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TIINA KARPPINEN
ANAEROBIC DIGESTION OF SEDIMENTED FIBER FROM PULP
INDUSTRY FOR HYDROLYSIS AND BIOGAS PRODUCTION

Master of Science Thesis

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ABSTRACT

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Sedimented fiber is the accumulated waste from pulp and paper industry from the time before wastewater treatment. In this case, it consists mainly of wood fibers that left the pulping process in the effluent waters and sedimented at the bottom of the receiving waterbody. As the old industrial sites are rehabilitated, large quantities, in this case 1.5 million m³, of sedimented fiber require treatment. Anaerobic digestion is one option to stabilize the sediment while utilizing its energy content. Biogas produced in anaerobic digestion can be utilized in heat and power production or upgraded into vehicle fuel. Anaerobic digestion produces also digestate. Digestate can be utilized in soil amendment or construction as long as it does not consist harmful substances.

After promising results from previous research, this study was carried out to examine more efficient ways and different technologies of anaerobic treatment of the material. The continuous anaerobic mono-digestion of sedimented fiber was studied in a CSTR and hydrolysis in LBRs.

In the anaerobic digestion in a CSTR (OLR=2.5 kg VS/(m³ d) and HRT=48 d), high concentrations of VFAs (13 g/L SCOD) and SCOD (14 g/L) were produced, yet methanogenesis was struggling. Methane production was highest (240 m³ CH₄/t VS), when inoculation was still stabilizing the process. Even with buffering and nitrogen supplementation, the methane production decreased to 43–100 m³ CH₄/t VS by the end of operation. The failure of the process was most likely due to inadequate buffering and imbalance between the hydrolyzing/acidogenic and methanogenic microbial groups. The OLR was possibly too high for the methanogens, and the accumulation of propionic acid further inhibited methanogenesis. Recovery of the process was visible after one month, yet degradation of propionic acid in particular was slow. Higher buffering (> 0.57 g bicarbonate/(L d)) as well as possibly lower OLR or higher HRT is required.

Two-stage reactor configuration may allow better optimization of the anaerobic digestion process. One option is to treat sedimented fiber in an LBR, where hydrolysis and acidogenesis take place. After that, the leachate that has the sub-products, such as VFAs, can be directed to a high-rate liquid digester, where methanogenesis takes place. Increase in temperature from mesophilic to thermophilic range, recirculation of the leachate, and inoculation or nitrogen supplementation had a positive effect on hydrolysis. Total SCOD production was 42 g SCOD/kg VS at the maximum. The total volume of the material decreased during the treatment in LBRs due to compaction of the material and leachate extraction.

All in all, sedimented fiber is a novel feedstock for anaerobic digestion. It is a promising feedstock due to its methane potential, capability to produce high concentrations of VFAs, large quantities of the biodegradable material available, and the emerging need for the rehabilitation of the old pulp and paper mill sites. Further research is required on long-term stability of the anaerobic digestion of sedimented fiber and optimization of the buffering of the process. Pre-treatment could be considered in order to improve the hydrolysis for optimized VFA production. Methanogenesis of the hydrolyzed leachate from the sedimented fiber LBRs, or possibly also the digestate of CSTR with high VFA concentrations, ought to be studied further.

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Sedimentoitunut kuitu on sellu- ja paperiteollisuuden jätevesien mukana vesistöön pääsyttä kiinteää jätettä, joka on aikojen saatossa sedimentoitunut vesistön pohjaan. Tampereen Hiedanrannan tapauksessa sedimentoitunut kuitu koostuu pääosin puukuiduista ja on kertynyt järveen ennen jätevedenpuhdistuksen ottamista käyttöön. Kun vanhojen metsäteollisuuden toimipaikkojen ympäristöä kunnostetaan esimerkiksi muunnettaessa tehdasalueita asumiskäyttöön, kunnostusta tarvitsevien sedimenttien määrät ovat suuria. Tässä tapauksessa sedimentoitunutta kuitua on arviolta 1,5 miljoonaa m³. Anaerobinen hajoaminen eli mädätys on yksi mahdollisuus, jolla sedimentoitunut kuitu voidaan stabiloida. Mädätyksessä muodostunutta biokaasua voidaan käyttää lämmön ja sähkön tuotantoon tai se voidaan jalostaa liikennepolttoaineeksi. Prosessi tuottaa lisäksi mädätejännöksen, jota voidaan käyttää tuotteen laadusta riippuen esimerkiksi lannoitteena tai maanrakentamisessa.

Aiempien tutkimusten lupaavien tulosten pohjalta tämän tutkimuksen tavoitteena oli tutkia tehokkaampia tapoja ja teknisiä vaihtoehtoja sedimentoituneen kuidun anaerobiseen käsittelyyn. Sedimentoituneen kuidun anaerobista käsittelyä tutkittiin jatkuvatoimisella täyssekoitteisella reaktorilla (CSTR). Lisäksi materiaalin anaerobista hydrolyysiä tutkittiin suotovesireaktoreilla (LBR).

CSTR-kokeessa mädätteen VFA- ja SCOD-pitoisuudet olivat korkeat (VFA 13 g/L SCOD ja SCOD 14 g/L), kun reaktoria oli ajettu yli kaksi kuukautta OLR:n ollessa 2,5 kg VS/(m³ d) ja HRT:n 48 d. Metanogeenisissä oli ongelmia kokeen edetessä. Metaanintuotanto oli korkeinta (240 m³ CH₄/t VS) kokeen alussa, kun ympin suuri osuus stabiloi prosessia. Metaanintuotanto laski 43–100 m³ CH₄/t VS tasolle kokeen loppua kohden, vaikka reaktoriin lisättiin typpeä ja puskuria. Prosessin ongelmat johtuivat todennäköisesti riittämättömästä puskuroinnista ja siitä seuranneesta epätasapainosta osaprosessien (hydrolyysin/asidogeenin sekä metanogeenin) välillä. OLR saattoi lisäksi olla liian korkea metanogeenisille. Alhainen pH suosi hydrolyysiä ja VFA:iden, kuten metanogeenisiä inhiboivan propionihapon muodostumista. Syötön lopettamisen jälkeen prosessi alkoi palautua noin kuukaudessa, mutta varsinkin propionihapon hajottaminen oli hidasta. Prosessin stabiilius edellyttää suurempaa puskurointia (> 0,57 g bikarbonaattia/(L d)) sekä mahdollisesti OLR:n alentamista ja HRT:n pidentämistä.

Kaksivaiheisessa käsittelyssä prosessiolosuhteita voidaan paremmin optimoida anaerobisen hajottamisen osaprosesseille erikseen. Eräs kaksivaiheisen prosessin sovellus on käyttää LBR-reaktoria hydrolyysiin ja asidogeneesiin. Tämän reaktorin suotovedet, joiden VFA- ja SCOD-pitoisuudet ovat korkeat, voidaan johtaa edelleen metaanintuotantoreaktoriin. Tässä tutkimuksessa havaittiin, että sedimentoituneen kuidun hydrolyysiä LBR-reaktoreissa voidaan tehostaa nostamalla lämpötilaa mesofiiliseltä tasolta termofiiliselle, kierrättämällä suotovettä sekä lisäämällä hydrolysoivaan reaktoriin ympäriä tai typpeä. Korkeimmillaan SCOD:n tuotto oli 42 g SCOD/kg VS. Sedimentoituneen kuidun tilavuus pieneni LBR-kokeissa materiaalin tiivistymisen ja suotoveden poistamisen myötä.

Sedimentoituneen kuidun anaerobista hajotusprosessia on tutkittu vasta vähän. Se on kuitenkin kiinnostava syöte, sillä sen metaanipotentiaali on hyvä, siitä voidaan tuottaa korkeita VFA-pitoisuuksia tässä tutkimuksessa kuvailluissa olosuhteissa, materiaalia on saatavilla suuria määriä ja anaerobista käsittelyä voidaan käyttää osana vanhojen metsäteollisuuden tehdasalueiden kunnostusta. Tutkimusta tarvitaan kuitenkin lisää sedimentoituneen kuidun anaerobisen hajotuksen pitkäaikaisesta stabiiliudesta ja puskuroinnin optimoinnista. Hydrolyysiä voitaisiin edelleen tehostaa tutkimalla kuidun esikäsittelyä. Suotovesien ja VFA-pitoisen mädätteen metanogeneesiä tulisi myös tutkia edelleen.

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ABBREVIATIONS

AD	Anaerobic digestion
CH ₄	Methane
CO ₂	Carbon dioxide
VS	Volatile solids
CSTR	Completely stirred tank reactor
OLR	Organic loading rate
HRT	Hydraulic retention time
LBR	Leach bed reactor
COD, SCOD	Chemical oxygen demand, soluble chemical oxygen demand
CTMP	chemi-thermo mechanical pulping
BOD	Biological oxygen demand
TS	Total solids
LCC	Lignin-carbohydrate complex
VFA	Volatile fatty acid
C:N ratio	Biodegradable carbon to nitrogen ratio, here estimation of biodegradable carbon is based on volatile solids content
UASB	Upflow anaerobic sludge blanket (reactor)
GC	Gas chromatograph
FID	Flame ionization detector
TCD	Thermal conductivity detector
TN _b	Total nitrogen bound (ammonia, ammonium salts, nitrite, nitrate, organic nitrogen compounds)
NH ₄ ⁺ -N	Ammonium nitrogen

1. INTRODUCTION

Around the world, near old pulp and paper industrial sites, there is sedimented solid waste and contaminants found in the receiving waterbodies (Kokko, et al., 2018; Guo, et al., 2016; Jackson, 2016; Munawar, et al., 2000; Munkittrick, et al., 1997). Sedimented fiber from one location consists mainly of wood fibers (Autiola & Holopainen, 2016) and is proven to be biodegradable (Kokko, et al., 2018). Since pulp and paper industry is often located near shoreline and other waterbodies, the industrial sites gain growing interest as prominent residential areas after the industrial activities have ceased (Ekman, et al., 2016). Rehabilitation is required as the old industrial sites with substantial amounts of sedimented fiber in the water bodies is taken into residential and recreational use (Ekman, et al., 2016).

One of the approaches in rehabilitation is to dredge the sediment from the bottom of the waterbody and treat it in anaerobic digestion (AD) for biogas production (Kokko, et al., 2018). After AD, the remaining solid fraction needs to be utilized, for an example in soil amendment (Al Seadi, et al., 2008). AD is a microbiological process in which biodegradable organic matter is degraded in a sequence of microbial processes, and a mixture of gases, mostly methane (CH_4) and carbon dioxide (CO_2), is produced (Al Seadi, et al., 2008). The methane potential of sedimented fiber is 250 ± 80 L CH_4/kg volatile solids (VS) in batch assays (Kokko, et al., 2018). Methane production of 180–210 CH_4/kg VS was reached in completely stirred tank reactor (CSTR) experiments with OLRs of 1.5 – 2.5 $\text{kg VS}/(\text{m}^3 \text{ d})$, HRTs of 30–60 d as well as nitrogen and buffer supplementation (Lahtinen, 2017).

The methane potential of sedimented fiber is higher or in a similar range than the reported methane potentials for primary and biosludges from pulp and paper industry (150–170 L CH_4/kg VS (Bayr & Rintala, 2012) and 230 L CH_4/kg VS (Ekstrand, et al., 2016)). It is also in a similar range as the methane potential of primary sludge (190–240 L CH_4/kg VS (Bayr & Rintala, 2012)). The wood fibers may have received a sort of a pretreatment during the 30–100 years of storage at the bottom of the lake (Kokko, et al., 2018; Pearson, 1980). However, further studies on capabilities of AD of sedimented fiber is required due to small amount of research carried out with this feedstock.

There are numerous technical options for the AD of waste materials (Zhang, et al., 2016). In addition to wet processes, such as a CSTR, AD can also be carried out as dry process (Al Seadi, et al., 2008). Dry AD offers many benefits, such as smaller reactor volumes and energy consumption, but also the drawbacks, like longer retention times, compared to wet processes (Li, et al., 2011). A leach bed reactor (LBR) is a dry digester, where the AD process is controlled by recirculation of the percolating liquid extracted from the material, or the leachate (Riggio, et al., 2017; Chan, et al., 2002).

Dividing an AD process into two separate stages offers an opportunity to separately optimize the different microbial sub-processes (Ghosh, 1986). Combining a dry digester for the initial steps of AD (hydrolysis and acidogenesis) with a high-rate reactor for rapid methane production is a commonly presented approach for the treatment of lignocellulosic materials in the literature (Jagadabhi, et al., 2017; Jagadabhi, et al., 2011; Nizami & Murphy, 2011; Xu, et al., 2011; Nizami, et al., 2009; Lehtomäki, et al., 2008; Demirel & Yenigün, 2002). Solid-liquid separation of sedimented fiber was also studied in the research by Kokko et al. (2018) and Lahtinen (2017). Most of the nutrients (Lahtinen, 2017) and methane potential (Kokko, et al., 2018) is in the solid fraction after the separation. However, the chemical oxygen demand (COD) of the liquid fraction is ca. 8.8 g/L, suggesting a need for a treatment (Kokko, et al., 2018).

These findings support the approach of coupling LBRs for the hydrolysis and solid-liquid separation of the sedimented fiber with high-rate liquid reactors for the methanogenesis of the liquid fraction. The main benefits of the approach include increasing the rate of hydrolysis and decreasing retention times as well as gaining savings in reactor technology. In this case, the amount of the sedimented fiber to be treated is large, ca. 1.5 million m³ (Kokko, et al., 2018). An increase in the rate of the process leads to reduction in the time required for the remediation of the site, yet also decreases the need for reactor volumes.

In this research, the aim is to study the anaerobic mono-digestion of sedimented fiber further in CSTR experiments. Another aim of this research is to study the factors affecting the potential of hydrolysis or SCOD extraction of sedimented fiber in LBRs.

2. SEDIMENTED FIBER FROM PULP AND PAPER INDUSTRY

Pulp and paper industry utilizes large amounts of water (van Oel & Hoekstra, 2012). Over the decades of activities without efficient wastewater treatment, the industry was flushing untreated wastewater into the nearby waterbodies around the world (Guo, et al., 2016; Jackson, 2016; Munawar, et al., 2000; Munkittrick, et al., 1997). In one of the sites examined, the deposition consists mainly of wood fibers that can be utilized in AD for biogas production (Kokko, et al., 2018).

2.1 Effluents from pulp and paper industry

Global production of paper and board is 409 million tons a year (2016) (Finnish Forest Industries, 2018). In the recent years, 10 million tons of paper and board (2016) and almost 8 million tons of pulp (2017) has been produced in Finland alone (Finnish Forest Industries, 2018). Finland is the world's 4th largest pulp producer after USA, Canada, and China (van Oel & Hoekstra, 2012). Currently, there are 31 paper and board mills and 19 pulp mills operating in Finland (Finnish Forest Industries, 2018).

Pulp and paper industry is a water intensive practice that has required 300–2600 m³ of fresh water per ton of paper produced or 2–13 L per an A4 sheet of paper, depending on the location and process configuration (van Oel & Hoekstra, 2012). Earlier, before the extensive process development and the stringent environmental regulation, the water consumption was even higher (van Oel & Hoekstra, 2012). The excess process waters end up in large quantities of wastewaters (Vepsäläinen, et al., 2011).

The wastewaters from pulp and paper mills contain both solid and soluble components (Pokhrel & Viraraghavan, 2004) (Table 1). Many of those components, such as chlorinated compounds from bleaching, resin acids, and tannins are potentially harmful for the environment (Lindholm-Lehto, et al., 2015). Some of the contaminants, such as chlorinated compounds (Sponza, 2003) and lignin derivatives (Benner, et al., 1984), are recalcitrant and may cause toxicity even after decades from deposition (Hynynen, et al., 2004). There has also been considerable amounts of wood fibers and particulates that has escaped the processing and ended up in wastewaters, particularly before the extensive wastewater treatment actions (Pearson, 1980).

Table 1. Potential pollutants in the pulp and paper industry effluents. (Modified from Pearson (1980) based on Poole et al. (1978) and Walden (1976).

Pollutant	Origin
Suspended solids	Fiber, bark, ash, lime, clay
Dissolved organics	Lignin, carbohydrate, organic acids, alcohols
Toxicants	Resin acids, chlorinated organic compounds, phenolic compounds, unsaturated fatty acids, diterpene alcohols, lignin degradation products (esp. lignosulfonates), Hg & Zn compounds
Coloring agents	Lignin, paper dyes, fibers

In the early decades of pulping and papermaking in Finland, there was little or no treatment for the mill effluents (Luonsi, et al., 1988). Consequently, by 1970s and 1980s many waterbodies in Finland were highly polluted by the effluent wastewaters from pulp and paper industry. The remediation of the waterbodies started in the 1980s after environmental regulation took force and wastewater treatment technology was introduced in the pulp and paper mills (Hynynen, et al., 2004; Meriläinen, et al., 2001; Kähkönen, et al., 1998). Until today, there are deposits of sedimented solid waste and contaminants near pulp and paper industrial locations around the world (Guo, et al., 2016; Jackson, 2016; Munawar, et al., 2000; Munkittrick, et al., 1997).

2.2 Pulp and paper industry in Finland

Pulp and paper industry arrived in Finland in the 1870s along with the development of sulfite pulping process. Pulping and paper making has played a central role in Finnish industry ever since (Kuisma, 1993). For example, pulp and paper companies had been operating in the city district of Lielähti, 6 kilometers west from the city center, on the shore of lake Näsijärvi in Tampere, Finland nearly 100 years, since the 1910s (Figure 1). Currently, the municipality of Tampere plans building a new residential area, Hiedanranta, there for ca. 25 000 inhabitants (Väliharju & Toivonen, 2017). Rehabilitation of the bay area is required in order to utilize the old industrial area in recreation (Pyykkö & Lehtovaara, 2011).

