From Waste to Wealth: Transforming Biorefineries into Platforms to Valorize Low-Quality Residues

Sanchis Sebastia, Miguel

2021

Document Version:
Publisher's PDF, also known as Version of record

Link to publication

Citation for published version (APA):
Sanchis Sebastia, M. (2021). *From Waste to Wealth: Transforming Biorefineries into Platforms to Valorize Low-Quality Residues*. [Doctoral Thesis (compilation), Division of Chemical Engineering]. Chemical Engineering, Lund University.

Total number of authors:
1

From Waste to Wealth:
Transforming Biorefineries into Platforms to Valorize Low-Quality Residues

Miguel Sanchis Sebastiá

DOCTORAL DISSERTATION

by due permission of the Faculty of Engineering, Lund University, Sweden.
To be defended at the Centre for Chemistry and Chemical Engineering,
Naturvetarvägen 14, Lund, on the 4th of June at 9:00.

Faculty opponent
Antonio David Moreno García, Centro de Investigaciones Energéticas,
Mediomabientales y Tecnológicas (CIEMAT)

Organization
LUND UNIVERSITY
Department of Chemical Engineering

Document name
DOCTORAL DISSERTATION

Date of issue 2021-06-04

Author(s)
Miguel Sanchis Sebastiá

Sponsoring organization
Energimyndigheten

Title and subtitle
From Waste to Wealth: Transforming Biorefineries into Platforms to Valorize Low-Quality Residues

Abstract

The transition to a bioeconomy, in which resources from biowaste could be reclaimed for the production of chemicals, materials or fuels, could contribute to the establishment of a sustainable society by replacing fossil fuels as raw materials. The core of this bioeconomy would be biorefineries, which are similar to petroleum-based refineries, but are facilities where biowaste is processed and refined through different chemical and/or biochemical processes. There are already some commercial-scale biorefineries that utilize edible biomass, such as corn or sugarcane, to produce mainly fuels. However, edible biomass can hardly be considered a biowaste, and there is an ethical conflict between the use of such resources for food and the production of energy. An alternative strategy has been developed to utilize non-edible materials such as forest and agricultural residues in biorefineries, but the relatively high price of these residues has prevented biorefineries from being economically viable.

This thesis presents a framework for the redesign of current biorefineries so that they can use low-value waste as feedstock. This shift in feedstock would improve not only the economics of biorefineries, as feedstock cost accounts for almost one third of the total production cost, but also their environmental performance, as it would be possible to valorize residues that are otherwise incinerated or landfilled. The framework was developed based on research on animal bedding, i.e., a mixture of manure and straw, and cotton-based waste textiles.

The first step involves the adaptation of analytical methods so that they can be applied to these high-complexity and variable low-value residues. Since low-value waste is usually unsorted, fractionation will be required to separate the different materials so that each fraction can be analyzed separately with conventional methods. An example of such fractionation is presented for the case of animal bedding, where manure and straw were separated by washing with water. Secondly, conversion technologies must be adapted to the special characteristics of low-value residues to avoid compromising the efficiency of the operation. This was demonstrated by the redesign of steam treatment for the pretreatment of animal bedding, and concentrated-acid hydrolysis for the depolymerization of waste textiles. In both cases, the overall efficiency of the biorefinery would be around 70%, which is similar to that achieved with higher quality residues. Finally, process concepts must be redesigned to transform complex low-value residues into opportunities to further improve biorefineries. This was demonstrated by the exploitation of synergies between simultaneous biogas and bioethanol production from animal bedding, and the possibility of establishing industrial symbiosis between the depolymerization of waste textiles and pulping for paper production.

The superior economic performance of a low-value waste biorefinery, after appropriate adaptation, was illustrated through a biorefinery based on animal bedding. Such a biorefinery could deliver bioethanol to the market 40% cheaper than a biorefinery based on wheat straw; the higher quality counterpart of animal bedding. This example showed that the conditioning cost associated with low-value residues could potentially be compensated for by savings in the cost of feedstock, leading to an economic advantage in the biorefinery. Thus, low-value waste biorefineries could provide early market opportunities for the transition to a bioeconomy.

Key words
Biorefinery, Animal Bedding, Waste Textiles, Biomass, Biotechnology, Process Design

Classification system and/or index terms (if any)

Supplementary bibliographical information

Language
English

ISBN (print)
978-91-7422-804-5

ISBN (pdf)
978-91-7422-805-2

Recipient’s notes

Number of pages 142

Price

Security classification

Signature Miguel Sanchis

Date 2021-04-20

From Waste to Wealth:
Transforming Biorefineries into Platforms to Valorize Low-Quality Residues

Miguel Sanchis Sebastiá

LUND
UNIVERSITY

Cover photo by Raquel Marquino Fernández

Faculty of Engineering
Department of Chemical Engineering

ISBN (print) 978-91-7422-804-5
ISBN (pdf) 978-91-7422-805-2

Printed in Sweden by Media-Tryck, Lund University, Lund 2021

Printed matter
3041 0903

Media-Tryck is a Nordic Swan Ecolabel
certified provider of printed material.
Read more about our environmental
work at www.mediatryck.lu.se

MADE IN SWEDEN

There are those that look at things
the way they are, and ask why?
I dream of things that never were,
and ask why not?

George Bernard Shaw

Abstract

The transition to a bioeconomy, in which resources from biowaste could be reclaimed for the production of chemicals, materials or fuels, could contribute to the establishment of a sustainable society by replacing fossil fuels as raw materials. The core of this bioeconomy would be biorefineries, which are similar to petroleum-based refineries, but are facilities where biowaste is processed and refined through different chemical and/or biochemical processes. There are already some commercial-scale biorefineries that utilize edible biomass, such as corn or sugarcane, to produce mainly fuels. However, edible biomass can hardly be considered a biowaste, and there is an ethical conflict between the use of such resources for food and the production of energy. An alternative strategy has been developed to utilize non-edible materials such as forest and agricultural residues in biorefineries, but the relatively high price of these residues has prevented biorefineries from being economically viable.

This thesis presents a framework for the redesign of current biorefineries so that they can use low-value waste as feedstock. This shift in feedstock would improve not only the economics of biorefineries, as feedstock cost accounts for almost one third of the total production cost, but also their environmental performance, as it would be possible to valorize residues that are otherwise incinerated or landfilled. The framework was developed based on research on animal bedding, i.e. a mixture of manure and straw, and cotton-based waste textiles.

The first step involves the adaptation of analytical methods so that they can be applied to these high-complexity and variable low-value residues. Since low-value waste is usually unsorted, fractionation will be required to separate the different materials so that each fraction can be analyzed separately with conventional methods. An example of such fractionation is presented for the case of animal bedding, where manure and straw were separated by washing with water. Secondly, conversion technologies must be adapted to the special characteristics of low-value residues to avoid compromising the efficiency of the operation. This was demonstrated by the redesign of steam treatment for the pretreatment of animal bedding, and concentrated-acid hydrolysis for the depolymerization of waste textiles. In both cases, the overall efficiency of the biorefinery would be around 70%, which is similar to that achieved with higher quality residues. Finally, process concepts must be redesigned to transform complex low-value residues into opportunities to further improve biorefineries. This was demonstrated by the exploitation of synergies between simultaneous biogas and bioethanol production from animal bedding, and the possibility of establishing industrial symbiosis between the depolymerization of waste textiles and pulping for paper production.

The superior economic performance of a low-value waste biorefinery, after appropriate adaptation, was illustrated through a biorefinery based on animal bedding. Such a biorefinery could deliver bioethanol to the market 40% cheaper than a biorefinery based on wheat straw; the higher quality counterpart of animal bedding. This example showed that the conditioning cost associated with low-value residues could potentially be compensated for by savings in the cost of feedstock, leading to an economic advantage in the biorefinery. Thus, low-value waste biorefineries could provide early market opportunities for the transition to a bioeconomy.

Popular Scientific Summary

Bringing low-value waste back into circulation: Redesigning waste management to prevent the incineration and landfilling of animal bedding and waste textiles

Human consumption generates a huge amount of waste worldwide. Until now, this low-value waste has mostly been discarded, leading to negative effects on the environment. The research in this thesis exploits the potential in this dead-end economy and brings now the waste back into circulation.

Time for action

The consequences of climate change are no longer a warning, they are a reality. Coral reefs are dying and the ice in the Arctic Sea is melting. Recently, my hometown in Spain experienced serious floods, which seem to become worse every year. At the same time, Madrid was paralyzed by the heaviest snowfall in decades.

It is still possible for us to stop climate change if we take action now. One means of attaining sustainability could be to use plants and other organic materials instead of fossil resources (coal, natural gas and oil) to produce products such as plastics, fuels and clothes. In fact, there are already several industrial plants around the world where bioethanol, a fuel that can replace gasoline, is produced from cereals such as maize. However, utilizing edible resources for the production of chemicals poses an ethical dilemma, as these resources could have been used to feed people instead.

Why have earlier attempts failed?

New ways of utilizing biological materials were being developed before concerns were raised about the use of edible feedstocks. Researchers focused mainly on processes based on forest and agricultural residues, for example, tree branches and straw. However, the utilization of these materials has also been questioned, and there are heated debates on the sustainability of these strategies. Nevertheless, processes based on these materials are not economically feasible, due to the relatively high price of these residues.

Bringing waste back to life

This thesis addresses this issue by sourcing organic material from other waste streams that have a lower value. The lowest possible cost is associated with residues that are currently incinerated or landfilled as they have no application, and there is therefore no competing interest for these materials. Utilizing these materials could not only bring economic benefits, due to their lower price, but also environmental benefits, as waste would be diverted from landfills and enter the economy as renewable products or energy. Two examples of such residues were investigated in this research: animal bedding, a mixture of straw and manure from farms, and discarded clothing.

The problem with low-value residues is that their lower value is associated with lower quality. For instance, residues that are landfilled are typically unsorted, and they therefore contain a mixture of different materials, which complicates their transformation into new products. Existing industrial plants must therefore be adapted in several ways before they can handle this kind of residue efficiently. A procedure was established for this transformation, which consisted of adapting the analytical procedures used to characterize low-value waste, redesigning technologies to transform the waste into new products, and developing new process concepts that define how different operations are connected with each other. The successful implementation of these processes led to a high yield of products, despite the lower quality of the feedstock, and thus improved the economic performance. For example, it was estimated that an industrial plant based on low-value residues (animal bedding) could deliver its products to the market 40% cheaper than conventional industrial plants based on agricultural waste.

Low-value waste – high-value potential

The concepts presented in this thesis could improve the economic performance of industrial plants using residual organic material as raw material to produce fuels and chemicals, providing the opportunity to reduce environmental impact. This was achieved by developing a methodology that allows low-value waste to be valorized with the same efficiency as other residues of higher quality. Moreover, this strategy would divert these residues from incineration or landfilling, leading to a more sustainable society with lower waste generation.

Populärvetenskaplig Sammanfattning

Återanvändning av lågvärdigt avfall: Förändring av avfallshantering för att minska förbränning och deponering av djupströ och textilier

Mänsklig konsumtion skapar en enorm mängd avfall över hela världen. Hittills har detta lågvärdiga avfall oftast kasserats, vilket har lett till negativa effekter på miljön. Forskningen i denna avhandling syftar till att utnyttja potentialen i denna återvändsgrändekonomi för att återföra avfallet till förnyad användning.

Dags att agera

Konsekvenserna av klimatförändringarna är inte längre en varning, de är en verklighet. Korallreven dör och isen i Arktiska havet smälter. Nyligen upplevde min hemstad i Spanien allvarliga översvämningar, som verkar bli värre varje år. Samtidigt förlamades Madrid av det kraftigaste snöfallet på decennier.

Det är fortfarande möjligt för oss att stoppa klimatförändringen om vi vidtar åtgärder nu. Ett sätt att uppnå hållbarhet kan vara att använda växter och andra organiska material istället för fossila resurser (kol, naturgas och olja) för att producera produkter som plast, bränsle och kläder. Det finns faktiskt flera industrianläggningar runt om i världen där bioetanol, ett bränsle som kan ersätta bensin, produceras av spannmål, till exempel majs. Att använda ätbara resurser för produktion av kemikalier utgör emellertid ett etiskt dilemma, eftersom dessa resurser kunde ha använts för att föda människor istället.

Varför har tidigare försök misslyckats?

Nya sätt att använda biologiska material utvecklades innan problemen med användningen av ätbara råvaror väcktes. Forskare fokuserade främst på processer baserade på skogs- och jordbruksrester, till exempel trädgrenar, halm och andra växtrester. Användningen av dessa material har emellertid också ifrågasatts och hållbarheten i dessa strategier har lett till heta diskussioner. I vilket fall, för närvarande är processer baserade på dessa material inte ekonomiskt genomförbara på grund av det relativt höga priset på dessa rester som utgör råvaran i processerna.

Att ge ett nytt liv till avfall

Denna avhandling behandlar denna problematik genom att utnyttja organiskt material från andra avfallsströmmar som har lägre värde. Lägsta möjliga råvarukostnad är förknippad med rester som för närvarande förbränns eller deponeras eftersom de inte har någon annan tillämpning och det finns därför inget konkurrerande intresse för dessa material. Att använda dessa restmaterial kan inte bara medföra ekonomiska fördelar på grund av det lägre priset utan också miljöfördelar, eftersom avfall kommer att ledas bort från deponier och komma in i ekonomin som förnybara produkter eller energi. Två exempel på sådana rester undersöktes i denna forskning: djupströ, en blandning av halm och gödsel från gårdar och kasserade kläder.

Problemet med lågvärdiga avfall är att deras lägre värde är förknippat med lägre kvalitet. Till exempel är restprodukter som deponeras vanligtvis osorterade och de innehåller därför en blandning av olika material, vilket försvårar deras omvandling till nya produkter. Befintliga industrianläggningar måste därför anpassas på flera sätt innan de kan hantera denna typ av rester effektivt. En metod utvecklades för denna anpassning, som bestod av att förändra de analytiska procedurerna som används för att karakterisera lågvärdigt avfall, omforma tekniker för att omvandla avfallet till nya produkter och utveckla nya processkoncept som definierar hur olika operationer är kopplade till varandra. Genomförandet av dessa framgångsrika anpassningar ledde till ett högt utbyte av produkter, trots råvarans lägre kvalitet, och förbättrade därmed processekonomin. Till exempel uppskattades att en industrianläggning baserad på lågvärdiga restprodukter (djupströ) skulle kunna leverera sina produkter till marknaden 40% billigare än konventionella industrianläggningar baserade på jordbruksavfall.

Lågvärdigt avfall – högvärdig potential

De resultat som presenteras i denna avhandling skulle kunna förbättra processekonomin i industrianläggningar som använder restorganiskt material som råvara för att producera bränslen och kemikalier, vilket ger möjlighet att minska miljöpåverkan. Detta uppnåddes genom att utveckla en metod som gör det möjligt att värdera avfall med lågt värde med samma effektivitet som andra rester av högre kvalitet. Dessutom skulle denna strategi avleda dessa rester från förbränning eller deponering, vilket skulle leda till ett mer hållbart samhälle med lägre avfallsproduktion.

Resumen de Divulgación Científica

Devolver a la economía los residuos de bajo valor: Rediseño de la gestión de residuos para evitar la incineración y depósito en vertedero de lechos para animales y residuos textiles

El consumo humano genera una enorme cantidad de residuos alrededor del mundo. Hasta ahora, estos residuos de bajo valor han sido desechados, generando efectos negativos en el medioambiente. La investigación presentada en esta tesis aprovecha el potencial de mejora de esta economía de usar/tirar para traer de vuelta al sistema productivo estos residuos.

Hora de actuar

Las consecuencias del cambio ya no son una advertencia, sino una realidad. Los arrecifes de coral están muriendo y el hielo del Mar Ártico se está derritiendo cada vez más rápido. Mi ciudad natal fue afectada recientemente por inundaciones, las cuales son peores cada año. Al mismo tiempo, Madrid quedó paralizada por la peor nevada en varias décadas.

Todavía tenemos la posibilidad de detener el cambio climático si actuamos ahora. Una alternativa para alcanzar una sociedad sostenible sería la utilización de plantas y otros materiales orgánicos, en lugar de recursos fósiles (carbón, gas natural y petróleo), para fabricar productos como plásticos, combustibles o ropa. De hecho, existen varias plantas industriales alrededor del mundo que producen bioetanol, un combustible que puede reemplazar a la gasolina, a partir de cereales como el maíz. Sin embargo, la utilización de cultivos comestibles para la producción de químicos plantea un dilema ético, ya que estos recursos podrían haberse dedicado para la alimentación humana.

¿Por qué han fracasado los intentos previos?

Nuevas tecnologías para la utilización de materiales orgánicos estaban siendo desarrolladas incluso antes de que la preocupación por el uso de materiales comestibles fuese planteada. Los investigadores se centraron mayoritariamente en el desarrollo de procesos basados en residuos forestales o agriculturales, por ejemplo, ramas de árbol o paja de cereales. Sin embargo, la utilización de estos materiales también ha sido cuestionada y se han generado debates muy polémicos sobre la sostenibilidad de estas estrategias. En cualquier caso, los procesos basados en este tipo de materiales no son económicamente rentables, debido al precio relativamente alto de estos residuos.

Dar una nueva vida a los residuos

Esta tesis aborda esta problemática a través de obtener material orgánico de otros tipos de residuo con un valor menor. El valor más bajo posible está asociado a residuos que se incineran o depositan en vertedero, dado que no tienen ninguna aplicación conocida y por tanto no hay ninguna competencia por la obtención y utilización de dichos residuos. La utilización de estos residuos no solo generaría efectos positivos a nivel económico, debido al menor precio del material, sino que también tendría beneficios a nivel medioambiental, ya que los residuos volverían a entrar en la economía en forma de productos y/o energía renovable en lugar de ir a parar al vertedero. Dos ejemplos de dichos residuos han sido investigados en este trabajo: lecho para animales, una mezcla de estiércol y paja procedente de granjas, y ropa desechada.

El problema con los residuos de bajo valor es que su menor valor está asociado a una peor calidad. Por ejemplo, los residuos depositados en vertedero normalmente no están clasificados y por tanto contienen una mezcla de diferentes materiales, lo que dificulta su transformación en productos de valor. Las plantas industriales existentes deben en consecuencia ser adaptadas en diferentes aspectos para poder manejar estos residuos eficientemente. Un procedimiento para dicha adaptación ha sido establecido en esta investigación, el cual consiste en adaptar los métodos analíticos para caracterizar residuos de bajo valor, rediseñar las tecnologías existentes para transformar residuos en productos de valor y desarrollar nuevos conceptos de proceso, los cuales definen la relación entre las diferentes operaciones del proceso. La implementación de esta metodología permite obtener una eficiencia elevada, a pesar de la peor calidad de la materia prima, y en consecuencia generar un mejor rendimiento económico. Por ejemplo, se ha estimado que una planta industrial basada en residuos de bajo valor (lecho para animales) puede introducir sus productos en el mercado un 40% más barato que una planta industrial convencional basada en residuos agriculturales.

Residuos de bajo valor – potencial de alto valor

La investigación presentada en esta tesis podría mejorar el rendimiento económico de las plantas industriales que utilizan material orgánico como materia prima para la producción de combustibles o químicos, generando la oportunidad de reducir nuestro impacto ambiental. Esta mejora se consigue a través de una metodología que permite valorizar residuos de bajo valor con la misma eficiencia que otros residuos de mejor calidad. Además, esta estrategia evitaría que estos residuos acabasen en incineradoras o vertederos, dando lugar a una sociedad con menor generación de residuos y, por tanto, más sostenible.

List of publications

This thesis is based on the publications listed below, which will be referred to by their Roman numerals throughout the thesis.

I. **M. Sanchis-Sebastiá**, B. Erdei, K. Kovacs, M. Galbe, and O. Wallberg, "Analysis of animal bedding heterogeneity for potential use in biorefineries", *Waste and Biomass Valorization*, vol. 11, no. 6, pp. 2387-2395, 2019.

II. **M. Sanchis-Sebastiá**, B. Erdei, K. Kovacs, M. Galbe, and O. Wallberg, "Introducing low-quality feedstocks in bioethanol production: efficient conversion of the lignocellulose fraction of animal bedding through steam pretreatment", *Biotechnology for Biofuels*, vol. 12, no. 1, p. 215, 2019.

III. M. Victorin, **M. Sanchis-Sebastiá**, Å. Davidsson, and O. Wallberg, "Production of biofuels from animal bedding: biogas or bioethanol? Influence of feedstock composition on the process layout", *Industrial & Engineering Chemistry Research*, vol. 58, no. 48, pp. 21927-21935, 2019.

IV. **M. Sanchis-Sebastiá**, J. Gomis-Fons, M. Galbe, and O. Wallberg, "Techno-economic evaluation of biorefineries based on low-value feedstocks using the BioSTEAM software", *Processes*, vol. 8, no. 8, 2020.

V. **M. Sanchis-Sebastiá**, E. Ruuth, L. Stigsson, M. Galbe, and O. Wallberg, "Novel sustainable alternatives for the fashion industry: a method of chemically recycling waste textiles via acid hydrolysis", *Waste Management*, vol. 121, pp. 248-254, 2021.

VI. **M. Sanchis-Sebastiá**, V. Novy, L. Stigsson, M. Galbe, and O. Wallberg, "Towards circular fashion – transforming pulp mills into hubs for textile recycling", *RSC Advances*, vol. 11, no. 20, pp. 12321-12329, 2021.

My contributions to the studies

I. I planned and performed the experiments. The results were evaluated together with the co-authors, and I wrote the paper with input from the co-authors.

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

III. I planned and performed the experiments together with M. Victorin. I evaluated the results and wrote the paper together with M. Victorin and input from the co-authors.

IV. I developed the models, performed the simulations, and evaluated the results together with J. Gomis-Fons, with input from O. Wallberg. I wrote the paper with input from J. Gomis-Fons and the co-authors.

V. I planned the experiments with E. Ruuth, and E. Ruuth performed them. I evaluated the results together with E. Ruuth. I wrote the paper with input from the co-authors.

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

Acknowledgements

Although I stand as the only author of this thesis, this work has been far from a one-man job and I therefore want to express my gratitude to all those people that helped me get to this point. First, I want to thank my main supervisor, Ola Wallberg, for always providing and supporting me in every way I needed, no matter what. You have been even better than the best supervisor I could have dreamed of.

I am also extremely grateful to my co-supervisor Mats Galbe for being my biggest fan, the hero that saved the day when no one else could and simply putting a complicit smile when I was up to mischief. I want to thank my other co-supervisor, Krisztina Kovacs, for thinking me capable of doing anything, almost to the point of blind faith. Fulfilling your expectations has been the best driver I could have to become better and better.

I am obviously grateful to all my co-authors for the amazing collaborations that we had together. Starting in order of appearance, I want to thank Mirjam Victorin for making the work in the lab a joy, I never thought that I could laugh so much while washing manure away. I want to thank Joaquín Gomis Fons for always thinking that there is a fix to any problem, even when your simulations return error messages that are several pages long. I want to thank Edvin Ruuth for being my best master student and eventually becoming an amazing collaborator; I am sure that there is nothing that we cannot solve together. Finally, I want to thank Lars Stigsson for giving me a once-in-a-lifetime opportunity to change the world a little bit, even if only a very small part of it.

All my colleagues at the Department of Chemical Engineering played their small part in this work, in one way or another, but a special mention needs to go to some of them. I need to thank Borbála Erdei for being everything a colleague could be: mentor, co-author, collaborator, office mate, confidant and friend. I certainly will never forget you and your cheerful attitude. I also want to thank Herje Schagerlöf for being a human encyclopedia about chemistry, being always willing to help and provide assistance, and share your knowledge as well as your real-life tips and bad dad jokes.

I want to thank Balázs Frankó and Basel Al-Rudainy for being the best role models I could have wished for at the beginning and at the end, respectively, of my PhD studies. Your guidance has been invaluable to me and I can only hope that I developed to be a little bit like you because that means that I became a little bit better.

I want to thank Gregor Rudolph for being an amazing PhD buddy. It has been a pleasure to share our experiences as PhD students as we lived them. I cannot image anyone better to walk this path with.

I want to thank Rubén Juárez Cámara and Mariona Battestini Vives for bringing a piece of my homeland to the grey days in Sweden. Your friendship has been really warming and it helped keep my spirits up at the hardest moments.

There are also people outside the workplace that I would like to acknowledge in these pages. I want to thank Laura, Antonio and David for being an infinite source of laughing, typically with some nachos and beer. I want to thank Anja, Marco, Gunnar and Jonas for pushing my body as hard as the PhD studies pushed my brain; it has been awesome to train with you.

Last, but not least, I could not leave my family out of this because they have been fundamental during this time. I want to start with my partner, Lidia, who loves me so effortlessly that it seems like magic. I am the best version of myself when I am with you and there are not enough words in the world to express how grateful I am for that. These PhD studies have only been a small part of our love story because, even if it may not seem like it, our story together has just started and I am sure that we have plenty of new adventures ahead of us.

Sometimes it feels that children do not acknowledge their parents enough so I have only one thing to say to them: I remember everything and I could not be more grateful. You will always be shareholders of my successes.

Finally, I want to thank all my siblings, both by blood and by law. I am grateful to my brother, Alberto, for always supporting and rooting for me in spite of the distance. I want to thank my sister-in-law, Raquel, for the coolest cover I could have had for this thesis and baking my favorite cakes whenever I went home. I want to thank my brother-in-law, Alejandro, for thinking I am some sort of golden boy and all the hours of podcasting that always made me smile no matter how stressed I was.

Contents

1. Introduction

1.1. Climate change and the need for a paradigm shift

Human activities since the middle of the 20th century have led to a dramatic increase in the concentration of CO_2 in the atmosphere (Figure 1). In particular, the concentration of CO_2 has increased by 47% above pre-industrial levels over the past 170 years, which is greater than the natural increase over the past 20,000 years [1]. It is believed that this increase in the concentration of CO_2 has been mainly caused by the burning of fossil fuels, although other human activities have contributed to a lesser extent [2].

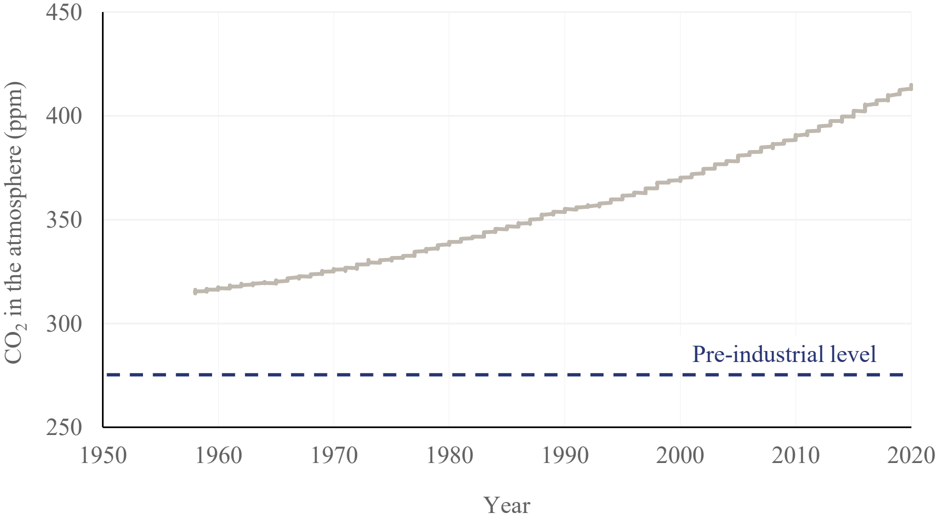


Figure 1. Concentration of CO_2 in the atmosphere over recent decades. (Original data from NASA [1].)

This increase in the concentration of CO_2 has resulted in the greenhouse effect, which is altering our planet's climate and ecosystems. For example, the global temperature has been steadily increasing over the past decades (Figure 2), which has led to a reduction in the Arctic Sea ice of 13% every decade [3]. This, in turn, is leading to an increase in sea level of 3.3 mm per year [4].

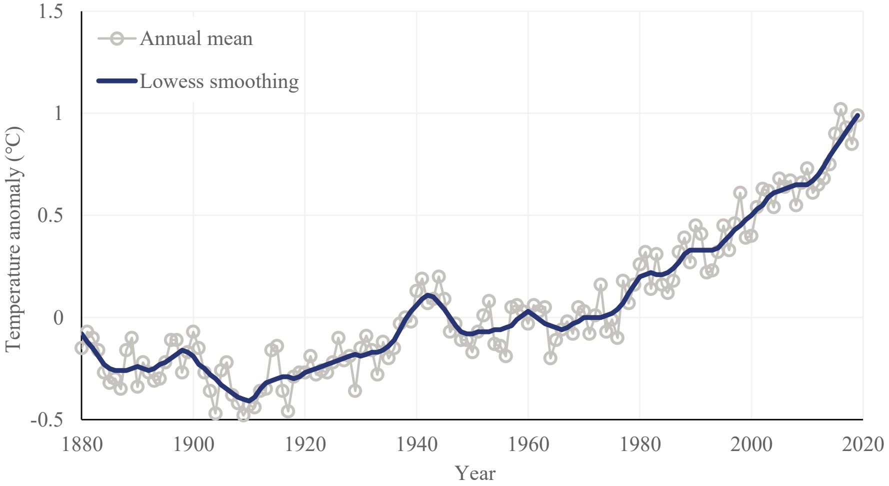


Figure 2. Change in global surface temperature relative to 1951-1980 average temperatures. (Original data from NASA [5]).

In 2015, many nations (196 when the text was negotiated) pledged to limit anthropogenic emissions in an attempt to mitigate climate change and its impacts through the Paris Agreement [6]. The aim of this agreement is to limit the increase in global temperatures to below 2 °C and, if possible, to below 1.5 °C [6]. In spite of this, world emissions increased in 2017 compared to 2016, following a three-year period of stabilization [7], and it appears that the world is on track to extract and use considerably more coal, natural gas and oil than are consistent with the goals of the Paris Agreement [8]. This means that emissions in the near future would have to be much lower than they are expected to be if the goals of the Paris Agreement are to be fulfilled; a difference referred to as “the emission gap”, and that can only be closed if actions are taken urgently [9].

It has been suggested that shifting to a bioeconomy, in which fuels and energy, as well as chemicals and materials, are produced from biomass instead of oil, will play a crucial role in the transition to a sustainable society. In fact, the development of a solid bioeconomy is central to the European Union’s strategy on sustainability [10]. Moreover, apart from the environmental benefits, it has been estimated that the bioeconomy could bring with it business opportunities worth US$7.7 trillion worldwide [11], and promote other necessary transitions, such as the development of a circular economy [12]. The bioeconomy has already been partly introduced in our society, for example, the bioenergy sector is the largest employer within renewable energy in Europe with 706,000 jobs [13], but it is necessary to expand the bioeconomy if we are to meet the climate goals set in the Paris Agreement.

1.2. Biorefineries: the workhorse of the bioeconomy

Industrial biorefineries have been identified as the most promising means of transitioning to a bioeconomy [14]. These facilities will become the engine of the bioeconomy as they will allow the transformation of biomass into an array of products, such as fuels, chemicals and energy, in a similar fashion to the way in which petroleum-based refineries extract products from crude oil [14]. Several technologies can be used to extract value from biomass, and biorefineries are therefore categorized as either thermochemical or biochemical.

Thermochemical biorefineries use high temperatures and/or pressures to transform biomass into valuable products. The main thermal conversion processes are combustion, gasification and pyrolysis, which differ mainly in the availability of air in the reactor [14]. Although considerable research efforts are focused on gasification and pyrolysis due to their higher level of sophistication [14], direct combustion of biomass currently accounts for 86% of the bioenergy used in the world [15]. Apart from these processes, thermochemical biorefineries also make use of other technologies that have not been widely applied at scale, such as liquefaction and supercritical fluid extraction [14].

In biochemical biorefineries, biomass is depolymerized into the sugars that constitute the material, and these are subsequently fermented to the desired product by an appropriate microorganism. Pretreatment is applied, if necessary, to reduce the recalcitrance of the biomass, after which enzymatic hydrolysis is used to depolymerize the carbohydrates into their respective sugars, followed by fermentation to transform the sugars into the desired product [14]. The most common product in biochemical conversion processes to date is bioethanol, the worldwide annual production of which amounted to 110 million m^3 in 2019, although most of it (84%) was concentrated in the USA and Brazil [16].

Biochemical biorefineries are currently based on sugar- and starch-containing biomass, such as sugarcane and corn, as these feedstocks are readily hydrolysable, and they can therefore easily be converted into the desired products. However, since these types of biomass are edible, concerns have been raised over the possible effect on food prices and availability; a debate that came to be known as the food vs. fuel debate [17]. This debate has been tremendously misleading, as food prices are more strongly correlated with crude oil prices than with biofuel production (Figure 3). For example, the dramatic decrease in oil prices in 2009 and 2015-2016 translated into lower food prices, despite increased biofuel production during these periods (Figure 3). Nevertheless, the research community had already started to investigate the possibility of using non-edible biomass as feedstock for biorefineries, and lignocellulosic feedstocks, such as forest residues, wheat straw and dedicated energy crops, became the most popular alternatives.

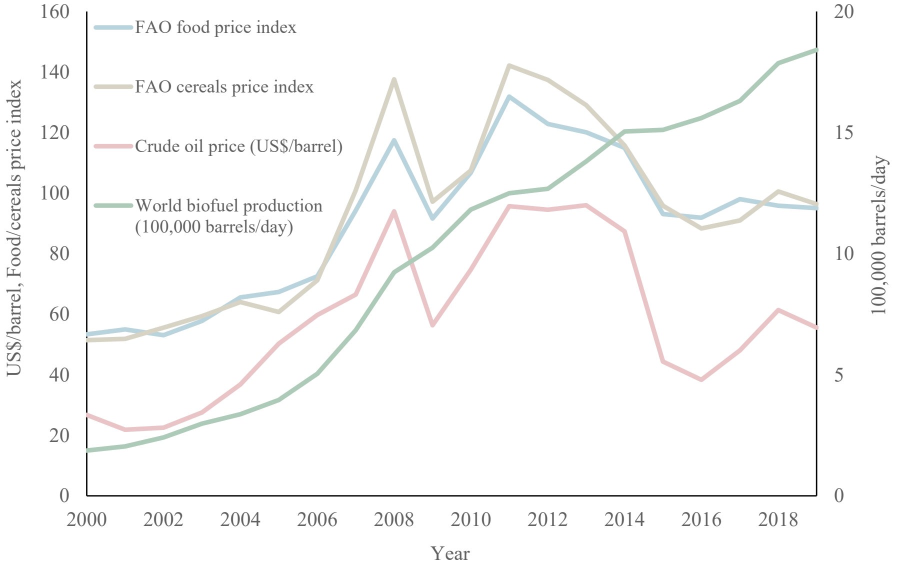


Figure 3. Evolution of food prices, crude oil price and biofuel production over recent years. (Original data from Food and Agriculture Organization [18], US Energy Information Administration [19], and I. Tiseo [20]).

A shift to non-edible biomass is desirable as biorefineries would then have a superior environmental performance to sugar- and starch-based biorefineries in many different respects, such as lower greenhouse gas emissions, higher soil quality, improved biodiversity, and lower land-use requirements [21, 22]. Consequently, various facilities were commissioned worldwide to demonstrate the use of lignocellulosic feedstocks in biorefineries [23]. Some of these demonstration plants are still operational. The Cellulonix Kajaani project in Finland managed by St1 [23], for example, is attempting to achieve profitability and take the next step towards commercialization. However, none of these projects was able to completely overcome the challenges to commercialization, and there are therefore currently no commercial biorefineries based (exclusively) on lignocellulosic feedstocks.

1.3. The role of unconventional feedstocks in commercialization

A number of hurdles must be overcome before biorefineries can be commercialized and introduced into the economy. The lowest production cost of lignocellulosic biofuels reported to date is in the range US$82-105 per MWh, which is almost twice the price of fossil-based alternatives [24]. This inability to achieve economic feasibility on a commercial scale does not seem to be a particular problem of biorefineries, as research efforts in other areas of green or sustainable chemistry, such as green catalysts and biodegradable polymers, have led to very few patents, and thus very few applications in the industrial world [25].

Initially, one of the greatest challenges in the commercialization of (biochemical) biorefineries was the high cost of the enzymes used in the hydrolysis step. However, enzyme costs have been dramatically reduced in the past two decades: from US$1.3 per liter ethanol in 2000 to US$0.1-0.2 per liter ethanol in 2019 [24]. This reduction was mainly the result of improvements in the efficiency of the enzymes, allowing a reduction in enzyme dosing in the process. For example, thanks to these advancements, the company DuPont was able to reduce the enzyme dosage in their ethanol production process by 75% between 2008 and 2012 [24].

The largest contributions to the production cost of biofuels at the moment are the capital cost and the feedstock cost [24]. In fact, feedstock costs can account for almost one third of the total production cost of lignocellulosic biofuels [26]. Thus, expert groups within the European Union and the International Energy Agency have suggested a transition to feedstocks with zero or negative value in biorefineries [24, 27]. It has been estimated that such a strategy could reduce production costs by 25-35%, which could be sufficient to provide early market opportunities for biorefineries [27]. The commercialization of biorefineries would then require a new generation of processes based on another shift of feedstock: from lignocellulosic biomass to low-value waste.

1.4. The role of unconventional feedstocks in sustainability

Low-value waste can be defined as residues that have no use or known application, hence their low value. Their low value usually arises from inadequate sorting, i.e. different residues are mixed in one stream, or low quality originating from several uses, i.e. materials that have been reused or recycled several times. Examples of these residues are animal bedding, waste textiles and municipal solid waste.

Low-value residues are usually landfilled or, in the best case, incinerated due to the lack of alternatives to extract and valorize the resources contained in them. For example, 132,000 tons of textiles was introduced into the Swedish apparel market in 2008, of which only 26,000 tons was collected by charity organizations for reuse or resale [28]. In spite of second-hand initiatives at the individual level, it has been estimated that most of these textiles ended up in waste streams that were landfilled or incinerated, probably the latter due to waste management policies in Sweden [28].

Biorefineries based on low-value waste offer the opportunity to address the environmental impact of these residues, as they would remove them from landfills or other unsustainable management strategies. This displacement of waste from unsustainable practices would also improve the environmental performance of the biorefinery. For example, it has been shown that bioethanol production from animal manure has an improved environmental performance as the emissions mitigated in the disposal of the manure counterbalance the emissions of the ethanol production process [29]. Moreover, such a biorefinery would also deliver biofertilizer with better amendment and fertilizing properties than the original manure [30], which would contribute to improved soil quality and conservation.

A shift to low-value waste in biorefineries would improve both their economic and environmental performance. The improved economic performance arises from the lower feedstock cost, while the improved environmental performance is attributed to the fact that the biorefinery would contribute towards a sustainable society through the provision of renewable products as well as the valorization of the residues used as feedstock.

1.5. Scope and outline of this thesis

The aim of the work presented in this thesis was to design new biorefining processes based on low-value waste as feedstock, which will henceforth be referred to as *unconventional feedstocks*. The research was restricted to biochemical processes, and thermochemical processes are therefore not further discussed. Two low-value residues, animal bedding and waste textiles, were used, so the findings and discussion are applicable to these specific materials. However, the general principles and the conclusions inferred from the results could be applied to other low-value residues.

This thesis is divided into several chapters that cover different aspects of the process design. Chapter 2 describes the properties of animal bedding and waste textiles, and discusses the challenges in the characterization of unconventional feedstocks. This chapter combines information from the literature with the results presented in Paper I, and the compositional analysis described in the other papers. Chapter 3 presents different principles involved in the design of individual steps of the biorefining process through adapting existing technologies, while Chapter 4 focuses on aspects of the design of the process as a whole, both within the biorefining process and in combination with other industrial processes. These two chapters are based on the experiments described in Papers II, III, V and VI. Chapter 5 introduces the economic perspective to the discussion, as it presents the results of the techno-economic simulations presented in Paper IV. The final chapters (Chapters 6 and 7) present the overarching conclusions drawn from the research presented in this thesis and perspectives on future research.

2. The Characterization of Unconventional Feedstocks

2.1. Feedstock complexity and variability

Unconventional feedstocks typically consist of a mixture of different types of biomass, as in the case of animal bedding where straw is mixed with manure, or a mixture of biomass with other types of materials, as in the case of waste textiles where cotton fibers are mixed with synthetic fibers. The properties of these fractions in the mixture are far from homogeneous as no resources are devoted to preserving the quality of the residue, since no value is attributed to the material. As a result, unconventional feedstocks exhibit high variability in their properties.

Biomass variability has been recognized as one of the greatest challenges to the successful implementation of biorefineries [31], as variability has a significant influence on the robustness of the process, its conversion efficiency, and even the feedstock cost (due to the rejection of feedstock with unacceptable properties) [32]. Traditionally, biomass variability has focused on ash and moisture content [31, 33, 34], but it has been suggested that a broader range of physicochemical properties be used for improved determination of biomass variability [35].

The determination of the physicochemical properties of unconventional feedstocks is more challenging than that of traditional feedstocks due to the higher complexity of the material. However, appropriate analysis methods are necessary in a biorefinery as accurate and reliable determination of the properties of the biomass and its variability is crucial to ensure good performance of the process [35]. The following sections therefore describe some ways in which analysis methods can be adapted to unconventional feedstocks, together with the physicochemical properties determined for animal bedding and waste textiles.

2.2. Characterization of animal bedding

2.2.1. Main components of animal bedding

The second largest application of wheat straw in Sweden, after soil incorporation, is its use as bedding for animals on farms (800,000 tons destined to this application annually) [36]. After it has served its purpose, the material, known as animal bedding, is a mixture of manure, urine, straw, soil and other minor impurities arising from its collection. In this work, animal bedding was considered to be a mixture of manure and straw, since these are the two main components of the material, and the other components are assigned to one of these fractions.

Animal manure is a nitrogen-rich biomass, whose composition varies widely depending on the type of animal that generated the residue. Despite this, the total nitrogen content of manure is typically 2.5-3% [37-40]. In the case of dairy cow manure, which was used in this work, a significant fraction of the nitrogen is in the form of proteins, the crude protein content of dairy manure being about 16-18% [37, 38] while the total ammonia nitrogen is less than 1% [37]. Apart from the nitrous compounds, animal manure contains undigested fibers, and the fiber content can be up to 40-50% of the dry weight [38, 39, 41]. As a result, animal manure contains 20-30% cellulose, 11-18% hemicellulose and 10-15% lignin. Other minor components are also present in manure, such as P, K, Ca, Na and other types of metals [38, 42].

The other main component of animal bedding is straw, which is a lignocellulosic material, composed mainly of cellulose, hemicellulose and lignin. Cellulose is the most abundant polymer in lignocellulosic biomass (30-50%), followed by hemicellulose (15-35%) and lignin (10-30%). Apart from these components, lignocellulosic biomass contains minor amounts of pectins, proteins, inorganic compounds and non-structural compounds referred to as extractives.

Cellulose is a linear polymer of D-glucose linked by β-1,4 glycosidic bonds, whose repeating unit (cellobiose) is a disaccharide consisting of two glucose molecules rotated 180° (Figure 4). The polymeric chains interact with each other through hydrogen bonds and van der Waals forces, creating a structure (microfibrils) with crystalline and amorphous regions. Cellulose is insoluble in most solvents due to the hydrophobic nature of the interactions between the polymer chains.

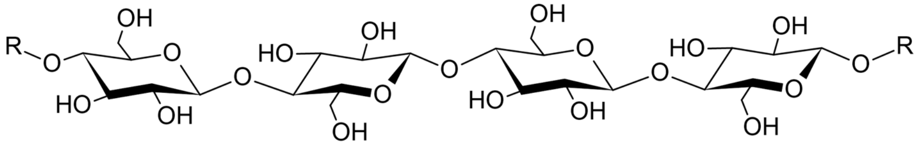


Figure 4. The chemical structure of cellulose.

Hemicellulose is a term that includes several branched polymers based on sugars with a number of substituents. These polysaccharides are made up of hexoses (sugars with 6 carbon atoms), pentoses (sugars with 5 carbon atoms) and sugar acids [43]. The composition and structure of hemicelluloses vary greatly as they can have different glycosidic bonds, side groups and degrees of polymerization depending on the plant species [44]. In the case of agricultural plants such as grasses and straw, the main hemicellulose present is L-arabino-D-xylan.

Lignin is an aromatic heteropolymer that is based on three basic monomers or monolignols: *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Figure 5). These units are connected by ether and carbon-carbon linkages, creating a complex structure that varies between different plant species. This high degree of heterogeneity has made it impossible to assign a defined structure to lignin [45]. The proportions of monolignols also depend on the plant species, and agricultural plants contain significant amounts of all three monolignols [46].

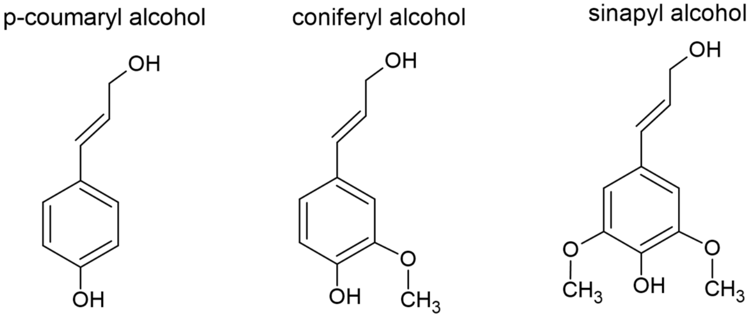


Figure 5. The three monolignols that provide the basis for lignin synthesis.

2.2.2. Challenges in the analysis of animal bedding

The analysis of lignocellulosic materials is typically based on the depolymerization of the carbohydrates via acid hydrolysis, and quantification of the resulting sugars in the solution. This is the basic principle of the analysis method developed by the National Renewable Energy Laboratory (NREL) [47] and Van Soest fiber analysis [48]. However, acid hydrolysis cannot be applied to the analysis of animal bedding as the nitrous compounds present in manure would react with the monosaccharides through a reaction known as the Maillard reaction [49]. The Maillard reaction is actually a complex combination of reactions, with many intermediate products, all of which lead to the production of melanoidins [50]. This reaction would lead to a loss of analyte, which would result in the underestimation of the carbohydrate content of animal bedding. It is therefore necessary to separate the two fractions of the material prior to analysis.

Apart from analyzing each fraction of the material separately, the analysis of animal bedding (and unconventional feedstocks in general) involves the additional challenge of quantifying the proportions of each fraction in the material. This is crucial for the reliable assessment of the physicochemical properties of the bulk material, as well as the efficiency of the biorefining process. The separation of manure from straw could also be used for this purpose, so the benefit of fractionating the material prior to its analysis is twofold: it allows the quantification of each fraction, and provides suitable substrates for the analysis of each fraction.

A method based on extensive washing of the material was developed to separate the manure from the straw prior to the analysis of animal bedding (Paper I). The material was subjected to consecutive washing cycles using fresh water in each cycle. After washing, no trace of manure could be seen in the remaining straw (Figure 6), and conventional fiber analysis methods could be used without the risk of losing analyte due to the Maillard reaction.

Figure 6. Animal bedding before (left) and after (right) extensive washing prior to the analysis of the material.

Washing produced liquid manure, with decreasing concentration in each washing cycle (Figure 7). The material was washed until the total solids (TS) content of the liquid manure was equal to or below 0.05%. Ten washing cycles were required to achieve this goal and the number of cycles did not vary although materials with different manure content collected on different occasions were analyzed in this work.

Figure 7. Manure samples resulting from the washing of animal bedding prior to the analysis of the material.

The manure content of the animal bedding could be determined by simply measuring the TS content of the washing liquid and recording the liquid volume after each washing cycle. The same principle could be applied to determine other physicochemical properties of the manure by further analysis of the liquid samples. For example, the volatile solids (VS) content was measured to obtain an estimate of the inorganic matter content of the manure, and the total carbon and total nitrogen were measured to estimate the C/N ratio (Paper I).

2.2.3. Composition and heterogeneity of animal bedding

Straw is the major component of animal bedding, as lignocellulosic fiber accounted for over half of the material (Papers I and II) and even for two thirds of the dry mass (Paper III) (Table 1). These findings appear to be consistent with the composition of animal bedding in other parts of the world, such as Italy [39], the USA [38] and China [51], the exception being Australia, where lignocellulosic fiber accounted for only 45% of the material [41].

