Enhancement of methane production from the anaerobic digestion of chemical pulp and paper mill effluents

Lourdes Rodriguez-Chiang

UNIVERSITAT POLITÈCNICA DE CATALUNYA
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Aalto University

DOCTORAL
DISSERTATION

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DOCTORAL DISSERTATION

Enhancement of methane production from the anaerobic digestion of chemical pulp and paper mill effluents

Lourdes Rodriguez-Chiang

A doctoral dissertation completed under the Erasmus Mundus Joint Doctorate program Environomical Pathways for Sustainable Energy Services (SELECT+) for the degree of Doctor of Science (Technology) from Aalto University and the degree of Doctor from the Universitat Politècnica de Catalunya.

Universitat Politècnica de Catalunya
Instituto de Técnicas Energéticas

Aalto University
School of Chemical Engineering
Department of Bioproducts and Biosystems
Clean Technologies

Supervising professors
Professor Olli Dahl, Aalto University, Finland
Professor Jordi Llorca, Universitat Politècnica de Catalunya, Spain

Preliminary examiners
Professor Jaakko Puhakka, Tampere University of Technology, Finland
Professor Jerry D. Murphy, University College Cork, Ireland

Opponents
Assistant Professor Dr. Aiduan Borrion, University College London, UK
Assistant Professor Dr. Lluís Soler Turu, Universitat Politècnica de Catalunya, Spain
Senior Scientist Dr. Johan Kristian Melin, VTT Technical Research Center of Finland

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Abstract

Aalto University, P.O. Box 11000, FI-00076 Aalto www.aalto.fi

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Abstract

Sustainability of resources such as energy, water and waste have become important drivers in our current economy. For large industries that are water and energy intensive like pulp and paper (P&P) mills, this is a specifically relevant issue. The large and heterogenous volumes of effluents in P&P mills make it a difficult task to properly treat before discharge. Anaerobic digestion is an efficient wastewater technology that cleans the effluent, reduces wasted sludge and simultaneously produces methane that can be further used as energy. The research work described in this thesis aims to enhance the methane production from the anaerobic digestion of different chemical P&P mill effluents. Through effluent characterization, variability of process parameters, promoting agents and the assessment of biochemical methane potential (BMP) tests, the benefits of three potential methods to enhance methane yields of wastewaters were evaluated. In addition, the conversion of chemical pulp fibers directly to methane was explored.

The attained results described the different improvements that can be made to enhance methane production. Easily degradable effluents such as hydrolyzed filtrates and evaporator condensates contain high concentrations of sugars and acetic acid respectively, which could encourage an oversaturation of acids during acidogenesis. An inoculum to substrate ratio (ISR) of 2 proved to be the optimal in order to add the required buffering capacity to neutralize the pH and produce significantly high methane yields of up to 333 mLCH4/gVS. The high productivity of these effluents can be then considered for co-digestion with harder to treat effluents such as lignin-rich streams. Lignin clearly hinders methane production indicated by the negative linear correlation found between lignin content and methane yield. The co-digestion of lignin-rich effluent with evaporator condensates from neutral sulfite semi-chemical (NSSC) pulping proved to enhance the overall methane productivity of the mill´s wastewater treatment.

Furthermore, the assessment of hydrotalcites (HT) addition indicated a contribution towards an increase in methane yield, faster production rates and a greater lignin removal. The poor performance of calcined HT suggests that the advantages of HT addition came from the layered sheet structure. Finally, besides establishing the methane potential in various P&P effluents, the examination of methane productivity of different pulp fibers and its products proved to be a promising new energy alternative to explore. Brown, oxygen delignified and bleached pulp gathered biodegradabilities of up to 90% and methane yields as high as 380 mLCH4/gVS. With the current changing bio-economy this last approach paves the way in exploring alternative and novel uses for chemical pulp mill products.

Keywords pulp and paper; methane, anaerobic digestion; wastewater treatment; industrial effluents

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Summary in Spanish

La sostenibilidad en la gestión de recursos como la energía, el agua y los residuos se ha convertido en un aspecto clave en nuestra economía actual. Para grandes industrias que consumen mucha agua y energía, como la industria de pulpa y papel (P&P) este es un tema especialmente relevante. Los grandes y heterogéneos volúmenes de efluentes que producen las fábricas de P&P hacen que su adecuado tratamiento sea una tarea difícil. La digestión anaerobia es una tecnología eficiente para el tratamiento de aguas residuales; que limpia el efluente, reduce el lodo producido y simultáneamente produce metano que puede usarse como fuente de energía. El objetivo del trabajo de investigación descrito en esta tesis es aumentar la producción de metano a partir de la digestión anaerobia de diferentes efluentes producidos en fábricas de pulpa. A través de la caracterización del efluente, determinación de parámetros del proceso, agentes promotores y la evaluación de pruebas de producción de metano bioquímico se evaluaron los beneficios de tres métodos potenciales para mejorar los rendimientos de metano de los efluentes. Asimismo, se exploró la conversión directa de fibras de pulpa química a metano.

Los efluentes fácilmente degradables, como los filtrados hidrolizados y los condensados del evaporador, contienen altas concentraciones de azúcares y ácido acético, respectivamente, lo que podría provocar una sobresaturación de ácidos durante la acidogénesis. Se ha demostrado que una relación de inóculo a sustrato de 2 resulta óptima para aumentar la capacidad tampón del sistema y neutralizar el pH y producir rendimientos de metano significativamente altos, de hasta 333 mLCH4/gVS. La alta productividad de estos efluentes hace que se puedan considerar para la co-digestión con efluentes más difíciles de tratar, como las aguas ricas en lignina. Claramente la lignina obstaculiza la producción de metano, tal y como indica la correlación lineal negativa encontrada entre el contenido de lignina y el rendimiento de metano. La co-digestión del efluente rico en lignina con el condensado de evaporadores de pulpa semi-química de sulfito ha demostrado mejorar la productividad de metano en el tratamiento de aguas residuales de la planta. Además, la adición de hidrotalcitas (HT) como catatlizadores contribuye hacia un aumento en la producción de metano, tasas de producción más rápidas y una mayor eliminación de lignina. Con HT calcinadas se han obtenido rendimientos bajos, lo que sugiere que las ventajas de la adición de HT provienen de su estructura laminar.

Finalmente, además de establecer el potencial de metano que existen en los efluentes de P&P, la evaluación de la productividad de metano de diferentes fibras de pulpa y sus productos ha demostrado ser una nueva alternativa energética para explorar. El uso de pulpa blanqueada, sin blanquear y deslignificada con oxígeno muestran biodegradabilidades de hasta 90% y rendimientos de metano de hasta 380 mLCH4/gVS. Con la cambiante bioeconomía actual, este último enfoque estimula la exploración de usos alternativos y novedosos para productos de plantas de pulpa química.

Preface

The work for this dissertation was conducted at the Department of Bioproducts and Biosystems (formerly known as Forest Products Technology) of Aalto University and at the Institute of Energy Technologies of Universitat Politècnica de Catalunya (UPC). The economic support of the Erasmus Mundus Joint Doctorate program Environomical Pathways for Sustainable Energy Services (SELECT+) and Stora Enso Oyj is gratefully acknowledged.

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My deepest appreciation goes to my family which supported me through the tough times and celebrated the good times. Finally thank you Shane for being the cherry on top of the perfect sundae that was my time in Finland.

Helsinki, November 2017

Lourdes Rodriguez-Chiang

List of publications

This thesis consists of an overview of the following papers, which will be referred to in the text by their corresponding Roman numerals. The corresponding author is marked in bold:

I **Rodriguez-Chiang, L.M.**, & Dahl, O. (2015) Effect of Inoculum to Substrate Ratio on the Methane Potential of Microcrystalline Cellulose Production Wastewater. *BioResources 10(1),* 898-911. DOI: 10.15376/biores.10.1.898-911

II **Rodriguez-Chiang, L.M.**, Llorca, J., & Dahl, O. (2016) Effect of Fe–Zn–Mg–Al hydrotalcites on the methane potential of synthetic sulfate-containing wastewater. *J Water Process Eng 10*, 120–127. DOI: 10.1016/j.jwpe.2016.03.001

III **Rodriguez-Chiang, L.**, Llorca, J., & Dahl, O. (2016) Anaerobic co-digestion of acetate-rich with lignin-rich wastewater and the effect of hydrotalcite addition. *Bioresour Technol 218,* 84-91. DOI: 10.1016/j.biortech.2016.06.074

IV **Rodriguez-Chiang, L.**, Vanhatalo, K., Llorca, J., & Dahl, O. (2017) New alternative energy pathway for chemical pulp mills: from traditional fibers to methane production. *Bioresour Technol 235, 265-273.* DOI: 10.1016/j.biortech.2017.03.140

The author’s contribution

I L. Rodriguez-Chiang carried out the planning and execution of the study. She was responsible of the substrate characterization, setup and running of the experimental plan consisting of three experiment sets, interpretation of the results and writing of the manuscript under the supervision of O. Dahl.

II The conceptual approach of the study was designed by L. Rodriguez-Chiang and J. Llorca. She was responsible of the synthesis and characterization of hydrotalcite materials and synthetic wastewater used in the experiments, as well as planning and running of the experimental plan consisting of four experiment sets and interpreting the results. The manuscript was written by L. Rodriguez-Chiang and reviewed by J. Llorca and O. Dahl.

III L. Rodriguez-Chiang carried out the planning and execution of the study. She was responsible of the synthesis and characterization of hydrotalcite materials, wastewater substrate characterization, planning and running of the experimental plan consisting of two experiment sets. She interpreted the results and wrote the manuscript under the supervision of J. Llorca and O. Dahl.

IV L. Rodriguez-Chiang designed the study in collaboration with K. Vanhatalo and O. Dahl. L. She was responsible of planning and running of the experimental plan consisting of three experiment sets, interpreting the results and wrote most of the manuscript except for the materials’ characterization section. K. Vanhatalo manufactured and characterized the materials investigated. J. Llorca and O. Dahl assisted in the manuscript review.

Nomenclature

Abbreviations

AD	anaerobic digestion
Adt	air dried ton
AF	anaerobic filter
AMPTS	automatic methane potential test system
AnMBR	anaerobic membrane bioreactor
ANOVA	analysis of variance
AOX	adsorbable organic halides
BD	anaerobic biodegradability
BMP	biochemical methane potential
BOD	biochemical oxygen demand
CI	crystallinity index
COD	chemical oxygen demand
CSTR	continuous stirred tank reactor
CTMP	chemical thermo-mechanical pulping
DAF	dissolved air flotation
DW	dry weight
EC	evaporator condensates
ECF	elemental chlorine free
FBR	fluidized bed reactor
FC	fiber circulation
FTIR	Fourier transform infrared spectroscopy
GPC	gel permeation chromatography
HPLC	high performance liquid chromatography
HRT	hydraulic retention time
HT	hydrotalcite

IC	inorganic carbon
ISR	inoculum to substrate ratio
LCC	lignin-carbohydrate complexes
LDHs	layered double hydroxides
MCC	microcrystalline cellulose
MPR	methane production rate
MWD	molecular weight distribution
NSSC	neutral sulfite semi-chemical
O2	oxygen delignified
OLR	organic load rate
P&P	pulp and paper
SA	soil amendment
SEM	scanning electron microscopy
SRB	sulfate-reducing bacteria
SS	suspended solids
STP	standard temperature and pressure
TCF	total chlorine free
TMP	thermo-mechanical pulping
TOC	total organic carbon
TS	total solids
TSAMBR	thermophilic submerged aerobic membrane bioreactor
UASB	up-flow anaerobic sludge blanket
UV/VIS	ultraviolet/visible spectroscopy
VFA	volatile fatty acids
VOC	volatile organic compound
VS	volatile solids
WWTP	wastewater treatment plant
XRD	X-ray diffraction

Chemical compounds and minerals

Al_2O_3	aluminium oxide
$Al(NO_3)_3$	aluminium nitrate
$CaCO_3$	calcium carbonate
CaO	calcium oxide
CH_4	methane
ClO_2	chlorine dioxide
CO_2	carbon dioxide
$FeSO_4$	ferrous sulfate
H_2O	water
H_2O_2	hydrogen peroxide
H_2S	hydrogen sulfide
H_2SO_3	sulfurous acid
H_2SO_4	sulfuric acid
HCl	hydrochloric acid
MgO	*periclase* (mineral)
$Mg(NO_3)_2$	magnesium nitrate
Na_2CO_3	sodium carbonate
Na_2S	sodium sulfide
Na_2SiO_3	sodium silicate
Na_2SO_3	sodium sulfite
$NaHCO_3$	sodium hydrogen carbonate
$NaOCl$	sodium hypochlorite
$NaOH$	sodium hydroxide
NH_3	ammonia
O_3	ozone
SO_3	sulfite
SO_4	sulfate

ZnO	zinc oxide
ZnS	zinc sulfide
$Zn(NO_3)_2$	zinc nitrate

Symbols

GJ	gigajoule
kDa	kilodalton
MJ	megajoule
R^2	correlation coefficient
TWh	terawatt-hour
°C	Celsius degree
€	Euro

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1 Introduction

In our current society, global concern has revolved over the negative impacts originating from the production and use of fossil fuels and the dependency of global economy towards them. This has caused a major impulse in the direction of alternative energies and the production of biofuels for transportation. Following the need to stimulate the increase of renewable energy use worldwide and move towards a carbon neutral pathway, the European Union has set an overall common target of 20% renewable energy use by the year 2020. Therefore, not only do industries have a legal environmental responsibility need due to national legislation and international strategies and protocols, but now the social acceptance on green products is growing and becoming a great driver for consumer goods.

The pulp and paper (P&P) industry is one of the world's largest energy and water consuming industries, therefore they face a great responsibility to increase their environmental input. This has directed P&P companies to increase efforts on energy efficiency and reducing their water footprint and waste. To tackle these issues, studies investigating and analyzing the recovery of energy from the anaerobic digestion (AD) of P&P waste have been increasingly emerging. AD is a widely applied technology for the treatment and stabilization of wastewaters, wastewater sludge and many different types of organic wastes. Its energetic output has given it an economic and environmental advantage which has development through the years keeping it as one of the major contributors of the global energy supply. Waste and wastewater can be anaerobically digested and produces biogas, a mixture of mostly methane and carbon dioxide. It also reduces the volume of sludge for disposal and presents a positive energetic balance using methane to recover energy as heat and electricity (Mao et al. 2015). Hence AD of pulping wastewater presents a practical answer to the P&P industry's energy and water concerns; where water is efficiently treated, waste sludge production is decreased and bioenergy produced.

In the last decade, AD of P&P wastewater has gained increasing attention which has made the number of operating anaerobic digesters double worldwide. Pulp and paper mill effluents are rich in chemical oxygen demand (COD) typically ranging between 1-10 g/L. This characteristic alone makes them a suitable candidate for energy conversion, assuming 25% of the COD load can potentially be converted to biogas this would generate 1-100 TWh of electricity (Meyer and Edwards 2014). However, besides their high COD concentrations; P&P wastewaters are known for their inherent lignocellulosic nature which make them difficult to degrade. Their difficulty to treat includes the presence of suspended solids, fatty acids, tannins, resin acids, lignin and its derivatives, sulfur compounds and other xenobiotic compounds (Ali and Sreekrishnan 2001).

In addition, each mill has a unique wastewater composition that depends on the type of raw material, chemicals and process used, which makes it a hard task to assess their anaerobic degradability. Various studies have reported on the methane potential of different P&P mill wastewaters and have concluded on an overall positive yield of methane ranging from 40 to 60% of the theoretical potential (Bayr and Rintala 2012; Ekstrand et al. 2013; Hagelqvist 2013). The heterogeneity of the wastewaters makes them have a large range of anaerobic degradability; therefore, in practice only a few selected wastewater streams with easily degradable organic content are treated in anaerobic reactors. Meyer and Edwards (2014) reviewed the methane potential of many different pulping wastewaters their conclusion enveloped the fact that contrary to common opinion, most pulping effluents; even the highly inhibiting ones; were to some degree anaerobically treated. This suggests the numerous possibilities to encourage the enhancement of methane production of these effluents by means of catalysis, pre-treatments, co-digestion or other process control methods.

The key focus of the research work in this thesis was to explore different approaches for a chemical pulp mill to enhance its energy value (methane) output. Enhancing the methane potential in the treatment of chemical P&P wastewaters was explored following three approaches. Firstly, evaluating the effects of changing a key process parameter such as inoculum to substrate ratio (ISR). Secondly, by introducing a promoter such as hydrotalcites (HT) which have high ion-exchange capacities for adsorption of pollutants (Gillman 2006). Lastly, the benefits of co-digestion of two in-mill effluents was addressed. Co-digestion between pulping effluents is uncommon, however their diverseness can improve digester stability and dilute hard to treat streams to provide substantial methane production (Meyer and Edwards 2014). In addition, an alternative option was explored that evaluated the potential of converting chemical pulp fibers directly to methane. In order to maintain their competitiveness and changing markets, the P&P industry is evolving and integrating new high added-value products where bioenergy seems a profitably investment (Machani et al. 2014). Hence, investigating an energy pathway from traditional fiber to methane production can help shed some light into the diversification of the market portfolio of chemical pulp mills.

2 Background

2.1 Pulp and paper effluents and their characteristics

As a water-intensive industry, P&P companies have produced great efforts and technological advances to reduce their water consumption. They have managed to lower the water usage from 200 m^3/ton paper to a current usage of 13-30 m^3/ton paper produced (Kamali and Khodaparast 2015). Their wastewater however, has particular characteristics that make them highly polluted and toxic to aquatic environments, hence their treatment is necessary.

Table 1. *Summary of typical characteristics of wastewater from pulp and paper processes.*

Source/process	**pH**	**COD (mg/L)**	**BOD (mg/L)**	**SS (mg/L)**	**Other (mg/L)**	**References**
Debarking and chipping	7	5000-40,000	12,000	6100-7150	Tannins: 200-1600	Meyer and Edwards 2014; Ashrafi et al. 2015
TMP[a]	4.2	3300-5500	2800	500-810	Nitrogen: 12	Ashrafi et al. 2015; Kamali et al. 2016
CTMP[b]	6.2	7500-25,000	2500-6800	500-3000	Sulfate: 500-1500	Ashrafi et al. 2015; Kamali and Khodaparast, 2015
Kraft cooking	10-13	1000-2440	460	40	Sulfides: 200	Ekstand et al. 2013
ECF[c]/TCF[d] Bleaching	6-8	1500-4000	352	60-950	Chloride: 1200-1600	Meyer and Edwards 2014; Ekstand et al. 2013
NSSC[e] composites effluents	7	13,000-19,000	-	120-940	Acetic acid: 500	Meyer and Edwards 2014; **Paper III**
Papermaking	6.5-8.5	1000-3000	560-641	645-1844	-	Ekstand et al. 2013; Kamali et al. 2016
Recycled paper mill	6.2-7.8	3380-4930	1650-2565	1900-3138	-	Zwain et al. 2013

[a] Thermomechanical pulping; [b] Chemical thermomechanical pulping; [c] Elemental chlorine free; [d] Total chlorine free; [e] Neutral sulfite semi-chemical pulping

The differences in raw materials, pulping processes and chemical usage in each mill have resulted in the production of a very diverse range of wastewaters. **Table 1** reviews the typical characteristics of wastewater from various pulping processes. For every type of pulp and paper production process two typical raw materials can be used; virgin or recycled material. Depending on the type of mill, the raw fibers are treated by chemical, mechanical or a combination of both methods with the aim to dissolve lignin and release the fibers from the wood matrix (Manskinen et al. 2011). Chemicals and other additives are used in each step of the P&P production process, hence besides compounds found in the raw material itself (cellulose, hemicellulose, lignin, resins, waxes) part of the chemicals used also end up in the wastewater streams. **Figure 1** depicts several of the chemicals added in the P&P processes and the most common contaminants that are found in the process wastewater. As environmental and efficient strategies grow, a common practice taking place in mills is recycling and recovery of aqueous streams. Cooking chemicals and other effluents can be recycled in a chemical recovery unit. For example, the process water made of lignin and spend inorganic chemicals is separated from the cellulose fibers and then concentrated by evaporation where it becomes black liquor. Burning this black liquor can generate much of the steam and energy used in the mill. Other compounds are also reduced and form smelt or green liquor which is reused in the pulping process (Rapp and Pfromm 1998). Chemical recovery can significantly reduce the toxicity of P&P wastewaters; however, many contaminants are still present in high concentrations and finish in the common wastewater treatment plant (WWTP) of the mill.

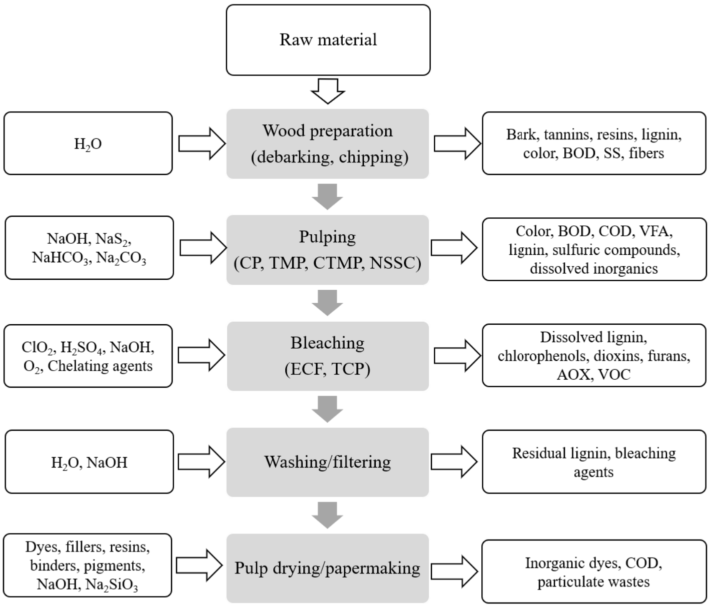

Figure 1. *Common additives and contaminants from different sources of the P&P process (adapted from Kamali et al. 2016)*

Besides high loads of organic material, color and suspended solids (SS), pulping effluents maintain certain contaminants that are important to mention. Lignin is a complex aromatic polymer found in the lignocellulosic matrix of wood, hence it is a common pollutant providing a major source of COD and color in most P&P wastewaters. It is highly recalcitrant towards chemical and biological degradation caused by its high molecular weight and chemical heterogeneity (Ruiz-Dueñas and Martinez 2009). In addition, the delignification treatment applied in the pulping process will further modify and depolymerize the lignin into a mixture of lignin fractions that have different chemical and physical characteristics (Sierra-Alvarez and Lettinga 1991). Studies have investigated different treatments to target lignin degradation or removal such as coagulation-flocculation techniques (Nawaz et al. 2014); combination of chemical precipitation using sulfuric acid followed by ozonation (De los Santos Ramos et al. 2009); thermochemical pre-treatment using alkali (Koyama et al. 2015) and other depolymerisation methods (Sturgeon et al. 2014). However, the limited understanding of the lignin polymer and its different variations makes tackling lignin degradation still a difficult task.

Sulfur compounds are widely present in P&P effluents as they are the main cooking reagents in chemical pulping processes. The kraft process is a commonly used pulping technique that uses a mixture of sodium hydroxide (NaOH) and sodium sulfide (Na_2S). The sulfite process cooks woodchips in a solution of sulfurous acid (H_2SO_3) and bisulfide ion (HSO^-_3) to dissolve lignin (Pokhrel and Viraraghavan 2004) and NSSC pulping cooks woodchips in a mixture of sodium sulfite (Na_2SO_3) and sodium carbonate (Na_2CO_3) (Manskinen et al. 2011). Consequently, high concentrations of sulfur compounds (i.e. sulfate) are bound to be found in the pulping effluents and need to be properly treated before discharged to the environment. The biological treatment of sulfate-rich effluents consists mainly of sulfate anaerobically reduced to sulfide by sulfate-reducing bacteria (SRB) and subsequently sulfide can be partially oxidized to elemental sulfur by sulfide-oxidizing bacteria. However, the production of sulfide can lead to corrosion, bad odor, toxicity towards human health and inhibition in anaerobic digestion (Blazquez et al. 2016). Other methods used for the removal of sulfur compounds from wastewaters consist of membrane filtration (Silva et al. 2012), chemical precipitation (Silva et al. 2010), and ion exchange/adsorption (Dou et al. 2017).

Pulp bleaching was initially done with chlorine (Cl_2), then it was replaced by ECF reagents such as ClO_2 and NaOCl or by TCF reagents such as H_2O_2, O_2 and O_3 (Monje et al. 2010). ECF bleaching is the most common technique used worldwide. This means that the majority of effluents originating in the bleaching process contain chlorinated compounds measured as adsorbable organic halides (AOX) (Sharma et al. 2014). Chlorine reacts with other organic material producing many toxic substances like chlorate (Malmqvist and Welander 1994), chlorolignin, chlorinated resin acids and chlorinated phenols, of which pentachlorophenol is the most toxic causing 50% inhibition in methanogenesis (Sierra-

Alvarez et al. 1994). Most of the organochlorinated compounds in P&P effluents are high molecular weight chlorolignins (>100 kDa) which are biologically inactive and most probable have little effect on their toxicity. On the contrary, low molecular weight chlorinated compounds contribute to mutagenicity and bioaccumulation caused by their hydrophobicity and cell penetration, making them highly toxic to the environment (Ali and Sreekrishnan 2001). TCF effluents show the least methanogenic toxicity of all bleaching effluents. Although they mostly lack chlorinated compounds, they have also been found to induce toxicity; this indicates that other substances (besides organohalogens) found in bleaching effluents contribute to their toxicity. Alkaline extraction used in bleaching produced wood resins that are also toxic.

2.2 Overview of pulp and paper effluent treatments

The significant variety in the characteristics of pulp and paper mill effluents have made it an increasingly hard task to design an efficient wastewater treatment process. Each WWTP is different in terms of design, operating costs, flow, environmental regulation and most importantly effluent characteristics which will determine the most suitable type of treatment to deliver the best results in which some cases can be a combination of processes to ensure the minimal impact of their wastewater.

2.2.1 Physicochemical treatment

Physicochemical methods are usually the primary or tertiary treatment option for P&P WWTPs. Most of these treatments methods should be combined with secondary (biological) methods to accomplish the most efficient results. Physicochemical treatments are proven methods that remove turbidity, biochemical oxygen demand (BOD), suspended solids (SS), color and other contaminants (Kamali and Khodaparast 2015). Some of the most commonly applied techniques are the following:

Sedimentation and flotation techniques are used as a primary clarification process. This is the most common process used for P&P effluents which consist of a high volume of suspended and colloidal particles (bark and woodchip particles, fibers, fillers, sand). These can be efficiently removed (up to 80%) with sedimentation/flotation methods. Dissolved air flotation (DAF) is the most used treatment option in pulp mills, it can remove 95% of SS and is cost effective for large volumes of water (Bhattacharjee et al. 2007; Pokhrel and Viraraghavan 2004).

Coagulation/flocculation and precipitation methods involve the use of metal salts to promote the production of large flocs of particles in the wastewater that can later precipitate and be removed. These methods are not very commonly applied in P&P mills because of high costs and additional sludge production, however when applied they are usually tertiary treatments for additional polishing of the final effluent. Flocculants such as polyaluminium chloride (PAC), chitosan, polymeric phosphate-aluminum chloride, cationic and anionic poly-acrylamides (PAMs) and polydiallyldimethylammonium chloride (polyDADMAC) have been proven to remove turbidity and lignin in P&P effluents (Wang et al. 2011; Kamali and Khodaparast 2015). However, it was found that the efficiency of the process depends greatly on many parameters such as type and dosage of coagulant/flocculant, pH, temperature and retention times (Wang et al. 2011).

Adsorption treatments consists of using an adsorbent to remove organic molecules by mechanisms such as ion exchange, covalent bonding, van der Waals forces, dipole-dipole interactions etc. (Andersson et al. 2011). Although adsorption methods are less applied in P&P mills than sedimentation or coagulation methods, the use of activated carbon had been proved to be highly effective for polishing the final effluent. Pokhrel and Viraraghavan (2004) reviewed the use of activated carbon, charcoal and coke, fuller's earth and coal ash as absorbents and reported up to 90% removal of COD, AOX and color from bleached wastewater. Andersson et al. (2012) found that fly ash is capable to absorb up to 90% of lignin from mechanical pulping wastewaters. Hydrotalcites (HTs) are ionic clays that are gaining much attention in wastewater treatment for their high ion exchange capacities for adsorption of different pollutants found in wastewaters (Wimonsong et al. 2013). Although this new technique has not been tested in P&P wastewaters, they have successfully treated mining and metallurgy effluents.

Membrane technologies such as microfiltration, ultrafiltration, nanofiltration and reverse osmosis can be employed as tertiary treatment for additional improvement of water quality. In the past decade, membrane technologies have gained attention in P&P effluents and investigation and testing has resulted in stimulating results. However, the applicability of these technologies in P&P mills is less observed. This is due to high investment in equipment, the high volumes of wastewater in P&P mills require an increased number of modules to produce an efficient permeate. Also, the maintenance of membranes is expensive (membrane fouling is common in highly polluted waters with high loads of sediments such as P&P). Pizzichini et al. (2005) tested all these processes in pulp and paper wastewater and obtained good performances especially with microfiltration, however high index fouling was a common issue. Pokhrel and Viraraghavan (2004) reviewed the application of membrane filtration in P&P effluents and found these technologies suitable for removing AOX, COD, color and SS including heavy metals. They also reviewed other physicochemical treatments such as ozonation and other advanced oxidation processes which held efficient removal of pollutants. The use of oxidation processes before the biological treatment was regarded as highly effective, as these increased the biodegradability of bio-refractory organic substances.

2.2.2 Biological treatment

Biological treatment is the current most common method applied for P&P effluents. This involves the use of microorganisms such as bacteria, archaea, algae, fungi and enzymes for the degradation of organic material. The most common biological processes are described as followed:

Aerobic treatment implements bacteria and addition of oxygen to remove organic contaminants. Activated sludge, aerated lagoons and aerated stabilization basins are the aerobic processes used in P&P wastewater treatment, the most common wastewater treatment currently applied in mills is the activated sludge process. These techniques have several advantages such as ease in operation, relatively low operating costs and high COD and BOD removal rates (Ashrafi et al. 2015). Pollutants such as chlorinated compounds have also been efficiently removed using aerobic techniques (Pokhrel and Viraraghavan 2004). The main setback in aerobic treatments is the large production of sludge compared to other treatments (i.e. AD) which needs further processing to discard. However, the high efficiencies in removal of pollutants (from 60-95%) maintain it as a popular treatment used for P&P effluents.

Anaerobic treatment is less applied for P&P effluents but is becoming more employed due to various advantages like lower sludge production (30-70% less than aerobic treatments) (Ekstrand et al. 2013); smaller area requirements, applicability at different scales and most importantly the production of renewable energy in the form of biogas. The most used reactors for the treatment of P&P effluents are the up-flow anaerobic sludge bed reactor (UASB), fluidized bed reactor (FBR), anaerobic filter (AF), completely stirred tank reactor (CSTR) and the anaerobic membrane bioreactor (AnMBR). Kamali et al. (2016) and Meyer and Edwards (2014) have thoroughly reviewed the AD of different types of P&P wastewaters and concluded in COD removal rates (between 30-90%) and methane yields (0.30-0.40 m^3/kg). Anaerobic reactors have also been efficient in removing AOX, chlorinated organics, sulfate and additionally Cu, Zn, Ni, As and Fe. Although anaerobic treatment presents many benefits, in most cases anaerobic digestion alone does not provide an effluent of sufficient quality for release into a body of water. Hence, it is usually followed by and aerobic treatment to provide further purification.

Fungal treatment is less applied in full scale and mostly coupled with aerobic treatments, it has been proven to remove color, lignin and COD in P&P effluents. Pohkrel and Viraraghavan (2004) reviewed several studies where white-rot fungi was commonly used for the efficient removal of color (35-94%), COD (41-79%) and lignin content (16-66%). White-rot fungi was able to remove lignin (77%) and COD (48%) from black liquor even under strong alkaline condition (Wu et al. 2005). It has also been demonstrated that the addition of ligninolytic fungi in anaerobic reactors improved the removal of COD and color (Ortega-Clemente and Poggi-Varaldo 2007).

2.2.3 Integrated treatment

Integrated treatment is the option with most potential in terms of efficiency and quality of the final effluent. Also known as a hybrid/combined system; integrated treatment combines two biological or two physicochemical or a biological and physicochemical treatment (Ashrafi et al. 2015). Physicochemical treatments are usually considered as the primary and/or tertiary treatment, while biological processes (AD, aerobic treatment or a combination of both) are the secondary treatment. Buyukkamaci and Koken (2010) and Ashrafi et al. (2015) made comprehensive studies to determine the optimum treatment options for P&P effluents. As a common result, they found the most technically and economically optimal process to be the application of two biological treatments (**Figure 2**). The use of UASB followed by an activated sludge process or aeration basin was the most effective in treating medium and high strength P&P effluents. The inclusion of a chemical treatment was found to be uneconomic, however it was necessary for the removal of specific contaminants that biological processes could not treat. The use of a combination of biological and physicochemical treatments can also deliver excellent results. Qu et al. (2012) treated thermomechanical pulping effluents with an aerobic membrane reactor followed by electrochemical oxidation that resulted in high quality treated effluent with COD removal of 96-98%. Jaafarzadeh et al. (2016) tested the treatment of P&P effluents with combined physicochemical treatment of electrocoagulation and UV/oxidant system which achieved a 61% COD removal and a further 40-50% removal after UV treatment. **Table 2** shows the performance of different integrated systems used to treat P&P effluents.