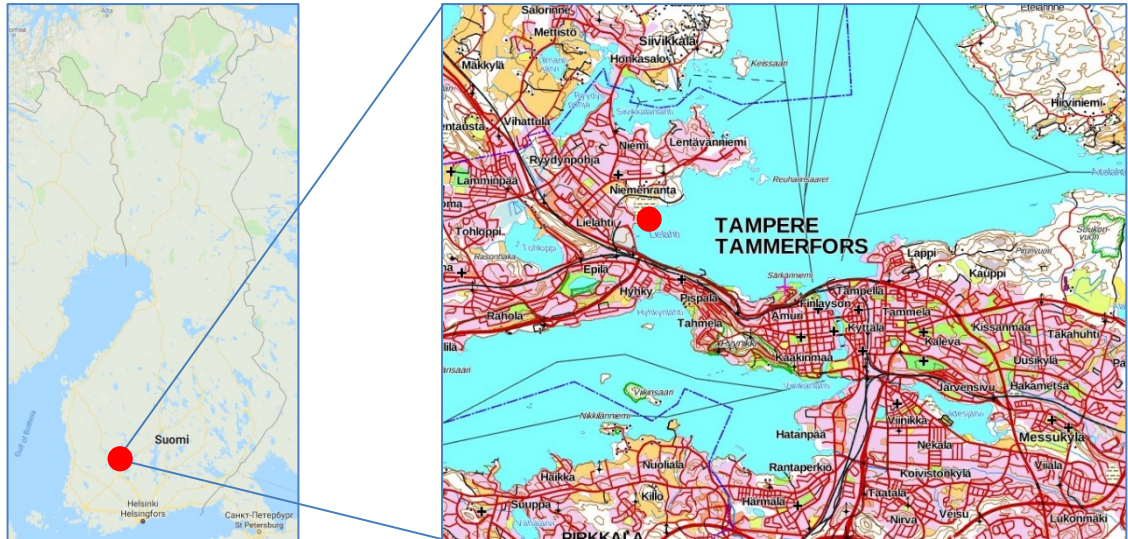


Figure 1. The site is located in Tampere, Western Finland (Google Maps; National Land Survey of Finland).

In this case, sulfite pulping and chemi-thermo mechanical pulping (CTMP) processes were carried out in 1913–1985 and 1985–2008, respectively. (Pyykkö & Lehtovaara, 2011) In addition to pulping processes, there was also lignin processing from 1960s to 2008 (Ekman, et al., 2016) and a sawmill operating in 1889–1965 at the same site (Pyykkö & Lehtovaara, 2011). The (pollution) history of the site is more closely explained in a work by Lahtinen (Lahtinen, 2017).

Sulfite pulping produces comparably large volumes waste effluents that have high concentrations of suspended solids and organic material (55 kg SS/t pulp and 350 kg biological oxygen demand (BOD)/t pulp) compared to other pulp and paper processes. The effluent volumes from CTMP processes are less than half in volume compared to sulfite pulping. In addition, the organic solids loads are smaller (45 kg SS/t pulp or 190 kg BOD/t pulp) (Pearson, 1980). A study by Hofsten & Edberg (1972) suggests that sulfite pulp is more readily degraded in water than mechanical pulp, which still contains lignin.

The lignin mill has produced chromium loading to the lake (Pirkanmaan ympäristökeskus, 2006). According to categorization of pulp and paper industry pollution sites by Pearson (1980), Hiedanranta bay is categorized as a site of gross pollution, as the sediment is composed of fiber blanket.

Before construction of a sedimentation basin for the wastewaters in the 1950s, all the effluents from the mills were discharged into the lake untreated (Ekman, et al., 2016). The effluents contained wood material and pollutants, such as mercury, chlorobenzene, and toluene (Autiola & Holopainen, 2016). The organic loading was decreased during the time of operation of the lignin factory, since it utilized the pulp mill wastewaters (Ekman, et al., 2016). The waste loading decreased in the 1980s as activated aerobic sludge process

was put into operation in 1985 (Luonsi, et al., 1988). Majority of the wood fibers that are now in the sediments originate from the sulfite pulping before wastewater treatment.

Majority of the solid waste discharged to the recipient lake settled in close vicinity of the discharge point (Hynynen, et al., 2004) sedimenting for an area of 20 ha in the bay. It is estimated that today at the bottom of the lake there are ca. 1.5 million m³ of sedimented fibers, forming a layer up to 10 meters high (Ramboll Finland Ltd, 1984). There are also pollutants bound to the solid particulates (Kähkönen, et al., 1998) and sedimentation of material from other sources, such as runoff during snowmelt and potential changes in land use (Meriläinen, et al., 2001).

In a study by Autiola & Holopainen (2016), sedimented fiber from Hiedanranta was found to contain considerable concentrations of harmful substances such as metals and organic pollutants (Table 2). Concentrations of metals like arsenic, mercury, copper, and cobalt as well as organic pollutants like toluene, fluoranthene, naphthalene, hexa-chlorobenzene, dioxins, and furans exceeded the maximum permissible limit for soil contamination (Autiola & Holopainen, 2016; Council of State of Finland, 2007). The samples were collected from different depths and sampling points, since the consistency and content of contaminants varies based on the depth of the sediment, or time of discharge (Autiola & Holopainen, 2016). Similar contamination results were reported by Hoffman et al., (2017) from sediments in the vicinity of a kraft pulp mill operated for 50 years in Nova Scotia, Canada.

Table 2. Contents of pollutants, nitrogen, and phosphorus in sedimented fiber from Hiedanranta in Tampere, Finland (Autiola & Holopainen (2016), table translated and modified from Lahtinen (2017)).

Substance	Concentration (mg/kg TS)			Concentrations for Contaminated Soil in Finnish Legislation* (mg/kg TS)		
	Minimum	Maximum	Average	Threshold	Lower Limit	Higher Limit
Arsenic (As)	<i>b.d</i>	7.7	<i>n.d</i>	5.0	50	100
Mercury (Hg)	0.12	<u>2.8</u>	0.8	0.5	2.0	5.0
Copper (Cu)	13	59	27	10	150	200
Cobalt (Co)	<i>b.d</i>	36	<i>n.d</i>	20	100	250
Toluene	<i>b.d</i>	<u>6.7</u>	<i>n.d</i>	<i>n.d</i>	5.0	25
Fluoranthene	<i>b.d</i>	1.0	<i>n.d</i>	1.0	5.0	15
Naphthalene	<i>b.d</i>	2.6	<i>n.d</i>	1.0	5.0	15
Hexachlorobenzene	0.002	<u>0.055</u>	0.0094	0.01	0.05	2.0
Phosphorus	130	370	273	<i>n.d</i>	<i>n.d</i>	<i>n.d</i>
Nitrogen	2100	5800	3627	<i>n.d</i>	<i>n.d</i>	<i>n.d</i>

The values in bold exceed the threshold value and the bold and underlined values exceed the lower limit set in the *act of the Council of State 214/2007 (Council of State of Finland, 2007).

b.d. = below the level of detection, *n.d.* = not determined.

2.3 Characteristics of the organic material in sedimented fiber

Majority of the bulk volume of the sedimented wastes from pulp and paper mill effluents in Hiedanranta case are wood-based solids (Autiola & Holopainen, 2016). Wood is comprised of mainly three chemical components: cellulose (40–45 % of wood TS), hemicellulose (20–35 %), and lignin (20–30 %). In addition, wood also contain pectin, starch, and proteins, as well as small amounts of extractives and soluble substances are also present (Fardim, 2011).

Cellulose is the most abundant naturally occurring polysaccharide in the world. It is a structural polysaccharide, giving support to the structures of plants (Chawla, et al., 2014). The ringed glucopyranose units are bound together via strong 1-4 glycosidic bonds forming a stable, linear polymer (Figure 2) (Fardim, 2011). Compared to cellulose, hemicelluloses are shorter (with 100–300 monomeric units), branched, and less stable polysaccharides with a more complex chemical structure. Hemicellulose consists of C5 and C6 sugars: hexoses, pentoses, or deoxyhexoses (Fardim, 2011).

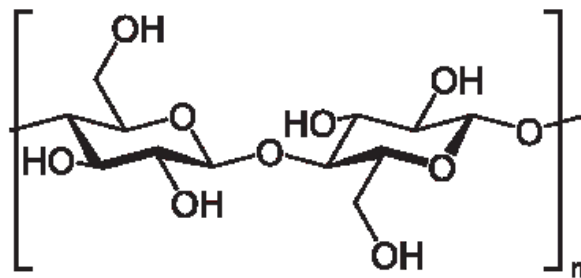


Figure 2. Stereochemical structure of cellulose.

Lignin is a polymer that consists of different types of aromatic phenylpropane units bound together via ether or carbon-carbon bonds in an irregular order (Mulat, et al., 2018; Fardim, 2011). Unlike the carbohydrates cellulose and hemicellulose, lignin is generally considered recalcitrant to biodegradation (Mulat, et al., 2018; Benner, et al., 1984). Lignin acts as a thermoplastic glue binding cellulose and hemicellulose together in bundles that form fibers in lignocellulose materials. The breakdown of these lignin-carbohydrate complexes (LCCs) is necessary in order to access the main energy content of wood, the carbohydrates (Fardim, 2011). In pulping lignin is removed to as high extent as techno-economically viable. Lignin removal method differs between pulping technologies. In sulfite pulping, the lignin in the wood material is transformed into liginosulfonates (Fardim, 2011).

In addition to biopolymers providing structure and strength for the wood material, there is a number of other substances present in wood. These substances are collectively called

as extractives. They are involved in biological functions such as providing energy storage for the plant cells (lipids) and protection against insects and microbial attacks (e.g. resins). Some of them are lipophilic and more prone to be deposited in the sediments of the effluent-receiving water bodies (Fardim, 2011).

Some of the extractives are toxic to aquatic organisms (Figure 3). Plant sterols resemble hormones found in animals and alternate the hormonal functions in aquatic fauna (Stahlschmidt-Allner, et al., 1997). According to Mahmood-Khan & Hall (Mahmood-Khan & Hall, 2003), the most common plant sterols found in pulp and paper mill effluents in Canada were β -sitosterol, β -sitostanol, and campesterol. Resin acids are considered the most toxic compounds for aquatic life that are found in pulp and paper mill effluents (Oikari, et al., 1982). The most common resin acid in wood, dehydroabietic acid has been found to severely affect the enzymatic functions in fish (Pandelides, et al., 2014; Oikari, et al., 1982). Toxic responses of such compounds may arise also in low concentrations (Mattson, et al., 2001).

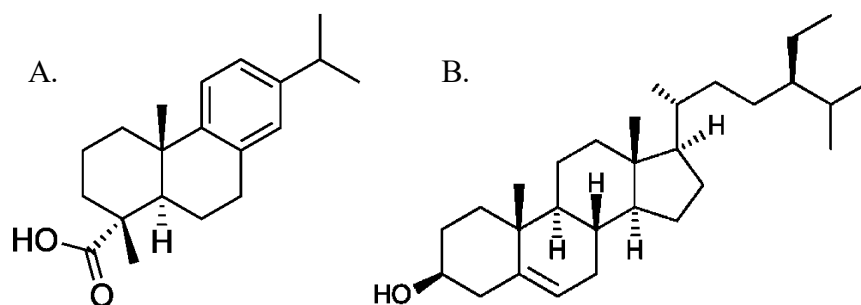


Figure 3. Examples on the extractives found in coniferous wood. A. Dehydroabietic acid is toxic to aquatic organisms and the most common resin acid found in wood (Oikari, et al., 1982). B. β -Sitosterol is one of the most commonly found plant sterols in wood (Mahmood-Khan & Hall, 2003).

3. ANAEROBIC DIGESTION (AD)

AD is described as “a microbial process of decomposition of organic matter in absence of oxygen” (Al Seadi, et al., 2008). In the following chapters, fundamentals of AD, such as the biological background, process design and using sedimented fiber as the feedstock of AD, are discussed.

3.1 Fundamentals of AD

The main products of the process include biogas, a gas mixture consisting of methane and carbon dioxide, and digestate, a slurry or process remainder with high nutrient content (Al Seadi, et al., 2008). AD provides means to stabilize organic waste while simultaneously producing energy and recovering the nutrient content of the feedstocks (Al Seadi, et al., 2008). As the European Union is setting increasingly ambitious targets on circular economy and renewable energy production, biogas production from AD is receiving increasing attention (Grando, et al., 2017).

In Finland, there are 40 fueling stations for methane (Gasum, 2018) and 4000 methane-fueled passenger vehicles (Pro Agria Pohjois-Karjala, 2018). National gas network covers the South-Eastern Finland from the eastern border to Helsinki and Tampere (Gasum, 2018). According to the National Energy and Climate Strategy for 2030 by government of Finland, the share of renewable energy in the energy end use is over 50 % in the 2020s and there should be 50 000 biomethane-powered vehicles in Finland by 2030 (Huttunen, 2017).

3.1.1 AD for biogas production

Biogas consists mainly of methane (ca. 60 %) and carbon dioxide (ca. 40 %) with trace impurities such as water vapor or hydrogen sulfide and some oxides of nitrogen, volatile organic compounds etc. Biogas can be utilized as such in direct combustion for heat production, upgraded for combined heat and power production (Al Seadi, et al., 2008), or upgraded further into value-added products like pure gases (Chen, et al., 2018). Biogas can be upgraded most to produce methane with the same quality as natural gas for transportation fuel (Al Seadi, et al., 2008). Upgraded biomethane can be pressurized or liquefied for storage and transportation (Al Seadi, et al., 2008). Biogas production can also be combined into a larger biorefinery system in the transition towards circular economy (Chen, et al., 2018).

The digestate from anaerobic digester can be used as such for fertilization and soil amendment, if the quality of the digestate is good and it does not contain harmful substances (Al

Seadi, et al., 2008). In addition, the digestate can be further processed, separated in fractions or valuable components can be extracted from it (Al Seadi, et al., 2008). Utilization of the digestate is a central question in the assessment of the viability of AD processes. Digestate can be utilized in fertilization, soil amendment or land construction (Al Seadi, et al., 2008). Due to conservation of mass, the mass of digestate is only decreased by the mass of biogas released, so the digestate forms a significant side stream from the biogas plant (Al Seadi, et al., 2008).

Life cycle assessments have shown that AD of waste to produce biogas, while replacing fossil energy sources and inorganic fertilizers, reduces greenhouse gas emissions compared to fossil fuel use (Whiting & Azapagic, 2014) or other waste treatment options (Evangelisti, et al., 2014). Upgraded biomethane that is produced from waste-derived resources and used as a transportation fuel has the lowest life cycle emissions of the biofuels (Börjesson & Mattiasson, 2008).

AD is currently a mature technology that has variety of implementations in waste treatment and energy production (Mao, et al., 2015). Still, improvement in the AD technology and adoption of new substrates has taken place in the recent years (Zhang, et al., 2016). Further optimization of the process is needed to reach higher stability of the process (Mao, et al., 2015), particularly with novel feedstocks, such as lignocellulosic materials (Sawatdeenarunat, et al., 2015).

3.1.2 Microbiology of AD

AD takes place in a series of consequent microbial processes in which the substrate is stepwise decomposed to simpler components (Al Seadi, et al., 2008). There are 4 main process phases in AD: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 4). In hydrolysis, the large molecules present on the feedstock are degraded to smaller molecules, such as monosaccharides or amino acids. In acidogenesis, the simpler organic molecules are converted to volatile fatty acids (VFAs) and alcohols or straight to acetic acid, hydrogen and carbon dioxide that are the substrates for methanogenesis (Al Seadi, et al., 2008). In acetogenesis, VFAs and alcohols are transformed into the substrates of methanogenesis: acetic acid, hydrogen, and carbon dioxide (Al Seadi, et al., 2008). Finally, methane, carbon dioxide and water is produced in methanogenesis (Al Seadi, et al., 2008).

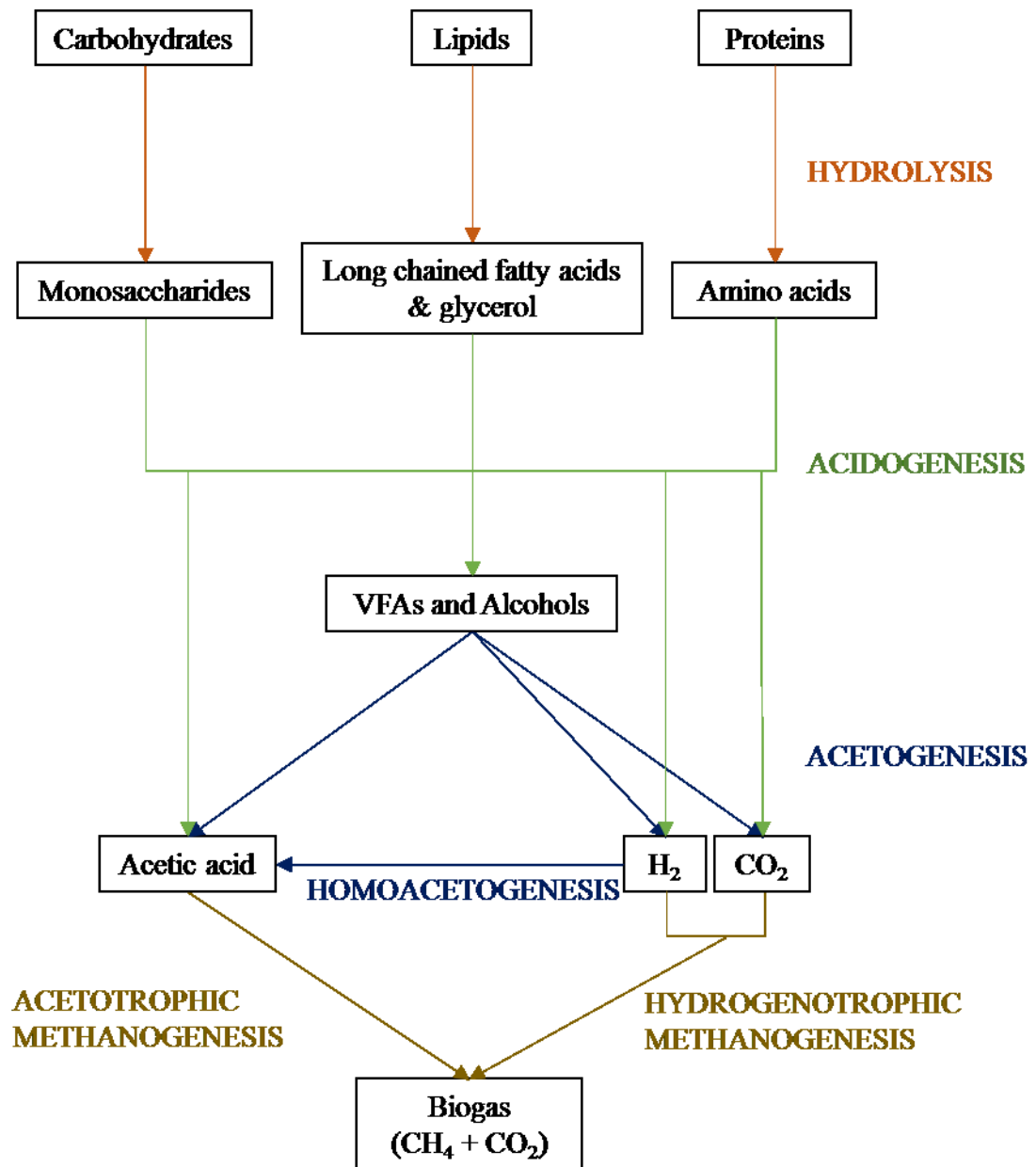


Figure 4. The main processes in AD (modified from Al Seadi et al. (2008)).