Table 1. Composition of animal bedding (Papers I-III)

Content (%TS bedding)	Papers I & II	Paper III
Manure	**43.4**	**33.9**
Organic	29.7	23.0
Inorganic	13.7	11.0
Straw	**56.6**	**66.1**
Glucan	24.4	26.4
Xylan	11.7	15.1
Galactan	0.5	0.4
Arabinan	1.2	-
Mannan	0.6	-
Lignin	12.0	14.9
Extractives	4.3	3.5
Ash	1.9	5.7

The manure present in animal bedding was mainly composed of organic compounds; organic matter accounting for two thirds of the manure in all cases (Table 1). However, significant amounts of inorganic compounds were found which, together with the inorganic compounds contained in the straw, led to a high ash content in the material. For example, wheat straw has been reported to contain about 4.5-6% ash [52, 53], while the ash content in wood is below 1% [54, 55]. The animal bedding used in this work contained about 15% total ash (from both the manure and the straw).

The physicochemical properties of animal bedding exhibited considerable variation depending on where in the bed the sample was collected. Three different sampling levels were defined in order to evaluate the physicochemical properties of different layers from the top of the bedding to the bottom (in contact with the floor). Not only did the proportions of manure and straw differ between the layers, but the individual fractions also had different properties. For example, the top layer of straw contained 7% more glucan than the bottom layer, while there was 3% more lignin in the bottom layer than in the top layer (Table 2). These spatial variations in the fiber composition are greater than the variations in wheat straw collected on different occasions [56] and the variations between different sources of straw [53, 56], illustrating the considerable variability in the physicochemical properties of animal bedding.

Table 2. Composition of the straw at different layers in animal bedding

Content (%TS fiber)	Top	Middle	Bottom
Ash	3.0±0.1	5.0±1.6	3.2±0.2
Extractives	6.2±0.1	3.7±0.3	3.3±0.3
Glucan	44.6±1.1	39.3±2.6	37.4±1.5
Xylan	22.0±0.5	21.3±1.3	21.0±0.8
Galactan	1.1±0.0	0.1±0.0	0.2±0.0
Arabinan	2.7±0.1	1.1±0.1	1.3±0.1
Mannan	1.2±0.0	-	-
Lignin	20.6±0.3	22.2±0.5	23.5±0.9

The study described in Paper I showed that the properties of different layers can be used to predict the bulk properties of the material. In fact, the bulk properties of animal bedding are a linear combination of the properties of each layer. This applies not only to the main chemical components, but also to other physicochemical properties, such as the behavior of the material during washing or the total solids content. This implies that the quality of animal bedding depends on the proportion of each layer in the material, as this would affect the amount of substrate available for the biorefining process.

2.3. Characterization of waste textiles

2.3.1. Main components of waste textiles

There is a wide variety of textile fibers on the market as they are used in very different applications. Waste textiles therefore contain a mixture of different fiber types. The fibers most commonly used in the production of textiles, and therefore found in waste textiles, are cellulosic, protein, regenerated and synthetic fibers. A brief description of these fiber types is given in this section, while a complete classification of textile fibers is presented in Figure 8. More information on both the major and minor fiber types can be found in the book by R. R. Mather and R. H. Wardman [57].

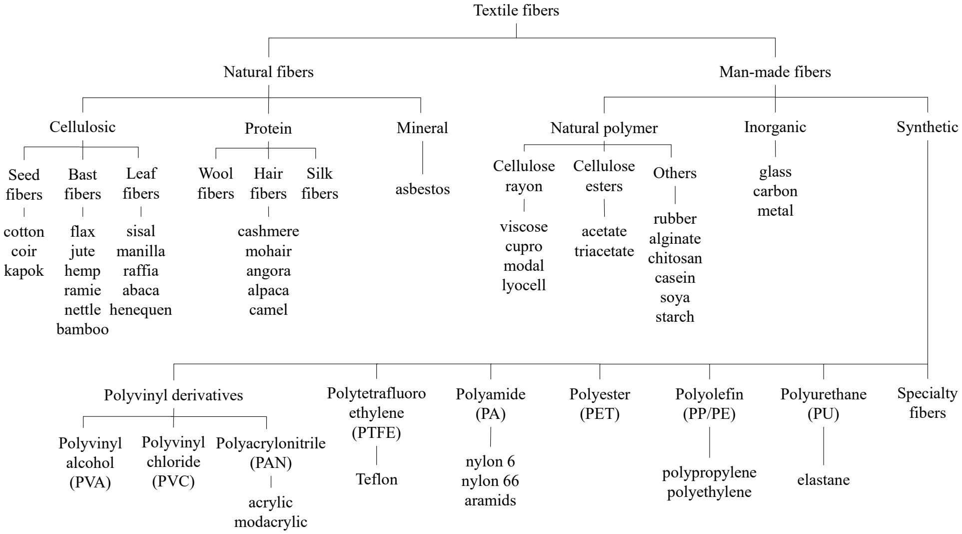


Figure 8. Classification of textile fibers (adapted from the book by R. R. Mather and R. H. Wardman [57]).

Cellulosic fibers are derived from plants and can be obtained from any of the three components of a plant: the seeds, the stem or leaves. The main seed fiber is cotton, which is the predominant natural fiber in the textile market. For example, cotton accounted for 82% of the natural fiber production in 2013 [57]. Cotton is mainly composed of cellulose, and has one of the highest molecular weights and structural orders among all plant fibers [58]. In contrast to cotton, bast or stem fibers, such as flax, hemp and ramie, contain hemicellulose and lignin, which complicates the extraction of the cellulosic fibers. Finally, leaf fibers have limited commercial applications as they are even coarser than bast fibers.

Protein fibers are mostly derived from animal hair, the most important example being wool. The principal protein in hair fibers is keratin, which has a high content of the aminoacid cysteine. However, the predominant protein in silk, another popular protein fiber, is fibroin. Apart from these, other protein fibers present in the textile market are cashmere and spider silk, although to smaller extents.

Regenerated fibers are man-made fibers based on polymers that are obtained through chemical processing of natural materials. Chemical processing is used to extract the fiber-forming polymer and impart new properties to the regenerated fibers. Viscose is a common example of regenerated fibers, which is produced by dissolving cellulose (usually extracted from wood pulp) followed by derivatization to cellulose xanthate and subsequent regeneration of the cellulose solution. Another example of this type of fiber is lyocell, whose production is also based on the dissolution of cellulose, but in this case an organic solvent, N-methylmorpholine-N-oxide (NMMO) is used to achieve solubilization. Although these fibers are cellulosic, they have a lower molecular weight than cotton fibers as the dissolution/regeneration process lowers the degree of polymerization of the cellulosic chains [59].

Synthetic fibers are man-made fibers based on different polymers derived from petroleum-based chemicals. The most important example of this type of fiber is polyester (PET), which accounted for almost 80% of the total production of synthetic fibers in 2013 [57]. However, a wide variety of polymers are used for the production of synthetic fibers, which are the building blocks of very well-known fibers such as nylon, Lycra and Teflon.

2.3.2. Analysis of waste textiles

A series of textile analysis methods based on ISO norms are available to identify the proportions of different fiber types in (waste) textiles, and these are commonly used to characterize products in the textile industry [60]. These methods cover a wide spectrum of textile mixtures, and can be used to characterize 24 different combinations of textile fibers, mostly through partial dissolution followed by gravimetric measurements. As in the case of animal bedding, the identification of the different fractions in the material is crucial in its characterization, and these procedures therefore provide an important first step in the analysis of waste textiles.

The problem with these ISO procedures is that they cannot provide any information on the composition of each individual fiber type since gravimetric methods are not sufficient to obtain information on chemical composition. For example, the proportion of cotton in waste textiles can be determined using these procedures, but determination of the cellulose content in the cotton would require further analysis. Thus, textile analysis based on ISO norms must be combined with other techniques that can provide information on the chemical composition of the textile fibers.

The procedure developed by the NREL for the analysis of carbohydrates and lignin in lignocellulosic biomass [47] was successfully applied to determine the cellulose content of cotton-based waste textiles (Papers V and VI). These textiles contained 93% cellulose, which is consistent with the fact that the most typical cellulose content of cotton is 94% [61]. Virgin cotton is not composed exclusively of cellulose; the secondary cell wall contains only 55% cellulose, and significant amounts of proteins, pectins and waxes are present in this part of the fiber [62].

The waste textiles used in the present studies contained compounds other than cellulose that accounted for approximately 4.5% of the dry mass (Papers V and VI). These compounds were non-cellulosic, since they did not dissolve during the acid hydrolysis procedure used to determine the cellulose content (Figure 9). Although most non-cellulosic components are removed during the processing of cotton, it has been shown that non-cellulosic components nevertheless account for 4% of processed cotton fibers [63].

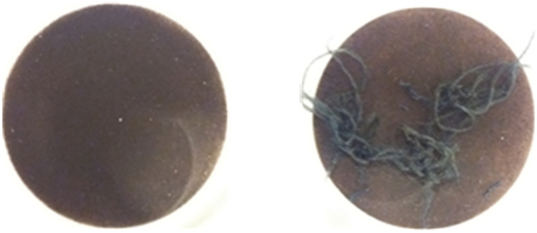


Figure 9. Solid residue after the analysis of cotton-based waste textiles without seams (left) and with seams (right) using acid hydrolysis.

The chemical composition of the non-cellulosic compounds was not determined in this work, as this would have required the development of other analysis methods. However, apart from dyes and other additives that might have been used during the processing of the textiles, fibrous matter was observed in the solid residue when seams were included in the sample analyzed (Figure 9). Thus, it appears that cotton-based waste textiles contain small amounts of non-cellulosic fibers in the seams to reinforce the union between different pieces of textiles, despite the fact that they are labelled 100% cotton.

3. Adaptation of Existing Technologies to Unconventional Feedstocks

Technologies developed for the conversion of lignocellulosic materials could be used in biorefineries based on unconventional feedstocks. However, these technologies would have to be adapted to cope with the higher complexity and heterogeneity of unconventional feedstocks. This chapter presents two examples of such adaptations: one related to the pretreatment of animal bedding, and the other related to the depolymerization of cotton in waste textiles.

3.1. Steam pretreatment of animal bedding

Biorefining processes based on animal bedding have been reported to have lower yields than processes based on wheat straw; the higher quality counterpart of animal bedding. For example, several studies reported ethanol yields of about 70% from wheat straw, while the yields from animal bedding reached barely 50% (Figure 10). The reason for this could be the low sugar recovery after the pretreatment step, since recoveries below 80% were reported for the pretreatment of animal bedding with sulfuric acid or sodium hydroxide [37, 41], whereas sugar recoveries following the pretreatment of wheat straw are well above 90% [53].

Although steam treatment dominates the pretreatment technologies developed for wheat straw, acid hydrolysis has been used extensively in the pretreatment of animal bedding, which may explain the poorer performance observed with animal bedding. Both technologies are based on the treatment of the material with acid, which depolymerizes the carbohydrates in hemicellulose and, to a lesser extent, in cellulose [64]. However, after depolymerization of the carbohydrates, these reactions continue with the conversion of monomeric sugars into furfurals and, subsequently, organic acids [65], having a negative effect, as the substrate for the subsequent process steps is degraded. Steam treatment reduces the degradation of carbohydrates in the material as the residence times are typically shorter, as result of the increased mass and energy transfer arising from the gaseous form of the water.

Thus, the application of steam treatment could improve the conversion efficiencies in processes based on animal bedding, as a result of increased sugar recoveries in the pretreatment step, while reducing both the environmental impact and the cost of pretreatment [66].

In spite of these advantages, steam pretreatment suffers from the same problem as acid hydrolysis: the addition of acid and high temperatures would lead to substrate losses due to the Maillard reaction. For this reason, the procedures developed for steam pretreatment of lignocellulosic biomass must be modified before they can be successfully applied to animal bedding. The solution is similar to that for the analysis procedures: namely, reducing the manure content of the material prior to pretreatment. Steam treatment was complemented with a conditioning step, which consisted of a washing step, in which the animal bedding was washed with 20 times its weight of water. This reduced the manure content of the material by approximately 75%, and the actual manure content during pretreatment was even lower due to the effect of the soaking step typical of steam treatment of agricultural residues. As a result, the bioethanol yield from animal bedding increased considerably (Paper II), reaching the same level as that obtained with wheat straw (Figure 10). This clearly illustrates that unconventional feedstocks do not reduce the efficiency of the biorefinery provided the conversion technologies are appropriately adapted to the feedstock.

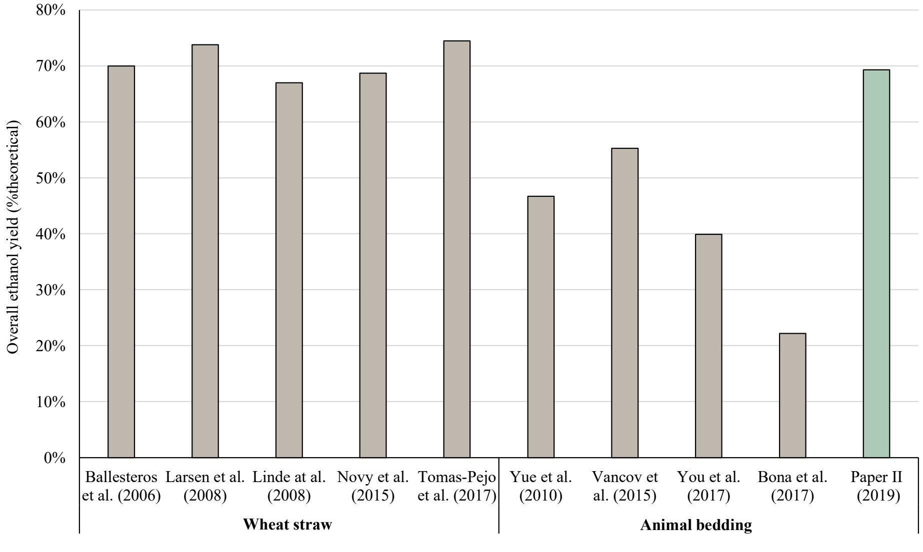


Figure 10. Bioethanol yields from wheat straw and animal bedding reported in several studies in the literature and Paper II. (The original data from the previous studies can be found in the following references [37, 39, 41, 51, 53, 67-70]).

The improved bioethanol yield was partly the result of the good sugar recoveries in the pretreatment step. Thanks to conditioning, the recovery of glucose and xylose were 90-100% and 80-95%, respectively, over a wide range of pretreatment conditions. This represents a significant increase compared to the (maximum) sugar recoveries of around 80% reported for pretreatment technologies without prior conditioning of the material [37, 41]. These results show that avoiding the Maillard reaction is crucial to achieve high conversion efficiencies from animal bedding, and underline the importance of adapting the processes in the biorefinery to the particular characteristics of unconventional feedstocks.

Although the sugar recoveries after pretreatment were high, carbohydrate degradation took place during steam treatment, and degradation products such as furfural and 5-hydroxymethylfurfural (HMF) were present in the pretreated material (Figure 11). The formation of HMF was lower than that in steam treatment of wheat straw reported by Ballesteros et al. [67], which indicates a smaller loss of substrate in the treatment of animal bedding. However, the formation of furfural was much higher than in steam treatment of wheat straw. A possible explanation of this is that furfural is an intermediate of the Maillard reaction and therefore, apart from carbohydrate degradation, reactions with the residual manure present in the material contribute to its formation. The quantities of these degradation products indicate that there was residual manure present in the pretreatment reactor, and that it had an effect on the operation, although this effect was insignificant in the overall process.

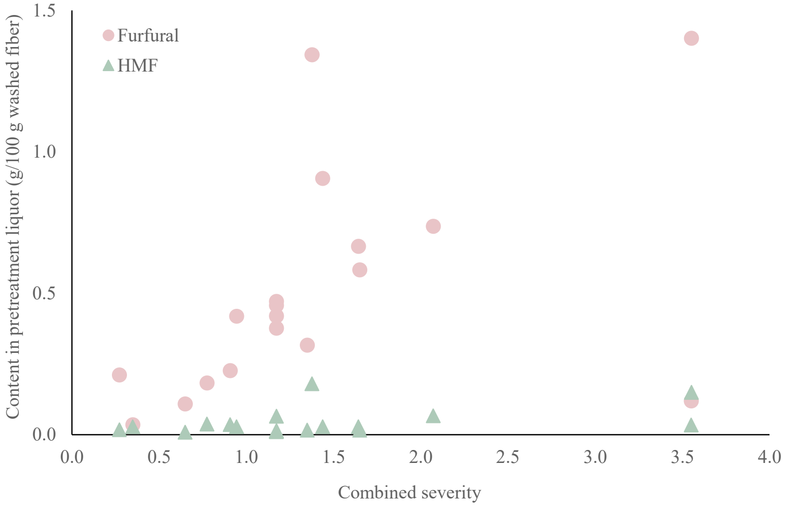


Figure 11. Formation of degradation products at different combined severities in steam treatment of washed animal bedding.

In spite of the residual manure present in the material, the results of steam treatment were similar to those of wheat straw. In fact, the composition of the pretreated material was very similar to that obtained in the steam treatment of wheat straw and the operating parameters (temperature, residence time and pH) had a similar influence on the results of pretreatment; pH having the highest influence on the final outcome (Paper II). For these reasons, the optimal conditions for the pretreatment of animal bedding were the same than those for the pretreatment of wheat straw. This demonstrates the strong influence of conditioning on pretreatment, as well as the overall biorefining process, and shows that a simple modification, washing, allowed a procedure developed for lignocellulosic materials to be applied to unconventional feedstocks, without any changes in the way in which the operation was performed or the final outcome.

3.2. Concentrated acid hydrolysis of waste textiles

In contrast to the pretreatment of animal bedding, acid hydrolysis appears to be a promising technology for the depolymerization of cotton-based waste textiles into glucose, as it has been demonstrated that it is possible to completely hydrolyze virgin cotton by treatment with 55% sulfuric acid at room temperature [71]. Unfortunately, waste textiles proved to be more recalcitrant than virgin cotton. No conditions were found under which one-step acid hydrolysis led to high glucose production from this material, and no glucose was produced under many of the conditions tested (Figure 12) (Paper V).

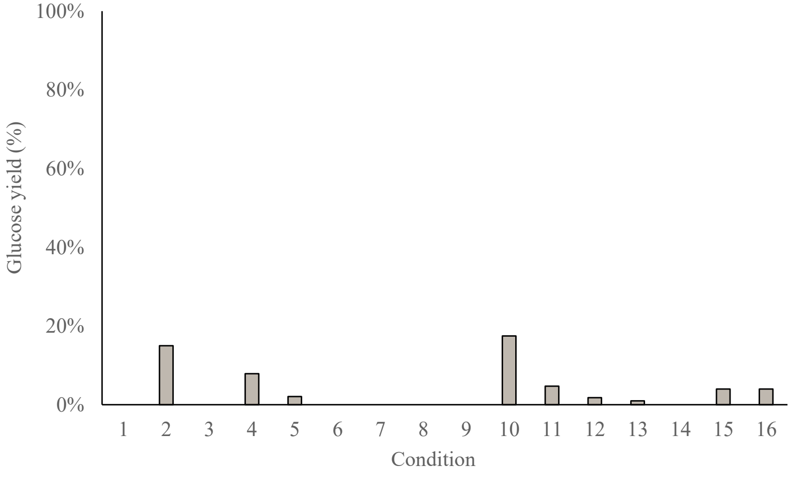


Figure 12. Glucose yield from cotton-based waste textiles treated with one-step acid hydrolysis under different conditions.

Concentrated acid hydrolysis could not completely depolymerize cotton-based waste textiles, as the degree of polymerization decreased exponentially, and levelled off as the operation continued. Thus, the degree of polymerization never reached one, but reached the so-called levelling-off degree of polymerization. For example, the levelling-off degree of polymerization of discarded cotton sheets is 120 [72], which is very far from complete hydrolysis. For this reason, many researchers have combined acid hydrolysis with other treatments, such as enzymatic hydrolysis [73] or mechanical treatment [74], in an attempt to completely depolymerize waste textiles.

Although there is no procedure in which stand-alone acid hydrolysis is used to depolymerize cotton-based waste textiles, the analysis procedure developed by the NREL successfully depolymerizes the carbohydrates in lignocellulosic biomass using only sulfuric acid, but in a two-step procedure in which the material is treated at different acid concentrations [75]. This procedure was applied to the depolymerization of cotton-based waste textiles, resulting in a glucose yield above 90% (Paper V). It therefore appears that two-step acid hydrolysis with concentrated and dilute acid can be used to successfully depolymerize waste textiles.

The problem with this procedure is that it has been optimized for analysis purposes, and therefore provides a high yield, to avoid the loss of analyte during the analysis, while other performance indicators that would be relevant in an industrial process are neglected. For example, the glucose solution obtained with this procedure had a concentration of about 3 g/L, which would be insufficient for profitable valorization of the solution (unless the targeted product is extremely valuable). Thus, the procedure must be adapted to provide an industrially relevant glucose solution. The method adopted in this work (Paper V) was to increase the solids loading during treatment, which resulted in an increase in the glucose concentration to 40 g/L, while maintaining the yield above 80% (Figure 13).

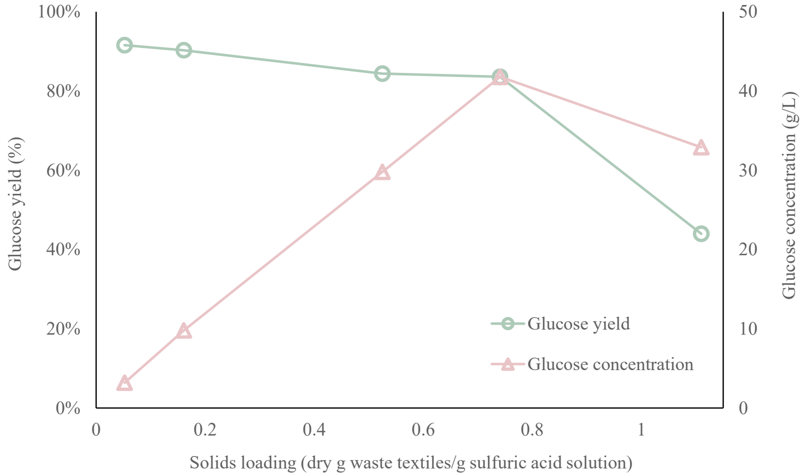


Figure 13. Effect of solids loading on the glucose yield and concentration in the depolymerization of cotton-based waste textiles via acid hydrolysis.

The glucose concentration cannot be indefinitely increased by increasing the solids loading as this would lead to a decrease in the mass and energy transfer, which would eventually hinder the depolymerization process (Figure 14). For example, the dramatic decrease in the yield at a solids loading of 1.1 dry g waste textiles/g sulfuric acid resulted in a decrease in the glucose concentration (Figure 13), despite the fact that a higher amount of textiles was available at the beginning of the treatment. Further modifications of the procedure would thus be required to obtain glucose concentrations above 40 g/L. One possible strategy to further increase the glucose concentration would be to reduce the amount of water used in the second step of the acid hydrolysis treatment. Initial tests showed that this strategy could increase the glucose concentration to well above 100 g/L, but at the expense of the glucose yield.

Figure 14. Treatment of waste textiles with 80% sulfuric acid at 30 °C at low (left) and high (right) solids loading.

Apart from achieving a higher glucose concentration, an industrially relevant procedure would also require purification of the solution to recover the sulfuric acid, as it is unlikely that a process without acid recirculation would be economically feasible. Moreover, neutralization of the acid (without salt removal) could compromise the efficient valorization of the glucose solution, as high salt concentrations would exert a high osmotic pressure on the fermenting microorganism, limiting its ability to transform glucose into the desired product. Thus, the two-step procedure presented in Paper V would have to be combined with acid recovery technologies, such as ion-exclusion chromatography [76], electrodialysis [77] or solvent extraction [78], prior to the implementation of this technology on commercial scale.

4. Process Design and Industrial Symbiosis

Biorefineries based on unconventional feedstocks would likely be used to treat different fractions of the residue in cases when the feedstock is composed of materials of similar nature, whereas residues composed of very different materials could probably not be processed in the same facility. For example, manure and straw contained in animal bedding could be treated in the same facility, whereas cotton and synthetic fibers from waste textiles are likely to be treated in separate facilities. This implies that the process designs of biorefineries based on unconventional feedstocks would be different depending on whether the facility is to process all the material or only a fraction of it. This chapter shows that, in cases where all the material is treated, the design would focus on exploiting synergies between the different processing lines, while in cases where only one fraction is treated, the design would focus on identifying synergies with existing facilities in order to develop an industrial symbiosis.

4.1. Synergies within the same process

Chapter 3 described how the straw in animal bedding could be converted into ethanol with high efficiency, provided the feedstock is conditioned. Complete valorization of the feedstock would require valorization of the other stream generated in conditioning, namely the liquid manure. Biogas production is an attractive alternative, since this technology has a high maturity level. However, the production of biogas from manure is rather inefficient as this material has a low methane potential. For example, the methane potential of dairy manure has been reported to be 204 NmL/g VS [79], which could be increased to approximately 250 NmL/g VS depending on the diet of the animals [80]. This should be compared with methane potentials of 450-500 NmL/g VS that have been achieved using food waste [81, 82], another residue typically treated by anaerobic digestion. Thus, separating the manure from the straw helps to increase the conversion efficiency in the production of ethanol, but it cannot improve the efficiency of the production of biogas by itself.

The problem associated with the anaerobic digestion of manure is that the nitrogen content of the material is excessive in relation to the carbon content, which can cause ammonia inhibition of the methanogens [83]. In fact, it was found that the liquid manure generated in the conditioning of animal bedding had a C/N ratio of 8 (Paper III), which is far from the optimal value of 25 [84]. As a result, the methane production from the liquid manure was about 300 NmL/g VS after 42 days (Figure 15), which is slightly higher than the values reported in the literature [79, 80], and 25% higher than the methane production from the whole animal bedding. Thus, the anaerobic digestion process benefits to some extent from the fractionation of the material, but it is not sufficient for the efficient valorization of the manure fraction.

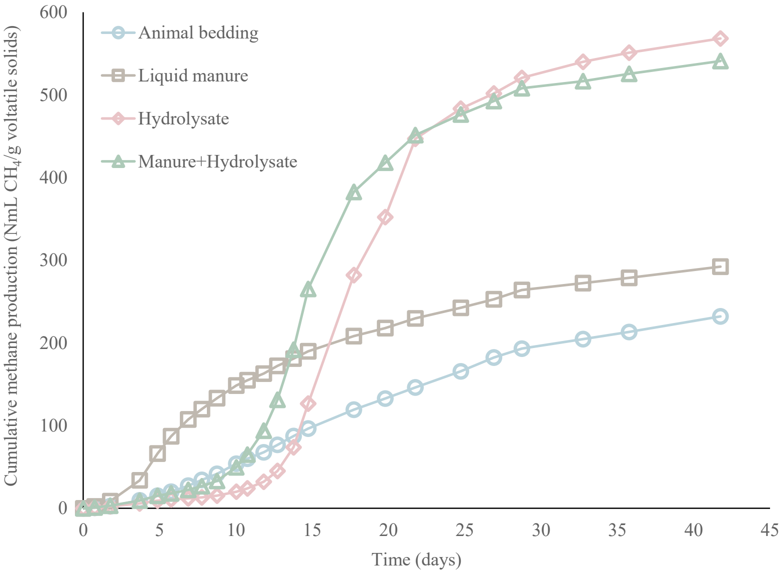


Figure 15. Cumulative methane production from different streams of a biorefinery based on animal bedding.

Efficient valorization of the manure through anaerobic digestion would require co-digestion of this stream with another substrate rich in carbon, to achieve a better C/N ratio in the digester. This offers the possibility of creating and exploiting a synergy between the ethanol and biogas processing lines as the liquid fraction of the steam-pretreated straw is rich in pentoses. Pentoses cannot be fermented into ethanol by naturally occurring yeast, but they are a suitable substrate for the mixed cultures used in anaerobic digestion. Diverting the liquid fraction after steam pretreatment (hydrolysate) to the anaerobic digestion line would increase the efficiency of biogas production to above 500 NmL/g VS (Figure 15), while it would remove the need to use genetically modified microorganisms in ethanol production, leading to benefits in both processing lines. Although many previous studies on co-digestion have focused on the C/N ratio and the balance between nutrients and substrate in the reactor, it appears that the pentose-rich liquid from steam pretreatment considerably increased the methane production as a result of high carbon accessibility, rather than the adjustment of the C/N ratio. A small test was conducted to compare the effect of the C/N ratio and carbon accessibility on the co-digestion process (Paper III). It was found that mixtures of liquid manure and wheat straw at very different C/N ratios had the same methane potential, around 300 NmL/g VS (Figure 16). A similar trend was observed when co-digestion was performed with a mixture of glucose and xylose, although the methane potential increased to 500 NmL/g VS (Figure 16). This illustrates the important role of carbon accessibility, as the small differences resulting from the C/N ratio were insignificant compared to the greater effect of the nature of the carbon source.

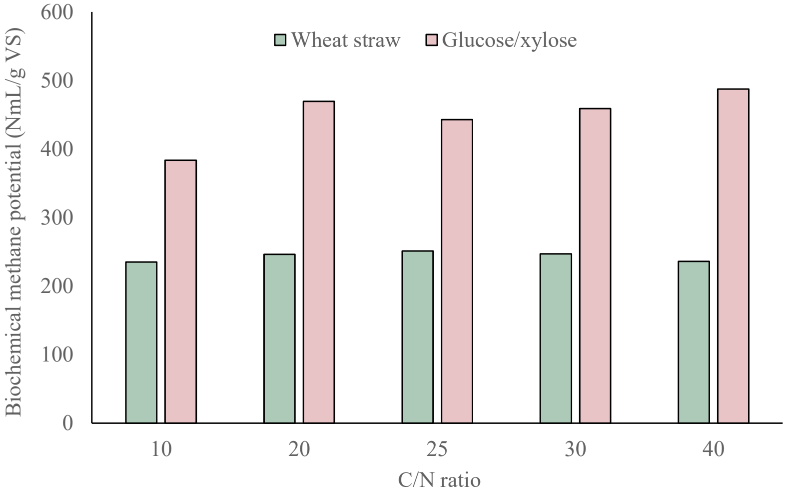


Figure 16. Methane potential in the co-digestion of liquid manure derived from animal bedding with wheat straw, or a mixture of glucose and xylose at different C/N ratios.

The results presented in Paper III indicate that there is an optimal level of carbon accessibility in the anaerobic digester at which the efficiency is maximized, while the C/N ratio may only be important in ensuring the stability of large-scale reactors during continuous operation. Thus, the initial conditioning and fractionation of the material allows only the necessary amount of carbon to be diverted to the anaerobic digestion line while providing a carbon source with higher accessibility to the microorganisms. The rest of the carbon can be safely converted into ethanol, without the risk of the Maillard reaction, which increases the overall efficiency of the process, and therefore maximizes the valorization of the feedstock.

4.2. Synergies with other processes

The opportunity for synergy in a biorefinery based on waste textiles is limited as the processing technologies for natural and synthetic fibers differ considerably. For example, a natural material like cotton fibers can be processed with biochemical technologies, while the valorization of synthetic fibers has more in common with the recycling of plastic. In fact, it is plausible that after sorting the different types of fiber, they would be treated at separate facilities or in completely independent processing lines at the same facility. For this reason, the strategy presented in Section 4.1 to increase the overall efficiency cannot be applied to a biorefinery based on waste textiles.

An alternative for biorefineries processing only one fraction of a complex residue would be to find synergies with other, existing processes. Paper VI presents an example of such synergies: namely, the recycling of cotton-based waste textiles in pulp mills. Expertise and know-how in the treatment of cellulose are already available at pulp mills, which would facilitate integration. The pulp mill would benefit from an expansion of the portfolio to include new recycled products, transforming the pulp mill into a true biorefinery. The key factors in such an integration would be the utilization of the excess residual heat from the pulp mill to provide energy for the pretreatment of waste textiles, together with the provision of green liquor, an alkaline solution that can be used in pretreatment to activate the material prior to enzymatic hydrolysis.

Alkaline pretreatment can increase the amenability of cotton fibers to enzymatic hydrolysis. It was demonstrated in this work that pretreating cotton-based waste textiles with Na_2CO_3, the main alkaline species in green liquor, increased the enzymatic hydrolysis yield by more than 2.5 times compared with untreated textiles (Figure 17) (Paper VI). The inclusion of other alkaline species in the pretreatment had disparate effects: Na_2S had a negative effect on the enzymatic hydrolysis yield, while NaOH had a positive effect. Although green liquor contains a higher amount of Na_2S than NaOH [85, 86], the stronger effect of NaOH on the process cancels out the effect of additional alkaline species, and therefore pretreatment with a mixture containing all three alkaline species (prepared in the laboratory) led to the same enzymatic hydrolysis yield as with pure Na_2CO_3 (Figure 17). Pretreatment with green liquor from a pulp mill led to similar results as the alkaline mixture prepared in the laboratory, and it therefore appears that heavy metals and other impurities present in green liquor do not significantly affect the enzymatic depolymerization of cotton-based waste textiles.

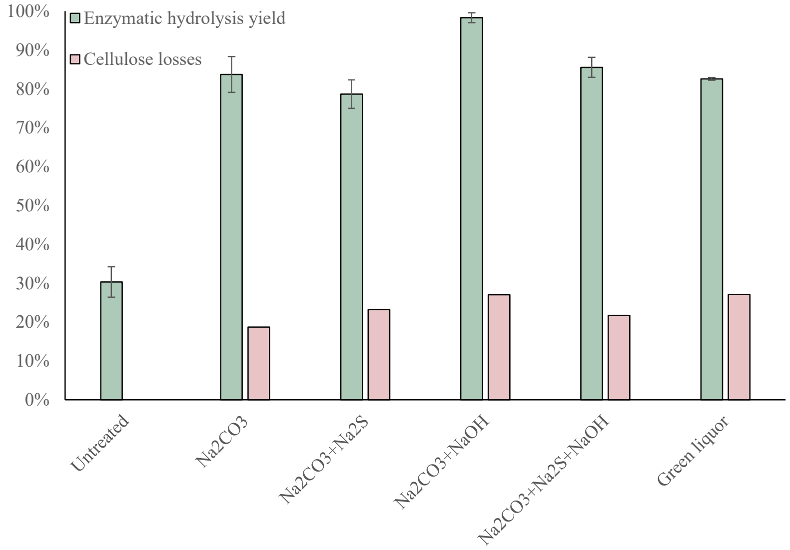


Figure 17. Enzymatic hydrolysis yield and cellulose losses after pretreatment of cotton-based waste textiles with different alkaline solutions.

The enzymatic hydrolysis yield obtained with green liquor (83%) was similar to that reported in another study in which Na_2CO_3 was used as the pretreatment agent [87]. However, it has been reported that pretreatment with NaOH at near- or sub-zero temperatures provides higher enzymatic hydrolysis yields, of around 90% [88] and above [89], possibly due to the stronger basicity of NaOH than Na_2CO_3. Pretreatment with green liquor could not outcompete other types of technologies in terms of process efficiency either. For example, enzymatic hydrolysis has been reported to give a yield of 91% following pretreatment with NMMO [90], while pretreatment with an imidazolium-based solvent led to an even higher yield of 94% [91]. However, these studies are not directly comparable to the current study (Paper VI) as the materials and methods used were not exactly the same. Despite the lower yield, pretreatment with green liquor might be able to outcompete these technologies on the techno-economical level as the poorer performance would be compensated for by the fact that integration with the pulp mill would provide the energy and chemicals needed for pretreatment.

The main problem associated with the enzymatic depolymerization of cotton fibers is that there are very few sites for the enzymes to attack due to the small distance between the cellulose chains and their high structural order [58]. Pretreatment with green liquor increases the enzymatic hydrolysis yield because it increases the distance between the cellulose chains (Figure 18a and c), which increases accessibility and thus the amenability of the material to depolymerization [92]. Moreover, this pretreatment is able to expand and modify the structure of the visible fiber (Figure 18b and d), providing the additional benefit of creating more attack sites on the fibers.

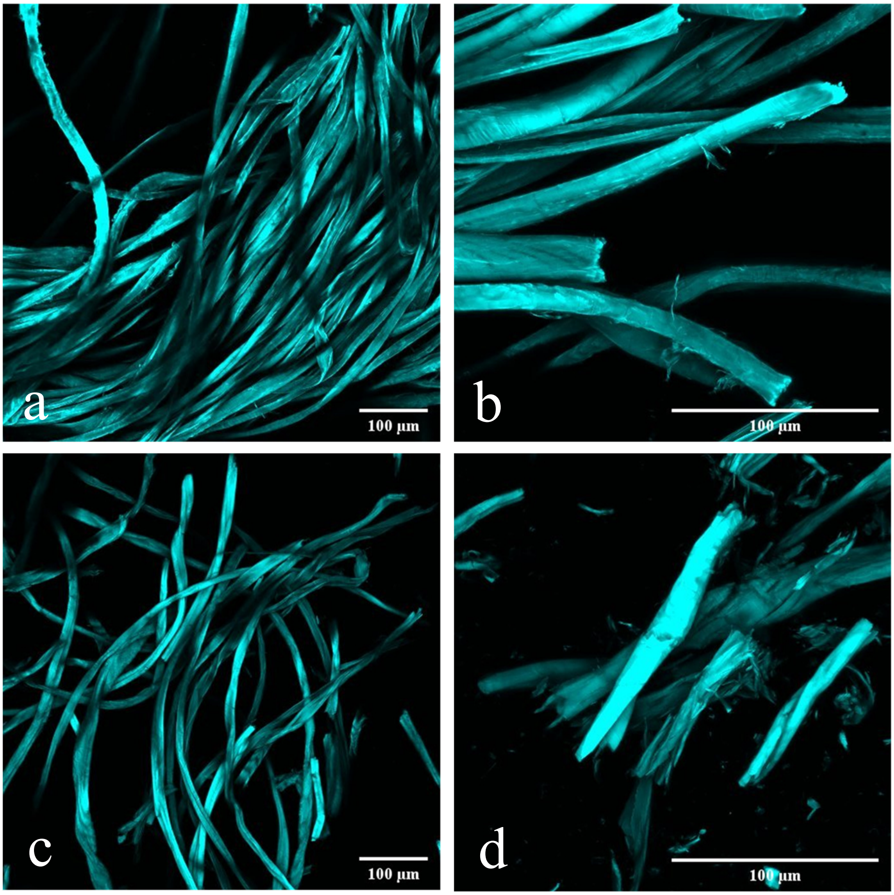


Figure 18. Confocal laser scanning microscopy images of cotton-based waste textiles before (a, b) and after (c, d) treatment with green liquor at 200 °C for 8 h. Images were taken with 20x (a, c) and 40x (b, d) magnification. Cellulose was stained with calcofluor as dye. The color was assigned arbitrarily.

The structural changes in the cotton fibers resulting from treatment with green liquor could have been caused by the titration of the -OH groups in the cellulose chains due to the presence of the alkaline compounds [93]. This titration generates surface charges on the chains that, due to repulsion forces and the breakage of the hydrogen bond network, increase the distance between the chains (Figure 19). This mechanism is very similar to that used in the derivatization of cellulose [94], with the exception that a derivatizing chemical that can interact with the surface charges is introduced into the system instead of enzymes that depolymerize the cellulose chains.

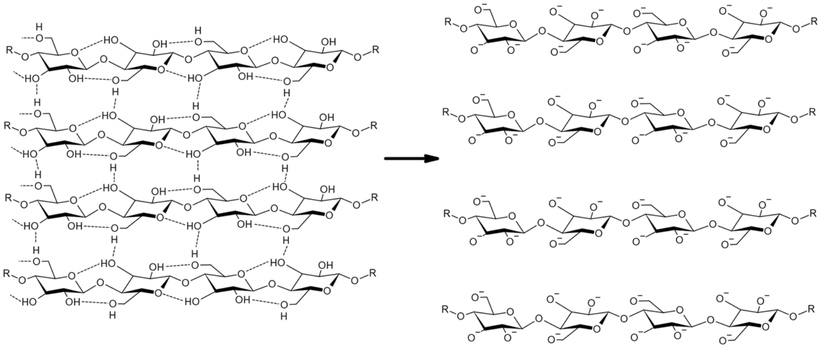


Figure 19. Illustration of the creation of surface charges and structural modifications during the pretreatment of cellulose with green liquor.

Alkaline compounds can even produce a change in the crystallographic structure of the material, leading to a transition between different cellulose allomorphs, which is the basic mechanism of other processes such as mercerization [95]. The extent of structural changes can be such that they cause the dissolution of the material, due to the elimination of the hydrogen bond network and the hydrophobic interactions between chains [96, 97]. However, changes in allomorphs have been reported to take place at room temperature [98], while the dissolution of cellulose in alkali requires lower temperatures [96]. Thus, the underlying mechanism governing the pretreatment of waste textiles with green liquor appears to be the creation of surface charges, rather than crystallographic changes or partial dissolution.

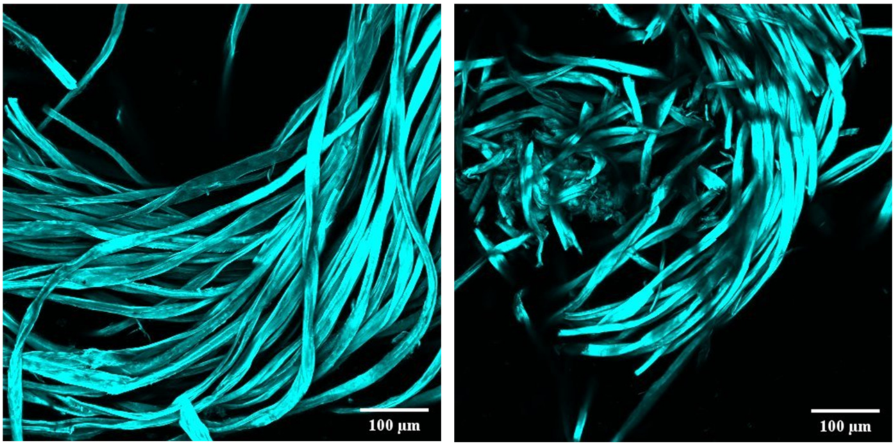


Figure 20. Confocal laser scanning microscopy images of cotton-based waste textiles before (left) and after (right) pretreatment with green liquor, where fragmentation of the fibers can be observed. Images were taken with 20x magnification. Cellulose was stained with calcofluor as dye. The color was assigned arbitrarily.

Apart from structural changes, degradation reactions can take place in an alkaline environment when cellulose is treated above 170 °C [99]. These reactions generate random scissions of the glycosidic bonds, releasing short-chain molecules into the solution [99]. Such scissions were observed after pretreating cotton-based waste textiles with green liquor at 200 °C for at least 6 h (Figure 20). The enzymatic hydrolysis yield increased due to the increased surface area and the creation of new attack sites for the enzymes. Thus, it is beneficial to design the pretreatment step such that scission reactions take place to some extent, in order to maximize the efficiency of depolymerization.

5. Techno-Economics of Unconventional Feedstocks

The previous chapters have described how biorefineries based on unconventional feedstocks can be designed and their application to the examples of animal bedding and waste textiles. It was shown that biorefineries based on unconventional feedstocks can perform at the same technical level as those based on conventional feedstocks, provided they are appropriately designed and adapted to the special characteristics of unconventional feedstocks. This chapter introduces an economic perspective to show that biorefineries based on animal bedding can compete with, or even outcompete, those based on conventional feedstocks from a techno-economic perspective.

5.1. Flowsheeting: mass and energy balances through modelling

The economic evaluation of a (potential) biorefinery requires estimation of the mass and energy flows in the process, as these are needed for the quantification of both the capital and the operational costs. Although experimental data provide invaluable information on the performance of a process, they rarely describe the complex interdependence between different process steps, and therefore cannot completely describe the process. For this reason, process simulation is often combined with experimental data to estimate the mass and energy flows in a process prior to economic evaluation.

Commercial process simulators have been used extensively to conduct techno-economic assessments of biorefineries. For example, Aspen Plus® has been used by the NREL to develop models to estimate the thermodynamic properties of biomass components [100], and the same software was later used for the techno-economic assessment of biorefineries based on forestry residues [101], as well as agricultural residues such as corn stover [102], wheat straw [103] and olive tree prunings [104]. However, the software BioSTEAM was used for the techno-economic assessment presented in Paper IV.

BioSTEAM is freeware intended to provide a fast and flexible platform for the design and simulation of biorefineries, and it has already been shown that the results obtained are comparable to those with Aspen Plus® [105]. Moreover, researchers are encouraged to upload their models to the development platform in order to facilitate review, the development of new models and the simulation of already existing models. The models presented in Paper IV were revised in collaboration with the developers of the software, and have been uploaded to BioSTEAM's development platform as one of the case studies that demonstrate the capabilities of the software [106].

Most of the models used to solve the mass and energy balances of the process were already available in the BioSTEAM software. Models for the conventional process units were taken from Seider et al. [107], while models for specific biorefining units were developed by Humbird et al. [102]. However, no model was available for the conditioning of animal bedding (washing with water) as biorefineries based on unconventional feedstocks had not previously been simulated. Thus, a new model for conditioning was developed to simulate the animal bedding biorefinery in BioSTEAM (Paper IV). This model was based on a series of dissolution reactions that simulate the transfer of manure to the liquid phase according to the experimental observations presented in Papers I-III. Despite the limited scope of the model, it can be claimed that it expands the capabilities of BioSTEAM in terms of simulating unconventional feedstocks, and provides a framework for the introduction of conditioning associated with such feedstocks in process simulations.

5.2. Economic estimates for animal bedding

Unconventional feedstocks usually require a conditioning step (e.g. washing of animal bedding or sorting of textile fibers) due to the low quality of these residues, which will introduce additional costs in the biorefinery. However, their lower quality also leads to savings for the biorefinery, since the price of the feedstock is usually lower. Thus, the techno-economics of unconventional feedstocks revolve around the question of whether the conditioning costs are less than or greater than the feedstock savings.

In the case of animal bedding, the biorefinery had a better economic performance than the benchmark (a biorefinery based on wheat straw), provided that the water used in conditioning was recycled. The use of animal bedding as feedstock increased the operational cost by 20% when the cost of the feedstock was not considered, partly due to the necessity of the conditioning step (Figure 21). However, the use of animal bedding could provide savings in the feedstock cost of up to 80% (assuming that the only cost is for transportation of the material), which can more than compensate for the increase in the other operational costs. Although conditioning also increased the capital cost by 17%, the overall cost of the biorefinery based on animal bedding with water recycling was approximately 15% lower than that for the biorefinery based on wheat straw (Figure 21). As a result, the minimum ethanol selling price decreased from US$0.61 per liter to US$0.38 per liter, a reduction of almost 40%, which illustrates the potential of unconventional feedstocks to improve the economics of biorefineries.

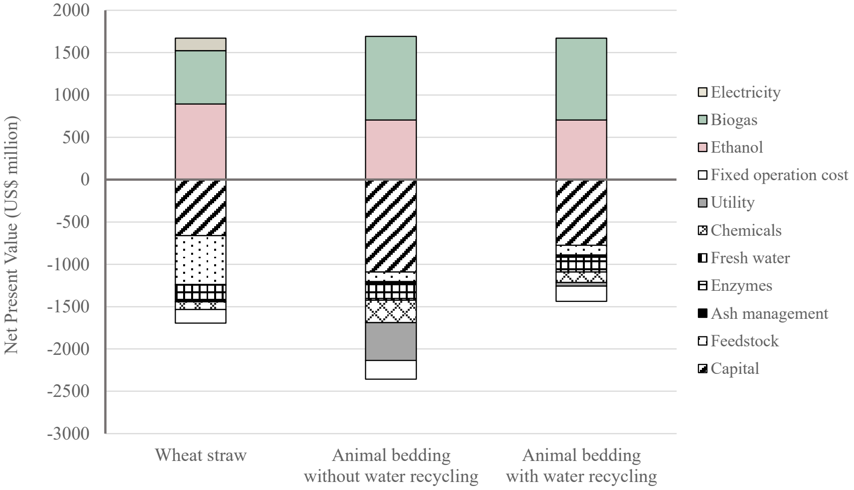


Figure 21. Net present value of revenues, capital and operational costs in a wheat biorefinery and an animal bedding biorefinery, with and without water recycling in the conditioning step.

