis, but it should be in a free state, without overly strong interactions with the solid surface. Smaller particle sizes of straw increase the amount of free pore water and thus its availability for hydrolysis (Dumas et al., 2015), facilitating the transportation of hydrolytic enzymes through the liquid. He et al. (2013) reported that the internal surface area of rice straw is more sensitive to anaerobic digestion by the hydrolytic bacteria

*Clostridium Thermocellum* than the external surface area, which was attributed to the protective layer of silica on the outer layers of rice straw.

In conclusion, the hydrolysis of lignocellulosic material during anaerobic digestion is affected by several physiochemical parameters, the sum of which determines its bioaccessibility to hydrolytic bacteria.

## Acidogenesis/fermentation

The monomers and dimers that are produced during hydrolysis are digested by hydrolytic bacteria and other fermenting/acidogenic bacteria. Depending on the feedstock composition and environmental conditions, the resulting compounds of this step vary, but the most common products are referred to as volatile fatty acids (VFAs). Acidogenesis is the most rapid step during anaerobic digestion of lignocellulosic biomass, with greater abundance and biodiversity of active microorganisms than in other conversion steps (Schnürer, A., 2016). Fermentation of carbohydrates is associated with ethanol and lactate production at higher hydrogen pressure and with the formation of butyrate, propionate, and acetate at lower hydrogen pressures. Protein degradation leads to greater fractions of propionate during the anaerobic digestion of manure (**Paper III**).

VFAs accumulate when their production outpaces their consumption. Such accumulation can inhibit other bacteria in the digester.

$$VFAH \overset{pKa}{\longleftrightarrow} VFA^{-} + H^{+}$$

This inhibition by VFAs is believed to depend on cross-membrane transport of non-charged VFAs into the intracellular liquid. After absorption, the acid dissociates, releasing its proton, which lowers the pH inside of the cell and thus disrupts its function. Because the dissociation of VFAs is pH-dependent, their inhibitory effects also depend on pH and the buffering capacity of the digester liquid.

## Acetogenesis

Acetogens are slow-growing bacteria (Schnürer and Jarvis, 2009) that convert VFAs into acetate, carbon dioxide, and hydrogen (Mata-Alvarez, 2003). As discussed, 2 of the main products of the acidogenesis of lignocellulose are propionate and butyrate (Hu et al., 2005). This conversion is not thermodynamically favorable at excessive hydrogen partial pressures, with $\Delta G = +76.1$ and $+48.3$ kJ/mol, respectively (Lens et al., 1998). Thus, acetogens have a syntrophic relationship with hydrogen-consuming bacteria, such as hydrogenotrophic methanogens that reduce the partial pressure of hydrogen.

This syntrophic relationship can also exist with sulfate-reducing bacteria that consume hydrogen. Because these organisms also consume acetate, they might compete with acetotrophic methanogens for substrate (Thauer et al., 1977; Lide et al., 1993). Energetically, sulfate is the preferred electron acceptor over carbon dioxide.

### Methanogenesis

The final step is methanogenesis, which generates the final product, methane. This step is performed by *archaea* using precursor compounds, such as acetate, carbon dioxide, hydrogen, formate, and methanol. In co-digestion systems, the dominant species is *methanosarcea,* which utilizes acetate as its main food source.

$$CH_3COO^- + H_2O \rightarrow HCO_3^- + CH_4$$

$$HCO_3^- + H_2 \rightarrow H_2O + CH_4$$

Methanogens are slow-growing bacteria (depending on the species) and are sensitive to variations in environmental conditions. Thus, methanogenesis can be inhibited due to many factors, such as the accumulation of VFAs, a decrease in pH, and fluctuations in temperature. One of the main competitors of methanogens are sulfate-reducing bacteria, affecting the selection of pretreatment methods for lignocellulosic materials. The product of the reduction of sulfate is hydrogen sulfide, an odorous, corrosive gas that should be avoided in biogas production.

## Key operational parameters affecting anaerobic digestion

Stable anaerobic digestion requires the maintenance of a healthy microbial community and the growth of essential bacteria and archaea. This growth depends on the availability of food/substrate, thermodynamics that favor the conversion of these substrates into energy and building blocks, and low concentrations of toxic compounds. Further, the operational conditions under which anaerobic digestion is performed can be varied and used to target the growth or dominance of specific groups of bacteria. Various parameters can be used as controls to monitor the stability of the process and obtain early indications of eventual complications.

### Organic loading rate and hydraulic retention time

Organic loading rate (OLR) is the feeding rate of volatile solids into the digester. It depends on the content of volatile solids in the feedstock, the size of the digester, and hydraulic retention time (HRT)—i.e., how long biomass stays in the digester on average. Agricultural biogas plants normally operate at an OLR of 1-5 kg VS $m^{-3}$ $day^{-1}$ and an HRT of 15-60 days, depending on substrate type and process configuration. The more substrate that is processed in a biogas plant per year, the more methane can potentially be produced. Therefore, it is desirable to increase the OLR as much as possible. Raising the total solids content in the feed can increase the HRT in a digester with a fixed volume. Longer retention times correlate with increased degrees of degradation, in turn improving specific methane yields (Linke et al., 2013).

## Temperature

Anaerobic digestion processes can be categorized by the range of temperatures at which they are run: thermophilic (52-55°C), mesophilic (35-42°C), and psychrophilic (<25°C). Although thermophilic anaerobic digestion effects higher degradation rates (Maharaj and Elefsiniotis, 2001) and subsequent methane production (Lokshina and Vavilin, 1999), most biogas plants in Sweden operate under mesophilic conditions, because the temperature range for optimal methanogenic activity is much narrower in thermophilic digesters, due in part to the lower microbial biodiversity (Schnürer, A., 2016). Any fluctuation in temperature inside of the digester can thus have severe consequences.

Biogas plants that operate primarily using nitrogen-rich substrate, such as manure (especially pig and chicken manure) can experience inhibition by ammonia, because the equilibrium between non-toxic ammonium and inhibitory free ammonia (FAN) is temperature-dependent. However, previous studies on anaerobic digestion of cattle manure have shown that unadapted mesophilic reactors are more sensitive to the accumulation of FAN than thermophilic reactors (Hashimoto et al., 1986) and that methane production is disrupted at a total ammonia concentration ($NH_3 + NH_4^+$) of >2.5 g total ammonia nitrogen (TAN) L-1.

Nevertheless, most centralized manure-based anaerobic digesters, as in Denmark, are run under thermophilic conditions. Adaptation of the inoculum will increase the tolerance to TAN levels, and depending on the buffering capacity of the medium, the FAN concentration can also be reduced. Angelidaki et al. (1993) reported that thermophilic digestion of cow manure was affected at >3.0 g TAN $L^{-1}$ and that maintained biogas production could be achieved at >4.0 g TAN $L^{-1}$, albeit at a biogas yield of 75% of that of an uninhibited control digester.

Psychrophilic digesters are used primarily as storage for digestate, with a module for biogas collection but no temperature control.

## C/N ratio

Because anaerobic digestion is a microbial treatment, its feasibility depends entirely on the health and growth of its microbes. To maintain a balanced supply of macronutrients, the incoming feedstock mixture should aim for a carbon-to-nitrogen (C/N) ratio of 20-30, for example (Igoni et al., 2008). Excessive nitrogen increases the risk of that the concentrations of ammonia will become elevated, and because manure generally has a low C/N ratio of approximately 10 (Risberg et al., 2017), it can be combined with a carbon-rich feedstock that has a low nitrogen content.

Zhao et al. (2018) studied the co-digestion of oat straw and cow manure at various C/N ratios (18-33) and TS contents (4% to 10%) and observed synergistic effects on methane yield and greater decreases in VS (19% to 54%) with all substrate mixtures. Arias et al. (2020) concluded that co-digestion of corn stover and swine manure increases lignocellulytic enzyme activities but that TS contents above 2% prolong the inhibition/adaptation phase of methanogenesis. The exact optimal C/N ratio depends on the rate at which carbon and nitrogen become available and the uptake rate of growing microorganisms.

Breure et al. (1986) claimed that complete protein degradation is frequently unobtainable in the presence of carbohydrates during anaerobic fermentation. They showed that addition of glucose severely inhibited the hydrolysis of gelatin during mesophilic anaerobic fermentation and concluded that this inhibition was not due to increased VFA concentrations. Instead, the fermenting organisms preferred glucose as substrate.

### pH level

The pH in an anaerobic digester influences the bacteria in the digesters to various degrees, as well as reaction equilibria (e.g., between ammonium and ammonia). Lower pH levels (4.5–6.0) promote the hydrolytic activity of fermentative bacteria, whereas methanogenic activity is optimal at higher pHs (7.5–8.0). Thus, ideally, anaerobic digestion processes are run at neutral pH levels. Typically, VFAs accumulate in response to a disruption in the stability of the digestion, due to substrate overload, temperature fluctuations, or the presence of inhibitors. Although higher concentrations of acids per se can inhibit methanogenesis, their accumulation also decreases the pH, creating a negative feedback loop. To control pH in an anaerobic digester, the alkalinity should be monitored. In large-scale operations, such additives as NaOH and lime are used to neutralize pH.

# Chapter 3. Methods

Anaerobic digestion can be assessed using many approaches. Because biogas production from various substrates and substrate mixtures was evaluated primarily by experimental batch test, its limitations should be considered, because they influence the analyses of the results and their implications.

## Biogas batch test

Anaerobic digestion was assessed by batch test (**Paper I-IV**), primarily through biochemical methane potential (BMP) test but also under other conditions.

The BMP tests were designed to study the degradation of a specific substrate or substrate mixture. At a high inoculum load, the limiting factor becomes the characteristics of the substrate and maximum degradation is achieved. In this work, all BMP tests were performed at an inoculum-to-substrate ratio (I/S) of 2:1, based on the VS contents of the materials. The BMP tests were generally performed at a substrate load of 20 g VS $L^{-1}$ (**Papers I, II, IV**) per Hansen et al. (2004) and at the lower end of the range of recommended doses (20-60 g VS $L^{-1}$) per Holliger et al. (2016). Wang et al. (2015) confirmed that methane production from cellulose decreases significantly at substrate loads below 10 g VS $L^{-1}$.