Table 2. *Performance of integrated treatment of pulp and paper effluents.*

Treatment	**Type of effluent**	**COD removal (%)**	**Other parameter**	**Removal (%)**	**References**
Anaerobic + aerobic sequential reactor	Composite P&P effluent	83.9	Color Lignin	87.7 76.5	Chuphal et al. 2005
TSAMBR + Electrochemical oxidation	TMP	96-98	Color	100	Qu et al. 2012
Activated sludge + hydrogenation	Kraft pulp effluent	95	BOD Color	98 97.5	Ghoreishi and Haghighi 2007
AnMBR + ozonation	Recycled paper mill	90	Color	95	Merayo et al. 2013
Coagulation + Flocculation	Blending black liquor	n.a.	Turbidity Lignin	99.6 88.4	Wang et al. 2011

TSAMBR: thermophilic submerged aerobic membrane bioreactor
n.a. information not available

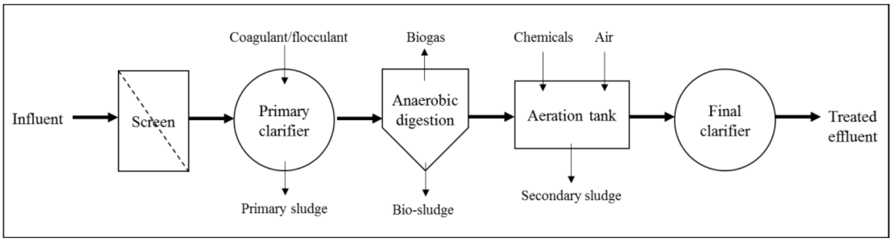

Figure 2. *A generalized schematic diagram of the wastewater treatment process in a P&P mill.*

2.3 Anaerobic digestion

AD is the biological process in which organic material is degraded by microorganisms in the absence of oxygen and converted to biogas; a mixture of methane, carbon dioxide and small quantities of H_2S, H_2 and NH_3 (Angelidaki and Sanders 2004). The remainder after AD is the digestate. AD is characterized by four phases starting with hydrolysis (**Figure 3**). In this phase, complex organic molecules are simple converted into a soluble form that can be utilized by the microbial communities. This is often considered as the rate-limiting step (Parkin and Owen 1986). The hydrolyzed organic molecules are then converted to organic acids in the acidogenesis phase. The compounds formed during acidogenesis are further digested by acetogens in the acetogenesis phase where they are converted mainly into acetic acid and CO_2 and H_2. The low partial pressure of hydrogen in the mixture plays a key role in this conversion. Methanogenesis is the last phase, where methanogens convert acetic acid into CH_4 and CO_2. Around 70% of methane is produced this way where acetic and butyric acids are the main precursors; while the rest 30% is converted from methanogens using hydrogen as electron donor and CO_2 as acceptor to produce methane (Appels et al. 2008).

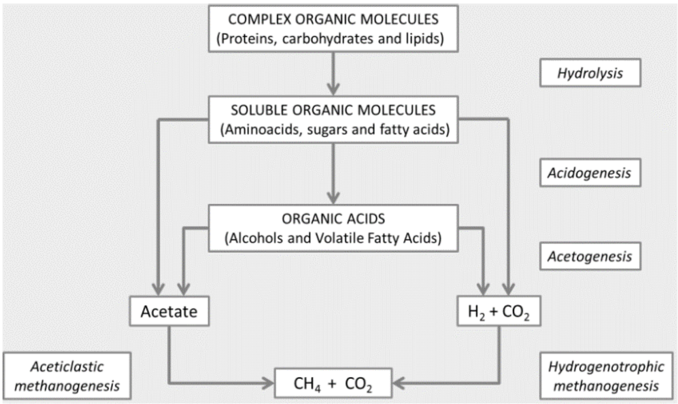

Figure 3. *A schematic illustration of the AD process (adapted from Appels et al. 2008)*

Biogas production is a complex process and therefore needs optimal conditions for the necessary chemical reactions to take place and the different microorganisms needed in all stages to be present. To keep this process running efficiently, several parameters that influence the AD process need to be monitored for a stable biogas production. Temperature is a primary parameter that affects the physiochemical properties of the substrate and influences the growth rate and metabolism of microorganisms as well as the partial pressure of H_2, hence the overall kinetics of the process (Boe 2006). AD can occur at several temperature ranges; thermophilic (50-60 ºC) and mesophilic (32-35 ºC) are the most commonly used. Although thermophilic temperatures favor higher methane generation and COD removal, it also increases the fraction of free ammonia that can cause inhibition and makes the AD process more sensitive to sharp fluctuations of temperature (Van Lier 1993). Moreover, the use of higher temperatures requires more energy input; this makes mesophilic temperature the most attractive option offering high methane yields and better process stability.

The pH of a system has a direct effect on the methane production. Each group of microorganisms present in the AD process function at different optimum pH ranges. Mao et al. (2015) observed that the optimum range for methane production was 6.8-7.4 which was explained by the pH ranges of acidogenic and methanogenic activity which are 5.5-6.5 and 6.5-8.2 respectively. Low pH may cause acid toxicity due to the free VFA while high pH may cause inhibition due to free ammonia concentrations (Boe 2006).

The inoculum to substrate ratio (ISR) is one of the most important factors that may influence the efficiency of AD when doing biochemical methane potential (BMP) tests (Raposo et al. 2006). Having an optimum ISR in the digester ensures the presence of all the microbial communities needed for the digestion of primary and intermediate products of AD. Previous studies suggest that higher ISR positively affect the ultimate methane yield with the optimal being 2 (Eskicioglu and Ghorbani 2011; Dechrugsa et al. 2013). Conversely lower ISR (< 2) gave higher specific methane production rates due to the greater availability of organic at a given time; however, this is associated to the accumulation of long chain acids within the system that lowers the pH and could inhibit methanogenic activity.

Nutrients are essential for microbial cell growth. C, H_2, O_2, N_2, P and S are nutrients required for microorganisms to thrive in a digester. Most nutrients are found within organic material however N_2 and P are found in shorter amounts therefore addition of material rich in these nutrients is recommended to keep a proper balance (Jain et al. 2015). The C/N ratio is a good indicator of the nutrient level in the AD system. Carbon is used for energy and nitrogen for the formation of cell structure in bacteria. Studies recommend using an optimal C/N ratio of 25/1(Mao et al. 2015). Low C/N ratio increase the possibilities of ammonia inhibition and low carbon utilization resulting in lower degradability. Meanwhile a high ratio provides deficient nitrogen amounts to maintain biomass growth that may lead to VFA accumulation and potential inhibition of the process.

Other important factors to take into account are substrate composition, mixing and toxic substances. The type of substrate has an evident direct effect on the methane yield. The organic material in a substrate is usually measured as COD or volatile solids (VS) content. These can be of available degradable nature such as VFA, lipids, proteins and carbohydrates or of inert or recalcitrant nature like lignin. Raposo et al. (2011a) summarized the methane yield of many solid organic substrates i.e. fat-pork, cellulose, tomato, tea residue and found a variety of methane yields like 900, 400, 218, 67 mL CH_4/gVS_{added} respectively. Mixing intensity has been proven to affect the methane production in a reactor. Adequate mixing is needed to prevent stratification and temperature gradients as well as avoid the accumulation of intermediates in certain areas of the digester. Inefficient mixing stimulates scum and foam formation and excessive deposition of solids while intensive mixing has been found to result in acidification and process failure (Boe 2006; Parkin and Owen 1986). Toxic compounds that have a negative effect on the methane production can either be produced in the digestion process itself or be found in the substrate. The most common inhibitors that are produced during degradation are VFA, ammonia and sulfide; which when found in high concentrations are toxic to methanogenic archaea. P&P effluents contain many types of inhibiting compounds due to the natural polymers found in wood and the various chemical present in their process wastewater. Common toxic/inhibiting organic substances in P&P effluents are resin compounds, chlorinated phenolics, tannins and lignin derived compounds (Vidal and Diez 2005). Toxic compounds are found to be less inhibitory when using adapted biomass.

2.3.1 Biochemical methane potential

The BMP test is a commonly used procedure used to determine the anaerobic degradability of a substrate. It is a relatively simple and reliable method to obtain the extent and rate of organic matter conversion to methane. The BMP provides information on how much and how fast a substrate is degraded under optimal batch condition. This are valuable indicators for optimizing the design, economics and functioning of a full-scale anaerobic digester (Raposo et al. 2011b). The correlation between results from a BMP test and the operation of a full-scale digester has been studied and successfully demonstrated (Batstone et al. 2009; Holliger et al. 2017). Reports concluded that the BMP can be used to estimate with confidence the methane production for the design and operation of full-scale installations, given that the substrate is well characterized. It is also a good tool to identify potential pre-treatments and other methane enhancing techniques.

The basic principle for a BMP is to gather organic waste and mix it with inoculum, incubate it in a closed vial at a specific temperature and measure the gas production volume and composition. The inoculum is seed sludge from and operating anaerobic digester that guarantees the presence of the microbial population needed for AD. The duration of the digestion test depends on the biodegradability rate of the substrate. The methane potential is expressed in terms of standard temperature and pressure (STP) in units of mL of CH_4 per gram of VS added (mL CH_4/gVS); it can also be expressed per gram of COD (Hansen et al. 2004).

Throughout the literature available, the technical approaches and experimental setups vary significantly. Many authors suggest the need of standardization of a protocol or test procedure for the BMP, for this purpose Angelidaki et al. (2009) have proposed a protocol that is commonly applied in the field of research. Inoculum and sample preparation, ISR and nutrient requirements are some of the factors that are specific to the substrate in question. In terms of quantifying gas volumes, they can be measured as an increase of volume under constant pressure (volumetric methods), as the increase in pressure in constant volume (manometric methods), or the measurement of methane formation by specialized equipment such as gas chromatography or automatic online methane devices (Angelidaki et al. 2006).

2.4 Methane production from pulping effluents

As an economical and advantageous wastewater treatment option, the application of AD in the P&P industry has been increasing in recent year mainly due to the added value of sludge reduction and methane production. Since the 1990's and after the invention of the UASB, the application of AD systems in P&P wastewater and sludge has rapidly grown and been found effective in treating the waste and producing methane (Kamali et al. 2016). Worldwide, there are now nearly 400 full-scale applications of anaerobic treatment in the P&P industry (**Figure 4**). Most of these applications are treating selected in-mill streams, such as paper mill effluents, mills using recycled fibers and evaporator condensates from chemical pulping (NSSC) while in the meantime most other effluents are excluded (Meyer and Edwards 2014).

Due to the special characteristics in P&P effluents such as recalcitrant contaminants and halogenated compounds, there is need of improvement in the matter of process stability, inhibition problems and enhancement of efficiency. Rintala and Puhakka (1994) studied the AD of P&P effluents concluding that effluents from mechanical pulping and secondary fiber pulping as well as NSSC condensates were found to be non-toxic and easily degradable. On the other hand, chemical bleaching, debarking and CTMP effluents are likely to be inhibitory and have low degradability (see **Table 3**). Ekstrand et al. (2013) tested 62 wastewater effluents from different mills from which bleaching effluents were the most toxic. Composite NSSC effluents gave the highest methane yields while effluents from a TMP mill had the best average yield (between 40-65% of the theoretical potential).

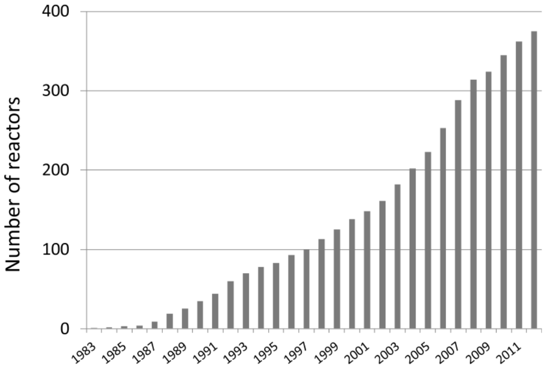

Figure 4. *Global cumulative anaerobic reactors installed in P&P mills by September 2012 (Meyer and Edwards 2014)*

Meyer and Edwards (2014) found similar results in their review on the methane potential of different pulping wastewaters finding that condensates streams from NSSC pulping have the highest COD removal rates and methane production, owed to the high acetic acid concentrations that contribute to the direct formation of methane. Lowest degradabilities correspond to debarking effluents from mechanical pulping; mostly due to high concentration of lignin and resins.

Table 3 summarizes the methane production and AD performance for several types of effluents originating from the P&P industry. A common observation found in studies is the fact that most P&P effluents are to some extent anaerobically biodegradable, this is considering the suitable anaerobic reactor configuration, operating conditions, pre-treatments and other techniques like co-digestion to improve the AD performance and enhance methane production.

2.4.1 Co-digestion

Co-digestion between different organic wastes has been a common practice to enhance methane production, process stability and overall biodegradability of the substrates. Regarding P&P wastes, co-digestion of sludge produced from the wastewater treatment in mills has been previously studied. Hagelqvist (2013) reviewed co-digestion of P&P biosludge with different organic waste such as municipal biosludge; manure and grass silage (55% methane increase) and pig and dairy manure (short start-up time). All co-digestion processes presented promising results with higher methane yields, stable wasted sludge and overcoming nutrient deficiency (common issue found in P&P sludge).

Table 3. *Summary of the methane potential of various P&P effluents. (Adapted from Meyer and Edwards 2014; Kamali et al. 2016 and references therein)*

Effluent	**Methane production (mL/gCOD)**	**AD system**	**COD removal (%)**	**Other parameters/info**
TMP composite	300-400	n.a.	50-70	Carbohydrates (1230-2700 mg/L)
TMP whitewater	380-410	AnMBR	75-90	Mesophilic, alkaline pH shocks
TMP + peroxide bleaching	330-400	Full scale UASB	50-55	Limited sludge growth
CTMP	180-310	Pilot scale	45-66	Sulfate (500-1500 mg/L)
Kraft evaporator condensates	350	AnMBR	97-99	Methanol (375-12,000 mg/L)
Kraft evaporator condensates	330	Full scale internal circulation	80-85	Lack of micronutrients
Kraft woodroom effluents	350-400	n.a.	92-100	COD (2.1-4 g/L)
Kraft alkaline bleaching effluent	0-140	n.a.	15-90	Chloride (1200-1400 mg/L)
Kraft hydrolysis liquor[a]	333	Batch	n.a.	Carbohydrates (55 g/L)
Sulfite evaporator condensate	290-310	Full scale CSTR	65-70	Poor sludge settling
NSSC evaporator condensates[b]	333	Batch	83	Acetic acid (510 mg/L)
NSSC composite	192	Batch	64	Lignin negatively affected the BMP
NSSC spent sulfite liquor	310	Pilot plant	45-50	Dilution required due to toxicity
Paper mill effluents	274-291	AF	77-80	6 h HRT
Recycled paper mill effluents	300-350	Full scale UASB	65-75	COD (0.14-3.3 g/L)
Chlorine bleaching	0-380	n.a.	20-67	Chloride (1300-1600 mg/L)

n.a. information not available

[a] **Paper I**

[b] **Paper III**

Co-digestion of P&P sludge and food waste obtained high bioconversion efficiency resulting in higher methane yield, COD removals and buffering capacity (Lin et al. 2012). Co-digestion of in-mill sludges (primary and secondary sludge) was tested by Bayr and Rintala (2012). In their study, thermophilic digestion of primary sludge obtained a maximum methane yield of 240 m^3/tVS_{fed} at an organic load rate (OLR) of 1 $kgVS/m^3d$, while co-digestion of primary and secondary sludge at the same conditions yielded a maximum of 170 m^3/tVS_{fed}. Although lower methane yields are observed in co-digestion of the sludges, it is still considered a feasible process as the mono-digestion of secondary sludge yields an average of 75 m^3/tVS_{fed} and nevertheless the secondary sludge needs to be treated one way or another.

On the other hand, the co-digestion of different in-mill effluents is somewhat less studied. Mata-Alvarez et al. (2013) observed that the diversification of substrates improved the resilience of the microbial communities. Hence, the diverseness in P&P effluents can improve long-term stability after acclimation is achieved. Co-digestion can also dilute the toxicity of known inhibiting effluents such as bleaching or lignin-rich effluents. Driessen and Wasenius (1994) addressed the concept of combining heavily polluted effluents with less concentrated effluents in an UASB. Their work describes the effective co-digestion of peroxide bleached TMP and CTMP effluents with addition of kraft condensate in an integrated P&P mill in Finland; with COD and BOD removal rates of 62 and 78%, respectively; and specific gas production of 0.4 m^3 biogas per $kgCOD_{rem}$. Lin et al. (2014) studied the co-digestion of 4 in-mill streams in a kraft paper mill, namely chlorine dioxide bleaching, alkaline extraction reinforced with oxygen and peroxide bleaching, kraft evaporator condensates and screw press liquor from wasted sludge dewatering. The packed bed anaerobic digester in their study removed 50-65% of COD and produced 0.22-0.34 m^3 CH_4 per kgCOD. The use of a subsequent activated sludge process only further polished the effluent to an overall COD removal of 70%. The poor performance of the aerobic process was affected by the presence of recalcitrant material; therefore, a pretreatment was suggested. Yang et al. (2010) tested the co-digestion of sulfite condensates and bleached CTMP effluents and found high degradability (>80%). Subsequently, they added different amounts of pulp-washing effluents to the cod-digestion experiments and observed that this addition did not influence the biogas production, meaning it did not inhibit or enhanced the production but rather just added the amount of biogas produced from the individual mono-digestion of the pulp-washing effluent. However, they did notice that the pulp-washing effluents degraded slower and could cause an accumulation of non-degradable compounds in a real-scale reactor. The application of co-digestion for the enhancement of methane production of in-mill effluents from a NSSC mill is addressed in the third approach of this thesis (**Paper III**).

2.4.2 Hydrotalcites

Hydrotalcites; also known as layered double hydroxides (LDHs) or anionic clays; are materials with a wide range of applications. They are characterized for their high capacities for adsorption, ion exchange and catalysis (Tao et al. 2006). Their simplicity, versatility, low cost and tailored-made properties have made these inorganic materials practical as neutralizers, polymers stabilizers, anion scavengers and many other potential applications (Othman et al. 2009). Choy et al. (2007) made a comprehensive review of the diverse biological and pharmaceutical applications of LDHs. HTs have a brucite-type structure, where layers or metal cations are stacked on top of each other and help together by hydrogen bonding. The sizable interlayer spaces and large quantity of exchangeable anions have made HTs great adsorbents of pollutants from water. Many studies have described the efficient removal of different pollutants from solutions; such as fluoride (Wang et al. 2007), arsenate and vanadate (Palmer and Frost 2010), chromium (Martinez-Gallegos et al. 2005); bromide (Wajima 2014) and other heavy metals. They have also been known to remove contaminants present in pesticides (Sampieri et al. 2011), mining and acidic wastewaters (Douglas et al. 2010), high-nitrate effluents (Cheng et al. 2014) and carbon nanotube production effluents (Alves et al. 2011).

Besides their efficiency in removing pollutants from water, HTs have also been used to improve the production of hydrogen in biological processes. Wimonsong et al. (2013) tested the effect of different Mg-Al HTs on the hydrogen production in dark fermentation. They found that Fe-Zn-Mg-Al HT could enhance the hydrogen production by 44%. High doses of the HT however, showed poor performance due to sharp increases in the pH. In a subsequent study Wimomsong et al. (2014) tested the same Fe-Zn-Mg-Al HT with an Au support and reported a further 19% increase in hydrogen yield, the behavior was assumed to be the combination effects of the metallic dispersion of Au on the support surface containing Zn, which can act as the active sites of hydrogenase enzyme. Le and Nitisoravut (2015) reported similar results where the addition of Ni-Mg-Al HT showed 80% improvement in hydrogen yield. The enhancement in gas production is attributed to the basicity of HTs and the facilitation of Mg and Ni. Although the effect of HT in methane production has not been explored yet, the notable characteristics of HTs make them an interesting material to possible enhance methane production. The methane promoting effect of Fe-Zn-Mg-Al HT in P&P-like effluents is addressed in **Papers II and III** of this thesis.

3 Thesis objectives

The central objective of this thesis was to develop assessments to produce high methane yield biogas from the anaerobic digestion of chemical pulping waste streams to potentially recover energy. So, how can we enhance the methane production of chemical P&P streams? Three different approaches were followed to address this question: the effect of a change in process parameter, the addition of a promoter and the symbiosis of co-digestion. Finally, an alternative approach was assessed were different types of fibers were directly tested for methane production with the aim to potentially diversify the end-product portfolio of chemical pulp mills. **Figure 5** illustrates the relationship between the papers presented in this thesis. The first approach was directed towards testing the effect caused by the change of a key parameter in the AD process. Using microcrystalline cellulose (MCC) production wastewater, the influence of different inoculum to substrate ratios were analyzed and the most suitable ISR for higher methane production was determined. The second approach studied the addition of a promoter to enhance the AD process. Hydrotalcites were synthesized and tested to determine their effect on the methane production of synthetic sulfate-containing wastewater which resembled pulping wastewaters. A third approach evaluated the effect of co-digestion of two pulping effluents from a neutral sulfite semi-chemical (NSSC) pulp mill. Additionally, the influence of HT is assessed. The fourth and final approach attempts to elucidate on a new potential alternative option for chemical pulp mills. The methane potential of traditional fibers is explored to estimate their energy value and consider methane production as an alternative end-product. The first approach is addressed in **paper I**, second approach in **paper II**, third approach in **paper III** and fourth approach is addressed in **paper IV**.

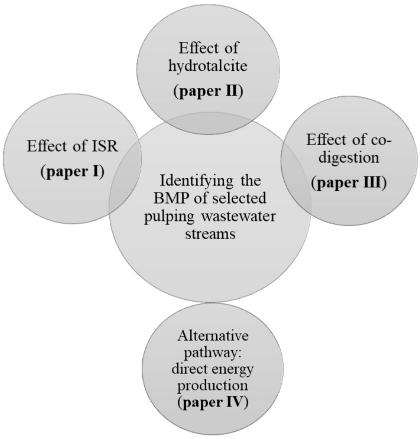

Figure 5. *Relationship of the research work presented in this thesis.*

4 Materials and methods

This chapter briefly describes the materials and the most essential methods used throughout this study. A more detailed description can be found in the **papers I – IV.**

4.1 Characterization of inoculum and substrates

The inoculum used to seed the reactors of all the experiments done for this thesis was collected periodically from Suomenoja municipal WWTP located in Espoo, Finland. Approximately 10 liters of fresh inoculum was collected directly from their mesophilic anaerobic digester some days prior to the start of each experiment. After collection, the bottle was purged with nitrogen gas and stored in an incubator at 37 ºC ± 2 until further use. As new and fresh inoculum was collected at the start of each experiment, its characteristics resulted in some variability considering the heterogeneity of the wastewater in the treatment plant. Using average values of triplicates, the main characteristics of the inoculum sludge resulted in: pH (7.1-7.6); total solids (TS) % (1.7-2.8); VS% (0.9-1.5); COD (19,057 mg/L); alkalinity (6.3-6.8 g$CaCO_3$/L) and conductivity (6.3-6.6 mS/cm).

The main characteristics of the different substrates used for methane production in this thesis are summarized in **Tables 4** and **5**. The choice of substrates included in the study was partly based on the theoretical potential of their methane production and their industrial availability.

AaltoCell^{TM} hydrolyzes

The AaltoCell^{TM} process is a new simplified MCC production process (Vanhatalo and Dahl 2014) that uses mild acid hydrolysis and produces hydrolyzed filtrates. These hydrolyzes are rich in sugars and have no inhibiting compounds making them perfect substrates for methane production. Sampling was done directly after the hydrolysis process (**paper I**).

Synthetic sulfate-containing wastewater

Sulfuric compounds are common in most P&P process wastewater, therefore a synthetic sulfate-containing wastewater was prepared in the laboratory and used as substrate in BMP experiments. The synthetic wastewater consisted primarily of 5000 mg/L of COD and 200 mg/L of sulfate and the addition of nutrients (reagent grade) to achieve a ratio of COD:N:P of 100:5:1; a detailed description can be found in **paper II**.

Table 4. *Summary of the characteristics of the wastewater (liquid) substrates used in all experiments.*

Substrate	Origin/Type	pH	VS (%)	COD (mg/L)	Other	Paper
AaltoCell™ hydrolyzes	Kraft acid hydrolysis	1.75	7.82	n.a.	Carbohydrates (55.82 g/L)	I
Sulfur-rich wastewater	Synthetic wastewater	7.66	0.35	5000	Conductivity (6.98 mS/cm)	II
Evaporator Condensates	NSSC	2.98	0.04	2303	VFA (510 mg/L); TOC (961 mg/L)	III
Fiber circulation	NSSC	5.90	0.52	11,572	TOC (4529 mg/L); Lignin (195.5 mg/L)	III
Brown filtrate	Kraft MCC	1.9	1.23	14,765	Carbohydrates (7379 mg/L); VFA (38 g/L)	IV
O2 filtrate	Kraft MCC	1.8	1.46	18,514	Carbohydrates (7562 mg/L); VFA (40 g/L)	IV
Bleached filtrate	Kraft MCC	1.4	0.81	9187	Carbohydrates (6691 mg/L); VFA (2.7 g/L)	IV

TOC: Total organic carbon

NSSC effluents

Two different NSSC effluents were collected from a Stora Enso Fluting mill located in Heinola, Finland. Sampling was carried out in the mill, where individual daily samples were combined to form a composite sample that was stored in plastic containers and delivered to the laboratory. Evaporator condensates; from now on denoted as EC; is process water generated in the sulfite evaporation plant. Its key characteristics is a high concentration of VFA that are readily degradable. Fiber circulation effluents; from now on denoted as FC; is recycled effluent from the debarking and chipping processes. Its main component is a high COD concentration which is mostly composed of low-degradable lignin compounds. Further characterization of the effluents is described in **paper III**.

Kraft pulp substrates

As opposed to **papers I – III**, the approach in **paper IV** includes the methane potential of the raw pulping material as well as the wastewaters generated after its further processing. Three different pulp fibers were used as raw material and tested for methane production; namely brown fiber (BRFiber), oxygen delignified fiber (O2Fiber) and bleached fiber

(BLFiber). After processing of each fiber, three substrates were further obtained namely solid MCC fraction, liquid fraction (filtrate) and a mix of both fractions; all of which were also tested for methane production. **Table 5** summarizes the characteristics of the solid substrates used. A description of the pulping and MCC manufacturing processes used to obtain all four tested substrates as well as their molecular weight distribution (MWD) and crystallinity index (CI) are further discussed in **paper IV**.

4.2 Experimental set-up and process parameters

All the experimental tests were carried out using an automatic methane potential test system (AMPTS II). This is a laboratory-scale batch testing system used to measure and analyze the methane volume produced from the AD of substrates. Measurements are based on the principle of liquid displacement and buoyancy. The instrument setup can be divided into three units (**Figure 6**).

Table 5. *Main characteristics of the pulp (solid) substrates used in experiments (**See paper IV**).*

Substrate	Origin/Type	VS (%)	Carbohydrate (% DW)	Total lignin (% DW)
Brown fiber	Kraft softwood	29.7	95.4	4.2
Brown MCC	Kraft softwood (AaltoCell^{TM} process)	31.2	95	4.6
O2 fiber	Kraft softwood	33.4	97.6	2.3
O2 MCC	Kraft softwood (AaltoCell^{TM} process)	31.4	96.8	3.2
Bleached fiber	Kraft softwood	44.7	99.2	0.7
Bleached MCC	Kraft softwood (AaltoCell^{TM} process)	44.4	99.4	0.5

DW: dry weight

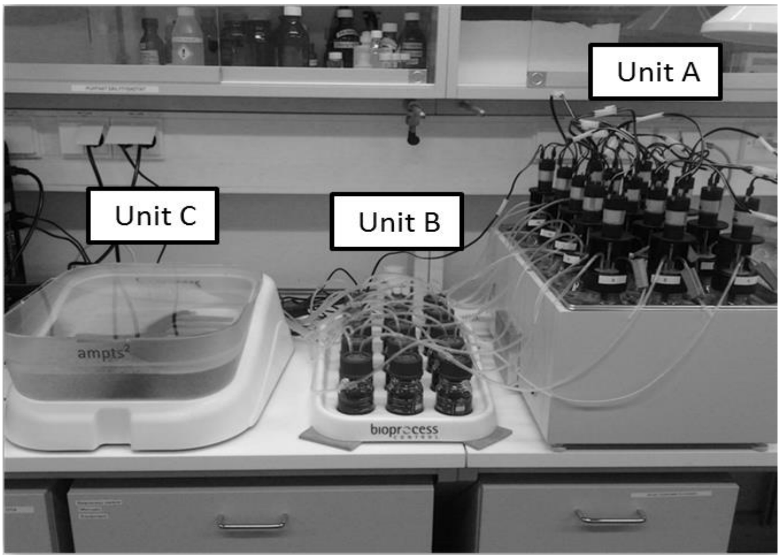

Figure 6. Set-up for batch BMP experiments in the laboratory.

Unit A is a thermostatic water bath with a capacity to incubate 15 individual reactors in a varied range of temperatures. Each reactor consists of a glass bottle with a 500 mL working volume where an amount of substrate and inoculum are incubated at a desired temperature, sealed with a hermetic rubber stopper and mixed by a rotating bent rod. Biogas is produced in unit A and passes to unit B by tygon tubbing. Unit B is a CO_2 fixing step, the biogas produced in each reactor goes through an individual vial containing 80 mL of 3 molar alkali solution (NaOH). Gases such as CO_2 and H_2S are chemically removed and CH_4 passes through unchanged to the next unit. Unit C is the gas volume measuring device, after CH_4 passes through the alkali solution it is measured and analyzed using a wet gas-flow measuring device that works by the principle of liquid displacement. After a defined calibrated volume of gas is accumulated under each flow cell, it lifts open and releases the trapped methane gas through the water and clicks back shut. The click sends a digital pulse that is recorded in the computer program. Each flow cell is connected to a data acquisition unit that together with a computer records the methane production.

All BMP tests were conducted using the AMPTS II operated at a mesophilic temperature of 37 ºC ± 0.5 with 400 mL of working volume and 250 mL headspace in all reactors for biogas accumulation. Each sample was tested in triplicate for statistical significance. Triplicate blank samples with no added substrate were run in each experiment to determine the background gas production originating for the inoculum alone. Different ISR were tested in **paper I** where a ratio of 2:1 (VS based) was identified as the optimal, consequently that was ratio maintained for the rest of experiments conducted in **papers II** and **IV**. In **paper III**, the ISR was set to 1:1 as it was COD based. According to the characteristics of each substrate tested different additional measures were taken to improve the process stability. In **paper I**, the substrate was highly acidic (pH 1.75) therefore before the beginning of the experiments the pH of the substrate was adjusted to 7 with a 20% NaOH solution. In **papers III** and **IV**, the substrates were either acidic or alkaline, therefore reactors were adjusted to a neutral pH using HCl or NaOH/CaO when needed. In **paper II** the substrate was synthetic wastewater therefore different nutrients were added to ensure their presence. The addition of 4 g/L sodium bicarbonate ($NaHCO_3$) was implemented to increase the buffering capacity in reactors where substrates with initial low pH were used (**papers I** and **III**).

4.3 Hydrotalcite promoters

The HT used in **papers II** and **III** were synthesized using the co-precipitation method described in Salomão et al. (2011). HTs followed the general formula presented in Palmer and Frost (2010) of $[M_{1-x}{}^{2+}\ M_x{}^{3+}\ (OH)_2]\ [A_{x/m}]^{m-} \cdot nH_2O$, where M^{2+} is a divalent cation, M^{3+} is a trivalent cation, A an interlamellar anion with charge m^- and x is ratio of $M^{+3}/(M^{+2} + M^{+3})$ (**Figure 7**). Three HTs of M^{2+} – Mg – Al were prepared in which M^{2+} was Fe^{2+} ; Zn^{2+} and $Fe^{2+} + Zn^{2+}$. The molar fraction of divalent and trivalent cations was 3:1. Briefly described; the preparation method consisted of two solutions. The first aqueous solution was a 300 mL mixture of metal nitrates: $Mg(NO_3)_2 \cdot 6H_2O$ and $Al(NO_3)_3 \cdot 9H_2O$ and/or $FeSO_4 \cdot 7H_2O$ and $Zn(NO_3)_2 \cdot 6H_2O$ in the desired molar ratios. The second solution was prepared by dissolving NaOH (2.25 M) and Na_2CO_3 (0.45 M) in 300 mL of distilled water. The second solution was added dropwise to the first solution under vigorous stirring until the pH reached around 9-10. The mixed solutions formed a thick slurry that was aged in a thermostatic bath for 18 hours at 60 ºC. Afterward, the slurry was filtered and washed with deionized water in order to remove the alkaline metals and nitrate ions until the filtrate effluent reached a neutral pH. Finally, the filter cake was dried at 110 ºC for 24 hours and ground in a mortar until powder-like form. To obtain calcined HTs (mixed Mg-Al oxides), the powdered HTs were placed in a muffle furnace at 500 ºC for 4 hours.

The cations chosen as M^{+2} (Fe^{+2} and Zn^{+2}) were picked for their potential in promoting methane production in the AD process. Fe is essential from microbial growth in methanogens and is an important component of the enzymes involved in metabolic reactions (Jackson-Moss and Duncan 1990). Although it is an abundant element, its bioavailability is limited, therefore incorporating it to the HT structure could enhance its accessibility to microorganisms in AD. Zn^{+2} was chosen for its potential removal properties of sulfur compounds (Sekhavatjou et al. 2014; Wu et al. 2011). Full characterization of HTs is detailed in **paper II**.