Different microorganisms are responsible for each of the steps, the previous step providing substrate to the next one until the biodegradable fraction of the feedstock is decomposed to biogas (Al Seadi, et al., 2008). The degradation reactions are enzymatic (Al Seadi, et al., 2008). Each of the process steps is inhibited by accumulation of its products. Thus, all the process steps must proceed in balance, and the rate of degradation is limited by the slowest of the process steps (Al Seadi, et al., 2008).

Bacteria are involved in hydrolysis, acidogenesis, and acetogenesis, whereas archaea are responsible for methanogenesis (Ziganshin, et al., 2013). The bacterial and archaeal communities present in the anaerobic digesters differ between different types of digesters,

feedstock (Abendroth, et al., 2015; Ziganshin, et al., 2013), and operation conditions (Ziganshin, et al., 2013).

Hydrolysis of cellulose is considered to be the rate-limiting step of AD of lignocellulosic materials, such as wood fibers (Adney, et al., 1991). Hydrolysis is the enzymatic conversion of macromolecules, such as polysaccharides, lipid compounds and proteins into monomers, oligomers and other compounds with smaller molecular mass. Different groups of microorganisms excrete enzymes that are targeted to hydrolysis of a specific type of compounds (Al Seadi, et al., 2008). Some organic material is more readily hydrolyzed than others. Lignocellulosic materials are particularly challenging to be hydrolyzed (Sawatdeenarunat, et al., 2015). Lignin content and to smaller extent cellulose crystallinity affects the methane production from lignocellulosic material (Liew, et al., 2012; Monlau, et al., 2012).

Hydrolysis of cellulose requires cellulose-hydrolyzing enzymes: cellulases (Sawatdeenarunat, et al., 2015). However, cellulose and hemicellulose are tightly bound with lignin in lignin-carbohydrate complexes that hinder hydrolysis (Fardim, 2011). Hence, a variety of enzymes is needed to hydrolyze lignocellulosic material (Cirne, et al., 2007). A study by Cirne et al. (2007) suggests that due to the large variation in lignocellulosic materials, there are different microorganism groups that are best suited for their hydrolysis. Generally, the hydrolysis of polysaccharides, proteins and lipids require cellulase, protease, and lipase enzymes, respectively. These enzymes are commonly produced by bacteria, such as species from groups *Cellulomonas*, *Bacillus* and *Mycobacterium* (Gerardi, 2003). Hydrolysis of cellulose can be monitored from the production of SCOD (Lai, et al., 2001).

3.2 AD process

Designing an AD process requires microbiological and technical understanding. In the following chapters, the design of an AD process is discussed in terms of microbiological considerations, operational conditions as well as some technical options.

3.2.1 Operational parameters of AD

The main process parameters in AD for biogas production are organic loading rate (OLR), hydraulic retention time (HRT), pH, temperature, as well as the concentrations of nutrients, trace elements and inhibiting substances (Al Seadi, et al., 2008). OLR is a parameter of the level of loading of the substrate into the process. OLR is measured as mass of organic material fed per cubic meter of the digester a day (Al Seadi, et al., 2008). OLR, HRT and reactor volume are linked together (Al Seadi, et al., 2008). The range of suitable OLR depends on the quality of the feedstock, process design and the adaptation of the microorganisms (Al Seadi, et al., 2008).

$$OLR = \frac{VS \times m_{\text{substrate fed per day}}}{V_{\text{digester}}}, \quad \text{Eq. 1}$$

where OLR = organic loading rate [kg VS/m³ d],
 VS = volatile solids content of the substrate [%],
 $m_{\text{substrate fed per day}}$ = the mass of substrate fed per unit time [kg/d] and
 V_{digester} = working volume of the digester [m³].

HRT describes the retention time of the material in a continuously operated process. HRT must be long enough to provide the microorganisms time to multiply in order not to flush them away from the process. HRT is dependent on the OLR, feedstock biodegradability, and process conditions (Al Seadi, et al., 2008). More recalcitrant feedstock requires longer HRT and high moisture content of the feedstock typically decreases HRT (Al Seadi, et al., 2008).

$$HRT = \frac{V_{\text{digester}}}{V_{\text{substrate fed per day}}}, \quad \text{Eq. 2}$$

where HRT = hydraulic retention time [d] and
 $V_{\text{substrate fed per day}}$ = the volume of substrate fed per day [m³/d].

In the AD process, pH affects not only the chemical reactions taking place but also the activity of microorganism present (Montañés, et al., 2014; Cysneiros, et al., 2012; Al Seadi, et al., 2008). Adjustment of pH to a suitable range of 6.5–8.0 (in mesophilic digestion) (Al Seadi, et al., 2008) has been found to increase methane production and stabilize the AD process (Montañés, et al., 2014; Cysneiros, et al., 2012). Too high or low pH may lead to inhibition of AD (Yenigün & Demirel, 2013). The optimum pH is higher in thermophilic digestion due to increasing carbon dioxide solubility with increasing temperature. For mesophilic AD inhibition occurs at the pH below 6.0 or over 8.3 (Al Seadi, et al., 2008). However, the optimum pH range for acidogenic (Al Seadi, et al., 2008) and hydrolyzing microorganisms is somewhat lower, in the range of 5–7 (Montañés, et al., 2014) than for other microorganisms in AD.

Over the digestion process, accumulation of ammonia increases the pH, whereas accumulation of VFAs leads to decrease in pH. Addition of a buffer stabilizes the changes in pH over AD process if the feedstock has low buffering capacity (Al Seadi, et al., 2008).

Temperature is an essential process parameter in AD, since the microorganisms needed in the process only function in a specific temperature range (Madigan, 1999). Typically, AD is operated at mesophilic (30–42 °C), thermophilic (43–55 °C), or less frequently

psychrophilic (< 20 °C) temperature range. Increasing temperature also increases the metabolic rate of the microorganisms thus increasing the decomposition of the feedstock (Al Seadi, et al., 2008). However, higher temperature processes are more susceptible towards inhibition of certain substances, such as free ammonia than processes with more moderate temperature (Chen, et al., 2007).

3.2.2 Nutrients and trace elements

A number of elements are crucial for the growth of microorganisms associated in AD. The most fundamental elements for life, carbon, nitrogen, phosphorus, and sulfur, are called macronutrients. The other crucial elements, yet required in smaller amounts, are called micronutrients or trace elements. Trace elements include cobalt, iron, molybdenum, nickel, selenium, and tungsten (Al Seadi, et al., 2008; Zandvoort, et al., 2006).

Nutrients and trace elements should be available in suitable concentrations, since too high or low concentrations inhibit the process (Al Seadi, et al., 2008; Hinken, et al., 2008; Zandvoort, et al., 2006). Macronutrients should be available at a ratio of 600:15:5:1 (C:N:P:S) (Al Seadi, et al., 2008). However in the recent literature, the carbon to nitrogen (C:N) ratio of 25 is most commonly promoted as the optimum in AD (Mao, et al., 2015).

Particularly, low nitrogen content have been associated with decreasing the methane potential in the AD of the sludges from pulp and paper industry (Bayr & Rintala, 2012) as well as sedimented fibers from pulp and paper industry (Lahtinen, 2017). The knowledge of the effects of nutrient addition on dry anaerobic mono-digestion is still limited (Jagadabhi, et al., 2017). Not much research focuses on the effects of nutrient addition on hydrolysis of lignocellulosic substrates in mono-digestion or dry processes (Jagadabhi, et al., 2017). A stable mono-digestion process using lignocellulosic feedstock with inherently low nutrient content requires nutrient supplementation (Nges, et al., 2012; Scherer, et al., 2009). Suitable concentrations of elements are often pursued by mixing a number of substrates that complement each other. However, this is not always possible and chemical additions may be required (Hinken, et al., 2008).

The trace elements are crucial for microorganisms, such as methanogens. Their availability affects methane production in AD (Jones, et al., 1987). Many of the trace elements are metals that act as cofactors of enzymes (Zandvoort, et al., 2006). Hinken et al. (2008) found 35 % increase in methane production from mono-digestion of maize silage by adding 205 µg Fe/g COD, 11 µg Ni/g COD, and 9 µg Co/g COD. Similarly, Pobeheim et al. (2010) observed 30 % increase in methane production from synthetic model of maize silage after addition of trace element solution containing iron, zinc, manganese, boron, cobalt, copper, nickel, selenium, molybdenum, and tungsten.

3.2.3 Microbiological factors

As discussed in the previous chapters, the microorganisms require certain environmental conditions in order to live and grow. The AD process must be provided with adequate nutrients in right proportions (Al Seadi, et al., 2008; Hinken, et al., 2008; Zandvoort, et al., 2006). Process conditions such as pH and temperature must be in a suitable range for the microorganisms associated in the process (Chen, et al., 2007). Too high loading of the process (Alvarez & Lidén, 2008), lack of nutrients (Bougrier, et al., 2018) or buffering of the pH (Meng, et al., 2018; Alvarez & Lidén, 2008) may lead to process failure.

A number of substances such as ammonia (Yenigün & Demirel, 2013; Procházka, et al., 2012), sulfide (Gerardi, 2003), metal ions, heavy metals and organic pollutants may inhibit AD process. The susceptibility towards inhibition is dependent on the feedstock, process conditions, and microbial acclimation (Yenigün & Demirel, 2013).

In the sedimented fiber that originates from pulp and paper industry there are a number of substances that may inhibit AD (Table 3). Tannins, the wood extractives that originate in the bark of trees, are inhibitory to microorganisms such as methanogens (Kostamo, et al., 2004; Field, et al., 1988). Lignin processing has produced chromium loading to the waterbody (Pirkanmaan ympäristökeskus, 2006). Chromium is considered a harmful heavy metal in AD (Jin, et al., 1998). Lignin, a basic component of wood is transformed into lignosulfonates in sulfite pulping (Fardim, 2011). Lignosulfonates have been found to cause toxicity inhibiting the functions of enzymes and other biological systems, also methanogenesis (Sierra-Alvarez & Lettinga, 1991; Naess & Sandvik, 1973). Remnants of sulfur-containing chemicals used in sulfite pulping, such as CaHSO_3 and SO_2 (Fardim, 2011), may be found in the sedimented fiber (Meriläinen, et al., 2001).

Table 3. *Potentially inhibiting substances present in the sedimented fiber.*

Potentially inhibiting substance	Source of the substance	Reference
Lignosulfonates	Sulfite pulping	Sierra-Alvarez (1991) & Naess & Sandvik (1973)
Tannins	Bark of wood	Kostamo et al. (2004) & Field et al. (1988)
Chromium	Lignin processing	Jin et al. (1998)
Organic pollutants	Pulping processes	Oikari et al. (1982)
VFAs (esp. propionic acid)	AD process	Wang et al. (2009)

In addition, a balance between the microorganisms involved in the different steps of anaerobic digestion must be ensured at all times. In the operation of AD, a balance in the activities of the different microbial groups must be obtained by suitable environmental conditions (pH, temperature), OLR and HRT (Demirel & Yenigün, 2002). For an example, the acetogens and methanogens co-operate in a way that the acetogens produce acetic acid and hydrogen that is taken up by the methanogens. Accumulation of acetic acid and hydrogen inhibits acetogenesis (Gerardi, 2003). Concentrations of the intermediate products are monitored in order to follow the process and avoid the inhibitory effects (Al Seadi, et al., 2008).

Especially propionic acid that is one of the VFA species is considered highly toxic to methanogens (Wang, et al., 2009). According to Wang et al. (2009), a propionic acid concentration of 900 mg/L causes inhibition of methanogens leading to further accumulation of VFAs. Similarly to ammonia, pH also affects the form of VFAs in water solutions. When pH is low, the non-ionized forms are predominant causing more severe inhibition (Kymäläinen & Pakarinen, 2015). The main options for overcoming inhibition include co-digestion with other substrates or dilution to decrease the accumulation of inhibitors, providing time for microbial adaptation or acquiring appropriate inoculum, and removal of the inhibitors (Chen, et al., 2007).

3.3 AD process technology

The type of the anaerobic digester should be selected according to the properties of the feedstock, most importantly the total solids (TS) content (Igoni, et al., 2008). Digesters can be operated either continuously or in batches (Al Seadi, et al., 2008). There are numerous applications of the different types of operation and digester design. AD process can also be divided into two or more stages taking place in different digesters (Al Seadi, et al., 2008).

3.3.1 Wet and dry digestion

AD processes can be dry or wet digestion based on the TS content of the feedstock. A process with a TS content over 15 % (Li, et al., 2011), typically 20–40 % is considered dry digestion (Al Seadi, et al., 2008). Correspondingly, wet AD takes place at the TS range of 0.5–15 % (Li, et al., 2011). At high TS content (>30 %) liquid to gas mass transfer is limiting the AD process (Abbassi-Guendouz, et al., 2012). Along with increasing TS content, the rate of hydrolysis decreases and methanogenesis is eventually inhibited due to the hindered mass transfer, taking place in solid matter (Abbassi-Guendouz, et al., 2012).

Dry digestion of lignocellulosic materials has been found to have a number of attributes, such as better performance at higher OLR and higher volumetric biomethane production (Yang, et al., 2015). The dry reactors also require less moving parts and typically consume less energy, both due to lack of mixing, compared to wet processes. In addition, dry processes require less water input for feedstocks with high TS content, and less energy for heating (Li, et al., 2011). Treatment of the solid fraction with subsequent higher TS content may lead to lower capital costs due to smaller digester volume required (Ge, et al., 2016). Dry digestion is claimed to be more robust regarding the quality or homogeneity of the feedstock (Li, et al., 2011).

Increasing the TS content of the feedstock up to 15–20 % may lead to decreasing methane yield and methane production rate (Yang, et al., 2015; Xu, et al., 2014) due to prohibition of diffusion (Ge, et al., 2016) and recalcitrance of the feedstock (Yang, et al., 2015). Dry digestion also requires large amounts of inoculum and longer HRTs (Li, et al., 2011).

3.3.2 Continuous and batch operation

Anaerobic digester can be operated either in batch or continuously. In batch operation, the feedstock is placed in the digester that is operated and finally emptied after the process is complete. Batch operation is most often applied to dry digestion (Al Seadi, et al., 2008). Often there is no mechanical mixing equipment, yet the percolating liquid is recirculated in the digester for material transfer (such as in an LBR) (Riggio, et al., 2017).

In continuous process, the feedstock is fed and removed throughout the operation. The gas production is continuous, since there is no pause due to loading and emptying of the digester. The main types of continuous digesters are mechanically mixed vertical digesters (such as a CSTR) and horizontal plug-flow digesters. In plug-flow digesters the feed pushes the material forward in the digester (Al Seadi, et al., 2008). In semi-continuous operation, the feeding is not continuous, yet it is frequent over the operation and not carried out only when starting of the reactor (Al Seadi, et al., 2008).

3.3.3 Digester types

A CSTR is a first-generation digester type for high-rate AD process. It is a common digester design for liquid AD of slurries, such as manures (Al Seadi, et al., 2008). It is used in 90 % of the newly built wet AD processes. The design consists of a tank and a mixer (Figure 5). The complete stirring of the reactor contents allows good microbial contact to the substrate, yet consumes energy (Mao, et al., 2015). As the feed and removal of digestate is continuous, CSTR is more labour-intensive than the digesters operated in batch (Al Seadi, et al., 2008).

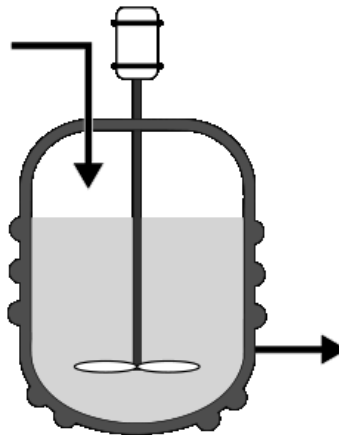


Figure 5. A schematic on a CSTR digester (public domain).

An LBR is a dry digester that has no moving parts and where the mass transfer is carried out via circulation of the percolating liquid (Figure 6) (Riggio, et al., 2017). LBRs are often operated in batch. The AD process can be optimized and gas production enhanced by recirculation of the percolating liquid, the leachate (Riggio, et al., 2017; Chan, et al., 2002). Multiple LBRs can also be operated in a sequence so that leachate from previous reactor is directed to the next one allowing different process steps of AD to take place in each reactor (Nkemka & Hao, 2018; Riggio, et al., 2017). Replacement of leachate partially or entirely with fresh water has also been found to improve the hydrolysis in AD of

lignocellulosic materials (Jagadabhi, et al., 2010). Leachate recirculation may improve methane yield in dry AD, however, it requires precise optimization (Ge, et al., 2016).

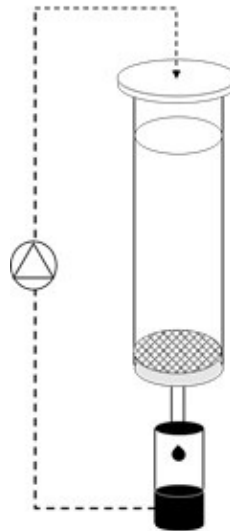


Figure 6. A schematic on an LBR digester.

3.3.4 Two-stage AD

When AD process takes place in one digester, all the microorganisms involved in the process must be able to function in the same conditions. Dividing the process in two stages allows separate optimization of the hydrolysis-acidogenesis and methanogenesis stages (Ghosh, 1986). It may also reduce the need for water additions for dilution as well as homogenization of the feedstock (Ghosh, 1986).