Cumulative specific methane production, *B*, over time, *t*, was modeled according to first-order kinetics (Eq. 1, Fig. 4a) or a modified Gompertz's model (Eq. 2, Fig. 4b) (Zwietering et al., 1990):

$$B = B_1 \cdot (1 - \exp\{-k_H \cdot (t - \lambda_1)\}) \quad (1)$$

$$B = B_1 \cdot \exp\left\{- \exp\left(\frac{R_m \cdot e}{B_1} \cdot (\lambda_1 - t) + 1\right)\right\} \quad (2)$$

where $B_1$ is the maximum specific methane yield (NmL $CH_4$ $g^{-1}$ VS), $k_H$ is the hydrolysis constant ($day^{-1}$), $\lambda_1$ is the initial lag time (days) before methane production begins, and $R_m$ is the maximum daily methane production (NmL $CH_4$ $g^{-1}$ VS $day^{-1}$). The modified Gompertz model was applied when cumulative methane production followed a Monod-like curve (Figure 4b). The hydrolysis constant, however, can also be estimated from these curves if the measurements during the lag time are excluded—i.e., by starting the modeling when daily methane production peaks (Hafner et al., 2022).

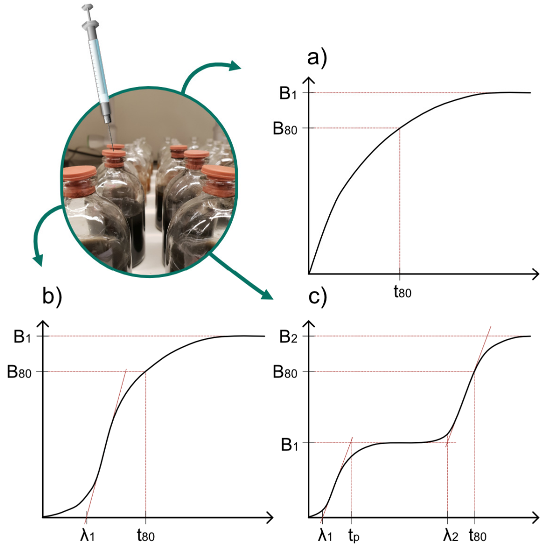


**Figure 4** The cumulative methane production over time, following the a) first-order kinetics, b) Monod-like kinetics, and c) diauxic Monod-type kinetics.

In **Paper III**, the inoculum load was lower (I/S = 0.55–1.4) than recommended for BMP tests, and the results thus mirrored the limitations of the substrate and inoculum. This approach was chosen to capture how anaerobic digestion responds to overloads and determine the impact of carbohydrate accessibility. However, a low inoculum load and improper I/S ratio can lead to the maximum specific methane yield being underestimated (Koch et al., 2019), which is why the result of these batch tests should not be regarded as an evaluation of the maximum degradability or accessibility of substrate mixtures. In cases in which inhibition occurred mid-methane production (Fig. 4c), a 2-phase modified Gompertz's equation was applied (Eq. 3):

$$B = \sum_i^n B_i \cdot \exp\left\{-\exp\left(\frac{Rm_i \cdot e}{B_i} \cdot (\lambda_i - t) + 1\right)\right\} \qquad (3)$$

where $B_i$ is the maximum methane yield (NmL $CH_4$ $g^{-1}$ VS) pre-inhibition (i=1) and post-inhibition (i=2). The total cumulative methane production was calculated by adding the methane production of both phases ($B_1 + B_2$). The start of the inhibition plateau, $t_p$, was defined as the intersection point of the line $y = B1$ and the line that crosses the x-axis at $\lambda_l$ and the inflection point $d^2y/dt = 0$ (Fig. 4c) (Gomes et al., 2021).

Batch tests are limited, in that the substrate concentration is initially high and then decreases over time. Thus, the conversion rates of substrate to its final form, methane, are dynamic. In full-scale applications, however, the mode of operation is continuous or semi-continuous. Consequently, the methane production rate will be lower than the maximum rate from batch tests ($k_H$ or $R_m$). Further, when studying the impact of co-digestion on the risk of instability (**Paper III**), the preferred method is a continuous experiment, in which the inoculum can adapt to the feedstock.

# The inoculum

Many factors affect the outcome of biogas trials—the origin and age of the inoculum and the heterogeneity of the substrates will impact the results of an experiment, despite a standard protocol being followed. Depending on the origin of the inoculum for a thermophilic BMP test, the outcome will differ, as illustrated in Figure 5, in which cellulose, wheat straw, and pig manure were used as substrates for a BMP test using 2 inocula—1 each from an anaerobic digester at a municipal wastewater treatment plant (Kävlinge, Sweden) and a co-digestion plant (Lemvig, Denmark) that processes fish, household, and slaughterhouse waste.

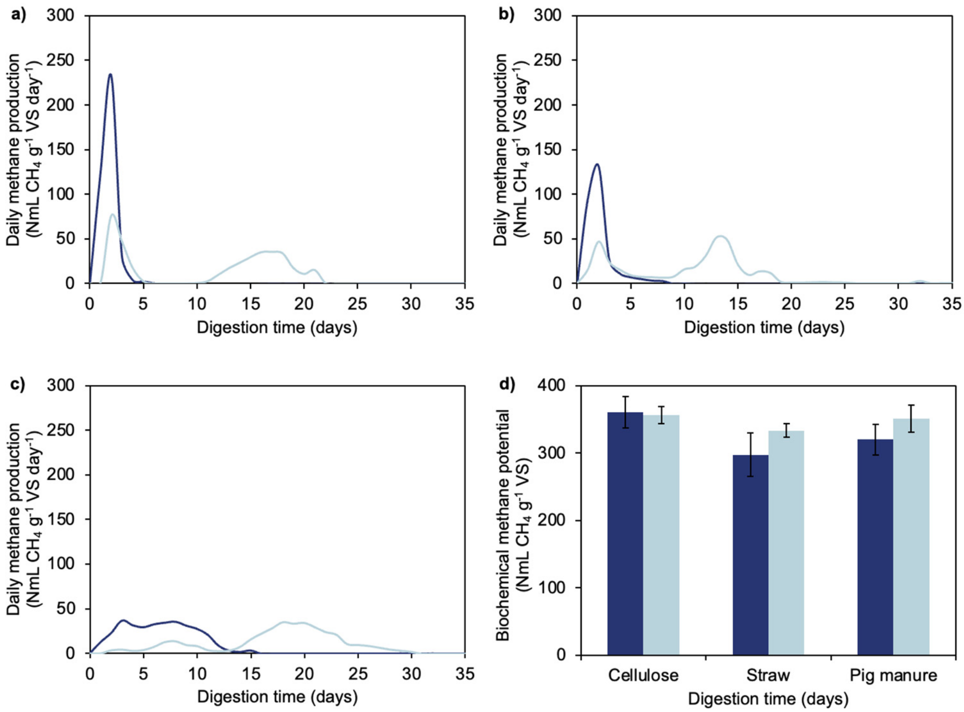


**Figure 4** Daily methane production during thermophilic anaerobic digestion of a) cellulose, b) wheat straw, and c) pig manure using two inocula: one from a wastewater treatment plant (dark blue) and one from a co-digestion plant (light blue). The biochemical methane potential from digestion of the different samples with the two inocula is also shown (d). VS=volatile solids.

Although the BMP of the cellulose was similar between the two inocula those of the wheat straw and pig manure differed with the inoculum from the co-digestion plant generating higher values. It is possible that this disparity was affected by the heterogeneity of the substrates, given that the largest differences were observed for wheat straw, compared with the smallest difference for cellulose. However, similar observations have been made by other groups, wherein the origin of the inoculum influenced BMP values (Yu et al., 2014). The inhibition phase during digestion with the inoculum from the co-digestion plant did not lead to a lower yield.

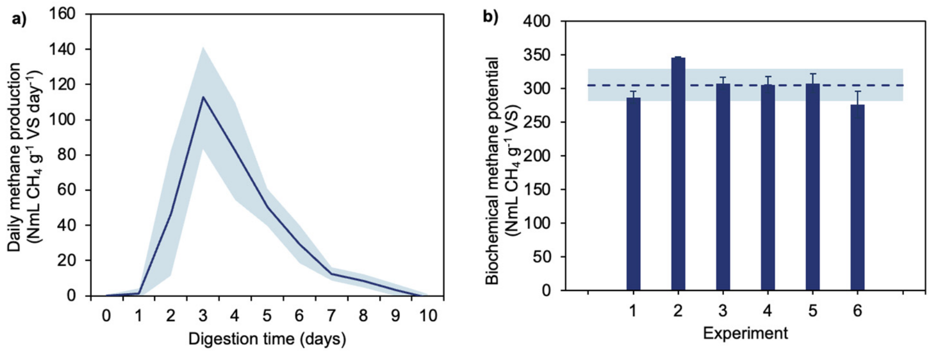


**Figure 5** The figure shows a) the daily methane production, and b) the biochemical methane potential from six different mesophilic BMP tests performed on cellulose (Avicel PH-101) with inoculum from a digester at a wastewater treatment plant. VS=volatile solids.

The daily methane production rate from mesophilic digestion of cellulose (Figure 6a) and the calculated BMP (Figure 6b) were calculated based on six BMP tests that were run under the same conditions with inoculum from the same source (a digester processing sludge from a wastewater treatment plant), albeit at various times. The inoculum had a large effect on the outcome of the BMP test, and the variation between tests was greater than that between triplicates that were run in parallel. Thus, comparisons of numerical results should be avoided between tests (Hafner et al., 2022).

# Equipment for measuring biogas production

For experiments with few samples (**Paper I** and **Paper IV**), an automatic methane production system (AMPTS II, Bioprocess Engineering) was used to log volumetric methane production, converting it automatically to normal conditions (0°C, 1 atm, dry). This system is limited, being unable to record the actual gas composition of the biogas. Instead, integrated carbon dioxide traps (3 M NaOH) are used under the well-founded assumption that all carbon dioxide is dissolved.

The AMPTS II is also limited by the number of samples that can be run in parallel. When many conditions were tested and compared in **Papers II** and **III**, it was more suitable to perform the batch tests in smaller glass serum bottles (120 mL) in an incubator. The gas that was produced here was logged by measuring the pressure with a manometer, equalizing the headspace pressure to room pressure and analyzing the gas composition on a gas chromatograph. The 2 methods differed with respect to several parameters that could have affected the results. With regard to mode of agitation, a stirring rod was used in the AMPTS II trials, whereas the serum bottles in the incubator were mixed on a shaker. The efficiency of agitation impacts methane production due to the release of entrapped bubbles (Wang et al., 2017).

Further, the serum bottles experienced increasing headspace pressure between each measurement, in contrast to the AMPTS II system. Higher headspace pressures (>600 to 1000 mbar) affect methane production during batch tests (Valero et al., 2016) due to the solubilization of carbon dioxide in the liquid. In addition, the cumulative methane production was only corrected for temperature and pressure in the headspace of the reactors (0°C, 1 atm)—not for water vapor pressure—which can lead to overestimation of the gas production by 2% to 8% (Strömberg et al., 2014).