The high consumption of fresh water, and its associated cost, could at first be thought to be the reason why water recycling is needed in the animal bedding biorefinery. Although the cost of fresh water was increased 13 times compared to the benchmark, it represented approximately only 1.5% of the operational costs, and was in fact the smallest contribution (Figure 21). However, conditioning without water recycling had a dramatic impact on the capital cost, which increased by 65% compared to the benchmark, due to the larger units required after conditioning (for anaerobic digestion and wastewater treatment) as a result of a larger volumetric flow rate in that part of the biorefinery. The effect was so marked, that it also significantly increased the utility costs of the biorefinery (Figure 21) due to the heating required for such a large anaerobic digester. Thus, water recycling was necessary in the animal bedding biorefinery due to the impact of conditioning on other process units, highlighting the complex interdependence of different process steps.

An animal bedding price of US$12 per ton was assigned in the initial simulations, which was assumed to cover the cost of transportation, but no cost for the material itself was included. However, the study presented in Paper IV shows that a higher price could be paid for the feedstock without compromising the competitiveness of the biorefinery. The animal bedding biorefinery (with water recycling) could outcompete the benchmark refinery provided animal bedding could be sourced 35% cheaper than wheat straw (Figure 22), a price considerably higher than that assumed in the initial simulations. This means that the biorefinery based on animal bedding would still be competitive with a wheat straw biorefinery if the bedding costs up to US$39 per ton. Although such a high feedstock price could compromise the marketability of the biorefinery, it might be beneficial to pay for the material as this could increase biomass availability, since more farmers would want to deliver material to the facility [108].

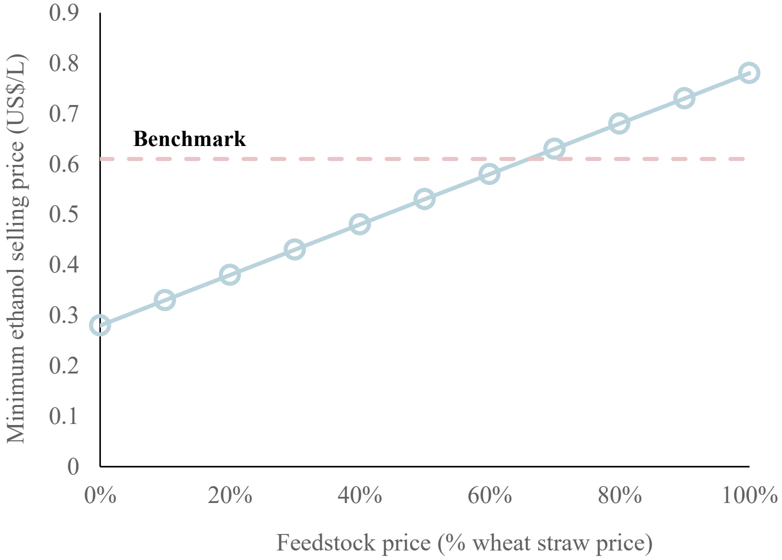


Figure 22. Minimum ethanol selling price as a function of feedstock price in an animal bedding biorefinery with water recycling.

Animal bedding can have considerably different properties as these are affected by a variety of factors, such as the type and number of animals, diet and animal housing [109]. Thus, assigning the same price to materials of varying quality could drive materials of higher quality off the market [110]. The reason for this is that materials with higher quality would have higher value than the average price, while materials with low quality would have lower value, making it profitable to sell only the low-quality materials at the average price [110]. A price scheme based on the characteristics of the material could incentivize farmers to ensure high quality of the material, which would in turn increase the revenues of the biorefinery as higher production rates can be achieved from feedstocks with higher quality (as shown in Paper I).

6. Conclusions

The research presented in this thesis has shown that (biochemical) biorefineries can be transformed into platforms that divert low-value waste from unsustainable management practices (e.g. landfilling or incineration) and turn it instead into resources for the production of fuels, chemicals or materials. This transformation could potentially bring economic advantages to the facility, due to the lower price of the feedstock, as well as improved sustainability, as the emissions avoided in the management of the waste could counterbalance the emissions generated in the production process. In order to reap these benefits, several aspects of the biorefinery must be adapted so that low-value residues can be utilized without disrupting the operation of the biorefinery.

Analysis methods must be adapted to cope with the higher complexity and variability of low-value wastes, since current methods are typically not suitable for the analysis of mixtures of different types of residue. It is therefore necessary to introduce fractionation steps into the analytical procedures to separate the various fractions making up the material, so that each fraction can be analyzed separately with the most appropriate method, and the proportion of each fraction quantified. For example, a washing procedure was developed to separate the straw from manure in animal bedding, so that conventional fiber analysis methods could be applied to the straw without the risk of losing analyte due to the Maillard reaction.

The analytical procedures revealed that straw was the major component of animal bedding, as it represented between half and two thirds of the dry mass. Since straw is a lignocellulosic material, this resulted in contents of fermentable carbohydrates and lignin of about 40% and 15%, respectively. However, the material also contained a considerable proportion of inorganic compounds, since the ash content accounted for approximately 15% of the dry mass. In contrast, most of the mass in cotton-based waste textiles (over 90%) proved to be cellulose, an expected result given the high cellulose content of virgin cotton. However, waste textiles contained small amounts of non-cellulosic compounds due to non-cellulosic fibers in the seams.

The physicochemical properties of animal bedding and waste textiles varied considerably. For example, the carbohydrate content of the lignocellulosic fiber of animal bedding exhibited a 45% higher variability than that in wheat straw collected on different occasions and from different sources. The physicochemical properties of the low-value residues presented in this thesis should thus be interpreted with caution, and the high variability associated with these residues should be borne in mind in planning any future biorefinery.

Conversion technologies must also be adapted so that low-value residues can be used in biorefining operations without any loss of process efficiency. This was exemplified by the adaptation of steam treatment to the pretreatment of animal bedding by the introduction of a conditioning step, based on washing with water, prior to steam treatment, thus avoiding the Maillard reaction during the pretreatment of the material. Applying the proposed technology led to a much higher efficiency than reported previously in the literature, reaching the same as for wheat straw (around 70% overall yield), a material with a higher value but similar characteristics. Moreover, the washed animal bedding behaved in almost the same way as wheat straw during pretreatment, which means that, apart from increasing the efficiency, a simple modification allowed the transfer of knowledge and methods currently applied to conventional lignocellulosic materials to a low-value residue such as animal bedding.

Another example of a successful adaptation is the modification of an analysis procedure for the quantification of carbohydrates in lignocellulosic biomass for the depolymerization of cotton-based waste textiles into glucose. Two-step acid hydrolysis gave a glucose solution with high concentration and a high yield through an increase in the initial substrate concentration, constituting a more industrially relevant procedure. Further modifications, such as less dilution in the second hydrolysis step, indicate that this technology could provide glucose concentrations well above 100 g/L, which are considerably higher than those typically reported for lignocellulosic materials.

Process concepts must be adapted in order to transform the complexity of low-value residues into an opportunity to increase the efficiency of biorefineries through synergies within the facility. For example, a biorefinery based on animal bedding could benefit from co-digestion of the liquid manure generated in the conditioning step with the pentose-rich liquid generated in the pretreatment of the straw. The pentose-rich liquid increased the methane potential of the substrate by almost 70%, due to its high carbon accessibility, which allowed the valorization of the manure with high efficiency. At the same time, the removal of pentoses removed the need to use genetically modified yeasts in the ethanol production line, creating a positive effect in this processing line.

The efficiency of a biorefinery that treats only one fraction of a low-value residue that has been conditioned elsewhere, for example, cotton fibers sorted from waste textiles, could be increased through synergies with other processes. It was demonstrated that a biorefinery treating cotton-based waste textiles could be integrated with a pulp mill as treatment with green liquor followed by enzymatic hydrolysis allowed the cotton fibers to be depolymerized into glucose with an overall yield of about 70%. As a result of such integration, utility and chemical costs could be avoided in the pretreatment of the textile recycling process, while the pulp mill would benefit from a broader portfolio of products and operations.

Biorefineries based on unconventional feedstocks could provide early market opportunities for the bioeconomy, provided that the biorefining operations are appropriately adapted to the characteristics of low-value residues. For example, a biorefinery based on animal bedding could sell bioethanol at a minimum ethanol selling price of US$0.38 per liter, which is 40% lower than a biorefinery based on wheat straw. The superior performance of the animal bedding biorefinery was rather robust, since only a reduction of 35% in the feedstock price was required for the biorefinery to be competitive with the wheat straw biorefinery. This illustrates that additional conditioning costs do not reduce the competitiveness of biorefineries based on low-value residues, as the savings from the lower feedstock price more than compensate for the additional costs.

7. Future Work and Outlook

The biorefineries proposed in this thesis could be subject to technical developments to further improve the production process. The conditioning step in the animal bedding biorefinery could be investigated in greater detail in order to optimize the water flow and residence time, and to determine the most suitable operation mode (cocurrent or countercurrent flow) in continuous conditioning. The process would be uneconomical if performed as it was in the laboratory due to the high dilution in the enzymatic hydrolysis and fermentation steps. It is thus necessary to reevaluate these steps at higher solids loadings to increase the ethanol concentration in this part of the biorefinery.

The biorefinery based on waste textiles also has some underdeveloped engineering aspects. The acid hydrolysis process requires further investigation into acid/sugar separation technologies that can be used to purify the glucose solution after the depolymerization of the textile fibers and to recover the acid so that it can be reused in the process. The green liquor process could benefit from a product with a higher concentration of cellulases, rather than the enzymatic cocktail intended for lignocellulosic biomass used in the present work. This would allow the enzyme loading in the process to be reduced. Apart from these improvements, it would be interesting in both cases (acid hydrolysis and green liquor followed by enzymatic hydrolysis) to investigate the possibility of further increasing the overall yield through recirculation of the residual solids that have not been depolymerized.

Although the economics of converting waste textiles appears to be as promising as that of animal bedding, the techno-economic performance of the waste textile biorefinery must be further investigated. The study of the acid hydrolysis process should investigate the question of whether the lack of pretreatment and enzymes compensates for the need to purify and recover the acid at high concentrations, while the study of the green liquor process should determine whether the sodium losses during pretreatment could be compensated for by the additional revenues from textile recycling.

Apart from the economic benefits, the potential environmental benefits motivate the transition towards low-value waste biorefineries. Techno-economic analysis must thus be complemented with life cycle assessments that confirm these environmental benefits, and demonstrate that low-value waste can outcompete conventional lignocellulosic feedstocks in this regard. These assessments must consider both the impact of the production process and the change in management practices for the residue.

The implementation of low-value waste biorefineries would be associated with technical challenges not only in the biorefinery itself, but along the whole value chain. For example, there are currently no logistic chains for the collection of these types of residues and their delivery to the biorefinery, which could pose a challenge. There is also a need to develop new chemistries that exploit the higher oxygen content of biomass, and the building blocks that can be produced from it.

The transition to a bioeconomy, and thus the development of biorefineries, poses challenges outside the disciplines of science and engineering. One example of this is the economic barrier. It has been estimated that achieving a low-carbon economy in Europe by 2050 would require an investment in clean technologies of US$317 billion [111]. Although this investment would easily be repaid, such large investments would require the commitment of both public and private economic agents.

Social barriers can also impede the transformation of the economy, since a sustainable economy can only be achieved by a sustainable society [112]. This means that societal changes are necessary, apart from technological advancements and economic incentives. For example, new definitions of value must be created so that corporations and entrepreneurs incorporate ecological and social values into their business models [113], while consumption patterns must be modified so that consumption can be kept within planetary boundaries [112]. These barriers highlight the important role that politics and governance will play in the transition to a bioeconomy, indeed, inconsistent policy is often cited by stakeholders in the bioeconomy as one of the greatest hurdles for its implementation [114].

Transformation at so many levels could lead to unexpected negative outcomes. For example, it has been suggested that an increase in waste valorization could lead to greater waste generation, the so-called rebound effect [115], or that the transition in one sector, or even one niche, of the economy could negatively affect the transition in other sectors, creating a complex network of interdependencies [116]. This underlines the importance of applying systems thinking and analysis to this transition in order to promote a holistic perspective and avoid unexpected outcomes.

In spite of future advancements in research, technological progress alone will never be sufficient to bring about the transition to a bioeconomy through the use of biorefineries. This transformation requires a multi-disciplinary approach that allows all aspects of sustainability (economic, social and environmental) to be aligned.

References

[1] NASA. “Global Climate Change. Vital signs of the planet - Carbon dioxide”. https://climate.nasa.gov/vital-signs/carbon-dioxide/ (accessed 9 Nov, 2020).

[2] NASA. “The causes of climate change”. https://climate.nasa.gov/causes/ (accessed 9 Nov, 2020).

[3] NASA. “Global Climate Change. Vital signs of the planet - Artic sea ice minimum”. https://climate.nasa.gov/vital-signs/arctic-sea-ice/ (accessed 9 Nov, 2020).

[4] NASA. “Global Climate Change. Vital signs of the planet - Sea level”. https://climate.nasa.gov/vital-signs/sea-level/ (accessed 9 Nov, 2020).

[5] NASA. “Global Climate Change. Vital signs of the planet - Global temperature”. https://climate.nasa.gov/vital-signs/global-temperature/ (accessed 9 Nov, 2020).

[6] United Nations Framework Convention on Climate Change, “Report of the Conference of the Parties on its twenty-first session, held in Paris from 30 November to 13 December 2015. Addendum. Part two: Action taken by the Conference of the Parties at its twenty-first session.”, United Nations Office, Geneva, Switzerland, 2015.

[7] M. Muntean *et al.*, “Fossil CO2 emissions of all world countries - 2018 Report”, EUR 29433 EN, 2018. [Online]. Available: https://edgar.jrc.ec.europa.eu/overview.php?v=booklet2018

[8] SEI, IISD, ODI, Climate Analytics, CICERO, and UNEP, “The Production Gap”, 2019. [Online]. Available: http://productiongap.org/

[9] United Nations Environment Programme (UNEP), “The emissions gap report 2017”, Nairobi, ISBN: 978-92-807-3673-1, 2017.

[10] European Commission, “A sustainable bioeconomy for Europe: strengthening the connection between economy, society and the environment”, Luxembourg, ISBN: 978-92-79-94144-3, 2018. [Online]. Available: https://ec.europa.eu/research/bioeconomy/pdf/ec_bioeconomy_strategy_2018.pdf

[11] World Business Council for Sustainable Development, “CEO guide to the circular bioeconomy”, 2019. [Online]. Available: https://www.wbcsd.org/contentwbc/download/7723/122348

[12] J. Sherwood, “The significance of biomass in a circular economy”, *Bioresource Technology,* vol. 300, p. 122755, 2020.

[13] International Renewable Energy Agency (IRENA), “Renewable Energy and Jobs - Annual Review 2020”, Abu Dhabi, ISBN: 978-92-9260-266-6, 2020. [Online]. Available: https://www.irena.org/publications/2020/Sep/Renewable-Energy-and-Jobs-Annual-Review-2020

[14] A. Demirbas, *Biorefineries: For biomass upgrading facilities* (Green Energy and Technology). London, UK: Springer, 2010.

[15] World Bioenergy Association, "Global bioenergy statistic 2019", Stockholm, Sweden, 2019. [Online]. Available: https://worldbioenergy.org/uploads/191129%20WBA%20GBS%202019_HQ.pdf

[16] Renewable Fuels Association. "Annual Ethanol Fuel Production". https://ethanolrfa.org/statistics/annual-ethanol-production/ (accessed 10 Nov, 2020).

[17] R. W. Harrison, "The Food versus Fuel Debate: Implications for Consumers", *Journal of Agricultural and Applied Economics,* vol. 41, no. 2, pp. 493-500, 2009.

[18] Food and Agriculture Organization of the United Nations (FAO). "FAO Food Price Index". http://www.fao.org/worldfoodsituation/foodpricesindex/en/ (accessed 11 Nov, 2020).

[19] US Energy Information Administration. "Petroleum & Other Liquids". https://www.eia.gov/dnav/pet/hist/LeafHandler.ashx?n=PET&s=F000000__3&f=A (accessed 11 Nov, 2020).

[20] I. Tiseo. "Global biofuel production from 2000 to 2019". Statista. https://www.statista.com/statistics/274163/global-biofuel-production-in-oil-equivalent/#:~:text=In%202019%2C%20global%20biofuel%20production,that%20was%20produced%20in%202000.&text=The%20global%20biofuels%20market%20is,billion%20U.S.%20dollars%20by%202024 (accessed 11 Nov, 2020).

[21] R. Sims, M. Taylor, J. Saddler, and W. Mabee, "From 1st- to 2nd-generation Biofuel Technologies: An Overview of Current Industry and RD&D activities", IEA, Paris, France, 2008. [Online]. Available: http://task39.sites.olt.ubc.ca/files/2013/05/From-1st-to-2nd-generation-biofuel-technologies.pdf

[22] G. P. Robertson *et al.*, "Sustainable Biofuels Redux", *Science,* vol. 322, no. 5898, pp. 49-50, 2008.

[23] IEA Bioenergy. "Database on facilities for the production of advanced liquid and gaseous biofuels for transport". https://demoplants.bioenergy2020.eu/ (accessed 11 Nov, 2020).

[24] Sub group on Advanced Biofuels, "Building up the future. The cost of biofuels", MI-06-17-107-EN-N, 2017. [Online]. Available: https://publications.europa.eu/en/publication-detail/-/publication/13e27082-67a2-11e8-ab9c-01aa75ed71a1/language-en/format-PDF

[25] D. J. C. Constable, "What Do Patents Tell Us about the Implementation of Green and Sustainable Chemistry?", *ACS Sustainable Chemistry & Engineering,* vol. 8, no. 39, pp. 14657-14667, 2020.

[26] V. Balan, "Current Challenges in Commercially Producing Biofuels from Lignocellulosic Biomass", *ISRN Biotechnology,* vol. 2014, p. 463074, 2014.

[27] International Energy Agency (IEA), "Advanced Biofuels - Potential for Cost Reduction", 2020. [Online]. Available: https://www.ieabioenergy.com/wp-content/uploads/2020/02/T41_CostReductionBiofuels-11_02_19-final.pdf

[28] N. Tojo, B. Kogg, N. Kiørboe, B. Kjær, and K. Aalto, "Textile flow in three Nordic countries", in *Prevention of textile waste.* Copenhagen, Denmark: Nordic Council of Ministers, 2012, pp. 41-47.

[29] A. de Azevedo, F. Fornasier, M. da Silva Szarblewski, R. D. C. D. S. Schneider, M. Hoeltz, and D. de Souza, “Life cycle assessment of bioethanol production from cattle manure”, *Journal of Cleaner Production,* vol. 162, pp. 1021-1030, 2017.

[30] F. Tambone *et al.*, “Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost”, *Chemosphere,* vol. 81, no. 5, pp. 577-583, 2010.

[31] J. R. Hess. “Variability — Understanding the True Complexity”. Office of Energy Efficiency & Renewable Energy. https://www.energy.gov/eere/bioenergy/articles/variability-understanding-true-complexity (accessed 05 Oct, 2020).

[32] D. S. Hartley, D. N. Thompson, L. M. Griffel, Q. A. Nguyen, and M. S. Roni, “Effect of Biomass Properties and System Configuration on the Operating Effectiveness of Biomass to Biofuel Systems”, *ACS Sustainable Chemistry & Engineering,* vol. 8, no. 19, pp. 7267-7277, 2020.

[33] J. H. Leal *et al.*, “Impacts of Inorganic Material (Total Ash) on Surface Energy, Wettability, and Cohesion of Corn Stover”, *ACS Sustainable Chemistry & Engineering,* vol. 8, no. 4, pp. 2061-2072, 2020.

[34] D. A. Sievers *et al.*, “Throughput, Reliability, and Yields of a Pilot-Scale Conversion Process for Production of Fermentable Sugars from Lignocellulosic Biomass: A Study on Feedstock Ash and Moisture”, *ACS Sustainable Chemistry & Engineering,* vol. 8, no. 4, pp. 2008-2015, 2020.

[35] A. E. Ray *et al.*, “Multiscale Characterization of Lignocellulosic Biomass Variability and Its Implications to Preprocessing and Conversion: a Case Study for Corn Stover”, *ACS Sustainable Chemistry & Engineering,* vol. 8, no. 8, pp. 3218-3230, 2020.

[36] T. Prade, L. Björnsson, M. Lantz, and S. Ahlgren, “Can domestic production of iLUC-free feedstock from arable land supply Sweden’s future demand for biofuels?”, *Journal of Land Use Science,* vol. 12, no. 6, pp. 407-441, 2017.

[37] Z. Yue, C. Teater, Y. Liu, J. Maclellan, and W. Liao, “A sustainable pathway of cellulosic ethanol production integrating anaerobic digestion with biorefining”, *Biotechnology and Bioengineering,* vol. 105, no. 6, pp. 1031-9, 2010.

[38] S. Chen *et al.*, “Studies into using manure in a biorefinery concept”, *Applied Biochemistry and Biotechnology,* vol. 124, no. 1, pp. 999-1015, 2005.

[39] D. Bona *et al.*, “The Biorefinery Concept Applied to Bioethanol and Biomethane Production from Manure”, *Waste and Biomass Valorization,* vol. 9, no. 11, pp. 2133-2143, 2018.

[40] Z. Wen, “Hydrolysis of animal manure lignocellulosics for reducing sugar production”, *Bioresource Technology,* vol. 91, no. 1, pp. 31-39, 2004.

[41] T. Vancov, R. C. Schneider, J. Palmer, S. McIntosh, and R. Stuetz, “Potential use of feedlot cattle manure for bioethanol production”, *Bioresource Technology,* vol. 183, pp. 120-128, 2015.

[42] G. M. Ward, T. V. Muscato, D. A. Hill, and R. W. Hansen, “Chemical composition of feedlot manure”, *Journal of Environmental Quality,* vol. 7, no. 2, pp. 159-164, 1978.

[43] X. Zhao, L. Zhang, and D. Liu, "Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose", *Biofuels, Bioproducts and Biorefining,* vol. 6, no. 4, pp. 465-482, 2012.

[44] T. W. Jeffries, "Biodegradation of lignin and hemicelluloses", in *Biochemistry of microbial degradation*, C. Ratledge Ed. Dordrecht, Netherlands: Springer, 1994, pp. 233-277.

[45] M. Ek, G. Gellerstedt, and G. Henriksson, *Wood chemistry and biotechnology*. Berlin, New York: de Gruyter, 2009.

[46] S. P. S. Chundawat, G. T. Beckham, M. E. Himmel, and B. E. Dale, "Deconstruction of Lignocellulosic Biomass to Fuels and Chemicals", *Annual Review of Chemical and Biomolecular Engineering,* vol. 2, no. 1, pp. 121-145, 2011.

[47] A. Sluiter *et al.*, "Determination of structural carbohydrates and lignin in biomass. Laboratory Analytical Procedure (LAP)", Golden*,* Colorado: National Renewable Energy Laboratory*,* 2008.

[48] H. K. Goering and P. J. Van Soest, "Forage fiber analyses: apparatus, reagents, procedures, and some applications", in *Agricultural Handbook No. 379*. Washington DC, USA: Agricultural Research Service, US Department of Agriculture, 1970, pp. 1-12.

[49] O. Theander and D. A. Nelson, "Aqueous, High-Temperature Transformation of Carbohydrates Relative to Utilization of Biomass", in *Advances in Carbohydrate Chemistry and Biochemistry*, vol. 46, R. S. Tipson and D. Horton Eds. Academic Press, 1988, pp. 273-326.

[50] J. E. Hodge, "Dehydrated Foods, Chemistry of Browning Reactions in Model Systems", *Journal of Agricultural and Food Chemistry,* vol. 1, no. 15, pp. 928-943, 1953.

[51] Y. You *et al.*, "Bio-ethanol production by Zymomonas mobilis using pretreated dairy manure as a carbon and nitrogen source", *RSC Advances,* vol. 7, no. 7, pp. 3768-3779, 2017.

[52] B. Erdei, Z. Barta, B. Sipos, K. Réczey, M. Galbe, and G. Zacchi, "Ethanol production from mixtures of wheat straw and wheat meal", *Biotechnology for Biofuels,* vol. 3, no. 1, p. 16, 2010.

[53] M. Linde, E.-L. Jakobsson, M. Galbe, and G. Zacchi, "Steam pretreatment of dilute H2SO4-impregnated wheat straw and SSF with low yeast and enzyme loadings for bioethanol production", *Biomass and Bioenergy,* vol. 32, no. 4, pp. 326-332, 2008.

[54] B. Frankó, M. Galbe, and O. Wallberg, "Influence of bark on fuel ethanol production from steam-pretreated spruce", *Biotechnology for Biofuels,* vol. 8, no. 1, p. 15, 2015.

[55] K. E. Saarela *et al.*, "Elemental analyses of pine bark and wood in an environmental study", *Science of The Total Environment,* vol. 343, no. 1, pp. 231-241, 2005.

[56] B. Erdei, B. Frankó, M. Galbe, and G. Zacchi, "Separate hydrolysis and co-fermentation for improved xylose utilization in integrated ethanol production from wheat meal and wheat straw", *Biotechnology for biofuels,* vol. 5, no. 1, p. 12, 2012.

[57] R. R. Mather and R. H. Wardman, *The chemistry of textile fibres*. Cambridge, UK: Royal Society of Chemistry, 2015.

[58] Y. L. Hsieh, “1 - Chemical structure and properties of cotton”, in *Cotton*, S. Gordon and Y. L. Hsieh Eds. Abington, UK: Woodhead Publishing, 2007, pp. 3-34.

[59] S. K. David and M. T. Pailthorpe, “Classification of textile fibres: production, structure, and properties”, in *Forensic examination of fibers*, J. Robertson and M. Grieve Eds., 2nd ed. Boca Ratón, Florida: CRC Press, 1999.

[60] International Organization for Standardization (ISO), “Textiles – Quantitative chemical analysis – Part 1: General principles of testing (ISO 1833-1:2006, including Cor 1:2009)”, 2010.

[61] E. R. McCall and J. F. Jurgens, “Chemical composition of cotton”, *Textile Research Journal,* vol. 21, no. 1, pp. 19-21, 1951.

[62] R. Mitchell, C. M. Carr, M. Parfitt, J. C. Vickerman, and C. Jones, “Surface chemical analysis of raw cotton fibres and associated materials”, *Cellulose,* vol. 12, no. 6, pp. 629-639, 2005.

[63] J. Buchert, J. Pere, L. S. Johansson, and J. M. Campbell, “Analysis of the Surface Chemistry of Linen and Cotton Fabrics”, *Textile Research Journal,* vol. 71, no. 7, pp. 626-629, 2001.

[64] F. Carvalheiro, L. Duarte, and F. Gírio, “Hemicellulose biorefineries: A review on biomass pretreatments”, *Journal of Scientific & Industrial Research,* vol. 67, pp. 849-864, 2008.

[65] J. Li, G. Henriksson, and G. Gellerstedt, “Carbohydrate reactions during high-temperature steam treatment of aspen wood”, *Applied Biochemistry and Biotechnology,* vol. 125, no. 3, pp. 175-188, 2005.

[66] B. K. Avellar and W. G. Glasser, “Steam-assisted biomass fractionation. I. Process considerations and economic evaluation”, *Biomass and Bioenergy,* vol. 14, no. 3, pp. 205-218, 1998.

[67] I. Ballesteros, M. J. Negro, J. M. Oliva, A. Cabanas, P. Manzanares, and M. Ballesteros, “Ethanol production from steam-explosion pretreated wheat straw”, *Applied Biochemistry and Biotechnology,* vol. 130, no. 1, pp. 496-508, 2006.

[68] J. Larsen, M. Østergaard Petersen, L. Thirup, H. Wen Li, and F. Krogh Iversen, “The IBUS Process – Lignocellulosic Bioethanol Close to a Commercial Reality”, *Chemical Engineering & Technology,* vol. 31, no. 5, pp. 765-772, 2008.

[69] V. Novy, K. Longus, and B. Nidetzky, “From wheat straw to bioethanol: integrative analysis of a separate hydrolysis and co-fermentation process with implemented enzyme production”, *Biotechnology for Biofuels,* vol. 8, p. 46, 2015.

[70] E. Tomás-Pejó *et al.*, “Valorization of steam-exploded wheat straw through a biorefinery approach: Bioethanol and bio-oil co-production”, *Fuel,* vol. 199, pp. 403-412, 2017.

[71] C.-Y. Chu, S.-Y. Wu, C.-Y. Tsai, and C.-Y. Lin, “Kinetics of cotton cellulose hydrolysis using concentrated acid and fermentative hydrogen production from hydrolysate”, *International Journal of Hydrogen Energy,* vol. 36, no. 14, pp. 8743-8750, 2011.

[72] A. Palme, H. Theliander, and H. Brelid, “Acid hydrolysis of cellulosic fibres: Comparison of bleached kraft pulp, dissolving pulps and cotton textile cellulose”, *Carbohydrate Polymers,* vol. 136, pp. 1281-1287, 2016.

[73] C. Sasaki, T. Nakagawa, C. Asada, and Y. Nakamura, “Microwave-Assisted Hydrolysis of Cotton Waste to Glucose in Combination with the Concentrated Sulfuric Acid Impregnation Method”, *Waste and Biomass Valorization*, vol. 11, no. 8, pp. 4279-4287, 2020.

[74] A. Ouchi, T. Toida, S. Kumaresan, W. Ando, and J. Kato, “A new methodology to recycle polyester from fabric blends with cellulose”, *Cellulose,* vol. 17, no. 1, pp. 215-222, 2009.

[75] A. Sluiter *et al.*, “Determination of sugars, byproducts, and degradation products in liquid fraction process samples. Laboratory analytical procedure (LAP)”, Golden, Colorado: National Renewable Energy Laboratory*,* 2008.

[76] R. P. Neuman, S. R. Rudge, and M. R. Ladisch, “Sulfuric acid-sugar separation by ion exclusion”, *Reactive Polymers, Ion Exchangers, Sorbents,* vol. 5, no. 1, pp. 55-61, 1987.

[77] I. S. Goldstein, F. Bayat-Makooi, H. S. Sabharwal, and T. M. Singh, “Acid recovery by electrodialysis and its economic implications for concentrated acid hydrolysis of wood”, *Applied Biochemistry and Biotechnology,* vol. 20, no. 1, pp. 95-106, 1989.

[78] J. L. Gaddy and E. C. Clausen, "Method of separation of sugars and concentrated sulfuric acid," USA Patent 4,608,245, 1986.

[79] G. K. Kafle and L. Chen, “Comparison on batch anaerobic digestion of five different livestock manures and prediction of biochemical methane potential (BMP) using different statistical models”, *Waste Management,* vol. 48, pp. 492-502, 2016.

[80] H. B. Møller, V. Moset, M. Brask, M. R. Weisbjerg, and P. Lund, “Feces composition and manure derived methane yield from dairy cows: Influence of diet with focus on fat supplement and roughage type”, *Atmospheric Environment,* vol. 94, pp. 36-43, 2014.

[81] A. M. Viturtia, J. Mata-Alvarez, F. Cecchi, and G. Fazzini, “Two-phase anaerobic digestion of a mixture of fruit and vegetable wastes”, *Biological Wastes,* vol. 29, no. 3, pp. 189-199, 1989,.

[82] J. P. Lee, J. S. Lee, and S. C. Park, “Two-Phase Methanization of Food Wastes in Pilot Scale”, in *Twentieth Symposium on Biotechnology for Fuels and Chemicals. Applied Biochemistry and Biotechnology*, B. H. Davison and M. Finkelstein Eds. Totowa, NJ: Humana Press, 1999, pp. 585-593.

[83] I. Angelidaki and B. K. Ahring, “Thermophilic anaerobic digestion of livestock waste: the effect of ammonia”, *Applied Microbiology and Biotechnology,* vol. 38, no. 4, pp. 560-564, 1993.

[84] D. J. Hills, “Effects of carbon: Nitrogen ratio on anaerobic digestion of dairy manure”, *Agricultural Wastes,* vol. 1, no. 4, pp. 267-278, 1979.

[85] A. Wensley and P. Champagne, "Effect of Sulfidity on the Corrosivity of White, Green, and Black Liquors", presented at the CORROSION 99, San Antonio, Texas, 1999.

[86] B.-H. Um and G. P. van Walsum, “Acid Hydrolysis of Hemicellulose in Green Liquor Pre-Pulping Extract of Mixed Northern Hardwoods”, *Applied Biochemistry and Biotechnology,* vol. 153, no. 1, pp. 127, 2009.

[87] E. Hasanzadeh, S. Mirmohamadsadeghi, and K. Karimi, “Enhancing energy production from waste textile by hydrolysis of synthetic parts”, *Fuel,* vol. 218, pp. 41-48, 2018.

[88] E. Gholamzad, K. Karimi, and M. Masoomi, “Effective conversion of waste polyester–cotton textile to ethanol and recovery of polyester by alkaline pretreatment”, *Chemical Engineering Journal,* vol. 253, pp. 40-45, 2014.

[89] A. Jeihanipour and M. J. Taherzadeh, “Ethanol production from cotton-based waste textiles”, *Bioresource Technology,* vol. 100, no. 2, pp. 1007-1010, 2009.

[90] A. Jeihanipour, K. Karimi, C. Niklasson, and M. J. Taherzadeh, “A novel process for ethanol or biogas production from cellulose in blended-fibers waste textiles”, *Waste Management,* vol. 30, no. 12, pp. 2504-2509, 2010.

[91] F. Hong, X. Guo, S. Zhang, S.-f. Han, G. Yang, and L. J. Jönsson, “Bacterial cellulose production from cotton-based waste textiles: Enzymatic saccharification enhanced by ionic liquid pretreatment”, *Bioresource Technology,* vol. 104, pp. 503-508, 2012.

[92] T. Jeoh, C. I. Ishizawa, M. F. Davis, M. E. Himmel, W. S. Adney, and D. K. Johnson, “Cellulase digestibility of pretreated biomass is limited by cellulose accessibility”, *Biotechnology and Bioengineering,* vol. 98, no. 1, pp. 112-22, 2007.

[93] M. Gubitosi *et al.*, “Stable, metastable and unstable cellulose solutions”, *Royal Society Open Science,* vol. 4, no. 8, p. 170487, 2017.

[94] E. Sjöström, *Wood chemistry: fundamentals and applications*. 2nd ed. New York, New York: Academic Press, 1993.

[95] T. Okano and A. Sarko, “Mercerization of cellulose. II. Alkali–cellulose intermediates and a possible mercerization mechanism”, *Journal of Applied Polymer Science,* vol. 30, no. 1, pp. 325-332, 1985.

[96] B. Medronho, A. Romano, M. G. Miguel, L. Stigsson, and B. Lindman, “Rationalizing cellulose (in)solubility: reviewing basic physicochemical aspects and role of hydrophobic interactions”, *Cellulose,* vol. 19, no. 3, pp. 581-587, 2012.

[97] M. Bergenstråhle-Wohlert, T. Angles d’Ortoli, N. A. Sjöberg, G. Widmalm, and J. Wohlert, “On the anomalous temperature dependence of cellulose aqueous solubility”, *Cellulose,* vol. 23, no. 4, pp. 2375-2387, 2016.

[98] A. C. O'sullivan, “Cellulose: the structure slowly unravels”, *Cellulose,* vol. 4, no. 3, pp. 173-207, 1997.

[99] P. J. Wakelyn *et al.*, “Cotton fibers”, in *Handbook of Fiber Chemistry*, M. Lewin Ed. Boca Raton, Florida: CRC Press, 2006, pp. 607-608.

[100] R. J. Wooley and V. Putsche, “Development of an ASPEN PLUS Physical Property Database for Biofuels Components”, Golden, Colorado, NREL/TP-425-20685, 1996. [Online]. Available: https://www.nrel.gov/docs/legosti/old/20685.pdf

[101] B. Frankó, M. Galbe, and O. Wallberg, “Bioethanol production from forestry residues: A comparative techno-economic analysis”, *Applied Energy,* vol. 184, pp. 727-736, 2016.

[102] D. Humbird *et al.*, “Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol”, Golden, Colorado, NREL/TP-5100-47764, 2011. [Online]. Available: https://www.nrel.gov/docs/fy11osti/47764.pdf

[103] E. Joelsson, D. Dienes, K. Kovacs, M. Galbe, and O. Wallberg, “Combined production of biogas and ethanol at high solids loading from wheat straw impregnated with acetic acid: experimental study and techno-economic evaluation”, *Sustainable Chemical Processes,* vol. 4, no. 1, p. 14, 2016.

[104] A. Susmozas, A. D. Moreno, J. M. Romero-García, P. Manzanares, and M. Ballesteros, “Designing an olive tree pruning biorefinery for the production of bioethanol, xylitol and antioxidants: a techno-economic assessment”, *Holzforschung,* vol. 73, no. 1, pp. 15-23, 2019.

[105] Y. Cortes-Peña, D. Kumar, V. Singh, and J. S. Guest, “BioSTEAM: A Fast and Flexible Platform for the Design, Simulation, and Techno-Economic Analysis of Biorefineries under Uncertainty”, *ACS Sustainable Chemistry & Engineering,* vol. 8, no. 8, pp. 3302-3310, 2020.

[106] Y. Cortes-Peña. “Bioindustrial-Park: BioSTEAM's Premier Repository for Biorefinery Models and Results”. https://github.com/yoelcortes/Bioindustrial-Park (accessed Jan 2020).

[107] W. D. Seider, D. R. Lewin, J. D. Seader, S. Widagdo, R. Gani, and K. M. Ng, *Product and process design principles: synthesis, analysis, and evaluation*, 3rd ed. Hoboken, New Jersey: Wiley, 2017.

[108] S. Kim and B. E. Dale, “All biomass is local: The cost, volume produced, and global warming impact of cellulosic biofuels depend strongly on logistics and local conditions”, *Biofuels, Bioproducts and Biorefining,* vol. 9, no. 4, pp. 422-434, 2015.

[109] T. Wallace, “Sampling liquid manure for analysis”, Alberta Agriculture and Food, Canada, Agdex 538-2, 2008.

[110] G. A. Akerlof, “The Market for "Lemons": Quality Uncertainty and the Market Mechanism”, *The Quarterly Journal of Economics,* vol. 84, no. 3, p. 488, 1970.

[111] European Commission, “Roadmap for moving to a competitive low carbon economy in 2050”, Brussels, Belgium, COM(2011) 112, 2011. [Online]. Available: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2011:0112:FIN:EN:PDF

[112] M. Jaeger-Erben, C. Jensen, F. Hofmann, and J. Zwiers, “There is no sustainable circular economy without a circular society”, *Resources, Conservation and Recycling,* vol. 168, p. 105476, 2021.

[113] U. A. Cullen and R. De Angelis, “Circular entrepreneurship: A business model perspective”, *Resources, Conservation and Recycling,* vol. 168, p. 105300, 2020.

[114] J. Grafström and S. Aasma, “Breaking Circular Economy Barriers”, *Journal of Cleaner Production,* vol. 292, p. 126002, 2021.

[115] T. Siderius and K. Poldner, “Reconsidering the Circular Economy Rebound effect: Propositions from a case study of the Dutch Circular Textile Valley”, *Journal of Cleaner Production,* vol. 293, p. 125996, 2021.

[116] S. Wydra, B. Hüsing, J. Köhler, A. Schwarz, E. Schirrmeister, and A. Voglhuber-Slavinsky, “Transition to the bioeconomy – Analysis and scenarios for selected niches”, *Journal of Cleaner Production,* vol. 294, p. 126092, 2021.

Paper I

Waste and Biomass Valorization (2020) 11:2387–2395
https://doi.org/10.1007/s12649-018-00578-6

ORIGINAL PAPER

Analysis of Animal Bedding Heterogeneity for Potential Use in Biorefineries Based on Farmyard Manure

Miguel Sanchis-Sebastiá[1] · **Borbála Erdei**[1] · **Krisztina Kovacs**[1] · **Mats Galbe**[1] · **Ola Wallberg**[1]

Received: 30 July 2018 / Accepted: 30 December 2018 / Published online: 5 January 2019

Abstract

Purpose Animal bedding, a mixture of straw and manure, could be used as a feedstock for bioenergy production, but its heterogeneity poses new challenges in its use in biorefineries. We have investigated the origin of this heterogeneity and quantified it, and discuss its impact on bioenergy production.

Methods Samples were collected from the bedding at different points and depths and analysed by first separating the manure from the straw by means of washing with water, and then determining the chemical composition of the liquid and solid fractions.

Results The results supported our hypothesis that animal bedding behaves as a combination of several layers at different stages of degradation. Analysis revealed that the layers with higher organic content in the manure exhibited a poorer performance during the washing, since the residence time in the barn alters the washing profile of the organic fraction in the manure. It was also found that the variability in the composition of animal bedding was much greater than in other agricultural feedstocks: the manure content in animal bedding varied from 26 to 41%, and the content of fermentable carbohydrates varied by 20%. Total carbon and total nitrogen analyses showed that these changes in composition also affected the C/N ratio of the material, and thus its suitability as a feedstock for anaerobic digestion.

Conclusions This implies that the residence time in the barn affects not only the heterogeneity of the properties of animal bedding, but also the best way to process it in a biorefinery.

Keywords Animal bedding · Manure · Compositional analysis · Bioenergy

Statement of Novelty

Our work presents a new methodology for the analysis of animal bedding and studies its heterogeneity for the first time, which affects the design of biorefineries and the managing of the farm.

Introduction

Since the industrial revolution, Western economies have been based on the linear consumption of natural resources [1], which has had both economic and environmental effects. Resource depletion, especially since 2000, has resulted in a marked increase in the price of natural resources [1], while unsustainable practices are degrading our environment [2, 3]. To remedy this situation, many governments and companies are taking steps to establish a circular economy, to replace current practices [4]. Such a shift towards a circular economy calls for renewable energy sources, where biomass will play an important role in changing the current energy production paradigm, in which 75% of the energy generated worldwide currently originates from non-renewable sources [4]. The workhorse of this new bioeconomy will be biorefineries [5], i.e., facilities where biomass is converted into a range of products, including biofuels, to maximize raw material usage [6].

✉ Miguel Sanchis-Sebastiá
miguel.sanchis_sebastia@chemeng.lth.se

Borbála Erdei
borbala.erdei@chemeng.lth.se

Krisztina Kovacs
krisztina.kovacs@chemeng.lth.se

Mats Galbe
mats.galbe@chemeng.lth.se

Ola Wallberg
ola.wallberg@chemeng.lth.se

[1] Department of Chemical Engineering, Lund University, P.O. Box 124, 221 00 Lund, Sweden

An interesting agricultural residue in this context is animal bedding, which is a mixture of straw and manure. This residue has advantages as a potential feedstock for biofuel production over other agricultural residues more widely investigated, such as wheat straw, corn stover and sugarcane bagasse. In particular, the cost of animal bedding is lower than that of other residues, and it would also reduce the environmental impact of the agricultural sector in terms of greenhouse gas emissions from the manure itself, nutrient leaching from its use as a fertilizer, and waste generation [7].

Despite its considerable potential, no attempt has yet been made to use animal bedding for biofuel production, on demonstration scale or commercial scale, although some research has been carried out on laboratory scale [8–10]. One possible reason for this is the heterogeneity of animal bedding, which complicates its use as a feedstock in biorefineries [11, 12]. The problems associated with this heterogeneity are feedstock variability and the Maillard reaction.

The chemical and physical properties of manure vary considerably between different animals and farms [13]. Furthermore, changes take place in its composition with time after excretion due to combination with urine, bacterial decomposition, ambient temperature and moisture, and admixture with soil [14]. This would be especially the case in animal bedding in some areas of Denmark, where the technique of spreading fresh straw in the barn every day increases the heterogeneity. Feedstock variability leads to problems in a biorefinery, as a homogeneous feedstock is desirable. Studies on bioenergy production from manure are usually based on the average composition obtained from several samples [9, 10, 14], thus information is lacking on the variability in composition, and how this may affect energy production.

Regarding the Maillard reaction, manure contains a higher amount of nitrogen than other agricultural waste, such as wheat straw [15], which leads to the risk of the loss of reducing sugars due to their reaction at elevated temperatures with nitrogen compounds (e.g. ammonia, primary and secondary amines) once they are released from the carbohydrates contained in animal bedding [16, 17]. The best way to design a biorefinery so as to minimize the loss of substrate through the Maillard reaction is currently the subject of debate in the scientific community. Some authors have proposed the use of animal bedding in an anaerobic digester to produce biogas, and subsequent processing of the digestate for ethanol or fertilizer production [10, 18]. Others have proposed washing with water to separate the manure from the straw, so that the fibre can be hydrolysed separately without the risk of reducing sugars reacting with nitrous compounds [9, 11, 15]. However, no consensus has been reached on the best way to use animal bedding in a biorefinery.

The aim of this study was to explain the variability of animal bedding by proposing the following hypothesis: animal bedding can be described as the linear combination of several layers at different stages of degradation. Each of these layers was analysed separately, as opposed to previous studies where only values for the bulk were presented, to quantify the differences in terms of the washing profile and composition. In addition, we contribute to the discussion on the processing of manure-containing feedstocks by proposing a novel approach based on comparing the C/N ratios in animal bedding with optimal values for biogas production. Based on the implications of this study, it could be said that our new analysis methodology has the potential to be extrapolated to analyse similar materials and investigate other farms.

Materials and Methods

Animal Bedding Collection and Sampling

Animal bedding from a dairy farm at Lille Skensved, a small town close to Køge (Denmark), was studied. The barn has a rectangular shape, approximately 600 m^2, hosts 150 dairy cows in loose housing regime and approximately 500 ton of straw per year are used as bedding. Samples were collected on three different occasions in September 2016, January 2017 and September 2017. The material was stored frozen before being analysed, according to previous recommendations [19].

On the first two occasions, samples were taken from an outdoor pile where animal bedding was stored after cleaning the barn. Material from these two samples was used to create artificial mixtures of bedding at different stages of degradation in order to test the hypothesis of an ideal mixture. The material collected in September 2016 (S16) had remained in the barn considerably longer than that collected in January 2017 (J17), and can therefore be expected to show a higher degree of degradation.

The samples collected in September 2017 (S17) were taken from several places in the barn after the accumulation of bedding for 5 weeks. Non-agitated systems can exhibit considerable variability in manure composition [20], and it has therefore been recommended that at least around 40 samples be collected to obtain representative mean values [21]. Following these recommendations, 13 sampling positions were established: 8 around the perimeter of the barn, 1 in the centre and 4 along the diagonals (Fig. 1), where the greatest variability was expected [22]. Three different samples were collected at each sampling position: bedding from the top layer, bedding from the middle layer and bedding from the bottom layer. This gives 39 samples in total, which should ensure representability.

After the sample collection, a subsample was taken from each of the 13 positions after thorough mixing of the material in a concrete mixer. The 13 subsamples were then

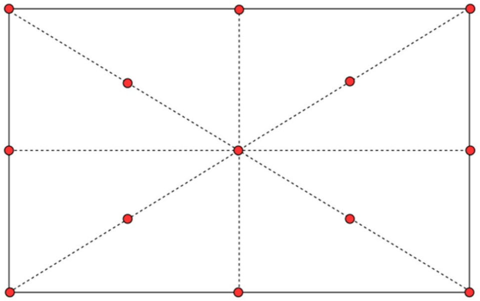


Fig. 1 Sampling positions in the barn

mixed to create an average sample that is representative of the whole barn. This procedure was repeated for each of the three samples from different layers of the bedding, rendering three average samples: top layer, middle layer, and bottom layer.

Characterization of the Washing: Washing Profile and Washing Efficiency

Four hundred g of wet animal bedding was mixed with 1 L distilled water and then pressed in a filter press at 6 bar to remove the liquid. The procedure was repeated until a total of ten washing cycles had been performed. The weight of the expressed liquid was recorded, and liquid samples were taken for further analysis after each washing cycle.

The results of the washing were characterized by two parameters: the mass removed and the washing efficiency. The first is the accumulated mass removed during the ten washing cycles, while the second is the ratio of the mass removed in the nth cycle to the total mass removed in all ten washing cycles (see Eqs. 1 & 2).

$$\text{Mass removed}_n = \frac{\sum_{i=1}^{i=n} M_{liquid,i} \cdot TS_i}{\text{bedding mass} \cdot TS_{bedding}} \tag{1}$$

$$\text{Washing efficiency}_n = \frac{M_{liquid,n} \cdot TS_n}{\sum_{i=1}^{i=10} M_{liquid,i} \cdot TS_i} \tag{2}$$

where $M_{liquid,i}$ is the weight of the expressed liquid after washing cycle i; TS_i the total solids content of the liquid after washing cycle i; bedding mass is the initial animal bedding weight in the first washing cycle; $TS_{bedding}$ the total solids content of the bedding; $M_{liquid,n}$ is the weight of the expressed liquid after washing cycle n; and TS_n the total solids content of the liquid after washing cycle n.