Typically, in the first stage of the two-stage AD, the feedstock is placed in a dry digester, such as an LBR, where the hydrolysis and acidogenesis take place and the liquid fraction is separated. The LBR can be coupled with a high-rate digester, such as up-flow anaerobic sludge blanket (UASB) reactor, for the treatment of the liquid fraction rich in the products of acidogenesis. Methanogenesis takes place in this second phase. In addition, there may be recirculation of the liquids. In this way, the AD process can be divided in two separate stages that can be further optimized for optimum pH (Jagadabhi, et al., 2017; Jagadabhi, et al., 2011; Nizami & Murphy, 2011; Xu, et al., 2011; Nizami, et al., 2009; Lehtomäki, et al., 2008; Demirel & Yenigün, 2002).

In two-phase digestion, more time can be allocated for hydrolysis of solid or semi-solid, cellulosic feedstock (Ghosh, 1986). Lee et al. (2009) suggest that two-stage process may enable better optimization of temperature conditions for the AD process. The best process

performance was achieved by carrying hydrolysis and acidogenesis steps out in thermophilic temperature and methanogenesis at mesophilic level (Lee, et al., 2009). However, Jiang et al. (Jiang, et al., 2013) found mesophilic temperature (35 °C) to be optimum for hydrolysis and VFAs production.

3.4 AD of fiber sediments and pulp and paper industry sludges

There is little research available on AD of sedimented fiber from pulp and paper industry. According to Kokko et al. (2018), sedimented fiber (from the same source that was used in this thesis) is anaerobically biodegradable. The methane production in AD batch assays was rapid and high: methane production was 250 ± 80 L CH₄/kg VS and 80 % of the methane potential was gained during the first 14 d (Kokko, et al., 2018). The total methane potential of the entire 1.5 million m³ of sedimented fiber present in the bay area was estimated to reach 40 million m³ of methane (Kokko, et al., 2018).

After using a filter press type of solid-liquid separation, 90 % of the methane potential was in the solid fraction, yet also the liquid fraction contained ca. 8.8 g COD/L (Kokko, et al., 2018). Thus, also the liquid fraction requires treatment before it can be discharged, yet it also has significant value when considering the substantial total volume of the material to be treated (1.5 million m³) (Kokko, et al., 2018). The liquid fraction could be treated using a high-rate anaerobic digester, such as an upflow anaerobic sludge blanket (UASB) reactor. The solid fraction, on the other hand, could be treated separately in a different anaerobic dry digester.

A study by Lahtinen (2017) found that supplementation of nitrogen to sedimented fiber treated in a CSTR improved methane production and buffering stabilized the process. Co-digestion of sedimented fiber with municipal wastewater sludge produced 70 % higher methane production compared to mono-digestion of sedimented fiber (Lahtinen, 2017).

Primary sludge from pulp and paper industry is another waste fraction the properties of which resemble those of sedimented fiber. Majority of the research carried out on the AD of pulp and paper industry sludges consider mostly utilization of secondary sludge or co-digestion of different sludges or other feedstock (Kamali, et al., 2016). Secondary sludge is comprised of bacterial biomass from activated sludge wastewater processing and cannot be compared with sedimented fiber. Primary sludge, on the other hand, is more similar to sedimented fiber, since it is mostly comprised of wood fibers (Fardim, 2011).

Methane yield from the AD of sedimented fiber can be higher than the methane yield of primary sludge (Table 4). The increase in methane potential from pulp and paper mill sludges is often achieved via pretreatment (Kamali, et al., 2016). In the case of sedimented fiber, the long (30–100 years), storage in the sediments of a lake has given the material a slow pretreatment, which may enhance the AD (Kokko, et al., 2018).

Table 4. Methane potentials of pulp and paper sludges and sedimented fiber. (Modified from Kokko et al. (2018).)

Substrate	Mode of Operation	OLR (kg VS/m ³ d)	HRT (d)	Methane Yield (L CH ₄ /kg VS)	Reference
Primary sludge	CSTR	1–1.4	16–32	190–240	Bayr & Rintala (2012)
Primary and biosludge	CSTR	1	25–31	150–170	Bayr & Rintala (2012)
Primary and biosludge	CSTR	4	4	230	Ekstrand et al. (2016)
Sedimented fiber	Batch	-	-	250±80	Kokko et al. (2018)
Solid fraction of sedimented fiber	Batch	-	-	270±40	Kokko et al. (2018)
Sedimented fiber	CSTR	1.5–2.5	30–60	180–280	Lahtinen (2017)

Sedimented fiber is an abundant resource that is readily available in a spot location (Autiola & Holopainen, 2016). Remediation of the site is required in any case (Ekman, et al., 2016). AD provides means to recover energy of the material while stabilizing it (Al Seadi, et al., 2008). Pulping of the wood fibers and decades long storage at the bottom of the lake has pretreated the material and it is more biodegradable than virgin wood fibers (Kokko, et al., 2018; Pearson, 1980). On the other hand, sedimented fiber has low nutrient content, hence a high C:N ratio and it contains metals and pollutants (Autiola & Holopainen, 2016). The lignocellulosic material may benefit from pretreatment (Sawatdeenarunat, et al., 2015).

Mixing of the fibrous feedstock can be energy-intensive (Sawatdeenarunat, et al., 2015). Hence, the dry digester reactor design without mechanical mixing can be a viable option.

Many lignocellulosic feedstocks require supplementation of macro and micro nutrients and adjusting pH (Sawatdeenarunat, et al., 2015).

A variety of pretreatments is suggested for lignocellulosic materials (Ge, et al., 2016; Menon & Rao, 2012). Pretreatments are often studied in improving the methane production or hydrolysis of the lignocellulosic material (Ge, et al., 2016; Sawatdeenarunat, et al., 2015). The aim of the pretreatment of lignocellulosic material is to increase the availability of cellulose for the hydrolyzing enzymes and thus increase the rate and degree of hydrolysis (Jönsson, et al., 2013). Thermal pretreatment has been found to increase the biomethane yield of pulp and paper mill sludges by making the material more readily biodegradable (Kinnunen, et al., 2015; Wood, et al., 2009). Pretreatment is a key factor in increasing the rate of hydrolysis in the recent literature of VFA production from lignocellulosics (Zhou, et al., 2018).

There is high potential in the AD and biogas production from lignocellulosic materials (Sawatdeenarunat, et al., 2015). In the recent reviews dealing with AD of lignocellulosic materials (Ge, et al., 2016; Sawatdeenarunat, et al., 2015), the sedimented fibers accumulated over the decades from pulp and paper industry effluents are not yet mentioned as a potential resource.

4. MATERIALS AND METHODS

In this study, sedimented fiber originating from pulp and paper industry was digested anaerobically in laboratory scale reactors. Digestion in two different types of reactors was carried out. The origin, sampling, and consistency of the raw materials as well as details from the reactor studies and analyses carried out to monitor the experiments are explained in the following sub-chapters.

4.1 Sedimented fiber

The feedstock was sedimented fiber originating from pulp and paper industry. The sedimented fiber samples were collected from the bottom of Lake Näsijärvi on 26.6.2017 and 3.7.–5.7.2017. The sampling was carried out by using an excavator from 3 sampling points (Figure 7). The points were determined based on previous studies in order to get most representable sample, although the consistency of the sediment changes both by depth and by distance to shoreline (Ramboll Finland Oy, Infra & Liikenne, 2017). Samples taken from the same points one year earlier were used in the previous studies by Lahtinen (2017) and Kokko et al. (2018).

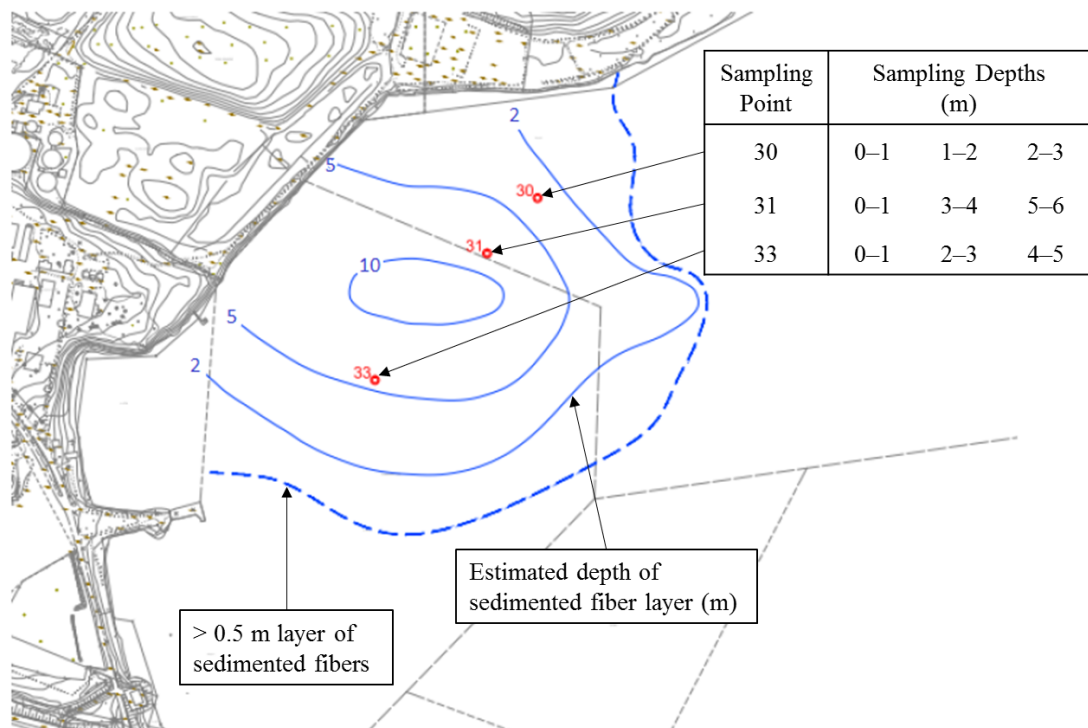


Figure 7. Sampling of sedimented fiber in Näsijärvi lake outside Hiedanranta area in Tampere, Finland. Modified from (Ramboll Finland Oy, Infra & Liikenne, 2017).

From each of the points, two samples from three depths were taken. The consistency of the sediment changes by depth (Table 5). In all of the sampling points, the layers closest to the surface of the sediment (0–1 m from the bottom of the lake) had the highest TS (22.32 ± 3.10 %) and VS (21.60 ± 3.02 %) content. Sediment from deeper layers had higher moisture content (TS = (11.54 ± 1.24) % and VS = (20.73 ± 1.36) %) and was more homogenous in its macro-structure. In addition, the color and visual appearance of the material changed by depth as well (Figure 8).

Table 5. Sampling of sedimented fiber in 2017. * (Ramboll Finland Oy, Infra & Liikenne, 2017).

Sampling Point*	Sampling Depth (m from sediment surface)*	TS (%)	VS (%)	VS/TS (%)
30	0–1	23.36 ± 0.91	22.84 ± 0.94	97.80
30	1–2	11.14 ± 0.39	10.54 ± 0.39	94.61
30	2–3	11.03 ± 0.03	9.55 ± 0.20	86.59
31	0–1	18.73 ± 1.48	17.99 ± 1.41	96.09
31	3–4	11.80 ± 0.19	11.23 ± 0.18	95.17
31	5–6	11.81 ± 0.24	11.19 ± 0.24	94.73
33	0–1	24.88 ± 2.27	23.96 ± 2.10	96.30
33	2–3	12.32 ± 2.74	11.74 ± 2.72	95.26
33	4–5	11.13 ± 0.36	10.12 ± 0.27	90.92



Figure 8. The visual appearance of the sedimented fibers. The layers closest to surface are rich in wood pieces and solids (on the left), whereas deeper the sediment has higher moisture content and the fibers are softer (in the center and on the right), forming a felt-like structure (on the right).

Sedimented fiber samples were stored at 4 °C, in gastight containers before use for experiments. A mixed sample was prepared for laboratory experiments by combining equal volumes of each 18 samples (every sampling point in duplicate). Before sampling, the sediment was vigorously mixed in a sturdy bucket with a cement mixer connected to a power drill. The mixed sample was again thoroughly mixed and stored in gastight containers at 4 °C.

The TS content of the compilation was (14.16 ± 0.37) % and VS content (13.36 ± 0.40) %. The mixed sample had a structure that resembled a paste rather than solid material. The pH of the mixed sample was analyzed by inserting a pH probe into a container of thoroughly mixed and lightly compressed sedimented fibers.

4.2 Inoculum

The inoculum was taken from the mesophilic digester of a municipal wastewater treatment plant Viinikanlahti in Tampere, Finland. The substrate of the digester is mixed municipal wastewater sludge. The inoculum was collected from the plant 2 weeks before starting the experiments and was stored in tightly closed containers at 4 °C. TS and VS of the inoculum were 2.88 % and 1.53 %, respectively. The pH of the inoculum was 7.43.

4.3 Completely stirred tank reactor (CSTR) set-up

Sedimented fibers were treated in a CSTR at 35 °C (Figure 9). Total volume of the reactor was 5 L with a liquid volume ca. 4 L. The reactor was heated using a heating mantle and the contents was continuously mechanically stirred. Biogas from the reactor was collected via gas tubes into 10 L aluminum gasbags (Supelco, SigmaAldrich).

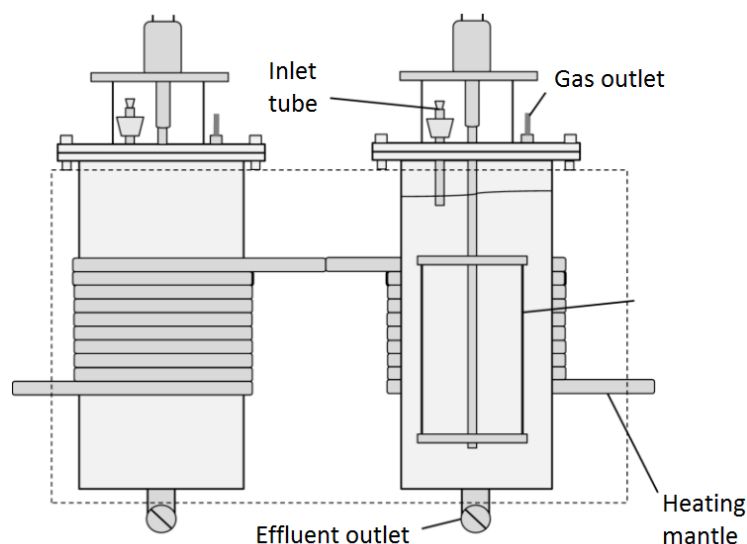


Figure 9. A schematic on the CSTR design. (Modified from Ylä-Outinen (2014).)

The experiments were started by adding 4 L of inoculum. Feeding was started on day 5 with an OLR of 2.5 kg VS/(m³×d) and an HRT ca. 48 d, both as weekly averages. The reactor was fed from Monday to Friday by allocating the weekly feed for 5 working days.

Initially, the mixed fiber without any screening was fed in the CSTR for 60 days. Later (after day 60) the fiber samples were manually screened in order to remove larger wooden particles that were causing clogging of the parts of the reactor. Approximately 4 % of the mass of the sedimented fibers were larger wooden particles that were removed before feeding. Digestate with a volume corresponding ca. 90 % of feed volume was removed prior to feeding according to the substrate level in the reactor. Initially, no buffering or nutrient supplementing was carried out.

The digestate was analyzed for pH, 5 days a week as well as soluble COD (SCOD) and VFAs three times a week. In addition, TS and VS were determined once in two weeks and total COD at the end of the experiment. Methane and carbon dioxide contents in biogas were analyzed three times a week in the morning, followed by biogas volume determination.

Nitrogen (NH₄Cl) supplementation was started on day 31 to reach C:N ratio of 20 or 6.7 g N/L in reactor. 130 mL of 119 g NH₄Cl/L solution was added weekly. In addition, buffering of the substrate with bicarbonate was started on day 54 as a decline in digestate pH was observed. 200 mL of 80 g/L bicarbonate solution was added weekly, however only 60 g/L on the first week.

The reactor was operated in semi-continuous mode for 105 days. After that, the reactor was operated for 29 more days in batch mode without feeding or removing digestate (with the exception of sampling). Finally, after total of 134 days of operation, the experiments were stopped, and the reactor content was used in a brief batch assay.

4.3.1 Batch assays to determine trace element sufficiency

Batch assays were carried out with the CSTR digestate. Four assays were prepared: two controls containing only the digestate and two assays with digestate supplemented with trace elements. For each of the assays, 500 g of CSTR digestate was added to a 1000 mL Pyrex bottle. The pH values were 7–8. To the both of the sample assays, 2 mL of trace element solution (DSMZ anaerobacter medium no.144) was added (Table 6) in order to reach similar additions of trace elements as in a study by Hinken et al. (2008).

Table 6. Contents of trace element solution used in the batch assays after CSTR experiments (Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, 2015).

Ingredient	c (g L⁻¹)
Nitrilotriacetic acid	12.80
FeCl ₂ × 4 H ₂ O	0.20
MnCl ₂ × 4 H ₂ O	0.10
CoCl ₂ × 6 H ₂ O	0.17
CaCl ₂ × 2 H ₂ O	0.10
ZnCl ₂	0.10
CuCl ₂	0.02
H ₃ BO ₃	0.01
Na ₂ MoO ₄ × 2 H ₂ O	0.01
NiCl ₂ × 6 H ₂ O	0.03
NaCl	1.00
Na ₂ SeO ₃ × 5 H ₂ O	0.03
Distilled water	

Headspace (ca. 500 mL) were flushed with nitrogen gas for 5 minutes. The bottles were tightly capped with double-line caps that enabled sampling from the headspace as well as gas collection to gasbags. The assays were placed in a water bath at 35 °C. Gas composition and volume were determined once to twice a week. The experiment was run for 43 days.

The gas concentrations and volumes as well as the appearance of the substrate in one of the assays with trace element supplementation differed from all of the others. The pH, COD, SCOD, and VFA concentrations results of that assay were excluded from the inspection.

4.4 Leach bed reactor (LBR) set-ups

Three LBRs constructed using acrylic tubes (height: 600 mm, diameter: 150 mm) were used (Figure 10). Round plastic meshes (mosquito net, pore size ca. 3 mm) were fitted at the bottom of the reactors. In addition, a 2 cm layer (ca. 0.77 kg) of washed gravel was placed on the mesh. The reactors were filled with sedimented fibers from the top and the lids were sealed. At the bottom of a reactor, there was an outlet with a valve for leachate collection, as well as two openings at the top for gas collection and leachate recirculation or water addition, respectively.