The flexibility of the smaller bottles also allowed us to sample the liquid and analyze VFA concentrations during the experiments. Because samples of the liquid were drawn with narrow syringes, the volume that was removed was assumed not to have significantly affected the solid substrate content in the reactors. Nevertheless, the total volume of liquid that was withdrawn from each reactor was limited to a maximum of 10% of the total active reactor volume.

# Chapter 4. Pretreatment of straw fibers

## Pretreatment strategies for lignocellulosic fibers

Lignocellulosic fiber is an acceptable feedstock for biogas production plants if the material is first pretreated prior to anaerobic digestion, to allow the material to be fed to the digester, stirred inside of the digester, and, most importantly, digested. When these criteria are met, the pretreatment should be optimized to increase methane production and digestibility and decrease energy consumption.

In this work, two types of pretreatments were applied: mechanical and hydrothermal (Figure 7). The mechanical pretreatments comprised roll milling, extrusion, hammer milling, and pelletization of wheat straw (**Paper I**). These methods were chosen to encompass a wide range of comminution techniques to study their effects on anaerobic digestion. The hydrothermal pretreatment was performed by acid-catalyzed steam explosion.

**Figure 6** Mechanically pretreated straw. From left to right: chopped, roll milled, hammer milled, extruded, and pelletized.

### Roll milling

Roll mills are typically used during liquid extraction or densification of biomass (Tumuluru et al., 2011). Biomass is fed to a defined gap between 2 equally sized rollers that rotate counterclockwise to compress the material, increasing its bulk density. The roll mill that was used in **Paper I** was customized for the pretreatment of straw.

## Hammer milling

A hammer mill primarily uses impact forces to reduce the particle size of biomass, applied by rotating hammers that crush and press the material against a screen (Mayer-Laigle et a., 2018; Himmel et al., 1985). Smaller screens consume more energy due to the lower rate of biomass throughput (Adapa et al., 2010). The use of screens allows the size of the resulting particles to be specified but also places limits on the moisture content of the biomass to prevent them from becoming clogged. Further, higher moisture contents increase the energy requirement due to the greater shear strength of the biomass (Mani et al., 2004), to approximately 20-40 kWh/ton straw (Himmel et al., 1985; Cadoche and Lopéz, 1989; Mani et al., 2004; Bitra et al., 2009; Adapa et al., 2010).

## Extrusion

Extrusion has a wider application range with regard to the dry matter content of the feed. This pretreatment method consists of a single or twin screw that conveys the material forward and builds local pressure by altering the opening of the output and selecting various types of screw elements (e.g., kneading, reverse) (Hjorth et al., 2011; Wahid et al., 2015). In addition to breaking up fibers, the extrusion of straw raises the friction-induced temperature from 33°C (Hjorth et al., 2011) to 84-104°C (Wahid et al., 2015), correlating positively with dry matter content.

In contrast to hammer milling, a higher moisture content during extrusion decreases the consumption of electrical power (Hjorth et al., 2011; Kupryaniuk et al., 2020) due to reduced friction. The reported energy consumption varies widely—10-300 kWh/ton—and depends largely on the scale of the equipment (Hjorth et al., 2011; Wahid et al., 2015).

## Pelletization

Pelletization was examined as a potential mechanical pretreatment for wheat straw. By compressing wheat straw at a high pressure and slightly elevated moisture content, its density can be increased. There are various types of mills (pellet mill, piston press) that can be used to produce straw pellets. In our study, a pellet mill increased the density of wheat straw from 33 to 652 kg $m^{-3}$ (**Paper I**). No binder was added, because the lignin in the straw acts as a natural binder when it melts during compression (Tumuluru et al., 2011). Previous studies have reported that the pelletization of wheat straw requires approximately 80-170 kWh/ton at a moisture content of 17% to 18% (Adapa et al., 2010; Wilson et al., 2014) but that 15-40 kWh/ton is needed for commercial pellet mills (Tumuluru et al., 2011). Energy consumption by pelletization increases with applied pressure but also with lower initial particle sizes.

### Steam explosion

Steam explosion of lignocellulosic biomass entails subjecting pre-soaked material to high temperatures (160°C to 230°C) for a short period (5 to 20 minutes) in a closed reactor chamber. These temperatures are achieved by introducing steam into the reactor chamber, to solubilize and thus fractionate the lignocellulose. Next, the pressure is suddenly released, causing explosion of the material. If an acid catalyst is used during the pre-soaking (impregnation) step, the polysaccharides in the biomass are hydrolyzed. Steam explosion of wheat straw generates a xylose-rich liquid stream due to the solubilization and hydrolysis of the hemicellulose. The resulting fraction of solids contains most of the cellulose and lignin.

In this study, sulfuric acid was selected as the catalyst due to its low cost. However, it might be a suboptimal acid for biogas processes, because sulfuric compounds inhibit methane production due to competition for acetate. Moreover, the digestion of sulfate effects the formation of $H_2S$, a corrosive and hazardous compound.

## Effect of mechanical pretreatment on anaerobic digestion of wheat straw

### The handling properties of straw

Despite the well-known issue of the accumulation of floating layers inside of the digesters, due to the intake of fiber-rich feedstock by the process (Nielsen, et al., 1997), few studies have taken it into account (Finck and Goma, 1984; Mönch-Tegeder et al., 2013). Tian et al. (2015), however, concluded that an increasing floating layer reduces biogas production in lab-scale experiments.

Although straw is made up of compounds with high true densities—with a crystalline cellulose of 1.6 g $cm^{-3}$ (Daicho et al., 2020)—its porous structure (46% to 84% porosity; Zhang et al., 2012) renders it a bulky material. The particle density of straw and manure fibers is thus much lower than their true density. Due to the hydrophobic wax layer and presence of lignin, untreated straw has low wettability, preventing existing inner pores from being fully occupied by water (Teghammar et al., 2012).

In **Paper I**, extrusion and pelletization significantly reduced the tendency of wheat straw to form floating layers—i.e., the floating index—and increased its bulk density (Table 2). The floating index was determined by adding a known amount of wheat straw to a measuring cylinder that was filled with water and calculating the volumetric quotient between the floating layer, $V_{float}$, and the total volume of wheat straw in the water, $V_{tot}$. Both pretreatment methods are more energy-intensive and subject the material to higher local temperatures than roll milling and hammer milling, which could explain the higher wettability. Moreover, extrusion and pelletization led to higher bulk densities but mean particle sizes of 0.7±0.1 and 1.2±0.0, respectively.

**Table 2** Bulk density, floating index, and mean particle size of mechanically pretreated wheat straw (**Paper I**).

| | **Bulk density ($kg/m^3$)** | **Floating index ($V_{float}/V_{tot}$)** | **Mean particle size (mm)** |
|---|---|---|---|
| **Chopping (untreated)** | 33 | 1.00 | 3.1±0.3 |
| **Roll milling** | 91 | 1.00 | 3.0±0.2 |
| **Hammer milling** | 152 | 0.91 | 0.6±0.1 |
| **Extrusion** | 334 | 0.04 | 0.7±0.1 |
| **Pelletization** | 652 | 0.29 | 1.2±0.0 |

There is no standard method for assessing the tendency of a material to form floating layers in a digester. It is possible that a high floating index is acceptable in large-scale digesters due to their long retention times and adequate mixing. Nonetheless, extrusion has greater potential in mitigating the floating problem and rendering wheat straw bioavailable for biogas production compared with the other mechanical pretreatments in this study. The handling properties of straw can thus be improved via mechanical pretreatment.

## Improved methane production rate by extrusion or pelletization

Extrusion and hammer milling generated similarly sized particles (0.7±0.1 mm and 0.6±0.1, respectively; Table 2) but yielded differing methane production rates of 52±2 and 43±1 NmL $CH_4$ $g^{-1}$ VS $day^{-1}$ (Table 3). In contrast, pelletization proceeded at similar kinetics as extrusion but resulted in a larger average particle size of 1.2±0 mm. The biochemical methane potential of roll-milled and hammer-milled straw increased 21% and 14%, respectively, compared with untreated wheat straw.

**Table 3** The biochemical methane potential (BMP), the maximum daily methane production ($DMP_{max}$), and the technical digestion time (T80) of mechanically pretreated wheat straw (**Paper I, IV**). VS=volatile solids.

| Pretreatment | BMP (NmL $CH_4$ $g^{-1}$ VS) | DMPmax (NmL $CH_4$ $g^{-1}$ VS $day^{-1}$) | T80 (days) |
|---|---|---|---|
| **Paper I** | | | |
| **Chopping (untreated)** | 237±16 | 41±5 | 12±4 |
| **Roll milling** | 287±24 | 41±4 | 14±1 |
| **Hammer milling** | 269 ± 10 | 43±1 | 13±2 |
| **Extrusion** | 237±26 | 52±2 | 6±1 |
| **Pelletization** | 239±4 | 49±0 | 7±1 |
| **Paper IV** | | | |
| **Chopping (untreated)** | 279±28 | 21±0 | 18±1 |
| **Hammer milling** | 287±31 | 33±0 | 10±1 |

Higher methane production rates theoretically shorten hydraulic retention times in large-scale biogas production and thus increase the available capacity, whereas a higher methane yield implies more efficient use of material. Depending on the objective of the pretreatment optimization, the ideal mechanical pretreatment approach can vary. None of the mechanical pretreatments that were examined was optimized for anaerobic digestion; thus, it is possible that other configurations of the individual pretreatments will yield different results. Moreover, pretreatment of another batch of wheat straw by hammer milling did not significantly affect the final methane yield (increase of 3%), whereas the maximum daily methane production rate rose from 21 to 33 NmL $CH_4$ $g^{-1}$ VS $day^{-1}$.

## Altering methane production rates by size reduction

The most apparent effect of mechanical pretreatment on wheat straw properties is size reduction. Although the mean particle size was not a determinant of methane production rate when comparing mechanical pretreatments, it could still impact the optimization of an individual pretreatment method. To examine the effect of size reduction on anaerobic digestion, additional tests were performed on size-fractionated straw.

Particle size was an important factor with regard to the efficacy of anaerobic digestion—maximum daily methane production (DMPmax) doubled when particle size decreased from approximately 3 mm to <1 mm (Figure 9a, **Paper I**), corresponding to hydrolysis reaction rate constants of roughly 0.15 to 0.35 $day^{-1}$, respectively. Larger wheat straw particles are generally more porous than smaller particles. Consequently, the total particle surface becomes less dependent on particle size, explaining the impact of the latter in decreasing methane production rates (Figure 9a).