Compositional Analysis

Animal bedding was considered to be a mixture of two components: manure and straw. The manure content in the animal bedding was assumed to be equal to the proportion of mass removed during washing, while the rest was assumed to be straw. Soluble organic carbon from the straw is a source of error in this methodology, but to a minor extent since only a small portion of the soluble organic carbon would be transferred to the liquid when using room temperature water. In fact, the National Renewable Energy Laboratory (NREL) analysis methods recommend the use of much higher temperature (around 100 °C) to successfully extract all these components from the straw [23]. Thus, we consider that our assumption is true as the mentioned effect is negligible.

Each of these fractions was further analysed to determine its chemical composition, and each analysis was performed in triplicate. The composition of the animal bedding was then obtained by multiplying the composition of manure and straw by their respective content in the mixture.

Analysis of the Washed Straw

The solid material remaining after the ten washing cycles was dried at 45 °C and milled to a particle size of 1 mm prior to analysis using the methodology described by NREL. The ash content was determined by incineration at 575 °C for 3 h [24]. The extractives content corresponded to the mass removed by 24 h of water extraction followed by 24 h of ethanol extraction [23]. Double-step acid hydrolysis was performed on the extracted fibre to determine the content of structural carbohydrates and lignin [25].

The sugar content in the structural carbohydrates and lignin analysis was determined using high-performance anion-exchange chromatography coupled with pulsed amperometric detection. A Dionex system with a Carbo Pac PA1 column, a GP50 gradient pump and an AS50 autosampler were used. The flow rate was 1 mL/min and the solutions used as eluents were: deionized water, 200 mmol/L sodium hydroxide, and 200 mmol/L sodium hydroxide mixed with 170 mmol/L sodium acetate.

Samples of the washed fibre were sent to an external laboratory, where total carbon and total nitrogen were analysed with a Vario Max CN elemental analyser (Elementar, Langenselbold, Germany). The operating principle of this equipment consists of combusting the sample at 850–1150 °C and analysing the exhaust gas.

Analysis of the Washing Liquid

The total solids (TS) in the washing liquid after each cycle was analysed by drying an aliquot at 105 °C overnight. The resulting solids were incinerated at 575 °C for 3 h to determine the organic matter content. The inorganic matter content was calculated as the difference between the TS and the organic matter content.

Lange cuvette tests (LCK 338 and LCK 138) were used to determine the total nitrogen in the washing liquids. This analysis consists of converting the nitrogen compounds to one species through chemical treatments and subsequently measure the absorbance of this species to determine the nitrogen content. The spectrophotometer used was a Hach Lange DR2800. Total carbon was analysed with a TOC-5050A Shimadzu total organic carbon analyser, equipped with an ASI-5000A autosampler. This analysis is based on combusting the sample at 680 °C and then determining the CO_2 content in the exhaust gas.

Results and Discussion

Validation of the Ideal Mixture Hypothesis

Animal bedding was collected on two occasions from an outdoor pile after the barn had been cleaned. The material collected in September 2016 showed a higher degree of degradation than the material collected in January 2017. This is indicated by the higher manure content, as can be seen in Fig. 2a: S16 contained 52% manure while J17 contained 38%. S16 not only contained more manure, the fibre in this material also showed a higher degree of degradation. For example, S16 contained 18.4% hexoses, while the content in J17 was 27.2% (Table 1). The chemical composition of the samples thus corroborates the visual assessment of the degree of degradation of the materials.

Table 1 Predicted and experimental compositions of the 50:50 mixture of S16 and J17 and the experimentally determined compositions of materials S16 and J17

Content (%TS)	S16 (%)	J17 (%)	50:50 S16 & J17 Experimental (%)	50:50 S16 & J17 Predicted (%)
Total solids	28.2	29.3	26.8	28.8
Manure	52.0	38.1	43.9	44.9
Fibre	48.0	61.9	56.1	55.1
Glucan	17.8	26.3	21.3	21.9
Xylan	11.4	16.6	13.9	13.9
Galactan	0.1	0.3	1.3	0.2
Arabinan	0.7	1.3	2.0	1.0
Mannan	0.5	0.6	1.2	0.5
Lignin	13.8	13.6	13.5	13.9
Ash	2.8	1.8	2.1	2.4
Extractives	2.5	3.8	4.3	3.1

The degree of degradation had a clear effect on the washing of the materials. Manure was removed from S16 at a slower rate than from J17 (Fig. 2). J17 showed a high initial washing efficiency, which decreased rapidly after a few washing cycles. 80% of the manure was removed during the first two washing cycles, and only residual amounts were removed in the subsequent cycles. The slower decrease in washing efficiency for S16 indicates that manure was not removed as quickly as from J17 (60% removal in the first two cycles), but that removal was distributed over more washing cycles. This can be seen in Fig. 2b.

Since S16 was more degraded than J17, mixing the two materials may provide a mixture that resembles animal bedding in the barn. Figure 3 shows the results obtained for

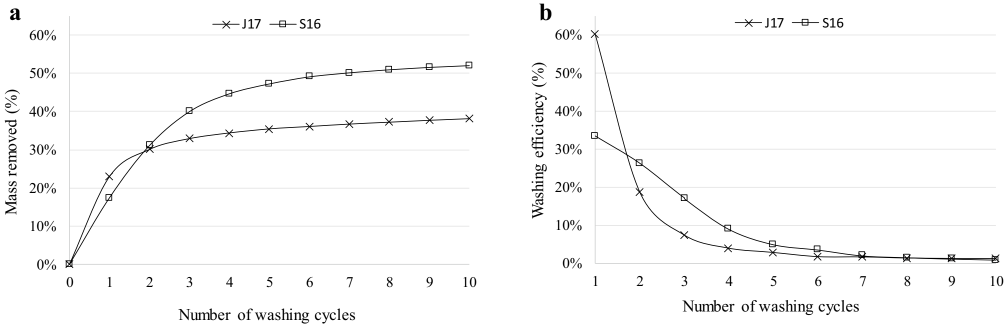


Fig. 2 Mass removed (**a**) and washing efficiency (**b**) after each washing cycle for materials S16 (more decomposed) and J17 (less decomposed)

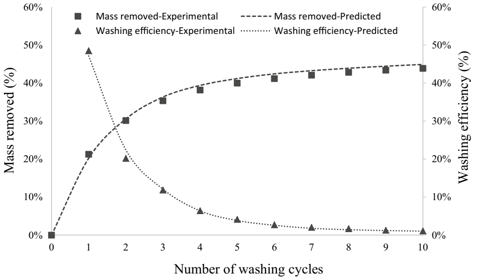


Fig. 3 Predicted and experimental values of the mass removed and washing efficiency for a 50:50 mixture of S16 and J17 as a function of washing cycle

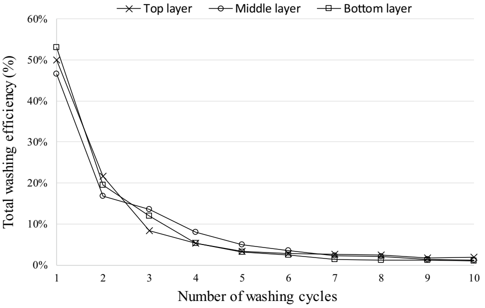


Fig. 4 Washing efficiency in each of the washing cycles for the top, middle and bottom layers in material S17

a 50:50 mixture of S16 and J17 during washing, and the predicted values based on the ideal mixture hypothesis (i.e. linear combination). It can be seen that there is good agreement between the experimental and predicted values, which means that the profiles for the 50:50 mixture are the same as the average of the profiles for S16 and J17, thus proving that our hypothesis was correct. A similarly good fit was obtained when performing the same experiment with a 25:75 mixture of S16 and J17 (data not shown), which further confirms our hypothesis.

The results of the chemical analysis provided some evidence that the ideal mixture hypothesis may also apply to the chemical composition (Table 1). However, accurate values were only obtained for the main components in animal bedding: manure, glucan, xylan and lignin, while the determination of minor carbohydrates (galactan, arabinan and mannan), ash and extractives was not sufficiently accurate to confirm this.

The verification of the ideal mixture hypothesis describes the heterogeneity of animal bedding: the properties of animal bedding result from the mixing of several fractions with different properties due to different stages of degradation. The proportion of each of these fractions in the material would be key in assessing the quality of the material, and therefore in establishing its value and possible price. In principle, our findings suggest that it is possible to measure the properties of each fraction and those of the mixture, and then calculate the amount of each fraction in animal bedding using the washing profile and the content of glucan, xylan and lignin. However, due to practical problems during the sampling procedure, it is difficult to validate this principle empirically. In order to validate the principle, it would be needed to develop a sampling method that does not modify the amount of each fraction during the collection, i.e. the amount of each fraction in the sample is the same as in the native material.

Effect of Degree of Degradation on Washing

After our hypothesis had been verified, material S17 was collected to identify the different fractions in animal bedding and to quantify the differences in their properties. It was observed that each of the layers had a slightly different washing efficiency profile (Fig. 4), which confirms that the degree of degradation influences the behaviour of the material during washing. The reproducibility of the analysis was investigated by washing the bottom layer of material S17 twice, where greater variability was expected due to its higher degree of degradation. Due to the lack of replicates in the analysis of other samples, errors could not be calculated, but the small difference between the two replicates from the bottom layer of S17 indicates that these should be small. Thus, the differences are significant compared to the standard deviation, i.e. the different curves do not overlap when considering the standard deviation and therefore it can be said that pure experimental error is not enough to explain such differences, even if they are small.

It can then be said that manure was removed more efficiently from the top layer than from the middle and bottom layers, which indicates that manure becomes more attached to the straw during degradation of animal bedding, making the washing more difficult. Interestingly, the middle layer showed a lower removal rate than the bottom layer. For example, after two washing cycles, 63% and 73% of the manure had been removed from the middle and bottom layers, respectively. Thus, loss of performance during washing was not proportional to the residence time in the barn. In other words, it is not necessarily true that the longer animal bedding stays in the barn, the more difficult it is to separate the manure from the straw.

Figure 5 shows the washing efficiency for the inorganic and organic fractions of manure for each of the layers. The removal of inorganic mass was similar in all the layers, but

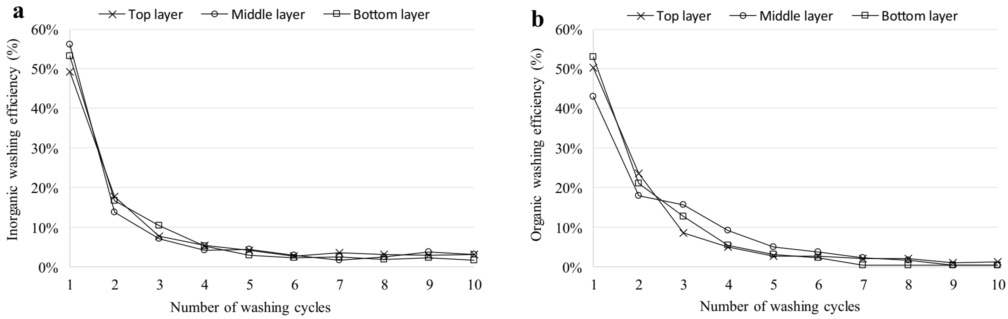


Fig. 5 Washing efficiency for the inorganic (**a**) and organic (**b**) fractions of manure in each of the washing cycles for the top, middle and bottom layers in material S17

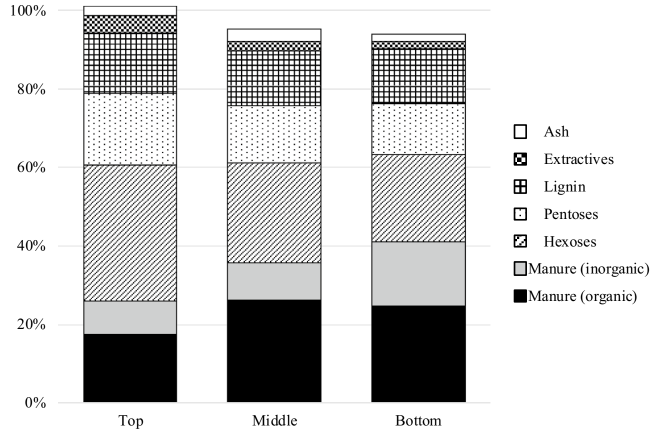
differences were seen in the profiles for organic mass. Furthermore, the patterns for the organic fraction were very similar to those obtained for the washing efficiency based on total mass (Fig. 4). These results imply that the heterogeneous behaviour of the layers during washing is due to different degrees of change in the organic fraction of manure. A longer residence time in the barn, and thus a higher degree of degradation, leads to changes in the organic fraction of manure that modify its behaviour during washing, however, differences in the overall removal rate are counterbalanced by the similar behaviour of the inorganic fraction in all three layers.

Effect of Degree of Degradation on Bedding Composition

Residence time in the barn affects animal bedding composition, as shown by analysis of the fibre and washing liquid after ten washing cycles. The manure content in the bedding was 26%, 36% and 41% in the top, middle and bottom layers (average samples), respectively (Fig. 6). This increase in manure content is probably due to the sinking of manure towards the bottom due to gravity. These values are in agreement with compositions reported previously [11, 12], and show that the longer the bedding remains in the barn, the higher its manure content or, the lower its fibre content.

Apart from affecting the content of manure in the mixture, residence time also had an effect on the composition of the manure. The manure composition in each layer was different as residence time did not affect inorganic and organic

Fig. 6 Chemical composition of each of the layers in material S17, expressed as % TS



compounds in the same way. Inorganic compounds increased with increasing residence time, but the highest organic fraction was found in the middle layer, which means that the organic compounds in manure started to decrease after a certain residence time (Fig. 6). This could explain the observed pattern in the washing of the layers, i.e. the middle layer was the most difficult to wash because the manure in this layer contained the highest fraction of organic compounds, the washing of which was modified by residence time. The total manure content in the middle layer was approximately 10% more than in the top layer, the main reason being an increase in organic compounds, 9% more than in the top layer (Fig. 6). However, the origin of the 5% higher amount of manure in the bottom layer than in the middle layer was that the inorganic fraction increased by 7% while the organic fraction decreased by 2%. A possible explanation of this may be that the methanogenic bacteria present in the manure degrade organic matter to methane and carbon dioxide [26], while inorganic matter (e.g., sand, gravel, stones, etc.) increases due to mixing with soil from the barn [14].

The composition of the other component in animal bedding, straw, also underwent changes with increased residence time. The top, middle and bottom layers had hexose contents of 35%, 25% and 22%, and pentose contents of 18%, 14% and 13%, respectively (Fig. 6), which are similar to values reported previously [11, 12]. This accounts for a variation of almost 20% in the content of fermentable carbohydrates: 13% difference in hexose content and 5% in pentose content. This decrease in fermentable carbohydrates resulted from the combination of two factors: a lower amount of straw in the mixture and a lower amount of fermentable carbohydrates in the straw. The decrease in hexose and pentose contents in the straw could be due to the high cellulolytic activity of microorganisms in rumen fluid, which makes these bacteria very efficient in degrading lignocellulosic material [27, 28].

An interesting observation is that the variability in the composition of the animal bedding found in this study was much greater than that in other agricultural waste investigated for bioenergy production. Wheat straw from the same source showed only an 8% variation in fermentable carbohydrate content between different occasions [29, 30], which is the same variation as in wheat straw from another source [31, 32]. The variations between wheat straw from several sources are also smaller than the variation in animal bedding from the same occasion. Thus, the variability in the composition of feedstock for bioenergy production when using animal bedding can be expected to be more than twice that when using wheat straw. This will have important implications on the planning of biomass supply to the biorefinery as the amount of substrate available for bioenergy production per ton of biomass could vary considerably.

These compositional changes strongly affect the bioenergy potential of animal bedding. During the time spent in the barn, fresh bedding, consisting of straw with some manure (top layer), is transformed into degraded straw with a high manure content (bottom layer). Initially, the loss of bioenergy potential of the straw due to a decrease in its fermentable carbohydrate content would be counteracted by the higher potential of the manure, due to the increase in its organic fraction. However, the organic fraction in manure starts to decrease after a few weeks residence time, which means that the potential of both the straw and manure would decrease, seriously compromising the usability of this feedstock in a biorefinery. This suggests that farmers could increase the value of their waste by cleaning the barn more often, since this would reduce the residence time in the barn, thus avoiding the formation of low-quality fractions, leading to a decrease in bioenergy production, and thus revenue. However, this would lead to higher costs for the farmer, both in terms of raw material and labour.

C/N Ratio as an Indication of the Best Processing Technology

The analysis of the chemical composition of each layer was complemented with the determination of the total carbon and total nitrogen content in order to estimate the C/N ratio. It can be seen from Table 2 that residence time in the barn had little effect on the total carbon and total nitrogen contents of the straw in animal bedding. However, it affected the nitrogen content of the manure; the total nitrogen content almost doubling between the top and bottom layers. This means that the nitrogen content in animal bedding increases with increasing residence time in the barn, due, not only, to an increased manure content, but also to a higher nitrogen content in the manure.

This variation in nitrogen content results in a modification of the C/N ratio in animal bedding, being 56, 38 and 30 for the top, middle and bottom layers, respectively. This difference in C/N ratio affects the suitability of the material for anaerobic digestion, since this process is best operated at a C/N ratio of 25–30 [33, 34]. Thus, the bottom layer of animal bedding, with the highest degree of degradation, is

Table 2 Total carbon and total nitrogen contents in each of the layers in material S17

Content (%TS)	Top layer (%)	Middle layer (%)	Bottom layer (%)
Manure			
Total carbon	22.4	24.3	24.7
Total nitrogen	1.3	2.0	2.4
Straw			
Total carbon	45.6	46.7	46.5
Total nitrogen	0.5	0.5	0.5

suitable for anaerobic digestion, in terms of the C/N ratio, while the top and middle layers contain too much carbon.

We therefore suggest that high-quality animal bedding (short time in the barn) be processed using a biorefinery concept in which carbon and nitrogen are separated, for example, by removing the manure through washing with water. If correctly designed, this would render a stream containing mainly carbon with little nitrogen, and the carbohydrates could be hydrolysed without the risk of the Maillard reaction, even if the process is performed at elevated temperatures [15], and another stream with an optimal C/N ratio for anaerobic digestion, which could be used for biogas production. However, above a certain degree of degradation, it seems more suitable to feed the material directly to an anaerobic digester. Further research should be directed towards establishing more appropriate indicators for the quality of animal bedding, and using them together with techno-economic calculations to determine this threshold.

Conclusions

In this study, we have investigated and quantified the heterogeneity of animal bedding, and discussed its impact on bioenergy production. The results show that the heterogeneous nature of animal bedding originates from the fact that the material is a combination of several layers at different stages of degradation. These layers exhibit differences in both their washing and composition. It appears that washing is modified as a result of changes in the removal rate of the organic fraction of the manure. Regarding compositional differences, increasing the residence time in the barn not only increases the manure/straw ratio in animal bedding, but also modifies the composition of both the straw and manure: altering the organic fraction in manure and decreasing the content of fermentable carbohydrates in the straw. These changes result in a reduction in the quality of the material that influences its potential for bioenergy production, and suggest that farmers could increase the value of their residue by cleaning the barn more often. Furthermore, changes in the composition of animal bedding will also affect the best way to process the material, which means that operations at the farm would affect the design of the biorefinery. In terms of the C/N ratio, the results of this study suggest that high-quality bedding could be processed in a biorefinery where the carbon and nitrogen are separated, however, above a certain degree of degradation it would be more suitable to use the material directly for biogas production.

Acknowledgements This work was financially supported by the Swedish Energy Agency and BESTF3 (Grant No. P42674-1). The authors are also grateful to TK Energy A/S and Niels Lundager for their help in the collection of the animal bedding and to Margaréta Rozbach for her participation in the experimental work.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Ellen MacArthur Foundation (EMAF): Towards the circular economy. https://www.ellenmacarthurfoundation.org/assets/downloads/publications/Ellen-MacArthur-Foundation-Towards-the-Circular-Economy-vol.1.pdf (2013)
2. Millennium Ecosystem Assessment: Ecosystems and Human Well-Being: Synthesis. Island Press, Washington, DC (2005)
3. Millennium Ecosystem Assessment: Ecosystems and Human Well-being: Current State and Trends. Island Press, Washington, DC (2005)
4. Korhonen, J., Honkasalo, A., Seppälä, J.: Circular economy: the concept and its limitations. Ecol. Econ. **143**, 37–46 (2018). https://doi.org/10.1016/j.ecolecon.2017.06.041
5. Demirbas, A.: Biorefineries: For Biomass Upgrading Facilities, vol. 26. Green Energy and Technology. Springer, London (2010)
6. Rabaçal, M., Ferreira, A.F., Silva, C.A., Costa, M.: Biorefineries: Targeting Energy, High Value Products and Waste Valorisation, vol. 57. Springer, Cham (2017)
7. Liebig, M.A., Franzluebbers, A.J., Follett, R.F.: Agriculture and climate change: mitigation opportunities and adaptation imperatives. In: Managing Agricultural Greenhouse Gases, pp. 3–11. Academic Press, San Diego (2012)
8. Vancov, T., Schneider, R.C., Palmer, J., McIntosh, S., Stuetz, R.: Potential use of feedlot cattle manure for bioethanol production. Bioresour. Technol. **183**, 120–128 (2015). https://doi.org/10.1016/j.biortech.2015.02.027
9. Woldesenbet, G.A., Shiferaw, G., Chandravanshi, B.S.: Bioethanol production from poultry manure at Bonga Poultry Farm in Ethiopia. Afr. J. Environ. Sci. Technol. **7**(6), 435–440 (2013). https://doi.org/10.5897/ajest2013.1443
10. Elumalai, S., Roa-Espinosa, A., Markley, J.L., Runge, T.M.: Combined sodium hydroxyde and ammonium hydroxide pretreatment of post-biogas digestion dairy manure fiber for cost effective cellulosic bioethanol production. Sustain. Chem. Process. **2**(12), 1–13 (2014). https://doi.org/10.1186/2043-7129-2-12
11. Chen, S., Wen, Z., Liao, W., Liu, C., Kincaid, R.L., Harrison, J.H., Elliott, D.C., Brown, M.D., Stevens, D.J.: Studies into using manure in a biorefinery concept. Appl. Biochem. Biotechnol. **124**(1), 999–1015 (2005). https://doi.org/10.1385/ABAB:124:1-3:0999
12. Bona, D., Vecchiet, A., Pin, M., Fornasier, F., Mondini, C., Guzzon, R., Silvestri, S.: The Biorefinery Concept Applied to Bioethanol and Biomethane Production from Manure.

Waste Biomass Valoriz. **9**(11), 2133–2143 (2017). https://doi.org/10.1007/s12649-017-9981-2

13. Wallace, T.: Sampling Liquid Manure for Analysis. Alberta Agriculture and Food, Alberta (2008)
14. Ward, G.M., Muscato, T.V., Hill, D.A., Hansen, R.W.: Chemical composition of feedlot manure. J. Environ. Qual. **7**(2), 159–164 (1978)
15. Liao, W., Liu, Y., Liu, C., Chen, S.: Optimizing dilute acid hydrolysis of hemicellulose in a nitrogen-rich cellulosic material–dairy manure. Bioresour. Technol. **94**(1), 33–41 (2004). https://doi.org/10.1016/j.biortech.2003.11.011
16. Collins, P.M., Ferrier, R.J.: Monosaccharides: Their Chemistry and Their Roles in Natural Products. Wiley, Chichester (1995)
17. Hodge, J.E.: Dehydrated foods, chemistry of browning reactions in model systems. J. Agric. Food Chem. **1**(15), 928–943 (1953). https://doi.org/10.1021/jf60015a004
18. Yue, Z., Teater, C., Liu, Y., Maclellan, J., Liao, W.: A sustainable pathway of cellulosic ethanol production integrating anaerobic digestion with biorefining. Biotechnol. Bioeng. **105**(6), 1031–1039 (2010). https://doi.org/10.1002/bit.22627
19. Peters, J., Combs, S.M., Hoskins, B., Kovar, J.L., Watson, M.E., Wolf, A.M., Wolf, N.: Recommended Methods of Manure Analysis. University of Wisconsin System, Cooperative Extension Publishing Operations, Madison (2003)
20. Tasistro, A.S., Kissel, D.E., Bush, P.B.: Sampling broiler litter: how many samples are needed? J. Appl. Poult. Res. **13**(2), 163–170 (2004)
21. Dou, Z., Galligan, D.T., Allshouse, R.D., Toth, J.D., Ramberg, C.F., Ferguson, J.D.: Manure sampling for nutrient analysis: variability and sampling efficacy. J. Environ. Qual. **30**(4), 1432–1437 (2001)
22. Roberts, S., Xin, H., Swestka, R., Yum, M., Bregendahl, K.: Spatial variation of manure nutrients and manure sampling strategy in high-rise laying-hen houses. J. Appl. Poult. Res. **25**(3), 428–436 (2016). https://doi.org/10.3382/japr/pfw013
23. Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D.: Determination of Extractives in Biomass. Laboratory Analytical Procedure (LAP). National Renewable Energy Laboratory, Golden (2008)
24. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D.: Determination of Ash in Biomass. Laboratory Analytical Procedure (LAP). National Renewable Energy Laboratory, Golden (2008)
25. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D.: Determination of Structural Carbohydrates and Lignin in Biomass. Laboratory Analytical Procedure (LAP). National Renewable Energy Laboratory, Golden (2008)
26. Ozbayram, E.G., Ince, O., Ince, B., Harms, H., Kleinsteuber, S.: Comparison of rumen and manure microbiomes and implications for the inoculation of anaerobic digesters. Microorganisms **6**(1), 15 (2018)
27. Hu, Z.-H., Yu, H.-Q.: Application of rumen microorganisms for enhanced anaerobic fermentation of corn stover. Process. Biochem. **40**(7), 2371–2377 (2005). https://doi.org/10.1016/j.procbio.2004.09.021
28. Yue, Z.-B., Li, W.-W., Yu, H.-Q.: Application of rumen microorganisms for anaerobic bioconversion of lignocellulosic biomass. Bioresour. Technol. **128**, 738–744 (2013). https://doi.org/10.1016/j.biortech.2012.11.073
29. Erdei, B., Barta, Z., Sipos, B., Réczey, K., Galbe, M., Zacchi, G.: Ethanol production from mixtures of wheat straw and wheat meal. Biotechnol. Biofuels **3**(1), 16 (2010)
30. Linde, M., Jakobsson, E.-L., Galbe, M., Zacchi, G.: Steam pretreatment of dilute H_2SO_4-impregnated wheat straw and SSF with low yeast and enzyme loadings for bioethanol production. Biomass Bioenerg. **32**(4), 326–332 (2008). https://doi.org/10.1016/j.biombioe.2007.09.013
31. Erdei, B., Frankó, B., Galbe, M., Zacchi, G.: Separate hydrolysis and co-fermentation for improved xylose utilization in integrated ethanol production from wheat meal and wheat straw. Biotechnol. Biofuels **5**(1), 12 (2012)
32. Erdei, B., Hancz, D., Galbe, M., Zacchi, G.: SSF of steam-pretreated wheat straw with the addition of saccharified or fermented wheat meal in integrated bioethanol production. Biotechnol. Biofuels **6**(1), 169 (2013)
33. Kayhanian, M.: Ammonia inhibition in high-solids biogasification: an overview and practical solutions. Environ. Technol. **20**(4), 355–365 (1999). https://doi.org/10.1080/09593332008616828
34. Wang, X., Yang, G., Feng, Y., Ren, G., Han, X.: Optimizing feeding composition and carbon–nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. Bioresour. Technol. **120**, 78–83 (2012). https://doi.org/10.1016/j.biortech.2012.06.058

Paper II

Sanchis-Sebastiá *et al. Biotechnol Biofuels* (2019) 12:215
https://doi.org/10.1186/s13068-019-1558-9

Biotechnology for Biofuels

RESEARCH

Open Access

Introducing low-quality feedstocks in bioethanol production: efficient conversion of the lignocellulose fraction of animal bedding through steam pretreatment

Miguel Sanchis-Sebastiá*, Borbála Erdei, Krisztina Kovacs, Mats Galbe and Ola Wallberg

Abstract

Background: Animal bedding remains an underutilized source of raw material for bioethanol production, despite the economic and environmental benefits of its use. Further research concerning the optimization of the production process is needed, as previously tested pretreatment methods have not increased the conversion efficiency to the levels necessary for commercialization of the process.

Results: We propose steam pretreatment of animal bedding, consisting of a mixture of straw and cow manure, to deliver higher ethanol yields. The temperature, residence time and pH were optimized through response-surface modeling, where pretreatment was evaluated based on the ethanol yield obtained through simultaneous saccharification and fermentation of the whole pretreated slurry. The results show that the best conditions for steam pretreatment are 200 °C, for 5 min at pH 2, at which an ethanol yield of about 70% was obtained. Moreover, the model also showed that the pH had the greatest influence on the ethanol yield, followed by the temperature and then the residence time.

Conclusions: Based on these results, it appears that steam pretreatment could unlock the potential of animal bedding, as the same conversion efficiencies were achieved as for higher-quality feedstocks such as wheat straw.

Keywords: Animal bedding, Steam pretreatment, Bioethanol, Response-surface modeling

Background

Lignocellulosic ethanol is still too expensive to compete with fossil fuels on the commercial scale, due to its relatively high production cost, the main contributions being the cost of the feedstock and the capital cost [1]. In fact, the cost of the feedstock can represent as much as one-third of the total production cost [2], and the use of new feedstocks with zero or negative value will be required to achieve cost competitiveness [1]. Animal manure is one example of such a low-value feedstock, and ethanol production could offer a way of valorizing a biomass source that is usually lost otherwise [3]. The use of this material as feedstock would reduce the cost of the raw material in the ethanol production process and, at the same time, alleviate the problem of waste disposal, which would counterbalance the environmental impact of the ethanol production process [3]. Animal manure is thus an attractive feedstock from both the economic and environmental perspectives.

In spite of these advantages, animal manure has been little explored as a resource in bioenergy production [4], although a few studies have been carried out on ethanol production from animal manure. For example, Gomaa et al. concluded that this feedstock had potential as a raw material for biogas and bioethanol production [5]. However, the ethanol yields obtained in their study were low, and they pointed out the need for further research to optimize the production process. Some

---

*Correspondence: miguel.sanchis_sebastia@chemeng.lth.se
Department of Chemical Engineering, Lund University, P.O. Box 124, 221 00 Lund, Sweden

studies have found that a pretreatment step is necessary to enhance the release of sugars from animal manure, and thus improve the ethanol yield [6, 7]. This would be especially the case for animal manure with a high fiber content, such as farmyard cow manure [8]. The effect of acid concentration, pretreatment time and cellulase dosage in pretreatment involving acid hydrolysis followed by enzymatic hydrolysis, on the fermentability of farmyard cow manure has been studied by Vancov et al. [9]. They reported the highest ethanol yield to date of 55% of the theoretical maximum based on the glucose in the raw material. Although this is a significant improvement compared to previous yields of 20% [8], the authors stated that further development was needed to realize the potential of cow manure as a feedstock for bioenergy production [9].

This study was carried out to investigate the effect of steam pretreatment instead of acid hydrolysis. This technology would reduce both the environmental impact and the cost of pretreatment [10], and we hypothesized that higher ethanol yields could be obtained from the fibrous fraction of animal bedding, which is a mixture of straw and cow manure. To validate this hypothesis, we tested several operating conditions in a steam pretreatment reactor to identify the maximum ethanol yield obtainable and compared the results with the yields obtained previously from similar materials. In addition, we modeled the effect of temperature, residence time and pH in the pretreatment step on the ethanol yield to identify trends that explained the results obtained, which could be extrapolated to the design of other processes based on similar materials.

Results and discussion

Raw material composition

Table 1 gives the composition of the raw material and the fiber fraction after washing in a concrete mixer with deionized water at room temperature. Fermentable carbohydrates accounted for almost 40% of the dry mass of the unwashed bedding, which proves that this material could become an important source of substrate for bioethanol production. Moreover, 30% of the dry mass of the unwashed bedding (the organic part of the manure) could potentially be used as a substrate for biogas production, which illustrates the high potential of animal bedding as a resource for bioenergy production, since approximately 70% of its dry mass could be used for this purpose.

The composition of the animal bedding presented in this study is similar to that reported by Bona et al. [8]. However, the manure content is lower, and the fermentable carbohydrate content is higher, than those reported by Vancov et al. [9], while the opposite is true compared with the composition reported by Chen et al. [6]. This variation can be expected, as the composition of such material is affected by many factors, such as the kind and number of animals, their diet, animal housing and time spent in the stable [11].

Table 1 Composition of the animal bedding before and after washing

Content (%DM bedding)	Animal bedding	Fiber after washing
Manure	43.4	10.5
Organic matter	29.7	7.2
Inorganic matter	13.7	3.3
Fiber	56.6	89.5
Glucan	24.1	38.1
Xylan	11.6	18.3
Galactan	0.5	0.8
Arabinan	1.2	1.9
Mannan	0.6	0.9
Lignin	11.8	18.7
Extractives	4.2	6.7
Ash	1.9	2.9

Washing reduced the manure content of the material from 43 to 10% (Table 1), as the average washing efficiency was 75.8% with a standard deviation of 3.6%. After washing, the material has a composition very similar to that of wheat straw [12, 13], despite the fact that the washed fiber still contains a small fraction of manure. Although the residual manure could give rise to the Maillard reaction during pretreatment [14], it can be expected that the washed fiber would behave similarly to wheat straw during steam pretreatment, as the materials have very similar compositions.

Pretreatment

The fiber fraction obtained after washing the material with water was pretreated with steam and subsequently analyzed before its conversion to bioethanol. Rather than discussing the complete composition of all the materials, which can be found in Additional file 1, the intention of this section is to validate the data through checking its consistency with the chemistry of steam pretreatment reported previously in the literature, and comparing the results with those obtained when performing steam pretreatment on wheat straw.

The fiber fraction of the pretreated materials contained 43–59% glucan, 4–14% xylan and 32–38% lignin, depending on the pretreatment conditions, while the liquid fraction contained mainly xylose, at concentrations between 21 and 41 g/L, and only minor amounts of glucose and other sugars. This implies that cellulose

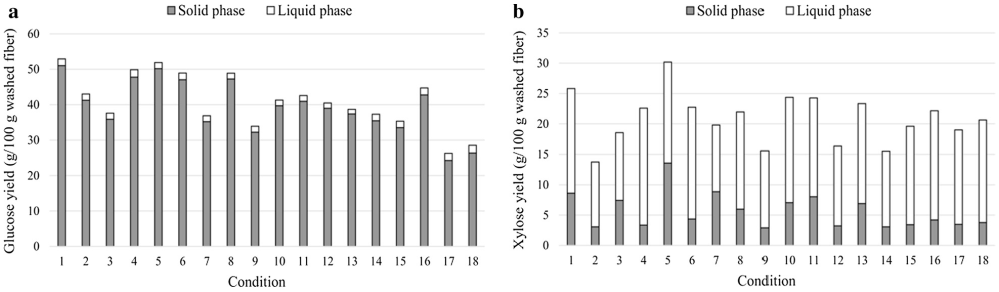


Fig. 1 Yield of glucose (**a**) and xylose (**b**) for each set of conditions in the steam pretreatment of the fiber fraction of animal bedding

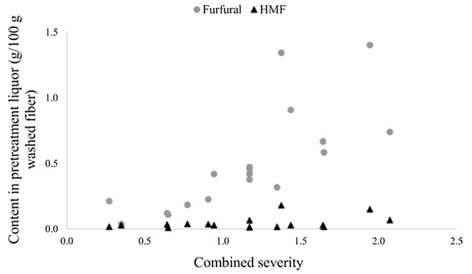


Fig. 2 Furfural and HMF contents in the pretreatment liquor after steam pretreatment of the fiber fraction of animal bedding

and lignin remained mostly in the solid phase after pretreatment, while hemicelluloses were solubilized (Fig. 1), which is consistent with the chemistry of pretreatment performed at low pH [15]. Moreover, these compositions are similar to those reported for steam-exploded wheat straw in previous studies [16, 17], which indicates that washed animal bedding behaves similarly to wheat straw during steam pretreatment.

A fraction of the sugars released during pretreatment was degraded into other by-products, such as furfural and hydroxymethylfurfural (HMF); this effect became more pronounced as the severity of the pretreatment was increased (Fig. 2). This is consistent with the conclusions about carbohydrate degradation during steam pretreatment presented by Li et al. [18]. The generation of by-products during steam pretreatment does not follow the same pattern as for wheat straw, as the furfural production was higher and the HMF production lower than the results reported by Ballesteros et al. [19]. It thus appears that, although sugars are recovered in a similar fashion, carbohydrate degradation during steam pretreatment differs between animal bedding and wheat straw, possibly due to the presence of residual manure in the material that can trigger various degradation mechanisms, such as the Maillard reaction [14].

Despite the differences in by-product generation, the amount of furfural generated during pretreatment is not high enough to compromise the efficacy of fermentation, since furfural concentrations over 3 g/L are necessary to affect the performance of *S.* cerevisiae [20]. Thus, by-product formation does not appear to be critical when pretreating the fiber fraction of animal bedding, as the concentrations of the by-products obtained are not toxic to the fermenting microorganism. However, this may be a problem when using pretreatment techniques that produce a material with a higher dry matter content, as the resulting inhibitor concentrations may be higher.

Simultaneous saccharification and fermentation

The material steam pretreated at each of the conditions tested was converted into ethanol through simultaneous saccharification and fermentation (SSF), and the yield obtained in each case is given in Table 2. The ethanol yield ranged from 36.3 to 69.3% depending on the pretreatment conditions, and the maximum error between duplicates, obtained for conditions 2 and 8, was 0.03 g ethanol/g glucose in the washed fiber.

The maximum yield obtained in this study (69.3% for condition 4) was higher than those previously reported for acid hydrolysis pretreatment, although the results are not strictly comparable since the methods used to perform the biological steps were not the same. For example, a yield of 55.3% has previously been achieved using acid hydrolysis [9], while a lower yield of 22.2% was reported in another study using the same technology [8]. The results obtained with steam pretreatment also compare well to those from other technologies based on high pH, such as the NaOH pretreatment applied by You et al. [21], with which the authors achieved a yield of 39.9%. The

Table 2 Ethanol yield after SSF for each of the conditions tested in the steam pretreatment

Condition	Ethanol yield (g/g)	Ethanol yield (% max theoretical)
1	0.302	59.1
2	0.279	54.7
3	0.228	44.6
4	0.354	69.3
5	0.253	49.5
6	0.318	62.3
7	0.185	36.3
8	0.284	55.7
9	0.262	51.3
10	0.215	42.2
11	0.222	43.4
12	0.288	56.4
13	0.202	39.5
14	0.301	59.0
15	0.248	48.6
16	0.263	51.5
17	0.204	42.7
18	0.300	58.9

Table 3 ANOVA for the model developed to relate ethanol yield to the operational parameters in the pretreatment step

Source	Degrees of freedom	Sum of squares	Mean square
Total	18	48,853.0	2714.1
Mean	1	47,535.0	47,535.0
Corrected	17	1318.6	77.6
Factor effects	10	999.1	99.9
Residuals	8	319.5	39.9
Lack of fit	4	183.5	45.9
Purely experimental uncertainty	3	136.0	45.3

outcome is also favorable when compared to radically different technologies, such as pretreatment by anaerobic digestion followed by NaOH treatment, proposed by Yue et al. [22]. They obtained a highly digestible fiber, leading to high enzymatic hydrolysis and fermentation yields (90% and 72%, respectively) but, based on their mass balances, there is a cellulose loss of 24% during NaOH treatment, which lowers the combined sugar yield based on the original fiber to 46.7%. Thus, it seems that our initial hypothesis is valid, and steam pretreatment allows higher yields to be obtained than with previously tested technologies. However, to confirm the hypothesis irrefutably, it would be necessary to evaluate the performance of the different technologies with the same methodology to obtain results that can be directly compared.

The limitation on the ethanol yields obtained from farmyard cow manure could be attributed to the relatively low recoveries achieved in the pretreatment step, as the hydrolysis and fermentation yields are usually within an acceptable range. For example, acid hydrolysis provided 79% sugar recovery [9], which is very similar to that obtained using NaOH pretreatment [22]. This means that the excellent sugar recovery that characterizes steam pretreatment [18] might be the reason why this technology enables higher conversion efficiencies (approximately 90–100% recoveries were obtained in this study).

The maximum yield in our study is also in the same range as those reported for ethanol production from steam-exploded wheat straw [19, 23–26], which indicates that fractionation with water (i.e., washing) followed by steam pretreatment allows the same conversion efficiencies to be achieved as for higher-quality residues. This implies that the technology proposed in this study could help to unlock the potential of cow manure as a resource for bioenergy production, since the same conversion efficiencies can be achieved, but at a reduced feedstock price.

Modeling and optimization

Model development and validation

We developed a model that relates the ethanol yield to the operational parameters in the pretreatment step using multiple linear regression (Eq. 1). The model was developed based on the coded variables, which implies that the coefficients in the model are a measure of the significance of each of the terms included in the model [27], i.e., a larger coefficient in Eq. 1 means that the term has a greater influence on the response (ethanol yield).

$$Y_{\mathrm{EtOH}}(\%) = 50.8 + 4.1T + 3.0t - 4.8\mathrm{pH} + 0.4T^2 + 0.6t^2 - 0.3\mathrm{pH}^2 - 2.5T\cdot t + 1.5T \cdot \mathrm{pH} + 4.5t\cdot\mathrm{pH} + 2.8T\cdot t\cdot\mathrm{pH}. \quad (1)$$

To validate the model, the variance was disaggregated into several fractions through an ANOVA analysis (Table 3). The variance due to the residuals, i.e., the variance not explained by the model, can be used to calculate the value of R^2 for the model, which was 0.758. Although this value may seem low, R^2 is not sufficient to evaluate the goodness of fit of a model, since it does not consider the degrees of freedom, and contains no information on the source of the error in the prediction [28]. In fact, when considering the degrees of freedom using a test for the significance of the regression, it can be said that there is an 85% probability ($p = 0.1405$) that at least one of the coefficients is different from zero or, in other words, that the model is significant, which is acceptable for this kind of system.

ANOVA analysis deals with this apparent inconsistency through further separating the variance due to the residuals into: (i) the variance due to the lack of fit, which corresponds to that originating from bad fitting of the coefficients, and (ii) the variance due to experimental uncertainty. Based on these variances, a test for the lack of fit was applied, and the result showed that there was only a 66% probability ($p = 0.3394$) that the lack of fit is significant, which is low compared to the usually applied 95% confidence level. It can then be said that the effects of temperature, residence time and pH on the ethanol yield are correctly fitted, even though the predictive power of the model is low due to the relatively large experimental errors and possible uncontrolled factors.

The practical meaning of these results in terms of the ethanol production process is that the operating conditions in the steam pretreatment determine the ethanol yield of the process to a large extent, but not completely. This implies that the ethanol yield cannot be predicted based solely on the conditions chosen for pretreatment. Small fluctuations can be expected due to random errors in the overall process, and larger errors may arise from uncontrolled changes in factors deemed constant, such as the composition of the feedstock, the activity of the enzymes and the vitality of the yeast.

Size of the effects

The influence of the pretreatment variables on the ethanol yield was further investigated by performing a test for a set of parameters to determine the significance of each part of the model. The test showed that there is a 98% ($p = 0.0159$) probability that the linear terms are significant, while this probability is only 36% ($p = 0.6426$) for the quadratic terms, and 76% ($p = 0.2343$) for the interaction terms. The reason for this is that, of the 75% variance explained by the model, 69% is explained by the linear terms, 2% by the quadratic terms and 29% by the interaction terms. From this it can be seen that the pretreatment variables have a strong linear effect on the ethanol yield in the range studied, and that there are relevant interactions between them, while the curvature due to quadratic effects is minimal.

It is possible that the small size of the quadratic terms is a result of the range of conditions included in the study, which is relatively small, and not necessarily because these effects do not exist. A function with a curvature may appear linear when analyzed over a small range and, therefore, larger quadratic effects could have been found if the pretreatment variables had been studied over larger ranges. However, a larger range in the pretreatment variables would lower the precision in the fitting of the model [28, 29]. Thus, the model presented provides a more accurate representation of the effects near the optimal operating conditions, although it may not be valid for extreme conditions at much lower or higher combined severity, as defined by Chum et al. [30], than those tested in this study.

The curvature in the model is the result of interaction effects, which means that increasing the severity by changing one of the variables limits the severity that can be achieved through changing the others. This result is consistent with the fact that the optimal pretreatment severity is governed by carbohydrate degradation [18], and also with the results reported by Vancov et al. [9], where interactions between the pretreatment variables were also found for acid hydrolysis pretreatment of cow manure. The existence of these interactions makes the prediction of the outcome of pretreatment more complex, because the pretreatment variables are not completely interchangeable, i.e., different results could be obtained when increasing the severity by raising the temperature than by increasing the residence time.

To understand which of the pretreatment variables is more significant, it was necessary to analyze the model as a whole, rather than for just a set of parameters. Response surfaces were used for this purpose, in particular three surfaces at the value of each pretreatment variable giving the best conditions tested, i.e., $T = 200$ °C, $t = 5$ min and pH = 2 (Fig. 3). The more significant a variable is, the more it can compensate for suboptimal values of the other variables; therefore, the change in ethanol yield represented by the response surface can be used as an indication of the significance of the variable. For example, when the time is at its optimal value but the other variables are not, the ethanol yield decreases to 35% (Fig. 3b), but in the analogous situation for the pH, the yield is only reduced to 55% (Fig. 3c), which indicates that the pH has a greater influence on the yield than the residence time. Based on this, it can be said that the residence time influences the ethanol yield to a much lower extent than the temperature and the pH, which have a similar degree of influence, although that of the pH is slightly higher.

Model-based optimization

Based on the optimization of the model, the best conditions for steam pretreatment are 200 °C, for 5 min, at pH 2, which is one of the tested conditions, so no further validation was required. The optimal condition found for animal bedding was the same as that previously reported for wheat straw [19, 24], which shows that the time the bedding is in the stable does not help overcome the recalcitrance of the material, as the same severity is needed in its pretreatment.

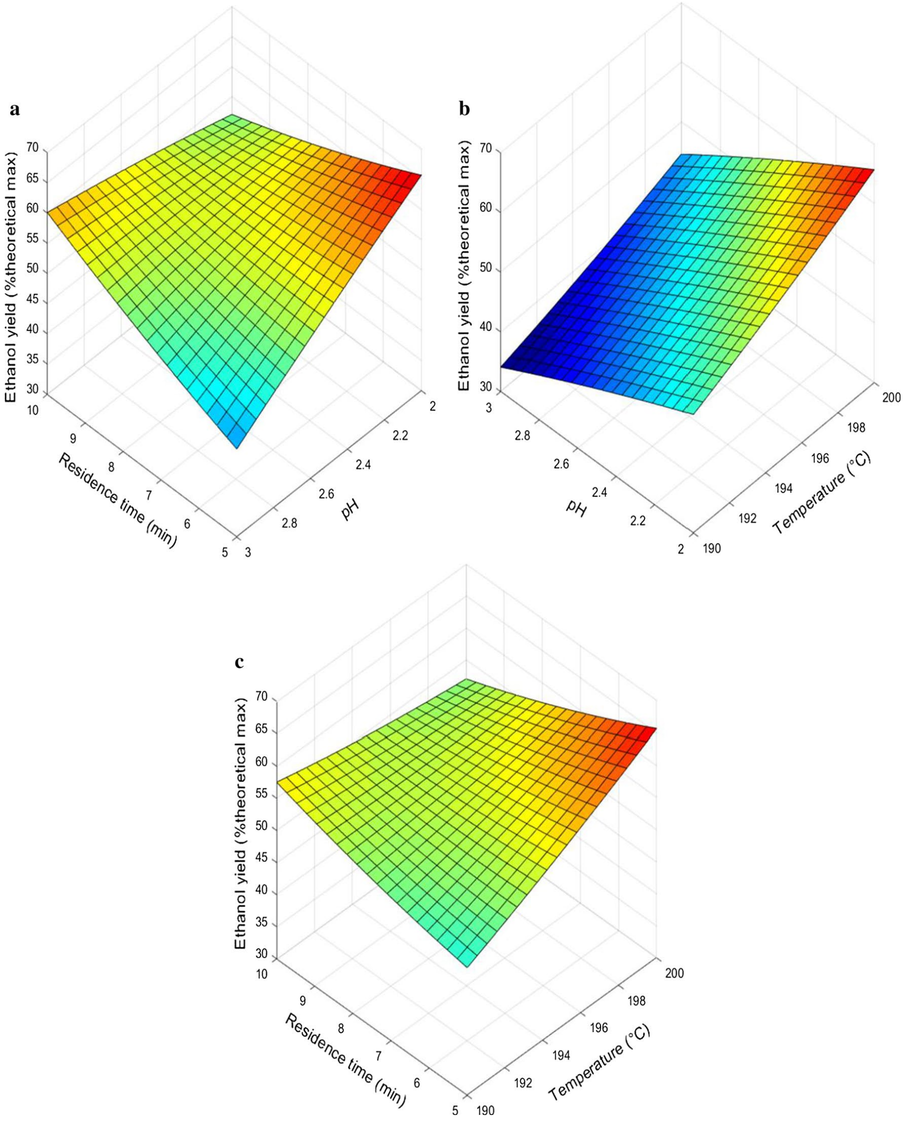


Fig. 3 Response surfaces at 200 °C (**a**), 5 min (**b**) and pH = 2 (**c**), based on the predictions of the model

Due to the relatively low predictive power of the model, the optimum may instead be at 190 °C, for 10 min, at pH 2. Other authors have reported that these two conditions gave very similar results in terms of ethanol yield in the subsequent biological processes [19], and it is therefore difficult to reach a level of accuracy that allows differentiation between them. In spite of this, the optimum does not lie outside the tested range, since the best yields were not obtained for either the lowest or the highest severity, although a more accurate estimate might be obtained through further experimentation in the vicinity of the reported optimum.