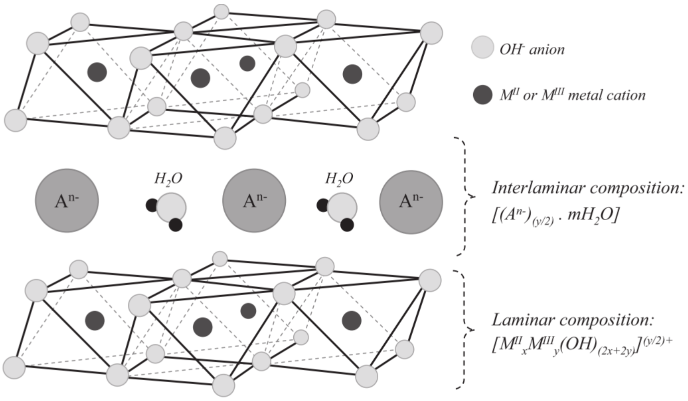

Figure 7. *Graphic representation of the hydrotalcite structure (Salomão et al. 2011).*

4.4 Analytical methods

TS and VS content in substrates and inoculum were determined gravimetrically following Standard Methods described in APHA (1998). COD was measured by closed reflux titration, Standard Method 5220. Total alkalinity to pH 4.4, Standard Method 2320 B. pH measurements were taken with a Thermo-Scientific model Orion 2-star pH-Benchtop pH meter and conductivity with a conductivity meter Orion Model 150. Elemental analysis was determined using a Perkin Elmer Model 2400 Series II CHNS Elemental Analyzer.

Methane production was measured using AMPTS II from Bioprocess Control AB (Sweden) using system version 2.0 V1.08; with corrected measurements to STP conditions at 273 K and 1013 mbar air pressure. VFA and furfural analysis were done after sample centrifugation and filtration, using high performance liquid chromatography (HPLC) with Dionex UltiMate 3000 HPLC equipped with refractive index and UV diode array detectors and HyperREZ XP Carbohydrate Ca+ column.

TOC and inorganic carbon (IC) were measured using a TOC-VCPH analyzer (Shimadzu) according to SFS-ISO 8245, Total P and Total N measurements were conducted in a FIAstar 5000 analyzer according to ISO 15681-1, 11905 and 13395. SO_3 and SO_4 were measured by ion chromatograph Dionex ICS-1500. Carbohydrate and total lignin (acid insoluble and soluble) were analyzed after two-step acid hydrolysis according to Standard NREL/TP-510-42623 using HPAEC-PAD Dionex ICS-3000 and UV- Vis spectroscopy UV-2550 Shimadzu.

The Quantitative real-time PCR technique with 16S rRNA-targeted oligonucleotide probes was employed to sample digested sludge pellets after centrifugation for determination of microbial community (total methanogens).

MWD of fibers was analyzed using a gel permeation chromatography (GPC) technique in a Dionex Ultimate 3000 system with a guard column, four analytical columns and refractive index detection. CI was calculated based on X-ray diffraction (XRD) done with X'pert Pro MPD Alpha-1difractometer recorded at 45 kV and 40 mA, Cu radiation (λ = 0.154056 nm).

To confirm a well crystallized HT structure there were characterized by XRD, using a Bruker D8 equipped with a Cu target and a graphite monochromator (recorded at 40 kV and 40 mA, Cu radiation (λ = 0.15406 nm) and Fourier transform infrared spectroscopy (FTIR) using a Nicolet 6700 FTIR spectrometer over the wavenumber range 4000 - 400 cm^{-1}. Crystal sizes were estimated from full width at half maxima of the XRD peals using Scherrer's equation. HT morphologies were examined in a Neon40 scanning electron microscope (SEM) Crossbeam Station Zeiss equipped with a field emission electron source. Size distribution was determined by measurements of 100 particle histograms. Further details regarding the analyses procedure and the instrumentation used throughout can be found in **papers I-IV.**

4.5 Data analysis and calculations

The methane potential (BMP) of substrates was calculated as the accumulated methane (mL) produced per gram of VS or COD added to each reactor, as determined in **Equation 1** (Strömberg et al. 2014).

$$BMP = \frac{V_{sample} - V_{inoc}\frac{gVS\ is}{gVS\ ib}}{gVS_{substrate}} \qquad \mathbf{(1)}$$

where BMP is the accumulated normalized volume produced per gram of VS of substrate added ($mLCH_4/gVS$), V_{sample} is the mean value of accumulated methane produced from the reactor with both inoculum and substrate, V_{inoc} is the mean value of the accumulated volume produced by the blanks with only inoculum, gVS_{is} is the mass of VS of the inoculum added in the sample, gVS_{ib} is the mass of VS of the inoculum added in the blanks, and $gVS_{substrate}$ is the mass of VS of the substrate added in the reactor. Calculation can also be done based on COD where VS in replaced by COD in **Equation 1**.

The theoretical methane potential (BMP_{th}) of a substrate can be calculated by its atomic composition using the stoichiometric conversion equation of Buswell and Mueller (1952). Triolo et al. (2011) developed a modified version where Buswell's equation was implemented on model compounds of organic fractions (carbohydrates, proteins, lipids and lignin). Therefore, **Equation 2** can be applied when the composition of the organic fraction of the substrate is known. Alternatively, the BMP_{th} can be calculated knowing the ratio between grams of COD and VS contained in the samples and the assumption that the theoretical methane yield of 1 gram of COD is 350 mL CH_4 at STP (Buffiere et al. 2006).

$$BMP_{th} = 415 \cdot \%\, carbohydrates + 496 \cdot \%\, proteins + 1014 \cdot \%\, lipids + 727 \cdot \%\, lignin \qquad (2)$$

The anaerobic degradability (BD) was determined as the ratio between the experimental methane yield (BMP) and the theoretical methane yield (BMP_{th}) of a given substrate. Further details on the above-mentioned calculations and additional equations used are found in **papers I-IV.**

4.6 Reliability and validity

Each sample in every experimental set was conducted in triplicate and results expressed as mean ± standard deviation when indicated in order to minimize experimental error. One-way analysis of variance (ANOVA) was performed on all data with significance level set at $p < 0.05$ to determine the statistical difference between replicas.

The anaerobic inoculum used to seed the reactors in all the experiments was collected periodically from a WWTP treating municipal wastewater. Hence, there is an inherent variability in its characteristics. Blanks determining the methane production of the inoculum were run in parallel in each experimental trial to minimize the error associated to the microbial activity in the inoculum at the time of collection. The use of acclimated sludge as inoculum could have increased the methane potential of the substrates, however its availability was limited.

Measurements are susceptible to human and systematic error. In order to minimize these, all experiments were prepared with proper and periodic calibration of equipment.

5 Results and discussion

5.1 Influence of different inoculum to substrate ratios (ISR) on the methane production

The selection of inoculum and the ISR are integral settings in a BMP test that determine the proper anaerobic degradability of a substrate in batch test mode. As discussed in **paper I**, the effect of different ISR (0.5, 0.8, 1 and 2) on the methane potential of pulping process waters was evaluated in batch tests. Results revealed that ISR of 0.8, 1 and 2 presented similar ultimate methane yields of 325.0, 297.4 and 332.9 mLCH_4/gVS; respectively. The methane production profiles (**Figure 8**) illustrates how each ISR presents a different type of curve. ISR 0.5 clearly shows an inhibiting effect. Initial methane is produced (from the hydrolysis of easily degradable fractions) until it stops abruptly on day 5 reaching merely 59.4 mLCH_4/gVS, which is noticeable lower than the other tested samples (81% decrease in methane yield). The inhibition of methanogenesis is caused by an accumulation of acids. The lower volume of inoculum present means a decreased number of working methanogenic archaea for the high load of organic material. The MCC hydrolyses have high concentrations of sugars (see **Table 4**) which are easily and rapidly degradable. The microbial population is then insufficient, thus the production of VFA from the easily degradable fraction is higher than the consumption of these VFAs by the acetogenic bacteria, and in consequence the accumulation of VFAs lowers the pH of the environment that greatly reduced the activity of methanogens. This is corroborated by the lower pH and alkalinity (5.6 and 3.1 respectively) compared to the other ISR samples where pH ranged from (7.3-7.8) and alkalinity (7900-8900 mg $CaCO_3$/L) (**paper 1**). Other studies have found similar results when using ISR of 0.5 and lower where high substrate concentration causes acid accumulation and low pH resulting in an inhibition phenomenon (Eskicioglu and Ghorbani 2011; Raposo et al. 2009)

The pH of the reactor is greatly influenced by the alkalinity which controls the capacity of a system to buffer and absorb rapid or significant changes in pH and neutralize acids (Raposo et al. 2011b). The bicarbonate buffering capacity is formed by the reaction of CO_2 from the biogas with the natural mineral alkalinity of the substrate. Buildup of acids neutralize the mineral alkalinity causing the pH to drop from 7.5 to 6.5 which in return releases CO_2 back into the environment (McCarty and Mosey 1991). However, when there is an excessive acid formation that exceeds the mineral alkalinity, the pH will drastically drop inhibiting the AD process. To avoid this, direct bicarbonate addition is recommended.

For this reason, each of the reactor of all ISR tested were supplied with 4000 mg/L of $NaHCO_3$ to increase their buffering capacity. Raposo et al. (2011b) suggest that an alkalinity of 2500-5000 mg $CaCO_3$/L would accommodate large increases in VFA and avoid a drop in pH. However in the case of ISR 0.5, the additional buffering capacity initially available was still insufficient to level out the pH, which caused the abrupt stop of methane production.

As illustrated in **Figure 8b,** ISR 0.8 and 1 both observed a sigmoid behavior where an initial increase in methane is followed by a lag phase lasting from days 4 to 8. By day 10 both ISR curves resumed methane production plateauing at day 18. The behavior of ISR 0.8 and 1 is explained by the buffering capacities in the reactors. As in ISR 0.5, by day 4 the methane production of ISR 0.8 and 1 suffers a stagnation period due to an accumulation of acids. However, the difference in these cases is that the buffering capacity initially added and formed in the reactors was able to neutralize the pH imbalance and reestablish a stable environment, thus restarting a methane production until reaching its maximum by day 18. Alkalinity measurements were 8900 and 8700 mg $CaCO_3$/L for ISR 0.8 and 1 respectively, which express a healthy buffering capacity. The lag phase observed is commonly found in substrates that create a stress situation such as high organic loadings (Meyer and Edwards 2014).

ISR 0.8 (which had a lower amount of inoculum) has a 9% increment over ISR 1. This observation suggests that as long as this minimal amount of inoculum is present, then the potential for degradation of the substrate will take place; however, the degradation kinetics will vary. This is observed in the latency between both ISR 1 and 0.8 (**Figure 8b**), where ISR 1 accumulates methane at a faster rate until it plateaus. It may be observed that to find a more narrow or specific threshold of minimal inoculum before inhibition occurs, further studies are needed to test the methane productivity between ISR 0.5 and 0.8.

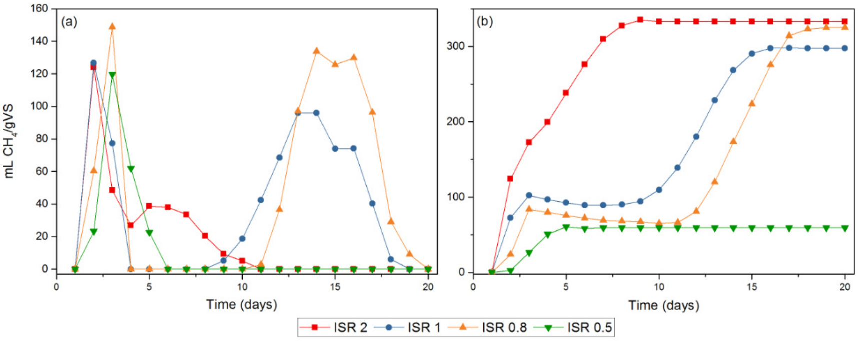

Figure 8. *Methane production at different ISR expressed as a function of time (**a**) daily methane production (**b**) accumulated methane production (modified from **paper I**)*

ISR 2 demonstrated a rapid initial methane production rate (MPR) that started immediately lasting until day 8 after which it plateaus reaching its ultimate yield. This follows the first-order kinetic model, where gas was produced in an exponential rise until a maximum (**Figure 9a**). This behavior represents a healthy and stable AD process which is also expressed in the biodegradability (BD) of the substrate. BD for ISR 0.8, 1 and 2 were 89.3, 81.7 and 91.5% whereas for ISR 0.5 it was 16.3%. Raposo et al. (2011b) described the BMP of naturally degradable substrates (starch, cellulose, gelatin and mung bean) and reported BD between 85-88% using an ISR of 2. For mung bean an ISR of 1 was also tested that resulted in an inhibition phenomenon which decreased the MPR by 26% however the final accumulated methane yield was not affected. This is similar to the results found in this study for ISR 0.8 and 1. Boulanger et al. (2012) also demonstrate that increasing the ISR increases methanogenic activity and decreases latency, nevertheless using an ISR over 4 showed no improvement over using an ISR of 2. This means that at ISR 2 the latency value is already minimal and the MPR and rate of substrate consumption are maximal.

Comparable curve representations were found in other experiments done for this thesis; when using an ISR of 2 in substrates with easily degradable organic matter. **Figure 9b** shows the methane production from synthetic wastewater (further detailed in **paper II**) where the same initial exponential increase is observed until reaching a maximum after around 45 hours of digestion. The substrate was rich in degradable COD and ISR of 2 was used. Additionally in **paper IV**, both brown and bleached chemical pulp and their fractions produced similar curves where most of the methane was produced during the first 5 days of digestion. This shows that applying an ISR of 2 (which avoids accumulation of intermediates) with substrates that have degradable fractions and low inhibiting compounds, follow the first-order kinetic model (**Figure 9a** further detailed in **paper I**) where the hydrolysis stage is the rate limit step in the AD process.

In conclusion, the ISR plays an important role in the BMP. Maximum methane potential, methane production rate and kinetic evaluation suggest that an ISR of 2 provided the most efficient and stable process where the maximum methane production was reached in the fastest time with no latency. Lower ISR of 0.8 and 1 had a methane production spanned over a longer period of time however achieved similar accumulated methane yields as ISR 2 over time. Lower ISR such as 0.5 are not recommended as a clear inhibition was observed which lead to a complete interruption of methane production. It is important to mention that all the BMP tests described in this thesis were performed using fresh municipal digested sludge (as defined in chapter 4.1) due to its metabolically active microbial composition and ease of access. Therefore, results are applicable for BMP testing using municipal digested sludge as seed sludge. However, when using acclimated or enriched sludge that has previously adapted to a certain substrate a lower ISR can be used to perform BMP tests. The adapted sludge provides a specific suitable set of microbial composition for the substrate's degradation, allowing for less inoculum to be used as seed.

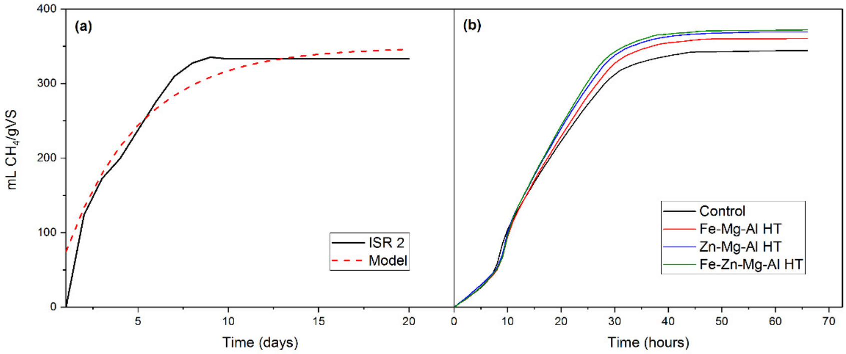

Figure 9. ***(a)*** *Experimental data obtained from applying ISR 2 fit to the first-order kinetic model (modified from **paper I**).* ***(b)*** *Accumulated methane production after addition of different HTs expressed as a function of time.*

5.2 Effect of hydrotalcite addition on the methane production

5.2.1 HT characterization

As discussed in **paper II**, three hydrotalcites were synthesized using the co-precipitation method as described in Section 4.3. HTs and their calcined form (mixed oxides) were characterized by XRD, FTIR and SEM. Based on the XRD patterns of HTs (**Figure 10a**) a well-crystallized HT structure is observed with the corresponding peaks and patterns described in the literature (Miranda et al. 2014; Cocheci et al. 2010). Sharp peaks at low diffraction angles corresponding to the crystal planes of (003), (006) and (012) are observed; these are characteristic of hydrotalcite-like materials. The peaks observed in HTs that contained Fe were somewhat broader, which expresses that these were smaller than the Zn HT. This is also observed from the estimated average crystal size calculated with Scherrer's equation.

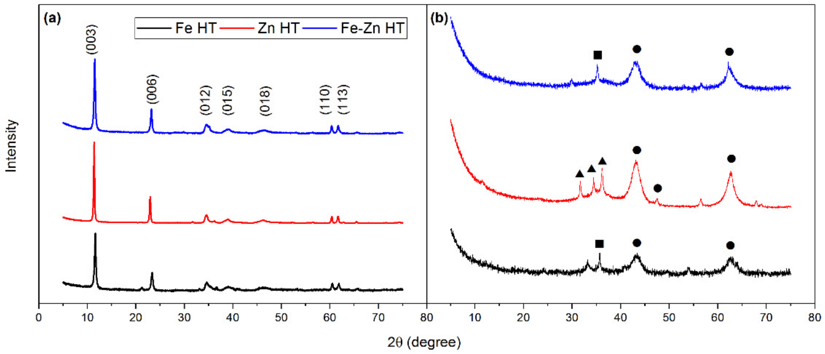

Figure 10. *X-ray diffractograms of* ***(a)*** *Layered HTs.* ***(b)*** *Calcined HTs where (●) is MgO periclase; (■) is Al_2O_3; and (▲) is ZnO (modified from **paper II**).*

In **Figure 10b**, the XRD patterns of calcined HTs indicate the loss of the (003) peak which reflects the collapse of the layered structure (Tao et al. 2006). The presence of diffraction peaks that correspond to MgO (periclase) and ZnO can also be observed. The calcination process stimulates the formation of Mg-Al oxides and the removal of hydroxyl groups and interlayer anions. This forms a more amorphous structure rather than the layered structure of non-calcined HTs. This is also observed in the SEM images of HTs (discussed in **paper II)**. The average aggregate size was between 50-60 nm for layered HTs and 40-50 nm for calcined HTs. Further details regarding the FTIR spectra and SEM images of the HTs are provided in **paper II**.

5.2.2 Influence of HT in methane production and AD efficiency

Additions of each HT at a concentration of 500 mg/L were tested in batch BMP tests. The substrate used was synthetic wastewater prepared with easily degradable material (sodium acetate and sucrose) and with the addition of sulfate. Sulfate was added to the mix in order to test the effect of the HTs in reducing sulfate-inhibiting properties and possibly enhance methane production; without the presence of any other pollutant. After 65 hours of digestion, all three HTs showed an increase in methane compared to the control with no HT addition (**Table 6**). HTs containing Zn had the highest increment of methane production compared to the control. Fe-Zn HT and Zn HT had corresponding increments of 8.1 and 7.4% respectively. Fe HT had a 4.8% increment. As expected, the better performance of Zn-containing HTs can be credited to zinc interaction with sulfide (S^{2-}). Zn^{2+} reacts with S^{2-} and forms zinc sulfide (ZnS) avoiding S^{2-} to form H_2S. Sulfate reducing bacteria (SRB) are commonly found in the AD process and are natural competitors of methanogens. Organic matter can be removed via methanogenesis or via sulfate reduction. SRB reduce sulfate to S^{2-}, which is toxic for many microbial communities (especially methanogens). The reduced dissolved S^{2-} is present in the unionized H_2S form that can reduce the COD removal rate, reduce the methane yield in biogas and negatively influence the reactor pH inducing process failure (Pol et al. 1998). This would be the case for the substrate in this study, which is sulfate-rich synthetic wastewater. The positive effect of Zn and Fe is also observed in the COD reduction (80-84%) compared to the control (65.5%).

Calcined HTs provided no significant influence when compared to the control. The addition of calcined HTs neither positively nor negatively affected the methane production (MPR or accumulated methane yield). Same behavior was found for COD removal rates and BD where calcined HTs obtained similar results to that of the control (**Table 6**). This makes the effect of calcined HTs negligible and the improvements found when layered double HT were added corresponding to their distinct structure. Fe^{+2} and Zn^{+2} cations incorporated and immobilized in the layered sheet structure would give way to the advantages of both (further described in section 4.3): zinc with its trapping abilities and iron with its enhancing capacities in the AD process. Results from Fe-Zn HT, which had the best performance, corroborated this. It is important to mention that both iron and zinc; although presenting possible promoting functionalities towards AD; have been also proven to be toxic to the AD process when present in high concentrations (Jackson-Moss and Duncan 1990; Luna-delRisco et al. 2011). However; when applied into the AD system chemically bonded in the HT layer structure; leaching is prevented and a rather controlled released of the metals is delivered.

Furthermore, the addition of different concentration of the most promising HT; which was Fe-Zn HT; were tested (ranging between 30-1000 mg/L). The optimal concentration found was the initial loading of 500 mg/L (**paper II**). Although the promoting effect in methane after the addition of HTs was merely an 8.1% increase, there is still an inherent improvement observed. This could be encouraging to further explore treatment options with HT. Different research pathways could be to test HTs under various process parameters (higher temperatures) to promote the AD process, or study the influence of HTs on the digested sludge properties and settleability. Furthermore, research on the application of HTs as pre-treatment or post-treatment methods in pulp and paper wastewater treatment would lead to cost-effective benefits, as HTs could be a more economical choice than the common polymer compounds used for coagulation and flocculation purposes.

Table 6. *AD efficiency parameters after the addition of HTs and their calcined form. Values expressed as mean ± standard deviation when indicated* ***(paper II)****.*

HT	Accumulated methane yield (mL CH_4/gVS)	Increment compared to control (%)	COD removal (%)	BD (%)
Control	343.9 ± 2.7	-	65.6	82.6
Fe HT	360.4 ± 4.2	4.8	80.1	86.5
Zn HT	369.3 ± 3.2	7.4	83.7	88.7
Fe-Zn HT	371.9 ± 1.5	8.1	84.0	89.3
Fe HT calcined	342.8 ± 6.1	-0.3	66.1	82.3
Zn HT calcined	345.8 ± 3.0	0.6	66.9	83.0
Fe-Zn HT calcined	347.7 ± 0.9	1.1	69.4	83.5

5.3 Enhancement of methane production by co-digestion of NSSC effluents

The neutral sulfite semi-chemical mill in Heinola, Finland has two mayor effluents. The evaporator condensates (EC), rich in degradable acetic acid; and fiber circulation effluent (FC), rich in lignin which poses and inherent restraint to anaerobic digestion treatment. EC are substrates that have been proven to have great potential for methane production (Larsson 2015), however both of the effluents need to be treated before discharging them to the nearest river. Thus, an efficient alternative to treat both of them together would be beneficial. **Paper III** discusses the co-digestion of these two effluents at different ratios, furthermore the addition of Fe-Zn HT was evaluated to determine if there was a promoting effect on AD process and methane production. Four different volume ratios (COD-based) were selected, these simulated the actual operating conditions and flow volumes in the Heinola mill. Ratios consisted of R1: 100/0; R2: 90/10: R3: 80/20 and R4:70/30 of EC/FC respectively. Additionally; R1, R2 and R3 were tested with the addition of HT, denoted as R1+HT, R2+HT and R3+HT. The HT was added to each reactor corresponding to 2% of its initial COD load concentration.

5.3.1. The effect of co-digestion on methane production

As illustrated in **Figure 11a** and discussed in **paper III,** all samples with different ratios showed viability to produce methane. Highest accumulated methane yields were found to be in the following order R1 > R2 > R3 > R4 with yields of 324 ± 8, 250 ± 15, 212 ± 8, 192 ± 4 mL CH_4/gCOD. Therefore, the higher the proportion of EC in the mix the higher the methane yield. This is attributed to the presence of acetic acid in EC (510 mg/L). R1 and R1+HT which had only EC, were the ones most prone to inhibition due to the possible accumulation of intermediates in the acetogenesis phase. However no serious inhibition was observed, but a minor lag phase during days 5 to 9 took place which gave the curves a semi-sigmoid behavior. This lag phase is validated by an accumulation of VFAs during day 5 observed in **Figure 11c**, R1 and R1+HT had the highest VFA concentrations (2005-2064 mg/L). This in turn made the pH of R1 and R1+HT slightly decrease on day 7 (**Figure 11d**) nonetheless the buffering capacity produced managed to rapidly stabilize and increase the pH from day 10 onwards. After this, a great spike of methane production occurred for both R1 samples which were the maximum daily production rates (day 13) observed from all the tested samples (38-52 mL CH_4/gCOD).

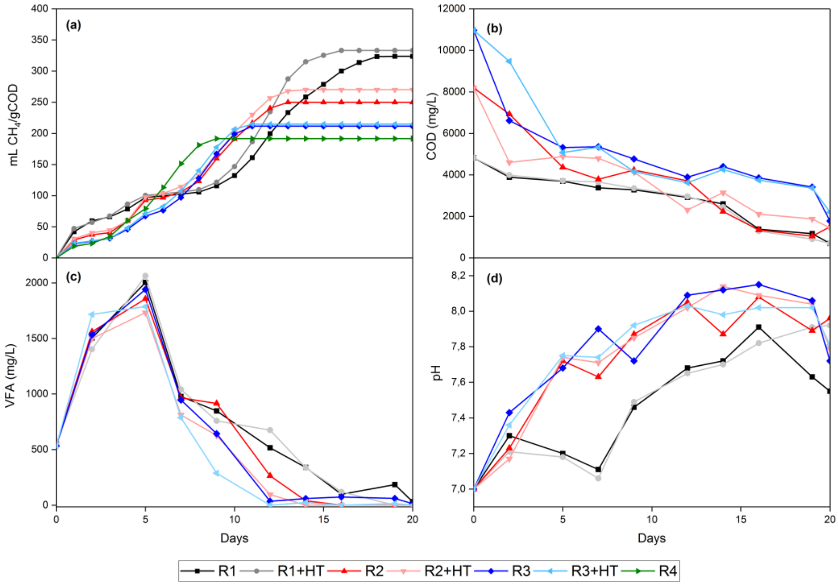

Figure 11. *Evolution of the AD process of all samples (with and without HT addition) expressed as a function of time (a) accumulated methane yield, (b) COD concentrations, (c) VFA concentrations, (d) pH variation (modified from* ***paper III****).*

Curves for R2, R3 and R4 presented in **Figure 11a** did not show a sigmoid-behavior but instead a decreased production of methane. Nonetheless; the lowest yield which was R4 (192 mL CH4/gCOD); is still between the average methane potential of NSSC effluents found in literature previously described (140-340 mL CH4/gCOD) regardless of the high amount of lignin present in the FC samples. This suggests that even when introducing into the AD process the theoretical highest amount of volume of FC effluent produced (considering Heinola's in-mill conditions); methane production is still feasible.

Table 7 reports the final AD efficiency parameters for all samples. COD removal rates ranged between 64-83% which express an efficient treatment in all samples (**Figure 11b**). Likewise, biodegradability followed a similar pattern. However, due to the high acetic acid content in EC, biodegradability is likely overestimated. Calculations were made with the assumption that 1 gram of COD theoretically yields 350 mL CH_4 at STP. However, this is assuming a carbohydrate substrate; which would produce a lower theoretical methane production than substrates containing intermediate products of AD that are much easily degradable such as methanol and acetic acid (Ekstrand et al. 2013). VFA behavior followed a similar trend in all samples, where a spike of production is observed around day 5 and later decreases until no VFA concentrations were measured (**Figure 11c**). Throughout the experiment, pH slowly increased in all samples ranging from 7 - 8.15 (**Figure 11d**). R1 and R1+HT experienced a slight drop between days 5-8 due to the VFA accumulation identified previously.

Table 7. *AD efficiency parameters for different ratios of NSSC effluents. Values expressed as mean ± standard deviation when indicated (**paper III**).*

Sample	Accumulated methane yield (mL CH_4/gCOD)	Soluble lignin removal (%)	COD removal (%)	BD (%)
R1	324 ± 8	-	82	105
R1+HT	333 ± 5	-	83	108
R2	250 ± 15	25 ± 3	76	65
R2+HT	270 ± 17	28 ± 6	78	70
R3	212 ± 8	41 ± 1	75	46
R3+HT	215 ± 3	44 ± 2	71	47
R4	192 ± 4	n.a.	64	37

n.a. information not available

Based on the nature of NSSC pulping, its effluents are rich in sulfur-containing compounds. This may lead to competition between SRB and methanogens. Sulfur (i.e. in evaporator condensates) is partially reduced to sulfide and released into the gas phase as H_2S. Sulfate (SO_4) and sulfite (SO_3) are known as electron sinks in AD and compete with methanogens for hydrogen. Studies have found that even at high concentrations of 3000 mg/L of sulfate and high COD organic load rates; AD was efficient achieving sulfate removal between 28-42% and COD removal rates above 80% (Jing et al. 2013). For R1 and R2 samples, initial SO_4 concentration was 67 mg/L and for R3 samples, it was 141 mg/L. After AD sulfate removal was 99%, this means it did not play an inhibiting role. SO_3 initial and final concentrations were low (between 4-30 mg/L) which was considered to not play any role.

As a concluding statement, the co-digestion of both NSSC effluents is evidently more energetically and anaerobically favorable than the hypothetical mono-digestion of EC or FC effluents. Unpublished results showed that biogas production from EC effluents alone had a low content of methane (30-37%) meaning the remaining gas was composed of CO_2 and H_2S. This would mean issues regarding biogas scrubbing and its utilization. Mono-digestion of FC would lead to a strong inhibition caused by the lignin accumulation. In full-scale application of co-digestion however, the presence of recalcitrant lignin could also lead to an accumulation of lignin compounds over time. This issue will have to be addressed by means of removal of these compounds before or after AD. Nevertheless, FC effluents are wastewaters that cannot be excluded and need to be treated in Heinola mill. Therefore, co-digestion of both effluents produce a synergistic effect where the methane yield of co-digestion samples is higher than the average individual substrates' methane yield.

5.3.2 The effect of lignin content on methane production

As discussed in **paper III**, the results demonstrated a linear decay in methane production when lignin was increased in the feed which resulted in a 41% decrease in methane yield when the lignin fraction had a 2.5-fold increase. The negative correlation between total lignin content and methane yield or biodegradability is represented in **Figure 12**. A correlation coefficient (R^2) of 0.95 fitted both experimental data sets. Statistical analysis showed a p-value of ≤ 0.01 for both curves. Relatively high values of R^2 indicate a reasonable predictability for methane production and biodegradability when lignin content is known. Even though in this study only 4 data sets were evaluated, similar trends in which lignin and methane yield or biodegradability shared the same negative correlation can be identified (Labatut et al. 2011; Li et al. 2013; Triolo et al. 2011). In each study, lignin content was found to be a good predictor for methane production of a substrate. Some key findings were the fact the dairy manure was an outlier when compared to cellulose substrates and that lignin content negatively affects the methane production more significantly in energy crops than in animal manure. This may be due to the nature of the lignin present in the substrate. In the P&P industry, industrial lignin (which is generated as a by-product from chemical pulping of wood) differs significantly from native lignin in its structure and chemical composition. This leads to the formation of lower molecular weight lignin and other impurities (Alekhina et al. 2015). Sierra-Alvarez and Lettinga (1991) discovered that the lower molecular weight fractions of lignin are specifically responsible for the inhibitory effect on methanogens. This makes the study of the behavior and composition of industrial lignin in the AD process even more so important.

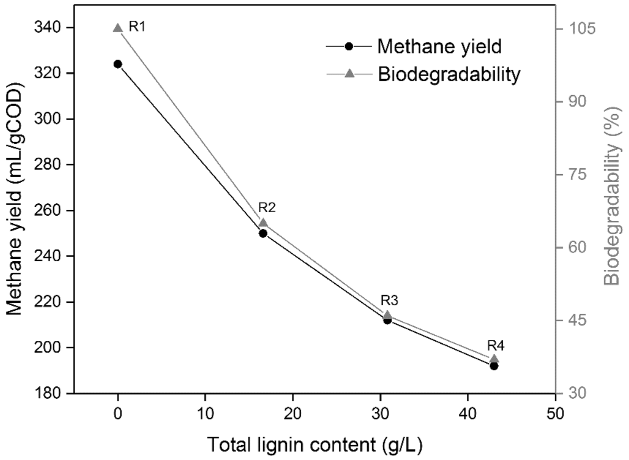

Figure 12. *Correlation between lignin content, methane yield and biodegradability in NSSC experiments (corrected and modified from* ***paper III****).*

A microbial population analysis was performed to estimate the population growth rate of methanogenic archaea in the reactors. This would provide a preliminary idea of the inhibiting or toxic effect of lignin in microbial growth. DNA extraction and Quantitative real-time PCR were used to quantify the initial and final concentrations of methanogens in the samples after the AD process. Results showed that R1 and R1+HT (reactors with no lignin presence) obtained a significant growth of 253 and 293% respectively. This rapid increase in microbial growth is assumed to be by the *Methanosarcinaceae* gene which are known to have high growth rates and outcompete other species at high acetate concentrations (Lee et al. 2009). Their predominance in anaerobic treatment of P&P wastewaters has been previously studied and it has shown that *Methanosarcina* is more flexible to changing substrate conditions and the presence of inhibitors. Their growth rate had doubling times in the order of 1–1.2 days and they maintain tolerance to sudden changes in pH of around 0.8–1.0 units (Walter et al. 2016). R2 had the lowest growth rate of 33% while R2+HT, R3 and R3+HT had respective populations growths of 86, 83 and 92%. Apart from the inhibiting effect of lignin on microbial growth, studies have also reported on its antimicrobial properties (Gregorova et al. 2011; Erakovic et al. 2014). Hence there are many paths of lignin that can lead to inhibition. A clear difference is observed between reactors with lignin and no lignin presence, thus an assumption that lignin has a direct effect on the population growth of methanogens can be presumed.