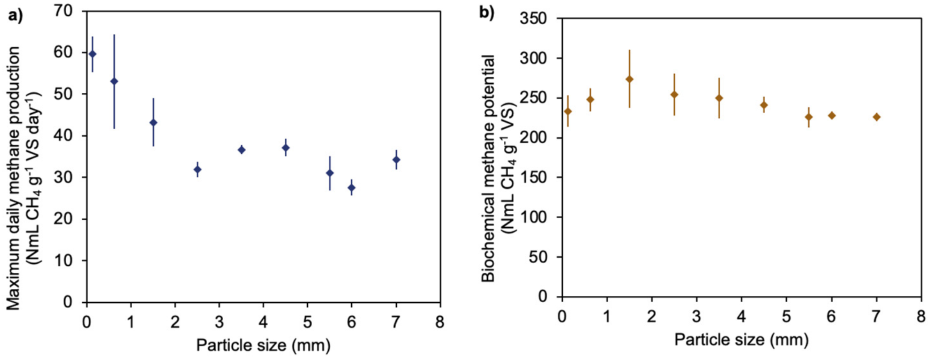


**Figure 7** The data points show the average of results from two separate BMP tests run on two different arrays of roll milled, size fractioned wheat straw. In both BMP tests, each sample was analyzed in triplicates. a) Maximum daily methane production rate (DMPmax) and b) biochemical methane potential (BMP) versus particle size. The error bars show the sample standard deviation. VS=volatile solids.

The maximum methane yield of the straw was unaffected, given that the BMP values of all size-fractionated samples remained unchanged (Figure 9b, **Paper I**), indicating that smaller particles do not grant access to unavailable organic matter. Instead, BMP depended more on the chemical composition, which does not differ significantly between particle sizes (Dumas et al., 2015). Sharma et al. (1988), however, observed a difference in methane yield, from 162 mL $g^{-1}$ VS to 227 and 249 mL $g^{-1}$ VS, when reducing the particle size of wheat straw from 30 mm to 6.0 and 0.088 mm, respectively, although the difference between the latter 2 fractions, which were comparable with those in this work (**Paper I**), was small.

All of the monodigestion tests on wheat straw led to immediate methane production and consistent dependence of methane production rates on the rate of hydrolysis, as indicated by the first-order kinetics. Extensive particle size reduction of wheat straw can effect the accumulation of VFAs due to the increased content of soluble organic matter (Dumas et al., 2015). However, no inhibition phases were observed during any experiment on mechanically pretreated straw.

# Effect of steam explosion on the anaerobic digestion of wheat straw

To improve the efficiency of digestion, the dependence of methane production rate on the rate of hydrolysis must be decreased. As the slowest hydrolysis reaction is that of the solubilization of polymers and their conversion into soluble oligomers Steam explosion of wheat straw was not assessed with regard to mean particle size but resulted in liquid (hydrolysate) and solid fractions, constituting 24% and 76%, respectively, of the initial VS content in the straw. As the hydrolysis of oligormers into monomers is deemed faster than the hydrolysis of solid polymers tin o soluble oligomers, the production of the hydrolysate should decreased the impact of the rate of hydrolysis on the methane production rate.

Due to the increased fraction of readily available organic matter in the feedstock and the lower recalcitrance of the solids, it was possible to improve the maximum daily methane production rate (Figure 10a). Chopped straw and hammer milled straw yielded maximum daily methane production rates of 21 and 33 NmL $CH_4$ $g^{-1}$ VS $day^{-1}$, respectively, compared with 45 and 147 NmL $CH_4$ $g^{-1}$ VS $day^{-1}$ with the fiber fraction and hydrolysate, respectively.

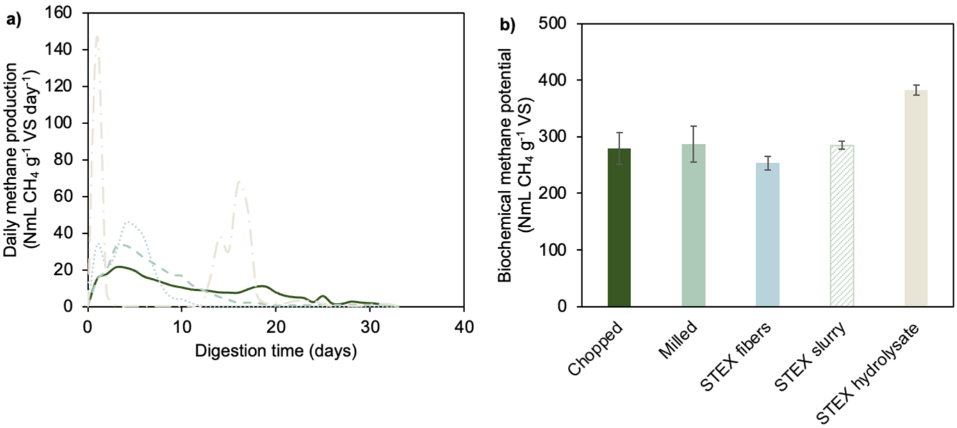


**Figure 8** Daily methane production (a) and biochemical methane potential (b) from anaerobic digestion of chopped wheat straw (dark green), hammer-milled wheat straw (light green, dashed), and the fiber fraction (blue, dotted) and hydrolysate (beige, dash-dot) from steam-pretreated wheat straw. The biochemical methane potential of the STEX slurry (fibers and hydrolysate prior to fractionation) was estimated based on the results from the STEX fibers and STEX hydrolysate. STEX=steam explosion; VS=volatile solids.

The hydrolysate yielded two peaks in daily methane production, separated by an inhibition phase during which no methane was produced. Adaptation of the inoculum to the substrate and its primary metabolic derivatives (likely the accumulation of VFAs) led to the reinitiation of methanogenesis and thus a high final methane yield. Thus, the anaerobic digestion of hydrolysate was not dependent on the rate of hydrolysis. Moreover, when the steam pretreated straw was introduced into water, no floating layer was observed, similar to what was reported by Risberg et al. (2013). Similar to extrusion and pelletization, steam explosion also fulfils the criterium of reducing the formation of floating layers.

# Energy gain from increased methane yield

Roll milling, hammer milling, extrusion, and pelletization were applied to wheat straw that had been chopped to a particle length of approximately 50 mm. Based on the obtained BMP values (Table 3), the energy gain from the application of the pretreatment could be estimated by calculating the difference between the untreated (chopped) and the pretreated samples (Table 4).

**Table 4** The biochemical methane potential (BMP) of pretreated wheat straw (Paper I, IV) and the net energy gain from the different pretreatments compared to untreated (chopped) wheat straw. The energy demand of the pretreatment methods was based on literature review. VS=volatile solids; BMP=biochemical methane potential.

| **Pretreatment** | **Energy gain (kWh/ton DM)** | **Energy demand** | | **Reference** |
|---|---|---|---|---|
| | | **(kWh/ton DM)** | **(% of BMP)** | |
| **Paper I** | | | | |
| **Chopping (untreated)** | 0 | 2-3 | 0.1 | Adapa et al., 2010 |
| **Roll milling** | 360 | 7-15 | 0.3-0.6 | Bojanić et al. 2021 |
| **Hammer milling** | 0 | 20-40 | 0.8-1.6 | Himmel et al., 1985<br>Cadoche and Lopéz, 1989<br>Bitra et al., 2009<br>Adapa et al., 2010a |
| **Extrusion** | 14 | 100-300 | 4.5-13 | Hjorth et al., 2011<br>Wahid et al., 2015<br>Kupryaniuk et al., 2020 |
| **Pelletization** | 230 | 15-40 | 0.7-1.8 | Tumuluru et al., 2011 |
| **Paper IV** | | | | |
| **Chopping (untreated)** | 0 | 2-3 | 0.1 | |
| **Hammer milling** | 57 | 20-40 | 0.7-1.5 | |
| **Steam explosion** | 43 | 250-500 | 7.3-18 | Zhu et al., 2010a,b<br>**Paper IV** |

Based on the results in Table 3, it is evident that even modest improvements in digestibility (BMP) have significant effects on the net energy balance of the mechanical pretreatments. Thus, making decisions based solely on such results is not advisable. The primary aim must be to render the wheat straw available—i.e., to fulfill the criteria with regard to its handling properties. For example, extrusion is an energy-demanding process (100–300 kWh/ton DM), especially under dry conditions, but if it reduces the floating layer of straw and generates a pumpable slurry when mixed with a liquid medium, the energy investment might be warranted.

Admittedly, an electrical consumption of 4.5-13% of the total biogas production is high compared with a total electricity demand for a biogas plant, at 2% to 3% (IRENA, 2018), and wetting of the straw might therefore be a more interesting approach to decrease the energy demand. If mechanical pretreatment contributes to heating the feedstock—a reported effect of extrusion (Wahid et al., 2015)—it is possible that the increased electricity demand could be compensated for by lower heating requirements. The average heating demand of an agricultural plant is 5% to 10% of the biogas that is produced.

# Chapter 5. Co-digestion of straw and manure

The use of wheat straw for anaerobic digestion requires the addition of nutrients. Thus, co-digestion with manure is a frequently considered option for balancing the C/N ratio and supplying important nutrients for the microorganisms. A common drawback of biogas production from manure is the high water content in most manures (85-95%). The high water content leads to high transportation costs. Animal bedding, however, has a higher total solids content of 25% to 40% (Sanchis-Sebastiá et al., 2019; **Paper II**).

This chapter examines co-digestion between wheat straw and animal bedding (**Paper II**) and between wheat straw and solid cow manure (**Paper IV**), in terms of overall process design (conditioning, pretreatment) and the effects on degradation. In-depth studies on the impact of carbohydrate accessibility on co-digestion with manure are also discussed (**Papers II, III**).

## Washing of animal bedding for pretreatment of fibers

The pretreatment of wheat straw was examined in the previous chapter. Using manure as co-substrate can also pose challenges regarding fiber accumulation in floating layers, feeding and mixing problems, and slow degradation due to the recalcitrant nature of the material. To render animal bedding available for processing, its handling properties must be improved.

A method for washing animal bedding was developed by Sanchis-Sebastiá et al. (2019), in which water is added at a liquid:solids ratio of 20:1 in a concrete blender, after which the washed fibers are separated from the liquid that is generated—i.e., the washing liquid—via filtration at 13 bars. By removing the manure from the fibers in the animal bedding, the fibers can be treated separately to improve their handling properties and decrease their recalcitrance.

In **Paper II**, the manure content in the animal bedding was varied. The washing efficiency, calculated as the fraction of manure that was removed from the raw material, was found to linearly increase with initial manure content (Figure 11). Due to the efficient washing step, the manure content in the fiber fraction of the animal bedding was lowered from 14–43% to 9–12%—that is, the washed fraction did not differ in composition as much as the raw material. This homogenizing effect resulted in the consistent output of material by the sulfuric acid-catalyzed steam pretreatment (**Paper II**).