Conclusions

Design of experiments together with response-surface modeling was used to optimize the pretreatment conditions to maximize the ethanol yield from animal bedding. The optimal conditions were 200 °C, for 5 min, at pH 2, at which an ethanol yield of 69.3% was obtained. The yield obtained when using steam pretreatment was higher than that obtained with other pretreatment technologies previously tested and was in the same range as that for steam-exploded wheat straw. This means that steam pretreatment may provide a means of unlocking animal bedding as a resource for bioenergy production, as the same conversion efficiencies can be obtained as for higher-quality feedstocks.

Further analyses of the model showed that pH has the greatest influence on the ethanol yield, followed closely by the temperature, and that residence time has considerably less influence. Although the effects were properly fitted, the predictive power of the model may be low due to the high experimental variability, and the possible existence of uncontrolled factors. This implies that, in an ethanol production process based on animal bedding, it would not be possible to predict the yield of the process based only on the pretreatment conditions, although they determine it to a large extent.

Materials and methods

Animal bedding collection and preparation

Animal bedding was collected from a dairy farm at Lille Skensved, a small town close to Køge, in Denmark. The barn is approximately 600 m^2, has a rectangular shape, hosts 150 dairy cows in a loose house regime, and approximately 500 ton of straw is used per year as bedding. Samples were collected from 13 different positions in the barn and stored frozen until further use, according to previous recommendations [31].

After sample collection, the animal bedding from each of the sampling positions was washed with deionized water to separate the manure from the straw. Washing was performed by mixing 4 kg of animal bedding (approximately 1 kg dry animal bedding) with 10 L of deionized water at room temperature in a concrete mixer for 2 min. The material was subsequently pressed in a filter press to remove the liquid, which contained most of the manure.

After washing, a subsample of the washed animal bedding was taken from the material collected at each of the 13 sampling positions after thorough mixing of the material in the concrete mixer. The 13 subsamples were then mixed to produce an average sample that is representative of the whole barn. This average material was used in the pretreatment and fermentation experiments.

Steam pretreatment

The washed animal bedding was impregnated with sulfuric acid by soaking in a dilute sulfuric acid solution (0.3–0.6 wt% depending on the pretreatment conditions) for 1 h. Soaking was performed at a solid-to-liquid ratio of 1:20, and sulfuric acid was added progressively until the desired pH was reached. Different pH levels, from 1.6 to 3.4, were tested according to the experimental design described in Sect. "Experimental design and statistical analysis". The material was pressed in a filter press at 13 bar to remove the liquid, and the soaked fiber was left overnight at room temperature in a sealed container prior to steam pretreatment.

The soaked fiber was then subjected to steam pretreatment in a 10 L reactor (Process & Industriteknik AB, Kristianstad, Sweden), which has been described elsewhere [32]. Steam pretreatment was performed at various conditions, from 186 to 204 °C, for 3–12 min, according to the experimental design described in Sect. "Experimental design and statistical analysis". At each condition, 600 g dry soaked fiber was pretreated and the pretreated materials were stored at 4 °C before further use for analysis or experiments.

Simultaneous saccharification and fermentation

SSF experiments were performed on the whole pretreated slurry in 2 L Labfors bioreactors with a working weight of 1 kg. Prior to running the experiments, the fermenters with the slurry were sterilized (after correcting the pH of the material to 5). A water-insoluble solid (WIS) load of 8%, Cellic CTec2 (Novozymes, Denmark) enzyme cocktail at a load of 0.05 g enzyme/g WIS (which corresponds approximately to 10 FPU/g WIS) and Ethanol Red (Lesaffre Advanced Fermentations, France) yeast at a dry weight concentration of 3 g/L were used during the experiments. Due to severe mixing difficulties at the start of SSF, mixing at 400 rpm was applied 1 h after adding the enzymes and the yeast, when the material had become sufficiently liquefied to be mixable. SSF was performed at 35 °C and the pH was maintained at 5 through the automatic addition of 10% NaOH solution. The SSF media were supplemented with 0.5 g/L $(NH_4)_2PHO_4$, 0.025 g/L $MgSO_4$, 1 g/L yeast extract and, to avoid the risk of infection, 10 mg/L streptomycin and 10,000 U/L penicillin. All the SSF experiments were performed in duplicate.

Samples obtained from the SSF experiments were centrifuged in 2 mL Eppendorf tubes at 13,000 rpm for 5 min. The supernatant was filtered through 0.2 μm syringe filters (GVS North America, Sanford, USA) and stored at −20 °C prior to high-performance liquid chromatography (HPLC) analysis. Ethanol, organic acids and other by-products were analyzed using a Shimadzu LC-20 AD HPLC system equipped with a Shimadzu RID 10A refractive index detector (Shimadzu Corporation, Kyoto, Japan). The chromatography column used was an Aminex HPX-87H, with a Cation-H Bio-Rad Micro-Guard column (Bio-Rad Laboratories, Hercules, United States) at 50 °C, and a 5 mM sulfuric acid solution was used as eluent at a flow rate of 0.5 mL/min.

Compositional analysis

Animal bedding is considered to be a mixture of two components, manure and straw, and the manure content is assumed to be equal to the mass removed after ten washing cycles [33]. The manure was further analyzed by incinerating a sample at 575 °C for 3 h to determine the organic matter content, and the inorganic matter content was calculated as the difference between the total solids and the organic matter content. The straw was analyzed following the protocols from the National Renewable Energy Laboratory (NREL) [34–37].

The manure content of the washed animal bedding was calculated as the product of the manure content in the native material and one minus the average washing efficiency of the 13 samples (Eqs. 2 and 3). The rest of the composition of the washed animal bedding was calculated assuming that the composition of the manure and the straw remained constant during washing and are therefore the same as that in the native material.

$$\text{Manure}(\%\text{TS}) = \text{manure}_{\text{native}} \cdot (1 - \text{wash}_{\text{eff}}), \qquad (2)$$

$$\text{Wash}_{\text{eff}} = \frac{\sum \frac{M_{\text{liquid}} \cdot \text{TS}_{\text{liquid}}}{\text{manure}_{\text{native}} \cdot M_{\text{bedding}}}}{N_{\text{samples}}}, \qquad (3)$$

where $\text{manure}_{\text{native}}$ is the manure content in the native material, expressed as %TS; M_{liquid} the mass of the expressed liquid after washing; $\text{TS}_{\text{liquid}}$ the total solid content of the expressed liquid after washing; M_{bedding} the dry mass of the animal bedding washed; and N_{samples} the number of samples that were washed (13 in this study).

The WIS content of the pretreated materials was determined using the non-wash method described by Weiss et al. [38]. The structural carbohydrates and lignin content of the solid fraction and the composition of the liquid fraction were analyzed following NREL protocols [37, 39]. Monomeric sugars in the liquid fraction were analyzed using the HPLC system described above, using an Aminex HPX-87P chromatography column with a De-Ashing Bio-Rad Micro-Guard column at 85 °C, using reagent-grade water as eluent at a flow rate of 0.6 mL/min. Pretreatment by-products in the liquid fraction were analyzed using the same HPLC system, chromatographic column, and conditions as described in "Simultaneous saccharification and fermentation".

Sugar samples generated during the analyses of structural carbohydrates and lignin were analyzed using high-performance anion-exchange chromatography coupled with pulsed amperometric detection. A Dionex system with a Carbo Pac PA1 column, a GP50 gradient pump and an AS50 autosampler were used. The flow rate was 1 mL/min, the temperature was 30 °C and the solutions used as eluents were: deionized water, 200 mmol/L NaOH, and 200 mmol/L NaOH mixed with 170 mmol/L sodium acetate.

Yield calculations

The ethanol yield was calculated based on the total available glucose in the washed fiber, which corresponds to 1.11 times the amount of glucan in the fiber (due to the addition of water during hydrolysis). The yield is presented as g ethanol/g glucose in the raw material (washed fiber), and also as a percentage of the theoretical stoichiometric ethanol yield (0.51 g/g), which are the values used in the development of the model described in Sect. "Experimental design and statistical analysis".

Experimental design and statistical analysis

The effects of the three pretreatment variables, temperature (T), residence time (t) and pH during soaking (pH), on the ethanol yield were investigated using response-surface modeling. A spherical central composite design was chosen due to its improved performance [40], and four replicates were performed at the center point (195 °C, 7.5 min, pH 2.5), which was chosen based on previously reported optimal conditions for wheat straw [19]. The variables were coded to prevent scale effects from influencing the modeling. The coding was based on centering so that the zero value was assigned to the values of the variables at the center point, and the rest of the values were calculated based on the following conversion factors: 5 °C/coded unit, 2.5 min/coded unit and 0.5 pH units/coded unit. Table 4 gives the value of the variables in each of the 18 runs in both uncoded and coded units.

An empirical model was constructed through multiple linear regression, as described previously by Brereton et al. [27] The model includes an intercept term, linear effects, quadratic effects and interaction terms (Eq. 4). The interaction terms account for the possibility that the value of one variable may influence the effect of another on the response [27]. For example, the effect of

Table 4 Experimental design used to investigate the pretreatment step

Condition	Temperature (°C)	Residence time (min)	pH[a]	Coded units		
1	200	10	3	1	1	1
2	200	10	2	1	1	− 1
3	200	5	3	1	-1	1
4	200	5	2	1	-1	− 1
5	190	10	3	− 1	1	1
6	190	10	2	− 1	1	− 1
7	190	5	3	− 1	− 1	1
8	190	5	2	− 1	− 1	− 1
9	195	7.5	1.6	0	0	− 1.8
10	195	7.5	3.4	0	0	1.8
11	195	3	2.5	0	− 1.8	0
12	195	12	2.5	0	1.8	0
13	186	7.5	2.5	− 1.8	0	0
14	204	7.5	2.5	1.8	0	0
15	195	7.5	2.5	0	0	0
16	195	7.5	2.5	0	0	0
17	195	7.5	2.5	0	0	0
18	195	7.5	2.5	0	0	0

[a] pH in the impregnation step

the pH may be different at low temperatures than at high temperatures.

$$
\begin{aligned}
Y_{\mathrm{EtOH}}(\%) = {} & a_0 + a_1 T + a_2 t + a_3 \mathrm{pH} + a_4 T^2 + a_5 t^2 \\
& + a_6 \mathrm{pH}^2 + a_7 T \cdot t + a_8 T \cdot \mathrm{pH} + a_9 t \\
& \cdot \mathrm{pH} + a_{10} T \cdot t \cdot \mathrm{pH}.
\end{aligned}
\tag{4}
$$

To evaluate the validity of the model, the value of R^2 was complemented with further significance analyses based on the ANOVA methodology, as described elsewhere [28]. First, a test for the significance of the regression was performed to evaluate the effectiveness of the model as a whole, i.e., to determine whether at least one of the terms in the model was significant. Since this test offers no insight into which terms are significant, a test for a set of parameters was used to determine the significance of each part of the model (linear, quadratic and interaction), and to calculate their respective contributions to R^2. Finally, a test for the lack of fit was performed to identify whether the terms in the model are fitted correctly. This test provides insight into the source of errors in the prediction, as it specifies whether the lack of fit originates from poor fitting of the model or from experimental error. All these statistical tests were performed according to the methodology described by Deming et al. [28].

All the calculations required for the development of the model and further statistical analyses were performed in MATLAB, and the probability density for the F function was calculated using the built-in MATLAB command *fpdf*.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13068-019-1558-9.

Additional file 1. Composition of the pretreated materials at each of the conditions tested for the steam treatment.

Acknowledgements
The authors are grateful to TK Energy A/S and Niels Lundager for their help in the collection of the animal bedding and to Margaréta Rozbach for her participation in the experimental work.

Authors' contributions
MSS designed the study with input from MG and OW. MSS and BE performed the experimental work. MSS analyzed the results together with BE, KK, MG and OW. MSS prepared the manuscript and BE, KK, MG and OW reviewed the text. All authors read and approved the final manuscript.

Funding
This work was financially supported by the Swedish Energy Agency and BESTF3 (Grant Number P42674-1). The funding bodies did not participate in the design of the study and collection, analysis and interpretation of data or in writing the manuscript.

Availability of data and materials
All data generated or analyzed during this study are included in this published article and its additional file.

Ethical approval and consent to participate
Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 27 May 2019 Accepted: 31 August 2019

References

1. Sub group on Advanced Biofuels. Building up the future. The cost of biofuels. 2017. Report No.: MI-06-17-107-EN-N.
2. Balan V. Current challenges in commercially producing biofuels from lignocellulosic biomass. ISRN Biotechnol. 2014;2014:31.
3. de Azevedo A, Fornasier F, da Silva Szarblewski M, Schneider RDCDS, Hoeltz M, de Souza D. Life cycle assessment of bioethanol production from cattle manure. J Clean Prod. 2017;162:1021–30.
4. Liao W, Liu Y, Hodge D. Chapter 13—Integrated farm-based biorefinery. In: Qureshi N, Hodge DB, Vertès AA, editors. Biorefineries. Amsterdam: Elsevier; 2014. p. 255–70.
5. Gomaa MA, Abed RMM. Potential of fecal waste for the production of biomethane, bioethanol and biodiesel. J Biotechnol. 2017;253:14–22.
6. Chen S, Wen Z, Liao W, Liu C, Kincaid RL, Harrison JH, et al. Studies into using manure in a biorefinery concept. Appl Biochem Biotechnol. 2005;124(1):999–1015.
7. Wen Z. Hydrolysis of animal manure lignocellulosics for reducing sugar production. Biores Technol. 2004;91(1):31–9.
8. Bona D, Vecchiet A, Pin M, Fornasier F, Mondini C, Guzzon R, et al. The Biorefinery concept applied to bioethanol and biomethane production from manure. Waste Biomass Valoriz. 2017;9(11):1–11.
9. Vancov T, Schneider RC, Palmer J, McIntosh S, Stuetz R. Potential use of feedlot cattle manure for bioethanol production. Bioresour Technol. 2015;183:120–8.
10. Avellar BK, Glasser WG. Steam-assisted biomass fractionation. I. Process considerations and economic evaluation. Biomass and Bioenergy. 1998;14(3):205–18.
11. Wallace T. Sampling liquid manure for analysis. Canada: Alberta Agriculture and Food, Division AS; 2008. Report No.: Agdex 538-2.
12. Nidetzky B, Steiner W, Hayn M, Esterbauer H. Enzymatic hydrolysis of wheat straw after steam pretreatment: experimental data and kinetic modelling. Biores Technol. 1993;44(1):25–32.
13. Erdei B, Barta Z, Sipos B, Réczey K, Galbe M, Zacchi G. Ethanol production from mixtures of wheat straw and wheat meal. Biotechnol Biofuels. 2010;3(1):16.
14. Liao W, Liu Y, Liu C, Chen S. Optimizing dilute acid hydrolysis of hemicellulose in a nitrogen-rich cellulosic material–dairy manure. Bioresour Technol. 2004;94(1):33–41.
15. Gírio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S, Bogel-Łukasik R. Hemicelluloses for fuel ethanol: a review. Biores Technol. 2010;101(13):4775–800.
16. Erdei B, Hancz D, Galbe M, Zacchi G. SSF of steam-pretreated wheat straw with the addition of saccharified or fermented wheat meal in integrated bioethanol production. Biotechnol Biofuels. 2013;6(1):169.
17. Nielsen F, Zacchi G, Galbe M, Wallberg O. Sequential targeting of xylose and glucose conversion in fed-batch simultaneous saccharification and co-fermentation of steam-pretreated wheat straw for improved xylose conversion to ethanol. BioEnergy Res. 2017;10(3):800–10.
18. Li J, Henriksson G, Gellerstedt G. Carbohydrate reactions during high-temperature steam treatment of aspen wood. Appl Biochem Biotechnol. 2005;125(3):175–88.
19. Ballesteros I, Negro MJ, Oliva JM, Cabanas A, Manzanares P, Ballesteros M. Ethanol production from steam-explosion pretreated wheat straw. Appl Biochem Biotechnol. 2006;129–132:496–508.
20. Navarro AR. Effects of furfural on ethanol fermentation by *Saccharomyces cerevisiae*: mathematical models. Curr Microbiol. 1994;29(2):87–90.
21. You Y, Liu S, Wu B, Wang Y-W, Zhu Q-L, Qin H, et al. Bio-ethanol production by Zymomonas mobilis using pretreated dairy manure as a carbon and nitrogen source. RSC Adv. 2017;7(7):3768–79.
22. Yue Z, Teater C, Liu Y, Maclellan J, Liao W. A sustainable pathway of cellulosic ethanol production integrating anaerobic digestion with biorefining. Biotechnol Bioeng. 2010;105(6):1031–9.
23. Larsen J, Østergaard Petersen M, Thirup L, Wen Li H, Krogh Iversen F. The IBUS process—lignocellulosic bioethanol close to a commercial reality. Chem Eng Technol. 2008;31(5):765–72.
24. Linde M, Jakobsson E-L, Galbe M, Zacchi G. Steam pretreatment of dilute H_2SO_4-impregnated wheat straw and SSF with low yeast and enzyme loadings for bioethanol production. Biomass Bioenergy. 2008;32(4):326–32.
25. Novy V, Longus K, Nidetzky B. From wheat straw to bioethanol: integrative analysis of a separate hydrolysis and co-fermentation process with implemented enzyme production. Biotechnol Biofuels. 2015;8:46.
26. Tomás-Pejó E, Fermoso J, Herrador E, Hernando H, Jiménez-Sánchez S, Ballesteros M, et al. Valorization of steam-exploded wheat straw through a biorefinery approach: bioethanol and bio-oil co-production. Fuel. 2017;199:403–12.
27. Brereton RG. Experimental design. Chemometrics. Boca Raton: Wiley; 2003. p. 15–117.
28. Deming SN, Morgan SL. Experimental design: a chemometric approach. Boca Raton: Elsevier; 1993.
29. Stenberg K, Tengborg C, Galbe M, Zacchi G. Optimisation of steam pretreatment of SO_2-impregnated mixed softwoods for ethanol production. J Chem Technol Biotechnol. 1998;71(4):299–308.
30. Chum HL, Johnson DK, Black SK, Overend RP. Pretreatment-catalyst effects and the combined severity parameter. Appl Biochem Biotechnol. 1990;24(1):1.
31. Peters J, Combs SM, Hoskins B, Kovar JL, Watson ME, Wolf AM, et al. Recommended methods of manure analysis. WI, United States; 2003. Report No.: A3769.
32. Palmqvist E, Hahn-Hägerdal B, Galbe M, Larsson M, Stenberg K, Szengyel Z, et al. Design and operation of a bench-scale process development unit for the production of ethanol from lignocellulosics. Biores Technol. 1996;58(2):171–9.
33. Sanchis-Sebastiá M, Erdei B, Kovacs K, Galbe M, Wallberg O. Analysis of animal bedding heterogeneity for potential use in biorefineries based on farmyard manure. Waste Biomass Valoriz. 2019. https://doi.org/10.1007/s12649-018-00578-6.
34. Sluiter A, Hames B, Hyman D, Payne C, Ruiz R, Scarlata C, et al. Determination of total solids in biomass and total dissolved solids in liquid process samples. Golden, CO: Laboratory Analytical Procedure (LAP), National Renewable Energy Laboratory; 2008.
35. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D. Determination of ash in biomass. Golden, CO: Laboratory Analytical Procedure (LAP), National Renewable Energy Laboratory; 2008.
36. Sluiter A, Ruiz R, Scarlata C, Sluiter J, Templeton D. Determination of extractives in biomass. Golden, CO: Laboratory Analytical Procedure (LAP), National Renewable Energy Laboratory; 2008.
37. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, et al. Determination of structural carbohydrates and lignin in biomass. Golden, CO: Laboratory Analytical Procedure (LAP), National Renewable Energy Laboratory; 2008.
38. Weiss ND, Stickel JJ, Wolfe JL, Nguyen QA. A simplified method for the measurement of insoluble solids in pretreated biomass slurries. Appl Biochem Biotechnol. 2010;162(4):975–87.
39. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D. Determination of sugars, byproducts, and degradation products in liquid fraction process samples. Golden, CO: Laboratory analytical procedure (LAP), National Renewable Energy Laboratory; 2008.
40. Myers R, Vining GG, Giovannitti-Jensen A, Myers S. Variance dispersion properties of second-order response surface designs. J Qual Technol. 1992;24(1):1–11.

Publisher's Note

Paper III

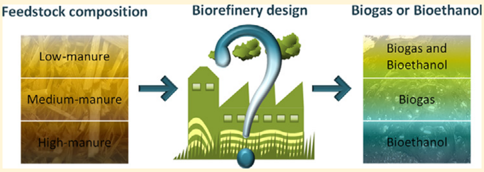
I&EC research
Industrial & Engineering Chemistry Research
Article
Cite This: *Ind. Eng. Chem. Res.* 2019, 58, 21927−21935
pubs.acs.org/IECR

Production of Biofuels from Animal Bedding: Biogas or Bioethanol? Influence of Feedstock Composition on the Process Layout

Mirjam Victorin,*,† Miguel Sanchis-Sebastiá,*,† Åsa Davidsson, and Ola Wallberg

Department of Chemical Engineering, Lund University, PO Box 124, SE-221 00 Lund, Sweden

Supporting Information



ABSTRACT: The design of biorefineries based on farmyard manure remains unknown, because various groups have obtained disparate results. These differences have been attributed to the composition of the material—an explanation that this study intends to develop. Several biorefinery layouts were tested in the laboratory for animal beddings with various manure contents, and the same result was obtained in all cases: fractionating the material and recycling part of the fiber, after pretreatment, to the anaerobic digester delivered the highest conversion efficiencies (540 N mL CH_4 g^{-1} VS). This result proves that the processing of animal bedding does not depend on the manure content of the material or, probably, on any other aspect of the composition. The process being unaffected by the composition of the material was attributed to fractionation of the material, because the output of this step was constant even though the feedstock differed. This result implies that fractionating animal bedding allows this material always to be processed through the same technique. This is because fractionation increases the conversion efficiencies compared with designs that lack this step (e.g., 15% higher ethanol yield), as it enables to unlock synergies between biogas and bioethanol production from this feedstock.

1. INTRODUCTION

Manure disposal is an intrinsic problem of livestock operations worldwide. In many cases, it is possible to dispose of manure through its use, with or without previous treatment, as fertilizer in nearby fields.[1] This strategy has a positive environmental impact, because it avoids the consumption of mineral fertilizers and the fossil fuels that are used in their production.[2] But, the high moisture content of manure constrains the operation, because it renders transport over long distances economically unfeasible.[2] This finding implies that areas with a surplus of manure experience difficulties in managing this residue, prompting new valorization methods to be developed.

Bioenergy production quickly became an alternative valorization technique for manure, especially biogas production through anaerobic digestion. For example, 20 years ago, there were already over 400 biogas plants throughout Europe, based on manure as feedstock,[3] with Germany and Denmark pioneering this change. In fact, Denmark has set a target of utilizing 50% of the manure that is produced in the country for energy purposes by 2020, compared with the 7−8% that was already used for this objective in 2014.[4]

Although anaerobic digestion of manure is an established technology, several groups have suggested that further valorization of this material can be achieved through its use in a multiproduct biorefinery, particularly in the coproduction of biogas, bioethanol, and biofertilizer.[5] The digestate after anaerobic digestion of farmyard manure can be treated with NaOH to decrease its recalcitrance and produce a suitable feedstock for bioethanol production.[6,7] Other studies have reported that farmyard manure, when subjected to acid hydrolysis, generates a material that can be subsequently hydrolyzed enzymatically and fermented to produce ethanol.[8,9] The solid residue that remains after ethanol production could be used to generate biogas through anaerobic digestion.

The proper design of biorefineries that are based on farmyard manure remains unknown—several groups favor biogas production before bioethanol production, whereas others favor the reverse concept. This apparent contradiction can be explained by the large differences in the composition of feedstock,[8] which originates from many factors, such as the type and number of animals, diet, animal housing, and time that is spent in the stable.[10]

We intend to develop this explanation by examining the manure content of the material as the cause of the apparently contradictory results. The manure contains most of the nitrogen that is present in farmyard manure;[11] i.e., to a large extent, the manure content defines the nitrogen content of the material, which, in turn, influences its suitability for anaerobic digestion, because this process is operated optimally at a C/N ratio of 25.[12] A higher or lower ratio leads to inhibition by accumulation of volatile fatty acids (VFAs) or ammonia, respectively. Several groups have demonstrated the positive effects of balancing the C/N ratio in the digester through

Received: September 5, 2019
Revised: October 16, 2019
Accepted: November 12, 2019
Published: November 12, 2019

DOI: 10.1021/acs.iecr.9b04945

codigesting several feedstocks with varying characteristics, such as manure and wheat straw, cattle manure and corn stover, and manure and sugarcane residues.[13−15] These studies implicate the balance in nutrients and the substrate (i.e., carbon) in the improvement in the performance of the digester, because it allows for better regulation of the bacterial consortium in the reactor.

Concurrently, the manure content affects the suitability of a material for bioethanol production, because the nitrous compounds (e.g., primary amines, secondary amines, and ammonia) could react with the reducing sugars that are released during the pretreatment step through a chemical mechanism, known as the Maillard reaction, or browning,[16] thus rendering the sugars unavailable for fermentation. The sugar yield during acid hydrolysis of farmyard manure can be improved significantly through removal of the nitrogen sources in the material.[17]

Because manure content affects the suitability of farmyard manure for biogas and bioethanol production, we aimed to demonstrate that this factor defines how this feedstock should be processed in a biorefinery. We chose animal bedding, a manure-containing residue that is typical of certain regions in Denmark, to test the hypothesis that the process layout of a biorefinery that is based on farmyard manure depends on the manure content of the material. Validation of this hypothesis will explain the apparent contradiction in the literature and provide guidelines that will help energy producers decide whether to produce biogas, bioethanol, or both from a given type of farmyard manure, possibly increasing the material efficiency of the process and valorizing this residue more extensively.

2. MATERIALS AND METHODS

2.1. Collection of Animal Bedding and Wheat Straw.

Animal bedding was collected from a dairy farm in a small town close to Køge, Denmark in February 2018. The barn was approximately 600 m^2, had a rectangular shape, hosted 150 dairy cows in loose housing, and used approximately 500 tons of straw per year as bedding. Samples were collected from 10 positions throughout the barn and stored at −18 °C until further use, per previous recommendations.[18]

A subsample was taken from each of the 10 positions after thorough mixing of the material in a concrete mixer and combined to produce an average sample that was representative of the entire barn. All experiments and analyses were performed using this average material.

Winter wheat straw was collected in Denmark, close to Køge, and air-dried to a moisture content below 10% before being fed to a chopper, which cut the straw into 5−10 cm pieces. The straw was stored at room temperature until further use. The composition of the straw is provided in Table S1 in the supporting information.

2.2. Preparation of the Process Streams.

A biorefinery process was reproduced on a laboratory scale to establish the process streams that were used in the bioethanol and biogas trials (Figure 1). We chose a bioethanol and biogas coproduction process that had a washing with water as its first step to separate the manure from the fiber, which was subsequently pretreated by acid-catalyzed steam explosion. The steam-exploded fiber was enzymatically hydrolyzed and fermented to produce ethanol, and the pretreatment hydrolysate was codigested with the washing liquid to produce biogas.

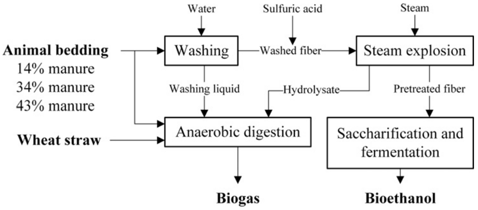


Figure 1. Layout of the biorefinery process, reproduced on a laboratory scale, to generate the process streams used in the bioethanol and biogas trials.

2.3. Preparation of Feedstocks.

The native animal bedding from the barn was used to produce 2 more materials: one each with lower and higher manure content. The materials were denoted low, medium, and high, according to their manure content and thus the native animal bedding from the barn corresponds to the material that is termed medium.

Washing with water was used to produce the material with lower manure content, because part of the manure is transferred to the liquid and thus removed. The washing was performed by mixing 1 kg of dry matter of animal bedding with 20 L room temperature deionized water in a concrete mixer for 2 min. The material was then drained and filtered in a filter press (Fischer, Germany) at 13 bar to remove the liquid, which contained part of the manure from the original material.

The liquid was then used to prepare the material with higher manure content. The volume of the liquid was reduced in a rotavapor (Fischer, Germany) at 55 mbar and 45 °C and dried in an oven at 45 °C until the total solids (TS) content of the original animal bedding was reached. The dried manure from this step was added to a sample of the animal bedding from the barn to simulate material with higher manure content.

2.4. Washing.

The three feedstocks were washed in a concrete mixer by mixing 1 kg of dry material with 20 L room temperature deionized water for 2 min. After the washing, the liquid was removed by first draining the material and then passing it through a filter press (Fischer, Germany) at 13 bar. The solid material after the washing and pressing was denoted as washed fiber, and the drained and expressed liquids, which were mixed in a unique sample, were termed as washing liquid. The efficiency of the washing was calculated as the ratio between the mass of material that had transferred to the liquid phase and the total amount of manure in the material before the washing (eq 1).

$$\mathrm{Wash}_{\mathrm{eff}} = \frac{M_{\mathrm{liquid}} \cdot \mathrm{TS}_{\mathrm{liquid}}}{\mathrm{Manure} \cdot M_{\mathrm{bedding}} \cdot \mathrm{TS}_{\mathrm{bedding}}} \quad (1)$$

where Manure is the manure content in the native material, expressed as % TS; M_{liquid} is the mass of the expressed liquid after the wash; $\mathrm{TS}_{\mathrm{liquid}}$ is the total solids content of the expressed liquid after the wash; M_{bedding} is the mass of the animal bedding that was used in the wash; and $\mathrm{TS}_{\mathrm{bedding}}$ is the total solids content of the animal bedding.

2.5. Steam Explosion.

The washed fiber was impregnated in dilute sulfuric acid solution, to which sulfuric acid was added to reach and maintain a pH of 2, for 1 h at a solid-to-liquid ratio of 1:20. The material was filtered in a filter press (Fischer, Germany) at 13 bar, and the soaked fiber was subjected to steam pretreatment in a 10 L reactor (Process & Industriteknik

AB, Kristianstad, Sweden) that has been described previously.[19] The steam pretreatment was performed at 200 °C for 5 min (based on previously optimized conditions[20]), and the pretreated material was passed through a filter press (Fischer, Germany) at 13 bar to separate the pretreatment slurry into a solid fraction, defined as pretreated fibers, and a liquid fraction, denoted the hydrolysate.

2.6. Ethanol Fermentations. The pretreated fibers were hydrolyzed enzymatically and fermented to produce ethanol. The trials consisted of a prehydrolysis step for 48 h, followed by simultaneous saccharification and fermentation (SSF) for 96 h, which were conducted in 2 L Labfors bioreactors (Infors HT, Switzerland) with a working weight of 1 kg. The bioreactors were loaded with 10% water insoluble solids (WIS), neutralized to pH 5, and sterilized prior to the trials. The prehydrolysis step was performed at 50 °C, 400 rpm, and pH 5, maintained through automatic addition of 10% NaOH solution, and Cellic CTec 2 enzyme cocktail (Novozymes, Denmark) was added at 0.05 g enzyme/g WIS. After 48 h, the temperature was decreased to 35 °C, and Ethanol Red yeast (Lessafre Advanced Fermentations, France) was added at a dry concentration of 3 g/L with 0.5 g/L $(NH_4)_2PHO_4$, 0.025 g/L $MgSO_4$, 1 g/L yeast extract, and, to prevent contamination, 10 mg/L streptomycin and 10 000 U/L penicillin.

The samples from the prehydrolysis were centrifuged in 2 mL Eppendorf tubes at 13 000 rpm for 5 min. The supernatant was passed through 0.2 μm syringe filters (GVS North America, Sanford) and stored at −20 °C prior to high-performance liquid chromatography (HPLC). Glucose, xylose, and other monomeric sugars were analyzed on a Shimadzu LC-20 AD HPLC system that was equipped with a Shimadzu RID 10A refractive index detector (Shimadzu Corporation, Kyoto, Japan). The chromatography column was an Aminex HPX-87P with a De-Ashing Bio-Rad microguard column (Bio-Rad Laboratories, Hercules) at 85 °C, and deionized water was used as the eluent at a flow rate of 0.6 mL/min.

The samples from the SSF were prepared as the prehydrolysis samples, and ethanol, organic acids, and other byproducts were analyzed on the same HPLC system, which was instead equipped with an Aminex HPX-87H column with a Cation-H Bio-Rad microguard column (Bio-Rad Laboratories, Hercules),at 50 °C and 0.5 mL/min with 5 mM sulfuric acid solution as eluent. Residual glucose, xylose, and other monomeric sugars were analyzed on the same HPLC system, with the same column and conditions as for the prehydrolysis samples.

The glucose yield in the prehydrolysis step and the ethanol yield in the SSF were calculated, on the basis of the total available glucose in the washed fiber, which corresponded to 1.11 times the amount of glucan in it (due to the addition of water during the hydrolysis). The yield was expressed as g ethanol/kg washed fiber and as the percentage of the theoretical stoichiometric ethanol yield (0.51 g/g glucose).

2.7. Anaerobic Digestion Trials. *2.7.1. Animal Bedding Codigestion Trials.* The biorefinery process that was described in Figure 1 was applied to each of the three feedstocks to produce separate process streams that were used to perform codigestion trials, in addition to wheat straw, which was included to consider the potential of adding an external lignocellulosic carbon source to the process or implementing a retrofit of the fiber fraction of animal bedding. Binary and ternary mixtures were evaluated, and the respective mixture ratios for each combination are provided in Table S5 in the supporting information. On the basis of the total carbon and nitrogen contents of the substrates, the process streams were combined such that the C/N ratio of the mixture was 30, with the exception of the washing liquid and hydrolysate mixture that was derived from the high-manure-content animal bedding, which could only reach a maximum C/N ratio of 20 due to the low C/N ratio of the hydrolysate. Each individual stream was also monodigested to provide a baseline for comparison and interpretation of the codigestion results.

The anaerobic digestion tests were performed in 120 mL glass serum bottles with a total liquid volume of 72 mL, allowing for 40% headspace (v/v). Digested sludge from a mesophilic anaerobic digester at a municipal wastewater treatment plant (Källby, Lund) was added at an inoculum/substrate ratio of 2, after it had been degassed in a heating cabinet at 37 °C for 14 days. Total solids were analyzed in all samples and the inoculum at 105 °C overnight, and the volatile solids (VS) content was measured by heating the dried samples in a furnace at 575 °C for 4 h. Due to the low TS content of the substrates, the bottles were only loaded with 2.3 g VS L^{-1}. The pH was brought to 7 with sodium hydroxide for the hydrolysates and sulfuric acid for the animal beddings and washing liquids. All bottles were sealed with a rubber cap and an aluminum crimp seal before they were flushed with pure nitrogen to ensure an anaerobic environment.

The overpressure was measured daily with a pressure meter for the first 14 days and then 1−3 times per week for the remaining trial period. Gas samples (0.2 mL) were taken with a pressure-locked syringe for compositional analysis (CH_4, CO_2, and H_2) on a gas chromatograph (Varian 3800) that was equipped with a thermal conductivity detector (TCD) at 50 °C and a 2.0 m HayeSep mesh column with a diameter of 3.2 mm. Nitrogen was used as a carrier gas at a flow rate of 10 mL/min.

2.7.2. C/N Ratio and Carbon Accessibility Trials. Two additional anaerobic digestion tests were performed to examine the impact of C/N ratio and carbon accessibility on the codigestion trials, according to the method in Section 2.7.1, except for an increase in substrate load to 4.3 g VS L^{-1}. The tests consisted of codigesting two carbon sources with different accessibilities with washing liquid at several C/N ratios (Table S6 in the supporting information) to determine whether the optimal C/N ratio of a substrate mixture differs, depending on carbon accessibility. In the first test, wheat straw was used as a carbon source with low accessibility, whereas in the second test, a blend of glucose and xylose (same proportions as in the wheat straw) was used to represent a carbon source with high accessibility.

2.7.3. Modeling the Anaerobic Codigestion Trials. The modified Gompertz equation (eq 2), first described by Zwietering et al.,[21] was fitted to the cumulative methane yield that was obtained in the anaerobic co-digestion trials in Section 2.4.1. The model was fitted by minimizing the residual sum of squares with the nonlinear solver GRG Nonlinear Solving method in Microsoft Excel 2013.

$$B = B_0 \exp\left[-\exp\left(\frac{R_m e}{B_0}(\lambda - t) + 1\right)\right] \tag{2}$$

where B is the cumulative methane yield (N mL CH_4 g^{-1} VS) at digestion time t (days), B_0 is the maximum methane yield (N mL CH_4 g^{-1} VS), R_m is the maximum methane production

DOI: 10.1021/acs.iecr.9b04945
Ind. Eng. Chem. Res. 2019, 58, 21927−21935

rate (N mL CH_4 g^{-1} VS day^{-1}), and λ is the estimated lag time (days) before methane production starts.

2.8. Compositional Analysis. *2.8.1. Analysis of Animal Bedding.* The native animal bedding was analyzed under the assumption that the material was a mixture of two components—manure and straw—and that the manure content corresponded to the cumulative mass that was removed after 10 washes with water.[11] The manure content of the low-content and high-content materials was determined, based on the washing efficiency when the material was prepared and the mass balance when the manure was added, respectively. The rest of the composition was calculated, assuming that the composition of the manure and straw remained constant and was thus the same as in the native material.

The chemical composition of the fiber was further analyzed per the National Renewable Energy Laboratory (NREL), as was the wheat straw that was used in the biogas trials. The extractives content corresponds to the mass that is removed after 48 h of extraction, the first 24 h of which is performed with water as solvent and ethanol for the second 24 h.[22] The structural carbohydrate and lignin contents were determined by two-stage acid hydrolysis.[23] The sugar samples that were generated during the analysis were examined by high-performance anion-exchange chromatography, coupled with pulsed amperometric detection. A Dionex system with a Carbo Pac PA1 column, GP50 gradient pump, and AS50 autosampler was used. The flow rate was 1 mL/min, the temperature was 30 °C, and the eluents were deionized water, 200 mmol/L NaOH, and 200 mmol/L NaOH that were mixed with 170 mmol/L sodium acetate.

2.8.2. Analysis of Steam-Exploded Material. The WIS, structural carbohydrate, and lignin contents of the pretreated fibers, and total sugar contents of the hydrolysate were analyzed per the NREL.[23−25] The sugar samples that were generated were analyzed with the same Dionex system, column, and conditions as with the fiber of animal bedding. The monomeric sugar contents in the hydrolysate were analyzed with the HPLC system, column, and conditions same as those for the prehydrolysis samples, whereas the by-product contents were analyzed with the same HPLC system, column, and conditions as with the SSF samples.

2.8.3. Determination of the C/N Ratio. The total carbon (TC) and total nitrogen (TN) contents of each process stream in the anaerobic digestion trials were analyzed to determine its C/N ratio (Table S3 in the supporting information). The solid materials were sent to an external laboratory, which measured TC and TN on a Vario Max CN (Elementar, Langenselbold, Germany). The operating principle of this equipment consists of combusting the sample at 850−1150 °C and analyzing the exhaust gas.

The TC content of the liquid materials was assessed on a Shimadzu TOC-5050A total organic carbon analyzer, equipped with an ASI-5000A autosampler. This analysis is based on combusting the sample at 680 °C and then determining the CO_2 content in the exhaust gas. The TN content of the liquid materials was analyzed with LANGE cuvette tests (LCK 338), which entails converting all of the nitrous compounds into one species and then measuring the absorbance of that species. Absorbance was measured on a Hach Lange DR2800 spectrophotometer.

3. RESULTS AND DISCUSSION

3.1. Composition of the Feedstocks. The native animal bedding from the barn contained 34% manure, 41% fermentable carbohydrates, and 15% lignin (Table 1). Thus, approximately 75% of the material represents a potential substrate for bioconversion, half of it being the manure itself, with the fermentable part of the lignocellulosic fiber constituting the other half. This result shows that the material is a combination of 2 disparate biomass sources, each of which requires different processing due to their individual characteristics, and thus indicates that the material should undergo fractionation to completely valorize each biomass source rather than the bulk material being used.

Table 1. Composition of the Three Feedstocks in the Bioethanol and Biogas Trials[a]

content (% TS bedding)	low	medium	high
manure	14.4	33.9	42.5
fiber	85.6	66.1	57.5
glucan	34.2	26.4	23.0
xylan	19.5	15.1	13.1
galactan	0.5	0.4	0.4
lignin	19.4	14.9	13.0
extractives	4.6	3.5	3.1

[a] Medium corresponds to the native animal bedding; low and high were simulated in the laboratory.

The native animal bedding was used to create two more materials: one each with a lower (14.4%) and higher manure content (42.5%) (Table 1). Although this range is seemingly small, it encompasses nearly the entire feasible spectrum for this residue: from fresh bedding that has spent several weeks or days in the barn to highly degraded bedding that has spent several months in it.[11] Thus, the manure range that is obtained after preparing the feedstocks could be representative of material that is collected from several farms with significantly different cleaning cycles.

The manure content of the native animal bedding is lower than what has been published for similar materials in other regions of the world: 41.8% in Italy, 42.5% in Australia, and 47.4% in the United States.[8,9,26] However, these values are similar to that of the high-content material, and no study has reported farmyard manure with a significantly higher manure content. Thus, the range of manure content in this study could also be applicable to similar residues in other regions worldwide, although the high-content material would likely be the native material in this case.

The mass balance closure in the compositional analysis was not 100% partly due to the presence of ash in the fiber fraction, which was not included in the analysis. However, even if all components were included, the mass closure in biomass analysis is usually not perfect due to the experimental error of the analysis methods and the fact that the analysis methods were developed for another biomass type (corn stover) and therefore errors could arise from the adaptation of the methods to the materials used in this study.

3.2. Validation of the Hypothesis. Codigesting the washing liquid and hydrolysate delivered the highest methane yield (501−540 N mL CH_4 g^{-1} VS) and methane production rate (42−49 N mL CH_4 g^{-1} VS day^{-1}) for all three feedstocks (Figure 2). Because the result was the same in all cases, our hypothesis could not be validated, disproving that feedstock

DOI: 10.1021/acs.iecr.9b04945

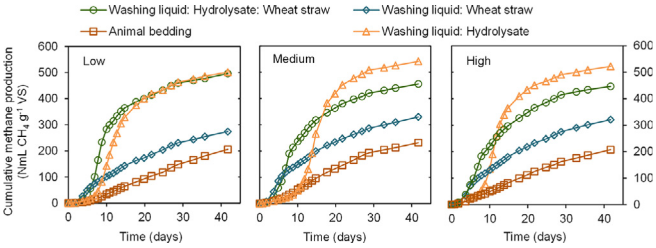


Figure 2. Cumulative methane production from codigestion of various process streams from animal bedding with low, medium, and high manure content.

composition affects the processing of animal bedding. However, this finding has positive implications, allowing the proposed biorefinery process to accept animal beddings with varying quality, in turn, increasing the availability of biomass and, consequently, the total methane potential and stability in the supply chain. Moreover, recycling the hydrolysate to the anaerobic digester unlocks synergies between biogas and bioethanol production from animal bedding: manure can be digested at high methane yields, and simultaneously, genetically modified microorganisms are not required in the ethanol production line to valorize the pentoses of the fiber.

These results represent a significant improvement in the performance of the anaerobic digestion, because the methane yield was approximately 2.5 times greater than that for animal bedding and the methane production rate was over 5-fold higher. The improvement could be attributed in part to the fractionation of the material because, in all cases, the methane yield that was generated by the animal beddings was markedly lower than for any other substrate or mixture, although animal bedding still produced gas by the end of the digestion period and thus failed to reach its final yield.

Another factor that explains the increase in performance is the bio-accessibility of the substrates, because the co-digestion mixtures that contained highly accessible carbon (i.e., hydrolysate) performed better than those with recalcitrant carbon, which also explains the low production rate of the animal bedding.

The highest methane yield was higher than those that have been reported for processing animal bedding with other strategies, although the results are not strictly comparable, because few groups have presented biogas and bioethanol yields together. For example, digesting the stillage after ethanol production of manure delivers a methane yield of 102 N mL g^{-1} TS,[9] and anaerobic digestion, followed by ethanol production, did not provide any comparable methane yields, as experienced by several groups,[6,8] although Yue et al. developed a commercial plug flow reactor that was estimated to produce 150 N mL CH_4 g^{-1} TS.[6] The increase in yield can not be attributed to an unusually low performance of the animal bedding, because its methane yield (206−231 N mL CH_4 g^{-1} VS) was in the same range as that reported by Gómez et al. (234 N mL CH_4 g^{-1} VS) and Riggio et al. (282 N mL CH_4 g^{-1} VS).[27,28] Thus, our paradigm (fractionating animal bedding prior to its bioconversion) allows this residue to be processed at increased conversion efficiencies compared with a sequential scheme when the production of one biofuel precedes the other.

Although the maximum methane yield and production rate were similar for all feedstocks, their varying compositions might have a greater influence during the scale-up of the process. For example, the hydrolysate from the high-manure-content animal bedding contained nearly twice the amount of nitrogen as the other 2 hydrolysates, decreasing the C/N ratio in the reactor to 20, in turn, elevating the ammonia concentration in the reactor and compromising the stability of the operation.[13] Thus, feedstock composition—in particular, manure content—could have a larger impact in large-scale operations and engender differences in the layout of the process, because the addition of straw or recirculation of washed fibers might be needed to maintain a stable microbial community, although this step would reduce the methane yield slightly (by approximately 15%).

3.3. Washing and Ethanol Production: the Homogenization Effect.

The fractionation of the material was vital with regard to the results of the anaerobic digestion trials; thus, the findings of the washing are central to understanding the biorefining of animal bedding. The manure content had a clear effect on the performance of the washing step, because the washing efficiency for the high-content material was twice that for the low-content material (Figure 3). This result indicates that the washing efficiency rises with increasing manure content; i.e., the higher the manure content of animal bedding is, the easier it is to separate the manure from the bulk of the material.

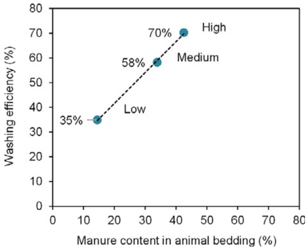


Figure 3. Effect of manure content of animal bedding on the efficiency of the washing steps.

This behavior has a significant consequence: the composition of the washed fiber is approximately the same, independent of the manure content of the starting material. For example, the largest difference in manure content of the materials was 5% after the washing (Table 2), despite the manure content differing by as much as 28% before it.