5.3.3 The effect HT addition on methane production

The addition of the Fe-Zn HT had an overall positive effect on the anaerobic digestion parameters of the samples. Final accumulative methane yields were increased by 1.4-8% after HT addition (**Table 7**). R1+HT had a faster methane production and other parameters such as COD removal, biodegradability and soluble lignin removal followed the pattern of slight increase in performance when HT was added. It is important to mention that the addition of HT did not affect negatively on the AD process of samples; results showed either no specific effect or a slight improvement. Through an extensive literature review there was no conclusive proof that associated the efficiency or inefficiency of the HT with regards to the lignin concentration in the sample. Kruger et al. (2016) reported on the effect of different HTs on the depolymerization of lignin. They found that the presence of both basic and nitrate anions in the interlayer increases the catalyst activity. On the other hand, other anions or transition metals did not present any effect.

Microbial population growth was greater in all samples containing HT. This is assumed to be by the higher surface are provided by HT nanoparticles as well as their catalytic action by the incorporation of Fe (Fe is a nutrient required for microbial growth in methanogens). HTs can possibly enhance microbial anchoring allowing for better environment for microbial growth.

5.4 Alternative option of methane production for chemical pulp mills

In **paper IV**, a completely different and new approach is addressed. In order to provide a larger diversity of end-products in chemical pulp mills, the direct production of methane from pulp substrates is an interesting option to explore. The methane potential of 3 raw pulping materials: brown fiber (BRFiber), oxygen delignified fiber (O2Fiber) and bleached fiber (BLFiber) and their respective processed products (denoted with the suffixes -MCC, -Filtrate and -Mix) were tested for methane production in batch reactors.

After initial characterization (**Table 4 and 5**), all substrates showed high amounts of carbohydrates, which is beneficial for methane production. Lignin content decreased by processing of the fiber in the order of BR > O2 > BL. This is due to the chemical pulping process where the key objective is the removal of lignin from the woody matrix. The MWD of solid samples showed that fiber substrates exhibited a bimodal behavior with higher MWD, on the other hand, MCC substrates have a singular peak shape at lower MWD due to the acid hydrolysis MCC substrates have gone through. Crystallinity index (CI) was also measured and demonstrated that the acid hydrolysis of MCC removed hemicelluloses and amorphous portions from the pulp fiber, thus displaying higher CI than fiber substrates and a much denser structure.

Table 8. *AD efficiency parameters for pulp substrates. Values expressed as mean ± standard deviation when indicated (**paper IV**).*

Sample	Accumulated methane yield (mL CH_4/gVS)	Theoretical methane yield (mL CH_4/gVS)	COD removal (%)	VS removal (%)	BD (%)
BRFiber	356.7 ± 3	427.0	35.7	42.0	83.5
BRMCC	332.5 ± 4	425.1	37.5	35.9	78.2
BRFiltrate	298.3 ± 8	421.2	86.0	31.5	70.8
BRMix	288.8 ± 27	429.7	49.6	34.5	67.2
O2Fiber	347.6 ± 4	421.8	25.5	34.7	82.4
O2MCC	298.1 ± 4	423.2	26.2	31.9	70.4
O2Filtrate	252.5 ± 8	443.8	78.6	27.2	56.9
O2Mix	261.3 ± 19	425.0	40.6	29.1	61.5
BLFiber	380.1 ± 4	416.4	34.1	39.2	91.3
BLMCC	349.5 ± 5	416.1	34.4	36.4	84.0
BLFiltrate	290.8 ± 6	397.5	83.2	32.0	73.2
BLMix	331.3 ± 32	410.6	47.9	36.7	80.7

The experimental methane yields and other AD efficiency parameters of each pulp samples are shown in **Table 8**. As can be seen from the results, all samples had a biodegradability above 55% of the theoretical methane potential which would consider them good candidates for energy production. Statistical analysis confirmed no significant variation between triplicates resulting in a *p*-value ≤ 0.05. The highest methane productions were observed in the fiber samples (380 mL/gVS) of each type of pulp (BL > BR > O2), followed by MCC samples (BL > BR > O2), Mix samples (BL > BR > O2), and the lowest yields (252 mL/gVS) were found in filtrates samples (BL > BR > O2).

Bleached substrates (fiber, MCC and mix) had the best methane yields compared to their respective brown and delignified substrates which was expected. This is because the requirement for bleached pulp is to have the purest cellulose content with the minimum amount of lignin. This would be the desired properties for a substrate when producing methane, as lignin has been previously discussed to hinder methane production. As discussed in **paper I**, a comparable result of 333 mL/gVS for bleached pulp filtrate from the Aaltocell^{TM} was attained. Steffen et al. (2016) reported on the methane yields of chemical and mechanical pulp fibers and fines fractions, discovering similar results to this study. They found that fiber samples and fines fraction of bleached kraft chemical pulp produced between 363 – 375 mL/gVS of methane. In contrast, when testing bleached mechanical pulp the methane yield was significantly reduced to as low as 21 mL/gVS. Recycled fiber pulp was also tested and reached 127 mL/gVS. They demonstrated that the low yields were not affected by the high ash content (up to 50%) but more by the content of lignin-rich fibers.

Substrates from O2 pulp had the most variation throughout the AD process and gave the lowest methane yields. The reason behind this is due to the structure of the lignin compounds in the oxygen delignification process. Even though BR pulp has a higher lignin content than O2 pulp, when the fibers go through the O2 process, the lignin fractions that are removed and the residual lignin fraction in the pulp suffer significant changes in their structure. Covalent linkages between lignin and carbohydrates are transformed into lignin-carbohydrate complexes (LCC) and lignin can contain free phenolic hydroxyl groups (Dence and Reeves 1996; Lawoko et al. 2004). As previously discussed, the industrial lignin formed leads to a higher toxicity than native lignin would, causing the organic material to be less accessible for anaerobic degradation.

As observed from **Figure 13,** BR and BL pulp substrates showed a similar pattern whereas O2 pulp substrates show greater fluctuation of methane production. Maximum daily production rates ranging between 127 – 192 mL CH_4/gVS were detected in BR and BL substrates achieved by day 3 after a short hydrolysis period with filtrate samples being the fastest to produce methane. The almost immediate production of methane is due to the healthy concentration of hydrolysable carbohydrates such as glucose, xylose and mannose found in all filtrates (concentrations of each sugar were between 1.2 – 3.4 g/L).

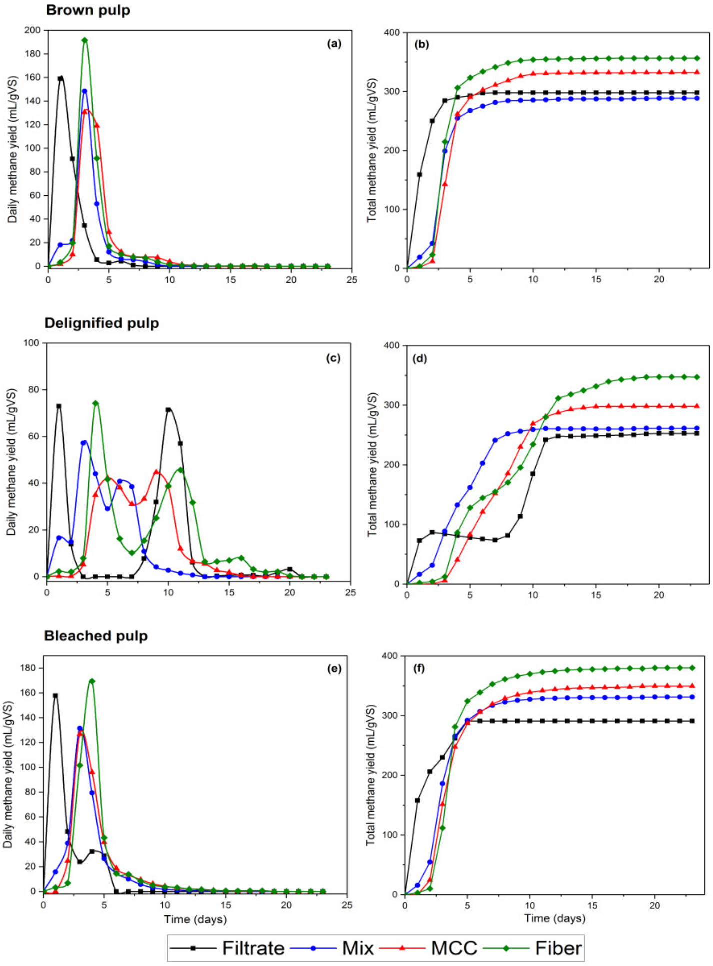

Figure 13. *Methane production evolution for all substrates expressed as a function of time. Brown pulp methane yields (a) daily (b) accumulated. Delignified pulp methane yields (c) daily (d) accumulated. Bleached pulp methane yields (e) daily (f) accumulated (modified from **paper IV**).*

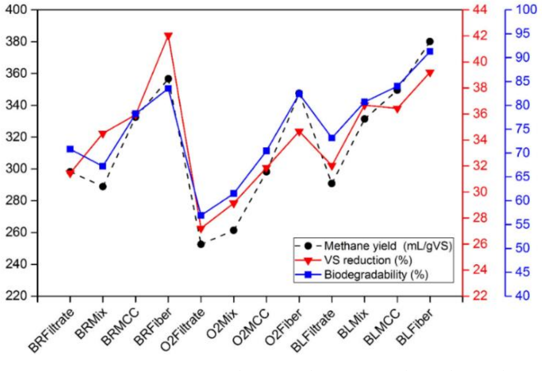

Figure 14. *Correlation between methane yield, VS reduction and biodegradability (BD) of all pulp substrates (**paper IV**).*

Xylose is an important monosaccharide found in hemicellulosic biopolymers which explains BL and BR filtrates containing higher contents, whereas O2 filtrates contain more furfural and hydroxymethylfurfural which come from the dehydration of the sugars (Barakat et al. 2012).

All BR and BL substrates achieved 90% of their ultimate methane yield by day 7 and plateaued by day 10 and 12 respectively. O2 substrates varied significantly as observed from **Figure 13c & d**, the production of methane was extended through a more spacious period of time plateauing by day 15. Each substrate had 2 separate peaks in maximum daily production with the highest corresponding to filtrate samples with 73 mL CH_4/gVS. The cumulative profiles show a slower semi-linear production compared to BR and BL curves. O2Filtrate displayed a sigmoid behavior with a clear and pronounced lag phase between days 2 and 8 were no methane was observed. The lag phase is attributed to an accumulation of acids after the hydrolysis phase. O2Filtrate consists of higher amounts of soluble and readily available organic material (COD, VFA, and formic acid) than other filtrates (**paper IV**) causing an unbalance in production/consumption of intermediates. Similarities were found in **paper I** when using ISR of 0.8 and 1 (**Figure 8**) where there was an accumulation of acids due to insufficient number of methanogens (amount of inoculum) to consume the produced intermediates. In both cases, the buffering capacity was sufficient to neutralize the pH and resume AD. In this study pH went from neutral so slightly alkaline (7.2-8.1).

Figure 14 shows a graphic representation of the correlation found between the substrates' methane yields, VS removal rates and BD. The parameters followed the order of BL > BR > O2 regarding the type of pulp and the order of Fiber > MCC > Mix > Filtrate regarding the type of substrate. COD removal efficiencies were found to be inversely correlated to BD; filtrates had higher removal (86-89%) and fibers had the lowest (25-35%). However, crystallinity of solid substrates had an apparent correlation with BD. Crystalline cellulose has a greater resistance to biological and chemical degradation (Zhao et al. 2012) so lower CI such as fibers' is more favorable for AD, validated on fibers' higher methane yields. Although there is a general agreement on the fact that lignin negatively affects the methane yield and BD of a substrate, data correlating lignin content and BD in this study was inconclusive. BR pulp had a higher lignin content (4.2-4.8%) than BL pulp (up to 0.9%) hence a much lower yield was expected from BR, however both pulp types have similar AD efficiency. In summary, the fact that all the substrates regardless of type of pulp had a BD > 50% and that raw fiber substrates had methane yields up to 380 mL/gVS, shows the great potential to produce energy (in the form of methane) as an alternative new use for fiber products and diversification of chemical pulp mills.

The 3 types of pulp that were used for methane potential experiments were of average paper pulp quality found in the kraft process. The methane produced correlated with the chemical composition of the pulp, mainly the carbohydrate %. However, to indentify a type of pulp as high quality for conventional pulp products (such as textile production, diapers, sanitary products, etc) other characteristics besides chemical composition must be taken into account. Fiber properties, bulk properties and water absorbency are some key characteristics; nevertheless it will also depend in the end-product wanted. For example, the textile industry needs high quality cellulose acetate which has the highest content of cellulose. In brief, pulp used in conventional products would need of additional assessment of quality parameters than would be needed for pulp used in methane production; this adds to the appeal of having a methane alternative.

5.4.1 Energy and cost estimation from methane production

The costs for investment and operation of methane production as well as the production cost and profit are key elements to determine the feasibility of its implementation. Considering that the production of methane has never been the priority product in a chemical pulp mill, a general overview of the potential economic benefits is imperative. Energy and cost estimations were considered in order to present an additional incentive towards an industrial application of methane production in chemical pulp mills and translate gas volumes into renewable energy amounts and economical profits. For this, two case studies were compared; a typical scenario where pulp is the main product and another scenario were the main product is methane. Firstly, before comparing costs, the methane yield previously found in pulp must be translated into energy units.

Methane and energy mass calculations were calculated using the **Equations 3** and **4**:

$$M_{amount} = O_{load} \times BMP \times M_{density} \qquad \textbf{(3)}$$

$$E_{amount} = M_{amount} \times M_{HHT} \qquad \textbf{(4)}$$

where M_{amount} is the amount of methane produced per one air-dried ton of substrate (kg/adt); O_{load} is the organic load of VS in the substrate (kgVS/adt); BMP is the experimental biochemical methane yield of the substrate; $M_{density}$ is the density of methane at STP assumed to be 0.716 kg/m^3. E_{amount} is the amount of energy produced per air-dried ton (MJ/adt) and M_{HHT} is the higher heating value of methane assumed to be 55 MJ/kg.

Table 9. *Values for the cost estimations for two softwood mill cases. First case scenario is pulp as the main product compared with the second scenario where methane is the main product (modified from **paper IV**).*

Sample	**Pulp case**[a]	**Methane case**	**Observations for methane case**
Pulp production (adt)	350,000	358,974	2.5% higher pulp yield
Wood (€/adt)	283	275.9	
Chemicals (€/adt)	33.0	3.0	no O2 or bleaching costs
Utilities (€/adt)	5.0	5.0	
Waste (€/adt)	2.0	0.5	no effluent treatment cost
By-products (€/adt)	-65.0	-65.0	
Total (€/adt)	258.0	219.4	
Cost of production (€)	90,300,000	78,767,949	
Unit price	600 (€/adt)	1450 (€/tn)	Price from Gasum (2017)
Income from main product (€)	210,000,000	118,832,958	228.3 kg CH_4 per adt of BRFiber, therefore 331 €/adt
Income from soil amendment (SA) (€)	0	4,813,846	Price of SA (assumed 100€/tn) 13.4% produced from AD
Total income (€)	210,000,000	123,646,804	
Estimated profit (€)	119,700,000	44,878,855	

[a] Values for pulp case taken from reference model in Kangas et al (2014).

Energy calculations (by application of **Equations 3** and **4**) suggest that using the fiber substrates of BR and BL as feedstock for AD would deliver the highest energetic outputs of around 12 GJ/adt (further energy and sludge calculation are referred to in **paper IV**). In order to estimate the economic potential of methane production in a chemical pulp mill, a comparison between two cases is shown in **Table 9.** From a practical point of view, the first case is a mill producing pure chemical pulp with paper grade (bleached) which would be a common type of mill. The second case is methane production coming from the AD of brown stock pulp without oxygen delignification stage which is the simplest form of fiber (BRFiber in this study). The investment costs in both cases was not taken into consideration.

Based on the aforementioned calculations expressed in **Table 9**, the pulp case is evidently the most profitable which was assumed as it is the basis of the P&P market. The direct income from selling the pulp makes the largest revenue. However, the methane case produces a substantial income which is 37.5% of the pulp case profit. This demonstrates how the alternative of producing and selling energy (in the form of biomethane) can be a potential cost-effective product in the case when chemical pulp mills have exhausted their markets or when the market price is running low.

6 Conclusions

Water and energy resources are the most fundamental necessities in our constantly growing society. Innovative approaches are continuously emerging both in water treatment and renewable energy production paving the way towards an environmentally friendly culture. Nevertheless, industries such as pulp and paper, still have much more effort to put as it is one of the largest water and energy consuming industries in the world. The increasing need to efficiently treat the large volumes of P&P wastewater generated should be perceived as a potential incentive to develop new process designs that treat waste and wastewater as valuable materials and align strategies towards sustainable resource use. AD is an efficient and mature waste-to-energy technology that tackles both water and energy needs. However, the different pollutants and sometimes non-degradable compounds found in P&P wastewaters make it a difficult task to efficiently degrade organic material into usable biomethane.

The different approaches to enhance the methane productivity of P&P wastewaters described in this thesis show the promise in developing substantial amounts of methane for energy recovery and simultaneously have high reductions of COD for an efficiently treated effluent. By the systematic characterization of the various types of wastewaters generated in the different processes taking place in a mill, a reliable hypothesis can be formed about its methane yields and biodegradability. However, the optimal selection of process parameters plays a key role. While it is commonly known that lignin compounds; which pose an inherent limitation in methane production; are present in most P&P effluents, there are also easily degradable wastewater streams. Hydrolyzed filtrates and NSSC evaporator condensate were two waste streams studied, that proved to be excellent substrates for methane production achieving as much as 333 mLCH_4/gVS. The high content of degradable sugars in filtrates and acetic acid in condensates were the major sources for initial hydrolysis to take place at a faster rate. However, this could imply an oversaturation of acids. The results from this study suggest that a high ISR and addition of buffering capacity offsets the possible pH decrease and subsequent inhibition of the process. An ISR of 2 was found to be the optimal with fast production rates and highest methane yields. Lower ISR of 1 and 0.8 also achieved comparable accumulated methane yields but presented long lag phases, whereas a low ISR of 0.5 was considered the threshold were total inhibition of the process took place.

P&P wastewaters with high lignin content such as fiber circulation water from NSSC, are streams that based on their characteristics are unsuitable for AD. Mono-digestion of these effluents would lead to a complete inhibition and a diminished methane yield but co-digestion with an easily degradable substrate could boost the process to average yields. To test this theory, fiber circulation water and evaporator condensates were co-digested at different ratios. The decrease in methane production when fiber circulation water was added indicated a negative linear correlation between lignin content and methane yield. Nevertheless, the lowest methane yield attained (ratio with the fraction of fiber circulation water that can be produced in the mill) was 192 $mLCH_4/gCOD$ which is still among the average yields for P&P substrates. Both wastewaters currently need to be treated before disposal to the environment, hence co-digestion of both wastewaters presented benefits in treating the lignin rich (hard to treat) effluent without significantly compromising the overall methane potential. Additionally, the added carbon source in the lignin-rich effluent can mitigate the susceptibility of a VFA accumulation in the AD of evaporator condensate. In summary, the co-digestion of both effluents is proposed to improve the overall biodegradability of NSSC wastewaters.

The addition of Fe and Zn HTs to enhance AD was a new technique never before tested in P&P wastewaters. The presence of Fe-Zn HT showed an increase of 8% in methane production, as well as faster production rates and higher lignin removal. HTs with Zn incorporated had slightly better methane yields which suggest that Zn played an important role in trapping sulfide present in the wastewater. The negligible effect of calcined HT in the methane potential indicate that the better performance of non-calcined HTs is the presence of Zn and Fe cations immobilized in the layered sheet structure of the HT allowing a controlled released of these into the anaerobic environment.

Finally, the last approach in this thesis showed that not only can P&P wastewater be anaerobically treated and produce methane but fibers and its products can also be efficiently used as feedstock to provide significant amounts. Brown, oxygen delignified and bleached pulp demonstrated high biodegradability (up to 91%) reaching methane yields as high as 380 $mLCH_4/gVS$. Fiber and MCC substrates corresponded to the higher range while filtrates had the lowest performance of all. Nevertheless, all the chemical pulp substrates had a biodegradability above 50% and high energy productions that could potentially generate a feasible profit. Based on the observations, methane can be a promising new alternative product in chemical pulp mills and give fiber products a novel additional use.

6.1 Significance of this work

The work presented in this thesis describes the importance in selecting conditions that provide the best suitability for P&P substrates to produce methane. Different approaches were chosen, each considering a different aspect and point of view to optimize the production of methane and recover energy. The use of optimal inoculum ratio, promoting agents, and co-digestion are some of the techniques presented in this study to aid on the hard degradability perception that follows P&P effluents. In general, the different P&P substrates all proved to be subjected to efficient AD and methane production. The high methane yields and COD removal efficiencies obtained from most substrates tested, substantiates the advantages of applying AD to P&P wastewaters.

The research on the application of HTs in P&P wastewater treatment reported in this thesis, is the first of its kind. The quantity and quality of P&P wastewaters have made it necessary for innovative treatment options to evolve in this field. This is why it is important to initiate curiosity in atypical treatments. Although the advantages in methane productivity when applying HTs are not extensive, the effects observed lead way to investigation on the different conditions and kinetics involved. This research work is intended to increase efforts and attention into the potential applicability of HT in P&P wastewater treatment.

Furthermore, in addition to promoting a waste-to-energy practice, this study sheds some light into the potential diversification of P&P end-products. Based on the high-energy output that pulp products (fibers and MCC) offered, an innovative alternative product can be considered. Methane in the form of heat, electricity or biomethane fuel could become a potential portfolio product for chemical pulp mills. The scale and importance of the P&P industry in Finland further supports the arguments underlying in this research work of working towards implementation of energy recovery processes and finding innovative uses for forest products. Therefore

6.2 Future research recommendations

Throughout the conducted research work for this thesis, some areas have been identified that entail further investigation.

The inoculum used in this thesis was digested sludge taken from a municipal mesophilic sludge digester. The ratio between different microbial communities (hydrolysis, acidogenesis and acetogenesis bacteria, and methanogenic archea) involved in the anaerobic degradation phases has not been measured and although municipal seed sludge is the most commonly used, it may not be the most adapted to the composition of P&P

effluents. Recalling that the rate and kinetic order of the reactions taken place in each phase depends on the ratio between the abundance of the releveant microbial community and the concentration and availability of the related substrate. Hence it would be of great interest to study the effect of using adapted sludge from an operating digester treating P&P effluents.

Although the presented work reports important findings in the use of HTs for P&P wastewater treatment, a clear gap for improvement could be identified. The use of HTs is a rather new practice, and the results of this study can be considered the first step towards exploring their applicability for P&P effluents. Further research is needed to fully understand the kinetics behind the HT reaction with specific components such as sulfur. In addition, continuous testing would allow further knowledge in operational parameter and most importantly sludge production properties. Characterization of the digested sludge molecules produced would aid in recognizing structural and chemical changes that occur after the pollutant has had contact with the HT. Furthermore, the addition of HTs could be tested as pre- or post-treatments separate from AD.

Co-digestion presented a clear benefit for hard to treat effluents that contain lignin. However, lignin is a slow-degrading compound meaning that it would most probably accumulate over time. Continuous testing for a longer period of time is necessary to assess the behavior of residual lignin in an anaerobic reactor. Furthermore, a mathematical model could be developed to select for future adequate methods for lignin removal and optimization of the process.

The production of methane was found to be a promising new alternative use for chemical pulp. As mentioned earlier, the quality of the pulp used was of the average quality found in kraft processes. It was tested in mono-digestion batch assays with the substrate in question and inoculum. An interesting pathway of investigation would be the co-digestion of the fiber and MCC substrates with mixed effluents from a P&P mill (simulating real condition in operating mills). The effect of adding solid waste into the AD process would be important to take note on. Additionally, further investigations would be needed in order to evaluate if lower quality pulp (than the chemical pulp used in kraft pulping) in any way affects the methane potential.

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Errata for appended publications

Paper I. In section 2.3, the chemical formula of sucrose reads $C_{12}O_{22}O_{11}$ when it should be $C_{12}H_{22}O_{11}$.

Paper II. Figure 5, the biodegradability axis shows a range from 30-200% and it should read from 0-105% as corrected in Figure 12 of this thesis.

Paper IV. In Table 5, the income of products should have a unit of € and not €/adt; additionally, the estimated profit for the pulp case reads 119,700,00 and it should be 119,700,000. Both errors were corrected in Table 9 of this thesis.

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Effect of Inoculum to Substrate Ratio on the Methane Potential of Microcrystalline Cellulose Production Wastewater

Lourdes M. Rodriguez-Chiang* and Olli P. Dahl

The methane potential and influence of the inoculum to substrate ratio of wastewater originating from the production of microcrystalline cellulose (MCC) were studied. Laboratory experiments were carried out in a continuously stirred batch multi-reactor at mesophilic temperature (37 °C). Inoculum to substrate ratios (ISRs) of 2.0, 1.0, 0.8, and 0.5 based on volatile solids (VS) were evaluated. The results demonstrate the suitability of MCC wastewater at ISRs of 2.0, 1.0, and 0.8 with ultimate methane potentials of 333, 297, and 325 mL CH_4 per gram of volatile solids added, respectively, which correspond to anaerobic degradabilities of 91.4, 81.7, and 89.3%, respectively, compared to the theoretical potential. The inoculum to substrate ratio of 2.0 provided a faster methane production rate and a kinetic constant of 0.24 d^{-1}, reaching its ultimate yield at day 8 of incubation. The lowest ISR of 0.5 showed the occurrence of process inhibition due to accumulation of acids. Energy estimation suggests that considering the volume and VS of wastewater produced in a MCC mill, a total energy amount of 44,105 GJ/year can be produced, which can be used to replace 29.4% of the natural gas demand.

Keywords: Anaerobic digestion; Biogas; Biochemical methane potential; Inoculum to substrate ratio; Microcrystalline cellulose; Wastewater

Contact information: Department of Forest Products Technology, Aalto University School of Chemical Technology, P.O. Box 16300, Vuorimiehentie 1, Espoo, Finland;
** Corresponding author: lourdes.rodriguezchiang@aalto.fi*

INTRODUCTION

The current global environmental concern focused on the increase in the emission of greenhouse gases has outlined the importance of studying alternative energies. As a result of the negative impacts of production and use of fossil fuels as well as the dependence of the global economy towards them, biogas technology has been one of the proposed energy solutions. Biogas is produced through anaerobic digestion (AD), which is a biological process in which organic material of a substrate is degraded by microorganisms in the absence of oxygen (Angelidaki *et al.* 2003). The result of this degradation is a mixture of methane, carbon dioxide, and some small quantities of H_2S, H_2, and NH_3. The composition of the biogas is dependent on the type of digested material and the operating parameters of the process (Rodriguez 2012).

The pulp and paper industry is one of the largest water consuming industries. It generates relatively large volumes and many types of wastewaters with different pollutants and characteristics depending on the process parameters and end product desired in each mill. Although technological advances have made the pulping process less water consuming, on average a pulp and paper mill will use 13 to 30 m^3 of water per ton of produced paper (Kamali and Khodaparast 2014). Anaerobic digestion of pulping wastewater and sludge has been evolving to analyze the potential recovery of energy in the form of biogas. This process presents an effective wastewater treatment that produces biogas and moreover minimizes the volume of sludge generated by 30 to 70% (Ekstrand *et al*. 2013). Such an approach can decrease additional problems associated with exponential generation of waste.

Anaerobic digestion helps break down biodegradable organic fraction present in wastewater by turning it into biogas with high methane content, thus having a high energy use and a stabilized final effluent. Hence, anaerobic digestion presents a positive energetic balance, allowing both pollution prevention and recovery of sustainable energy (De Baere 2000). This not only offers the potential use of biogas for heat and electricity, but also allows the possibility of upgrading biogas to biomethane and using it as vehicle fuel.

Biochemical methane potential (BMP) tests are the most used tool to provide a measure of the anaerobic degradability of a given substrate; this is a key parameter for assessing design, economics, and managing issues for the full scale implementation of anaerobic digestion processes (Angelidaki *et al*. 2009). This method is a simple and fast way to determine the suitability of a substrate for anaerobic digestion and the potential methane yield therein resulting in a quantitative measurement of methane production. Different inoculum to substrate ratios can be tested in order to achieve the maximum methane production within the substrate.

Many studies have reported on the methane potential of different substrates such as municipal sludge, food waste, energy crops, and animal slurry (González-Fernández and García-Encina 2009; Rodriguez 2012; Browne and Murphy 2013). However, fewer studies have reported on the methane potential of pulp and paper mill effluents and have concluded on a positive yield of methane ranging from 40 to 60% of the theoretical potential (Bayr and Rintala 2012; Ekstrand *et al*. 2013; Hagelqvist 2013). However, a setback in using AD for pulp and paper mill wastewaters is the fact that these effluents are highly loaded with different toxic compounds that can inhibit the AD process and also have a high amount of lignin and extractives that have low degradability. Therefore, there is a need to select and test different independent effluent streams that have a higher potential or easily degradable organic content that can be utilized to produce high methane volumes.

Microcrystalline cellulose (MCC) is a powder-like cellulose product which has a global market of more than 100,000 tons, with an annual increase of 5% (Ciechanska *et al*. 2010). Typically, MCC mills are equipped to handle relatively small throughputs (less than 10,000 tons/year) using a high amount of acid and low concentrations for cellulose cleavage. Due to these process characteristics, it is not economically viable to utilize released sugars from the process. Therefore, the generated wastewaters are normally led to the municipal wastewater treatment plant after pH neutralization in order to reduce organic load to the river and lake systems. However, Aalto University has developed a new method to produce MCC (Vanhatalo and Dahl 2014) by means of high volume, low acid dose, and high concentration; such an approach can lead to a high sugar content (30 to 80 g/L) in the process wastewater. These process conditions mean that the wastewater originating from the production of AaltoCell™ will have specific qualities that make it a suitable substrate for rapid biogas production.

The aim of this study is to analyze the stream of wastewater generated from the simplified production of MCC by the AaltoCell™ process invented by Aalto University, and also to test the most efficient methane production at different inoculum to substrate ratios. Additionally, the importance of produced methane as a source of energy for the AaltoCell™ process is estimated.

EXPERIMENTAL

Materials

The substrate used for this study was the hydrolyzed filtrate wastewater originating from the production of MCC by mild acid hydrolysis following the AaltoCell™ process, as detailed in earlier studies (Vanhatalo and Dahl 2014; Vanhatalo *et al*. 2014). Substrate sample was stored at –20 °C prior to its use. The original carbohydrate composition of the filtrate wastewater was as follows: arabinose 1.63 g/L, rhamnose 0.004 g/L, galactose 0.86 g/L, glucose 26.79 g/L, xylose 14.93 g/L, and mannose 11.61 g/L. The fresh substrate was analyzed prior to the start of the experiment using average values of triplicates, resulting in total solids (TS %) of 9.31, volatile solids (VS %) of 7.82, moisture content (%) of 90.69, and an initial pH value of 1.75. According to Angelidaki *et al*. (2009), the VS for acidic substrates can be underestimated due to volatile fatty acids (VFA) loss during the analysis of total solids. Therefore before TS and VS analysis, the pH of the substrate was adjusted to 7 using a NaOH 20% solution to decrease volatility of VFA during measurements. The elemental composition of the substrate was C (44.00%), H (4.45%), N (0.15%), and S (2.20%), from which the following empirical formula was determined: $C_{367}H_{445}O_{307}NS_7$.

The inoculum used for the batch tests originated from Suomenoja municipal wastewater treatment plant located in Espoo, Finland. It was taken fresh from their mesophilic anaerobic digester and degassed for 7 d at the same operating temperature (37.0 °C) prior to the start of the experiment. Using average values of triplicates, analysis resulted in total solids (TS%) of 1.7, volatile solids (VS%) of 0.9, and moisture content (%) of 98.3. A pH value of 7.6 was measured, with a total alkalinity (TA) of 6.8 g $CaCO_3$/L.

Methods

Experimental design

Experiments were carried out in an automatic methane potential test system (AMPTS), which is a laboratory scale batch methane potential analyzer developed for automatic real-time logging and measuring of methane production (Rodriguez 2012; Badshah *et al.* 2012; Browne and Murphy 2013; Browne *et al.* 2013). Measurements are expressed using the same unit for conventional BMP test found in literature, normalized mL of methane per gram of volatile solids added (N mL CH_4/gVS). It has a capacity for incubating 15 reactors of 500 mL each with an individual mixing motor and a defined carbon dioxide removal step in order to provide only methane yield.

Four different ISRs based on VS% were evaluated: 2.0, 1.0, 0.8, and 0.5. All sample tests were prepared in triplicates for statistical significance. The BMP tests were carried out using a working volume of 400 mL. In order to achieve the desired ISRs based on VS content, the volumes of inoculum and substrate were calculated (Table 1). Triplicate blank samples with no substrate were run to determine the produced background methane originating from the inoculum alone. Triplicate control samples containing Avicel® PH-101 pure cellulose (Sigma-Aldrich, USA) were also run to verify inoculum activity. Finally, to increase buffering capacity throughout the experiment, 4 g/L of sodium hydrogen carbonate ($NaHCO_3$) was added to each sample reactor at the beginning of the experiment (zero time) as suggested by Abdulkarim and Evuti (2010) and Raposo *et al*. (2011). After filling each reactor, bottles where sealed with a hermetic rubber stopper connected to a mechanical agitator and placed in a water incubator at 37.0 ± 0.5 °C.

Table 1. Substrate and Inoculum Volume Used for Each Reactor

Inoculum to substrate ratio (ISR)	Inoculum amount (mL)	Inoculum VS load (gVS)	Substrate amount (mL)	Substrate VS load (gVS)	Total volume (mL)	Total load (gVS)
2.0	378	3.41	22	1.71	400	5.12
1.0	359	3.24	41	3.24	400	6.64
0.8	350	3.16	50	3.95	400	7.11
0.5	308	3.17	92	6.34	400	9.51

To chemically remove carbon dioxide (CO_2) and hydrogen sulfide (H_2S) formed during anaerobic digestion, each reactor was individually connected to another small bottle containing 80 mL of an alkali solution of 3 M NaOH. Thymolphthalein pH indicator was added to each bottle to determine when the solution has been spent and needed replacement. Each alkali solution bottle was then connected to the measuring device and finally all reactors were flushed with pure nitrogen gas (N_2) for 5 min, to ensure anaerobic conditions.