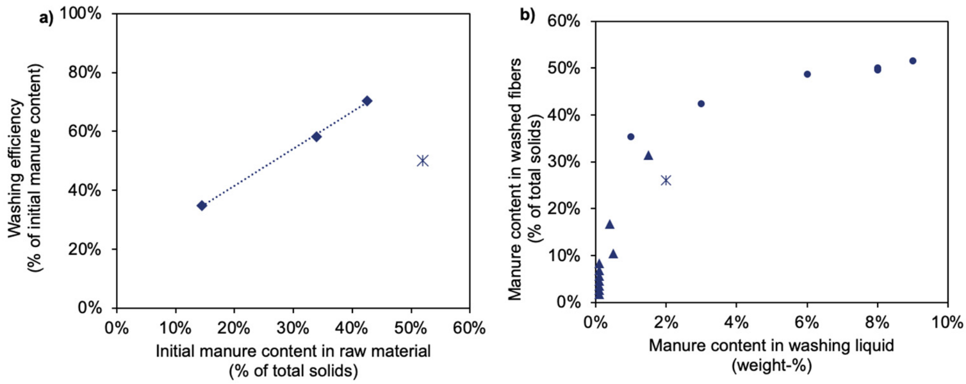


**Figure 11** Results of the washing experiments, showing a) the washing efficiency of the pilot-scale washing of animal bedding (diamonds) and cow manure (overlaid x) at a liquid:solids ratio of 20:1 and b) the manure content in the washed fibers versus the resulting washing liquid during bench-scale washing of cow manure, with recycling of fiber (triangles) or washing liquid (circles).

An insufficient washing step would have led to excessive manure—or more precisely, nitrogen content—in the washed fiber fraction, which in turn would have increased the loss of material due to Maillard reactions at higher temperatures with the solubilized sugars, yielding inert Maillard products. In that case, the anaerobic digestion of untreated animal bedding would have been the favored process design.

Such a pathway, however, did not occur, due to the large amount of water that was applied during the washing step. In a full-scale application, it would be reasonable to assume that the actual washing occurs in a countercurrent setup or that the washing liquid is recycled and reused to increase its total solids content before diverted toward anaerobic digestion. In this work, the washing step was not further optimized in terms of minimizing fresh-water usage or increasing the TS content in the liquid.

Additional washing experiments (*unpublished*) were, however, performed on solid cow manure, in which the washing liquid was reused to treat a new batch of fresh cow manure to increase the TS content in the washing liquid (Fig. 11b). The recycling of washing liquid increased the manure content in the outgoing liquid and solid fractions (Fig. 11b). It is possible, however, that because the wash of animal bedding was more efficient (Fig. 11a), more wash liquid could be recycled. Depending on the pretreatment of the fibers, the constraints for the wash procedure will likely vary.

# Biogas process designs

Agricultural residues can be incorporated into biogas production plants through various means. The most common type of agro-based process in Sweden is farm-based biogas production, in which primarily manure is fed to a 1-stage digester for local production of heat and electricity. The digestate can then be recirculated to local fields to produce animal feed. Because farm-based biogas solutions have lower capacity, they often fail to generate sufficient biogas for it to be economically sound to invest in upgrading units. However, there are cases in which farmers have combined their raw biogas production and installed local gas grids that transport the raw gas to a common upgrading unit.

Most agricultural residues that are used in anaerobic digestion in Sweden (64%), however, are digested in centralized co-digestion plants (Swedish Energy Agency, 2020). These types of plants produce 53% of all biogas in Sweden (Swedish Energy Agency, 2021). They usually have a higher capacity and more commonly upgrade the biogas to vehicle fuel compared to farm-based installments.

Further, any accepted feedstock that is classified as an animal by-product (Swedish Board of Agriculture, Sweden) must be hygienized prior to or after digestion—for example, animal manure. Hygienization is performed at 70°C for 1 h, and the material must have a particle size of less than 12 mm, although the latter requirement is usually impractical for certain types of animal manure. The Board of Agriculture has also cleared thermophilic digestion as a decontamination method if the material maintains a temperature of 52°C for a minimum of 10 hours during anaerobic digestion, at a minimum HRT of 7 days. These centralized co-digestion plants can run mainly on agricultural residues, as with several biogas plants in Denmark, or on a broader range of feedstocks by accepting food and slaughterhouse waste, for example.

Finally, it is also possible to integrate agricultural anaerobic digestion into existing industrial plants or biorefineries to process any available waste streams and co-produce biogas wit3h other fuels or chemicals. Because upgraded biogas has long been considered a low-value product due to the depressed prices of fossil gas, the co-production of bioethanol and biogas from animal bedding was examined in **Paper II**.

Bioethanol was generated from the fiber fraction of steam-exploded washed animal bedding at conversion yields of 60% to 66% of the theoretical maximum, and biomethane was produced from the manure-rich washing liquid and the hydrolysate at yields that ranged from 501-540 NmL $CH_4$ $g^{-1}$ VS. Biogas batch tests were performed at a C/N ratio of 30, given that it lies within the recommended interval of 20-30 (Hills, D.J., 1979), except for the washing liquid-hydrolysate mixtures that were co-digested at a C/N ratio of 20 (**Paper II**).

However, based on the mass flows from the washing and pretreatment of animal bedding, the resulting C/N ratios will range from 8-10 (Table 5). Thus, incorporation of an external carbon source into the process is a more realistic option for avoiding process failures due to elevated ammonia concentrations.

**Table 3** Process yields from streams directed toward anaerobic digestion—i.e., the wash liquid and hydrolysate—and the resulting C/N ratio in the feed to the digester. TS=total solids; DM=dry matter (in raw material, being animal bedding).

| Manure content (% of TS) | C/N ratio in feedstock | Washing liquid yield (kg TS/ton DM) | Hydrolysate yield (kg TS/ton DM) | Final C/N ratio |
|---|---|---|---|---|
| **15%** | 43 | 70 | 170 | 10 |
| **34%** | 28 | 200 | 110 | 9 |
| **42%** | 23 | 300 | 70 | 8 |

A balanced C/N ratio in the anaerobic digestion can be achieved by varying the process design of the agrowaste-based biorefinery as follows:

1) Adding biomass with a higher C/N ratio directly to the digester
2) Adding more fibers to the steam pretreatment to increase the hydrolysate flow

The process designs that were studied in **Paper IV** were created to increase the total biogas production by including three types of feedstocks in the process: wheat straw, animal bedding, and solid cow manure (Table 6). Notably, by increasing the diversity of feedstocks, more substrates will be available within a specific radius from the production plant. This study of process designs examined whether animal bedding should be washed and whether mechanical pretreatment or steam pretreatment should be applied to available fibers (wheat straw, washed animal bedding fibers), by estimating the energy demands and yields over the process steps based on measured mass balances. Further, the option of fractioning the steam pretreated fibers for the co-production of biogas and bioethanol was also included.

Dererie et al. (2011) reported that the overall energy yield from steam-pretreated oat straw was higher when ethanol production was introduced prior to the anaerobic digestion step, compared with the sole generation of biogas. However, first digesting straw and then producing ethanol from the pretreated digestate increases the likelihood of low ethanol yields due to sugar loss during anaerobic digestion, because biogas production converts hemicellulose and cellulose (Vancov et al., 2015). The incentive for introducing a high-value product, such as ethanol, would then be eliminated. Other studies on the co-production of multiple biofuels from the same feedstock have consistently reported higher energy yields compared with single-fuel production (Bauer et al., 2009; Kaparaju et al., 2009).

**Table 4** Main treatment steps in each process design investigated in **Paper IV**.

| Process design | Washing of animal bedding | Mechanical pretreatment | Steam pretreatment | Ethanol fermentation | Anaerobic digestion |
|---|---|---|---|---|---|
| **REF** | | | | | √ |
| **A** | | √ | | | √ |
| **B** | √ | √ | | | √ |
| **C** | | | √ | | √ |
| **D** | | | √ | √ | √ |
| **E** | √ | | √ | | √ |
| **F** | √ | | √ | √ | √ |

The criterion of maintaining a C/N ratio of 30 was included to consider the constraints of anaerobic co-digestion processes. Because the ratio between the animal bedding and cow manure that was received by the plant was held at 1:1, the C/N ratio criterion affected only the feeding rate of wheat straw. The animal bedding and cow manure in this study had C/N ratios of 28 (Table 6) and 23, respectively, which were within the acceptable range for maintaining a stable process (Chapter 2: C/N ratio). Without the addition of straw, the C/N ratio in the anaerobic digestion step would thus have been 23-25, except in Scenario F, in which the animal bedding was washed and the fibers were subjected to steam pretreatment, with subsequent fractionation and ethanol fermentation. In that scenario, the C/N ratio was 16, higher than if the process were to be run solely on animal bedding (Table 5).