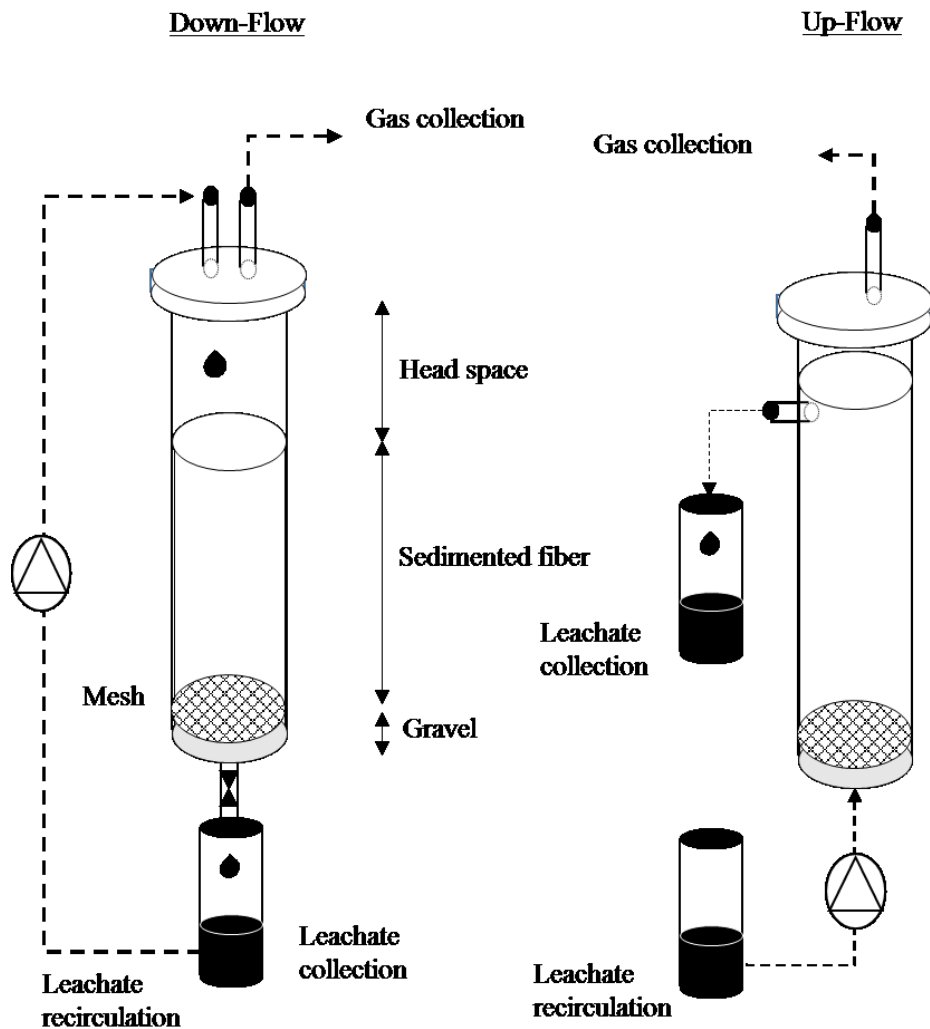


Figure 10. Design of the LBRs (height = 600 mm, inside diameter = 150 mm) used in down-flow and up-flow operation.

Up-flow and down-flow configurations of LBRs were used. In down-flow operation leaching was based on gravitation and leachate collection was controlled with a valve at the outlet. The outlet was connected to a leachate collection bottle (Pyrex) with Masterflex tubing. Leachate sampling was carried out straight from the outlet, however occasionally also from the collection bottle after mixing. A Masterflex L/S pump (Cole-Parmer) with Easy-Load II heads (1–2 pieces, model 77200-50, Cole-Parmer) and Masterflex Tygon (L/S 16, Cole-Parmer) tubing were used in leachate recirculation (rate: 3 mL/min). Gas outlet from the headspace was provided with a gas line via Masterflex Tygon tubing into aluminum gasbags. The up-flow LBR was operated semi-continuously by collecting leachate in a container and manually transporting it to recirculation tank for pumping back into the reactor. Additional water was initially pumped into the reactor from the bottom valve. Leachate collection commenced as liquid level reached the opening on the side of

the reactor at the top. Down-flow operation was selected for further studies due to lack of technical difficulties.

A series of 7 consequent studies were performed (Table 7). The first 3 experiments focused on extraction of leachate from the sedimented fiber in LBRs. The latter 4 experiments focused on hydrolysis of sedimented fiber. In the first 3 experiments, the reactors were operated at 22 °C. Heating was provided in the latter 4 experiments with external water circulation (tubing, ca. 50 coils from top to bottom).

Table 7. Overview on the LBR experiments. The superscripted letters combine the data of same reactors and the numbers in brackets refer to the number of reactors in question.

Topic	No. of Reactors	V _{sediment} / Reactor (L)	Compaction	Water Addition	Reactor Type	Duration	Study Question(s)
Leachate Extraction	8	2 ^a , 4 ^a , 6 ^a , 7 ^b , 9 ^d , 10 ^c	None (3) ^a , maximum (5) ^{bcd}	No (7) ^{abc} , yes ^d	Up-flow ^d , down-flow (7) ^{abc}	117 h (3) ^a + 24 h (3) ^b + 24 h ^c + 48 h ^d	Leachate formation and technical feasibility
	4	2 ^a , 4 ^a , 6 ^a , 10 ^c	None ^a , maximum ^c	No	Down-flow	117 h ^a + 24 h ^c	Effect of sediment volume on leaching
	3	7 ^b	None, light, maximum	No	Down-flow	24 h	Effect of compaction on leaching
Hydrolysis	3	7 ^e	Maximum	Yes	Down-flow	52 d	Effect of temperature on hydrolysis
	3	7 ^e	Maximum	Yes	Down-flow	52 d	Effect of nitrogen addition and inoculation on hydrolysis
	3	7 ^e	Maximum	Yes	Down-flow	52 d	Effect of leachate recirculation on hydrolysis
	3	7 ^e	Maximum	Yes	Down-flow	52 d	Effect of fresh water addition and recirculation on hydrolysis

Leachate extraction

In the first experiments, leachate extraction and technical feasibility of using LBRs for the treatment of sedimented fiber was studied. The effect of the volume and height of the sedimented fiber on total leachate volume was inspected. Total leachate volumes from reactors with different amounts (2, 4, 6, and 10 L) of sedimented fiber (mixed sample) were measured. Contents of the reactor with 10 L fiber were compacted and the others were not. The fibers were kept in the reactors over 24 hours allowing constant leaching. The effect of recirculation of the leachate on the total volume was also observed. The rate of leaching for the reactor with 10 L of fiber was monitored over the first 24 h of operation.

The effect of compaction on the leachate volume and the rate of leaching was studied by measuring the leachate volumes from 3 reactors with 7 L of sedimented fiber with different compactions in each of them (Table 8). The total leachate volumes were compared to the initial volumes of sedimented fiber. TS and VS were determined for the fiber before and after the experiments. The changes in sediment volumes over the experiments due to compaction and leaching were monitored.

Table 8. *Three degrees of compaction (gravitational packing, light compaction, maximum manpowered compaction) were used in the third LBR experiment to study the effect of compaction of the sediment on total leachate volume and the rate of leaching.*

Reactor	Uncompacted Sediment Volume (L)	Compacted Sediment Volume (L)	Compaction Method
1	7	4.5	Maximum compaction (pressing with a blunt object and poking with a spoon)
2	7	5.0	Light compaction with a blunt object
3	7	5.5	Packing the material to avoid void spaces and gravitational packing

Hydrolysis

The reactors were operated continuously for 51 days. Hydrolysis of sedimented fiber was promoted by providing heating, nutrient and inoculum supplementation, leachate recirculation, fresh water addition, and longer contact time in the reactor. The experiments were started by adding, 6.00 kg, corresponding 6 L, of sedimented fiber in each of the three airtight reactors with 2.5 L of 21 °C tap water. There was approximately 1 L airspace at

the top of each reactor. Reactors were left standing for 24 hours before opening the leachate valves at the bottom of the reactors (Figure 11). The reactors were drained from the leachates for another 24 hours and total leachate volumes were observed. Leachate recirculation was started with the flow rate of 3 mL/min after 48 hours of operation. The degree of hydrolysis was examined by following the SCOD and VFA concentrations as well as pH of the leachates throughout the operation. In addition, the volume and color of the collected leachates, heater and reactor temperatures, as well as leachate recirculation flow rate were monitored.

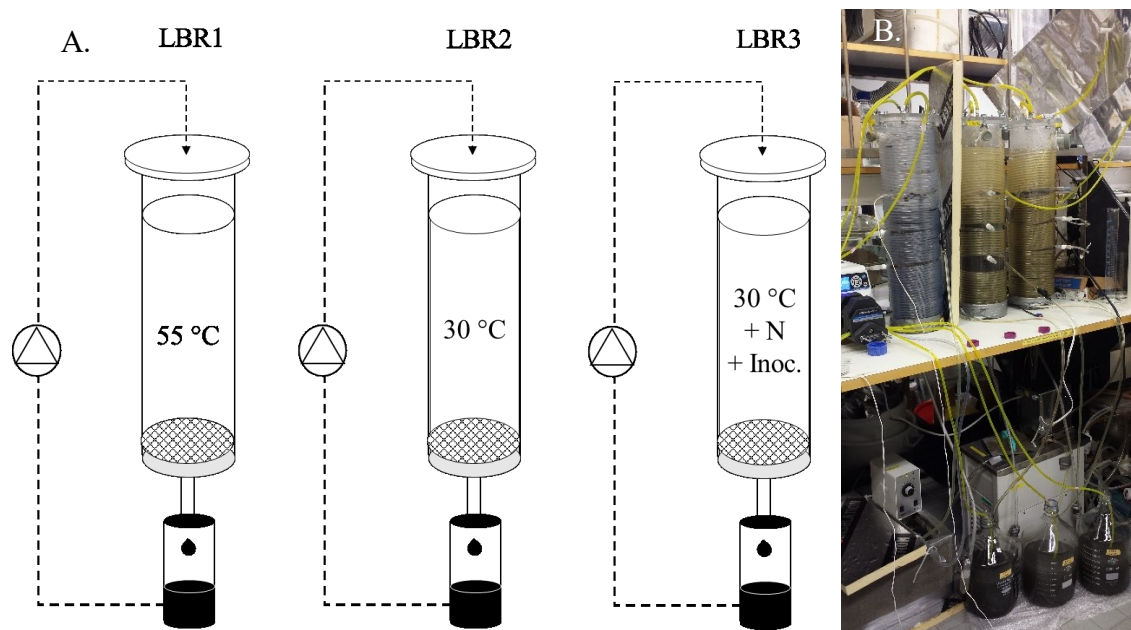


Figure 11. Experimental setup (A. a schematic, B. a photograph) of LBR experiments on hydrolysis of sedimented fiber.

The effect of temperature on the hydrolysis was studied by operating one reactor at 55 °C and two reactors at 30 °C for 51 days (Table 9). The effect of nitrogen addition and inoculation was studied by supplementing one of the mesophilic reactors with nitrogen and inoculum. Nitrogen was added after 4 days of operation. Target C/N ratio of 100 in the reactor was reached by adding 32.5 g of NH_4Cl in 274 mL water solution into the collected leachate after starting the leachate recirculation. The same reactor was also inoculated after 10 days of operation with 300 g of municipal wastewater sludge inoculum (described above). The substrate to inoculum ratio was 20.

Table 9. *The treatments used to promote hydrolysis of sedimented fiber in LBRs.*

Day	Treatment
0	Reactors heated to 55 (the thermophilic LBR) and 30 °C (the 2 mesophilic LBRs)
2	Leachate recirculation starts
4	Nitrogen added to one of the mesophilic reactors
10	Inoculation of the mesophilic reactor with nitrogen
28	Leachate recirculation rate is doubled
35	Leachate is replaced with tap water and recirculated (the 1 st time)
46	Leachate is replaced with tap water and recirculated (the 2 nd time)
50	Leachate is replaced with tap water and recirculated (the 3 rd time)
51	The experiment is ended

The effect of leachate recirculation (at the rate of 3 mL/min) on hydrolysis was studied for 33 days. Leachate recirculation rate was doubled (to 6 mL/min) for 7 days in order to determine, whether the rate of recirculation had an effect on the hydrolysis. The effect of fresh water addition on hydrolysis was studied by replacing the collected, maximum amount of leachate with fresh water. The replacement was carried out three times, allowing the process to stabilize in between.

4.5 Analyses

For SCOD and VFA analyses, the samples were centrifuged (Sigma 4K15, B.Braun Biotech International) at 4000 rpm and 21 °C for 15 minutes. The supernatant from the centrifuged sample was decanted and mixed with a Vortex stirrer prior to filtration with membrane syringe filters with a pore size of 0.45 µm (5 mL SOFT-JECT Luer syringe, HENKE SASS WOLF & CHROMAFIL Xtra PET-45/25 filter, Macherey-Nagel) into 5 mL Falcon tubes. In case the analysis was not carried out within 24 hours from sampling, the SCOD samples were stabilized with 4 M sulfuric acid as explained in the standard SFS 5504 (SFS, 1988) and stored at 4 °C. VFA samples were frozen and filtered again prior to analysis.

COD and SCOD analyses with dichromate oxidation were carried out according to the standard SFS 5504 (SFS, 1988). Mercury(II)sulfate solution was used in the analyses since 3.11.2017 (day 33 for CSTR and day 4 for LBR study) due to detection of chloride-ion inhibition. TS and VS were analyzed according to standards SFS-EN 14346 and SFS-EN 15169, respectively (SFS, 2007).

VFA concentrations were analyzed with a gas chromatograph (Shimadzu GC-2010 Plus, ZB-wax plus column: 27.6 m × 0.25 mm × 0.25 μm) with a flame ionization detector (FID) as explained by Kinnunen et al. (2015). Fatty acids that were detected were acetic, propionic, isobutyric, butyric, and valeric acid. VFA concentrations were converted mathematically to equivalent SCOD concentrations in order to compare the VFA and SCOD results. Sample pH was measured instantly after sampling with WTW pH meter (3210) with SenTix 41 electrode after daily three-point calibration.

Methane and carbon dioxide contents were determined with a gas chromatograph (Shimadzu GC-2014 TCD with Porapak N 80–100 MESH column: 2 m × 2.0 mm). Nitrogen was used as a carrier gas with flow rate of 20 mL min⁻¹. The default temperature for detector was 250 °C, injector 110 °C, and for column 80 °C. Biogas volumes were determined via water displacement method. Gas results were normalized by following the ambient pressure and temperature.

Both total bound nitrogen (TN_b, includes ammonia, ammonium salts, nitrite, nitrate, and organic nitrogen compounds) and ammonium nitrogen (NH₄⁺-N) were analyzed from cold stored, diluted samples without centrifugation or filtration using commercial nutrient analysis kits. For TN_b analysis, a total nitrogen analysis kit LCK 238 (HACH LANGE GMBH, range: 5–40 mg/L) was used. NH₄⁺-N was analyzed using an ammonium kit LCK 302 (HACH LANGE GMBH, range: 47–130 mg/L).

5. RESULTS

The feasibility of AD of sedimented fibers, originating from pulp and paper industry, was studied in CSTR LBR experiments. The aim of the CSTR experiments was to evaluate the long-term process performance of AD for biogas production from sedimented fibers. In the LBR experiments the goal was to study leachate extraction and optimize the hydrolysis of sedimented fibers.

5.1 Characteristics of sedimented fiber and inoculum

The sedimented fiber used for the study was a heterogeneous substrate with TS content of 14 %, VS content of 13 %, and pH of ca. 6.4 (Table 10). Sedimented fiber has total nitrogen concentration of 0.03 % and SCOD content of 3.6 g/L (Lahtinen, 2017). The inoculum had pH of 7.4 and the TS, VS, and SCOD concentrations of 2.9 %, 1.5 %, and 1.6 g/L, respectively.

Table 10. Characteristics of the inoculum and sedimented fiber.

Parameter	Sedimented Fiber	Inoculum
pH	6.41	7.43
TS (%)	14.4	2.88
VS (%)	12.9	1.53
VS/TS (%)	89.5	53.1
COD (g/L)	<i>n.d.</i>	1.7
SCOD (g/L)	<i>n.d.</i>	1.6
TN _b (mg/L)	<i>n.d.</i>	1.62 ± 0.06
NH ₄ ⁺ -N (mg/L)	<i>n.d.</i>	803

n.d. = not determined

5.2 Completely stirred tank reactor (CSTR) studies

The stability of the AD of sedimented fiber for biogas production was studied in mesophilic CSTR experiments lasting for 134 days. The reactor was first operated semi-continuously for 105 d. Later it was operated in batch mode for another 29 days, and the trace element sufficiency was tested in batch assays.

5.2.1 CSTR experiments

The reactor was operated for 105 d at an OLR of ca. 2.5 kg VS/(m³d) and HRT of ca. 48 d. Methane production increased after 28 d of operation reaching its highest weekly average value of 240 m³ CH₄/t VS on days 22–28 (Figure 12). Methane production started to decline and reduced to 135 m³ CH₄/t VS along with decreasing pH from 6.8 to 6.3 in 26–50 d. After one unfed day, due to suspicion of overloading (day 53), and starting the weekly bicarbonate addition to the reactor from day 51, the methane production settled around 170 m³ CH₄/t VS for days 42–77 with total VFA concentration ca. 1 g/L SCOD. At the same time, methane content in the biogas decreased slightly from over 50 % to 47 %.

A steep decline in methane production was observed after 77–105 days of operation, and production fluctuated in the range of 43–100 m³ CH₄/t VS. Also the methane content in the biogas declined reaching the level of less than 30 % from the total gas volume as pH dropped again to 6.4–6.6 (days 95–105) with accumulation of total VFAs from 4–5 g/L SCOD (days 87–89) to 9–12 g/L SCOD (days 95–105) (Figure 13).

Digestate pH was declining throughout the first 50 d of operation as the inoculum with good buffering capacity was gradually replaced by sedimented fibers with low pH. Bicarbonate addition (from 51 d) started to increase the pH. After reaching the highest value of 7.7 on day 87, the pH started to decline again due to accumulating VFAs, mostly acetic acid, resulting in a radical decline in methane production.