Table 2. Composition of the Washed Fiber in the Bioethanol and Biogas Trials

content (% TS bedding)	low	medium	high
manure	9.4	14.2	12.7
fiber	90.6	85.8	87.3
glucan	36.2	34.3	35.0
xylan	20.7	19.6	20.0
galactan	0.6	0.5	0.5
lignin	20.5	19.4	19.8
extractives	4.9	4.6	4.7

Similarly, the glucan content differed by as much as 11% before the washing versus 2% after it (Table 2), demonstrating that this pattern applies to other components of the material. Thus, the washing step has a homogenization effect in the biorefining process, because the output of this operation is constant, although the composition of the feedstock differs.

The homogenization effect of the washing could explain the results of the anaerobic digestion trials—i.e., because the washing mitigates the differences between feedstocks, the optimal layout is the same, irrespective of the starting composition of the feedstock. An important implication is that the washing—or fractionation, in general terms—allows material from various locations to be processed through the same operation, thus obviating the need for different processing lines that depend on the characteristics of the material. This approach could also resolve the contradiction in the literature, in which previous groups did not fractionate the material before bioprocessing it and causing the differences in the materials to be transferred further into the process, giving rise to the disparate results.

Table 3. Composition of the Pretreated Fiber for Each of the Feedstock in the Bioethanol and Biogas Trials

content	low	medium	high
WIS (%)	43.0	43.5	43.2
glucan (% WIS)	48.3	50.1	48.8
xylan (% WIS)	7.1	7.6	6.1
galactan (% WIS)	0.2	0.2	0.2
lignin (% WIS)	36.8	36.6	37.5

The results of the pretreatment step confirm the homogenization effect of the washing, because the pretreated fiber had the same characteristics for all three feedstocks (Table 3). Despite the differences in the composition of the feedstock, the constant output of the pretreatment reactor again demonstrates that the differences were virtually eliminated during the fractionation of the material and confirms that including a fractionation step allows to apply a unique design to treat animal beddings with distinct compositions. However, it may be possible that the homogenizing effect of the washing would have been less significant in pretreatment technologies that are not based on acid catalysts, since the risk of Maillard reactions is not relevant in such cases.[29]

Table 4. Glucose Yield in the Prehydrolysis and Ethanol Yield in the SSF for the Pretreated Fiber Corresponding to Each Feedstock in the Bioethanol Trials

yield	low	medium	high
EH (g glucose/kg washed fiber)	288	288	222
EH (% of theoretical)	72	76	57
SSF (g ethanol/kg washed fiber)	128	127	118
SSF (% of theoretical)	62	66	60

The performance of the pretreated fiber in the prehydrolysis and SSF was also similar in all cases, with the exception of slightly slower enzymatic hydrolysis for the material with the highest manure content (Table 4). Moreover, the ethanol yield was higher than that with technologies that lack a fractionation step, such as acid hydrolysis pretreatment that is followed by SSF (55.3%) and anaerobic digestion that is followed by NaOH treatment and ethanol production (46.7%).[6,8] These findings demonstrate that the homogenizing effect of the washing leads to one singular process design and higher yields in the bioconversion steps and thus increased valorization of the material. However, there would be an increased processing cost due to the water consumption in the washing, which points to the need of an economic study to determine whether this increase in conversion efficiencies offsets the additional cost of fractionating the material.

3.4. Synergy in Anaerobic Digestion: C/N Ratio and Carbon Accessibility. Fractionation of the material was why the same results were obtained for all feedstocks, but it does not explain why the binary and ternary mixtures performed better than the animal bedding. The aim of this section is to discuss why codigestion improved the efficiency, focusing primarily on the C/N ratio and carbon accessibility.

3.4.1. Synergy between Substrates in Codigestion. The three ternary mixtures had synergistic effects on methane yield and the production rate, whereas few to no interactions were detected for the binary mixtures (Figure 4), on the basis of an estimation of methane production, calculated as the linear combination of the cumulative methane yield from the monodigestion of each substrate. These synergies may originate from balancing the C/N ratio to its optimal value, because a robust microbial community is then maintained due to its sustained growth rate, explaining the differences in the anaerobic digestion trials. For example, the ternary mixture from the low-manure-content animal bedding, which exhibited greater synergy, performed much closer to methane yield of the washing liquid and hydrolysate mixture than the other two beddings.

The improvement in the ternary mixtures—for example, a 30% increase in the final methane yield for the ternary mixture of the low-manure-content animal bedding—was likely induced by synergistic effects between the two carbon sources, because the carbohydrates in the wheat straw, after their hydrolysis, are directed to the same metabolic pathway as the sugars in the hydrolysate. In fact, codigesting cellulose with xylose (the main sugar in the hydrolysate) increases the degradation of cellulose.[30] Further, in lignocelluloses, a high degradation rate of, for example, hemicellulose improves the degradation of the cellulose due to increased accessibility.[31] Thus, the synergy in the anaerobic digestion is most likely attributed to the blending of several carbon sources with disparate characteristics, rather than the presence of the washing liquid.

The improvement with the ternary mixtures occurred in terms of not only methane yield but also a shorter lag time, perhaps due to dilution, because the ternary mixtures contain less easily accessible carbohydrates than the binary mixtures with hydrolysate, yielding a more tolerable concentration of volatile fatty acids (VFAs) and thus inhibiting methanogenic

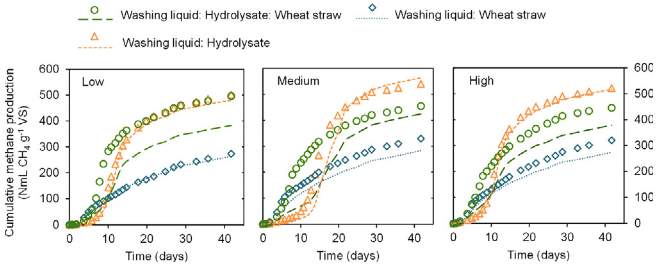


Figure 4. Modeled (dashed lines) and experimental (symbols) cumulative methane production from the substrate mixtures derived from the animal beddings with low, medium, and high manure content.

activity to a lesser extent.[32] Consequently, the inhibition and adaptation periods become shorter, in turn, decreasing the lag time in the ternary mixtures.

3.4.2. Relevance of the C/N Ratio.

Additional anaerobic digestion trials, in which washing liquid was mixed with wheat straw or a mixture of glucose and xylose, were performed to determine the effect of the C/N ratio and carbon accessibility on codigestion trials with animal bedding. The methane yield was approximately the same for all mixtures with wheat straw, approximately 300 N mL CH_4 g^{-1} VS, whereas those with glucose and xylose had a higher yield of roughly 500 N mL CH_4 g^{-1} VS, except for the mixture with a C/N ratio of 10, which had a yield of 428 N mL CH_4 g^{-1} VS (Figure 5). This result clearly shows the minimal impact of the C/N ratio on anaerobic digestion, although minor synergistic interactions might have occurred, because the methane yield would be expected to decline with decreasing C/N ratios.

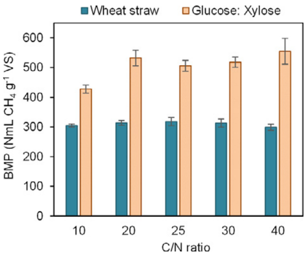


Figure 5. BMP generated from codigestion of washing liquid with wheat straw or glucose/xylose at several C/N ratios.

In contrast to the minute differences due to the C/N ratio, the bio-accessibility of the carbon source had a large effect on methane yield, wherein the biochemical methane potential (BMP) of the glucose and xylose blends was 40−85% higher than that of the wheat straw. This result explains why codigesting washing liquid with hydrolysate elicited the highest methane yield, because the carbon accessibility of the hydrolysate was much higher than that of animal bedding and wheat straw. It also strengthens the theory that the presence of manure in the ternary mixtures does not contribute much to the synergy with these mixtures, because balancing the C/N ratio could not have impacted the methane yield to a significant extent. In a broader context, this result indicates that increasing the bioaccessibility of the substrate to microorganisms—e.g., via pretreatment—is a more efficient strategy for achieving a high methane yield in the digester than balancing substrate mixtures to an optimal C/N ratio.

It is possible, however, that the C/N ratio is a larger factor in commercial-scale digesters, because the overload of inoculum, typical of the methods for measuring BMP, might have mitigated the effects of any possible limitations due to microbial growth. Moreover, the impact of the C/N ratio is lessened at low substrate loads,[33] such as those in this study, implicating a larger effect of the C/N ratio in a commercial setup, on the basis of the literature.

3.5. Relevance of Carbon Accessibility.

The anaerobic digestion trials in which wheat straw and a blend of glucose and xylose were codigested with washing liquid suggested that carbon accessibility had a larger effect on methane yield than the C/N ratio. To test this theory, we compared the daily methane production from the monodigestion of wheat straw, cellulose, and hydrolysate (Figure 6).

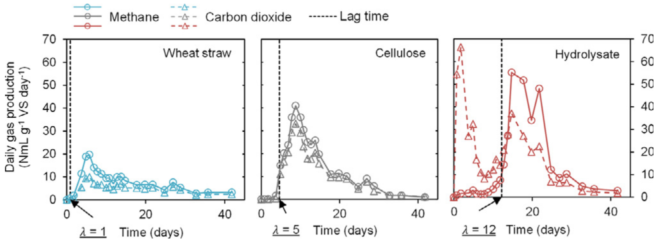


Figure 6. Daily methane production from monodigestion of wheat straw, cellulose, and hydrolysate.

Cellulose yielded a higher methane production rate than wheat straw, again demonstrating the positive effect of increasing carbon accessibility, attributed in part to the presence of inert lignin in the wheat straw. However, when increasing the carbon accessibility further (represented by the hydrolysate), the lag time was extended, perhaps due to a shift in the rate-limiting step of methane production: hydrolysis is not the rate-limiting step in substrates with low carbon accessibility, inducing a high initial conversion rate in acidogenesis and acetogenesis. This shift would also explain the wheat straw and cellulose simultaneously producing methane and carbon dioxide, whereas the hydrolysate produced only carbon dioxide at the beginning of the digestion.

There appears to be an optimal level of carbon accessibility for maximizing methane production with minimum risk of inhibition in the reactor. Diluting the hydrolysate with less accessible substrates decreased the lag time, because codigestion with washing liquid shortened the lag time by 14% and because codigestion with washing liquid and wheat straw nearly eliminated the lag time (Figure 7). Because the hydrolysate contains only easily accessible sugars and because acidification is generally a rapid conversion step, methane production depends almost exclusively on the performance of the methanogens, which are sensitive. Thus, the process might become more flexible when the metabolic flux is shifted to other bacterial species, as occurs when adding wheat straw because the hydrolysis activity increases. Codigestion of substrates generally promotes a more diverse and robust microbial consortium,[34] explaining why the strongest synergies were observed for ternary mixtures in the trials with animal bedding and suggesting that balancing carbohydrate and monomeric sugar content in the substrate optimizes the metabolic flux and thus methane production, because the initial hydrolysis and methanogenesis rates match.

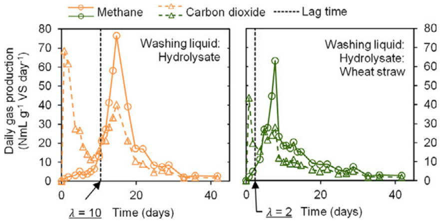


Figure 7. Daily methane production from codigesting a binary mixture of washing liquid and hydrolysate and a ternary mixture of washing liquid, hydrolysate, and wheat straw.

4. CONCLUSIONS

The processing of animal bedding does not depend on the manure content of the material, because the same biorefinery layout led to maximum conversion efficiencies for feedstocks with different manure contents. The selected layout consisted of washing the material to separate the manure from the fiber, which was subsequently pretreated by acid-catalyzed steam explosion. The liquid fraction of the pretreated material was codigested with the manure, whereas the solid fraction was used for bioethanol production. This technology delivered higher biogas and bioethanol yields, by approximately 3-fold and 1.2-fold, respectively, compared with other reported strategies.

The fractionation of the material appears to be why the composition of the material did not affect the results, because the output of the washing was the same in all cases, despite the ingoing material differing. This finding implies that fractionation has a homogenization effect on the process, allowing animal beddings with distinct characteristics to be treated with the same process layout.

The improved biogas yields were attributed to synergies between the substrates in the codigestion, which appeared to be related to the carbon accessibility of the resulting mixture. Our results demonstrate that higher carbon accessibility improves methane yields, although there is an optimal carbon accessibility with which the initial hydrolysis and methanogenesis rates of the bacterial consortium match. This effect explains why codigestion of the liquid fraction of the pretreated material with manure delivered the highest methane yields. It is also established that fractionating the material unlocks synergies between biogas and bioethanol production, in turn increasing conversion efficiencies compared with sequential production of the biofuels.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.iecr.9b04945.

> Composition of wheat straw, TS and VS of all process streams; TC, TN and C/N ratio of all process streams; composition of pretreatment hydrolysates, mixing ratios in anaerobic digestion trials based on animal bedding, and mixing ratios in anaerobic digestion trials based on wheat straw and a glucose/xylose blend (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: mirjam.victorin@chemeng.lth.se. Tel.: +46 722 51 78 71 (M.V.).
*E-mail: miguel.sanchis_sebastia@chemeng.lth.se. Tel.: +46 738 47 72 57 (M.S.-S.).

ORCID

Miguel Sanchis-Sebastiá: 0000-0003-4586-1229

Author Contributions

[†] M.V. and M.S.-S. contributed equally to this work.

Author Contributions

M.V. and M.S.S. designed the study with inputs from Å.D. and O.W. M.V. and M.S.S. performed all the experimental work together. M.V. and M.S.S. analyzed the results and wrote the manuscript with inputs from Å.D. and O.W. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported by the Swedish Energy Agency and BESTF3 (grant number P42674-1). The authors are also grateful to TK Energy A/S and Niels Lundager for their help in the collection of the animal bedding.

■ REFERENCES

(1) Hatfield, J. L.; Stewart, B. A. *Animal Waste Utilization: Effective Use of Manure as a Soil Resource*; CRC Press, 1997.

(2) Ceotto, E. The issues of energy and carbon cycle: new perspectives for assessing the environmental impact of animal waste utilization. *Bioresour. Technol.* **2005**, *96*, 191−196.

(3) Escobar, G.; Heillilä, M. Biogas Production in Farms, through Anaerobic Digestion of Cattle and Pig Manure. Case Studies and Research Activities in Europe. TEKES, TEKES/30.3.99/6, 1999.

(4) Jacobsen, B. H.; Laugesen, F. M.; Dubgaard, A. The economics of biogas in Denmark: a farm and socioeconomic perspective. *Int. J. Agric. Manage.* **2014**, *3*, 135−144.

(5) Liao, W.; Liu, Y.; Hodge, D. Integrated Farm-Based Biorefinery. *Biorefineries*; Elsevier: Amsterdam, 2014; Chapter 13; pp 255−270.

(6) Yue, Z.; Teater, C.; Liu, Y.; Maclellan, J.; Liao, W. A sustainable pathway of cellulosic ethanol production integrating anaerobic digestion with biorefining. *Biotechnol. Bioeng.* **2010**, *105*, 1031−9.

(7) Elumalai, S.; Roa-Espinosa, A.; Markley, J. L.; Runge, T. M. Combined sodium hydroxyde and ammonium hydroxide pretreatment of post-biogas digestion dairy manure fiber for cost effective cellulosic bioethanol production. *Sustainable Chem. Processes* **2014**, *2*, 12.

(8) Vancov, T.; Schneider, R. C.; Palmer, J.; McIntosh, S.; Stuetz, R. Potential use of feedlot cattle manure for bioethanol production. *Bioresour Technol* **2015**, *183*, 120−8.

(9) Bona, D.; Vecchiet, A.; Pin, M.; Fornasier, F.; Mondini, C.; Guzzon, R.; Silvestri, S. The Biorefinery Concept Applied to Bioethanol and Biomethane Production from Manure. *Waste Biomass Valorization* **2018**, *9*, 1−11.

(10) Wallace, T. *Sampling Liquid Manure for Analysis*. Final Report, Agdex 538-2; Alberta Agriculture and Food: Canada, 2008.

(11) Sanchis-Sebastiá, M.; Erdei, B.; Kovacs, K.; Galbe, M.; Wallberg, O. Analysis of Animal Bedding Heterogeneity for Potential Use in Biorefineries Based on Farmyard Manure. *Waste Biomass Valorization* **2019**, *143*, 37.

(12) Hills, D. J. Effects of carbon: Nitrogen ratio on anaerobic digestion of dairy manure. *Agric. Wastes* **1979**, *1*, 267−278.

(13) Wang, X.; Yang, G.; Feng, Y.; Ren, G.; Han, X. Optimizing feeding composition and carbon−nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. *Bioresour. Technol.* **2012**, *120*, 78−83.

(14) Li, X.; Li, L.; Zheng, M.; Fu, G.; Lar, J. S. Anaerobic Co-Digestion of Cattle Manure with Corn Stover Pretreated by Sodium Hydroxide for Efficient Biogas Production. *Energy Fuels* **2009**, *23*, 4635−4639.

(15) Mokomele, T.; da Costa Sousa, L.; Balan, V.; van Rensburg, E.; Dale, B. E.; Görgens, J. F. Incorporating anaerobic co-digestion of steam exploded or ammonia fiber expansion pretreated sugarcane residues with manure into a sugarcane-based bioenergy-livestock nexus. *Bioresour. Technol.* **2019**, *272*, 326−336.

(16) Hodge, J. E. Dehydrated Foods, Chemistry of Browning Reactions in Model Systems. *J. Agric. Food Chem.* **1953**, *1*, 928−943.

(17) Liao, W.; Liu, Y.; Liu, C.; Chen, S. Optimizing dilute acid hydrolysis of hemicellulose in a nitrogen-rich cellulosic material–dairy manure. *Bioresour. Technol.* **2004**, *94*, 33−41.

(18) Peters, J.; Combs, S. M.; Hoskins, B.; Kovar, J. L.; Watson, M. E.; Wolf, A. M.; Wolf, N. *Recommended Methods of Manure Analysis*. Final Report, A3769; University of Wisconsin-Madison: WI, 2003.

(19) Palmqvist, E.; Hahn-Hägerdal, B.; Galbe, M.; Larsson, M.; Stenberg, K.; Szengyel, Z.; Tengborg, C.; Zacchi, G. Design and operation of a bench-scale process development unit for the production of ethanol from lignocellulosics. *Bioresour. Technol.* **1996**, *58*, 171−179.

(20) Sanchis-Sebastiá, M.; Erdei, B.; Kovacs, K.; Galbe, M.; Wallberg, O. Introducing low-quality feedstocks in bioethanol production: efficient conversion of the lignocellulose fraction of animal bedding through steam pretreatment. *Biotechnol. Biofuels* **2019**, *12*, 215.

(21) Zwietering, M. H.; Jongenburger, I.; Rombouts, F. M.; van 't Riet, K. Modeling of the bacterial growth curve. *Appl. Environ. Microbiol.* **1990**, *56*, 1875−1881.

(22) Sluiter, A.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. Determination of Extractives in Biomass. Laboratory Analytical Procedure (LAP) Issue Date: 7/17/2005. NREL National Renewable Energy Laboratory, Technical Report NREL/TP-510-42619, January 2008.

(23) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. Determination of Structural Carbohydrates and Lignin in Biomass. Laboratory Analytical Procedure (LAP) Issue Date: 7/17/2005. NREL National Renewable Energy Laboratory, Technical Report NREL/TP-510-42618, January 2008.

(24) Sluiter, A.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. Determination of Insoluble Solids in Pretreated Biomass Material. Laboratory Analytical Procedure (LAP) Issue Date: 3/21/2008. NREL National Renewable Energy Laboratory, Technical Report NREL/TP-510-42627, March 2008.

(25) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples. Laboratory Analytical Procedure (LAP) Issue Date: 12/08/2006. NREL National Renewable Energy Laboratory, Technical Report NREL/TP-510-42623, January 2008.

(26) Chen, S.; Wen, Z.; Liao, W.; Liu, C.; Kincaid, R. L.; Harrison, J. H.; Elliott, D. C.; Brown, M. D.; Stevens, D. J. Studies into using manure in a biorefinery concept. *Appl. Biochem. Biotechnol.* **2005**, *124*, 0999−1015.

(27) Gómez, X.; Blanco, D.; Lobato, A.; Calleja, A.; Martínez-Núñez, F.; Martin-Villacorta, J. Digestion of cattle manure under mesophilic and thermophilic conditions: characterization of organic matter applying thermal analysis and 1H NMR. *Biodegradation* **2011**, *22*, 623−635.

(28) Riggio, S.; Hernandéz-Shek, M. A.; Torrijos, M.; Vives, G.; Esposito, G.; van Hullebusch, E. D.; Steyer, J. P.; Escudié, R. Comparison of the mesophilic and thermophilic anaerobic digestion of spent cow bedding in leach-bed reactors. *Bioresour. Technol.* **2017**, *234*, 466−471.

(29) Zhang, H.; Lopez, P. C.; Holland, C.; Lunde, A.; Ambye-Jensen, M.; Felby, C.; Thomsen, S. T. The multi-feedstock biorefinery - Assessing the compatibility of alternative feedstocks in a 2G wheat straw biorefinery process. *GCB Bioenergy* **2018**, *10*, 946−959.

(30) Xia, Y.; Cai, L.; Zhang, T.; Fang, H. H. P. Effects of substrate loading and co-substrates on thermophilic anaerobic conversion of microcrystalline cellulose and microbial communities revealed using high-throughput sequencing. *Int. J. Hydrogen Energy* **2012**, *37*, 13652−13659.

(31) Zhao, Y.; Sun, F.; Yu, J.; Cai, Y.; Luo, X.; Cui, Z.; Hu, Y.; Wang, X. Co-digestion of oat straw and cow manure during anaerobic digestion: Stimulative and inhibitory effects on fermentation. *Bioresour. Technol.* **2018**, *269*, 143−152.

(32) Astals, S.; Batstone, D. J.; Mata-Alvarez, J.; Jensen, P. D. Identification of synergistic impacts during anaerobic co-digestion of organic wastes. *Bioresour. Technol.* **2014**, *169*, 421−427.

(33) Wang, X.; Yang, G.; Li, F.; Feng, Y.; Ren, G. Response surface optimization of methane potentials in anaerobic co-digestion of multiple substrates: dairy, chicken manure and wheat straw. *Waste Manage. Res.* **2012**, *31*, 60−66.

(34) Mata-Alvarez, J.; Dosta, J.; Romero-Güiza, M. S.; Fonoll, X.; Peces, M.; Astals, S. A critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renewable Sustainable Energy Rev.* **2014**, *36*, 412−427.

Paper IV

processes

Article

Techno-Economic Evaluation of Biorefineries Based on Low-Value Feedstocks Using the BioSTEAM Software: A Case Study for Animal Bedding

Miguel Sanchis-Sebastiá *,†, Joaquín Gomis-Fons †, Mats Galbe and Ola Wallberg

Department of Chemical Engineering, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden;
joaquin.gomis_fons@chemeng.lth.se (J.G.-F.); mats.galbe@chemeng.lth.se (M.G.);
ola.wallberg@chemeng.lth.se (O.W.)
* Correspondence: miguel.sanchis_sebastia@chemeng.lth.se
† There is a shared first authorship.

Received: 10 July 2020; Accepted: 27 July 2020; Published: 31 July 2020

Abstract: Biofuels are still too costly to compete in the energy market and it has been suggested that low-value feedstocks could provide an opportunity for the production of low-cost biofuels; however, the lower quality of these feedstocks requires the introduction of a conditioning step in the biorefinery process. The aim of this study was to evaluate whether feedstock savings cover the cost of conditioning in the case of animal bedding. The BioSTEAM software was used to simulate a wheat straw biorefinery and an animal bedding biorefinery, whose economic performance was compared. The wheat straw biorefinery could deliver ethanol at a minimum selling price of USD 0.61 per liter, which is similar to prices in the literature. The cost of producing ethanol in the animal bedding biorefinery without water recycling was almost 40% higher, increasing the minimum selling price to USD 1.1 per liter of ethanol. After introducing water recycling in the conditioning step, the animal bedding biorefinery could deliver ethanol at a minimum selling price of USD 0.38 per liter, which is 40% lower than in the case of the wheat straw biorefinery. This demonstrates that low-value feedstocks can be used to reduce the biofuel price, as feedstock savings easily cover the additional conditioning cost.

Keywords: animal bedding; bioethanol; biorefinery; techno-economics; BioSTEAM

---

1. Introduction

In order to limit the global temperature increase to 2 °C, almost three-quarters of the global energy supply mix would need to be based on low-carbon technologies such as wind, solar or bioenergy, by 2050 [1]. The supply of bioenergy would be especially important in the transport sector, where the use of biofuels would need to triple by 2030, with advanced biofuels accounting for two thirds of this increase [2]. However, the production cost of biofuels is still too high for them to be commercially competitive with fossil fuels in the energy market [3]. The capital and feedstock costs have been identified as the main contributors to the total production cost of biofuels and are therefore the main hurdles to commercialization [3].

Zero- or negative-value waste could provide early market opportunities for the production of low-cost biofuels, although the most significant potential for cost reduction lies in reducing the capital cost through experience gained in deploying demonstration and early commercial plants [4]. In fact, biofuel researchers have started to investigate wastes that currently have no commercial use or value, such as municipal solid waste, food waste, slaughterhouse waste and industrial waste [5–8]. Another example of such a waste is animal bedding, consisting of straw mixed with manure, urine, and soil.

The problem in harnessing these early market opportunities lies in the lower conversion efficiency achieved with low-value wastes. For example, ethanol yields from wheat straw are typically around 70% [9,10], while the highest yield reported from animal bedding is 55% [11], and in many cases it is less than 50% [12,13]. This low conversion efficiency could eradicate the saving in feedstock costs, thus eliminating the opportunities associated with low-value waste. However, it has been shown that the introduction of a conditioning step, based on washing with water, in the biorefinery process allows the same conversion efficiency to be achieved with animal bedding as with wheat straw [14]. Similar conditioning processes, where the feedstock is fractionated into several streams, have also been proposed for other low-value wastes such as municipal solid waste and textile waste [15,16].

Even if conditioning allows the same conversion efficiency to be achieved as with high-value waste, it is still unclear whether the overall economics of the process would improve, as the conditioning step would increase both the capital and operational costs. To the best of our knowledge, no economic evaluation of such a process has been presented in the literature. This study was therefore undertaken to perform a techno-economic analysis of a biorefinery based on animal bedding as feedstock, and to compare the results to those for a biorefinery based on wheat straw, as this is a very similar facility at a higher maturity level. Our intention was to ascertain whether animal bedding actually represents an early market opportunity, by reducing the biofuel production cost. The software used to perform the techno-economic calculations was the open-source BioSTEAM suite, which means that this study also expands the portfolio of cases for which this software has been proven to be valid and reliable.

2. Materials and Methods

The modelling and economic estimations were performed according to the default settings in version 2.1.9 of the BioSTEAM software, written in Python v3.7 (Python Software Foundation, Wilmington, DE, USA, 2018), unless otherwise stated [17,18]. The models and economic estimations were taken from the biorefinery based on corn stover in the Bioindustrial-Park GitHub repository of examples provided by the developers of the software [19]. Thus, only the differences in modelling and the incorporation of new models are described in detail below.

Several tables (S1–S7) providing information on the specific reactions and conversion factors in each system (where applicable) together with block diagrams (Figures S1–S7) are given in the Supplementary Materials to facilitate the reproduction of the simulations without making the description here unduly long.

2.1. Modelling the Wheat Straw Biorefinery

The wheat straw biorefinery was based on 6 different systems: (i) pretreatment, (ii) hydrolysis and fermentation, (iii) ethanol purification, (iv) anaerobic digestion, (v) combined heat and power plant (CHP), and (vi) wastewater treatment. The first 3 systems model the production of ethanol from wheat straw. The liquid streams generated in these systems (apart from the final ethanol) are the input to the anaerobic digestion system, which models dedicated biogas production, while the solid stream (lignin residue) is the input to the CHP system, which models the production of steam and electricity. Finally, the residual water from anaerobic digestion is the input to the wastewater treatment system, which accounts for the cost of handling this residue.

2.1.1. Pretreatment System

A higher amount of water than in the original software [19] was used to model the soaking step, so the liquid-to-solid ratio in this step was increased to 20:1, and the sulfuric acid concentration in the liquid was set to 0.2 wt%. A filter press was therefore included after soaking to achieve a total solids (TS) content of 50% in the solid stream after this step. The soaking liquid removed by filtration was recycled to the soaking tank to minimize the consumption of fresh water and sulfuric acid.

Prior to steam pretreatment, the soaked biomass was preheated to 90 °C by mixing it with the residual and flashed steam after pretreatment. In order to do this, a split was included prior to the waste vapor condenser so that a fraction of the steam could be used for this purpose, while the rest of the residual steam was condensed for heat recovery.

Although the default model for steam pretreatment was retained [19], the temperature and residence time were assumed to be 190 °C and 10 min, and the conversion factors of the reactions were modified so that the simulation would match the experimental data presented by Erdei et al. [20]. A filter press was also included after the flash unit to fractionate the pretreated slurry into a solid fraction, used for ethanol production, and a liquid fraction, used for biogas production.

2.1.2. Hydrolysis and Fermentation System

The (solid) pretreated biomass was mixed with fresh water and ammonia in order to reduce the temperature to 50 °C and to obtain a pH of 5, in contrast to the original model [19], where a heat exchanger was used to reduce the temperature of the stream. This was done as it might be difficult to operate a heat exchanger with non-pumpable solid biomass in the stream. Fresh water is needed to dilute the stream to the water-insoluble solids (WIS) content at which enzymatic hydrolysis is carried out, so this strategy does not increase resource consumption, but avoids the need to design and operate a heat exchanger with solid biomass.

However, the amount of water required to cool the biomass to 50 °C is slightly greater than the amount needed to dilute the stream to the desired WIS content, which in this case was set to 20% (after the addition of the enzymes). Thus, a filter press was included prior to the addition of enzymes to remove the excess water, which was subsequently used in biogas production.

The flow of the enzyme stream (purchased externally) was modified to achieve a loading of 0.05 g enzymes/g WIS (assuming an activity of 200 FPU/g enzymes) in the enzymatic hydrolysis reactor, and the cultivation of yeast was designed to reach a yeast concentration of 3 g/L in the fermenter.

Although the hydrolysis reactions were not changed, the growth reactions in fermentation and cultivation were modified to match those of *S. cerevisiae*, as presented by Joelsson et al. [21], and the pentose fermentation reactions were removed, as naturally occurring *S. cerevisiae* was used as the fermenting microorganism. The new growth reactions required inclusion of an input stream of air in both cultivation and fermentation, and ammonia was assumed to be the nutrient source, instead of dihydrogen ammonium phosphate, as in the original software. The conversion factors of all reactions (hydrolysis and fermentation) were assigned to achieve an overall ethanol yield of 70% (0.35 g ethanol/g glucan in wheat straw), which corresponds to the experimental results presented by Ballesteros et al. [22].

2.1.3. Ethanol Purification System

Although the default distillation models were retained [19], the distillation scheme in the purification system was modified to increase the opportunities for energy integration and, therefore, reduce the energy consumption in the process. The new scheme included two strippers and a rectifier, the pressures of which were chosen so that the energy obtained in the condensers could be used in the reboilers. The pressure in the rectifier was set to 2 bar and the pressures in the strippers were designed to maximize the energy integration in the process, assuming a temperature difference of 5 °C in the heat transfer.

2.1.4. Anaerobic Digestion System

The dedicated biogas production system was modelled according to the anaerobic digestion model in the wastewater treatment system in the original software [19]. The default reactions and conversion factors were retained, but the biogas production was modified so that the methane flow would match the following experimental yields: 188 m^3 CH_4/ton TS from thin stillage and excess water (hydrolysis and fermentation system) [23], 0 m^3 CH_4/ton TS from condensed steam and rectifier stillage, and 350 m^3 CH_4/ton volatile solids from the liquid fraction of the pretreated slurry. The flow of biogas was then calculated under the assumption that the biogas had a molar methane content of 60%.

2.2. Modelling the Animal Bedding Biorefinery

The animal bedding biorefinery was based on the same systems as the wheat straw biorefinery, apart from the following three aspects: (i) a conditioning step was introduced in the pretreatment system, (ii) pretreatment was operated at 200 °C for 5 min and the acid concentration during soaking was 0.4 wt%, and (iii) it was assumed that the methane yield from the liquid fraction of the pretreated slurry was 550 m^3 CH_4/ton volatile solids [24], instead of 350 m^3 CH_4/ton volatile solids.

Conditioning and Pretreatment System

The conditioning step consisted of washing the animal bedding with water to transfer part of the manure to the liquid phase, to fractionate the different biomasses in the material. This was modelled as a series of dissolution reactions of the manure, assuming that the manure was insoluble in the starting material. The initial dissolution reactions took place in a new unit, the washing tank, where the animal bedding was mixed with 20 kg water/dry kg animal bedding, which was introduced after feedstock handling in the original system. This was followed by a filter press, which was designed to deliver a stream with a TS content of 50%. Only the solid fraction (washed fiber) was processed in the soaking step, while the liquid containing the manure (washing liquid) was directed to the anaerobic digester for biogas production. The conversion factors of the dissolution reactions were designed such that 60% of the manure in the starting material was removed during washing, according to the experimental data presented by Victorin et al. [24].

Additional dissolution reactions were introduced in the soaking tank, whose conversion factors were designed such that an additional 15% of the manure in the starting material was removed in this step. This corresponds to the manure removal that could be expected from an additional washing step with water, which means that the removal of manure in the actual process would be slightly higher due to the presence of the acid in the soaking water (animal bedding causes buffering in the soaking). Dissolution reactions were also introduced in steam pretreatment, so that all the remaining manure in the solid material was dissolved after this step, in accordance with our experimental observations. The conversion factors for the rest of the reactions in the pretreatment were designed such that the simulation would match the experimental data presented by Victorin et al. [24].

The possibility of recycling water in the washing step was investigated in a second version of the conditioning model. This model included a belt filter after the filter press to concentrate the washing liquid to a TS content of 5%. The filtered liquid (permeate) was subsequently mixed with fresh water to achieve the required flow, based on a value of 20 kg water/dry kg animal bedding, and the mixture was recycled to the washing tank. The concentrated liquid (retentate) was directed to the anaerobic digester for biogas production, as in the case of the washing liquid in the version without water recycling.

2.3. Economic Estimations

The majority of the economic estimations were the same as in the original BioSTEAM software, that is, the estimations presented by Seider et al. [25] for conventional units, and the estimations presented by Humbird et al. [26] for specific biorefining units. However, the economic estimations of the filter press and the distillation units under vacuum conditions were modified. The capital cost of the filter presses was estimated based on the required filtration area, instead of the volumetric flow rate, which was calculated based on a flux of 1220 kg/h m^2 for all units, except the filter press in the ethanol purification system, where a lower flux of 976 kg/h m^2 was assumed due to the high concentration of residual lignocellulosics in the stream. The capital cost of the distillation units under vacuum conditions was assumed to be the same as if the distillation had been performed at atmospheric pressure, since the vacuum applied was not sufficiently high to justify a cost increase [27].

New economic estimations were implemented for the models added to the original software: the soaking tank and its filter press, the anaerobic digester, the washing tank and its filter press, and the belt filter. The capital cost of the washing and soaking tanks was calculated based on the estimates of the capital cost of tanks, agitators, and transfer pumps in the fermenter model of the original software. The electricity consumption was assumed to be the same as that of the agitator in the fermenter tank in both cases.

The cost of the filter presses after the soaking and washing tanks could not be estimated with the same method as the filter press in the original software because of the large volumetric flow rate in these units, which required an initial draining step prior to filtration. Thus, the cost of the draining step, in which water was drained so that the resulting stream had a TS content of 20%, was estimated in the same way as the primary wastewater treatment cost presented by Seider et al. [25], while the cost of the filter press, where the stream was filtered to achieve the desired TS content, was estimated as in the original software (with modified fluxes as mentioned above).

The capital cost of the anaerobic digester was based on a personal quote of USD 14 million for an 18,000 m^3 digester (O. Wallberg, personal communication, Apr 2020), and it was assumed that this cost increases linearly with digester size. The other costs related to the anaerobic digester (heating, mixing, power consumption, etc.) were estimated with the same method as that used for the fermenter in the original software. An additional cost was also included in the economic estimation of the anaerobic digester to account for the upgrading of the biogas to vehicle fuel quality. This cost was calculated based on the assumption that the capital cost for such an installation would be USD 1430 per Nm^3/h of biogas produced, and that its electricity demand would be 0.25 kWh/Nm^3 of biogas produced [28].

The capital cost of the belt filter was based on a personal quote of USD 91,000 for a filter with a hydraulic capacity of 20 m^3/h (M. Sjölin, personal communication, May 2020), under the assumption that this cost increased linearly with the volumetric flow rate. In terms of operating costs, it was assumed that the belt filter would consume 80 W per m^3/h of liquid input.

3. Results and Discussion

3.1. Simulation of the Wheat Straw Biorefinery

Although a plant size of 2000 dry ton/day of feedstock was assumed in the economic estimations, simulations based on a plant size of 1000 dry kg/h of feedstock were performed in order to facilitate the interpretation of the results, comparison with the results of previous studies, and to confirm the validity of the models. Thus, the results presented and discussed in the two following sections are based on a plant capacity of 1000 dry kg/h, whereas the economic data presented in the final section are based on a plant capacity of 2000 dry ton/day.

The biorefinery based on wheat straw would produce 120 kg/h of ethanol, 60 kg/h of methane (approximately 170 kg/h of biogas) and 532 kW of electricity (Figure 1). Apart from the electricity, the combined heat and power plant would also produce enough steam to cover all the heating duties in the biorefinery. This means that the process based on wheat straw would not consume any utility, since the electricity consumed was less than the electricity produced in the combined heat and power plant; thus, the biorefinery would deliver a surplus of electricity, together with the biofuels, to the energy market.

The mass and energy flows for each individual system can be found in the Supplementary Materials (Figures S1–S7) and only the most relevant aspects in evaluating the validity of the models are discussed in this section. The pretreatment system consumed 426 kW of high-pressure steam (522 kg/h), of which 361 kW could be recovered in the waste vapor condenser. This result is consistent with previous models of biorefineries based on wheat straw [21] which, together with the fact that the composition of the streams matched the experimental data presented by Erdei et al. [20], proves the validity of this part of the model.

The fermentation broth in the saccharification and fermentation system had an ethanol concentration of 4.4 wt%, which is similar to the concentration obtained experimentally in fermentations based on (pretreated) lignocellulosic material. Apart from the production of ethanol, the (solid) pretreated biomass was used to produce the amount of yeast required in the process. It was necessary to divert 15% of the saccharified biomass for this purpose, a value similar to the 10% obtained in the example based on corn stover provided by the developers of the BioSTEAM software [29].

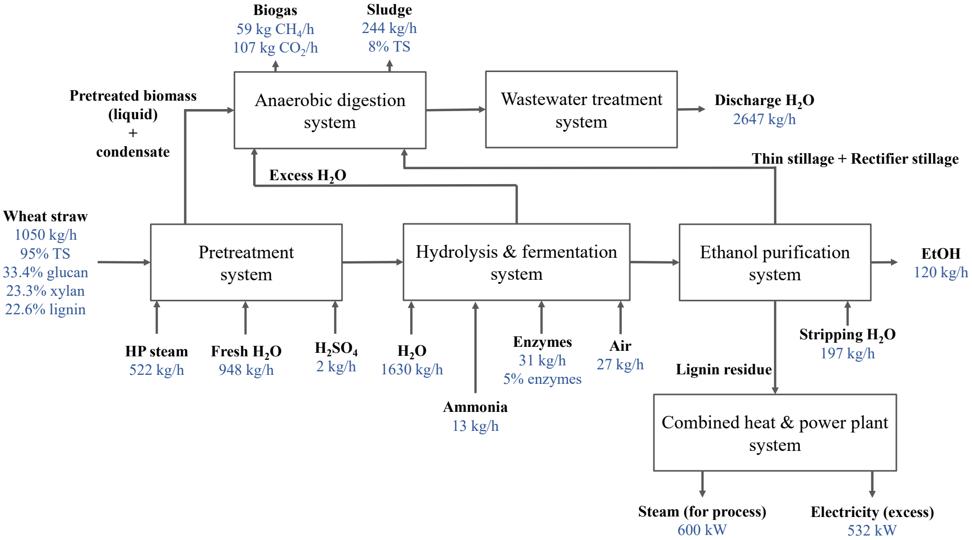


Figure 1. Mass and energy flows in the wheat straw biorefinery, assuming a plant capacity of 1000 dry kg/h of feedstock.

The energy consumed in distillation could be completely supplied through energy integration as the low-pressure stripper was supplied with energy by the high-pressure stripper (78 kW), which in turn was provided with energy from the rectifier and the cooling of the product after molecular sieving (90 kW). The energy recovered in the waste vapor condenser (361 kW) would be more than enough to supply the energy required by the rectifier (97 kW). Thus, the recovery of ethanol in the fermentation broth had no utility cost in terms of heating duty, which is consistent with the conclusions presented previously by Joelsson et al. [21].

Based on the above results, it was concluded that the model of the wheat straw biorefinery closely reflects the real process, provides similar results to previous models, and thus provides a valid benchmark that can be used to evaluate the biorefinery based on animal bedding.

3.2. *Simulation of the Animal Bedding Biorefinery*

The biorefinery based on animal bedding would deliver different amounts of the products: 94 kg/h of ethanol, 90 kg/h of methane (approximately 260 kg/h of biogas) and 145 kW of electricity (Figure 2). The reason for this is that the lignocellulosic fiber (straw) is a smaller fraction of the material, which implies that there is less substrate for ethanol production and less lignin available for combustion for heat and electricity production. However, the manure provides an additional substrate for biogas production, which means that this biorefinery would rely on biogas production considerably more than the biorefinery based on wheat straw.

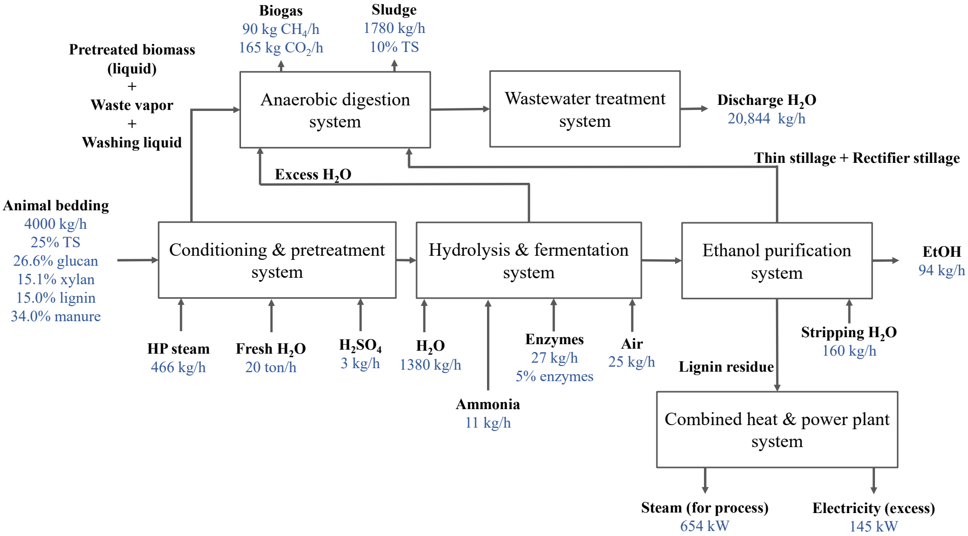


Figure 2. Mass and energy flows in the animal bedding biorefinery without water recycling, assuming a plant capacity of 1000 dry kg/h of feedstock.

The process for the production of ethanol from animal bedding was very similar to that for wheat straw, thanks to the introduction of conditioning. The composition of the (solid) pretreated material was very similar, and the ethanol concentration in the fermentation broth was in the same range (3.9 wt%). Distillation was operated at the same temperature, but the energy consumption of the boilers was slightly lower due to the smaller mass flow in this part of the process (222 kW, compared to 265 kW in the wheat straw model). The lignin stream used in the combined heat and power plant contained only 2 wt% manure, so its quality as a fuel would be approximately the same, and only a minor fraction of the manure would not reach the anaerobic digestion step and would be incinerated instead of being converted into biogas.

This process would require a slightly higher heating duty than the process based on wheat straw, as the combined heat and power plant was required to deliver 654 kW of steam (compared to 600 kW in the wheat straw model). The reason for this is that the heat recovered in the waste vapor condenser could not be used to supply the energy required by the rectifier, as it was instead used to heat the incoming stream to the anaerobic digester to the operating temperature. The introduction of the washing liquid in the anaerobic digestion step decreased the temperature of the incoming stream considerably, due to the large volumetric flow used in washing, which created a heating duty that did not exist in the process based on wheat straw.

The volumetric flow in the anaerobic digestion and wastewater treatment systems was almost eight times higher than in the wheat straw model due to the presence of the washing liquid. This led to a higher electricity consumption in these units (for both pumping and agitation) and a greater consumption of chemicals in the wastewater treatment system (mainly caustic soda). The electricity consumption was no longer less than the amount of electricity produced, which was further aggravated by the fact that less electricity was produced due to the lower lignin production. As a result of this, the biorefinery based on animal bedding would not be able to deliver electricity to the energy market. In fact, since it was necessary to supply electricity to the process, there was an additional utility cost compared to the wheat straw model.

The introduction of a belt filter to recycle water in the conditioning step reduced the additional flow in the anaerobic digestion and wastewater treatment systems considerably (approximately 4000 kg/h instead of 20,000 kg/h) and reduced the fresh water consumption of the conditioning step to 1700 kg/h. As a result of this, it was no longer necessary to heat the incoming stream to anaerobic digestion, so it was possible to supply energy to the rectifier through energy integration, as in the wheat straw model. However, although the electricity consumption decreased, the process still consumed more electricity than was produced in the combined heat and power plant, so water recycling did not completely eliminate the need for electricity supply.

Another negative effect created by water recycling was that the water used in conditioning contained 0.5 wt% manure, which might reduce the washing efficiency, and therefore modify the subsequent ethanol production, although this effect was not included in the model due to the lack of experimental data.

3.3. Techno-Economic Feasibility

The wheat straw biorefinery had revenues of approximately USD 1500 million, in terms of net present value (Figure 3). The largest source of revenue was from ethanol, which accounted for 53% of the total revenue, followed by biogas (38%) and, lastly, electricity (9%). The revenue from ethanol was calculated based on the minimum ethanol selling price (MESP), that is, the selling price at the break-even point, which was USD 0.61 per liter.

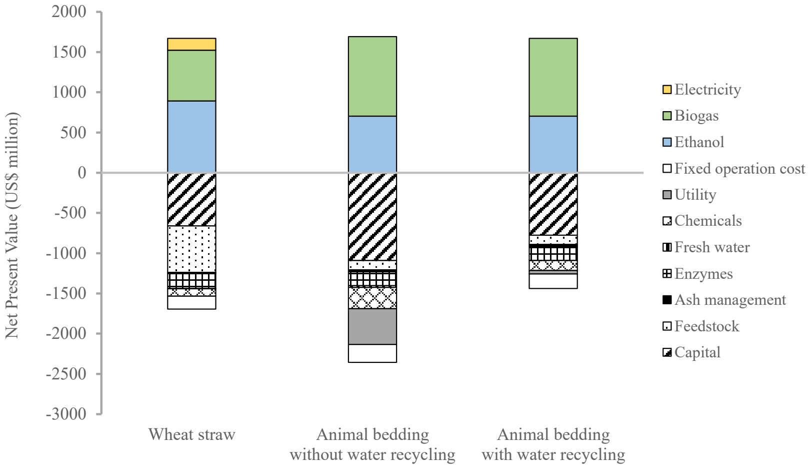


Figure 3. Net present value of revenues, capital cost, operational costs in the wheat straw biorefinery and the animal bedding biorefinery, with and without water recycling in the conditioning step.

In terms of costs, the three main contributors to the total cost of the wheat straw biorefinery were the capital cost (39%), the feedstock (34%), and the enzymes (11%). These findings are in accordance with previously reported results that the capital cost and the feedstock are the main contributors to the total cost [3], and that the feedstock usually accounts for a third of the total cost in production processes based on lignocellulosic material [30]. Thus, the economic estimations implemented in the model are valid. The large contribution of the feedstock to the total cost clearly illustrates the opportunity for cost reduction through the use of feedstocks with lower value.