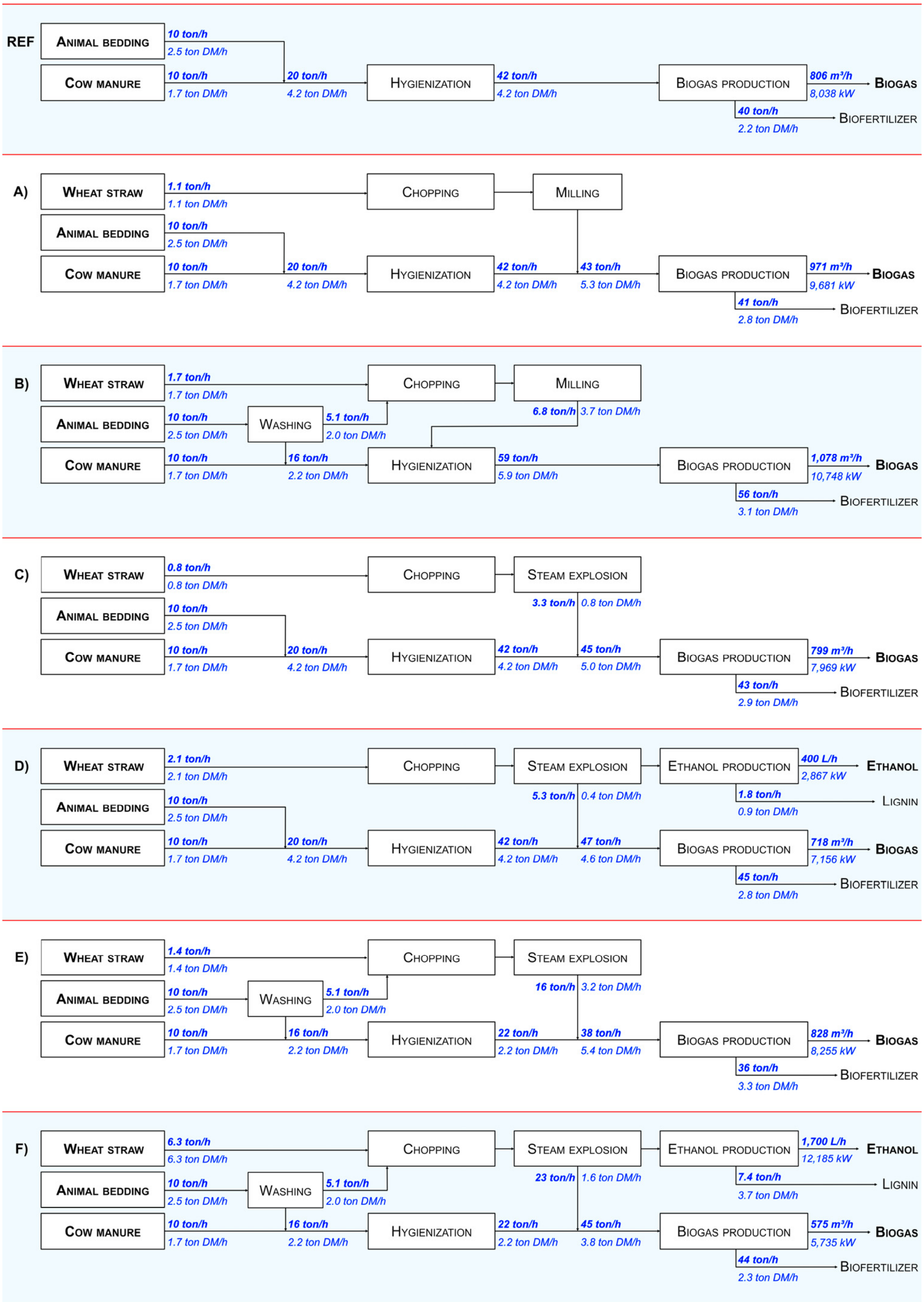


**Figure 9** Block flow charts of process designs, showing mass flows (ton/h) and biofuel energy outputs (kW). Water that flows in and out of the processes is not shown.

## Feedstock usage and biofuel production

Based on the mass balances over the various treatment steps that were obtained from **Papers II** and **IV** and the results of the BMP tests on the substrate mixtures in Scenario REF-F (**Paper IV**), biogas levels peaked when animal bedding was washed and the fibers were pretreated mechanically with wheat straw, hygienized, and co-digested with cow manure (Scenario B, Table 7). This process design yielded an annual biogas production rate of 93 GWh/year.

However, the analysis of the TC and TN mass balances over the wash step showed that the washed fiber and washing liquid that was generated had a lower C/N ratio than the initial animal bedding. This difference explains the higher rate at which wheat straw was fed to the plant, at 15 kton DM/year (Scenario B), compared with 9 kton DM/year when no washing was applied (Scenario A). The lowest amount of biogas was obtained when fractionation and ethanol fermentation were included in the process designs, leading to annual biogas production rates of 62 and 50 GWh/year, respectively. Notably, ethanol production provided an additional 25 and 105 GWh/year.

**Table 5** Annual feedstock flows into the biogas plants (kton DM/year) and annual biofuel production by the processes (GWh/year). Biogas production was calculated based on the experimental co-digestion (normal font) and mono-digestion (italic font in parentheses) methane yields and feeding rates of cow manure and animal bedding of 10 ton/h. $F_{tot}$=total feeding rate to the plant; $F_{Straw}$=straw feeding rate to the plant; AD=anaerobic digestion; SSF=simultaneous saccharification and fermentation.

| Scenario | $F_{Tot}$ (kton DM) | $F_{Straw}$ (kton DM) | $F_{AD}$ (kton DM) | Biogas (GWh) | $F_{SSF}$ (kton DM) | Ethanol (GWh) |
|---|---|---|---|---|---|---|
| **REF** | 37 | 0 | 37 | 70 *(65)* | 0 | 0 |
| **A** | 46 | 9 | 46 | 84 *(86)* | 0 | 0 |
| **B** | 51 | 15 | 51 | 93 *(97)* | 0 | 0 |
| **C** | 44 | 7 | 43 | 69 *(78)* | 0 | 0 |
| **D** | 54 | 17 | 41 | 62 *(72)* | 13 | 25 |
| **E** | 48 | 11 | 47 | 72 *(84)* | 0 | 0 |
| **F** | 89 | 53 | 33 | 50 *(58)* | 56 | 105 |

The total amount of biofuel that was produced depended on the total amount of feedstock that was treated in the plants. With regard to effecting the highest conversion rate of feedstock dry matter to biofuel, the process in which only animal bedding and cow manure were co-digested without any pretreatment steps other than mixing and hygienization (Scenario REF) was most efficient at 1.9 GWh/kton DM feedstock. The least efficient process design was Scenario E, at 1.5 GWh/kton

DM feedstock, in which steam explosion—but not fractionation—of wheat straw and animal bedding fibers was applied. These results are attributed to the lower methane potential (216 $Nm^3$ $CH_4$ $ton^{-1}$ VS) compared with Scenario A-C (231–263 $Nm^3$ $CH_4$ $ton^{-1}$ VS), in which all pretreated material was also directed to the anaerobic digestion step.

With regard to the material yields of the processes (Fig. 14a), most of the feedstock (approximately 40% to 60% of the DM) ultimately wound up in the digestate that exited the anaerobic digestion, indicating that the largest product of biogas production was biofertilizer. Although they are not energy carriers, biofertilizers are important products for decreasing the use of mineral fertilizers in agriculture, thus reducing GHG emissions. To validate the suitability of the different biofertilizers produced, further studies would have to be performed.

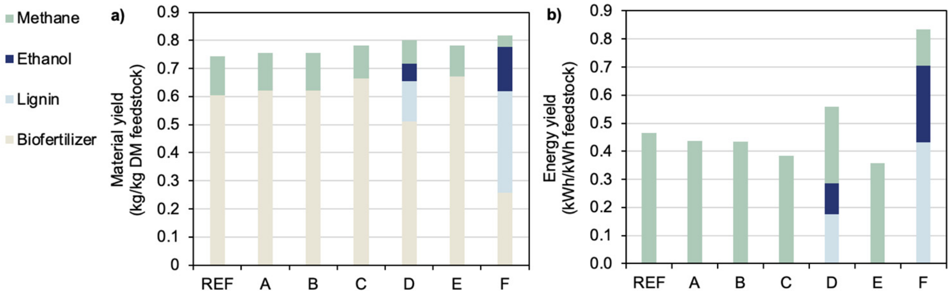


**Figure 10** Material yield (a) and energy yield (b), expressed as biofuel/energy carrier produced per treated amount of feedstock. The energy contents in the feedstocks were based on lower heating values.

One of the main products of Scenarios D and F was residual lignin cake that exited the ethanol production line. On being dried, the lignin was available as an energy carrier that can be sold or used internally for steam and electricity production, explaining the high energy yields of those process designs (Figure 14b).

## Energy demands and energy efficiencies

The heat and electricity requirements for biogas production in Scenarios REF-C and E were estimated as 0.14-0.17 and 0.08-0.1 kWh/kWh biogas produced (**Paper IV**), similar to the process energy demand that was estimated by Tufvesson et al. (2013)—0.14 kWh heat and 0.04 kWh electricity per kWh biogas produced. Angelidaki and Ellegaard (2003) reported that thermophilic co-digestion plants with an annual capacity of 100,000 tons substrate and no pretreatment steps or hygienization step can yield total energy efficiencies of 0.10-0.15 $kW_{IN}/kW_{OUT}$. Ahlberg-Eliasson (2015) recorded energy efficiencies of 0.15–0.84 $kW_{IN}/kW_{OUT}$ and an average of 0.44 $kW_{IN}/kW_{OUT}$ for 30 large-scale farm-based biogas plants in

Sweden. These plants had similar process configurations as in Scenarios A and B but with heat and electricity production instead of biogas upgrading.

The end use of the energy carriers that were produced was dependent on the energy demand of the processes. Assuming an efficiency of 95% over the gas turbine and assuming that 35% of the available energy can be recovered as electricity, with the remainder recovered as district heat (CHP), the total amount of biogas that is needed for internal use is an estimated 24% to 31% of the biogas that is produced (Table 8) for Scenarios REF-C and E—sufficient to meet the total electricity and heat demands.

Although fractionation of steam-exploded wheat straw increased the overall annual heat demand (32 and 94 GWh/year for Scenarios D and F, respectively), it also led to high amounts of available energy in the lignin fractions (40 and 166 GWh/year). To cover the electricity demand of these two scenarios, 80% of the available energy in the lignin cake was assumed to have been recovered in a boiler that produced high-pressure steam that was then used to generate electricity (20%) and heat (80%). In Scenarios D and F, 100% of the available lignin would be needed to meet the total electricity and heat demands. When only wheat straw is subjected to steam pretreatment and ethanol fermentation (Scenario D), the available lignin does not produce any surplus. Due to additional energy losses that are not accounted for in these estimations, extra energy will likely need to be inputted.

However, when the animal bedding is washed and included in the steam pretreatment and ethanol production line, it increases the need for wheat straw, to balance the C/N ratio in the anaerobic digester. Even after utilizing all the lignin to cover the total process energy demand, approximately 19 GWh electricity/year and 12 GWh heat/year will be available.

**Table 6** Heat and electricity demand for the process designs. Processes REF-C and E are fueled by internal use of the produced biogas in a CHP unit, and processes D and F are fueled by the available lignin in a steam turbine.

| Scenario | Heat demand (GWh/year) | Electricity demand (GWh/year) | % of produced biogas or lignin | Residual heat (GWh/year) |
|---|---|---|---|---|
| **REF** | 12 | 5.9 | 25 | 6.8 |
| **A** | 11 | 6.6 | 24 | 6.9 |
| **B** | 16 | 9.7 | 31 | 9.5 |
| **C** | 13 | 6.1 | 26 | 8.1 |
| **D** | 32 | 6.0 | 100 | 11 (+40) |
| **E** | 13 | 7.3 | 31 | 9.0 |
| **F** | 94 | 7.3 | 80 | 22 (+166) |

If the use of the residual heat that is available in the process is optimized (Table 8), the heat demand could likely be decreased. Most of the residual heat (80% to 100%) was derived from process streams with a maximum temperature of 37–80°C (Figure). One of the most energy-demanding processes was hygienization of the feedstock prior to anaerobic digestion, corresponding to 29 kWh/ton hygienized feed. Here, the hygienized feedstock was cooled to 37°C before being fed to the anaerobic digestion step and designed to preheat the feedstock that entered the hygienization step. Without the preheating step, the energy demand would have doubled. Other studies on large-scale biogas plants have reported similar values for hygienization, heating to 70°C for 1 h (26 kWh/ton in Lantz et al., 2009) or running a thermophilic process (15-25 kWh/ton in Angelidaki and Ellegaard, 2003).