BMP calculation

Results from the BMP tests were used to evaluate the anaerobic digestion rate and profile of the substrate in respect to different ISR over time. The methane potential was calculated as the accumulated methane produced per gram of VS added to each reactor, as determined in Eq. (1) (Strömberg *et al*. 2014),

$$BMP = \frac{V_{sample} - V_{inoc}\dfrac{gVS\ is}{gVS\ ib}}{gVS_{substrate}} \tag{1}$$

where *BMP* is the normalized volume produced per gram VS of substrate added (mLCH_4/gVS), V_{sample} is the mean value of accumulated methane produced from the reactor with inoculum and substrate mixed, V_{inoc} is the mean value of the accumulated volume produced by the blanks, gVS_{is} is the mass of volatile solids of the inoculum added in the sample, gVS_{ib} is the mass of volatile solids of the inoculum added in the blanks, and $gVS_{substrate}$ is the mass of volatile solids added of the substrate in the reactor.

Anaerobic degradability

The anaerobic degradability of a given substrate can be expressed by product formation (methane production). The anaerobic degradability achieved for each ISR tested is defined as the ratio between the experimental methane yield (M_{exp}) and the maximum theoretical potential (M_{th}) expressed as a percentage (Eq. 2) (Raposo *et al.* 2011):

$$\text{AD }(\%) = (M_{exp} \ / \ M_{th}) \ * 100 \tag{2}$$

Given the determined empirical formula for the substrate ($C_{367}H_{445}O_{307}NS_7$), by application of the Buswell equation (Eq. 3), the maximum theoretical potential (M_{th}) of methane formation can be calculated (Symons and Buswell 1933; Buswell and Mueller 1952):

$$\mathrm{C}_n\mathrm{H}_a\mathrm{O}_b\mathrm{N}_c + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right)\mathrm{H_2O} \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)\mathrm{CH_4} + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right)\mathrm{CO_2} + c\mathrm{NH_3} \tag{3}$$

Analytical methods

Total solids (TS), volatile solids (VS), and moisture content in fresh samples of substrate and inoculum were determined gravimetrically following standard methods described in APHA (2005). Total alkalinity (TA) to pH 4.5 was measured by Standard Method 2320 B. The pH measurements were performed using a pH meter model Thermo Scientific Orion 2-star (Singapore).

Methane production was measured using the AMPTS II from Bioprocess Control AB, Sweden (System Version 2.0 V1.08), which works by the principle of liquid displacement and buoyancy. Volumes of gas are corrected to standard temperature and pressure (STP) conditions at 273 K and 1013 mbar air pressure.

Elemental analysis of the substrate for C, H, N, and S was determined by duplicate samples using a Perkin Elmer Model 2400 Series II CHNS Elemental Analyzer (USA).

RESULTS AND DISCUSSION

Methane Potential

The ultimate methane production and flow rate from the substrate at ISRs of 2.0, 1.0, 0.8, and 0.5 were obtained. Methane production was monitored at a temperature of 37.0 ± 0.5 ºC for 21 d, which resembled time after which the methane production curves reached a plateau stage. The accumulated methane production of the different inoculum to substrate ratios tested is shown in Fig. 1. Daily methane production in each sample bottle was corrected by subtraction of the average background gas formed in the blank samples.

The values of cumulative methane production at STP, pH, and total alkalinity values after digestions are expressed in Table 2.

Table 2. Sample Characterization after Digestion

Inoculum to Substrate ratio (ISR)	Final VS (gVS)	Ultimate methane yield (mL CH_4/gVS)	Final pH	Final alkalinity (g $CaCO_3$/L)	Final conductivity (mS/cm)
2.0	2.00	332.9	7.6	7.9	11.5
1.0	2.69	297.4	7.8	8.7	12.0
0.8	2.60	325.0	7.8	8.9	12.1
0.5	5.19	59.4	5.6	3.1	16.7
All values are mean averages of triplicate samples					

For an ISR of 0.5, one can observe an accumulated methane yield of 59.4 mL/gVS, which is significantly lower than the other ISRs tested and implies a serious process imbalance and a severe inhibition of methanogenesis. Methane production ceased during day 5. This is mostly attributed to the excessive acid formation originating from the high initial load of easily available sugars in the substrate; this would in turn decrease the pH in the reactor, which is validated with the final pH value of 5.6 taken from the ISR 0.5 samples after the digestion experiment. The same behavior was also observed with sugarcane bagasse (Badshah *et al.* 2012). The pH value of the reactor is usually controlled by a bicarbonate buffer, which is formed by the reaction of carbon dioxide from the biogas with the natural mineral alkalinity of the wastewater.

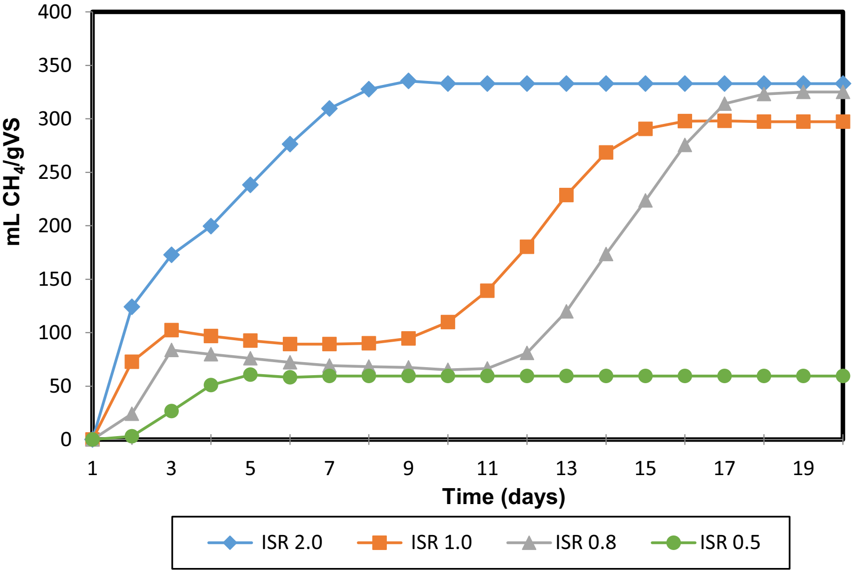

Fig. 1. Accumulated methane production at different inoculum to substrate ratios expressed as a function of time

Accumulations of acetic or other volatile acids tend to neutralize this mineral alkalinity, causing the pH value to fall from about 7.5 to about 6.5 which releases dissolved carbon dioxide (bicarbonate) back into the biogas. In severe cases, where the concentration of organic acids exceeds the concentration of mineral alkali, the pH value will fall to around 5.5 and be controlled by an acetate (or mixed volatile acid) buffer (McCarty and Mosey 1991); this is the case for the samples of the ISR of 0.5, which have a lower alkalinity and pH measurement than the rest of samples. Similar results were reported by Eskicioglu and Ghorbani (2011), where at a high organic load rate (0.46 ISR), reactors experienced volatile fatty acid accumulation and pH decrease.

The highest value of methane yield corresponds to the ISR of 2.0, with an ultimate yield of 332.9 mL/gVS, followed closely by ISRs 0.8 and 1.0, with values of 325.0 and 297.4 mL/gVS, respectively. From the ISR 2.0 curve, one can see the rapid methane production in a continuous semi-linear manner until day 8, after which it reaches a plateau stage corresponding to its ultimate yield. Both ISRs 1.0 and 0.8 curves have a sigmoid behavior where a lag phase is observed from day 4 to day 10 before reaching a plateau; this may be due to the accumulation of VFA during the first days. However, within this lag phase, the bicarbonate buffering formed in each reactor is sufficient to neutralize the acid formation and reestablish a neutral pH environment. After day 10 both curves showed a rapid methane production until they reached their ultimate yield at day 18. This lag phase can be called an acclimation period and is observed in substrates with inhibiting or toxic compounds and substrates that create a stress situation such as high organic loadings (Meyer and Edwards 2014). Before reaching a certain threshold, the microbial community can become tolerant to the chemical or physical stressor and adapt to metabolize the substrates. Similar acclimation periods were observed in Wiegant and Lettinga (1985), where easily degradable substrates such as glucose, sucrose, and volatile fatty acids experienced a lag phase during the first 8 to 9 days of digestion and after this period produced a significant amount of methane.

Cellulose control samples reached their ultimate yield of 315.0 mL/gVS on day 10, which can be compared to the maximum theoretical methane value of 414.0 mL/gVS calculated using the Buswell equation (Buswell and Mueller 1952); this ensures that the inoculum used to seed the reactors has the sufficient bacteria consortium to efficiently perform the anaerobic digestion process.

Methane Production Rate (MPR)

The methane production rate was calculated using the daily values of methane volume produced and applying the same formula from Eq. 1. Results (Fig. 2) show that for ISRs of 1.0 and 0.8, a similar pattern can be seen where there is an increased MPR over the first 4 days followed by a significant drop in MPR through days 4 to 10, and again another increase in MPR over days 11 to 17 followed by a gradual decrease towards a basal level. The reduced methane production from day 4 to 10 corresponds to the acclimation period described in the previous section. This means an estimated hydraulic retention time (HRT) of 18 to 19 days for ISRs of 1.0 and 0.8 when applied in a continuously fed system.

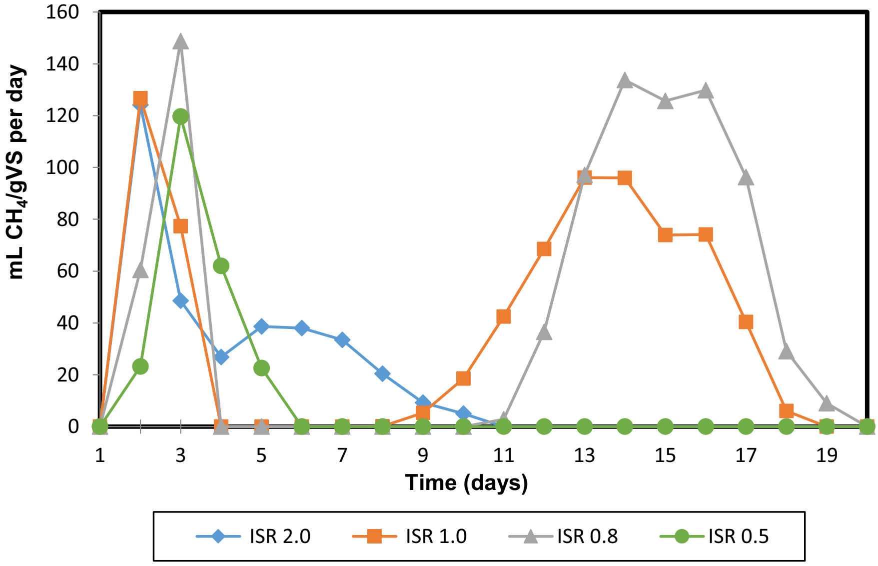

Fig. 2. Evolution of daily methane production rates for each inoculum to substrate ratio tested

The curve for ISR 2.0 shows an initial high MPR from the beginning peaking at day 3, which later slowly decreased and which finally ceased at day 11. This indicates a HRT of 8 to 11 days. Studies in pulp and paper mill residues (Elliott and Mahmood 2007; Bayr *et al.* 2013) state how these residues have long HRT of 20 to 30 days, making them less popular for anaerobic digestion. However several pretreatment methods which include microwave, ultrasonic, and hydrothermal were shown to reduce the HRT by 50% (10 to 15 days). Results in the present study show the efficiency of the digestion system at a shorter HRT of 8 to 11 days without the need of pretreatment, which provides higher techno-economic advantages such as reduced costs in pretreatments and smaller digester volumes.

The ISR 0.5 curve, which presented severe inhibition from day 5, exhibited the lowest peak in MPR. A tendency observed was that the lower the ISR, the longer time span over which methane was being produced. Also, the higher the ISR, the earlier the MPR peaks were observed. This may be due to the higher microbial activity present in the larger amount of inoculum used for higher ISRs. Increasing the ISR increases the amount of active methanogens in the inoculum, which moreover decreases the time required for the growth of sufficient methanogenic population to initiate methanogenic activity.

Alkalinity measurement was taken after digestion (Table 2) for all samples, with the exception of ISR 0.5, which ranged from 7.9 to 8.9 expressed as g $CaCO_3$/L. This exceeds the average buffering capacity needed to provide resistance to significant and rapid changes in pH (Raposo *et al.* 2011). However, the lag phase observed in curves of ISRs 1.0 and 0.8 can also be attributed to a slight drop in the pH due to VFA accumulation, and converting carbon dioxide to bicarbonate will require a time lag for gas equilibrium to occur.

Many authors (Chudoba *et al.* 1991; Neves *et al.* 2004; Raposo *et al.* 2006) clearly state that the ISR used is one of the most important parameters in batch tests. Previous research shows that the use of high ISR (2 to 4) is recommended because it ensures microbial activity, and there is low risk of overloading and inhibition because the substrate is more dilute. Similar results are found in Raposo *et al.* (2006) which reported that at different ISRs, the ultimate methane yields had slight variations; however a ISR of 1 resulted in higher maximum specific methane production rate.

Anaerobic Degradability

Knowing the empirical formula of the substrate utilized throughout the experiments and applying the Buswell equation (Buswell and Mueller 1952), the maximum theoretical methane production (M_{th}) was calculated giving 364 mL of CH_4/gVS. By applying the formula previously indicated (Eq. 3), the anaerobic degradation of the samples for each of the ISR tested was determined.

The highest degradability rate was for ISR 2.0, reaching 91.5%, followed closely by ISR 0.8, with 89.3%, and ISR 1.0, with 81.7% degradability. As previously stated, ISR 0.5 had an inhibition process and resulted in a low anaerobic degradability of 16.3%.

During anaerobic digestion, the organic fraction of the substrate is mostly converted to methane and carbon dioxide. However, other fractions are converted to sulfide by sulfate reducing bacteria, which compete with methanogens (Chynoweth *et al.* 2001), and it is estimated that about 5 to 15% of the organic fraction removed is used in the generation of new biomass (Owens and Chynoweth 1993). Therefore, an anaerobic degradability higher than 85% may be considered to be in the maximum range and implies a high degradable substrate/system for methane production. In this study, samples for ISRs of 2.0, 1.0, and 0.8 represent an efficient anaerobic degradation profile, and samples of ISR 0.5 are considered outliers.

The interlaboratory study performed by Raposo *et al.* (2011) describes the anaerobic degradability of substrates with high volatile solids content that are naturally biodegradable (starch, cellulose, gelatine and mung bean). Their results demonstrate the high anaerobic degradability achieved that ranges from 85 to 88% and assumes that the substrates can be fully degraded and hence the average amount of organic matter used for cell metabolism and new cells calculated by subtraction was around 12 to 15%. A similar behavior was found with the substrate in this study. Due to the specific production parameters of the AaltoCell™ process, the resulting wastewater was partially hydrolyzed, had a low sulfur content, and had a high content of naturally biodegradable sugars ranging from 30 to 80 g/L. Such a mixture led to a high anaerobic degradability and methane production.

However, comparing these results with other effluents from the pulp and paper industry, one can observe a vast difference. Ekstrand *et al.* (2013) carried out research with 62 samples of wastewater from 10 different processes in pulp and paper mills, from which only 19% of samples reached a degradability ranging from 50 to 65%. The reduced degradability may be attributed to the high content of lignin and sulfur compounds that are dissolved during the typical pulping processes into the wastewater. The results found in the present study show the potential and suitability for enhanced and rapid methane production of this particular MCC wastewater over other studied pulping effluents.

Kinetic Evaluation

Specific methane production is often modelled using the first-order kinetic model, a simple and useful model that has been commonly applied to anaerobic digestion systems (Raposo *et al.* 2011) and follows Eq. 4,

$$\mathrm{Y} = \mathrm{Ym}\,(1 - e^{-kt}) \tag{4}$$

where Y is the cumulative methane yield at time t, mL CH_4/gVS added; Ym is the ultimate methane yield, mL CH_4/gVS added; and k is the first-order rate constant, d^{-1}. The parameters Ym and k may be estimated using a nonlinear regression fit to the experimental yield data of a triplicate set.

This model however is recommended when hydrolysis is the rate limiting step in the anaerobic digestion process and there is an assumption that there is no accumulation of intermediate products such as VFA in the system (Veeken and Hamelers 1999; Yu *et al.* 2013). In this study, samples of ISR 0.5 showed total inhibition and therefore were considered as an outlier and not taken into further consideration. Curves of ISR 1.0 and 0.8 showed a lag phase, which is assumed to be caused by the slight accumulation of VFA; hence, this model does not present the best fit for the experimental data of ISR 1.0 and 0.8, generating very low rate constants of 0.0015 and 0.0007 d^{-1}, respectively.

For ISR 2.0, the experimental data showed an adequate fit to the model (Fig. 3), and by nonlinear regression using the Microsoft Solver tool, a rate constant of 0.24 d^{-1} was calculated. This rate constant is consistent with other studies using the same ISR of 2.0 for glucose-based substrates that rapidly degrade, such as starch and cellulose (Raposo *et al.* 2011).

The same substrate used in this study has not been tested in previous literature. For the MCC wastewater substrate, the kinetic rate constant was clearly affected by the ISR. Lower ISR (1 to 0.5), meaning a higher substrate concentration, resulted in lower rate constants and did not follow the first-order kinetic model.

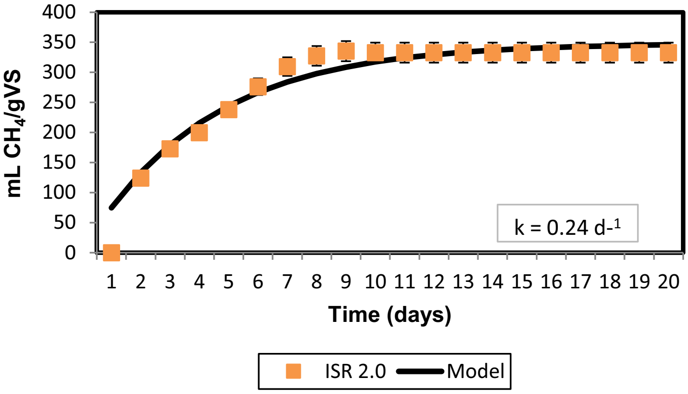

Fig. 3. Experimental data obtained from ISR 2.0 fit to the first-order kinetic model

Energy Estimation for the AaltoCell™ Process

As estimated by Vanhatalo *et al*. (2014), the MCC production process generates a total amount of 62,778 tons/year of wastewater, of which 3333 tons/year are dissolved sugars. Therefore, one can assume that there is a total weight of 3333 tonVS available for anaerobic digestion. The experimental BMP test results show that the ultimate methane potential using an ISR of 2.0 is 333 mL CH_4/gVS, which is equivalent to 333 m^3 CH_4/tonVS; therefore, an estimated amount of 1,109,889 m^3 of methane can be produced per year. Considering the higher heating value (HHV) of methane (55.5 MJ/kg) and its density at STP (0.716 kg/ m^3), the calculated amount of energy that can be generated in the MCC mill is 44,105 GJ/year.

The total amount of natural gas used per year in the MCC AaltoCell™ process is 5 GJ/BDt (Bone Dry tons); considering a mill capacity of 30,000 BDt of MCC per year (Vanhatalo *et al*. 2014), the annual natural gas demand amounts to 150,000 GJ/year. Therefore, the produced methane from the anaerobic digestion of the MCC wastewater can be used to replace 29.4% of the natural gas demand in a MCC mill.

A general theoretical calculation based on the sugar content of the wastewater can be estimated. Having a generated amount of 3,333,000 kg of sugars and knowing that 1 g of glucose is equivalent to 16,720 J, one can calculate a potential of 55,728 GJ/year. Another way of estimation is based on the theoretical yield of a molecule of glucose (Buswell equation), which renders a methane potential of 418 mL CH_4 per gram of glucose. This means 3333 tons of sugars produces 1,393,194 m^3, corresponding to 55,363 GJ/year.

A more specific calculation of the theoretical energy production is by application of the Buswell equation (Buswell and Mueller 1952) to the chemical formula of the wastewater determined by element analysis. As described in the "Anaerobic Degradability" section, the theoretical methane potential of the substrate wastewater was

364 mL of CH_4/gVS. This would therefore produce 1,213,212 m^3 of methane, which can potentially generate 48,211 GJ/year of energy.

The low deviations obtained between the theoretical and experimental energy yields (equal or lower to 21%) suggest that the proposed model in this study could effectively predict the energy production obtained in a reactor. These results could lead to a pilot scale operation that can be translated into industrial application and pave the way for a process change in wastewater treatment for pulp and paper mills. Benefiting from an energy recovery of 29.4% of the total energy consumption of a MCC mill would mean economic benefits and lower carbon footprint. Moreover, the application of anaerobic digestion can reduce costs incurred in the treatment of the effluents, where there is less sludge produced, lower retention time, and overall less costs for waste management.

CONCLUSIONS

1. Results of this study demonstrated success of an effective batch mesophilic anaerobic digestion of wastewaters originating from the production of MCC at ISR 2.0, 1.0, and 0.8. Ultimate methane yields of ISR 2.0, 1.0, and 0.8 after 21 d of incubation showed slight variations with values of 332.9, 297.4, and 325 mL CH_4/gVS, respectively. However, methane production rate curves indicate that ISR 2.0 achieved its ultimate methane yield in 44% of the degradation time of ISR 1.0 and 0.8.
2. The ISR of 0.5 showed a process imbalance due to accumulation of acids originating from the higher concentration of dissolved sugars in the substrate and causing a complete cessation of methane production after day 5 and a final pH value of 5.6.
3. Both curves of methane potential and MPR as well as kinetic evaluations suggest that an ISR of 2.0 is the adequate to achieve an efficient maximum methane yield with a high degradability of 91.5%. The amount of inoculum is then sufficient to have the suitable methanogen that are able to rapidly transform the organic acids into methane and create an equilibrium in production and consumption of intermediate products.
4. The ISR plays a critical element in the BMP test, and working with high ISR is the way to obtain a reproducible kinetic constant.
5. Considering the volume and VS content of wastewater produced in a MCC mill using the AaltoCell™ process, by anaerobic digestion of their wastewater a total energy amount of 44,105 GJ/year can be produced, which can be used to replace 29.4% of the natural gas demand.

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Effect of Fe–Zn–Mg–Al hydrotalcites on the methane potential of synthetic sulfate-containing wastewater

Lourdes Maria Rodriguez-Chiang[a,b,*], Jordi Llorca[b], Olli Pekka Dahl[a]

[a] Department of Forest Products Technology, School of Chemical Technology, Aalto University, P.O. Box 16300, Vuorimiehentie 1, Espoo, Finland
[b] Institute of Energy Technologies, Technical University of Catalonia, Barcelona, Spain

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ABSTRACT

Three hydrotalcites of M^{2+}–Mg–Al were synthesized using the co-precipitation method, where M^{2+} was Fe^{2+}, Zn^{2+} and $Fe^{2+} + Zn^{2+}$. The hydrotalcites and their calcined form of mixed oxides obtained by their thermal decomposition were characterized by FTIR, XRD and SEM. Subsequently their effect on the methane potential of synthetic wastewater comprised of sucrose and sulfur was evaluated in a multiple batch system at $37 \pm 0.5\,^{\circ}C$. The best methane potential was observed from the Fe–Zn–Mg–Al hydrotalcite at 500 mg/L yielding 372 mL CH_4/gVS which corresponds to an 8.1% increment against the control. The better performance of the Fe–Zn–Mg–Al hydrotalcite can be attributed to the Zn^{2+} ions. These react with S^{2-} in the substrate to yield zinc sulfide and therefore prevent it from forming H_2S by sulfate reducing bacteria, meanwhile reducing competition for methanogens to form methane. Calcined hydrotalcites neither stimulated nor inhibited the methane production which suggests that the enhancement of methane produced by the Fe–Zn–Mg–Al hydrotalcite was related to the presence of Fe^{2+} and Zn^{2+} cations incorporated and immobilized in the layered sheet structure of the hydrotalcite.

1. Introduction

The ongoing need to promote the production and use of renewable energy has opened way to research for improving the mature technologies found nowadays. Upgrading and optimization of industrial process design has led to increased energy efficiencies where a main focus is also energy recovery. Anaerobic digestion of organic waste and wastewater is a well-established treatment technology, it not only destroys pathogenic organisms and reduces problems associated with management and disposal of waste, but it also allows potential energy recovery in the form of methane-rich biogas. Methane derived via anaerobic digestion is often described as an ideal fuel [1,2]. It requires low energy to be produced, generates limited atmospheric pollutants and less carbon dioxide per unit energy. It will produce roughly 47% less carbon dioxide than coal and 24–27% less carbon dioxide than gasoline and diesel [3]. Methane can be easily distributed with existing pipelines making it available for domestic, municipal and industrial use and depending on its purity; it can be used for appliances, vehicle fuel, industrial applications and power generation.

Anaerobic digestion is widely applied as wastewater treatment in various types of industries and commonly in agriculture and the municipal sector. However for the pulp and paper industry it has received attention only in the recent past years. The pulp and paper industry is one of the most water consuming industries and although much effort has and is been implemented in reducing their water footprint it still generates high volumes of wastewaters, from 13 to 30 m^3 of water per ton of produced paper, with very particular characteristics [4]. One of the common characteristic is the presence of sulfur compounds due to the chemicals used in kraft pulping; which is the most widely used type of chemical pulping. Kraft pulping uses hydrogen sulfite (HSO_3^-) as the main chemical, resulting in sodium sulfide (Na_2S), sodium sulfate (Na_2SO_4), sodium sulfite (Na_2SO_3) and sodium thiosulfate ($Na_2S_2O_3$) present in the process effluent [4]. Wastewaters are also characterized by high biochemical oxygen demand (BOD), high chemical oxygen demand (COD), chlorinated compounds (measured as adsorbable organic halides AOX), suspended solids (mainly fibers), fatty acids, tannins, resin acids, lignin and other wood extractives [5,6].

Usually pulp and paper mills treat their wastewaters in aerobic activated sludge plants, which consequently create large production of waste sludge. This led to an increase of studies on various pretreatment methods in order to enhance the methane

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* Corresponding author at: Department of Forest Products Technology, School of Chemical Technology, Aalto University, P.O. Box 16300, Vuorimiehentie 1, Espoo, Finland.
E-mail address: lourdes.rodriguezchiang@aalto.fi (L.M. Rodriguez-Chiang).

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Table 1
Methane potential of different wastes from the pulp and paper industry.

Substrate	Methane yield (NmL/gVS)	Reference
Poplar biomass waste	230–320	[1]
Willow biomass waste	130–300	[1]
Pulp and paper mill biosludge	40–200	[2]
Bleached kraft secondary sludge	50	[7]
Thermomechanical secondary sludge	89–197	[7]
Sulphite secondary sludge	159	[7]
Kraft secondary sludge	145	[7]
Chemithermomechanical secondary sludge	97–199	[7]
Bleached kraft and paper secondary sludge	108	[7]
Microcrystalline cellulose wastewater	333	[1,27]

potential of pulp and paper sludge [2,7,8]. Buyukkamaci and Koken [9] explain the economic and effective alternative of combining anaerobic wastewater treatment with aerobic post treatment. This is due to the fact that in most cases the application of only anaerobic treatment does not deliver treated effluent of sufficient quality. However by implementing anaerobic treatment first, it reduces the organic load to the aerobic treatment; which in many cases is overloaded; and causes less sludge production. Moreover there is the potential of energy recovery which gives the economic incentive. Nonetheless, few literature is found on the anaerobic digestion and methane enhancement of pulp and paper wastewater.

Meyer and Edwards [2] reviewed the anaerobic digestion and methane potential of different effluents coming from pulp and paper mills. Depending of the type of pulping and the chemical used during the pulping process the COD removal and methane productions vary greatly (Table 1). They found that condensate streams from chemical pulping have the highest COD removal rates (75–90%). The lowest rates correspond to chemical sulfite pulping effluents (29–38%) and debarking effluents (44–70%) from the mechanical pulping; mostly due to sulfur inhibition and concentration of lignin and resins respectively. Methane production followed the same pattern. Bleaching effluents in general were found to have inhibitory compounds for anaerobic digestion having the lowest methane production (0–380 mL kg^{-1} COD removed). Effluents with the highest methane potential were from the neutral sulfite chemical pulping (NSSC) condensate (380–400 mL kg^{-1} COD removed), where there is high acetic acid concentrations contributing to the direct formation of methane. They concluded that the numerous studies conducted show that, contrary to common perception, most mill effluents were to some extent anaerobically treated even the difficult to digest streams. This suggests the possible enhancement of methane production of these effluents by means of pretreatment, co-digestion or catalysis.

Hydrotalcites (HTs) or layered double hydroxides are anionic clays that have a broad spectrum of applications such as catalysts, pharmaceuticals, absorbents, ion exchangers and many more applications arising due to the possibility of designing them tailored to specific reactions and/or substrates [10,11]. HTs and their calcined products have also gained attention in wastewater treatment and purification for its high ion-exchange capacities for adsorption of various anionic pollutants which include bromide, arsenic, lead, fluorine, chromate and other toxic anions [12–16]. Douglas et al. [17] studied the formation and effect of HTs in the treatment of mining wastewater and acidic wastewaters. They report results of optimal removal of a broad spectrum of contaminants from the wastewaters, including uranium. HTs have also been employed to increase hydrogen production in fermentation processes [11,18].

HTs compounds have a brucite-like structure with stacked layers of metal cations following the general formula of $[M_{1-x}{}^{2+} M_x{}^{3+} (OH)_2] [A_{x/m}]^{m-} \cdot nH_2O$, where M^{2+} is a divalent cation, M^{3+} is a trivalent cation, A an interlamellar anion with charge m^- and x is the ratio of $M^{+3}/(M^{+2} + M^{+3})$ [15]. For this study two anions (Fe^{2+} and Zn^{2+}) were chosen to be incorporated into the HT structure by co-precipitation; in order to assess their influence on the improvement of anaerobic digestion and methane potential of the substrate. The functionality of iron in the anaerobic process is well known [19]. Iron is essential for microbial growth and is an important component of many of the enzymes involved in the metabolic pathways of bacteria; it also enhances the granulation process. Methanogens have a specific growth requirement for iron, and concentrations of 2.6 g L^{-1} of $FeCl_2$ have increased conversion of acetic acid to methane. However if present in high concentrations (5.6 g L^{-1}) it becomes toxic [19,20]. Though iron is one of the most abundant elements, its bioavailability is limited since the majority is in the insoluble 3+ state. Casals et al. [21] studied iron oxide nanoparticles in anaerobic digesters and found an increase in biogas production of up to 40% when 0.15 mg/mL iron sulfate was added, concentrations higher than resulted in a dramatic decrease in biogas production. Zinc was chosen for its ability to remove sulfur components [22,23]. Another key characteristics is the nano-scale particle size of HTs, this provides large surface areas (20–120 m^2/g) that may possibly boost bacterial anchoring in the anaerobic process [24]. Nanoparticles have the advantage of reacting rapidly with the electron donors leading to kinetic improvements and also act as biocatalysts enhancing the activity of microorganisms [25]. This study addresses the importance of evaluating a new pretreatment for pulp and paper effluents. The idea of boosting methane production from these waste streams and generating energy is becoming more widespread through the pulping industry and the need for different pretreatment options is clearly noticeable. In this study, M^{2+}–Mg–Al HTs with combinations of Fe and/or Zn metal ions, were synthesized with the co-precipitation method and characterized. Most importantly, their effect on the methane potential of synthetic sulfate-containing wastewater was evaluated.

2. Materials and methods

2.1. Hydrotalcite preparation

Hydrotalcites of M^{2+}–Mg–Al were synthesized using the co-precipitation method as described in the literature [26], where M^{2+} was Fe^{2+}, Zn^{2+} and $Fe^{2+} + Zn^{2+}$. The ratio between the molar fraction of divalent and trivalent cations (M^{2+}/M^{3+}) was 3:1. In this method two solutions were prepared. The first aqueous solution consisted of a 300 mL mixture of metal nitrates of $Mg(NO_3)_2 \cdot 6H_2O$ and $Al(NO_3)_3 \cdot 9H_2O$ and/or $FeSO_4 \cdot 7H_2O$, and $Zn(NO_3)_2 \cdot 6H_2O$ in the desired molar ratios. The second solution was prepared by dissolving NaOH (2.25 M) and Na_2CO_3 (0.45 M) in 300 mL of distilled water. The second solution was added to the first solution dropwise at a rate of 50 mL/h under vigorous stirring until the pH reached around 9–10. The thick slurry formed was aged in a thermostatic bath for 18 h at 60 °C. Afterward, the slurry was filtered and washed thoroughly with deionized water in order to remove the alkaline metals and nitrate ions until the filtrate effluent reached a neutral pH. Finally, the filter cake was dried at 110 °C for 24 h and ground in a mortar. Calcined HTs were obtained by placing ground HTs in a muffle furnace at 500 °C for 4 h.