The sources of revenue in the animal bedding biorefinery without water recycling differed considerably from those in the wheat straw biorefinery (Figure 3), due to the different amounts of products mentioned above. Assuming an ethanol selling price of USD 0.61 per liter, the ethanol revenue decreased to USD 704 million (20% lower than the benchmark), while the biogas revenue increased to USD 987 million (57% higher than the benchmark). This means that biogas became the main revenue in the biorefinery based on animal bedding, which implies that the two kinds of biorefineries might not be competing in the same markets.

The total cost of the animal bedding biorefinery without water recycling was much higher than that of the benchmark: USD 2356 million, compared to USD 1694 million, which represents an increase of almost 40% (Figure 3). The implication of this is that the MESP almost doubled, to a value of USD 1.1 per liter, despite the savings in feedstock cost, which were assumed to be 80% compared to the benchmark. The main reason for this cost increase was a higher capital cost; an increase of 65% compared to the benchmark. Although the conditioning step increased the capital cost by USD 5.7 million, its contribution was insignificant compared to the increases in cost resulting from the need for a larger anaerobic digester and a larger wastewater treatment system, which amounted to USD 87.5 and 88.4 million more, respectively, than the corresponding units in the benchmark. Thus, water recycling in conditioning seems to be a promising alternative, as the increase in capital cost was due to the larger volumetric flow in the anaerobic digestion and wastewater systems, rather than the introduction of conditioning in the process.

The higher consumption of electricity and chemicals were additional reasons for the cost increase in the animal bedding biorefinery without water recycling. The utility cost was the second highest contributor to the total cost, USD 444 million (Figure 3), and the cost of chemicals was greater than the cost of enzymes (USD 265 million, compared to USD 173 million). The sources of these new contributions to the cost were again the anaerobic digester and the wastewater treatment system, which underlines the importance of water recycling in the conditioning system.

When implementing water recycling in the conditioning step, the total cost of the animal bedding biorefinery decreased to USD 1439 million (Figure 3), which is 15% lower than the benchmark. This means that the MESP decreased to USD 0.38 per liter as a result of water recycling, demonstrating that low-value feedstocks represent an opportunity for early production of low-cost biofuels, as this MESP was almost 40% lower than the benchmark. The main reason for this improvement was the decrease in the capital cost to USD 776 million (Figure 3), which was still 17% higher than the benchmark, but did not counterbalance the 80% savings in feedstock cost.

The reduction in the volumetric flow in the anaerobic digester and wastewater treatment systems reduced the consumption of chemicals considerably, so this contribution to the cost was in the same range as in the benchmark. The electricity consumption was also decreased, but not to a degree that the biorefinery could sell electricity to the energy market, and therefore the revenue structure remained the same as in the biorefinery without water recycling: biogas being the main source of revenue, followed by ethanol. However, despite the fact that the ethanol revenues remained the same, the biogas revenue decreased by 2% compared to the biorefinery without water recycling, due to the accumulation of manure in the water recycling loop. This was, nevertheless, an insignificant effect compared to the considerable cost savings resulting from water recycling.

Sensitivity Analyses

In the simulations described above, animal bedding was assigned a price that was 20% of that of wheat straw, which was assumed to cover transportation costs, but no additional cost for the material itself. However, it has been suggested that a higher price of the feedstock could have positive effects on the biorefinery, as more farmers would be willing to deliver biomass to the facility, increasing the biomass availability for the biorefinery [31]. We therefore conducted a sensitivity analysis to evaluate the effect of the price of the feedstock and to determine whether a higher price could be paid for animal bedding.

The MESP increased linearly with increasing price of the feedstock, from USD 0.28 per liter assuming no feedstock cost, to USD 0.78 per liter assuming the same cost as wheat straw (Figure 4). This means that the animal bedding biorefinery with water recycling requires a reduction of at least 35% in the feedstock price (65% of the wheat straw price) to compete with the benchmark in terms of process economics. This is considerably higher than our assumption in the simulations (20% of the wheat straw price), which means that the process would still outperform the benchmark if the price of animal bedding was higher than we initially assumed. For example, if the price of animal bedding was twice that assumed in the initial simulations, the MESP would still be 20% lower than the benchmark.

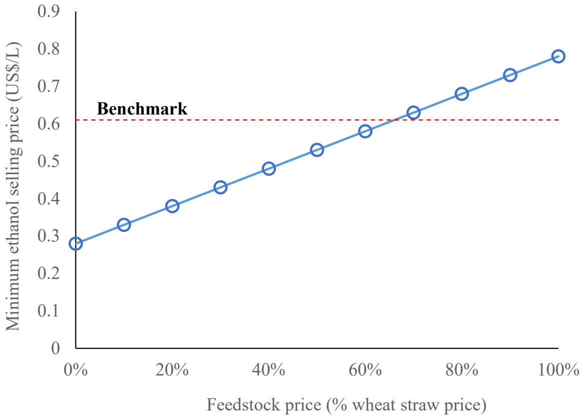


Figure 4. Minimum ethanol selling price as a function of the feedstock price in the animal bedding biorefinery with water recycling.

It is uncertain whether the efficiency of conditioning would remain the same in the system with water recycling, due to the accumulation of manure in the recycling loop, which might compromise the efficiency of the ethanol production process [32]. Although further studies are required on conditioning to quantify the effect of manure accumulation on the washing efficiency, we investigated the possibility of filtering and recycling only a fraction of the washing liquid. This would reduce the concentration of manure in the recycling loop, but would also have a negative impact on the process economics (Figure 5). The reason for this is that recycling only a fraction of the washing liquid increases the volumetric flow through the anaerobic digestion and wastewater systems, causing the process economics to shift towards that of the biorefinery without water recycling, although less manure would accumulate in the recycling loop due to the higher input of fresh water for conditioning.

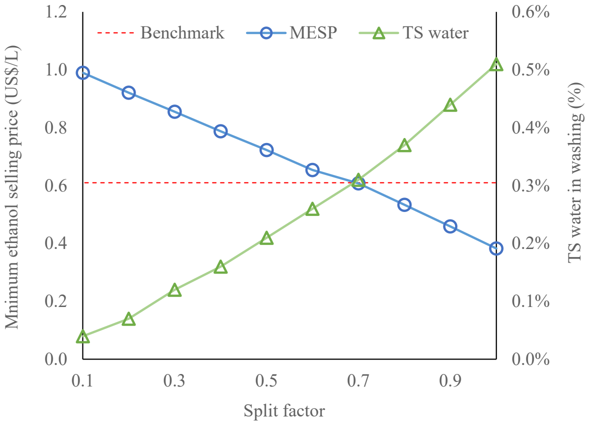


Figure 5. Minimum ethanol selling price (MESP) and total solids (TS) content in the water used in the conditioning step as a function of the split factor in the water recycling loop, expressed as the fraction of the washing liquid that was filtrated and recycled to conditioning. The feedstock price was assumed to be 20% of the wheat straw price.

It was found that the MESP would be the same as the benchmark when only 70% of the washing liquid was filtrated and recycled, which would reduce the manure concentration in the recycling loop from 0.5% to 0.3% (Figure 5). This implies that it would be possible to alleviate the problem of manure accumulation, but the benchmark would still economically outperform the process with water recycling if a manure concentration below 0.3% was required to maintain the efficiency of conditioning.

4. Conclusions

A biorefinery based on wheat straw would be able to deliver ethanol at a minimum ethanol selling price of USD 0.61 per liter, as a result of the additional revenues from biogas and electricity. Using a lower quality feedstock, such as animal bedding, would reduce the minimum ethanol selling price to USD 0.38 per liter, despite the introduction of a conditioning step in the biorefinery process. This demonstrates that low-value feedstocks could represent early opportunities to produce low-cost biofuels, as the feedstock savings easily overcome the conditioning cost. However, the animal bedding biorefinery required water recycling to the conditioning step to economically outcompete the wheat straw biorefinery. The reason for this was the large increase in volumetric flow in the subsequent steps of the process (anaerobic digestion and wastewater treatment) when water was not recycled, as the cost of conditioning itself was not significant. Thus, introducing a conditioning step in a biorefinery might affect other parts of the production process, which means that, apart from designing the conditioning technology, it would also be necessary to appropriately design its integration in the biorefinery.

Further sensitivity analyses showed that the animal bedding biorefinery with water recycling required a reduction of only 35% in the feedstock price, compared to straw, to economically outcompete the wheat straw biorefinery. This means that it would be possible to increase the price of animal bedding, which might have positive effects on feedstock availability, without compromising the competitiveness of the process. The sensitivity analyses also revealed that it would be possible to filtrate and recycle only 70% of the water used in conditioning, and still outperform the wheat straw biorefinery. This shows that the accumulation of manure in conditioning, which might compromise its efficiency, could be alleviated to a certain extent, if required.

Supplementary Materials: The following are available online at http://www.mdpi.com/2227-9717/8/8/904/s1, Figure S1. Block diagram of the pretreatment system in the wheat straw biorefinery, Figure S2. Block diagram of the hydrolysis and fermentation system in the wheat straw biorefinery, Figure S3. Block diagram of the ethanol purification system in the wheat straw biorefinery, Figure S4. Block diagram of the conditioning and pretreatment system (without water recycling) in the animal bedding biorefinery, Figure S5. Block diagram of the conditioning and pretreatment system (with water recycling) in the animal bedding biorefinery, Figure S6. Block diagram of the hydrolysis and fermentation system in the animal bedding biorefinery, Figure S7. Block diagram of the ethanol purification system in the animal bedding biorefinery, Table S1. Reactions considered in the steam pretreatment for the wheat straw biorefinery, Table S2. Reactions considered in the saccharification, yeast production and fermentation for the wheat straw biorefinery, Table S3. Reactions considered in the steam pretreatment (except for the manure dissolution reactions) for the animal bedding biorefinery, Table S4. Reactions considered in the saccharification, yeast production and fermentation for the animal bedding biorefinery, Table S5. Manure dissolution reactions considered in different parts of the animal bedding biorefinery, Table S6. Purchase and selling prices used in the economic estimations, Table S7. Financial parameters used in the economic estimations.

Author Contributions: † These authors contributed equally. Conceptualization, M.S.-S., J.G.-F. and O.W.; methodology, M.S.-S. and J.G.-F.; software, J.G.-F.; validation, M.S.-S., J.G.-F. and O.W.; investigation, M.S.-S., J.G.-F., M.G. and O.W.; resources, M.G. and O.W.; writing—original draft preparation, M.S.-S.; writing—review and Editing, M.S.-S., J.G.-F., M.G. and O.W.; supervision, M.G. and O.W.; project administration, O.W.; funding acquisition, O.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. International Energy Agency (IEA); International Renewable Energy Agency (IRENA). Executive Summary of Perspectives for the Energy Transition—Investment Needs for a Low-Carbon Energy System. 2017. Available online: https://www.irena.org/publications/2017/Mar/Perspectives-for-the-energy-transition-Investment-needs-for-a-low-carbon-energy-system (accessed on 20 April 2017).
2. International Energy Agency (IEA). Technology Roadmap—Delivering Sustainable Bioenergy. 2017. Available online: http://www.iea.org/publications/freepublications/publication/Technology_Roadmap_Delivering_Sustainable_Bioenergy.pdf (accessed on 18 January 2018).
3. Sub Group on Advanced Biofuels. Building up the Future. The Cost of Biofuels; MI-06-17-107-EN-N. 2017. Available online: https://publications.europa.eu/en/publication-detail/-/publication/13e27082-67a2-11e8-ab9c-01aa75ed71a1/language-en/format-PDF (accessed on 27 June 2018).
4. International Energy Agency (IEA). Advanced Biofuels—Potential for Cost Reduction. 2020. Available online: https://www.ieabioenergy.com/wp-content/uploads/2020/02/T41_CostReductionBiofuels-11_02_19-final.pdf (accessed on 13 March 2020).
5. Dabe, S.J.; Prasad, P.J.; Vaidya, A.N.; Purohit, H.J. Technological pathways for bioenergy generation from municipal solid waste: Renewable energy option. *Environ. Prog. Sustain. Energy* **2018**, *38*, 654–671. [CrossRef]
6. Uçkun Kiran, E.; Trzcinski, A.P.; Ng, W.J.; Liu, Y. Bioconversion of food waste to energy: A review. *Fuel* **2014**, *134*, 389–399. [CrossRef]
7. Sagastume Gutiérrez, A.; Cabello Eras, J.J.; Hens, L.; Vandecasteele, C. The energy potential of agriculture, agroindustrial, livestock, and slaughterhouse biomass wastes through direct combustion and anaerobic digestion. The case of Colombia. *J. Clean. Prod.* **2020**, *269*. [CrossRef]
8. Bakraoui, M.; Hazzi, M.; Karouach, F.; Ouhammou, B.; El Bari, H. Experimental biogas production from recycled pulp and paper wastewater by biofilm technology. *Biotechnol. Lett.* **2019**, *41*, 1299–1307. [CrossRef]
9. Tomás-Pejó, E.; Fermoso, J.; Herrador, E.; Hernando, H.; Jiménez-Sánchez, S.; Ballesteros, M.; González-Fernández, C.; Serrano, D.P. Valorization of steam-exploded wheat straw through a biorefinery approach: Bioethanol and bio-oil co-production. *Fuel* **2017**, *199*, 403–412. [CrossRef]
10. Larsen, J.; Østergaard Petersen, M.; Thirup, L.; Wen Li, H.; Krogh Iversen, F. The IBUS Process—Lignocellulosic Bioethanol Close to a Commercial Reality. *Chem. Eng. Technol.* **2008**, *31*, 765–772. [CrossRef]
11. Vancov, T.; Schneider, R.C.; Palmer, J.; McIntosh, S.; Stuetz, R. Potential use of feedlot cattle manure for bioethanol production. *Bioresour. Technol.* **2015**, *183*, 120–128. [CrossRef] [PubMed]
12. Yue, Z.; Teater, C.; Liu, Y.; Maclellan, J.; Liao, W. A sustainable pathway of cellulosic ethanol production integrating anaerobic digestion with biorefining. *Biotechnol. Bioeng.* **2010**, *105*, 1031–1039. [CrossRef] [PubMed]
13. You, Y.; Liu, S.; Wu, B.; Wang, Y.-W.; Zhu, Q.-L.; Qin, H.; Tan, F.-R.; Ruan, Z.-Y.; Ma, K.-D.; Dai, L.-C.; et al. Bio-ethanol production by Zymomonas mobilis using pretreated dairy manure as a carbon and nitrogen source. *RSC Adv.* **2017**, *7*, 3768–3779. [CrossRef]
14. Sanchis-Sebastiá, M.; Erdei, B.; Kovacs, K.; Galbe, M.; Wallberg, O. Introducing low-quality feedstocks in bioethanol production: Efficient conversion of the lignocellulose fraction of animal bedding through steam pretreatment. *Biotechnol. Biofuels* **2019**, *12*, 215. [CrossRef]
15. Ballesteros, M.; Sáez, F.; Ballesteros, I.; Manzanares, P.; Negro, M.J.; Martínez, J.M.; Castañeda, R.; Oliva Dominguez, J.M. Ethanol Production from the Organic Fraction Obtained After Thermal Pretreatment of Municipal Solid Waste. *Appl. Biochem. Biotechnol.* **2010**, *161*, 423–431. [CrossRef] [PubMed]
16. Hasanzadeh, E.; Mirmohamadsadeghi, S.; Karimi, K. Enhancing energy production from waste textile by hydrolysis of synthetic parts. *Fuel* **2018**, *218*, 41–48. [CrossRef]
17. Cortes-Peña, Y. BioSTEAM: The Biorefinery Simulation and Techno-Economic Analysis Modules. Available online: https://github.com/yoelcortes/biosteam (accessed on 17 January 2020).
18. Cortes-Peña, Y.; Guest, J.S. Documentation for BioSTEAM: The Biorefinery Simulation and Techno-Economic Analysis Modules. Available online: https://biosteam.readthedocs.io/en/latest (accessed on 17 January 2020).
19. Cortes-Peña, Y. Bioindustrial-Park: BioSTEAM's Premier Repository for Biorefinery Models and Results. Available online: https://github.com/yoelcortes/Bioindustrial-Park (accessed on 17 January 2020).

20. Erdei, B.; Hancz, D.; Galbe, M.; Zacchi, G. SSF of steam-pretreated wheat straw with the addition of saccharified or fermented wheat meal in integrated bioethanol production. *Biotechnol. Biofuels* **2013**, *6*, 169. [CrossRef] [PubMed]
21. Joelsson, E.; Dienes, D.; Kovacs, K.; Galbe, M.; Wallberg, O. Combined production of biogas and ethanol at high solids loading from wheat straw impregnated with acetic acid: Experimental study and techno-economic evaluation. *Sustain. Chem. Process.* **2016**, *4*, 14. [CrossRef]
22. Ballesteros, I.; Negro, M.J.; Oliva, J.M.; Cabanas, A.; Manzanares, P.; Ballesteros, M. Ethanol production from steam-explosion pretreated wheat straw. *Appl. Biochem. Biotechnol.* **2006**, *130*, 496–508. [CrossRef]
23. Bauer, A.; Bosch, P.; Friedl, A.; Amon, T. Analysis of methane potentials of steam-exploded wheat straw and estimation of energy yields of combined ethanol and methane production. *J. Biotechnol.* **2009**, *142*, 50–55. [CrossRef]
24. Victorin, M.; Sanchis-Sebastiá, M.; Davidsson, Å.; Wallberg, O. Production of Biofuels from Animal Bedding: Biogas or Bioethanol? Influence of Feedstock Composition on the Process Layout. *Ind. Eng. Chem. Res.* **2019**, *58*, 21927–21935. [CrossRef]
25. Seider, W.D.; Lewin, D.R.; Seader, J.D.; Widagdo, S.; Gani, R.; Ng, K.M. *Product and Process Design Principles: Synthesis, Analysis, and Evaluation*, 4th ed.; Wiley: Hoboken, NJ, USA, 2017.
26. Humbird, D.; Davis, R.; Tao, L.; Kinchin, C.; Hsu, D.; Aden, A.; Schoen, P.; Lukas, J.; Olthof, B.; Worley, M.; et al. *Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol*; NREL/TP-5100-47764; National Renewable Energy Laboratory: Golden, CO, USA, 2011.
27. Smith, R. *Chemical Process: Design and Integration*, 2nd ed.; Wiley: Chichester, UK, 2005.
28. Bauer, F.; Hulteberg, C.; Persson, T.; Daniel, T. *Biogas Upgrading—Review of Commercial Technologies*; SGC Rapport 2013:270; SGC Rapport: Malmö, Sweden, 2013.
29. Cortes-Peña, Y.; Kumar, D.; Singh, V.; Guest, J.S. BioSTEAM: A Fast and Flexible Platform for the Design, Simulation, and Techno-Economic Analysis of Biorefineries under Uncertainty. *ACS Sustain. Chem. Eng.* **2020**, *8*, 3302–3310. [CrossRef]
30. Balan, V. Current Challenges in Commercially Producing Biofuels from Lignocellulosic Biomass. *ISRN Biotechnol.* **2014**, *2014*, 463074. [CrossRef]
31. Kim, S.; Dale, B.E. All biomass is local: The cost, volume produced, and global warming impact of cellulosic biofuels depend strongly on logistics and local conditions. *Biofuels Bioprod. Biorefin.* **2015**, *9*, 422–434. [CrossRef]
32. Liao, W.; Liu, Y.; Liu, C.; Chen, S. Optimizing dilute acid hydrolysis of hemicellulose in a nitrogen-rich cellulosic material—Dairy manure. *Bioresour. Technol.* **2004**, *94*, 33–41. [CrossRef] [PubMed]

Paper V

Waste Management 121 (2021) 248–254

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Novel sustainable alternatives for the fashion industry: A method of chemically recycling waste textiles via acid hydrolysis

Miguel Sanchis-Sebastiá *, Edvin Ruuth, Lars Stigsson, Mats Galbe, Ola Wallberg

Department of Chemical Engineering, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden

ARTICLE INFO

Article history:
Received 3 September 2020
Revised 18 December 2020
Accepted 19 December 2020
Available online 31 December 2020

Keywords:
Cotton
Waste textiles
Recycling
Acid hydrolysis

ABSTRACT

The fashion industry has a considerable environmental impact, especially due to the increased generation of waste textiles as a result of fast fashion business models. Although fiber-to-fiber recycling processes are being developed, such a process is in reality a downcycling process, in which the mechanical properties of the textile fibers are impoverished with each cycle. Thus, new alternatives are required to completely close the fashion loop through chemically recycling textile fibers unfit for other types of recycling or resale due to their poor quality. We have evaluated the possibility of using acid hydrolysis to directly depolymerize the cotton fibers in waste textiles to produce a glucose solution, which could subsequently be used for the production of chemicals or fuels. Although a one-step procedure with sulfuric acid was unable to deliver high glucose production, it was possible to achieve a glucose yield over 90% through a two-step procedure, in which concentrated and dilute sulfuric acid were combined to exploit the benefits of both concentrations. Glucose concentrations around 40 g/L were achieved by increasing the solids loading in the two-step process, which might be sufficiently high for the fermentation of the solution into high-value products. Thus, this study demonstrates that it would be possible to chemically recycle (cellulose-based) waste textiles via acid hydrolysis, which, if correctly designed, could avoid the need to use enzymes to achieve high conversion efficiencies.

1. Introduction

The generation of waste textiles has been steadily increasing over the past decades due to the increase in the consumption of textiles worldwide. For example, the fiber consumption per capita in 1950 was about 3.7 kg, increasing to 10.4 kg in 2008 (Food and Agriculture Organization of the United Nations (FAO) and International Cotton Advisory Committee (ICAC), 2011). As a result, the world fiber demand surpassed 100 million tons in 2019, and is expected to increase to 121 million tons in 2025 (Ruiz, 2019). This, together with the fact that most waste textiles are landfilled or incinerated (Pensupa et al., 2018), implies that waste textiles are an increasing environmental problem that needs to be addressed.

New recycling processes are needed to introduce circularity in the fashion industry and reduce the environmental burden of waste textiles. Primary recycling technologies, in which fibers are converted to new fibers, are of course preferable, as the value of the product is maintained, and some such technologies have already been demonstrated on a small scale. For example, the company Re:newcell in Sweden is currently producing 7000 tons of cellulose pulp from used garments (Re:newcell AB, 2019). However, similarly to paper recycling, cellulosic fibers cannot be indefinitely converted to new fibers as the pulping and regeneration process usually reduces the degree of polymerization (David and Pailthorpe, 1999), which degrades the mechanical properties of the fibers. Moreover, not all used garments would be suitable for such a recycling process as the quality of the fibers is degraded during use, especially when industrial laundering has been applied (Wedin et al., 2019).

Primary recycling technologies would need to be combined with end-of-life strategies to completely close the fashion loop. These strategies could be based on depolymerizing cellulosic fibers with poor mechanical properties, resulting from use or multiple recycling cycles, to produce glucose and, subsequently, valuable chemicals or fuels. In fact, there has already been some research into the possibility of depolymerizing waste textiles to produce glucose, usually through some sort of pretreatment followed by enzymatic hydrolysis. For example, researchers have investigated pretreatment with acids such as sulfuric (Sasaki et al., 2019) and phosphoric acid (Kuo et al., 2010), with bases such as sodium hydroxide (Jeihanipour and Taherzadeh, 2009) and sodium

---

* Corresponding author.

E-mail addresses: miguel.sanchis_sebastia@chemeng.lth.se (M. Sanchis-Sebastiá), edvin.ruuth@chemeng.lth.se (E. Ruuth), novafiber@icloud.com (L. Stigsson), mats.galbe@chemeng.lth.se (M. Galbe), ola.wallberg@chemeng.lth.se (O. Wallberg).

https://doi.org/10.1016/j.wasman.2020.12.024
0956-053X/© 2020 The Author(s). Published by Elsevier Ltd.

carbonate (Hasanzadeh et al., 2018), and even with cellulose solvents (Jeihanipour et al., 2010).

Although the combination of pretreatment and enzymatic hydrolysis has been proven successful in depolymerizing lignocellulosic biomass, this technology might be too costly to process waste textiles as it relies on the incineration of lignin, which is an insignificant fraction of cotton (Buchert et al., 2001; McCall and Jurgens, 1951), to provide the energy required in the pretreatment step (Joelsson et al., 2016). We propose a novel process in which acid hydrolysis is used to directly depolymerize waste textiles into glucose, instead of as pretreatment prior to enzymatic hydrolysis. This process would considerably reduce the cost of valorizing waste textiles as fewer unit operations would be involved and there would be no need for enzymes, which could counterbalance the higher utility cost. The aim of this study was, therefore, to examine the design parameters and constraints of such a process, evaluate its efficiency and, ultimately, determine whether it is technically feasible.

2. Materials and methods

2.1. Collection and preparation of waste textiles

The waste textiles used in this study were discarded bed linens donated by the authors, the employees at the Department of Chemical Engineering at Lund University, and obtained from a collection point for Human Bridge, an organization that collects waste textiles in Sweden. We selected textiles that had been deemed unfit for resale and were labelled 100% cotton for the experiments. Prior to the experiments, labels, seams and buttons were removed from the bed linens and the material was cut into pieces of approximately 225 mm^2 with a textile knife (Ø 45 mm, Stoff & Stil). These were then thoroughly mixed to ensure the representability of the sample.

2.2. One-step acid hydrolysis experiments

One-step acid hydrolysis experiments were performed by exposing 40 dry g of waste textiles to 760 g of a sulfuric acid solution, at different concentrations, temperatures, and residence times, according to a central composite design (Table 1). The experiments performed at 130 ℃ were carried out in an autoclave, while the rest of the experiments were performed in a water bath. After the completion of acid hydrolysis, the mixture was vacuum filtered through a cloth with a pore size of 100 μm, generating a liquid and a solid fraction that were subjected to further analysis.

Table 1
Temperature, residence time and sulfuric acid concentration in the one-step acid hydrolysis experiments.

Condition	Temperature (°C)	Residence time (h)	H_2SO_4 (%wt)
1	130	6	60
2	130	6	5
3	130	1	60
4	130	1	5
5	30	6	60
6	30	6	5
7	30	1	60
8	30	1	5
9	80	3.5	5
10	80	3.5	60
11	80	6	32.5
12	80	1	32.5
13	130	3.5	32.5
14	30	3.5	32.5
15	80	3.5	32.5
16	80	3.5	32.5

2.3. Two-step acid hydrolysis experiments

Two-step acid hydrolysis experiments consisted of a dissolution step, in which waste textiles were dissolved in a concentrated sulfuric acid solution, followed by a glucose production step, in which a dilute sulfuric acid solution was used to completely depolymerize the cotton and form glucose. The dissolution step was performed in a water bath by exposing 15 dry g of waste textiles to 285 g of a concentrated sulfuric acid solution at 30 ℃ for 1 h. The concentration of the sulfuric acid solution in this step was varied between 60 wt% and 80 wt%. The glucose production step was performed by diluting the mixture to 5 wt% sulfuric acid and treating it at 121 ℃ for 1 h in an autoclave. After the completion of both steps, the mixture was vacuum filtered through a cloth with a pore size of 100 μm, generating a liquid and a solid fraction that were subjected to further analysis.

2.4. High solids loading experiments

The mass of waste textiles in the dissolution step was gradually increased to evaluate the effect of the solids loading on the two-step process. The solids loading in the dissolution step was increased from 0.05 to 1.1 dry g waste textiles/g sulfuric acid solution. The sulfuric acid concentration in the dissolution step was set to 80 wt% and the process was otherwise the same as that described in the previous section.

Two modifications were also separately tested to perform the process at high solids loading: fed-batch in the dissolution step, and increased acid concentration in the glucose production step. The first modification consisted of feeding the waste textiles gradually during the dissolution step, specifically, one fourth of the mass every 15 min. The second modification consisted of diluting the mixture to a concentration of 7.5 wt% sulfuric acid instead of 5 wt%, which means that less water was added to the mixture.

2.5. Compositional analysis

The total solids (TS) content of the waste textiles was analyzed by drying samples at 105 ℃ overnight in an oven, and the cellulose content was analyzed following the protocol developed by the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008a), with the exception that the textiles were cut into lengths of 10 mm instead of being milled through a 1 mm screen, according to ISO 1833–1 for quantitative chemical analysis of textiles (International Organization for Standardization (ISO), 2010). The fraction assigned to acid-insoluble lignin in the NREL protocol (Sluiter et al., 2008a) was denoted non-cellulosic residue, as the solid that remains after this analysis is an unidentified mixture of non-cellulosic threads, dyes, and other additives that may be present in the waste textiles. The abovementioned analyses were performed in triplicate.

Glucose and degradation by-products in the liquid samples generated during the cellulose analysis, as well as in the acid hydrolysis experiments, were analyzed using a Shimadzu LC-20 AD HPLC system equipped with a Shimadzu RID 10A refractive index detector (Shimadzu Corporation, Kyoto, Japan). The chromatography column used was an Aminex HPX-87H, with a Cation-H Bio-Rad MicroGuard column (Bio-Rad Laboratories, Hercules, CA, USA) at 50 °C, and a 5 mM sulfuric acid solution was used as eluent at a flow rate of 0.5 mL/min.

The solid samples generated in the acid hydrolysis experiments were dried overnight at 105 ℃ to determine the TS content, and the water-insoluble solids (WIS) content was determined by thoroughly washing the sample prior to drying at 105 ℃, according to the protocol developed by the NREL (Sluiter et al., 2008b). The

TS analysis was performed in triplicate, while the WIS analysis was performed in duplicate.

2.6. Calculations

The glucose yield was calculated based on the total available glucose in the waste textiles, which is 1.11 times the amount of cellulose in the material (due to the addition of water during the hydrolysis reaction). Apart from the glucose yield, three more parameters were used to evaluate the acid hydrolysis experiments: i) solid recovery indicates the fraction of the waste textiles that remained solid after the experiments (Eq. (1)), ii) glucose losses, which indicate the amount of glucose in the waste textiles that was degraded to other by-products (Eq. (2)), and iii) the degree of hydrolysis, which indicates the fraction of dissolved cellulose that was further hydrolyzed to glucose or other by-products (Eq. (3)).

$$Solid\ recovery = \frac{m_{residue} \cdot WIS_{residue}}{m_{textiles} \cdot TS_{textiles}} \tag{1}$$

where $m_{residue}$ is the wet mass of the solid fraction after the experiment, $WIS_{residue}$ the WIS content of the solid fraction after the experiment, $m_{textiles}$ the wet mass of waste textiles used in the experiment, and $TS_{textiles}$ the TS content of the waste textiles.

$$Glucose\ losses = \frac{C_{LeH} \cdot V \cdot \frac{M_{gluc}}{M_{LeH}} + C_{HMF} \cdot V \cdot \frac{M_{gluc}}{M_{HMF}}}{m_{textiles} \cdot TS_{textiles} \cdot Cell \cdot 1.11} \tag{2}$$

where C_{LeH} is the concentration of levulinic acid in the liquid fraction after the experiment, V the total volume of the liquid fraction after the experiment, M_{gluc} the molecular mass of glucose, M_{LeH} the molecular mass of levulinic acid, C_{HMF} the concentration of hydroxymethylfurfural (HMF) in the liquid fraction after the experiment, M_{HMF} the molecular mass of HMF, $m_{textiles}$ the wet mass of waste textiles used in the experiment, $TS_{textiles}$ the TS content of the waste textiles, and Cell the cellulose content of the waste textiles expressed as %TS.

$$\text{Degree of hydrolysis} = \frac{C_{gluc} \cdot V + C_{LeH} \cdot V \cdot \frac{M_{gluc}}{M_{LeH}} + C_{HMF} \cdot V \cdot \frac{M_{gluc}}{M_{HMF}}}{m_{textiles} \cdot (\text{1-Solid Recovery}) \cdot TS_{textiles} \cdot Cell \cdot 1.11} \tag{3}$$

where C_i is the concentration of compound i in the liquid fraction after the experiment, V the total volume of the liquid fraction after the experiment, M_i the molecular mass of compound i, $m_{textiles}$ the wet mass of waste textiles used in the experiment, $TS_{textiles}$ the TS content of the waste textiles, and Cell the cellulose content of the waste textiles expressed as %TS.

3. Results and discussion

3.1. Composition of the waste textiles

The waste textiles used in this study had a cellulose content of 93.0% on a dry basis (standard deviation 0.7%) and a non-cellulosic residue content of 4.6% on a dry basis (standard deviation 0.1%). These findings are consistent with the fact that raw cotton is not composed exclusively of cellulose. In fact, the secondary wall of raw cotton fibers consists of only 55% cellulose, as significant amounts of pectins, proteins and waxes are present in this region of the fiber (Mitchell et al., 2005). Although these non-cellulosic components are removed during the processing of cotton (Freytag and Donzé, 1983), it has been shown that non-cellulosic components still account for 3.6% of the processed cotton fibers prior to the addition of dyes (Buchert et al., 2001). Considering that the bed linens used in this study were dyed, the composition of these waste textiles was deemed consistent with that reported previously and provides a good basis for the calculation of glucose yield and other performance indicators.

3.2. One-step acid hydrolysis

One-step acid hydrolysis proved unsuccessful in producing glucose from waste textiles, since all the conditions tested yielded very little glucose, and in many cases glucose production was undetectable (Fig. 1). This shows that post-consumer waste textiles are considerably more recalcitrant than virgin cotton, as it has been shown that it is possible to hydrolyze cotton in 55% sulfuric acid at room temperature (Chu et al., 2011). The recalcitrance of waste textiles has also been demonstrated in previous studies, where treatment with 40% sulfuric acid at 95 ℃ decreased the degree of polymerization of mixed fabrics from 2200 to 160–170 (Ouchi et al., 2009), which is far from complete hydrolysis. In fact, treatment of waste textiles with sulfuric acid results in an exponential decrease of the degree of polymerization, but only until a certain level (known as the limiting degree of polymerization) is reached, upon which no further reduction takes place (Ouchi et al., 2009). This implies that the processing of cotton into fabrics complicates its depolymerization, due to the mechanical actions exerted during processing, the incorporation of dyes and additives, or possibly a combination of both.

It was impossible to model the acid hydrolysis process due to the similar, equally low, response obtained under all conditions, and thus identify which parameter had the greatest influence on the process. However, it was possible to construct response surfaces for other performance indicators to identify the cause of the poor glucose yield. For example, the solid recovery remained high under all conditions with dilute sulfuric acid (5 wt%), while concentrated sulfuric acid (60 wt%) was able to dissolve up to 80% of the waste textiles at higher temperatures (Fig. 2). This means that dilute sulfuric acid is unable to significantly reduce the degree of polymerization of processed cotton fibers, and the low glucose yield under these conditions was therefore caused by the inability of the acid solution to dissolve the waste textiles.

Although concentrated sulfuric acid was able to dissolve the waste textiles, the glucose yield was as low as in treatments with dilute sulfuric acid. The reason for this is that the glucose produced was rapidly degraded into other by-products (e.g. HMF and levulinic acid) when acid hydrolysis was performed with concentrated sulfuric acid (Fig. 3). In contrast, no glucose losses were observed for most of the conditions with dilute sulfuric acid, and the maximum glucose loss was less than 20% (Fig. 3). This implies that treatment with concentrated sulfuric acid was unable to deliver high glucose production because, due to the high concentration

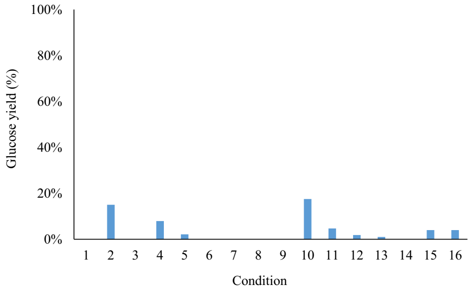


Fig. 1. Glucose yield under the conditions tested in the one-step acid hydrolysis experiments. The conditions of each experiment are defined in Table 1.

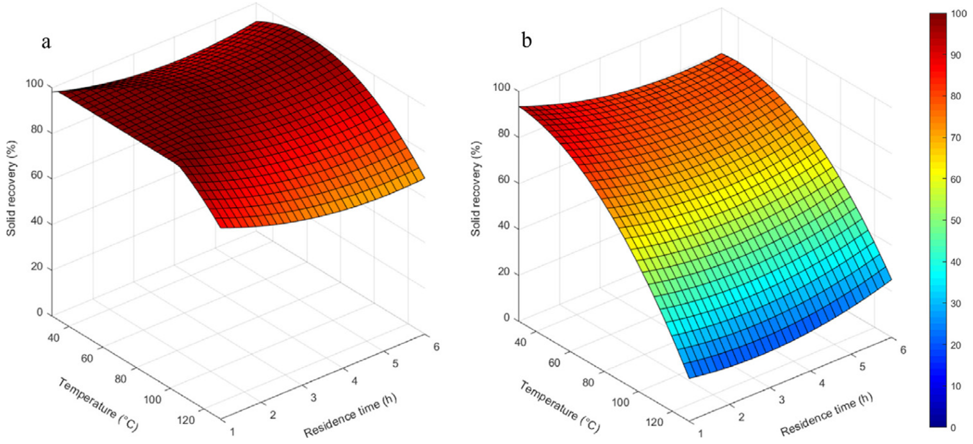


Fig. 2. Response surfaces for solid recovery using 5 wt% sulfuric acid (a) and 60 wt% sulfuric acid (b).

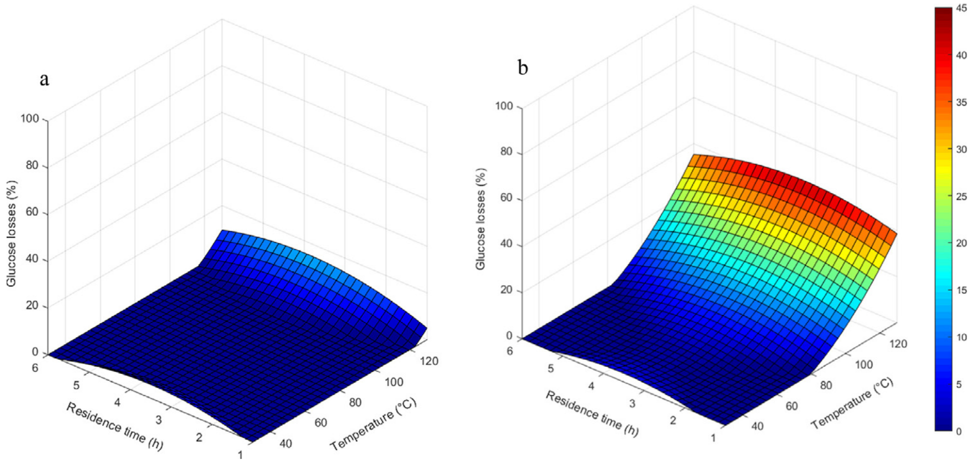


Fig. 3. Response surface for glucose losses at 5 wt% sulfuric acid (a) and 60 wt% sulfuric acid (b).

of protons, glucose continued to react to form HMF and, subsequently, levulinic acid. Thus, such treatment could be useful for the direct production of organic acids from waste textiles, but it would be unable to deliver glucose as a product.

These results prove that one-step acid hydrolysis is not a suitable technology to chemically recycle waste textiles via glucose production. However, it could provide an interesting means of separating cotton fibers from more complex fabrics. For example, using concentrated acid solutions, it would be possible to dissolve cotton fibers while the polyester fibers remain solid, and could then be separated by filtration (Shen et al., 2013). This technology could also be used to separate cellulosic fibers with different degrees of depolymerization, since it has been shown that rayon and cotton fibers can be separated by treating mixed fabrics with a 60% sulfuric acid solution at room temperature (Howlett et al., 1942).

3.3. Two-step acid hydrolysis

The differences in the results of acid hydrolysis depending on the acid concentration offer an attractive possibility to combine the advantages of each concentration, i.e. good dissolution at high acid concentrations and minor degradation at low acid concentrations, through two-step acid hydrolysis. This would be a very similar process to that used to depolymerize carbohydrates during the analysis of lignocellulosic biomass (Sluiter et al., 2008a). However, although the second step (glucose production) can be performed under similar conditions to those tested in the one-step experiments, the first step (dissolution) requires higher concentrations of sulfuric acid because it is performed at a low temperature (30 ℃), and 60 wt% sulfuric acid was not able to dissolve waste textiles sufficiently at lower temperatures (Fig. 1). For this reason, higher sulfuric acid concentrations were tested in the dissolution step, and it was found that a concentration of at least 70 wt% sulfuric acid was required to reduce the solid recovery to below 5%, and that waste textiles were completely dissolved when using 80 wt% sulfuric acid (Fig. 4).

Increasing the sulfuric acid concentration in the dissolution step also improved the degree of hydrolysis (Fig. 4). For example, although the solid recovery with 60 wt% sulfuric acid was around 70%, none of the dissolved cellulose was further hydrolyzed to glucose or its degradation products, whereas at 72 wt% sulfuric acid almost 90% of the dissolved cellulose was further hydrolyzed. Thus, the benefit of a high acid concentration in the dissolution step is twofold: it improves the dissolution of waste textiles and also improves the production of glucose in the subsequent step. In spite of this, it seems that a fraction of the dissolved cellulose remained unhydrolyzed in all cases as the degree of hydrolysis never reached 100% (Fig. 4), which means that part of the waste textiles would remain in solution as cellulose chains with a low degree of polymerization.

The good dissolution and high degree of hydrolysis led to glucose yields over 90% in two-step acid hydrolysis, provided that the sulfuric acid concentration in the dissolution step was higher than 70 wt% (Fig. 5). This efficiency is remarkably higher than that obtained with one-step acid hydrolysis, and could also outcompete other acid hydrolysis technologies and even acid hydrolysis combined with enzymatic hydrolysis. For example, a much lower glucose yield, of approximately 30%, has been reported using microwave-assisted acid hydrolysis (Sasaki et al., 2019), while a similar glucose yield was reported when using additional enzymatic saccharification (Kuo et al., 2014), which proves that the correct design of the acid hydrolysis process could eliminate the need for enzymes in the recycling of waste textiles, without compromising the efficiency of the process.

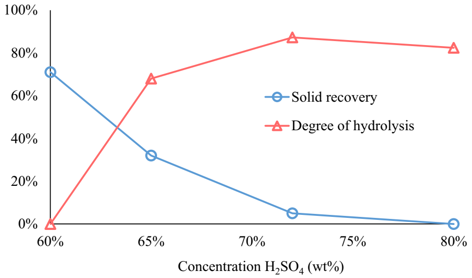


Fig. 4. Effect of sulfuric acid concentration in the dissolution step on solid recovery and degree of hydrolysis in two-step acid hydrolysis.

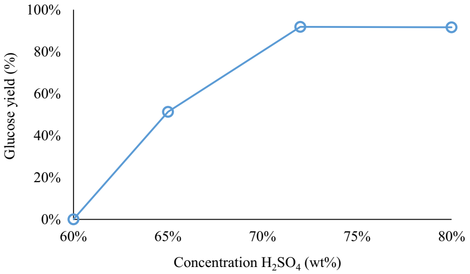


Fig. 5. Effect of sulfuric acid concentration in the dissolution step on glucose yield in two-step acid hydrolysis.

Table 2
Degree of hydrolysis in two-step acid hydrolysis at different sulfuric acid concentrations and temperatures in the dissolution step.

H_2SO_4 (%wt)	Temperature (°C)	Degree of hydrolysis (%)
60	30	0
60	80	37
65	30	68
65	80	65

The findings of the experiment with 60 wt% sulfuric acid in the dissolution step are particularly interesting because none of the dissolved waste textiles was further hydrolyzed (Fig. 4), which shows that the hydrolysis reaction stopped completely during the course of the experiment. A small increase in the acid concentration, from 60 wt% to 65 wt%, dramatically increased the degree of hydrolysis from 0% to almost 70% (Table 2). To further elucidate the cause of this effect, the dissolution step was performed at a higher temperature (80 ℃) and we found that the process behaved differently depending on the acid concentration. At 60 wt% sulfuric acid, the degree of hydrolysis increased to 37%, whereas at 65 wt% it remained approximately the same (Table 2). This points to the use of 60 wt% sulfuric acid in the dissolution step as the cause of hindered hydrolysis. A possible explanation of this could be that a molecule that hinders the hydrolysis process, which starts to decompose at higher temperatures, is formed when dissolving waste textiles in 60 wt% sulfuric acid, although further experiments and analyses would be required to confirm this hypothesis.

3.4. High solids loading experiments

The highest glucose concentration obtained in the two-step experiments described above was 3 g/L, which would not be sufficiently high to valorize the glucose solution cost-efficiently. For example, ethanol concentrations of at least 4 wt% are required to keep the purification costs within acceptable levels (Galbe et al., 2013), which would be almost impossible to achieve with such a dilute glucose solution. The solids loading in the dissolution step must therefore be increased above the 0.05 dry g waste textiles/g sulfuric acid solution used in the previous experiments, to achieve a higher glucose concentration.

Although using 70 wt% or 80 wt% sulfuric acid in the dissolution step provided similar results at low solids loading, at high solids loading (0.74 dry g waste textiles/g sulfuric acid solution) the glucose yield was considerably higher when the dissolution step was performed with 80 wt% sulfuric acid (80% glucose yield at 80 wt%, compared to 51% at 70 wt%). Thus, the slightly better dissolving properties of the 80 wt% sulfuric acid (Fig. 4) proved critical in a system with limited mass and heat transfer in the dissolution step, due to the higher solids loading, and it was therefore decided to perform all further high solids loading experiments with this acid concentration.

The glucose yield remained almost constant when increasing the solids loading from 0.05 to 0.74 dry g waste textiles/g sulfuric acid solution, which translated into an increase from 3 g/L to 42 g/L glucose in the final solution (Fig. 6). However, above this solids loading, the performance of the process decreased dramatically, and the glucose yield fell below 50%, which in turn reduced the

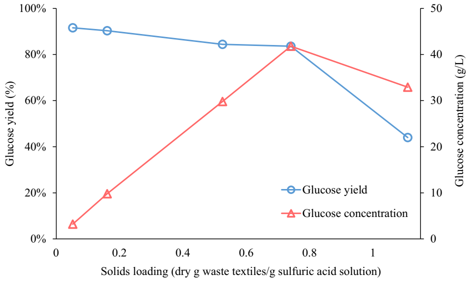


Fig. 6. Effect of solids loading on glucose yield and concentration in two-step acid hydrolysis.

glucose concentration in the final solution (Fig. 6), despite the higher mass of waste textiles used in the experiment. This high solids effect is in fact very similar to that exhibited in the enzymatic saccharification of lignocellulosic biomass (Weiss et al., 2019), again highlighting the similitudes between the depolymerization of cotton in waste textiles and carbohydrates in lignocellulosic biomass. A maximum glucose concentration of about 40 g/L could be achieved using the two-step acid hydrolysis process, which might be sufficiently high for the fermentation of the solution into high-value products, while it might not be sufficient for the production of low-value products such as bioethanol.

If higher glucose concentrations are required in the subsequent fermentation process, the acid hydrolysis procedure would have to be modified to further concentrate the final solution or a purification step of the sugar solution, such as acid recovery, could be implemented after the hydrolysis step. For example, using a fed-batch strategy in the dissolution step increased the glucose concentration at 1.1 dry g waste textiles/g sulfuric acid solution from 33 g/L to 40 g/L. Thus, a fed-batch process would have improved performance, albeit not sufficient to significantly alter the economics of the subsequent fermentation and purification. Another possibility is to use a higher concentration of sulfuric acid in the glucose production step, so that less water would be added to the process. For example, at 0.74 dry g waste textiles/g sulfuric acid solution, a glucose concentration above 50 g/L was obtained when the sulfuric acid concentration in the glucose production step was increased to 7.5 wt%, although the glucose yield was about 70% (compared to 80% when using 5 wt% sulfuric acid). Thus, reducing the dilution in the glucose production step seems to be the most promising strategy to achieve glucose concentrations above 40 g/L, although this would reduce the efficiency of the acid hydrolysis process, and therefore increase the proportion of waste textiles that is not depolymerized or, in other words, successfully recycled.

4. Conclusions

Acid hydrolysis proved successful in chemically recycling cotton-based waste textiles through their conversion into glucose, which could be further converted into valuable chemicals or fuels. This technology could thus help to completely close the fashion loop by valorizing textiles that are unfit for resale or fiber-to-fiber recycling. However, a two-step process was required to achieve a high glucose yield (around 80–90%) in the recycling process, since neither concentrated nor dilute sulfuric acid could deliver high glucose production by itself. Thus, a high glucose yield can only be achieved through combining the potent dissolving properties of concentrated sulfuric acid with the less degrading effects of dilute sulfuric acid.