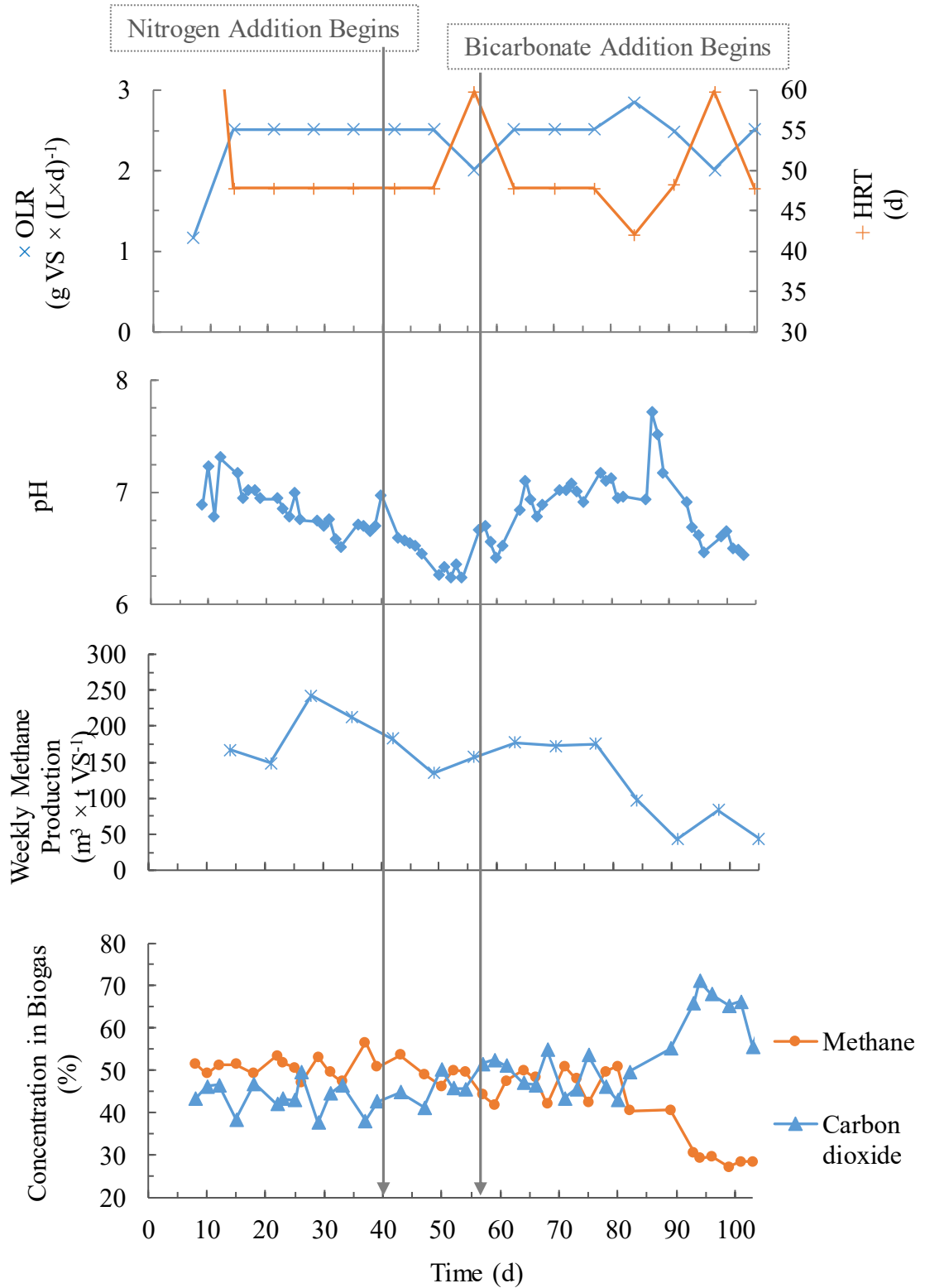


Figure 12. Process parameters, methane production, and biogas composition in AD of sedimented fibers in a semi-continuously operated, mesophilic CSTR. Methane production, OLR, and HRT are given as weekly averages.

There was an increase in digestate SCOD concentration after one HRT (ca. 48 d) from the beginning. During the days 1–45, 45–85, and 85–105 the SCOD concentration increased from 1.0–1.8 g/L to 2.6–6.8 g/L and finally to 9.2–14.2 g/L. By the end of the experiment (80–105 d), 80 % of SCOD content was VFAs.

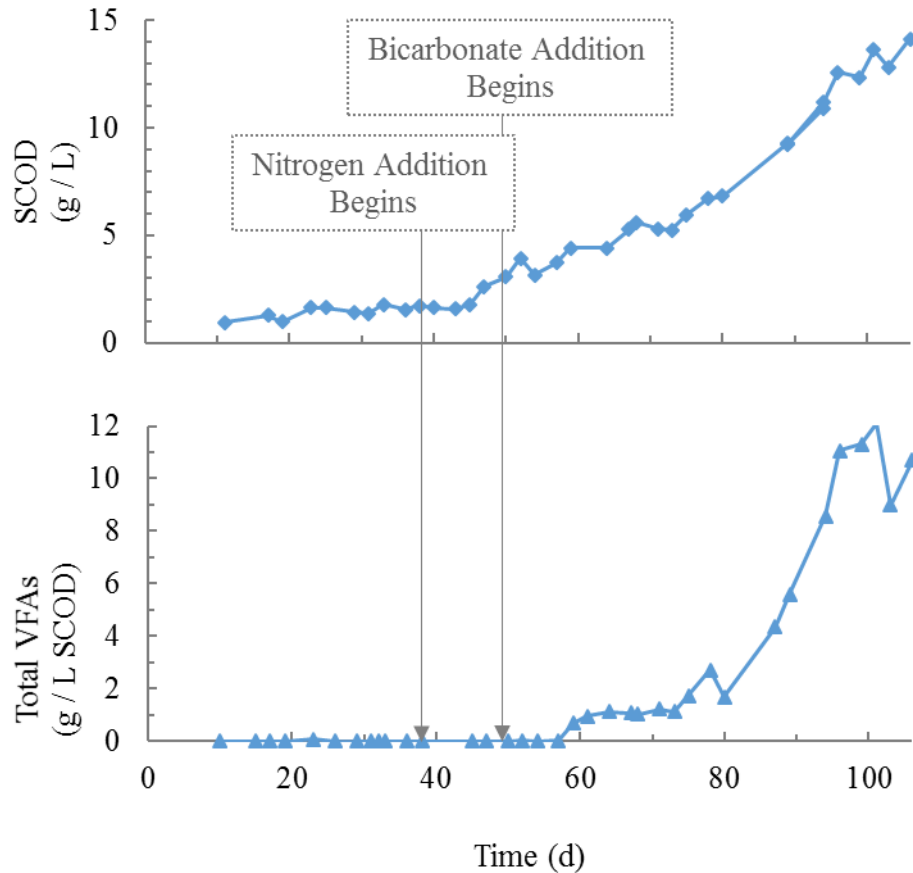


Figure 13. CSTR digestate SCOD and total VFA concentrations.

Total VFAs began to accumulate after 60 d of operation. At the time of 60–70 d, propionic acid was observed in concentration of 600–850 mg/L SCOD (Figure 14). Also minor concentrations of isobutyric (18–46 mg/L SCOD) and butyric acid (85–154 mg/L SCOD) were observed. Acetic acid was first observed during days 59–73 in concentrations of 62–182 mg/L SCOD. Later, after 89–105 d, acetic acid was the main constituent (80 %) of the total VFAs with the concentrations of 4000–9800 mg/L SCOD.

During the days 59–75, the propionic acid concentrations were in the range of 600–800 mg/L SCOD. After rapid decline and increase simultaneously with isobutyric and butyric acid, propionic acid levelled at the concentration of 1000–1400 mg/L SCOD during 89–105 d. Valeric acid, with the concentration of 40–130 mg/L SCOD, was only observed

after day 89. From 80th day of operation until the end, the concentration of SCOD followed closely total VFA concentration. Majority of SCOD was VFAs, and other soluble organic constituents contributed 2–3 g L⁻¹ of SCOD during the entire operation.

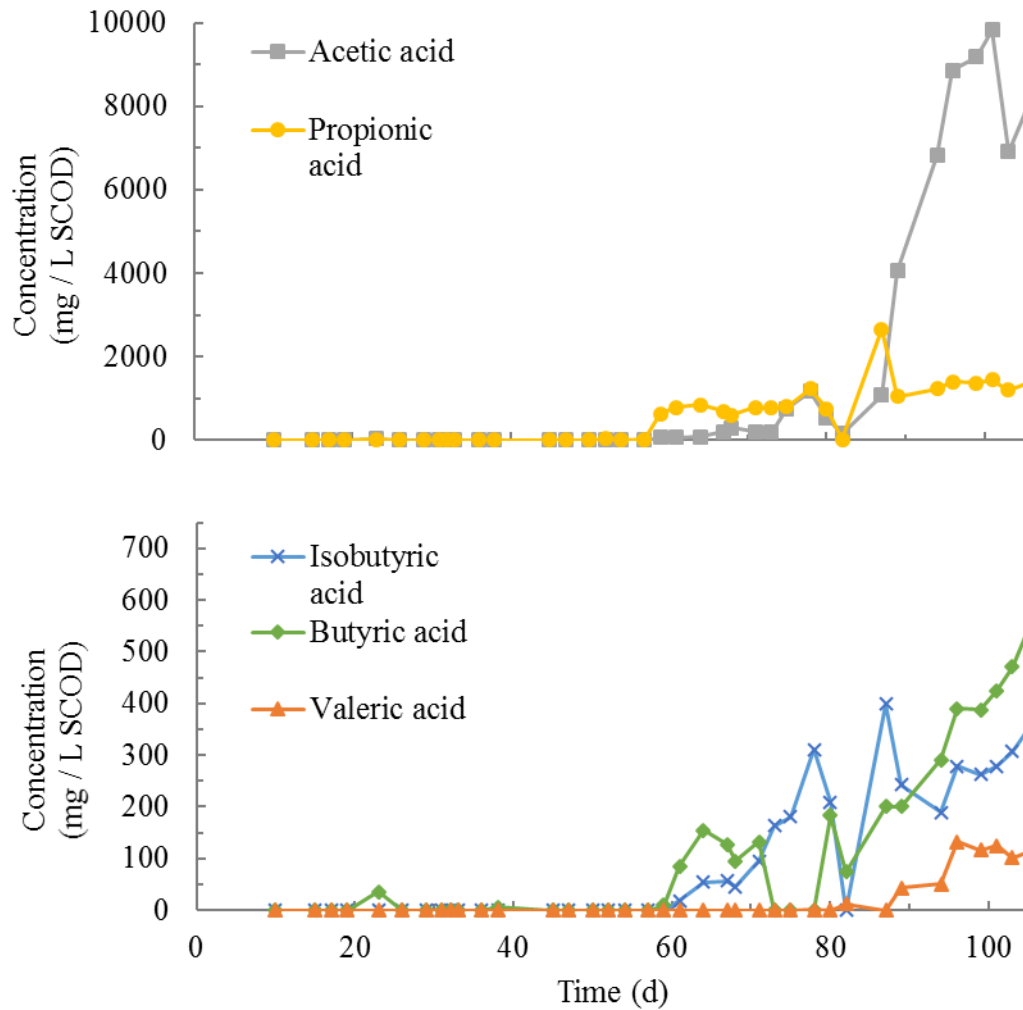


Figure 14. Concentrations of VFAs in AD of sedimented fiber in a CSTR.

Nitrogen concentrations were increasing over the operation, since 130 mL of 119 g NH₄Cl/L solution was added weekly. In the day 50 TN_b concentration of the digestate was 3.5 g/L and after the whole experiment (including batch operation) 4.1 g/L. In addition, NH₄⁺-N concentrations increased as well from 3.0 g/L (day 50) to 3.6 g/L (day 134).

Overall, a high VFAs yield, but low methane production was achieved in the semi-continuously operated CSTR over 105 d of operation with OLR of ca. 2.5 g VS/L d and HRT of 48 d.

5.2.2 Batch operation of CSTR

The CSTR reactor was operated in batch mode in order to provide the process time to recover from overloading. A decrease in VFA concentrations along with increase in bio-gas production was observed indicating the recovery of the process. However, methane production was still rather low and there were VFAs in the digestate.

In the beginning of the batch operation, methane production was low (1.8 L/week), but began to increase from days 127–134 d reaching the production of 4.2 L CH₄/week. The methane content began to increase from 30 % (day 115) to over 80 % (day 135) along with the increase in pH after two additions of bicarbonate buffer from 6.5 (day 115) to 7.5 (day 134). Simultaneously with the increase in pH and methane production, also a decrease in total VFAs from 12 g/L SCOD (days 113–120) to ca. 5 g/L SCOD (day 134) and a decrease in SCOD from 17 g/L (days 113–123) to 11 g/L (day 134) was observed (Figure 15).

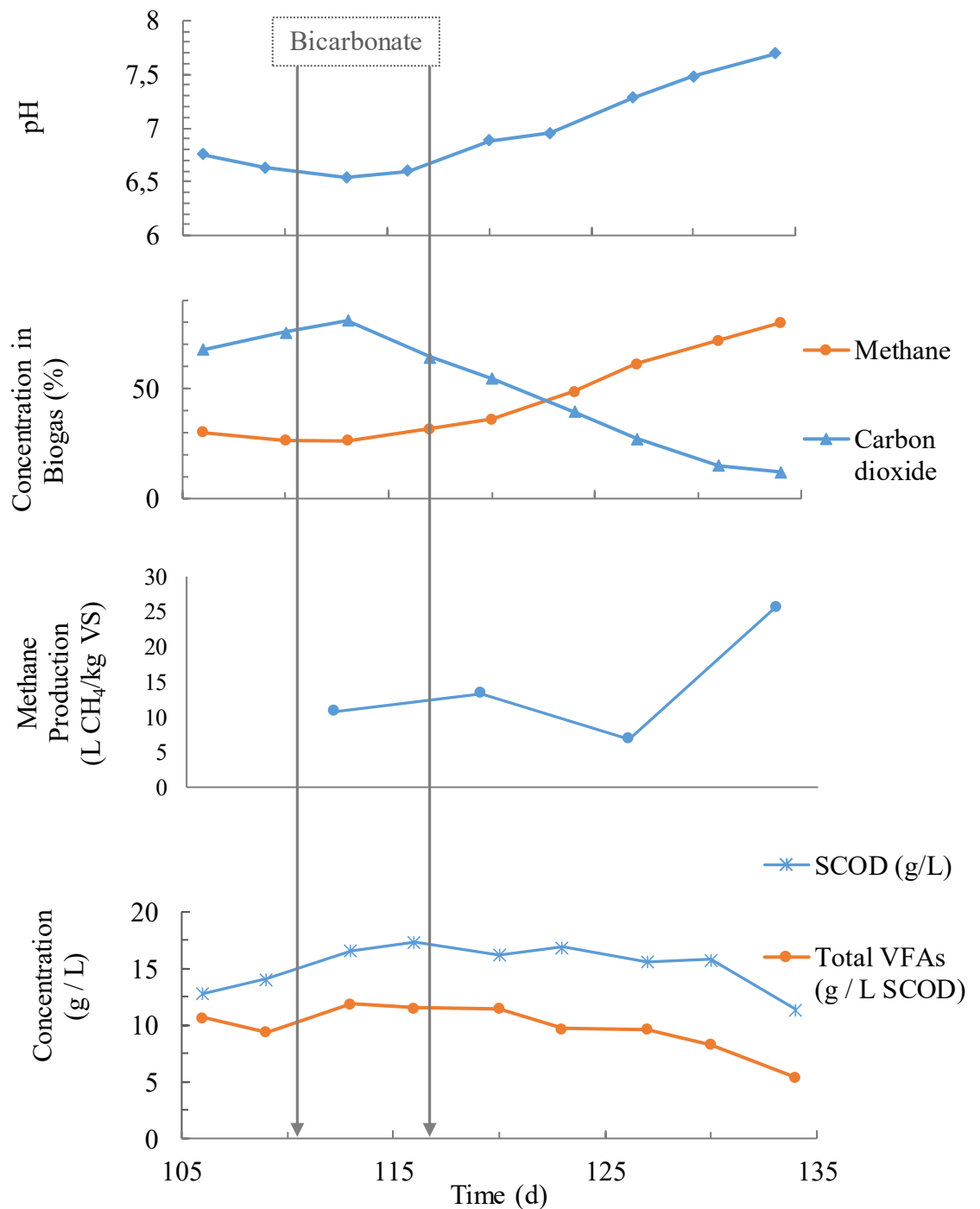


Figure 15. Digestate properties and methane production from sedimented fiber in a batch operation of a CSTR. Methane production is given as weekly average.

Total VFA concentration decreased from 11 g/L SCOD to 5 g/L SCOD in the batch operation. However, the decrease was mainly due to decrease in acetic acid concentration from over 9000 mg/L SCOD to 2000 mg/L SCOD (Figure 16). The concentration of propionic acid remained constant at 1500–2000 mg/L SCOD throughout the batch operation.

Concentrations of isobutyric and butyric acids first decreased to 350 and 450 mg/L SCOD (days 123–130) from the maximum values of 450 and 650 mg/L SCOD (day 113), but then increased to 650 and 750 mg/L SCOD by the end of the experiment. Valeric acid concentration was constant at 100 mg/L SCOD, yet increased to a maximum concentration of 200 mg/L SCOD on day 134.