The largest residual heat source was obtained by cooling the digestate from the main digester, which was operated at 37°C, to the storage unit, the incoming temperature of which was set to 20°C. If the digestate were to be used as a preheating step for the feedstock that was fed to hygienization or, alternatively, for the water that was used to dilute the feedstock prior to hygienization, the heating demand for the hygienization could be decreased by 40% for Scenario REF-D and by 80% and 100% for Scenarios E and F, respectively.

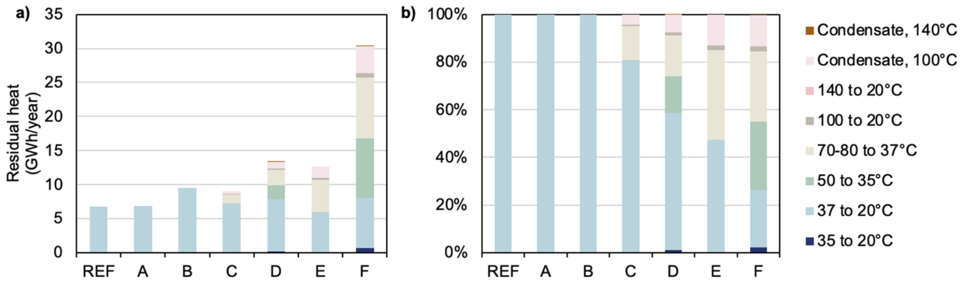


**Figure 11** Residual heat from various process streams, expressed as GWh/year (a) and share of total residual heat (b). The condensation and subsequent potential cooling of water vapor was split into two separate residual heat potentials. Cooling of streams carrying process heat was assumed to be possible to a minimum temperature of 20°C.

# The impact of co-digestion on methane yield

In **Paper II**, with regard to the impact of C/N ratio, mixing the washing liquid—a process stream that was derived from washing animal bedding—with wheat straw or a mixture of glucose and xylose did not alter the methane yield significantly (Figure 15). For the co-digestion of wheat straw and manure, this effect was expected, because previous tests have shown that the methane yields from the monodigestion of these substrates are similar. It is, however, not possible to determine whether the co-digestion had any synergistic or antagonistic effects on the methane yield. Co-digestion of soluble sugars with the manure stream increased methane production, compared to the wheat straw trials. This is not surprising due to the presence of lignin in the volatile solids of wheat straw. A slight increase in methane yield along with an increased C/N ratio (and thereof carbohydrate content) could be observed, although additional experiments would have to be performed to confirm the significance of the trend.

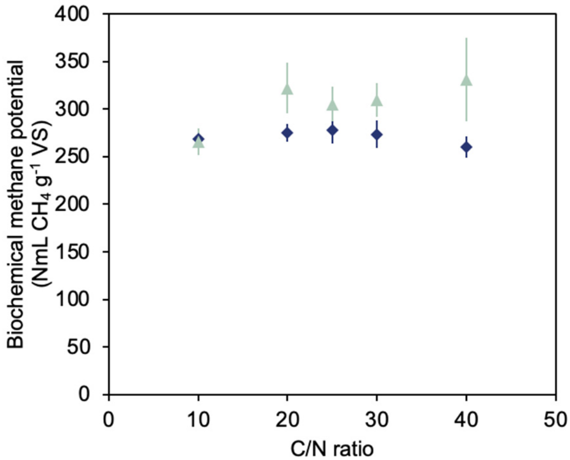


**Figure 15** The biochemical methane potential as a function of C/N ratio during co-digestion of wheat straw and washing liquid (dark blue diamonds) and co-digestion of a mixture of glucose and xylose and washing liquid (light green triangles).

The effects on methane yield during co-digestion between process streams from biogas plant designs that accepted wheat straw, animal bedding, and cow manure as feedstock (**Paper IV**) also differed at a C/N ratio of 30. No such substrate interactions were found, however, in similar co-digestion experiments on steam-pretreated animal bedding fibers and washing liquid, perhaps due to differences in the inoculum or initial substrate concentration (Fernández et al., 2001).

The co-digestion of filtered cow manure (FM) and the cellulosic substrates filter paper, cellulose, and glucose (Figure 16) in the biogas trials in **Paper III** yielded similar results. The effects clearly differed, regardless of the degree of carbohydrate accessibility. Based on these results, it was concluded that greater bioaccessibility (glucose) lowers the methane yield during monodigestion, compared with lower accessibility (filter paper and cellulose).

The decreased yields could be explained by greater biomass growth or impeded degradation. An analysis of final ammonium concentrations (**Paper III**) demonstrated lower levels in mixtures that contained manure compared with those without it, perhaps reflecting less protein degradation during co-digestion.

Alternatively, it could indicate nitrogen deficiency due to greater biomass growth (Sundh et al.). Wang et al. (2009) reported decreased methane yields from wet exploded versus untreated wheat straw, in which higher sugar release from the pretreatment coincided with lower methane yields.

Hills (1979) studied the mesophilic co-digestion of cow manure with cellulose and glucose at varying C/N ratios and organic loading rates in continuous lab-scale reactors. Their found that increasing the C/N ratio from 8 to 18 improved daily gas production and that this effect was more pronounced at higher organic loading rates and C/N ratios. At high loads, glucose increased gas production more than cellulose at high C/N ratios. However, at lower loads and C/N ratios, there was no difference with co-digestion between glucose and cellulose.

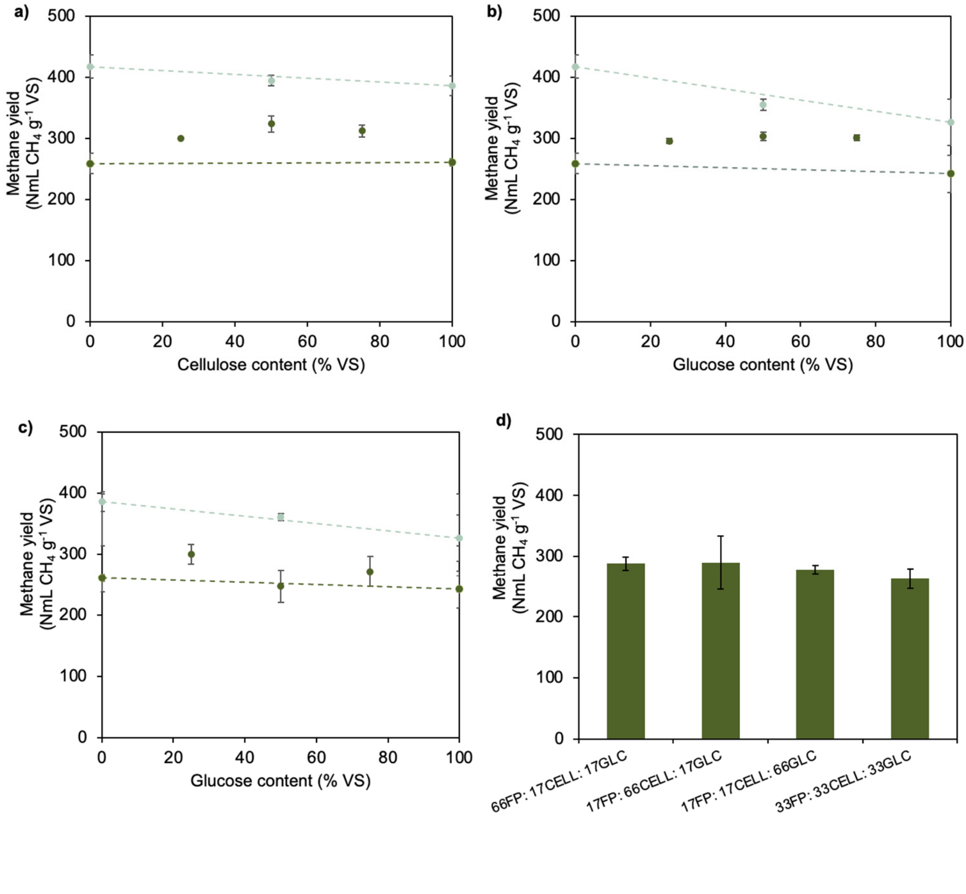


**Figure 12** The methane yield of a) filter paper and cellulose mixtures, b) filter paper and glucose mixtures, c) cellulose and glucose mixtures and d) ternary mixtures of filter paper, cellulose, and glucose. Monodigestion (light green) without and co-digestion with filtered cow manure (dark green), were performed. The dashed lines signifies when the mixing of carbohydrates does not influence the methane yield.

# The impact of co-digestion on kinetics

All co-digestion trials on hydrolysate and manure with wheat straw led to synergistic effects in reducing lag times (**Papers II and IV**), indicating that accessibility to the carbohydrate-rich compounds was an important determinant. In studies on the interactions between model compounds—filter paper, cellulose, and glucose—with varying degrees of accessibility during co-digestion with manure (**Paper III**), synergistic and antagonistic effects on the kinetics of methane production were observed. Mixing filter paper and glucose during co-digestion with filtered manure led to sustained methane production, as opposed to co-digestion of only glucose and filtered manure where severe inhibition was observed. However, using cellulose in mixture with glucose was not as effective in mitigating methane inhibition. Lower levels of accumulated VFAs and shortened adaptation phases were seen during co-digestion of filter paper and glucose with filtered manure, which increased methane productivity (**Figure**). The methane productivity was calculated as a quotient between 80% of the final methane yield and the digestion time to reach that yield, i.e., the technical digestion time, T80 (Figure 17). Conversely, cellulose worsened the acidification during co-digestion of glucose and manure. Cellulose was degraded marginally faster than filter paper but sufficiently for VFA formation to accelerate and reach inhibitory concentrations (>3.7 g TVFA L-1).

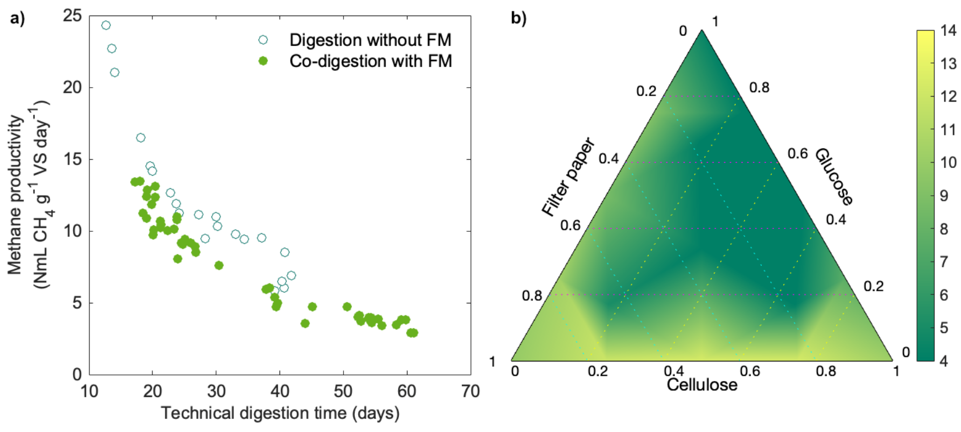


**Figure 13** The a) methane productivity (B80/T80) plotted against the technical digestion time (T80) for all carbohydrate mixtures with (full circles) and without (empty circles) manure. b) The effect of carbohydrate mixture ratios in co-digestion with manure on the methane productivity. FM=filtered manure.