2.2. Hydrotalcite characterization techniques

The structures of HTs as prepared and after calcination were analyzed by X-ray diffraction (XRD) technique using a Bruker D8 instrument equipped with a Cu target and a graphite monochromator. XRD patterns were recorded at 40 kV and 40 mA by using Cu radiation ($\lambda = 0.15406$ nm) at a rate of 0.02°/s from $2\theta = 5–75°$. The average crystals sizes (Dc) were estimated from full width at half maxima (FWHM) of the XRD peak with maximum intensity by using Scherrer Eq. (1).

$$Dc = \left(K\left(\lambda/\beta\right)\cdot\cos\theta^{-1}\right) \tag{1}$$

where K is the Scherrer constant (0.89), λ is the X-ray wavelength (1.54 Å), β is the line broadening at half the maximum intensity (FWHM) in radians, and θ is the diffraction angle. Fourier transform infrared spectroscopy (FTIR) was carried out with a Nicolet 6700 FTIR spectrometer. Spectra were recorded with a resolution of 4 cm^{-1} over the wavenumber range 4000–400 cm^{-1}. The morphologies of the HT samples were examined with a Neon40 scanning electron microscope (SEM) Crossbeam Station (Zeiss) equipped with a field emission electron source. For size distribution histograms of 100 particle measurements were taken.

2.3. Inoculum and substrate for BMP

Biochemical Methane Potential (BMP) tests were performed in batch experiments to determine the HTs effect on methane production. The inoculum used for experiments originated from Suomenoja municipal wastewater treatment plant located in Espoo, Finland. It was taken fresh from their mesophilic anaerobic digester and degassed at the same operating temperature (37.0 °C) prior to the start of the experiment. Analyses resulted in total solids (TS%) of 1.64 ± 0.05, volatile solids (VS%) of 0.88 ± 0.04, and moisture content (%) of 98.36 ± 0.05. Values represent the average ± STD of three samples. A pH value of 7.3 was measured at the start of the experiments, total alkalinity (TA) of 6.3 g $CaCO_3$/L and a conductivity of 6.32 mS/cm.

The substrate used for this study was synthetic wastewater simulating the composition of pulp and paper wastewater which includes average COD concentration of 5000 mg COD/L and 200 mg/L average concentration of sulfate. The synthetic wastewater used in the experiments comprised of (mg/L): sucrose ($C_{12}O_{22}O_{11}$) 3000; sodium acetate ($C_2H_3NaO_2$) 2000; magnesium sulfate ($MgSO_4$) 1540; ammonium chloride (NH_4Cl) 950; potassium dihydrogen phosphate (KH_2PO_4) 220; Calcium chloride ($CaCl_2$) 150 and sodium bicarbonate ($NaHCO_3$) 2000. Substrate was prepared to achieve a ratio of COD:N:P of 100:5:1 to ensure nutrient requirements, it was later stored at 4 °C prior to its use. The fresh substrate was analyzed using average values of triplicates resulting in total solids (TS%) of 0.73, volatile solids (VS%) of 0.35, moisture content (%) of 99.26, COD of 5000 mg/L, pH of 7.66 and conductivity of 6.98 mS/cm. The elemental composition of the substrate was C (25.21%), H (3.82%), N (0.10%), and S (2.35%).

2.4. Experimental design

Experiments in order to test the effect of HTs addition were carried out in an automatic methane potential test system (AMPTS II), which is a laboratory scale, multiple batch system developed for automatic real-time logging and measuring of methane production [27–29]. Measurements are expressed using the same unit for conventional BMP test found in literature; normalized mL of methane per gram of volatile solids added (N mL CH_4/gVS). It has a capacity for incubating 15 reactors of 500 mL each with an individual mixing motor and a defined carbon dioxide removal step in order to provide methane yield.

Three different hydrotalcites were evaluated: Fe–Mg–Al, Zn–Mg–Al and Fe–Zn–Mg–Al, as well as each one in its calcined form. Methane potential of samples were initially evaluated by adding 500 mg/L of HT, which was the optimal dose found by Wimonsong et al. [11] for the production of hydrogen and the same optimal dose suggested by Terry et al. [30] for the removal of Cr (VI) from aqueous solution with hydrotalcite. This concentration is equivalent to a ratio of HT:COD of 0.1:1. The HT that produced the highest methane increment was later tested at different ratios in order to evaluate the effect of HT concentration. All samples were prepared in triplicates for statistical significance. The BMP tests were carried out using a working volume of 400 mL and an inoculum to substrate ratio of 2, in order to ensure enough microbial population as suggested in Angelidaki et al. [31]. Triplicate blank samples with no substrate were run to determine the produced background methane originating from the inoculum alone. Triplicate control samples containing only inoculum and substrate and no hydrotalcite addition were run in parallel. After filling each reactor, bottles where sealed with a hermetic rubber stopper connected to a mechanical agitator and placed in a water incubator at 37.0 ± 0.5 °C. To chemically remove carbon dioxide (CO_2) and hydrogen sulfide (H_2S) formed during anaerobic digestion, each reactor was individually connected to another small bottle containing 80 mL of an alkali solution of 3 M NaOH. Thymolphthalein pH indicator was added to each bottle to determine when the solution has been spent and needed replacement. Each alkali solution bottle was then connected to the measuring device and finally all reactors where flushed with pure nitrogen gas (N_2) for 5 min, to ensure anaerobic conditions.

2.5. Analytical methods

Total solids (TS), volatile solids (VS), and moisture content in fresh samples of substrate and inoculum were determined gravimetrically following Standard Methods described in APHA [32]. COD was measured by Standard Method 5220. Total alkalinity (TA) to pH 4.5 was measured by Standard Method 2320 B. The pH measurements were performed using a pH meter Thermo Scientific model Orion 2-star pH-Benchtop. Conductivity was taken with a conductivity meter Orion Model 150. Elemental analysis of the substrate was determined by duplicate samples using a Perkin Elmer Model 2400 Series II CHNS Elemental Analyzer (USA).

Methane production was measured using the AMPTS II from Bioprocess Control AB, Sweden (System Version 2.0 V1.08), which works by the principle of liquid displacement and buoyancy. Volumes of gas are corrected to standard temperature and pressure (STP) conditions at 273 K and 1013 mbar air pressure. Biochemical methane potential was calculated as the accumulated methane produced per gram of VS added to each reactor, as determined in Eq. (2) [33]:

$$\mathrm{BMP} = \frac{V_{sample-}V_{inoc}\left(\mathrm{gVS}_{is}/\mathrm{gVS}_{ib}\right)}{\mathrm{gVS}_{substrate}} \tag{2}$$

where BMP is the normalized volume produced per gram VS of substrate added (mLCH_4/gVS), V_{sample} is the mean value of accumulated methane produced from the reactor with both inoculum and substrate, V_{inoc} is the mean value of the accumulated volume produced by the blanks with only inoculum, gVS_{is} is the mass of volatile solids of the inoculum added in the sample, gVS_{ib} is the mass of volatile solids of the inoculum added in the blanks, and $gVS_{substrate}$ is the mass of volatile solids of the substrate added in the reactor.

3. Results and discussion

3.1. Hydrotalcite characterizations

The X-ray diffraction profiles of the HTs prepared are shown in Fig. 1. All diffractograms showed a similar pattern, characteristic of a well crystallized HT structure described in the literature [11,34]. Sharp peaks were observed at low diffraction angles of 2θ: 11°, 23°, and 35°, corresponding to the crystal planes of (0 0 3), (0 0 6) and (0 1 2), respectively. Broader peaks were observed at 2θ: 39°, 46°, 61°, and 62° corresponding to the crystal planes of

Table 2
HTs lattice parameters and crystal sizes from XRD.

HT sample	Atomic ratio	Chemical formula	Lattice parameters		Crystal size
	M^{2+}:Mg^{2+}:Al^{3+}		a (Å)	c (Å)	(nm)
Fe–Mg–Al	0.5:2.5:1	$[Fe_{0.5}Mg_{2.5}Al\,(OH)_8](CO_3)_{0.5}\cdot2.5H_2O$	3.056	22.800	25
Zn–Mg–Al	0.5:2.5:1	$[Zn_{0.5}Mg_{2.5}Al\,(OH)_8](CO_3)_{0.5}\cdot2.5H_2O$	3.062	23.220	39
Fe–Zn–Mg–Al	0.25:0.25:2.5:1	$[Zn_{0.25}Fe_{0.25}Mg_{2.5}Al\,(OH)_8](CO_3)_{0.5}\cdot2.5H_2O$	3.064	22.971	28

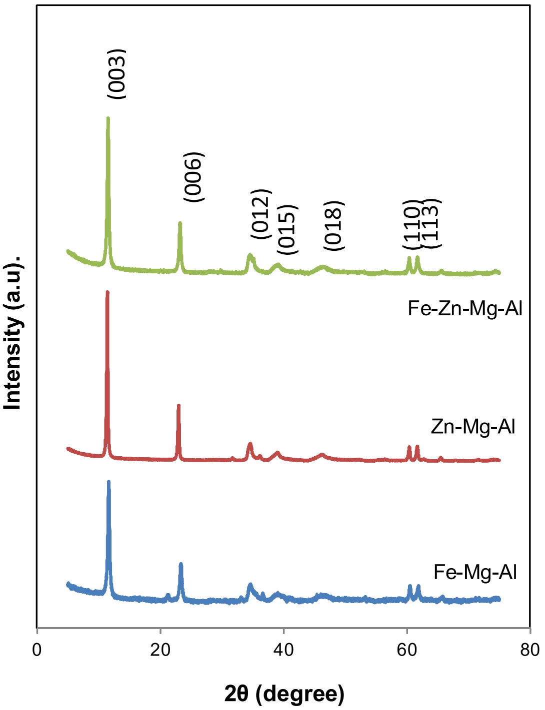

Fig. 1. X-ray diffractograms of HTs.

(0 1 5), (0 1 8), (1 1 0), and (1 1 3), respectively; also representative for hydrotalcite-like compounds. The characteristic peaks for the Fe–Mg–Al HT were somewhat broader, which indicated that the HTs samples containing Fe^{2+} were smaller in size. This result is validated by the estimated average crystal size using the Scherrer equation. The lattice parameters were calculated for a hexagonal unit cell on the basis of rhombohedral R−3 m symmetry. Crystal size and lattice parameters of HTs are shown in Table 2. The parameter “a” ($a = 2\cdot d_{110}$) was calculated as cation-cation distance within the brucite-like layer while the parameter “c” ($c = 3\cdot d_{003}$) is related to the thickness of the brucite-like layer and the interlayer distance [35]. The decrease in the lattice parameter for the Fe HT samples is due to the radius of the divalent metal (Fe^{2+} = 0.061 nm), which is smaller than the radius of Mg^{2+} = 0.072 nm and Zn^{2+} = 0.074 nm.

The XRD profiles recorded from calcined HTs can be observed in Fig. 2. Calcination of hydrotalcites induces the formation of the corresponding Mg-Al oxides as well as removal of hydroxyl groups and interlayer anions. The presence of diffraction peaks are clearly observed at $2\theta = 43°$ and 63° which correspond to a MgO-like phase (periclase) [36]. Peaks of Al_2O_3 phase are almost absent, indicating that Al^{3+} cations are dispersed in the structure of MgO or forming an amorphous phase. Calcined Zn–Mg–Al HT exhibited peaks corresponding to the formation of zinc oxide. By application of the Scherrer equation the crystal sizes of calcined HTs were determined: Fe–Mg–Al, 4 nm; Zn–Mg–Al, 5 nm and Fe–Zn–Mg–Al, 4 nm. This indicated the smaller size crystals compared to the uncalcined samples and which is also observed through SEM.

FTIR spectra of the HTs are shown in Fig. 3. In all the samples, a broad absorption band in the range of 3400–3500 cm^{-1} and 1610–1650 cm^{-1} are designated to the O-H stretching vibration and bending vibration of interlayer water molecules, respectively [16]. The strong peak at 1365 cm^{-1} indicates the presence of the carbonate anion in the interlayer region of the HTs. The bands in the interval of 500–800 cm^{-1} are attributed to a stretching of metal–oxygen bonds.

The morphology of the HTs was analyzed by SEM. Fig. 4. shows representative images; in all cases there was a very homogeneous distribution of crystal aggregates. For uncalcined HT samples the average aggregate size was 50–60 nm and in calcined samples aggregates ranged from 40–50 nm.

3.2. Methane production and hydrotalcite effect

The methane production of synthetic sulfate-containing wastewater was tested with the produced hydrotalcites. Initially, all three HTs and their calcined form were tested at a concentration of 500 mg/L. The respective accumulated methane yields of samples can be observed in Fig. 5.

All three hydrotalcites demonstrated an increase in production compared to the control sample with no HT addition (Table 3). Both HTs that contain Zn presented the highest methane yield; Zn–Mg–Al HT and Fe–Zn–Mg–Al HT had an increment of 7.4% and 8.1% respectively. Fe–Mg–Al HT had a 4.8% increment against the control. COD removal efficiencies presented the same pattern, ranging between 80–84%.

Anaerobic Biodegradability (BD) of the substrates can be estimated knowing the experimental BMP and the relation between COD/VS. Considering the substrate used in this study is composed of sucrose and acetate; a theoretical 1.19COD/VS ratio can be calculated by stoichiometric oxidation of the organic material [37]. Since it is known that the theoretical methane yield of 1 gram COD is 350 mL CH_4 at STP [38]. Eq. (3) expresses the relation of BMP and biodegradability:

$$BD = \frac{BMP\left(mLCH_{4,STP}/gVS\right)}{350 \times COD_{waste}\left(gCOD/gVS\right)} \quad (3)$$

Although this equation neglects the fraction of substrate used for generation of new biomass and assumes all COD is converted to methane [39], it is still a good assessment criterion for biodegradability. Table 3 shows the anaerobic degradability of samples. Uncalcined HT samples ranged from 86–89% degradability, whereas calcined samples had a degradability of 82–84%.

The purpose of sulfur addition to the synthetic wastewater used in the experiments was to determine if the different metal ions co-precipitated in the Mg-Al HT had any effect on the final methane potential. During anaerobic digestion, sulfate or sulfite is anaerobically reduced to sulfide by sulfate reducing bacteria (SRB). These bacteria use sulfate as a terminal electron acceptor for the degradation or organic material and hydrogen or organic matter as an electron donor [40]. SRB compete with methanogens for

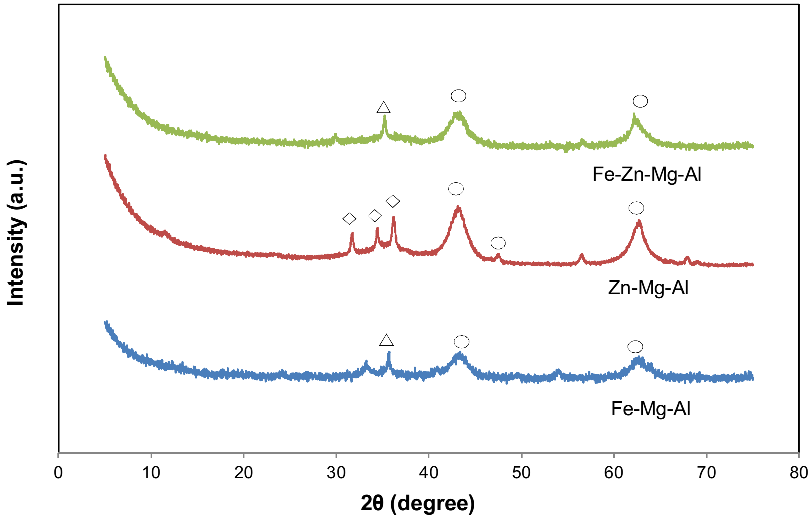

Fig. 2. X-ray diffractograms of calcined HTs, (●) MgO (periclase), (▲) Al2O3, (♦) ZnO.

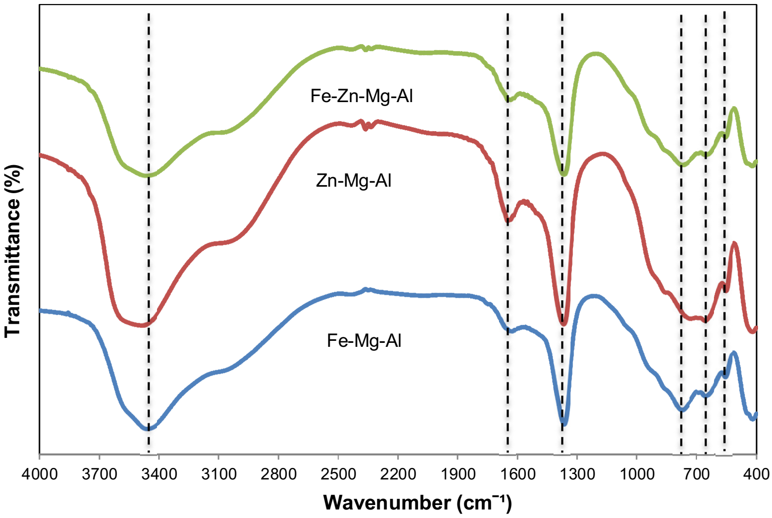

Fig. 3. FTIR spectra of HTs.

Table 3
Characterization of anaerobically digested samples with HTs and calcined HTs.

Sample HT	Initial pH	Final pH	Ultimate methane yield (mL CH_4/gVS)	COD removal (%)	Increment compared to control (%)	Anaerobic biodegradability (%)
Control	7.6	7.5	343.9	65.6	N.A.	82.6
Fe–Mg–Al	7.7	7.5	360.4	80.1	4.8	86.5
Fe–Mg–Al calcined	7.7	7.4	342.8	66.1	−0.3	82.3
Zn–Mg–Al	7.6	7.4	369.3	83.7	7.4	88.7
Zn–Mg–Al calcined	7.6	7.5	345.8	66.9	0.6	83.0
Fe–Zn–Mg–Al	7.7	7.4	371.9	84.0	8.1	89.3
Fe–Zn–Mg–Al calcined	7.8	7.5	347.7	69.4	1.1	83.5

intermediates (acetate and hydrogen). Increasing levels of sulfate result in an increased growth of SRB and consequently can decrease the methane production from 10% (at 100 mg/L SO_4^{-2}) up to 87% methane decrease at high sulfate concentrations of 10 g/L [41]. The reduced dissolved sulfide is then present as the unionized H_2S form and as HS^-. The presence of H_2S in the liquid phase reduces the COD removal efficiency and H_2S in the gas phase is an undesirable end-product of anaerobic digestion. The better performance of the HTs

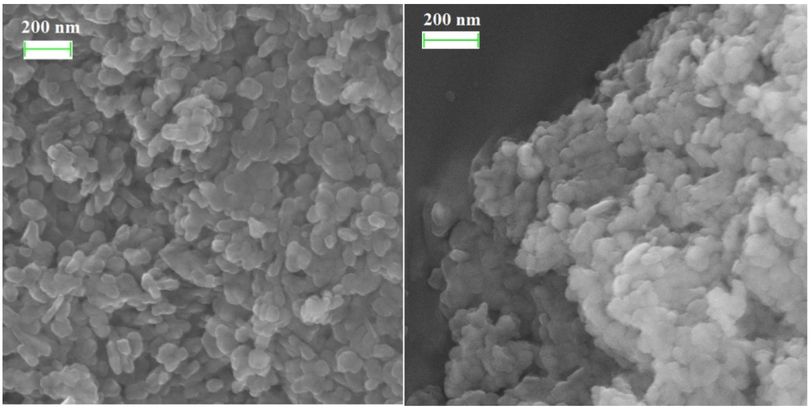

Fig. 4. SEM images for uncalcined Fe–Zn–Mg–Al HT sample (left) and calcined HT sample (right).

Energy dispersive X-ray analyses were carried out on different areas of the samples and in all cases the relative amount of M^{2+}:M^{3+} matched the nominal 3:1 value.

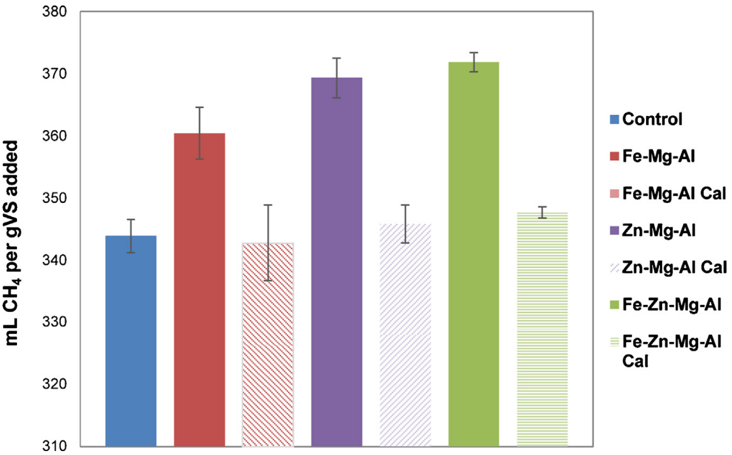

Fig. 5. Accumulated methane production of each HT at 500 mg/L dose after 90 h of digestion.

that contain Zn can be attributed to the fact that Zn^{2+} ions react with S^{2-} to yield zinc sulfide. Therefore Zn ions are more likely to react with sulfide and prevent it from forming H_2S. The sulfur trapping ability of zinc ions is, in fact, well known in the chemical industry, where it is used at a large scale in refining and reforming processes [23]. The main functionality of iron in the anaerobic digestion process is attributed to enhancing the conversion of acetic acid to methane in the methanogenesis phase [20]. It is interesting to note that several studies have proven the toxicity of Zn and ZnO nanoparticles on the anaerobic digestion process [42]. Similar results were found for iron; although it is required for microbial growth; if it is found in more than trace amounts it is generally considered as a toxic heavy metal [19]. In our study, in contrast, the Zn and Fe ions are chemically bonded in the hydrotalcite layer structure and, therefore, leaching of the metals into the system is prevented. Given the advantageous properties of Fe^{2+} and Zn^{2+} ions, a combination of both ions in the hydrotalcite structure would presumably be the most effective HT tested which is validated with the obtained results.

The calcined forms of each of HTs were also tested at the initial concentration of 500 mg/L. A minor decrease in methane production was observed for the calcined Fe–Mg–Al HT and a slight increase for the calcined Zn–Mg–Al and Fe–Zn–Mg–Al HTs (Fig. 5). COD removal efficiencies were similar to the control, between 65–70%. The thermal decomposition of the HT leads to a collapse in the layered structure and results in formation of mixed oxides with basic sites [43]. Consequently, Fe^{2+} and Zn^{2+} ions are converted to ZnO and Fe_2O_3, respectively. As observed from Fig. 5 all three uncalcined hydrotalcites have a noticeable increase in methane production compared to their calcined form, therefore their better efficiency is attributed to the presence of Fe^{2+} and Zn^{2+} cations incorporated and immobilized in the layered sheet structure of the hydrotalcite. Given that hydrotalcites and particularly their calcined form have strong basic properties, there was no apparent effect on the overall pH of each of the reactors. The final pH of all samples ranged between 7.4–7.5.

After determining that the Fe–Zn–Mg–Al HT presented the highest methane production, different concentrations of this HT were tested in order to determine the influence on the ultimate methane yield. In the same conditions as described previously, six concentrations were evaluated: 30, 60, 125, 250, 500 and 1000 mg/L (Fig. 6). The initial concentration of 500 mg/L proved to be the most effective. Concentrations of 30, 60 and 1000 mg/L presented no enhancement effect on the methane yield compared to the

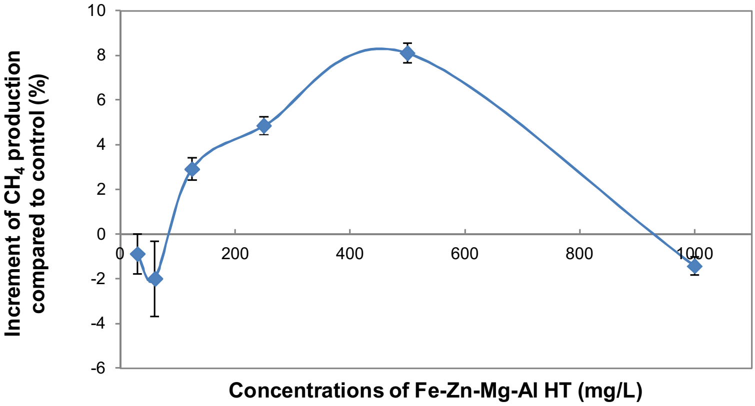

Fig. 6. Percent increments of methane production at different concentrations of Fe–Zn–Mg–Al HT after 90 h of digestion.

control. pH for all samples ranged from 7.1–7.7 except for 1000 mg/L samples where the pH was slightly higher at 7.9 assumed to be a result of the strong basic properties of HTs. From the concentrations evaluated in this study an optimal concentration of 500 mg/L Fe–Zn–Mg–Al HT can be identified. Further testing of concentrations between 500 and 1000 mg/L should be evaluated in order to determine a more exact range where methane production is boosted.

4. Conclusions

Three hydrotalcites of Fe–Mg–Al, Zn–Mg–Al and Fe–Zn–Mg–Al and their calcined form were prepared, characterized and tested in the anaerobic digestion of synthetic sulfate-containing wastewater in order to evaluate the effect on the methane potential. Results showed that Fe–Zn–Mg–Al hydrotalcite at 500 mg/L presented the highest methane production of 371.9 mL CH_4/gVS which is 8.1% higher than the control and corresponds to 89.3% anaerobic biodegradability. This is attributed to the fact that Zn^{2+} ions react with S^{2-} to yield zinc sulfide, trapping sulfur and preventing formation of H_2S. Second best on enhancing methane production was Zn–Mg–Al HT followed by Fe–Mg–Al HT. Calcined hydrotalcites did not show any significant effect on the methane potential of the substrate. This suggests that the enhancement of methane produced by the Fe–Zn–Mg–Al hydrotalcite was related to the presence of Fe^{2+} and Zn^{2+} cations incorporated and immobilized in the layered sheet structure of the hydrotalcite.

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Anaerobic co-digestion of acetate-rich with lignin-rich wastewater and the effect of hydrotalcite addition

Lourdes Rodriguez-Chiang [a,b,*], Jordi Llorca [b], Olli Dahl [a]

[a] Department of Forest Products Technology, School of Chemical Technology, Aalto University, P.O. Box 16300, Vuorimiehentie 1, Espoo, Finland
[b] Institute of Energy Technologies, Technical University of Catalonia, Barcelona, Spain

HIGHLIGHTS

- Different ratios of evaporator condensate and lignin-rich wastewater were co-digested.
- A negative linear correlation between lignin content and CH_4 potential was observed.
- The effect of hydrotalcite addition on the anaerobic digestion was evaluated.
- COD removal between 64 and 83% were obtained in all samples.
- Co-digestion improves the biodegradability of lignin-rich NSSC effluents.

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ABSTRACT

The methane potential and biodegradability of different ratios of acetate and lignin-rich effluents from a neutral sulfite semi-chemical (NSSC) pulp mill were investigated. Results showed ultimate methane yields up to 333 ± 5 mL CH_4/gCOD when only acetate-rich substrate was added and subsequently lower methane potentials of 192 ± 4 mL CH_4/gCOD when the lignin fraction was increased. The presence of lignin showed a linear decay in methane production, resulting in a 41% decrease in methane when the lignin-rich feed had a 30% increase. A negative linear correlation between lignin content and biodegradability was also observed. Furthermore, the effect of hydrotalcite (HT) addition was evaluated and showed increase in methane potential of up to 8%, a faster production rate and higher soluble lignin removal (7–12% higher). Chemical oxygen demand (COD) removal efficiencies between 64 and 83% were obtained for all samples.

1. Introduction

A common issue of pulp and paper mills is their wastewater treatment. Given that their water usage is among the most intensive in industry, efforts on reducing water consumption in pulping processes and enhancing efficiency in their wastewater treatment has become a prioritized objective. Pulp and paper mills produce large volumes of wastewaters with numerous types of contaminants that are dependent on the type of pulping process and desired end-product (Bayr and Rintala, 2012). Pulping effluents are characterized by high amounts of chemical oxygen demand (COD) and suspended solids. Furthermore, lignin and its derivatives generate a strong color and additional high COD and turbid effluent (Kamali and Khodaparast, 2014). Anaerobic treatment of pulp and paper mill effluents has gained increasing attention since the late 1980s; installed anaerobic reactors has more than doubled in the last decade (Meyer and Edwards, 2014). Nevertheless, many hurdles exist to increase its widespread application, such as the complexity to digest lignocellulosic material, the wide variations in effluent characteristics and the toxicity of some common compounds found in pulping effluents like sulfur, tannins, resin acids, and organochloride compounds.

Neutral sulfite semi-chemical (NSSC) is a type of pulping process where wood chips are initially cooked in a neutral solution of sodium sulfite and sodium bicarbonate. Sulfite ions react with the lignin in wood and the sodium bicarbonate acts as a buffer solution. The partly de-lignified soften wood chips are then mechanically disintegrated. This process is required to preserve high hemicellulose and lignin contents in order to retain the stiffness of the fibers in the semi-chemical fluting pulp (Manskinen et al., 2011). The wastewaters investigated in this study originate

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* Corresponding author at: Department of Forest Products Technology, School of Chemical Technology, Aalto University, P.O. Box 16300, Vuorimiehentie 1, Espoo, Finland.

E-mail address: lourdes.rodriguezchiang@aalto.fi (L. Rodriguez-Chiang).

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from a NSSC integrated pulp and board mill that produces high quality semi-chemical fluting. Fluting is the corrugated part between the surface liners in corrugated board. High quality fluting is needed to produce strong and durable corrugated board that is used in packaging applications.

Major effluents of the NSSC mill are the condensates from the evaporation plant, debarking plant and the circulation water with fibers (Fig. 1). Chemical recovery is practiced in the mill meaning there is no need to treat the spent liquor. Other minor effluents consist of collectable water from the liquor plant and power plant, leakage water from the landfill and sanitary water. All the effluents are collected, mixed and treated in an activated sludge plant followed by a sub-clarifier and flotation. Evaporator condensates may account for about 40% of the pulp mill's total effluent volume, 40% of the total COD load and 60% of the biochemical oxygen demand (BOD) load to the wastewater treatment plant. Evaporator condensates are acidic streams that contain mostly acetic acid (1.6–8.2 g/L), methanol (0.2–1.2 g/L), and furfural (0.2–1.0 g/L) as the major organic components (Rintala and Puhakka, 1994). As an effluent stream rich in acetic acid, it has an advantage of having readily degradable material for anaerobic digestion. Methanogenic bacteria can directly convert acetic acid into methane and carbon dioxide avoiding the initial hydrolysis step which is the rate-limiting step in the anaerobic digestion process. Different studies have investigated the anaerobic treatment of evaporator condensate (Ekstrand et al., 2013; Silva et al., 2009; Ferguson and Benjamin, 1985) and found that in spite of a general good running of the anaerobic digestion of this substrate, by itself it may cause inhibition and stability problems. Silva et al. (2009) also recognized that anaerobic treatment when an external carbon source is added to the reactor, specifically at high organic loads (2 kg COD/m^3 d^{-1} or higher) was beneficial and achieved a steady state of COD evolution.

The second largest effluent of the NSSC mill is the fiber circulation water, whose most significant characteristic is a high lignin content. The delignification treatment applied in the semi-chemical process modifies and depolymerizes the lignin resulting in a complex mixture of dissolved lignin fractions that considerably vary from native lignin in chemical composition, molecular weight distribution and physical characteristics (Sierra-Alvarez and Lettinga, 1991). Properties of lignin in wastewaters also vary according to the lignocellulosic raw material and the pulping process applied. The presence of lignin in wastewaters presents a major source of COD and organic matter however it is resistant to biological treatments. The microbial recalcitrance of lignin is due to its high molecular weight, chemical heterogeneity and the absence of regular hydrolysable intermonomeric linkages (Ruiz-Dueñas and Martínez, 2009). Lignin in aerobic conditions is depolymerized by lignolytic fungi which have the unique enzyme needed to stimulate nonspecific extracellular oxidative reactions that depolymerize lignin (Kato et al., 2015). Lignin degradation in anaerobic conditions is less studied; Kato et al. (2015) reviewed different anaerobic bacteria able to degrade lignin and lignin-derived aromatics. Acetogenic bacteria are able to utilize the O-methyl group as the carbon and energy sources, however they are unable to degrade the aromatic ring structure. Similarly, some sulfate-reducing bacteria and fermentative bacteria use the O-methyl group as carbon and energy sources, and some of them can also decompose the aromatic rings. Sierra-Alvarez and Lettinga (1991) study shows that lignin degradation is limited to the low molecular weight derivatives and only monomers and oligomers of lignin (<600 g mol^{-1}) have been shown to be mineralized whereas higher molecular weight lignin is not anaerobically degradable. Interestingly, they also discovered that the inhibitory effect of lignin towards methanogenic bacteria originated from compounds in the low molecular weight fraction. Moreover, various studies have proven the antimicrobial and antibiotic properties of lignin (Gregorova et al., 2011; Erakovic et al., 2014) in which polyphenol compounds of lignin are known to damage the cell membranes of microorganisms and cause lysis of the bacteria; this most likely influences the toxicity towards the anaerobic microbial community.