The processes described in this study could easily be modified to deliver glucose concentrations of around 40 g/L by increasing the solids loading. However, if the subsequent fermentation process required higher glucose concentrations, the efficiency of the process would decrease dramatically at solids loadings above 0.74 dry g waste textiles/g sulfuric acid solution, making it difficult to concentrate the final product above 40 g/L. In spite of this, we believe that further modifications of the procedure, such as less dilution in the glucose production step, could help to increase the glucose concentration above this threshold, although further research on a larger scale would be required to evaluate the effects of such modifications.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Buchert, J., Pere, J., Johansson, L.S., Campbell, J.M., 2001. Analysis of the Surface Chemistry of Linen and Cotton Fabrics. Text. Res. J. 71, 626–629.
Chu, C.-Y., Wu, S.-Y., Tsai, C.-Y., Lin, C.-Y., 2011. Kinetics of cotton cellulose hydrolysis using concentrated acid and fermentative hydrogen production from hydrolysate. Int. J. Hydrogen Energy 36, 8743–8750.
David, S.K., Pailthorpe, M.T., 1999. Classification of textile fibres: production, structure, and properties. In: Robertson, J., Grieve, M. (Eds.), Forensic examination of fibers. 2nd ed. CRC Press.
Food and Agriculture Organization of the United Nations (FAO), International Cotton Advisory Committee (ICAC), 2011. A summary of the world apparel fiber consumption survey 2005-2008. http://www.fao.org/fileadmin/templates/est/COMM_MARKETS_MONITORING/Cotton/Documents/World_Apparel_Fiber_Consumption_Survey_2011_-_Summary_English.pdf (accessed July, 2020)
Freytag, R., Donzé, J.-J., 1983. Handbook of Fiber Science and Technology, Vol. 1, Chemical Processing of Fibres and Fabrics, Fundamentals and Preparation, Part A. Marcel Dekker, NY.
Galbe, M., Wallberg, O., Zacchi, G., 2013. Cellulosic Bioethanol Production. Sep. Purificat. Technolog. Biorefineries, 487–501.
Hasanzadeh, E., Mirmohamadsadeghi, S., Karimi, K., 2018. Enhancing energy production from waste textile by hydrolysis of synthetic parts. Fuel 218, 41–48.
Howlett, F., Morley, M.J., Urquhart, A.R., 1942. 5—The chemical analysis of fibre mixtures. J. Textile Inst. Trans. 33, T75–T104.
International Organization for Standardization (ISO), 2010. Textiles – Quantitative chemical analysis – Part 1: General principles of testing (ISO 1833 1:2006, including Cor 1:2009).
Jeihanipour, A., Karimi, K., Niklasson, C., Taherzadeh, M.J., 2010. A novel process for ethanol or biogas production from cellulose in blended-fibers waste textiles. Waste Manage. 30, 2504–2509.
Jeihanipour, A., Taherzadeh, M.J., 2009. Ethanol production from cotton-based waste textiles. Bioresour Technol 100, 1007–1010.
Joelsson, E., Dienes, D., Kovacs, K., Galbe, M., Wallberg, O., 2016. Combined production of biogas and ethanol at high solids loading from wheat straw impregnated with acetic acid: experimental study and techno-economic evaluation. Sustain. Chem. Processes 4.
Kuo, C.-H., Lin, P.-J., Lee, C.-K., 2010. Enzymatic saccharification of dissolution pretreated waste cellulosic fabrics for bacterial cellulose production by Gluconacetobacter xylinus. J. Chem. Technol. Biotechnol. 85, 1346–1352.
Kuo, C.-H., Lin, P.-J., Wu, Y.-Q., Ye, L.-Y., Yang, D.-J., Shieh, C.-J., Lee, C.-K., 2014. Simultaneous saccharification and fermentation of waste textiles for ethanol production. BioResources 9, 2866–2875.
McCall, E.R., Jurgens, J.F., 1951. Chemical composition of cotton. Text. Res. J. 21, 19–21.
Mitchell, R., Carr, C.M., Parfitt, M., Vickerman, J.C., Jones, C., 2005. Surface chemical analysis of raw cotton fibres and associated materials. Cellulose 12, 629–639.
Ouchi, A., Toida, T., Kumaresan, S., Ando, W., Kato, J., 2009. A new methodology to recycle polyester from fabric blends with cellulose. Cellulose 17, 215–222.
Pensupa, N., Leu, S.-Y., Hu, Y., Du, C., Liu, H., Jing, H., Wang, H., Carol Sze Ki, L., 2018. Recent Trends in Sustainable Textile Waste Recycling Methods: Current Situation and Future Prospects, in: Lin, C.S.K. (Ed.), Chemistry and Chemical

Technologies in Waste Valorization. Springer International Publishing, Cham, pp. 189-228.

Re:newcell AB, 2019. Re:newcell. We make fashion sustainable. https://renewcell.com/ (accessed July, 2020).

Ruiz, L., 2019. Global textile fibre demand: trends and forecast. International Cotton Advisory Committee (ICAC). https://icac.org/Content/EventDocuments/PdfFiles4407c817_a379_45b7_b3ac_c33809c9ae4d/4OS-Global%20Textile%20Fibres%20Demand-%20Trends%20and%20Forecast.pdf (accessed July, 2020).

Sasaki, C., Nakagawa, T., Asada, C., Nakamura, Y., 2019. Microwave-Assisted Hydrolysis of Cotton Waste to Glucose in Combination with the Concentrated Sulfuric Acid Impregnation Method. Waste Biomass Valorization 11, 4279–4287.

Shen, F., Xiao, W., Lin, L., Yang, G., Zhang, Y., Deng, S., 2013. Enzymatic saccharification coupling with polyester recovery from cotton-based waste textiles by phosphoric acid pretreatment. Bioresour. Technol. 130, 248–255.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2008a. Determination of structural carbohydrates and lignin in biomass. Laboratory Analytical Procedure (LAP). Golden, CO: National Renewable Energy Laboratory.

Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2008b. Determination of insoluble solids in pretreated biomass material. Laboratory Analytical Procedure (LAP). Golden, CO: National Renewable Energy Laboratory.

Wedin, H., Lopes, M., Sixta, H., Hummel, M., 2019. Evaluation of post-consumer cellulosic textile waste for chemical recycling based on cellulose degree of polymerization and molar mass distribution. Text. Res. J. 89, 5067–5075.

Weiss, N.D., Felby, C., Thygesen, L.G., 2019. Enzymatic hydrolysis is limited by biomass–water interactions at high-solids: improved performance through substrate modifications. Biotechnol. Biofuels 12, 3.

Paper VI

RSC Advances

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Check for updates

Cite this: *RSC Adv.*, 2021, **11**, 12321

Towards circular fashion – transforming pulp mills into hubs for textile recycling†

Miguel Sanchis-Sebastiá, Vera Novy, Lars Stigsson, Mats Galbe and Ola Wallberg*

Received 8th January 2021
Accepted 24th March 2021

DOI: 10.1039/d1ra00168j

rsc.li/rsc-advances

Most waste textiles are currently incinerated or landfilled, which is becoming an increasing environmental problem due to the ever-increasing consumption of textiles in the world. New recycling processes are required to address this problem and, although textile-to-textile recycling would be preferable, many researchers have suggested implementing processes based on the depolymerization of the textile fibers. We suggest integrating textile recycling with pulp mills, which would reduce the cost of depolymerizing the textile fibers and, at the same time, would diversify the product portfolio of the pulp mill, transforming the facility into a true biorefinery. This integration would be based on using green liquor as the pretreatment agent in the textile recycling process, as well as energy integration between the two processes. Na_2CO_3 was used to identify the conditions under which this pretreatment should be performed. Temperature and residence time proved to be critical in the efficacy of the pretreatment, as suitable values were required to ensure partial solubilization of the waste textiles. The conditioning of the pretreated material also had an important effect on the process, as it ensured a suitable environment for the enzymatic depolymerization while maintaining the changes in the material caused by pretreatment. Pretreatment was then performed with industrial green liquor, showing that the efficiency of textile recycling was about 70% when integrated in a pulp mill.

1. Introduction

Waste textiles are becoming an increasing environmental problem as the fiber consumption per capita has almost tripled over the past six decades,[1] and waste textiles are usually landfilled or, in the best case, incinerated for energy recovery.[2] New textile recycling processes are needed to address the accumulation of waste textiles, and there are already some facilities recycling waste textiles on a small scale, such as the company Re:newcell in Sweden.[3] The textile-to-textile recycling implemented by Re:newcell is preferable as it maintains the value of the fibers. However, several researchers have suggested combining textile-to-textile recycling with end-of-life technologies based on the depolymerization of the fibers to produce valuable fuels or chemicals.[2,4,5] This could completely close the fashion loop and reduce the flow of waste textiles to landfills.

Cotton fibers can be depolymerized enzymatically, and the resulting glucose solution could then be fermented into different products such as ethanol,[6] hydrogen,[7] or bacterial cellulose.[8] However, enzymes cannot directly depolymerize cotton into glucose as this material has a high degree of polymerization and crystallinity, which makes the cellulose chains inaccessible to the enzymes.[9] In fact, cotton fibers have one of the highest molecular weights and structural order among all plant fibers.[10] Thus, in most of the studies reported in the literature, technologies based on pretreatment prior to enzymatic hydrolysis have been applied to overcome the high crystallinity of cotton,[11–14] in a similar fashion to the techniques used to depolymerize lignocellulosic biomass.

Pretreatment with alkaline solutions is known to reduce the crystallinity of cellulose significantly, through the conversion of cellulose I into cellulose II.[15] For this reason, several previous studies have been based on pretreatment with NaOH, usually at low temperatures, to improve the enzymatic digestibility of waste textiles.[5,11,12] Although NaOH has been the most widely used alkali, as the pretreatment mechanism is based on the titration of the –OH groups in the cellulose chains,[16] other alkaline compounds could be used to pretreat waste textiles. For example, it has been demonstrated that Na_2CO_3 can also enhance the enzymatic hydrolysis of waste textiles, although to a lesser extent than NaOH.[17]

Regardless of the alkali chosen, technologies based on pretreatment prior to enzymatic hydrolysis suffer from a fundamental problem when applied to waste textiles: namely, the material does not contain any lignin that can be incinerated to supply the energy required in the process, as in the case of lignocellulosic biomass.[18] Thus, novel solutions will be required

---

Department of Chemical Engineering, Lund University, P. O. Box 124, SE-221 00 Lund, Sweden. E-mail: miguel.sanchis_sebastia@chemeng.lth.se; vera.novy@t-online.de; novafiber@icloud.com; mats.galbe@chemeng.lth.se; ola.wallberg@chemeng.lth.se

† Electronic supplementary information (ESI) available. See DOI: 10.1039/d1ra00168j

to reduce the cost of depolymerizing waste textiles in order to implement this technology on a commercial scale.

A possible solution to this problem would be to integrate textile recycling in pulp mills, since these facilities usually produce excess heat that is sold as low-value energy.[19] This excess heat could instead be used to cover the energy needs of the textile recycling process, which would reduce the utility cost, and therefore improve the economics of textile recycling. Moreover, green liquor, one of the internal streams in a pulp mill, is rich in alkaline compounds such as Na_2CO_3, Na_2S, and NaOH.[20,21] At the beginning of the pulping process, the liquor contains only NaOH and Na_2S (in kraft pulping) but Na_2CO_3, which is subsequently recaustized in order to bring the liquor back to its original form, is formed in the recovery boiler after the combustion of the black liquor. This means that integration could also be implemented in terms of material flows, as green liquor could be used as the alkaline solution for the pretreatment of waste textiles. This could lead to an attractive industrial symbiosis, as textile recycling would in turn help the pulp mill to diversify its portfolio of both feedstocks and products, thus promoting its transformation into a true biorefinery, accomplishing an industrial change that has been widely encouraged in the academic literature.[22–24]

The aim of this study was to investigate the viability of integrating textile recycling in pulp mills by evaluating the pretreatment of waste textiles with green liquor. Experiments were performed with Na_2CO_3, the most abundant component in green liquor, to identify the optimal conditions and possible constraints, and to evaluate the efficiency of the process. The Na_2CO_3 solution was then replaced by industrial green liquor to assess the actual performance of the proposed integration.

2. Materials and methods

2.1. Collection and preparation of waste textiles

The waste textiles used in this study were donated by the authors and other employees at the Department of Chemical Engineering at Lund University. Shirts and T-shirts labelled as 100% cotton were used in the experiments. Buttons, labels, and printed patterns were removed, and the remaining material was cut into pieces measuring approximately 5 × 5 cm. These were then thoroughly mixed to ensure representability of the samples used in the experiments and analyses.

2.2. Pretreatment of waste textiles

The general procedure for pretreatment of waste textiles consisted of exposing 40 dry g waste textiles to 760 g of an alkaline solution at a certain temperature for a certain residence time, with occasional stirring during the treatment. The specific conditions at which the pretreatment was performed are described in the following sections, as they were varied differently in several series of experiments. Pretreatment was performed in a 2 L stirred tank reactor (Polyclave, Büchi AG, Switzerland), equipped with a stirrer unit (Cyclone 300, Büchi AG) and a thermostat (Unistat T305, Huber Kältemaschinenbau AG, Germany), and the resulting material was filtered in a filter press (Fischer, Germany). The solid fraction after filtration was used for the subsequent enzymatic hydrolysis experiments as well as for compositional analyses.

2.2.1. Evaluation of conditioning after pretreatment. The pretreated material had to be conditioned prior to enzymatic hydrolysis as pretreatment was performed in an alkaline environment, whereas the optimal pH for the enzymes used in this study was 5. Several conditioning strategies were investigated in order to evaluate the effect of this operation on the efficiency of the process. The strategies tested were: (i) enzymatic hydrolysis in citrate buffer at different concentrations; (ii) adjusting the pH in enzymatic hydrolysis prior to the addition of the enzymes; (iii) washing the pretreated material prior to the enzymatic hydrolysis experiments; and (iv) washing and drying the pretreated material prior to enzymatic hydrolysis. Each of these conditioning strategies was tested on four batches of material that had been pretreated differently, in order to assess the impact of the pretreatment conditions on the conditioning strategy. The four pretreatment conditions chosen for this evaluation were: 5% Na_2CO_3 at 150 °C for 2 h; 10% Na_2CO_3 at 150 °C for 2 h; 5% Na_2CO_3 at 120 °C for 4 h; and 5% NaOH at 150 °C for 2 h. These pretreatment conditions were chosen based on the study by Hasanzadeh *et al.*, where the authors determined that the optimal pretreatment conditions were 5% Na_2CO_3 at 150 °C for 2 h.[17]

The citrate buffers were prepared by mixing citric acid monohydrate (Merck, Germany) and sodium citrate dihydrate (VWR, Belgium) in water to final buffer concentrations of 0.1 M, 0.2 M, or 0.3 M, depending on the strategy being tested. After the addition of the citric acid and sodium citrate, small amounts of KOH or H_2SO_4 were added to the solution to adjust the final pH of the buffer to 5. In experiments were citrate buffer was not used, the pH was adjusted during enzymatic hydrolysis by adding H_2SO_4 to the reaction vessel, prior to the addition of enzymes, until a pH of 5 was reached.

The pretreated material was subjected to three washing steps in experiments where washing was included in the conditioning strategy. The material was washed with 60 times its weight of water, in the first and third washing steps, whereas the same amount of 0.05 M HCl was used in the second washing step. The washing liquid was removed by vacuum filtration between each washing step. In the experiments where drying was applied in combination with washing, the washed material was dried at room temperature for approximately 24 h.

2.2.2. Evaluation of the pretreatment conditions. Different temperatures and residence times were used to identify the pretreatment conditions that led to the best enzymatic digestibility of the waste textiles. The concentration of the alkaline solution was not modified during these experiments as alkaline pretreatment can only reduce cellulose crystallinity within a narrow window of alkali concentrations.[16] For this reason, all these experiments were performed with 5% Na_2CO_3 as the alkaline solution. The materials pretreated in these experiments were conditioned using 0.1 M citrate buffer in the enzymatic hydrolysis experiments.

2.2.3. Combination of several alkaline compounds. Prior to the experiments with green liquor, combinations of different alkaline compounds were investigated to evaluate the effects of each compound on the pretreatment. Two experiments were performed in which Na_2S and NaOH were combined separately with Na_2CO_3, after which all three compounds were combined, so that the alkaline solution would resemble industrial green liquor. The concentrations of the different alkaline compounds were based on the previously reported composition of green liquor.[20,21] The alkaline solutions used in each experiment were: 5% Na_2CO_3 with 1.75% Na_2S; 5% Na_2CO_3 with 0.4% NaOH; and 5% Na_2CO_3 with 1.75% Na_2S, and 0.4% NaOH. All these experiments were performed at 200 °C for 8 h, with 0.1 M citrate buffer as the conditioning strategy for the pretreated materials.

Finally, experiments were conducted with industrial green liquor containing a combination of different alkaline compounds. The green liquor was sourced from the pulp mill operated by Södra in Mörrum, in Southern Sweden, and its composition was: 95.8 ± 2.5 g L^{-1} Na_2CO_3, 49.5 ± 4.3 g L^{-1} Na_2S, and 12.0 ± 2.8 g L^{-1} NaOH. The composition of the green liquor was determined using the SCAN-N 30:85 methodology.[25] The green liquor was diluted prior to use so that the concentration of Na_2CO_3 was similar to that in the experiments with model solutions.

2.3. Enzymatic hydrolysis

Enzymatic Hydrolysis (EH) experiments were performed at 50 °C for 96 h in 50 mL Falcon tubes with a working mass of 20 g. A solids loading of 5% and Cellic CTec 2 (Novozymes, Denmark) enzyme cocktail at a loading of 0.15 g enzymes per g solids (approximately 30 FPU per g solids) were used in these experiments, so that the yield from the enzymatic hydrolysis of untreated waste textiles (negative control) would be similar to those reported previously in the literature.[11,12,17] The hydrolysis experiments were performed in a combi-H12 hybridization incubator (FinePCR, South Korea), which maintained the temperature and mixing during the experiment.

Samples obtained from the enzymatic hydrolysis experiments were centrifuged in 2 mL Eppendorf tubes at 13 000 rpm for 5 min. The supernatant was filtered through 0.2 μm syringe filters (GVS North America, Sanford, USA) and stored at −20 °C until HPLC analysis. Glucose was analyzed using a Shimadzu LC-20 AD HPLC system equipped with a Shimadzu RID 10A refractive index detector (Shimadzu Corporation, Kyoto, Japan). The chromatography column used was a CarboSep CHO 782 column (Concise Separations, San Jose, CA, USA) with a De-Ashing Bio-Rad Micro-Guard column at 80 °C, using reagent grade water as eluent at a flow rate of 0.6 mL min^{-1}.

2.4. Compositional analysis

2.4.1. Raw waste textiles. The total solids of the waste textiles was analyzed by drying the samples overnight at 105 °C. The cellulose content was analyzed according to the two-step acid hydrolysis procedure developed by the National Renewable Energy Laboratory (NREL),[26] with the exception that the samples were cut into lengths of 10 mm, according to ISO 1833-1 for quantitative chemical analysis of textiles,[27] due to difficulties in milling the samples through a 1 mm screen. The fraction assigned to lignin in the NREL procedure[26] was denoted non-cellulosic residue in this study as the material remaining after acid hydrolysis was an unidentified mixture of synthetic threads, dyes and other additives that may be present in waste textiles. All these analyses were performed in triplicate.

Glucose and degradation by-products generated in the liquid samples during the cellulose analysis, were analyzed using the same HPLC system as described above. However, the chromatography column used was an Aminex HPX-87H, with a Cation-H Bio-Rad MicroGuard column (Bio-Rad Laboratories, Hercules, CA, USA) at 50 °C, using a 5 mM sulfuric acid solution as eluent at a flow rate of 0.5 mL min^{-1}.

2.4.2. Pretreated waste textiles. The pretreated waste textiles were analyzed as-is after filtration following the alkaline pretreatment. The cellulose and non-cellulosic residue were determined using the same NREL procedure as for the raw waste textiles.[26] However, complete hydrolysis of the cellulose in the material may not be possible due to the presence of residual alkaline compounds in the textiles, which would neutralize part of the acid used in the procedure. For this reason, the analysis was also performed on washed samples of the pretreated materials, using the same washing scheme as described above, and the cellulose content in the material as-is was corrected based on the results obtained from the washed material. This ensured that the cellulose content of the material as-is, which was the one used in yield calculations, was obtained without imprecisions arising from incomplete cellulose hydrolysis.

Glucose and degradation by-products generated in the liquid samples in these analyses were analyzed using the same HPLC system, chromatography column and conditions as those used for the analysis of raw textiles. These analyses were also performed in triplicate.

2.5. Enzyme adsorption measurements

The accessibility of pretreated waste textiles to the enzymes was estimated through enzyme adsorption tests. These tests were performed under the same conditions as the enzymatic hydrolysis experiments, but the preparations were incubated in an ice bath for 6 h, in constant agitation with a magnetic stirrer. After the incubation time, the concentration of unbound proteins was measured in the liquid after vacuum filtration of the preparation. The concentration of unbound proteins was measured by incubating the samples with ready-to-use Bradford reagent (Sigma Aldrich, USA) for 5 min, measuring the absorbance at 595 nm and determining the protein concentration against an external BSA standard.

2.6. CLSM imaging

Confocal Laser Scanning Microscopy (CLSM) imaging was performed with a Nikon Ti-E/A1+ (Nikon Instruments Inc., USA) equipped with a 20× (air, N.A. 0.75) and a 60× (oil, N.A. 1.4) objective. For imaging, the fibers were mounted on the objective slide and covered in ~10 μL of calcofluor solution (#18909, Sigma-Aldrich, USA). After 5 min incubation in the dark, a drop of 10% (w/v) KOH was added, and the cover slide sealed with nail polish. Imaging was performed with a 409 nm fixed wavelength laser with an emission collection range of 425 to 475 nm. The laser power was kept constant for all images, where the imaging settings (*i.e.*, gain and offset) were adjusted to maximize the dynamic range of the detector. Each micrograph presented herein is a 2D maximum intensity projection of a 3D z-stack consisting of ~10 (20×) or ~20 (60×) optical slices. ImageJ (Fiji[28]) was used as processing software. 2 specimens were prepared for each fiber type and after manual screening of each specimen, 2 to 4 images were taken. Overall, the fibers were homogenous, and the micrographs shown herein are representative of the entire specimen.

2.7. Yield calculations

Cellulose losses resulting from alkaline pretreatment were calculated based on the amount of cellulose remaining in the solid material recovered after the pretreatment and the amount of cellulose available in the starting material. The enzymatic hydrolysis yield was based on the amount of glucose available in the pretreated textiles, which corresponds to 1.11 times the amount of cellulose contained in the material, due to the addition of water during the hydrolysis reaction.

3. Results and discussion

3.1. Composition of the waste textiles

The waste textiles contained 93.0% cellulose (standard deviation 2.0%) and 4.4% non-cellulosic residue (standard deviation 1.2%), prior to alkaline pretreatment. This result is reasonable, given that virgin cotton usually has a cellulose content between 88% and 96%; 94% being the most typical value.[29] Although non-cellulosic components present in virgin cotton, such as proteins, waxes, or pectins, might be removed during its processing,[30] it has been shown that non-cellulosic components nevertheless account for approximately 4% of processed cotton fibers.[31] It was therefore considered that the composition determined here was reliable for the determination of cellulose losses in the pretreatment step, as well as the yield from enzymatic hydrolysis.

The composition of the waste textiles after pretreatment can be found in ESI File 1 (Tables 1–5†), but no further discussion is included here since alkaline pretreatment causes mainly structural changes in cellulose, rather than changes in its chemical composition.[15] The discussion in the following sections thus concerns the yield from enzymatic hydrolysis obtained after each set of pretreatment conditions, which provides an indication of the accessibility of the cellulose to the enzymes and can therefore be interpreted as a measure of the pretreatment efficacy.

3.2. Conditioning of pretreated textiles

Conditioning of the pretreated material prior to enzymatic hydrolysis had a strong effect on the efficiency of enzymatic saccharification (Fig. 1). For example, drying the material had a marked negative effect on enzymatic hydrolysis, as the materials that were washed and then dried yielded the lowest glucose production in all cases (Fig. 1). In its hydrated form, cellulose II is an inflated structure, due to water molecules intercalated between the cellulose chains, but when the material is dried, the structure shrinks due to the removal of the water molecules, reducing the accessibility of the cellulose chains to the enzymes.[32,33] In spite of this, the material was dried as part of the conditioning of the pretreated textiles in some previous studies.[11,12] The yields reported in those studies may, therefore, be underestimated, and greater attention should be paid to the effect of conditioning on the enzymatic depolymerization process.

Washing the pretreated material without subsequent drying resulted in a similar enzymatic hydrolysis yield to that when adjusting the pH prior to the addition of the enzymes (Fig. 1). This means that similar and higher yields could be achieved without washing by adjusting the pH prior to enzyme additions. Thus, the goal of conditioning is to establish favorable

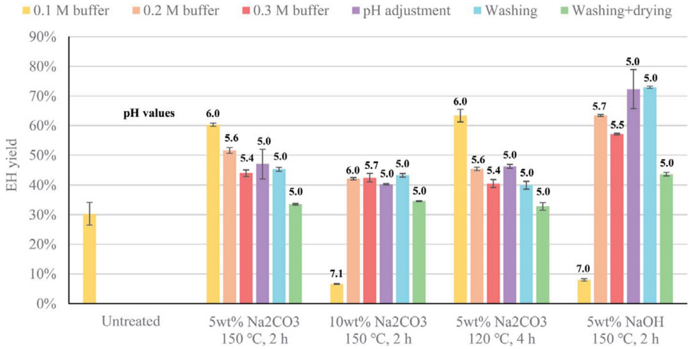


Fig. 1 Effect of various conditioning strategies on the yield from enzymatic hydrolysis of waste textiles pretreated with an alkaline solution, under 4 different conditions. Values in bold are the pH at which enzymatic hydrolysis was performed. Data represent mean values of 2 experiments; error bars indicate the spread.

conditions for the enzymes to act, rather than to remove residual compounds from the alkaline solution used in the pretreatment.

Adjusting the pH prior to the addition of the enzymes seemed to have a different effect depending on the strength of the alkaline solution. For the strongest bases (10% Na_2CO_3 and 5% NaOH), the best yield was obtained when enzymatic hydrolysis was performed at the optimal pH of the enzymes, that is, a pH of 5 (Fig. 1). However, for the weakest base (5% Na_2CO_3), higher yields were obtained when enzymatic hydrolysis was performed at a pH of 6 (Fig. 1). The reason for this might be that the strong acid used for pH adjustment neutralizes the titration effect of the weak base, reversing the structural changes that took place during pretreatment, thus reducing the accessibility of the cellulose chains to the enzymes, even though the high ionic strength of the solution might also play a role in the efficiency of the saccharification.

Additional enzyme adsorption tests showed that enzymes adsorbed differently to waste textiles pretreated with 5% Na_2CO_3 depending on the citrate buffer used to perform the enzymatic hydrolysis, while they adsorbed approximately the same to waste textiles pretreated with 5% NaOH (Fig. 2). This indicates that the conditioning of the pretreated material can revert the effect of pretreatment, but only when a weak alkali is used. Thus, if green liquor were to be used as a pretreatment agent, the accessibility of the material to the enzymes would depend on the conditions of pretreatment as well as the conditioning of the pretreated material prior to enzymatic hydrolysis.

Although adjusting the pH might reduce the accessibility of the cellulose in some cases, it would not be possible to carry out enzymatic hydrolysis without adjusting the pH to some extent, as the enzymes lost their activity at pH 7 (Fig. 1). This means that, if green liquor were to be used as the pretreatment agent, it is likely that setting the pH at a suboptimal value might benefit enzymatic hydrolysis, since there is a trade-off between high enzyme activity and maintaining the structural changes in the cotton fibers resulting from pretreatment. For this reason, it was decided to perform all subsequent experiments using 0.1 M citrate buffer, in order to ensure a pH of approximately 6 during enzymatic hydrolysis.

3.3. Influence of pretreatment conditions

Once the best conditioning strategy had been established, the effects of temperature and residence time were investigated to determine the conditions that provided a material susceptible to enzymatic hydrolysis. The highest enzymatic hydrolysis yield achieved at 100 and 150 °C was about 60–65%, while a yield of over 80% was achieved at 200 °C (Fig. 3). The improved enzymatic hydrolysis yield compared to untreated textiles (30% EH yield) arise probably from organizational changes at the macrofibril level, caused by the titration of the hydroxyl groups in cellulose.[16] These organizational changes likely reduced the degree of organization of microfibrils in the microfibril, and of the microfibril in the visible fiber. As a result, a less ordered arrangement was created, which probably increased the cellulose accessibility to enzymes and therefore aided the enzymatic hydrolysis of the pretreated fibers.

The reason for the higher yield observed at 200 °C might be that, in an alkaline environment, degradation reactions can only take place above 170 °C.[34] These reactions would generate random scission of the glycosidic bonds, releasing short-chain molecules into the solution, which would create new attack sites for the enzymes and therefore improve the accessibility to the enzymes.[34] Moreover, fiber fragmentation, induced by degradation of the more reactive paracrystalline cellulose regions,[33] leads to higher surface-to-bulk-ratios that, together with the increase in attack sites, aid the enzymatic hydrolysis. In spite of this, the fact that the best yield was obtained at the highest temperature and longest residence time would limit the size of the textile recycling plant, since the amount of excess heat available in the pulp mill is limited.

CLSM imagining was used to confirm the morphological and organizational changes caused by alkaline pretreatment. It was observed that the raw fibers were very smooth, with blunt ends, and were arranged tightly with each other (Fig. 4). On the contrary, the fibers treated with Na_2CO_3 exhibited a less ordered

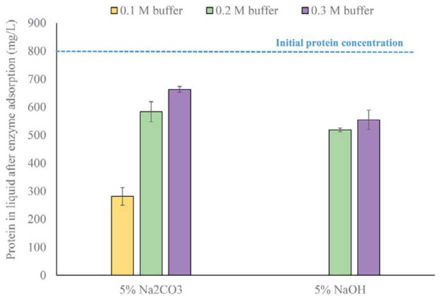


Fig. 2 Enzyme adsorption of waste textiles pretreated with sodium carbonate or hydroxide at 150 °C for 2 h, applying different citrate buffers as the conditioning strategy. Data represent mean values of 2 experiments; error bars indicate the spread.

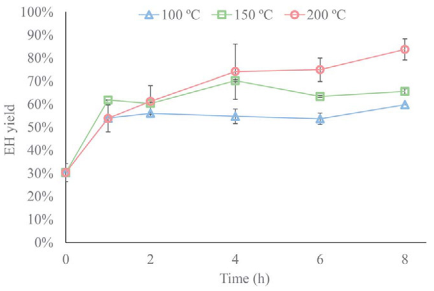


Fig. 3 Effect of the temperature and residence time on the yield from enzymatic hydrolysis of pretreated waste textiles using 5% Na_2CO_3 as the alkaline solution in pretreatment. Data represent mean values of 2 experiments; error bars indicate the spread.

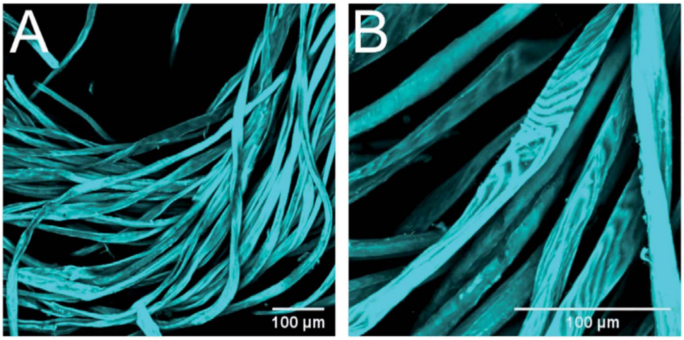


Fig. 4 CLSM images of waste textiles prior to alkaline pretreatment. Images were taken with 20× (A) and 40× (B) magnification. Cellulose was stained with calcofluor as dye. The color was assigned arbitrarily.

arrangement, with a larger distance between the fibers, and shorter fiber fragments were observed in the material as well as interfibrillar damages that allowed to observe macrofibrils at the end of the fibers (Fig. 5). These images confirm that the material treated with Na_2CO_3 presents many more attack sites for the enzymes, leading to an increased enzyme accessibility and therefore a higher enzymatic hydrolysis yield.

These degradation reactions had another effect on the pretreatment, which would have negative effects on the textile recycling process. Part of the cellulose contained in the waste textiles solubilized during pretreatment, which would be accounted for as a cellulose loss in the recycling process. For example, cellulose losses of about 3.5–6% were observed at 100 and 150 °C, which could be attributed to the mass losses due to experimental error, while cellulose losses of 15% and 20% were observed following pretreatment at 200 °C for 6 and 8 h, respectively. These losses confirm partial solubilization of the material during pretreatment, but they also indicate that the degradation reactions only take place after a contact time between the waste textiles and the alkaline solution of at least 6 h, as the cellulose losses at 200 °C were negligible for shorter residence times.

In spite of the cellulose losses, the overall yield of the process was higher at 200 °C than at 100 °C or 150 °C. The highest overall yield achieved at 200 °C was approximately 70%, while the overall yield at 100 or 150 °C, which is the same as the enzymatic hydrolysis yield since there were no cellulose losses at these conditions, never exceeded 65%. This shows that degradation reactions during pretreatment are beneficial to the process, to some extent, as the improved accessibility of the material to the enzymes compensated for the cellulose losses. Thus, the design of the alkaline pretreatment step consists of identifying the conditions under which a suitable degree of degradation reactions takes place, so that the improved accessibility to the enzymes counterbalances the cellulose losses.

3.4. Pretreatment with several alkaline species

The addition of sodium sulfide to the alkaline solution had a negative impact on the process, as the yield following

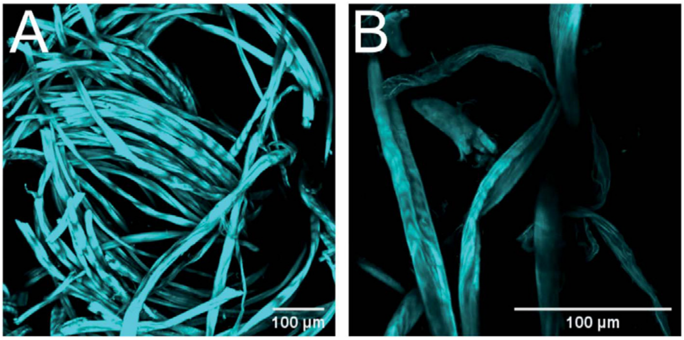


Fig. 5 CLSM images of waste textiles treated with 5% Na_2CO_3 at 200 °C for 8 h. Images were taken with 20× (A) and 40× (B) magnification. Cellulose was stained with calcofluor as dye. The color was assigned arbitrarily.

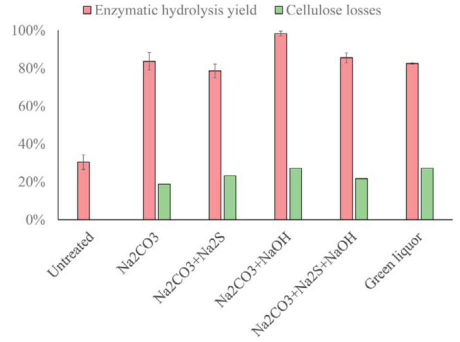


Fig. 6 Enzymatic hydrolysis yield and cellulose losses when waste textiles were pretreated at 200 °C for 8 h with different alkaline solutions. Data represent mean values of 2 experiments; error bars indicate the spread.

enzymatic hydrolysis decreased from 84% to 79% (Fig. 6). The problem associated with sodium sulfide is that, although the protonation of S^{2-} is almost immediate,[25] HS^- is a very weak base, which would contribute little to the titration of the cellulose chains. Although HS^- makes a small contribution to the titration, a second Na^+ ion is added to the solution because of it, which has a negative effect on the pretreatment due to the formation of cellulose–Na complexes,[15] which have been reported to cause the gelation of cellulose solutions,[16] and would thus reduce the accessibility of the material to the enzymes. It therefore appears that the negative effect of introducing additional Na^+ outweighs the increased titration capacity when Na_2S is added to the alkaline solution.

In contrast, the addition of NaOH increased the enzymatic hydrolysis yield from 84% to 98% (Fig. 6), which again demonstrates the important role of the strength of the base in the alkaline pretreatment. The OH^- ions were the strongest base added to the alkaline solution, which explains the improvement in the pretreatment efficacy, as these ions would significantly increase the extent to which the –OH groups in the cellulose chains are titrated.

The simultaneous addition of Na_2S and NaOH resulted in a similar enzymatic hydrolysis yield to that obtained with pure Na_2CO_3 (Fig. 6). This implies that the negative effect of Na_2S was compensated for by the positive effect of NaOH, and it could therefore be expected that the efficacy of the pretreatment would be approximately the same with green liquor as with a pure Na_2CO_3 solution. However, the amount of Na_2S added to the alkaline solution was more than 4 times the amount of NaOH added, which shows that NaOH had a greater effect on the pretreatment than Na_2S.

Pretreatment with the green liquor resulted in a similar yield to that obtained with pure Na_2CO_3 (Fig. 6), as was expected from the experiments with model solutions, but the cellulose losses were slightly higher than expected (27% instead of 21%). The higher cellulose losses could be attributed to the higher concentration of NaOH in the green liquor, which would increase the extent of the degradation reactions during pretreatment. Nevertheless, the overall yield was approximately the same as with pure Na_2CO_3; thus, the efficiency of textile recycling would be about 70% when this process is integrated with a pulp mill.

The material pretreated with green liquor was examined with CLSM and similar effects to those exerted by Na_2CO_3 pretreatment were observed: shorter fiber fragments, peeling reactions (dethreading) and increased surface to bulk ratio (Fig. 7). This proves that green liquor modifies the textile fibers in a similar fashion than pure Na_2CO_3, although to a larger extent. It seems that the images display increased extent of the degradation reactions during pretreatment with green liquor, which would explain the higher cellulose losses registered in this treatment.

The composition of the green liquor was changed during pretreatment although the concentrations of Na_2S and Na_2CO_3 remained the same (49.3 ± 1.1 g L^{-1} Na_2S and 93.1 ± 0.6 g L^{-1} Na_2CO_3 after pretreatment). However, the concentration of

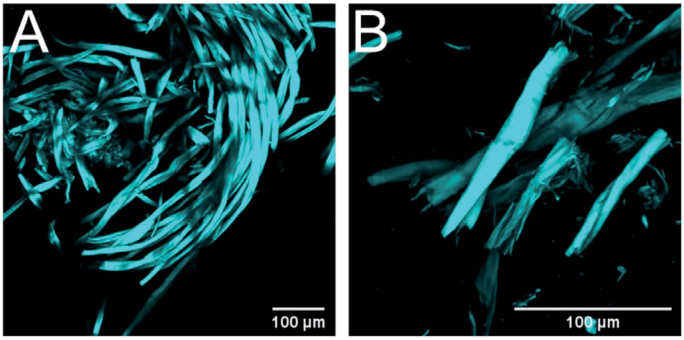


Fig. 7 CLSM images of waste textiles treated with green liquor at 200 °C for 8 h. Images were taken with 20× (A) and 40× (B) magnification. Cellulose was stained with calcofluor as dye. The color was assigned arbitrarily.

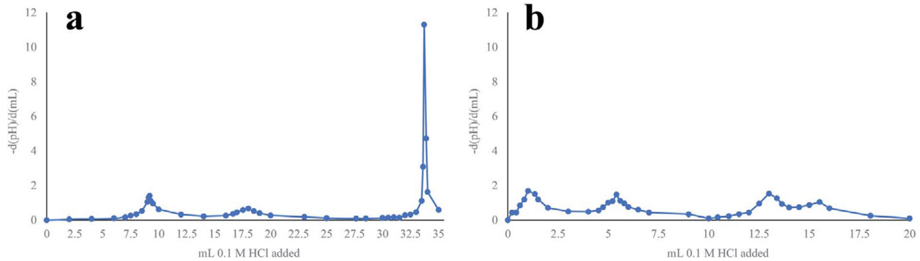


Fig. 8 Titration curves for the green liquor before (a) and after (b) pretreatment. The titration curves are presented as the first derivatives of the pH.

NaOH in the solution was negligible, which means that this component was completely consumed during pretreatment. This, together with the fact that some liquid was retained in the pretreated textiles, resulted in a sodium loss of 15% in pretreatment, which means that less sodium would be returned to the pulp mill. It would thus be necessary to compare the economic impact of this sodium loss with the revenue from textile recycling to ascertain the economic viability of the proposed integration.

The sodium loss estimated based on the titration of the green liquor might be an overestimate of the actual sodium loss during pretreatment as an additional inflexion point was observed in the titration curve when analyzing the green liquor after pretreatment (Fig. 8). This inflexion point suggests the presence of an additional extremely weak base (weaker than HS^-) in the solution, which could be the short-chain molecules generated in the degradation reactions. Since these molecules could establish ionic bonds with sodium, the presence of this base would reclaim sodium from the pretreatment, as the charge balance in the solution must be maintained, which implies that a higher amount of sodium would be returned to the pulp mill. Thus, the partial solubilization of the waste textiles during pretreatment, which translated into a cellulose loss that affected textile recycling negatively, would have a positive impact on the operation of the pulp mill, as more sodium is maintained in the alkaline solution.

4. Conclusions

This study shows that it would be possible to integrate textile recycling with pulp mills, by utilizing green liquor as the alkaline solution in the pretreatment of the textiles. An efficiency of about 70% could be expected in the textile recycling process, while sodium losses in the pulping process would be below 15%.

It was observed that the conditioning of the pretreated textiles had a significant impact on the efficiency of the process. Some conditioning strategies might reverse the changes that the material underwent during pretreatment, and it was concluded that, when green liquor is used as the alkaline solution, it might be beneficial to maintain the pH as high as the enzymes allow. The temperature and residence time also played an important role; temperatures of at least 200 °C and residence times longer than 6 h were required to partially dissolve the textiles through degradation reactions, which was necessary to achieve high enzymatic hydrolysis yields.

The effects of Na_2S and NaOH in green liquor counterbalance each other, and other impurities from the pulping process did not interfere with the pretreatment of the textiles. Thus, the same efficacy could be expected from a pretreatment process with green liquor, as from a pure Na_2CO_3 solution.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors are grateful to the employees at the Department of Chemical Engineering, Lund University for their contribution to the collection of raw material for this study. This research did not receive any funding from external sources.

References

1 Food and Agriculture Organization of the United Nations (FAO) and International Cotton Advisory Committee (ICAC), *A summary of the world apparel fiber consumption survey 2005–2008*, 2011.

2 N. Pensupa, S.-Y. Leu, Y. Hu, C. Du, H. Liu, H. Jing, H. Wang and L. Carol Sze Ki, in *Chemistry and Chemical Technologies in Waste Valorization*, ed. C. S. K. Lin, Springer International Publishing, Cham, 2018, pp. 189–228, DOI: 10.1007/978-3-319-90653-9_7.

3 Re:newcell AB, *Re:newcell. We make fashion sustainable*, https://renewcell.com/, accessed July 2020.

4 G. M. Peters, G. Sandin and B. Spak, *ACS Sustainable Chem. Eng.*, 2019, **7**, 11682–11690.

5 A. Palme, A. Peterson, H. de la Motte, H. Theliander and H. Brelid, *Text. Cloth. Sustain.*, 2017, **3**, 4.

6 A. Jeihanipour, K. Karimi, C. Niklasson and M. J. Taherzadeh, *Waste Manag.*, 2010, **30**, 2504–2509.

7 C.-Y. Chu, S.-Y. Wu, C.-Y. Tsai and C.-Y. Lin, *Int. J. Hydrogen Energy*, 2011, **36**, 8743–8750.

8 F. Hong, X. Guo, S. Zhang, S.-f. Han, G. Yang and L. J. Jönsson, *Bioresour. Technol.*, 2012, **104**, 503–508.

9 T. Jeoh, C. I. Ishizawa, M. F. Davis, M. E. Himmel, W. S. Adney and D. K. Johnson, *Biotechnol. Bioeng.*, 2007, **98**, 112–122.

10 Y. L. Hsieh, in *Cotton*, ed. S. Gordon and Y. L. Hsieh, Woodhead Publishing, 2007, pp. 3–34, DOI: 10.1533/9781845692483.1.3.

11 A. Jeihanipour and M. J. Taherzadeh, *Bioresour. Technol.*, 2009, **100**, 1007–1010.

12 E. Gholamzad, K. Karimi and M. Masoomi, *Chem. Eng. J.*, 2014, **253**, 40–45.

13 Y. Ma, B. Zeng, X. Wang and N. Byrne, *ACS Sustainable Chem. Eng.*, 2019, **7**, 11937–11943.

14 C.-H. Kuo, P.-J. Lin and C.-K. Lee, *J. Chem. Technol. Biotechnol.*, 2010, **85**, 1346–1352.

15 E. Sjöström, *Wood chemistry: fundamentals and applications*, Academic Press, 2nd edn, 1993.

16 M. Gubitosi, P. Nosrati, M. Koder Hamid, S. Kuczera, M. A. Behrens, E. G. Johansson and U. Olsson, *R. Soc. Open Sci.*, 2017, **4**, 170487.

17 E. Hasanzadeh, S. Mirmohamadsadeghi and K. Karimi, *Fuel*, 2018, **218**, 41–48.

18 E. Joelsson, D. Dienes, K. Kovacs, M. Galbe and O. Wallberg, *Sustainable Chem. Processes*, 2016, **4**, 14.

19 A. Erik, R. O. Marcus and B. Thore, *Nord. Pulp Pap. Res. J.*, 2006, **21**, 466–475.

20 A. Wensley and P. Champagne, *presented in part at the CORROSION 99*, San Antonio, Texas, 1999.

21 B.-H. Um and G. P. van Walsum, *Appl. Biochem. Biotechnol.*, 2009, **153**, 127–138.

22 S. González-García, A. Hospido, R. Agnemo, P. Svensson, E. Selling, M. T. Moreira and G. Feijoo, *J. Ind. Ecol.*, 2011, **15**, 568–583.

23 R. Fornell, T. Berntsson and A. Åsblad, *Energy*, 2013, **50**, 83–92.

24 M. Moshkelani, M. Marinova, M. Perrier and J. Paris, *Appl. Therm. Eng.*, 2013, **50**, 1427–1436.

25 Scandinavian Pulp Paper and Board Testing Comittee, *Total, active and effective alkali*, Stockholm, Sweden, 1985.

26 A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton D. Crocker and C. O. Golden, *Determination of structural carbohydrates and lignin in biomass. Laboratory Analytical Procedure (LAP)*, National Renewable Energy Laboratory, 2008.

27 International Organization for Standardization (ISO), *Textiles – Quantitative chemical analysis – Part 1: General principles of testing (ISO 1833-1:2006, including Cor 1:2009)*, 2010.

28 J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J. Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak and A. Cardona, *Nat. Methods*, 2012, **9**, 676–682.

29 E. R. McCall and J. F. Jurgens, *Text. Res. J.*, 1951, **21**, 19–21.

30 R. Freytag and J.-J. Donzé, Chemical Processing of Fibres and Fabrics, Fundamentals and Preparation, Part A, *Handbook of Fiber Science and Technology*, Marcel Dekker, NY, 1983, vol. 1.

31 J. Buchert, J. Pere, L. S. Johansson and J. M. Campbell, *Text. Res. J.*, 2001, **71**, 626–629.

32 M. Wada, M. Ike and K. Tokuyasu, *Polym. Degrad. Stab.*, 2010, **95**, 543–548.

33 V. Novy, K. Aïssa, F. Nielsen, S. K. Straus, P. Ciesielski, C. G. Hunt and J. Saddler, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 22545–22551.

34 P. J. Wakelyn, N. R. Bertoniere, A. D. French, D. P. Thibodeaux, B. A. Triplett, M.-A. Rousselle, W. R. Goynes, J. V. Edwards, L. Hunter, D. D. McAlister and G. R. Gamble, in *Handbook of Fiber Chemistry*, ed. M. Lewin, CRC Press, Boca Raton, FL, USA, 2006, pp. 607–608.