The maximum TVFA concentration increased with the fraction of glucose in the mixture (Figure 18, **Paper III**), as observed in the co-digestion trials with hydrolysate (**Paper II**). The design of anaerobic digestion processes on a large scale will thus require adaptation of the starting inoculum to the readily available feedstock. Notably, although the presence of manure increased TVFA concentrations, it also reduced the adaptation time, perhaps due to the added buffering capacity. In the batch tests that were performed here, the methanogenesis restarted at lower hydrogen partial pressures than at the outset of the inhibition phase.

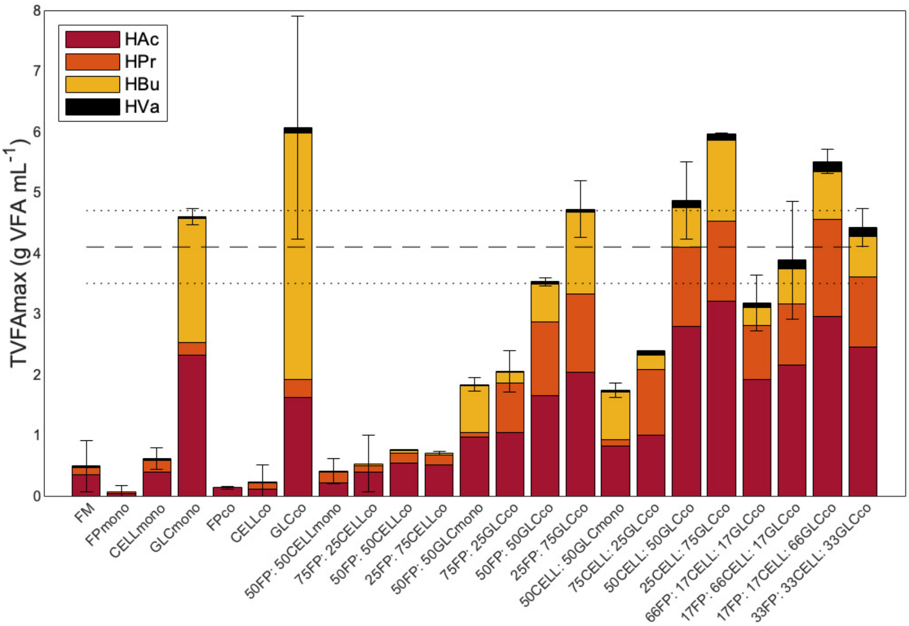


**Figure 14** Maximum total volatile fatty acid concentrations (TVFAmax) during co-digestion of filter paper (FP), cellulose (CELL), and glucose (GLC) with filtered cow manure (FM) at various mixture ratios. The suffices -co and -mono denote sample mixtures that were digested with and without manure, respectively.

To maintain robust anaerobic co-digestion, it is advisable to balance the degree of carbohydrate accessibility such that the rate of methane production does not depend entirely on the rate of hydrolysis or the rate of acetogenic/methanogenic biomass growth. Admittedly, the results from these batch tests were not validated against continuous experiments or full-scale trials.

# Realization of biogas solutions in Scania, Sweden

Implementation of technical solutions is key to mitigating the impact of human activity on climate. Such adoption per se, however, is often limited by factors that are beyond the scope of application-focused research. An uncertain biogas market, hesitant biogas actors, and the unpredictability of policy-makers and decision-makers are major contributors to the slow growth of biogas production in Sweden.

The southern region of Sweden—Skåne (Scania, in English)—holds a strong agricultural sector. The implementation of agricultural co-digestion plants that can upgrade raw biogas to fuel gas thus has high relevance for this region. To assess the impact of such biogas plants on the biofuel supply in Skåne, the total available wheat straw, animal bedding, and solid cow manure was estimated following the method by Björnsson et al., 2011, amounting to 381, 44, and 46 kton dry matter per year, respectively (Figure 19).

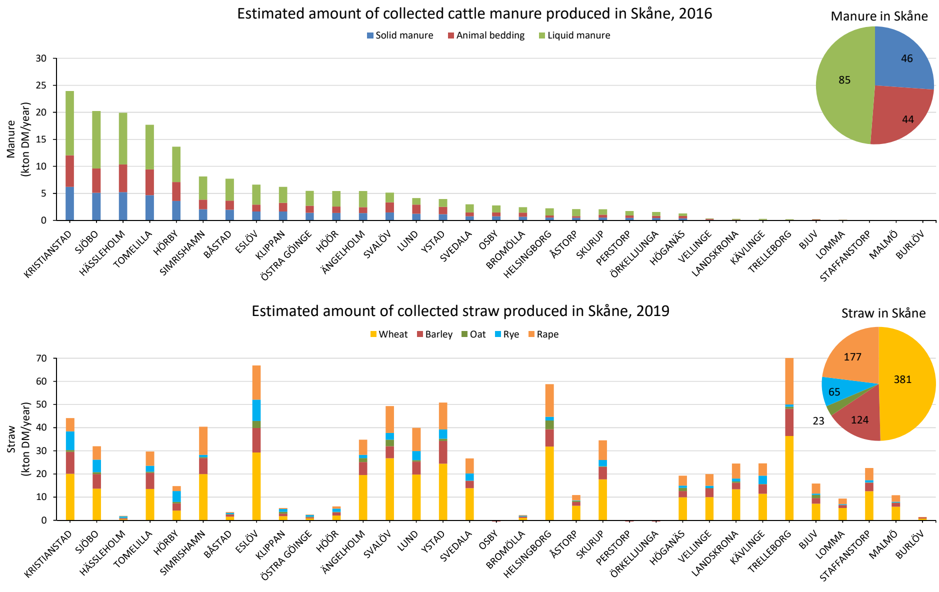


**Figure 15** Estimated amount of cattle manure and straw collected in Skåne in 2016 and 2019, respectively, per Björnsson et al. (2011) and applied to data from the Swedish Board of Agriculture.

Based on the regional production of animal bedding and solid manure from cattle, it would be possible to install two large biogas plants, on the scale of that in Paper IV, in Scania, Sweden, with a total biogas production of 100-180 GWh/year. However, only 2% to 30% of the available wheat straw would be needed to attain anaerobic digestion at a balanced C/N ratio. Because the availability of liquid manure is even higher than that of solid manure, it is likely that such a substrate at a reasonable transportation distance would also merit interest. Greater manure usage thus increases the need for straw and unlocks its biogas potential.

Naturally, there are other factors to consider to realize these biogas solutions. The investment will be higher for processes with larger equipment, and the operational costs will increase with the number of treatments steps. With higher prices for natural gas, however, the attraction of investing in biogas solutions is already increasing (Klimatklivet, 2023).

# Conclusions

The main conclusions of this body of work are as follows:

I. Mechanical pretreatment, particularly extrusion, mitigates the handling issues of straw as a feedstock, thus rendering it bioavailable for biogas production. Increased bioaccessibility also accelerates methane formation and improves kinetics.

II. Homogenization of animal bedding via washing enables the co-production of biogas and bioethanol with a consistent process design and thus the valorization of low-value feedstock.

III. Co-digestion of carbohydrates and manure has antagonistic effects on methane yield. Highly accessible carbohydrates accelerate the accumulation of VFAs, which can be prevented by adding a less accessible carbohydrate compound at maintained organic loads.

IV. Steam explosion of straw and subsequent co-digestion with animal bedding and cow manure maximizes annual biogas production per amount of feedstock and is the most energy-efficient process. Co-production of bioethanol and biogas leads to greater fluctuations in energy efficiency when the amount of wheat straw is varied, which can be adjusted as needed to keep the C/N ratio in the digester constant.

The underlying purpose of this research has been to contribute findings that can be used toward reducing GHG emissions and thus global temperatures.

# Future work

The work presented in this thesis only covers a fraction of possible research questions related to topic of developing biogas solutions from recalcitrant and unavailable lignocellulosic biomass.

The most energetically favorable process design investigated in this work was the washing of animal bedding with subsequent steam explosion of the fibers as well as of added wheat straw whereupon biogas was produced from raw cow manure, steam pretreatment hydrolysate and washing liquid. Ethanol was produced from the steam pretreated fibers. However, further washing experiments should be performed to optimize the water usage. Additional studies on the pretreatment of fibers containing manure should be done to determine the maximum allowable manure content. To be able to fully valorize the different product streams, the digestate should also be further characterized in order to determine its suitability as biofertilizer.

As discussed, the biogas trials performed during this work were all batch trials. Since the purpose of co-digestion is to achieve stability and a maintained biogas production, continuous biogas trials should be used to further study the behavior of the produced feedstocks. For example, the hypothesis that the optimal C/N ratio is dependent on carbohydrate and protein accessibility cannot be confirmed by batch tests. Most studies were conducted at a balanced C/N ratio. However, the total nitrogen load should also be considered since it may vary. In Paper III, batch trials were performed at a lower I/S ratio than normal which led to a methane production that was limited both by the substrate and by the inoculum. It would therefore have been advisable to repeat some of the samples of multiple different inocula and perform characterization of the inocula to determine why the different outcomes were observed. Further, validation towards a full-scale process is desirable.

Finally, to be able to fully assess the research outcomes of this work, they should be validated from other perspectives. For example, since biogas solutions will likely become more and more integrated into industrial plants, a system analysis that takes larger mass flows into consideration would be interesting. Exactly where in Sweden would the processes studied in this work be best applied, and in symbiosis with what other industries?

# Outlook

As we transition from an oil-dependent industry toward a biobased one, the development of biorefineries will evolve as we seek to replace existing chemicals with biochemicals. To this end, the recycling of biomass becomes an important component in realizing the vision of a circular economy. One can thus anticipate that future anaerobic digestion systems will be an essential element of such refineries for treating an ever-expanding range of different substrates.

Today, lignocellulosic biomass constitutes 90% of the potential resource for Swedish biogas production. However, biogas cannot be the sole answer for the global need for renewable energy. Such a shift must rely on a wide array of technical solutions. In doing so, we can improve the robustness of energy systems and secure our energy independence, strengthening our resistance to external events. Building a world in which the environment is regarded as a pillar, not a limitation, for welfare is the ultimate goal.

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