Numerous studies have stated the successful and beneficial result of co-digestion of easily degradable substrates with hard to degrade wastes. Specifically to pulp and paper wastes, (Lin et al., 2012; Hagelqvist, 2013; Bayr and Rintala, 2012) studies found that co-digestion improved the overall efficiency, resulted in higher methane yields and a more stable digestion process. These studies underline the importance of making highly polluted and hard to treat substrates into renewable energy recovery by co-digestion. In this investigation, the co-digestion of wastewaters rich in acetic acid and lignin was studied in batch biochemical methane potential (BMP) tests at different ratio mixtures, for the first time. Furthermore, the addition of hydrotalcite (double layered hydroxides) was tested in order to measure its effect on the ultimate methane potential of substrates. Hydrotalcites (HTs) are anionic clays that have been used in a wide range of applications because of the possibility of designing them tailored to specific reactions and/or substrates. In recent years they have been studied in the treatment of various industrial wastewaters, as they can

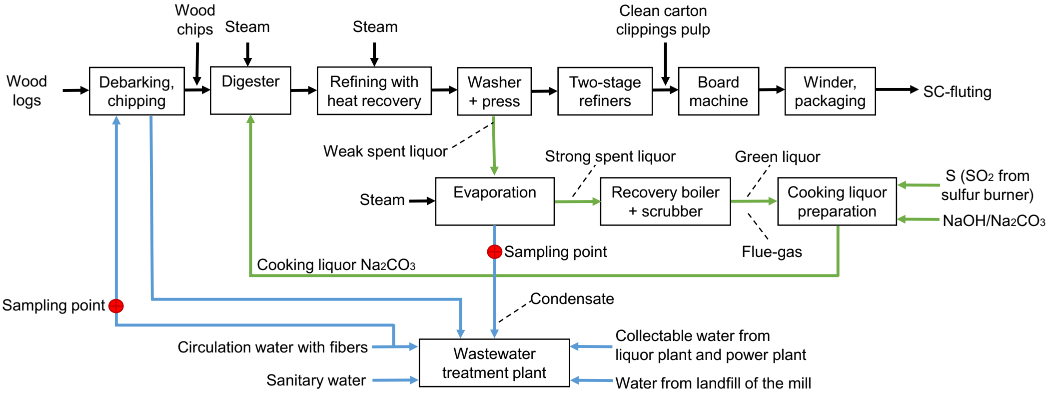

Fig. 1. Heinola NSSC process diagram with marked sampling points. Source: Stora Enso Oyj Heinola Fluting Mil.

tackle different contaminants in water due to their high ion-exchange capacities for adsorption (Wajima, 2014) and are able to depolymerize lignin (Kruger et al., 2016). HTs have also been proven to increase hydrogen production in fermentation processes (Wimonsong et al., 2014) and methane production in anaerobic digestion (Rodriguez-Chiang et al., 2016). The objective of this study is to evaluate the methane potential from the co-digestion of different ratios of acetic acid and lignin-rich wastewaters and assess the inhibitory effect of lignin, furthermore evaluate the effects after hydrotalcite addition.

2. Materials and methods

2.1. Inoculum and substrates

The inoculum used to seed the batch reactors for the BMP experiments originated from the mesophilic anaerobic digester in Suomenoja municipal wastewater treatment plant located in Espoo, Finland. Fresh inoculum was collected and degassed at the same operating temperature (37.0 °C) prior to the start of the experiments. Characterization of the inoculum is shown in Table 1.

Two different substrates were used for the co-digestion experiments; evaporator condensate effluent originating from the sulfite evaporation plant from now on denoted as EC and fiber circulation effluent from now on denoted as FC. All substrate samples were provided by Stora Enso's Heinola Fluting Mill in Finland which produces high quality semi-chemical fluting which is used as a raw material in the corrugated board industry. Fig. 1 shows Heinolas's fluting process and the different effluents that are treated in their wastewater treatment plant; sampling points are marked in the diagram. EC is characterized by having high concentrations of acetic acid and furfural, and low solids, providing readily degradable material for methane production (Rintala and Puhakka, 1994). FC is generally composed of high COD (usually above 8000 mg/L) provided from the high lignin content (25–50%). The fresh substrates were analyzed using average values of triplicates and subsequently stored at 4 °C prior to its use. Table 1 shows the substrates' characterization.

2.2. Hydrotalcite

Hydrotalcite (HT) of Fe^{2+}-Zn^{2+}-Mg-Al was added with each of the mixed ratios to a set of anaerobic reactors running in parallel. The HT was synthesized using the co-precipitation method and using a molar ratio of 3:1 of divalent and trivalent cations (M^{2+}/M^{3+}). The HT was then characterized by Fourier transform infrared (FTIR), X-ray diffraction (XRD) and scanning electron microscopy (SEM) in order to confirm characteristics of a well crystallized HT structure; full description can be found in previous research work (Rodriguez-Chiang et al., 2016).

2.3. Experimental set-up

BMP tests were performed using an automatic methane potential test system (AMPTS II), which is a laboratory scale multiple batch system (Badshah et al., 2012; Browne et al., 2013; Rodriguez-Chiang and Dahl, 2015). Four different volume distribution ratios (based on COD content) of substrates were tested. Specific ratios were selected in order to simulate the real volume conditions existing in the operating mill. Volumes consisted of R1: 100/0, R2: 90/10, R3: 80/20 and R4: 70/30 of substrates EC/FC respectively. Moreover ratios of R1 + HT, R2 + HT and R3 + HT were tested with HT addition to evaluate its effect on the digestion process and final methane production. Fe-Zn-Mg-Al hydrotalcite was added to the corresponding reactors in different quantities equivalent to 2% HT addition of the initial substrate COD loading.

All samples were prepared in triplicates for statistical significance. The BMP tests were carried in 500 mL glass bottles with a working volume of 400 mL and the inoculum to substrate ratio (ISR) was maintained at 1:1 (COD) for all samples. The pH values of all the reactors were adjusted to 7 using 1 M HCl or NaOH when needed. To increase the buffering capacity 4 g/L of $NaHCO_3$ was added to each of the reactors at the start of the experiments. Triplicate blank samples with no substrate were run to determine the produced background methane originating from the inoculum alone. After filling each reactor with the corresponding volumes of inoculum and substrates, bottles where sealed with a hermetic rubber stopper attached to an automatic stirrer and placed in a water bath at a mesophilic temperature of 37.0 ± 0.5 °C. Each reactor was then connected to a 3 M NaOH alkali solution with thymolphthalein pH indicator to chemically remove carbon dioxide (CO_2) and hydrogen sulfide (H_2S) from the biogas. Alkali solution bottles were attached to the measuring device and finally all reactors where flushed with pure nitrogen gas (N_2) for 5 min, to create anaerobic conditions before start-up. To monitor process stability, samples from each reactor were withdrawn three times a week for pH, COD and VFA analyses.

2.4. Analytical methods

Total solids (TS) and volatile solids (VS) content in fresh samples of substrate and inoculum were determined gravimetrically following Standard Methods (APHA, 1998). COD was measured by closed reflux titration, Standard Method 5220.

Table 1
Characteristics of inoculum and substrates. Values represent the average of triplicate samples and their standard deviation when indicated.

Parameter	Unit	Inoculum	Evaporator condensate	Fiber circulation
pH	–	7.36	2.98	5.90
Chemical Oxygen Demand (COD)	mg/L	19,057 ± 73	2303 ± 11	11,572 ± 26
Total Solids (TS)	%	2.79 ± 0.01	0.12 ± 0.002	0.98 ± 0.02
Volatile Solids (VS)	%	1.55 ± 0.01	0.04 ± 0.002	0.52 ± 0.007
Conductivity	mS/cm	6.66	0.56	3.58
Total Organic Carbon (TOC)	mg/L	NA	961.4	4529
Inorganic Carbon (IC)	mg/L	NA	0.75	5.95
Total-N	mg/L	NA	0.00	0.295
Total-P	mg/L	NA	0.11	7.56
Volatile fatty acids (VFA)	mg/L acetic acid	NA	510 ± 4	NA
Furfural	mg/L	NA	23 ± 0.2	NA
Lignin	g/L	NA	0.00	5.5[a] + 190[b]

NA: not available.
[a] Acid soluble lignin.
[b] Acid insoluble lignin.

The pH measurements were performed using a pH meter Thermo Scientific model Orion 2-star pH-Benchtop. Conductivity was taken with a conductivity meter Orion Model 150. TOC and IC were measured using a TOC-V_{CPH} analyzer (Shimadzu) according to SFS-ISO 8245, Total P and Total N measurements were conducted in a FIAstar 5000 analyzer according to ISO 15681-1 note 5241 and ISO 11905 and 13395 note 5202, respectively. SO_3 and SO_4 were measured by ion chromatograph Dionex ICS-1500 with column: ION PAC AS9-HC, 4 × 250 mm. VFA and furfural analysis was performed after centrifugation of samples at 11,000 rpm for 10 min after which the supernatant was filtered and the liquid samples were analyzed by High Performance Liquid Chromatography (HPLC) using a Dionex UltiMate 3000 HPLC (Dionex, Sunnyvale, CA, USA) equipped with refractive index (RI) and ultraviolet (UV) diode array detectors and HyperREZ XP Carbohydrate Ca^+ column (Thermo Scientific, Waltham, MA, SA). The eluent used was a 0.0025 mol L^{-1} sulfuric acid solution at a flow rate of 0.8 mL min^{-1}. The column and the RI detector temperatures were fixed to 70 °C and 55 °C, respectively. Acid-insoluble lignin or Klason lignin and acid-soluble lignin were analyzed after two-step acid hydrolysis according to Standard NREL/TP-510-42623. The total lignin content was defined as the sum of acid-insoluble and acid-soluble portions; the latter was measured by UV–vis spectroscopy (UV-2550, Shimadzu) at 280 nm and the former gravimetrically. The Quantitative real-time PCR technique with 16S rRNA-targeted oligonucleotide probes was employed to sample pellets after centrifugation for determination of microbial community (total methanogens).

2.5. Data analysis

Ultimate methane potentials and daily production rates of samples were measured using the AMPTS II from Bioprocess Control AB, Sweden (System Version 2.0 V1.08). By the principle of liquid displacement and buoyancy, volumes of gas are measured and corrected to standard temperature and pressure (STP) conditions at 273 K and 1013 mbar air pressure. BMP was calculated as the accumulated methane produced per gram of COD added to each reactor, as determined in Eq. (1) (Strömberg et al., 2014):

$$BMP = \frac{V_{sample-}V_{inoc}(gCOD_{is}/gCOD_{ib})}{gCOD_{substrate}} \tag{1}$$

where *BMP* is the normalized volume produced per gram COD of substrate added (mL CH_4/gCOD), V_{sample} is the mean value of accumulated methane produced from the reactor with both inoculum and substrate, V_{inoc} is the mean value of the accumulated volume produced by the blanks with only inoculum, $gCOD_{is}$ is the mass of COD of the inoculum added in the sample, $gCOD_{ib}$ is the mass of COD of the inoculum added in the blanks, and $gCOD_{substrate}$ is the mass of COD of the substrate added in the reactor.

Anaerobic Biodegradability (BD) of the substrates can be estimated knowing the relation between the experimental methane yield (BMP) and the COD concentration of the sample. It is known that the theoretical methane yield of 1 g COD is 350 mL CH_4 at STP (Buffiere et al., 2006). Eq. (2) expresses the relation of BMP and biodegradability:

$$BD = \frac{BMP(mL\ CH_{4,STP}/gCOD)}{350 \times COD_{waste}(gCOD)} \tag{2}$$

One-way analysis of variance (ANOVA) analysis was performed on all data with significance level set at $p \leqslant 0.05$ in order to determine if there is a statistical difference between replicas.

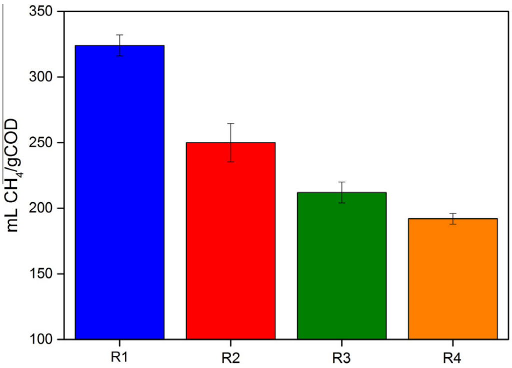

Fig. 2. Ultimate methane potential of mixed substrates and their standard deviation.

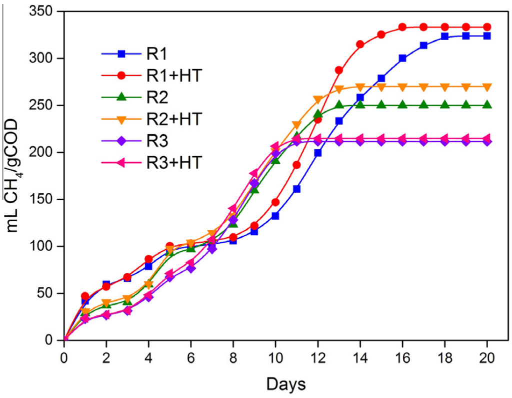

Fig. 3. Accumulated specific methane production of mixed substrates and mixed substrates with hydrotalcite addition, expressed as a function of time.

3. Results and discussion

3.1. Methane potential

Each of the samples demonstrated viability for methane production within the 20 days of digestion. Figs. 2 and 3 show the ultimate methane potential and gas production profiles for each ratio tested. Statistical analysis using ANOVA showed there was no significant variation with *p*-value $\leqslant$ 0.05 between triplicate samples. It can be observed that R1 had the highest yield (324 mL CH_4/gCOD) which is attributed to the higher VFA content (acetic acid) available in the EC substrate. Similar results for NSSC evaporator condensates ranging from 140 to 340 mL CH_4/gCOD were found in other studies (Meyer and Edwards, 2014; Ekstrand et al., 2013; Arshad and Hashim, 2012). A common setback of easily degradable substrates is the possible accumulation of intermediate products through the anaerobic degradation pathway. When high concentrations of organic acids are produced; specifically acetate; aceticlastic methanogens have insufficient capacity to rapidly convert the acetate into CH_4 and CO_2. This results in the accumulation of alcohols and VFAs that influence the pH and cause inhibition in methane production. An ISR of 1:1 applied in the experiments proved sufficient quantity of methanogenic bacteria available for conversion of VFA. In this study R1; which was the mixed sample most prone to process instability; did not develop any serious

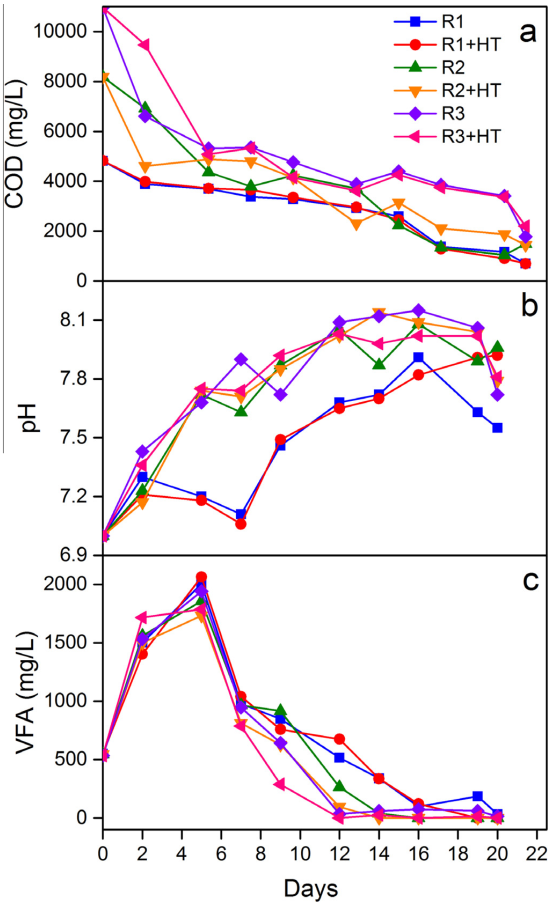

Fig. 4. Evolution of (a) COD, (b) pH variation and (c) volatile fatty acids concentration during anaerobic digestion in each reactor, expressed as a function of time.

process inhibition. However, a minor lag phase can me observed during days 5 through 8 (Fig. 3) followed by a spike of methane production until the ultimate yield is established. This is supported by the first and second maximum daily productions of R1 and R1 + HT. Both samples had a VFA concentration of 2005–2064 mg/L on day 5, this VFA accumulation is translated into the lag phase observed (Fig. 4c). After that, a spike is observed where the maximum daily production was 38–52 mL CH_4/gCOD day^{-1} on day 13, meaning a recovery of acid accumulation.

Table 2 reports the methane potential, biodegradability and lignin and COD removal of all samples. R2, R3 and R4 yielded 250 ± 15, 212 ± 8 and 192 ± 4 mL CH_4/gCOD respectively. Although lower than R1, methane yields were still among the average range of methane production of NSSC pulping effluents (140–340 mL CH_4/gCOD) considering the high content of lignin in the FC substrate. Methane potentials of co-digestion are much higher than the hypothetical mono-digestion of lignin-rich wastewater. R2 and R3 samples showed a somewhat similar pattern of degradation. Maximum daily production rates for R2 and R2 + HT was 28–32 mL CH_4/gCOD day^{-1} achieved on day 5, whereas for R3 and R3 + HT was 35–37 mL CH_4/gCOD day^{-1} reached on day 9. All four curves of R1 and R2 achieved around 90% of their ultimate methane potential by day 9 and a plateau stage by day 12. Samples with HT addition followed the same tendency as their original replica, however an increment in methane yield was observed in most of the HT samples ranging from 1.5 to 8%. R1 + HT also obtained a faster production rate while HT addition in R3 had no influence in methane production.

COD removal efficiencies between 64 and 83% were obtained for all samples. Samples with higher evaporator condensate content had higher COD removal (76–83%) which express an efficient biodegradability. Biodegradability of samples had a similar pattern to COD removal rates. However, due to the high acetic acid content of R1 and R1 + HT, the biodegradability of these samples (105 and 108% respectively) are likely overestimated, since the assumption from Eq. (2) that the COD consists of purely carbohydrates provides a lower theoretical maximum production of methane than acetate-rich substrates would deliver (Ekstrand et al., 2013). Biodegradability for R2, R2 + HT, R3, R3 + HT and R4 demonstrated a lower range of 37–70%.

3.2. Control parameters

COD removal of samples fluctuated for the first 5 days (Fig. 4a). From day 6 onwards each ratio and its replica with HT addition had the same reduction behavior. VFA measurements of samples all followed the same patterns however with difference in concentrations, R1 and R1 + HT being the highest. The maximum VFA production for all samples was on day 5 ranging from 1732 to 2064 mg/L (Fig. 4c). By the end of the experiments there was no VFA measured.pH measurements seemed to slowly increase throughout the experimental time between a range of 7–8.15 (Fig. 4b). For R1 and R1 + HT there is a slight drop starting at day 3 however increases by day 9, this behavior in pH validates the VFA accumulation and minor lag phase identified previously. HT addition, to some extent, influenced the pH. Samples with HT appeared to have slightly less fluctuation through time. This may be due to the fact that HT nanoparticles have stable basic sites (Rodriguez-Chiang et al., 2016), therefore reducing changes in pH.

Sulfate (SO_4) concentrations varied significantly at initial and final phases of the experiment. NSSC effluents are rich in sulfur-containing compounds making sulfate-reducing bacteria (SRB)

Table 2
Final characterization of all mixed samples. Values represent the average of triplicate samples and their standard deviation when indicated.

Sample	Mix ratio (EC:FC)	Methane yield (mL/gCOD)	Soluble lignin removal (%)	COD removal (%)	Biodegradability (%)	pH
R1	100:0	324 ± 8	NA	82	105	7.5
R1 + HT	100:0	333 ± 5	NA	83	108	7.9
R2	90:10	250 ± 15	25 ± 3	76	65	7.9
R2 + HT	90:10	270 ± 17	28 ± 6	78	70	7.8
R3	80:20	212 ± 8	41 ± 1	75	46	7.7
R3 + HT	80:20	215 ± 3	44 ± 2	71	47	7.8
R4	70:30	192 ± 4	NA	64	37	7.5

NA: not available.

outcompete methanogenic bacteria and produce toxic sulfide during sulfate reduction (Xu et al., 2012). Both sulfate and sulfite are known to act as electron sinks in anaerobic digestion and compete with methanogens for hydrogen. Jing et al. (2013) studied the anaerobic performance when treating sulfate-rich wastewater and found that at a concentration of 3000 mg/L of sulfate and high organic loading rates of 6–18 gCOD/L day^{-1} sulfate removals remained between 28 and 42% and COD removals were above 80%. In this study, initial sulfate concentrations for both R1 and R2 was 67 mg SO_4/L and for R3 was 141 mg SO_4/L. Final concentrations after anaerobic digestion in all reactors was reduced to a range of 0.1–0.7 mg SO_4/L making sulfate reduction over 99%. As initial concentration were not very high, sulfate may not have played an inhibiting role and there was most likely no hydrogen sulfide overproduction. Sulfite (SO_3) concentration at initial and final stages of the experiment had a negative correlation between each other. R1 had the highest concentration at the beginning of the experiment (23 mg/L) and the lowest at the end (10 mg/L); whereas R3 has the lowest concentration in the beginning (4 mg/L) and the highest by the end (30 mg/L). Sulfur in EC substrates is partially reduced to sulfide and released into the gas phase as H_2S, however some organic sulfonates and sulfite may persist through the process and other organic sulfur compounds may be produced in the way (Ferguson and Benjamin, 1985).

DNA extraction and Quantitative real-time PCR (qPCR) were adapted to quantify the methanogen concentration in each anaerobic reactor. Differences in initial and final concentrations show population growth in all batch reactors (Table 3). Both R1 and R1 + HT; where no lignin is present; demonstrate a significant methanogenic growth of 253 and 293% respectively compared to the samples were lignin is present in the feed. The large and rapid increase in methanogen concentration is assumed to be through the *Methanosarcinaceae* gene which is known to increase with the degradation of acetate. The *Methanosarcina* species have a high growth rate and outcompete other species at a high acetate concentration, hence the high population growth in acetate-rich reactors R1 and R1 + HT (Lee et al., 2009). R2 showed a reduced growth of 33%. For R2 + HT, R3 and R3 + HT methanogenic growth was between 83 and 92%. The predominance of *Methanosarcina* and *Methanosaeta* species has previously been reported during the anaerobic treatment of paper and pulp mill wastewaters, the latter known for having difficulties with growth and yield (Roest et al., 2005). The presence of lignin fractions, specially low molecular weight fraction, has been demonstrated to induce methanogenic toxicity as well inhibition on bacterial growth. Some studies have also reported antimicrobial properties of lignin (Gregorova et al., 2011; Erakovic et al., 2014), however lignin is a complex polymer that can change/transform into other compounds that have different toxicity in anaerobic digestion. Partial depolymerization of lignin polymers produce inhibitors like syringaldehyde and vanillin, dehydration of pentoses and hexoses produces furfural and 5-hydroxymethylfurfural (HMF) which also cause inhibition. Therefore there are many paths of lignin that can lead to inhibiting by-products (Barakat et al., 2012). It is important to highlight the distinct difference in population growth of samples with no lignin presence and sample where lignin was added, despite of the latter's lignin concentration.

Table 3
Microbial community population analysis of samples at initial and final stages of experimental testing.

Sample	Initial methanogen concentration[a]	Final methanogen concentration	Population growth (%)
R1	1.22E+07	4.31E+07	253.28
R1 + HT	1.22E+07	4.80E+07	293.44
R2	4.43E+07	5.87E+07	32.51
R2 + HT	4.43E+07	8.23E+07	85.78
R3	4.36E+07	7.99E+07	83.26
R3 + HT	4.36E+07	8.35E+07	91.51

[a] 16S rRNA gene copies/mL. Values are average of triplicates.

3.3. Effect of hydrotalcite addition

The addition of Fe-Zn-Mg-Al hydrotalcite (HT) presented a positive effect on the methane potential of the tested samples. Ultimate methane yields were slightly increased by 1.4–8% for samples with HT addition. R1 + HT achieved a faster production rate reaching its ultimate methane yield by day 16 whereas R1 at day 20. R2 + HT presented the highest increase in methane production up to 8% compared to R2 (Table 3). HT addition on R3 had no significant effect, the degradation profile and methane yield of both remained the same. Kruger et al. (2016) found that anions in the HT interlayers enhanced the catalytic activity of lignin depolymerization however they also reported no effect in different samples. Through extensive literature review no conclusive proof was found that could link the inefficiency of the HT in R3 with its lignin concentration. Both COD and lignin removal rates as well as biodegradability followed the same tendency, in which samples with HT were just slightly higher than samples with no HT addition. The acidity caused by VFA production may have been buffered by the stable surface basic sites of the HT nanoparticles therefore reducing changes in pH, all though it could not be clearly identified. It was observed that microbial growth was higher in all samples containing HT compared to the samples with no HT addition. Studies have found that some microorganisms take advantage of nanoparticles, especially in anaerobic conditions, by transferring more efficiently electrons to acceptors (Beckers et al., 2013). The increased surface area provided by HT nanoparticles and their action as biocatalysts leads to enhancement of microorganism activity and possible enhanced bacterial anchoring. This in turn, may be the cause of greater microbial growth in samples with HT addition (Rodriguez-Chiang et al., 2016; Beckers et al., 2013).

3.4. Influence of lignin content on BMP

Results demonstrated a linear decay in methane production when the fraction of total lignin was increased in the feed. A negative correlation between total lignin content and methane yield or biodegradability is shown in Fig. 5. The results show a linear corre-

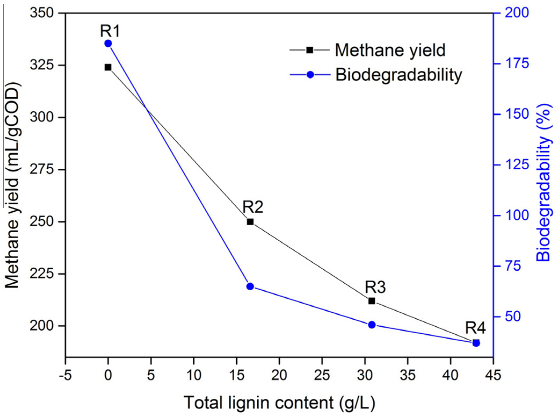

Fig. 5. Correlation between total lignin content, methane yield and biodegradability of NSSC effluents.

lation coefficient (R^2) of 0.94 for methane yield. For biodegradability a second-order polynomial model fitted the experimental results better with a R^2 of 0.95. Statistical analysis using ANOVA showed a p-value $\leqslant$ 0.01 for both curves. Li et al. (2013) found a similar negative linear correlation between lignin content and the biomethane yields of lignocellulosic and manure wastes.

Soluble lignin removal rates of samples ranged from 25 to 44%. R1 and R1 + HT had no lignin measured. Lignin removals for R2 + HT and R3 + HT were 12% and 7% higher than their replicas without HT addition, respectively. Lignin is widely known for its extremely low degradability and its ability to hinder hydrolysis of cellulose thus limiting its bioavailability. In this study, lignin presence had a detrimental effect on the methane production resulting in a 41% decrease in production when the fraction of lignin had less than a 3-fold increase. Triolo et al. (2011) studied the effect of lignin on the BMP of energy crops and animal manure and confirmed that the lignin fraction could be used to predict BMP for the combined model for energy crops and animal manures. Their findings included that lignin content negatively affects BMP more significantly in energy crops than in animal manure. However for industrial lignin; which is the lignin generated in large quantities as a by-product of chemical pulping of wood; conditions are different. This lignin differs significantly from native lignin in its structure and chemical composition, leading to formation of lower molecular weight lignin and other impurities such as sulfurous compounds or carbohydrates (Alekhina et al., 2015). Sierra-Alvarez and Lettinga (1991) have found that low molecular weight lignin fractions are the responsible of the inhibitory effect of lignin and its derivatives towards methanogenic bacteria. From the microbial population analysis performed in the final samples of the reactors it is assumed that lignin content and its derivatives had a negative influence in microbial growth of methanogens, this can be observed from the reduced growth in reactor R2 and R3 which include lignin fractions.

4. Conclusions

This study evaluated the methane potential of different ratios of effluents from a NSSC pulp mill. Methane yields ranged between 192 and 333 mL CH_4/gCOD where the greater the EC volume, the greater the methane yield. COD removal between 64 and 83% were obtained. A negative linear correlation between lignin content and methane yield was found, resulting in 41% decrease in methane when lignin had a 43 g/L increase. Addition of Fe-Zn-Mg-Al HT presented positive results such as faster production rate and higher soluble lignin removal. Co-digestion of acetate and lignin-rich effluents is suggested to improve the biodegradability of NSSC effluents.

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IV

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New alternative energy pathway for chemical pulp mills: From traditional fibers to methane production

Lourdes Rodriguez-Chiang [a,b,*], Kari Vanhatalo [a], Jordi Llorca [b], Olli Dahl [a]

[a] *Department of Bioproducts and Biosystems, School of Chemical Engineering, Aalto University. P.O. Box 16300, Vuorimiehentie 1, Espoo, Finland*
[b] *Institute of Energy Technologies and Barcelona Research Center in Multiscale Science and Engineering, Technical University of Catalonia, Barcelona, Spain*

HIGHLIGHTS

- Brown, oxygen delignified and bleached pulp were tested for methane potential.
- Highest methane yields correspond to fiber samples, particularly of bleached pulp.
- Degradability was found to be in the following order: bleached > brown > delignified.
- Energy and cost estimations were evaluated.

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ABSTRACT

Chemical pulp mills have a need to diversify their end-product portfolio due to the current changing bio-economy. In this study, the methane potential of brown, oxygen delignified and bleached pulp were evaluated in order to assess the potential of converting traditional fibers; as well as microcrystalline cellulose and filtrates; to energy. Results showed that high yields (380 mL CH_4/gVS) were achieved with bleached fibers which correlates with the lower presence of lignin. Filtrates from the hydrolysis process on the other hand, had the lowest yields (253 mL CH_4/gVS) due to the high amount of acid and lignin compounds that cause inhibition. Overall, substrates had a biodegradability above 50% which demonstrates that they can be subjected to efficient anaerobic digestion. An energy and cost estimation showed that the energy produced can be translated into a significant profit and that methane production can be a promising new alternative option for chemical pulp mills.

1. Introduction

Bio-economy markets are rapidly changing worldwide, therefore new alternative energy pathways, which use bio-based natural resources in a sustainable way will be needed in the future. European Union (EU) environmental regulations and energy directives have made renewable energy production an important topic to address. This ongoing trend can give a new potential to existing forest companies to also develop as significant biofuels producers in addition to producers of cellulose based products. Nowadays chemical pulp mills typically produce fibers for paper, board and viscose production and at the same time harvest great amount of bioenergy in the form of heat and power. So far fiber products have a good share in the market of traditional cellulose products, but due to global increasing capacities, the situation can dramatically change leading small production plants into financial difficulties. For these mills, the production of renewable energy in the form of biogas could become a potential alternative to the application/use of different types of pulps.

In recent years most of the anaerobic digestion (AD) studies in the forest industry have focused on primary and secondary sludge from the aerobic treatment of wastewaters mainly from paper industries (Bayr and Rintala, 2012; Hagelqvist, 2013; Lin et al., 2011). Sludge is produced in great amounts and disposal is costly; this is why studies have concluded that it is more effective to reduce sludge formation by changes in the wastewater treatment (such as including AD) than reducing the amount of sludge by post-treatment (Stoica et al., 2009). Little attention has been focused on the direct AD of streams of chemical pulping wastewater. Gavrilescu and Puitel (2007) describe the different processes and water flows in a chemical pulping line. Effluent emissions are generated in wood handling, debarking and chip washing, wood cooking, pulp washing and pulp bleaching. Bleaching efflu-

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[*] Corresponding author at: Department of Bioproducts and Biosystems, School of Chemical Engineering, Aalto University. P.O. Box 16300, Vuorimiehentie 1, Espoo, Finland.

E-mail address: lourdes.rodriguezchiang@aalto.fi (L. Rodriguez-Chiang).

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ents are the most important discharge of pollutants to water in a pulp mill. They are generally the main source of wastewater and chemical oxygen demand (COD) load (around 50–60% of the total load) in a chemical pulp mill, this underlines their potential for AD. However, they also contain many inhibiting compounds for methanogenic bacteria such as degradation products of lignin, polysaccharide, wood extractives and most importantly chlorine compounds that can produce absorbable organic halides (Monje et al., 2010). Different treatment processes of bleaching effluents have been reviewed and AD has been found to be most promising with COD removals ranging from 28 to 50% and maximum dechlorinating (Rintala and Puhakka, 1994; Savant et al., 2006). Ekstrand et al. (2013) elaborated a comprehensive study of the methane potentials of many different effluent streams in the pulping industry finding that kraft pulp effluents from the cooking and condensates had high yields at or above 50% of the theoretical potential. Larsson et al. (2015) also state that alkaline bleaching effluents from a kraft pulp mill were suitable for AD application, however significant lower yields were found when using softwood as raw material rather than hardwood.

Due to some of the challenges mentioned above, different sources for biogas production were assessed in order to improve the implementation of AD in chemical pulping. In this study, the biochemical methane potential (BMP) of brown, oxygen delignified and bleached pulp were evaluated in mesophillic batch reactors. The anaerobic degradability was monitored for each pulp fiber, acid hydrolyzed microcrystalline cellulose (MCC) manufactured from fibers, MCC + hydrolysis filtrate mixture and for filtrates alone. This research could provide an assessment of an alternative option to diversify the end-product portfolio of chemical pulp mills.

2. Materials and methods

2.1. Raw material

Softwood pulp fibers used in this study were collected from a kraft pulp mill located in north west Finland. The mill's cooking stage is done at 1000 H-factor and the bleaching stage works with elemental chlorine free (ECF) technique. Pulp fibers were taken after pulp mill digester, after oxygen delignification (O_2) stage and after final bleaching stage; from now on denoted as BRFiber, O2Fiber and BLFiber respectively. Fibers were washed prior to further use with distilled water in large Büchner funnel to attain wash filtrate conductivity value of 5 µS, subsequently they were centrifuged to dry consistency. Centrifuged pulps were used as raw material in MCC manufacturing and also employed in BMP tests.

MCC materials were manufactured using an acid hydrolysis procedure called AaltoCell™ described in Vanhatalo and Dahl (2014). In short, a defined amount of pulp was loaded in tube-like 2.5 dm^3 metal reactor using H_2SO_4 as hydrolyzing agent. The hydrolyzation was done with a 2.0% acid charge (calculated for oven dry cellulose weight) at 160 °C, 30 min, with a 10% pulp consistency. After reaction, reactor was cooled down to room temperature in cold water bath for 15 min. Content of reactor was poured to filter bag and liquid fraction was separated using a laundry centrifuge (UPO, Finland) at 4500 rpm. Liquid fractions were used as such in digestion experiments and denoted as BRFiltrate for brown pulp, O2Filtrate for oxygen delignification, and BLFiltrate for bleached pulp. Solid MCC fraction was washed three times using dilution thickening washing with dilution factor 10. Washed MCC's were used in BMP experiments and denoted as BRMCC, O2MCC, and BLMCC. Fourth material used for BMP denoted as BRMix, O2Mix, and BLMix was the reactor content after hydrolyzing procedure, but without filtrate separation and washing. Distilled water was used in all experiments, H_2SO_4 was 1 M and analytical grade.