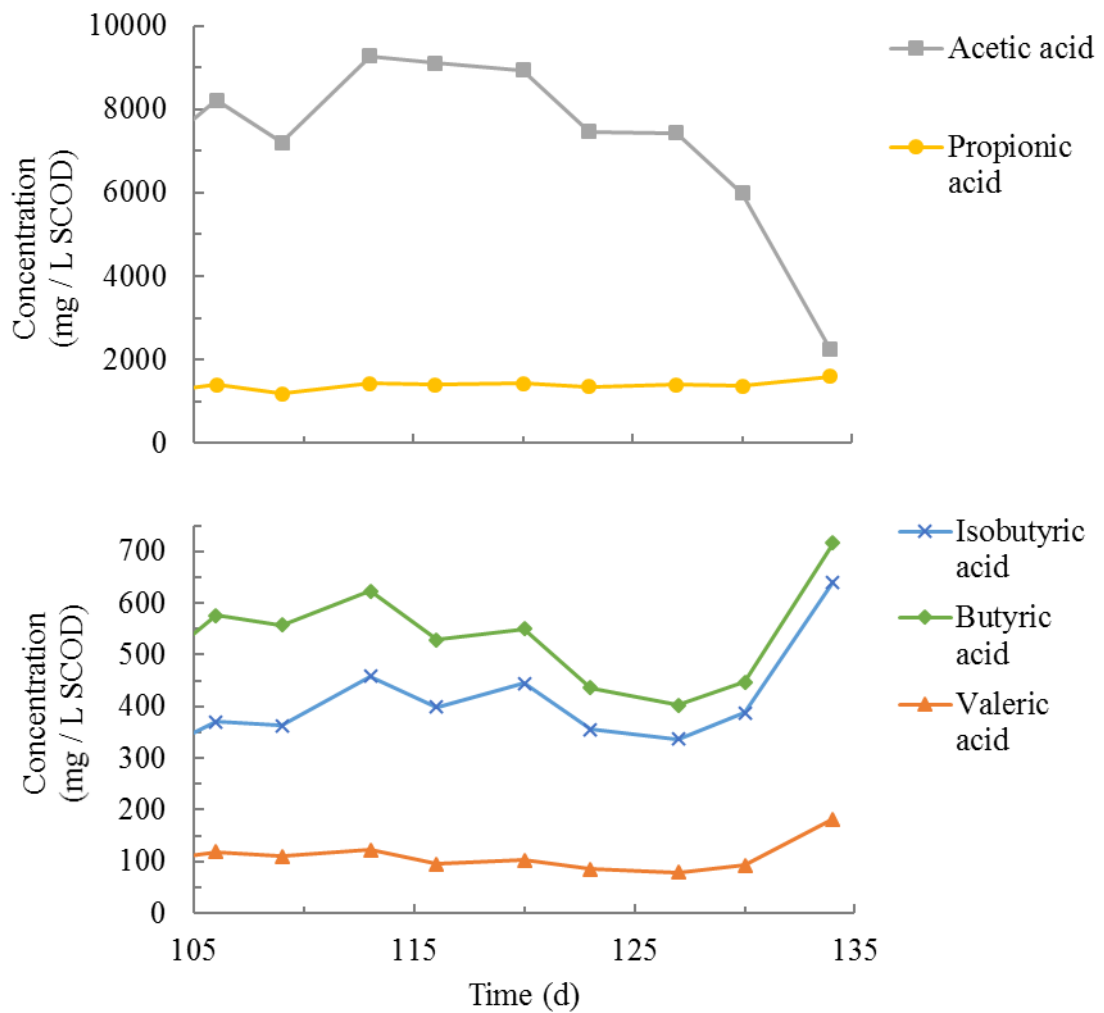


Figure 16. Concentrations of VFA species in batch operation of the CSTR with sedimented fiber.

5.2.3 Batch assays to determine trace element sufficiency

After operating the CSTR in batch mode without feeding for 29 d, the VFA concentrations were still rather high (5.4 g/L SCOD) and biogas production low (1.1 m³ CH₄/m³ reactor). A batch assay of 43 d, using 1 L Pyrex bottles, was carried out in order to find out whether the process was limited by trace element insufficiency.

There was no significant difference in cumulative methane production with (44.9 L CH₄/kg VS) or without (44.6 L CH₄/kg VS) trace element supplementation (Figure 17). COD concentration decreased more in the sample with trace element addition (35 % decrease) than in the control (13 % decrease) (Table 11). The difference in the decrease in SCOD concentrations was smaller: 26 % with trace elements and 22 % for the control, respectively.

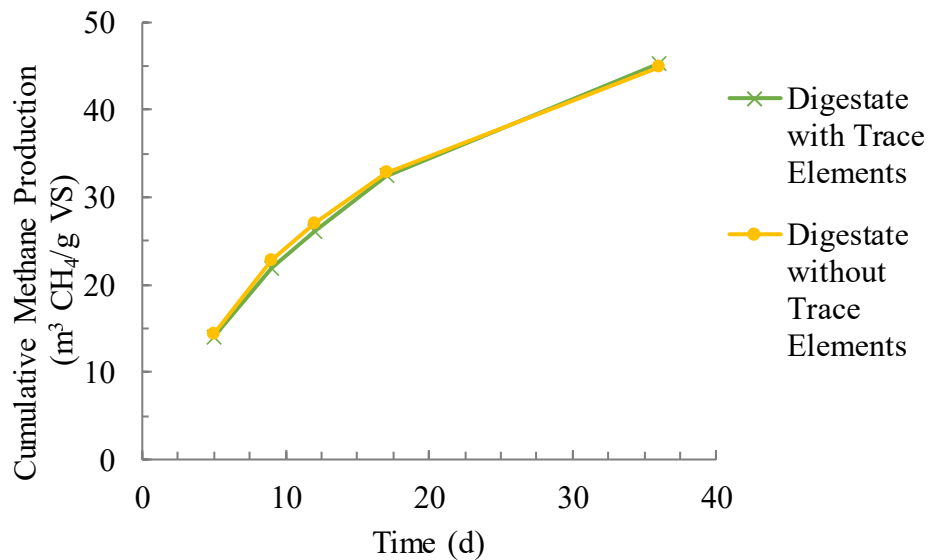


Figure 17. Cumulative methane production with and without the supplementation of trace elements in batch assays for the CSTR digestate.

Table 11. Decrease in COD, SCOD, and total VFAs of the batch assays with and without supplementation of trace elements.

Parameter	Assay with Trace Element Supplementation	Assay without Trace Element Supplementation
Decrease in COD (g/L)	15	5.4
Decrease in SCOD (g/L)	3.0	2.5
Decrease in total VFAs (g/L SCOD)	2.5	2.6

5.3 Characteristics of digestate

The consistency of the sedimented fiber material changed over the AD. The spongy texture of the raw sedimented fibers was transformed into a loose, brown slurry and containing some wooden particulates (Figure 18).



Figure 18. Sedimented fiber (on the left) and digestate from the AD of sedimented fiber (on the right).

Both TS and VS decreased over the treatment period (both semi-continuous and batch operation phases of CSTR) (Table 12). The TS content of the feed was 14 % and of the digestate 5.1 %.

Table 12. The properties of the feed (sedimented fiber) and digestate during the operation of CSTR as well as after batch assays.

Parameter	Feed	CSTR Digestate days 1–50 (^a day 75, ^b day 50)	CSTR Digestate days 51–105	CSTR Digestate days 106–134 (^c day 134)	After Batch Assays	
					With Trace Elements	Without Trace Elements
pH	6.4	6.8 ± 0.2	6.8 ± 0.3	7.0 ± 0.4	7.7	7.7
TS (%)	14.4	6.22 ± 0.07 ^a	<i>n.d.</i>	5.07 ± 0.04 ^c	4.96 ± 0.09	4.95 ± 0.09
VS (%)	12.9	4.62 ± 0.08 ^a	<i>n.d.</i>	3.18 ± 0.03 ^c	3.22 ± 0.09	3.20 ± 0.08
VS/TS (%)	89.5	74.4 ± 2.07 ^a	<i>n.d.</i>	62.8 ± 1.2 ^c	64.9	64.6
COD (g/L)	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	43.3 ± 3.2 ^c	27.5	36.7 ± 0.4
SCOD (g/L)	3.58	1.67 ± 0.53	7.43 ± 1.6	15.2 ± 1.9	8.45	8.94 ± 0.33
Total VFAs (g/L SCOD)	<i>n.d.</i>	<i>b.d.</i>	4.10 ± 4.27	9.78 ± 1.91	2.85	2.77 ± 0.03
TN _b (g/L)	<i>n.d.</i>	3.50 ^b	<i>n.d.</i>	4.13 ^c	<i>n.d.</i>	<i>n.d.</i>
NH ₄ ⁺ -N (g/L)	<i>b.d.</i>	2.98 ^b	<i>n.d.</i>	3.59 ^c	<i>n.d.</i>	<i>n.d.</i>

n.d. = not determined, *b.d.* = below the level of detection.

5.4 LBR experiments for sedimented fiber hydrolysis

Seven LBR experiments were carried out. Three experiments studied the technical feasibility of LBR configuration and leachate formation from sedimented fiber. The others examined the factors affecting hydrolysis of the sedimented fiber.

5.4.1 Screening experiments

LBRs can be used for the treatment of sedimented fiber. Leaching took place spontaneously as sedimented fiber was placed into an LBR. LBRs could be operated both up and down-flow. However, the running of the up-flow configuration was more prone to leakages and required water additions in the beginning to start the recirculation. Thus, down-flow LBRs were used in the further studies. Increasing the sedimented fiber volume (or the height of the sediment column) in the LBRs increased the relative leachate production (the volume of leachate per loose volume of sedimented fiber) (Figure 19). The highest relative leachate production (11 %) was achieved with the highest sedimented fiber volume tested (10 L). During the first 30 minutes of leaching, leaching was most rapid in the least compacted reactor and slowest in the most compacted one (Figure 20). Leaching slowed down after 1 h. Total leachate volume was less than 10 % of the initial, non-compacted leachate volume. Reaching over 50 % of total leachate volume took 25 min for the most compacted fibers, 15 min for the fibers with light compaction, and only 3 min for the fibers without compaction.

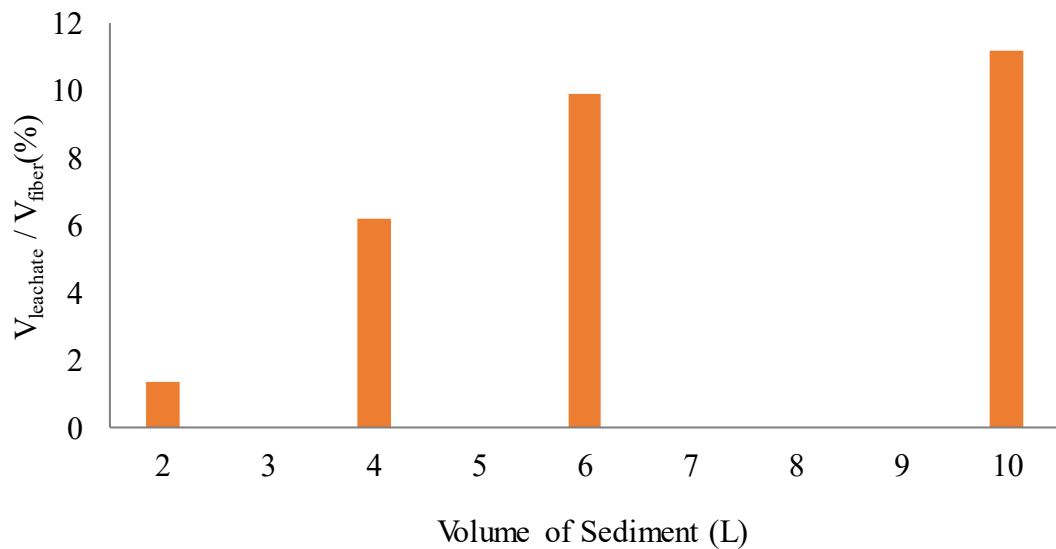


Figure 19. The leachate volume per sedimented fiber volume ratio for 2, 4, 6, and 10 L of sedimented fiber in the LBR.

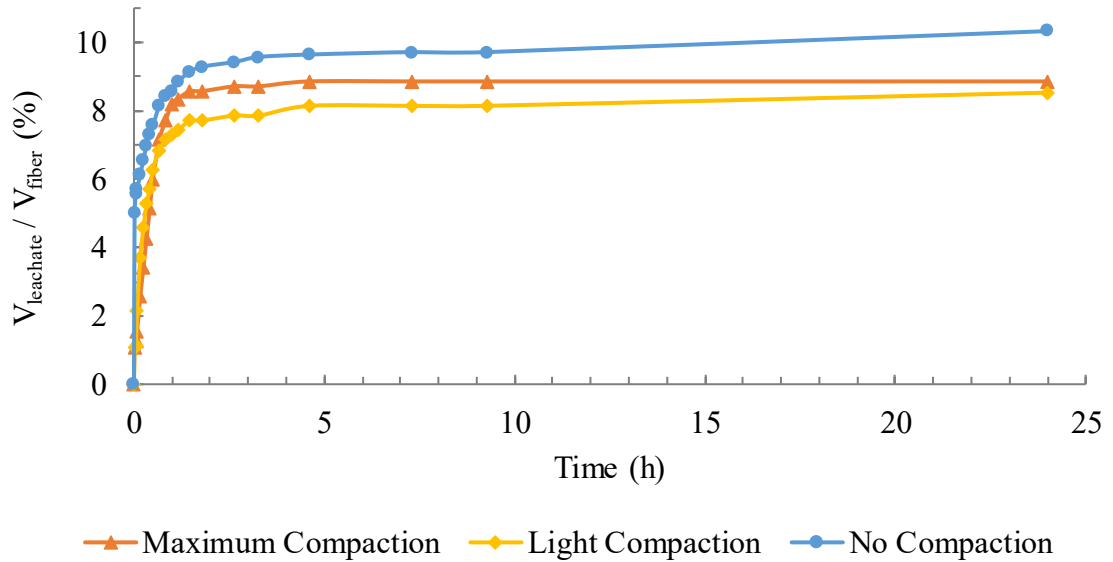


Figure 20. Leachate formation for 3 degrees of compaction from sedimented fiber in LBRs. Free volume of sedimented fiber in all of the reactors was 7 L.

The change in leachate properties over time was studied in an up-flow LBR. Leachate from sedimented fiber had an initial SCOD concentration up to 3 g/L (Figure 21). Most of the SCOD could be collected in the first 24 h of leaching. After 43 h, the SCOD concentration was only 1.5 g/L. The pH of the leachate decreased over time. After 20 h of operation, the pH was 5.5. Later, 24–43 h of operation, pH settled to below 5.1.

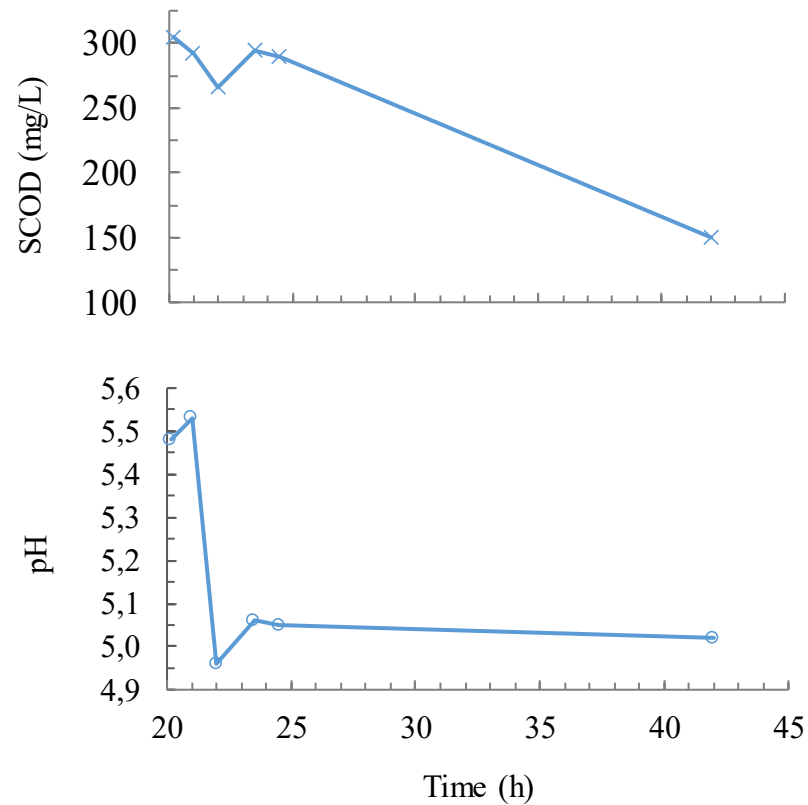


Figure 21. SCOD concentration and pH of the leachate from the treatment of sedimented fiber in an up-flow LBR during the first 20-42 h of operation.

5.4.2 Sedimented fiber hydrolysis

After the screening experiments that studied leachate formation, experiments on the hydrolysis of sedimented fiber were carried out for 52 d. The effect of temperature, nitrogen addition, inoculation, leachate recirculation, and fresh water addition on hydrolysis was examined. Three LBRs were operated in two different temperatures. One of the LBRs was operated at thermophilic temperature (55 °C, LBR1) and two of them at mesophilic temperature (30 °C, LBR2 & LBR3). One of the mesophilic LBRs was inoculated and supplemented with nitrogen (LBR3). Leachate recirculation was began in all of the LBRs on day 3, and all of the leachates were replaced with fresh water 3 times over the experiments.

Leachate pH

In the beginning of operation (day 1), the pH of the leachate was at 5.1–5.2 for thermophilic and mesophilic LBR without nitrogen supplementation (Figure 22). The pH dropped to 4.6 on day 2 and then increased to 4.8–4.9 after day 11. The pH of the leachate from the mesophilic reactor (LBR2) was constantly higher than the leachate from the

thermophilic reactor (LBR1). The final pH for the mesophilic reactor (LBR2) was 5.8 and for thermophilic (LBR1) 5.2.

The trend in pH was different for the leachate from the mesophilic reactor with nitrogen supplementation (LBR3). Initially pH was only at 4.6, yet it peaked to 3 on day 3. The drop in pH was observed 24 h later than in the other reactors. After the drop, the pH remained low, at 4.6–4.7, during the period of 3–10 d. The pH increased in 14–28 d to 5.2–5.3 as the total VFA concentrations dropped simultaneously. The pH turned to decline in 25–35 d, reaching the value of 5.0 as the total VFA concentration was at its highest. After replacement of leachate with fresh water, the pH increased again to the maximum of 5.3 as the VFAs and SCOD decreased.