2.2. Substrates and inoculum

The fractions from the production of the different MCC qualities were used as substrates for the BMP experiments. For each type of MCC: brown (BR), oxygen delignified (O_2) and bleached (BL); four substrates were tested for methane production. The substrates were namely Fiber which was used as a control, MCC, Mix (MCC + Filtrate) and the separated Filtrate. Each substrate was characterized (Tables 1 and 2) using average values of triplicates and subsequently stored at 4 °C prior to its use. Filtrates were stored at -20 °C to avoid any carbohydrate degradation before the beginning of experiments.

Fresh digested sludge from a mesophillic anaerobic digester of Suomenoja municipal wastewater treatment plant located in Espoo, Finland was used as inoculum for all the experiments. The inoculum was collected and degassed for 4 days prior to the start of each of the experiments. Using average values of triplicates, characterization of the inoculum resulted in total solids (TS) of

Table 2
Initial characteristics of pulp filtrates. Values represent the average of triplicate samples and their standard deviation when indicated.

Parameter	BRFiltrate	O2Filtrate	BLFiltrate
pH	1.9	1.8	1.4
TS (g/L)	14.8 ± 0.2	16.2 ± 0.2	8.4 ± 0.1
VS (g/L)	12.3 ± 0.2	14.6 ± 0.2	8.1 ± 0.1
Arabinose (mg/L)	483.1	268	704.4
Rhamnose (mg/L)	0	0	0
Galactose (mg/L)	297	164	260.9
Glucose (mg/L)	1962.7	3445.4	1165
Xylose (mg/L)	3373.9	2459.9	3404.9
Mannose (mg/L)	1262.3	1224.5	1155.8
Total carbohydrates (mg/L)	7379	7561.8	6691
COD (mg/L)	14,765.5 ± 97	18,514.3 ± 44	9187 ± 48
Soluble lignin (mg/L)	363	532	406
VFA (g/L)	38.1 ± 0.1	39.9 ± 0.3	2.7
Formic acid (mg/L)	4057.4	8800.2	113.7
Furfural (mg/L)	215.7	1649.2	46.9
Hydroxymethylfurfural (mg/L)	52.7	853.5	12.2

Table 1
Initial characteristics of solid substrates. Values represent the average of triplicate samples and their standard deviation when indicated.

Parameter	Brown pulp			Delignified pulp			Bleached pulp		
	Fiber	MCC	Mix	Fiber	MCC	Mix	Fiber	MCC	Mix
Carbohydrates (% DW[a])	95.4	95.0	94.6	97.6	96.8	96.2	99.2	99.4	97.8
Extractives (% DW)	0.3	0.8	0.3	0.1	0.2	0.3	0.2	0.1	1.4
Total Lignin[b] (% DW)	4.2	4.6	4.8	2.3	3.2	3.2	0.7	0.5	0.9
TS (%)	29.9 ± 0.2	31.2 ± 0.1	10.5 ± 0.5	33.4 ± 0.2	31.5 ± 0.1	9.8 ± 0.1	44.7 ± 0.2	44.4 ± 0.3	10.4 ± 0.2
VS (%)	29.7 ± 0.1	31.2 ± 0.1	10.3 ± 0.5	33.4 ± 0.2	31.4 ± 0.1	9.6 ± 0.1	44.7 ± 0.2	44.4 ± 0.3	10.4 ± 0.2

[a] DW: Dry weight.

[b] Reported as the sum of acid soluble and insoluble lignin fractions.

2.1%, volatile solids (VS) of 1.22%, pH of 7.1, conductivity of 6.6 mS/cm and a total alkalinity of 6.5 g $CaCO_3$/L.

2.3. Experimental set-up

All BMP experiments were carried out in an automatic methane potential test system (AMPTS II) from Bioprocess Control AB, Sweden; which is a laboratory scale multiple batch system designed for BMP determination (Rodriguez-Chiang and Dahl, 2015; Strömberg et al., 2014; Shen et al., 2014). BMP tests were performed in 3 experimental sets and each set was conducted in the same manner. An inoculum to substrate ratio of 2:1 (VS based) was maintained for all samples to ensure microbial activity. Triplicate blanks with only inoculum were also prepared for each experiment in order to determine the background gas to be subtracted from the sample gas. Each reactor was a 600 mL glass bottle with a working volume of 400 mL. After all reactors were filled with corresponding volumes of substrate and inoculum, the pH was measured and adjusted to neutrality with CaO when needed. Every reactor was then sealed with a hermetic rubber stopper where an automatic stirring rod was attached and subsequently placed in a water bath at mesophilic temperature of 37.0 ± 0.5 °C. Each reactor bottle was connected by Tygon tubing to an alkali solution bottle consisting of 80 mL of a 3 M NaOH solution with thymolphthalein pH indicator to chemically remove carbon dioxide (CO_2) and hydrogen sulfide (H_2S) from the biogas. Each alkali solution bottle was attached to the wet gas flow measuring device and finally all reactors were flushed with pure nitrogen gas (N_2) for 5 min to create an anaerobic environment. Each experiment was monitored for a period of 24 days after which the gas production was negligible.

2.4. Analytical methods

TS and VS of samples were determined gravimetrically following Standard Methods described in (APHA, 1998). Additionally, pH of filtrate samples was adjusted to neutrality before VS measurement to avoid underestimation caused by volatility of volatile fatty acids (Angelidaki et al., 2009). COD was measured by closed reflux titration using Standard Method 5220. Total alkalinity to pH 4.5 was measured using Standard Method 2320 B. A Thermo Scientific pH meter, model Orion 2-star pH-Benchtop was used for all pH measurements. Electrical conductivity was taken with a conductivity meter Orion Model 150. Ultimate methane production volumes and daily production rates of samples were measured using the AMPTS II from Bioprocess Control AB, Sweden (System Version 2.0 V1.08). Volumes of gas are measured by the principle of liquid displacement and buoyancy and corrected to standard temperature and pressure (STP) conditions at 273 K and 1013 mbar air pressure. Volatile fatty acids (VFA) and furfural analysis was performed after centrifugation of samples at 11,000 rpm for 10 min after which the supernatant was filtered and the liquid samples were analyzed by High Performance Liquid Chromatography (HPLC) using a Dionex UltiMate 3000 HPLC (Dionex, Sunnyvale, CA, USA) equipped with refractive index (RI) and ultraviolet (UV) diode array detectors and HyperREZ XP Carbohydrate Ca^+ column (Thermo Scientific, Waltham, MA, SA). The eluent used was a 0.0025 mol L^{-1} sulfuric acid solution at a flow rate of 0.8 ml min^{-1}. The column and the RI detector temperatures were fixed to 70 °C and 55 °C, respectively. Carbohydrate and lignin (acid insoluble and acid soluble) contents were analyzed after two-step acid hydrolysis according to the method from National Renewable Energy Laboratory procedure NREL/TP-510–42618, using HPAEC-PAD (Dionex ICS-3000, pulsed amperometric detector, CarboPac PA20 column, Dionex, Sunnyvale, USA). The lignin content was defined as the sum of acid-insoluble and acid-soluble portions; the latter was measured by UV–Vis spectroscopy (UV-2550, Shimadzu) at 280 nm and the former gravimetrically. Cellulose fraction molecular weight distributions were analyzed using a Gel permeation chromatography (GPC) technique. Samples were dissolved in 90.0 g l^{-1} lithium chloride (LiCl) containing N,N-dimethylacetamide (DMAc) and diluted according to the procedure described by Testova et al. (2014). GPC procedure was performed with a Dionex Ultimate 3000 system with a guard column (PLgel Mixed-A, 7.5 × 50 mm, Agilent Technologies, Santa Clara, USA), four analytical columns (PLgel Mixed-A, 7.5 × 300 mm), and refractive index detection (Shodex RI-101, Showa Denko K.K, Kawasaki, Japan). Chromatography eluent was 9.0 g l^{-1} LiCl/DMAc and samples were eluted with a flow rate 0.75 ml/min. Signal from RI detector was calibrated against a set of eleven narrow pullulan standards of known molecular weight (342 Da–710 kDa, Polymer Standard Service GmbH, Mainz, Germany, and 2560 kDa, Fluka GmbH, Germany). X-ray diffraction (XRD) of fiber and MCC samples were obtained with X'Pert Pro MPD Alpha-1 (PANalytical; Holland) diffractometer using CuKα radiation source ($\lambda = 0.154056$) operated at 45 kV and 40 mA. The samples were analyzed at a scan rate of 0.067512°s^{-1} and in a 2θ-range of 5–70°. Crystallinity index (CI) of each sample was calculated on basis of XRD data by peak height method according to Segal et al. (1959).

2.5. Data analysis and calculations

BMP was calculated as the accumulated methane produced per gram of VS added to each reactor, as determined in Eq. (1) (Strömberg et al., 2014):

$$BMP = \frac{V_{sample-}V_{inoc}\frac{gVS_{is}}{gVS_{ib}}}{gVS_{substrate}} \tag{1}$$

where *BMP* is the normalized volume produced per gram VS of substrate added ($mLCH_4$/gVS), V_{sample} is the mean value of accumulated methane produced from the reactor with both inoculum and substrate, V_{inoc} is the mean value of the accumulated volume produced by the blanks with only inoculum, gVS_{is} is the mass of VS of the inoculum added in the sample, gVS_{ib} is the mass of VS of the inoculum added in the blanks, and $gVS_{substrate}$ is the mass of VS of the substrate added in the reactor.

The anaerobic biodegradability (BD) of a substrate can be expressed by the ratio between the experimental methane yield (BMP) and the theoretical methane yield (BMP_{th}) of the sample. The theoretical methane yield of a given substrate can be calculated by atomic composition using Buswell and Mueller (1952) stoichiometric conversion equation or by organic fraction composition when the fraction of carbohydrates, proteins and lipids are known (Eq. (2)). The coefficients in this equation originate from the stoichiometric conversion of model compounds of each organic fraction using the former method. In this study, the BMP_{th} was calculated by organic fraction composition using the modified equation of Triolo et al. (2011) where lignin is included with the empirical formula of $C_{10}H_{13}O_3$. The theoretical methane production of lignin was calculated at 727.1 (CH_4 NL/kg lignin). Therefore to determine the total theoretical methane yield (BMP_{th}) the following equation was used:

$$BMP_{th} = 415 \cdot \%carbohydrates + 496 \cdot \%proteins + 1014 \cdot \%lipids + 727 \cdot \%lignin \tag{2}$$

The BMPth of liquid filtrate samples was calculated knowing the ratio between grams of COD/VS in the sample and the assumption that the theoretical methane yield of 1 g of COD is 350 mL CH_4 at STP (Buffiere et al., 2006).

Each experimental set was conducted in triplicate and results expressed as mean ± standard deviation when indicated. One-way analysis of variance (ANOVA) was performed on all data with significance level set at $p \leq 0.05$ in order to determine if there is a statistical difference between replicas.

Methane and energy mass calculations were calculated using the following equations:

$$M_{amount} = O_{load} \cdot BMP \cdot M_{density} \tag{3}$$

$$E_{amount} = M_{amount} \cdot M_{HHT} \tag{4}$$

where M_{amount} is the methane amount of kg produced per one air-dried ton of substrate (kg/adt), O_{load} is the organic load in each substrate expressed as kg of VS per air-dried ton (kgVS/adt), $M_{density}$ was assumed to be 0.716 kg/m^3. E_{amount} is the energy amount of mega joules produced per one air-dried ton (MJ/adt) and M_{HHT} is the higher heating value of methane (55 MJ/kg).

Estimates of sludge production were calculated by the assumption of Von Sperling and Concalves (2007) where 1 kg of VS/COD will produce and average of 0.15 kg of suspended solids during an anaerobic treatment.

3. Results and discussion

3.1. Raw material and substrate characterization

All the solid fractions (Fibers, MCCs and Mixes) showed high amount of carbohydrates and relatively low lignin portions (Table 1). Lignin portions decreased in the order BR pulp > O2 pulp > BL pulp. The order is substantiated by the fact that BR pulp fiber is taken after the digestion stage following oxygen delignification which further removes lignin in the O2 fiber samples. After oxygen delignification, bleaching stages remove the residual lignin components. MCC's and Mixes followed the same order. Molecular weight distribution (MWD) of Fiber fractions displayed a bimodal shape, whereas MCCs and Mixes have a singular peak form (Fig. 1). MCCs and Mixes have gone through an acid hydrolysis manufacturing process which caused a decrease in molecular weight, losing the bimodal shape and shifting the distribution to lower MWD areas. All the MCCs and Mixes within the same sample have nearly the same MWD, which was expected since the only difference is the separated filtrate liquid fraction. CI was measured for solids samples: BRFiber (0.75), BRMCC (0.88), O2Fiber (0.77), O2MCC (0.88), BLFiber (0.81) and BLMCC (0.89). Results demonstrate that the acid hydrolysis performed to MCC samples, removed the amorphous part and hemicelluloses from the raw pulp fiber and hence increased the MCC samples' CI which means a denser material structure.

3.2. Biochemical methane potential and production rates

The total accumulated methane yield from each of the samples was obtained after 24 days of digestion when the gas production decreased to a negligible amount. Fig. 2. shows the difference in final methane yield from each substrate and their standard deviation. Statistical analysis using ANOVA showed there was no significant variation with p-value ≤ 0.05 between triplicate samples. The highest values of methane production correspond to the fiber samples of each tested pulp with 357, 348 and 380 mL CH$_4$/gVS for BR, O2 and BL pulp respectively (Table 3). MCC samples followed, with methane yields between 298 and 350 mL CH$_4$/gVS and Mix samples with yields ranging from 261 to 331 mL CH$_4$/gVS. The lowest yields were found in Filtrate samples where O2Filtrate reached 253 mL CH$_4$/gVS, BLFiltrate 291 mL CH$_4$/gVS and BRFiltrate 298 mL CH$_4$/gVS. Rodriguez-Chiang and Dahl (2015) reported a comparable BMP of 333 mL CH$_4$/gVS from bleached filtrates, Walter et al. (2016) reported similar observations on the methane yield of pulp residues. Pulp residues with no pretreatment reached a methane yield of 323 mL CH$_4$/gVS, Steffen et al. (2016) also found comparable results from bleached kraft pulps, where the methane production measured was between 363 and 375 mL CH$_4$/gVS for both fiber sample and fines fraction. They also tested bleached mechanical pulps which showed significantly lower methane yields with values as low as 21 mL CH$_4$/gVS.

Most of the BL pulp substrates had the highest yields compared to BR and O2 pulp substrates. BRFiber gave 6.1% and O2Fiber 8.5% lower methane yield than BLFiber. The magnitude order of methane yield for MCCs and Mixes was the same than fiber samples. This can be attributed to the final properties required for

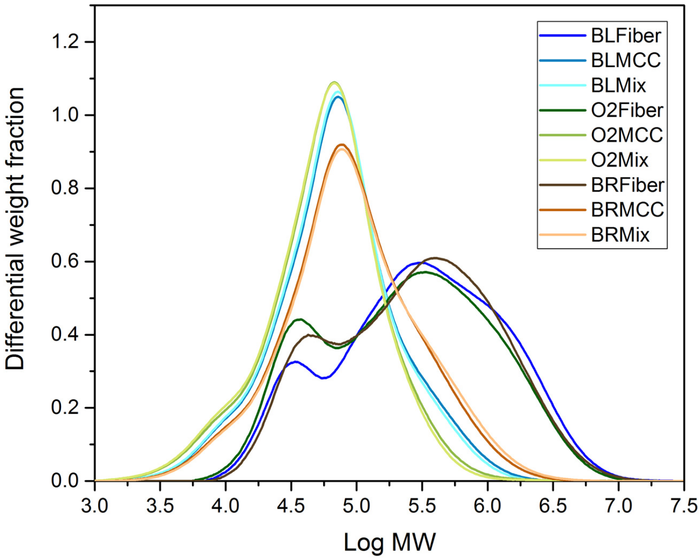

Fig. 1. Molecular weight distribution of pulp substrates.

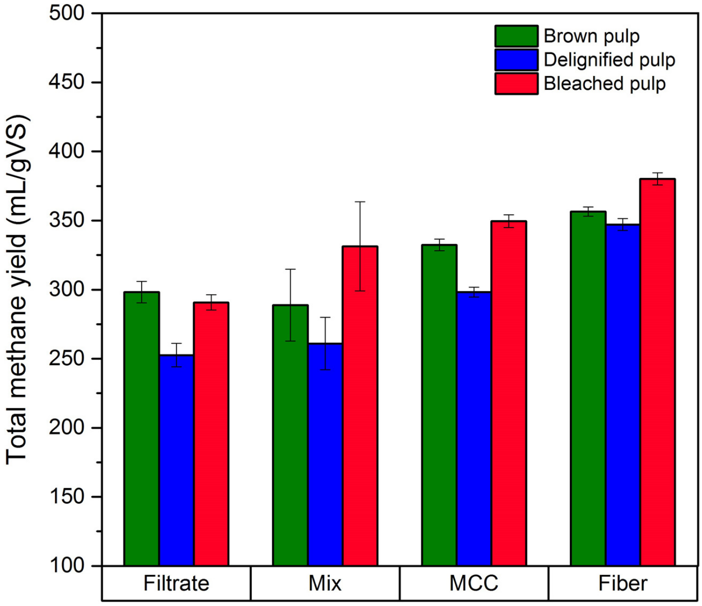

Fig. 2. Total accumulated methane yield of substrates after 24 days of anaerobic digestion.

Table 3
Final characterization of all pulp substrates. Values represent the average of triplicate samples and their standard deviation when indicated.

Sample	Methane yield (mL/gVS)	Theoretical methane yield (mL/gVS)	Biodegradability (%)	COD removal (%)	VS removal (%)	pH range
BRFiltrate	298.2 ± 8	421.2	70.8	86	31.5	7.2–7.5
BRMix	288.8 ± 27	429.7	67.2	49.6	34.5	7.5–8.1
BRMCC	332.5 ± 4	425.1	78.2	37.5	35.9	7.5–7.8
BRFiber	356.7 ± 3	427	83.5	35.7	42	7.5–7.9
O2Filtrate	252.5 ± 8	443.8	56.9	78.6	27.2	7.3–7.4
O2Mix	261.3 ± 19	425	61.5	40.6	29.1	7.2–7.7
O2MCC	298.1 ± 4	423.2	70.4	26.2	31.9	7.2–7.8
O2Fiber	347.6 ± 4	421.8	82.4	25.5	34.7	7.3–7.7
BLFiltrate	290.8 ± 6	397.5	73.2	83.2	32	7–2-7.4
BLMix	331.3 ± 32	410.6	80.7	47.9	36.7	7.3–7.5
BLMCC	349.5 ± 5	416.1	84	34.4	36.4	7.3–7.4
BLFiber	380.1 ± 4	416.4	91.3	34.1	39.2	7.3–7.5

bleached pulp; high concentrations of carbohydrates and volatile solids content in the bleached processed pulp and the lower concentration of total lignin after its delignification treatment make it a better suited substrate to undergo AD. The recalcitrance of lignin has been proven to hinder the overall anaerobic degradability of organic material (Rodriguez-Chiang et al., 2016; Ko et al., 2009), therefore lower concentrations of lignin are desired for faster degradation.

The O2 pulp substrates delivered the lowest yields due to a variety of factors. While BR pulp has a higher lignin content than O2 pulp; the oxygen delignification process which is implemented to remove lignin fractions in unbleached pulp; significantly changes the structure of the residual lignin in the O2 pulp. The residual lignin can contain many types of structural units that have free phenolic hydroxyl groups and the covalent linkages between lignin and carbohydrate compounds are also altered forming lignin-carbohydrate complexes (LCC) (Dence and Reeve, 1996; Lawoko et al., 2004). This may cause organic material to be less accessible for microbial degradation and can clearly retard digestion (Jeffries, 1990).

Ultimate methane yields for brown pulp substrates were reached between days 9 and 10 of digestion with the exception of BRFiltrate which reached its ultimate yield by day 4 (Fig. 3a) and a maximum daily methane yield of 159 mL CH_4/gVS on the first day of digestion. The immediate and fast production rate of gas is due to a healthy concentration of hydrolyzed carbohydrates common to all filtrate samples (Table 2). Sugars like glucose, xylose and mannose were found in high concentrations (between 1.2 and 3.4 g/L) in all filtrates. Xylose is the major monosaccharide present in hemicellulosic biopolymers (Barakat et al., 2012) which would explain BRFiltrate and BLFiltrate having larger fractions while O2Filtrate has larger fractions of furfural and hydroxymethylfurfural (HMF) which originate from the dehydration of these sugars. Maximum daily production rates for BRMCC and BRMix was 130–148 mL CH_4/gVS respectively achieved on day 3 after a short hydrolysis period. The highest maximum daily methane produc-

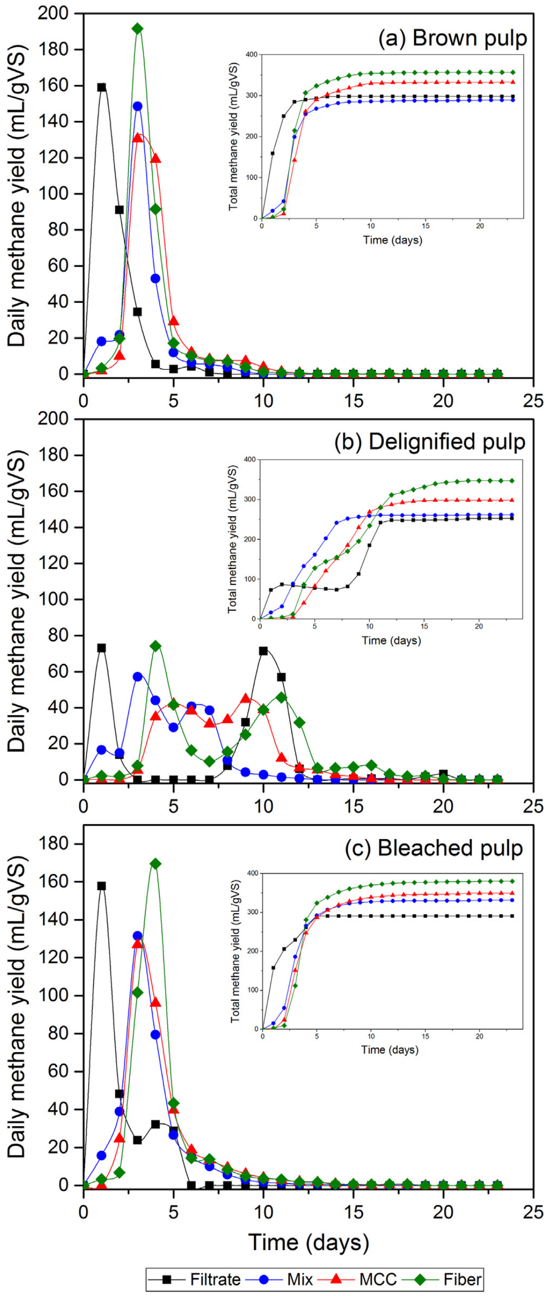

Fig. 3. Daily methane production of pulp substrates (large figure) and their total accumulated methane yield evolution (embedded figure) expressed as a function of time. (a) brown pulp, (b) delignified pulp and (c) bleached pulp.

tion of 192 mL CH_4/gVS was attained by the BRFiber on day 3. All brown pulp substrates achieved more than 90% of their ultimate methane potential by day 6 and a plateau stage by day 10. Bleached pulp substrates followed a similar pattern as brown pulp substrates (Fig. 3c). The highest maximum daily methane production was 169 mL CH_4/gVS from BLFiber on day 4, followed by 158 mL CH_4/gVS from BLFiltrate on day 1. Both BLMCC and BLMix samples reached peaks of 127 and 131 mL CH_4/gVS on day 3. All BL samples achieved 90% of their ultimate methane yield by day 7 and a plateau stage by day 12.

The degradation profile of O2 substrates varied significantly from those of brown and bleached pulp substrates (Fig. 3b). The ultimate methane yields were reached between days 9 and 15 for all samples. The maximum production rates were extended through a more spacious interval of time with each sample having two peaks of methane production. O2Fiber had a peak production of 74 and 46 mL CH_4/gVS on day 4 and 11 respectively. Initial gas production had a semi-linear tendency until reaching a plateau stage at day 15. O2MCC samples presented peak production of 42 and 45 mL CH_4/gVS on days 5 and 9 respectively and followed the same semi-linear tendency as the O2Fiber samples. O2Mix samples showed peaks of 57 and 41 mL CH_4/gVS on days 3 and 6 and reached an earlier plateau stage at day 8. O2Filtrate samples had 2 spaced out peaks, one the first day of digestion (73 mL CH_4/gVS) and another on day 10 (71 mL CH_4/gVS) before reaching a plateaus stage shortly after on day 12. A long lag phase of 6 days between peaks can be observed, where there was no methane production. Most likely an accumulation of acids occurred due to the high concentration of VFA and the availability of glucose found in O2Filtrates, that caused a drastic pH drop and furthermore an inhibition of methane production suggested by the higher presence of furfural and HMF, (Table 2). Both furfural and HMF are compounds originating from the dehydration of pentoses and hexoses and are considered main inhibitors of AD (Barakat et al., 2012), hence O2 Filtrates had the lowest methane yield of all samples. However as the methane production resumed on day 8 it is suggested that the bicarbonate buffering formed during the lag phase was sufficient to neutralize the acid formation and recover the neutral pH that was measures at the end of the experiments. Samples achieved more than 90% of their ultimate methane potential by day 12 and a plateau stage by day 15.

3.3. Anaerobic degradability

COD removal efficiencies varied between type of substrate. Filtrates had higher removal efficiencies (86–79%), whereas fiber samples had 25–35% (Table 3). This is inversely correlated to the biodegradability where fiber samples had the highest biodegradability and filtrates the lowest. Degradability regarding type of pulp was found to be in the following order: BL > BR > O2. The lower degradability of O2 pulp substrates may be linked to the chemical additions in the oxygen delignification process which cause modification in the fibers, hence the hydrolyzed filtrate from the O2 had the lowest degradability (Dence and Reeve, 1996). Degradability regarding type of substrate was found to be in the following order: Fiber > MCC > Mix > Filtrates. Degradability of samples had a similar pattern correlating to VS removal rates and their ultimate methane yield of samples (Fig. 4) which substantiates the efficiency of the AD process. Colberg and Young (1985) indicate that the smaller the size of the molecular weight fraction, the higher the degradation to biogas. Shown in Fig. 1; the MWD of Fiber samples had a bimodal shape where higher molecular weight fraction are apparent however the crystallinity index is higher. Crystalline cellulose is more resistant to chemical and biological degradation (Zhao et al., 2012), therefore it is more desirable to have lower CI substrates to increase biodegradability. Results in this study found correlation between CI and biodegradability between solid substrates. Fiber samples with lower CI (8–15%) compared with MCC samples, had higher biodegradability and methane yields.

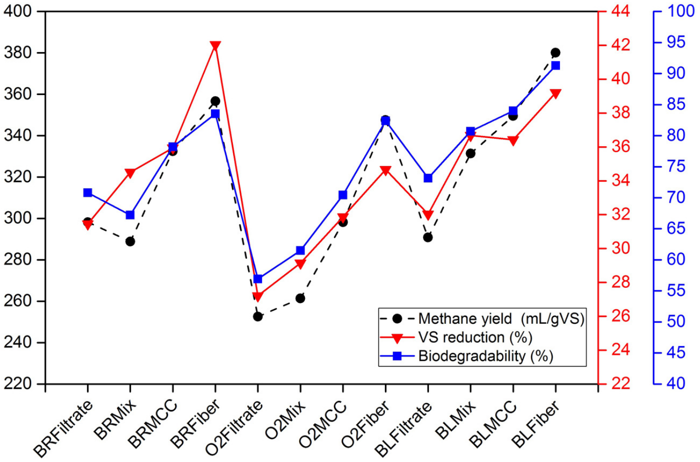

Fig. 4. Relationship between ultimate methane yield, VS reduction rates and biodegradability of brown, delignified and bleached substrates.

It is a common conclusion that lignin negatively affects the biodegradability of substrates due to the inhibiting and recalcitrant during AD. Steffen et al. (2016) compared degradabilities of different lignin content chemical pulps, 0–23%. The highest degradability corresponded to samples having the lowest lignin content. This supports the findings in this study where the highest degradabilities are shown in solid substrates with lower presence of lignin. For Filtrate samples, the high concentration of VFA (38.1 and 39.9 g/L for BRFiltrate and O2Filtrate respectively) may have caused an interrupted methane production where oversaturation occurs and is later compensated after a buffering period (Fig. 3). pH values increased from neutral to slightly alkaline (7.2–8.1), which showed sufficient buffering capacity in each batch reactor. This assured no total inhibition occurred.

3.4. Energy and economic estimation

The amount of energy from the produced methane of each substrate was calculated using Eqs. (3) and (4) in order to estimate the energetic output from a traditional fiber to an energy production scheme (Table 4). Energy estimations suggest that using BLFiber as feedstock for AD produces the highest amount of energy of 13.5 gigajoules per air dry ton (GJ/adt). BRFiber, BLMCC and O2Fiber follow with 12.6, 12.4 and 12.3 GJ/adt respectively. Fiber and MCC substrates were found to be high energy sources mainly because of their higher VS content compared to Mix and Filtrates substrates.

An estimated sludge production is important to compute and factor into the calculations. Management of the digested sludge accounts for a significant cost in a wastewater treatment plant, however simultaneously can be considered as a by-product used for soil amendment. The principal constituents in pulp and paper mill sludge include components of wood fiber such as cellulose, hemicellulose and lignin (Bayr and Rintala, 2012). Sludge production estimations ranged from 112 to 135 kg_{dry}/adt for all substrates where filtrates were in the lower range, however estimations did not vary much as they are expressed by air-dried ton. Sludge amount was then considered as an income in the cost estimation section, as it is assumed to be valued as soil amendment.

To estimate the economic potential of methane production in a chemical pulp mill it is first necessary to calculate the exisiting costs and incomes of the mill and compare them against the alternative option to produce pure biogas (methane). From a practical point of view we compare two cases (Table 5), one is pure chemical pulp production with paper grade and the second is methane production from brown stock pulp without oxygen delignification (BRFiber in this study). This would be the simplest form of raw pulp where the oxygen delignification stage stays in place, however there is no addition of chemicals or steam. The results from Table 5 compare the estimated profit from a pulp case and a methane case. The costs of initial investment for AD (which also

Table 4
Energy and sludge production calculations for all pulp substrate.

Substrate	Organic load (kgVS/adt[a])	Methane (kg_{CH4}/adt)	Energy (MJ/adt)	Sludge production (kg_{dry}/adt)
BRFiltrate	748.0	159.7	8783.5	112.2
BRMix	882.8	182.6	10040.7	132.4
BRMCC	900.0	214.3	11784.5	135.0
BRFiber	894.0	228.3	12557.6	134.1
O2Filtrate	811.1	146.6	8065.2	121.7
O2Mix	881.6	164.9	9072.0	132.2
O2MCC	897.1	191.5	10531.7	134.6
O2Fiber	900.0	224.0	12319.6	135.0
BLFiltrate	867.8	180.7	9938.4	130.2
BLMix	900.0	213.5	11741.9	135.0
BLMCC	900.0	225.2	12387.0	135.0
BLFiber	900.0	244.9	13471.5	135.0

[a] adt: air-dried ton.

Table 5
Cost estimations for two possible cases of a softwood mill.

	Pulp case	Methane case	Observations
Pulp production, adt	350,000	358,974	Methane case: 2.5% higher yield, no O2 or bleaching
Wood, €/adt	283.0	275.9	
Chemicals, €/adt	33.0	3.0	Methane case: No O2 or bleaching chemical costs
Utilities, €/adt	5.0	5.0	
Waste, €/adt	2.0	0.5	Methane case: No effluent treatment costs
By-products, €/adt	−65.0	−65.0	
Total price[a], €/adt	258.0	219.4	
Costs of production, €	90,300,000	78,767,949	
Price of main products, €/tn	600	1450[b]	Average market prices
Income from products, €/adt	210,000,000	118,832,958	22.83% methane from pulp
Income from soil amendment (SA), €/adt	0	4,813,846	Price of SA is 100 €/tn, 13.41% SA from pulp
Estimated profit, €	119,700,00	44,878,855	

[a] Chemical pulp production costs taken from the reference model in Kangas et al. (2014).
[b] Methane market prices taken from Gasum (2017).

include an aeration basin or activated sludge lagoon) can range between 1 and 2.3 million euros depending on the treated flow (Buyukkamaci and Koken, 2010). An average 1.5 million euros investment includes construction, mechanical equipment, electrical wiring, piping, transport and others costs. However for practical comparison purposes, investments costs of both cases were excluded from the calculations. In the pulp case, the direct income from the pulp makes this case the most profitable. However the methane case also makes a substantial income (37.5% of the pulp case profit) and can be a potential cost-effective product for a chemical pulp mill in the case where the mill has exhausted markets or when market prices for pulp drop. This shows that a chemical pulp mill can also become a platform for renewable energy production.

4. Conclusions

The results demonstrated that different types of chemical pulp (BL > BR > O2) are suitable for methane production. Fiber substrates showed higher yields due to their high carbohydrate and lower lignin content. Filtrates had fast production rates but the lowest yields (253 mL CH_4/gVS) mostly due to saturation of acids and washed out lignin compounds. Overall, all chemical pulp substrates had a biodegradability above 50% and a high energy production which in turn generates a profit (37.5% of the pulp case profit). This demonstrates their suitability for methane production and a promising new use for fiber products.

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