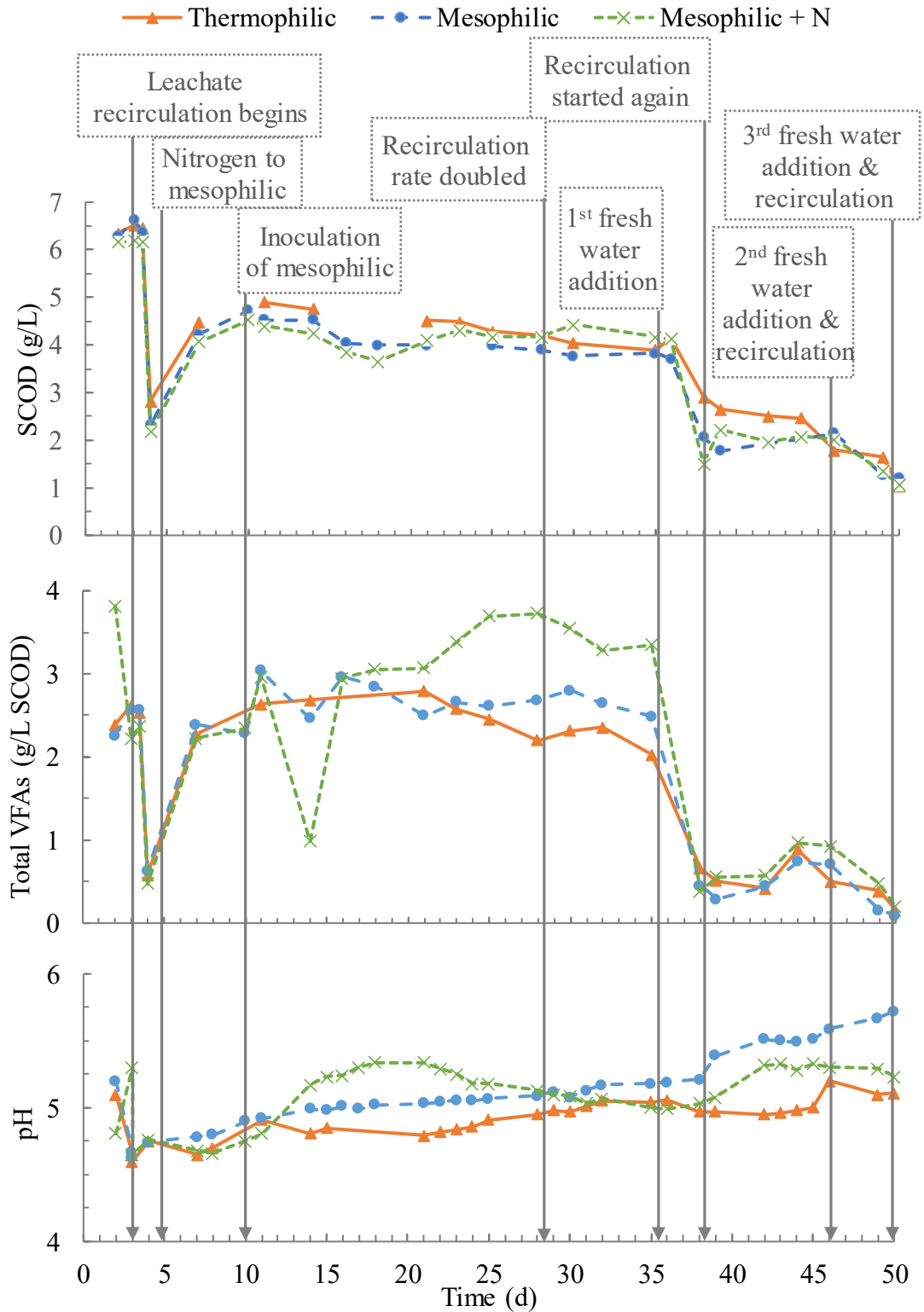


Figure 22. Soluble COD (SCOD) and total VFA concentrations as well as pH of the LBR leachates from sedimented fiber. The leachate was removed entirely prior to each of the fresh water additions.

Leachate SCOD concentrations

The SCOD concentration of leachates reached their maximum values on day 3 as the leachate recirculation was began. The mesophilic reactor without nutrient supplementation (LBR2) and the thermophilic reactor (LBR1) had the highest SCOD concentrations of 6.6 g/L and 6.5 g/L. The mesophilic reactor with nitrogen supplementation (LBR3) had slightly lower SCOD concentration of 6.2 g/L. On day 4, short-circuiting was observed in the substrate bed in the reactors and simultaneously the SCOD concentrations dropped. Later the entire substrate bed was visibly wet and the SCOD concentrations increased accordingly to their second highest values of 4.7 and 4.5 g/L for LBR2 and LBR3 on day 10 and up to 4.9 g/L in LBR1 on day 9.

The SCOD concentration of the leachates from the thermophilic and mesophilic reactors (LBR1 & LBR2) showed moderate decrease over the period of 10–36 d reaching the values of 4.1 and 3.8 g/L, respectively. The concentrations dropped along with the 1st fresh water addition and levelled down to 2.5 g/L (thermophilic) and 2.0 g/L (both mesophilic LBRs) during the days 42–44. The concentrations dropped again after the 2nd and 3rd fresh water addition. The final SCOD concentrations on day 50 were 1 g/L for the thermophilic reactor leachate (LBR1), 1.2 g/L for mesophilic (LBR2), and 1.1 g/L for the mesophilic reactor with nitrogen supplementation (LBR3).

The trend in the leachate SCOD concentration was more complex for the mesophilic reactor with nitrogen supplementation (LBR3). After the initial drop, the SCOD concentration recovered to over 4 g/L, yet turned into decline again during the days 14–20 while the total VFA concentration also dropped steeply. After the increase in total VFA concentrations, the SCOD concentration from LBR3 reached 4.4 g/L, the highest value after the initial peak. The trend was opposite to the one observed in pH of the leachate.

Throughout the hydrolysis experiments, the removed SCOD was the same or higher in the thermophilic than the mesophilic LBR (LBR1 & LBR2). However, the difference was small. The leachate from thermophilic reactor had on average (0.3 ± 0.3) g/L higher concentration of SCOD than the mesophilic reactor without nitrogen supplementation. The difference between the thermophilic reactor and the mesophilic reactor with nitrogen supplementation (LBR1 & LBR3) was equally small, 0.3 g/L. However, the standard deviation was higher, 0.4 g/L. There was no difference in SCOD concentrations throughout the experiments between the mesophilic reactors (with and without nitrogen supplementation, LBR2 & LBR3 (0.0 ± 0.3) g/L).

Most of the soluble organic matter that could be solubilized was collected in the beginning of the operation. From the total removed SCOD from LBR1, 55 % was collected in the beginning of operation before fresh water addition (Figure 23 & Figure 24). For LBR2 and LBR3 the contribution was 60 % and 61 %, respectively.

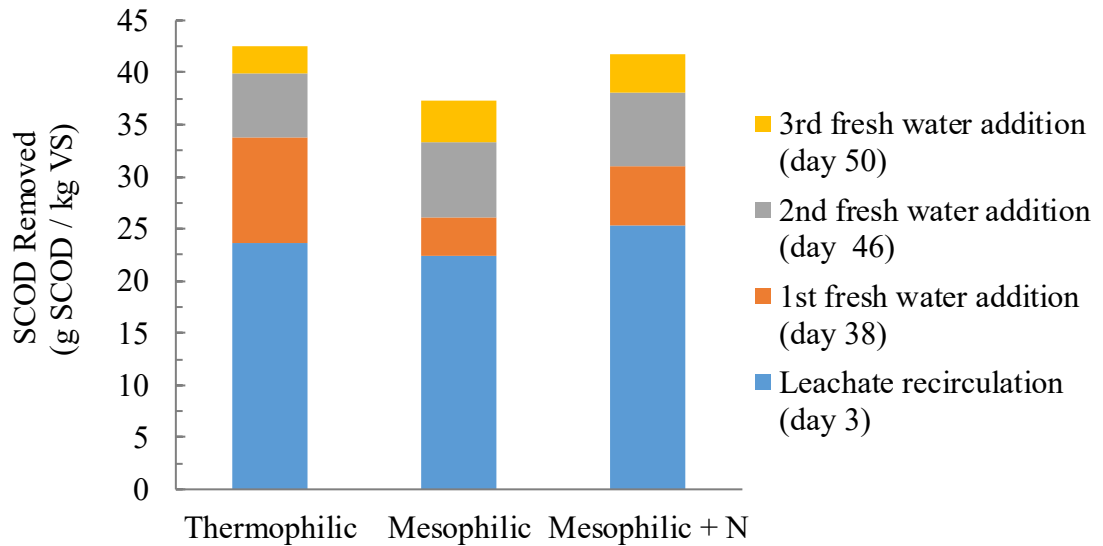


Figure 23. Mass of soluble organic matter (SCOD) removed from the reactors in the leachate per sedimented fiber VS after recirculation of leachate and after each of the fresh water additions.

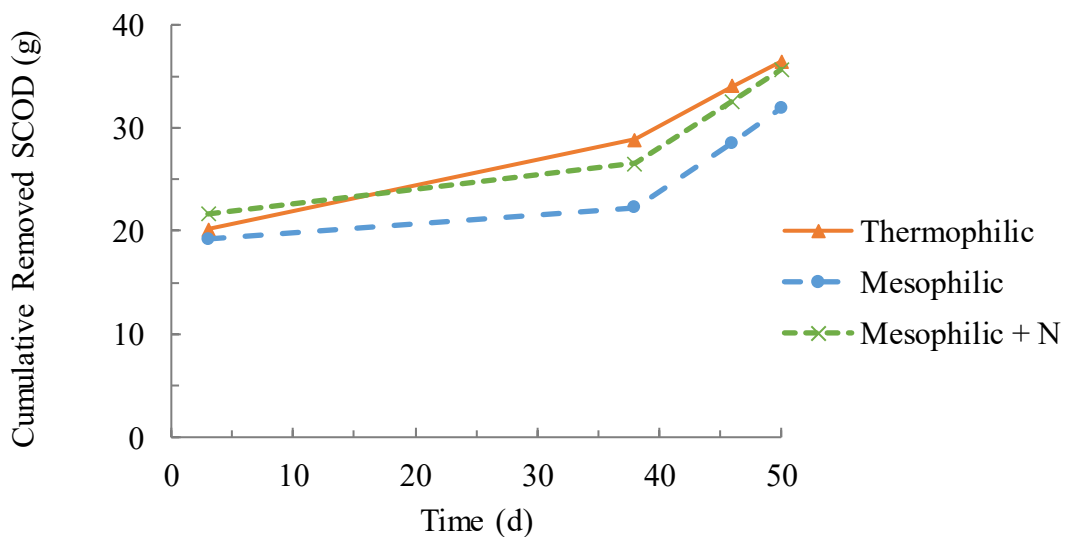


Figure 24. Cumulative SCOD removal from the LBRs. Ca. 3000 mL of leachate (and SCOD accordingly), was removed from each reactor before each of the 3 fresh water additions (3000 mL of fresh water/reactor).

Leachate VFA concentrations

The maximum total VFA concentrations for the LBRs 1, 2, and 3 were 2.8 g/L SCOD (day 21), 3.0 g/L SCOD (day 11), and 3.7 g/L SCOD (day 28), respectively. The highest

concentrations were observed in the mesophilic reactor that was supplemented with nitrogen and inoculum (LBR3). First, a decline in the total VFA concentrations from the level of 2.3–3.9 g/L SCOD to 0.5 g/L SCOD was observed in all of the reactors after a couple of days from starting leachate recirculation (day 4). The concentrations quickly recovered and somewhat stabilized in the range of 2–4 g/L SCOD, except in LBR3 that had more variation in the VFA concentrations.

Replacing leachate with fresh water (day 35) resulted in a sharp decrease, to 0.5 g/L SCOD, in the total VFA concentrations. The concentrations recovered in a couple of days reaching nearly 1 g/L SCOD. More moderate declines in the total VFA concentrations were observed also after the following two fresh water additions (days 46 and 50). At the end of the experiment, after replacing the leachate with fresh water 3 times, the total VFA concentrations were 0.1–0.2 g/L SCOD in all of the reactors.

For all of the LBRs, the concentrations and distribution of different VFAs was rather similar over the 53 d of operation and followed the same trend as the total VFA concentration (Figure 25). In the thermophilic reactor (LBR1), after the initial drop in the concentrations of all of the VFA species (day 4) the concentrations remained stable over the period of 5–35 d of operation. Butyric acid had the highest concentration, 1 g/L SCOD, followed by propionic acid with a concentration of 0.5–0.8 g/L SCOD for all the reactors. In the beginning, the concentration of acetic acid was slightly higher than the concentration of valeric acid. However, after 10 d of operation valeric acid stabilized at 0.4 g/L SCOD, whereas acetic acid concentration began to decline after 20 d from 0.4 to less than 0.2 g/L SCOD.

The concentration of different VFAs in the mesophilic reactor (LBR2) followed the same trend that was observed in the thermophilic reactor (LBR1). However, the maximum butyric acid concentrations were somewhat higher, 1200–1300 mg/L SCOD. The concentrations of the VFAs levelled during the period of 7–35 d to similar values than in the thermophilic reactor (LBR1). However, the concentration of propionic acid was lower (LBR2: 580 mg/L SCOD, LBR1: 790 mg/L SCOD on day 21) and acetic acid higher (LBR2: 270 mg/L SCOD, LBR1: 170 mg/L on day 30). On day 14 there was a drop (<200 mg/L SCOD) in the butyric, propionic, and valeric acid concentrations. Similar, yet much larger drop (for butyric acid 700 mg/L SCOD) was simultaneously observed in the mesophilic reactor with nitrogen supplementation (LBR3). As in the thermophilic reactor, there was a slight decrease in acetic acid concentrations from days 15–35.

After inoculation of the mesophilic reactor LBR3 (day 10), the same sharp decline that was observed in total VFA concentration was visible in the individual concentrations of all of the VFAs examined. Five days later the concentrations recovered. The concentrations of acetic, butyric, isobutyric, and valeric acid reached their maximum levels. Butyric acid stabilized at the highest concentration of 1.5 g/L. The concentrations of propionic and valeric acid stabilized at 700 and 500 mg/L. The acetic acid concentration began to

increase after 20 d of operation stabilizing finally at a maximum of 800 mg/L after 25–35 d of operation. Leachate replacement with fresh water decreased the VFA concentrations as in other reactors. After the first fresh water addition, recovery in the acetic acid concentration was observed prior to the increase in butyric or valeric acid concentrations, unlike in the other reactors.

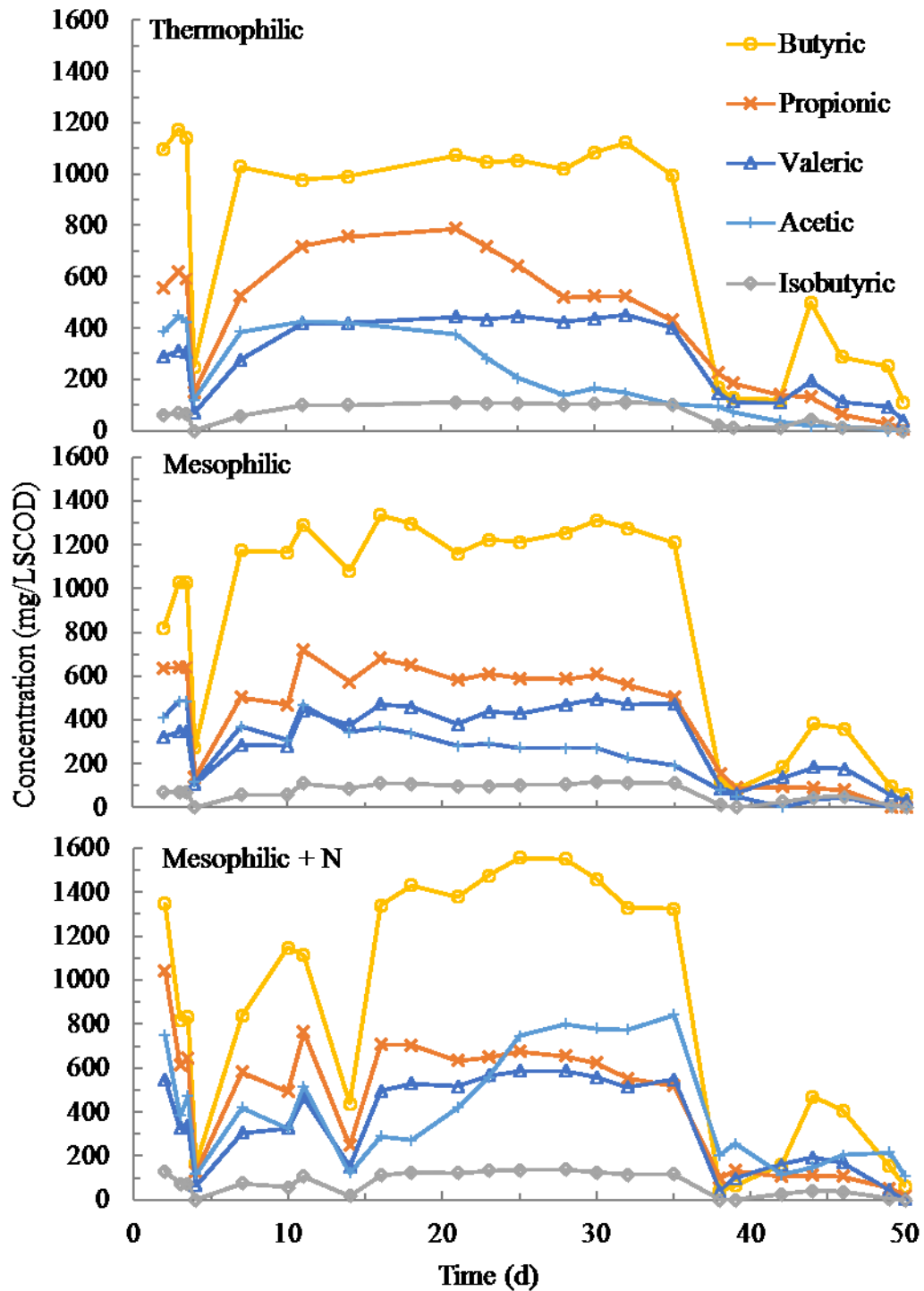


Figure 25. Concentrations of different VFAs in the leachates from LBRs in experiments 4-7.

Leachate total bound nitrogen (TN_b) concentrations

TN_b concentration before the 1st fresh water addition was 27 mg/L for thermophilic LBR1 and 7 mg/L for mesophilic LBR2. For LBR2, the concentration decreased below the level of detection (<5 mg/L) by day 50 (2 fresh water additions) and for LBR1 by the end of the experiments (day 52, 3 fresh water additions) (Figure 26). In the mesophilic reactor

with inoculation and nitrogen supplementation (LBR3), the leachate TN_b concentration was initially 2 orders of magnitude higher than in the other LBRs, 1100 mg/L. The concentration decreased to less than 200 mg/L by the end of the experiments (day 52, 3 fresh water additions).

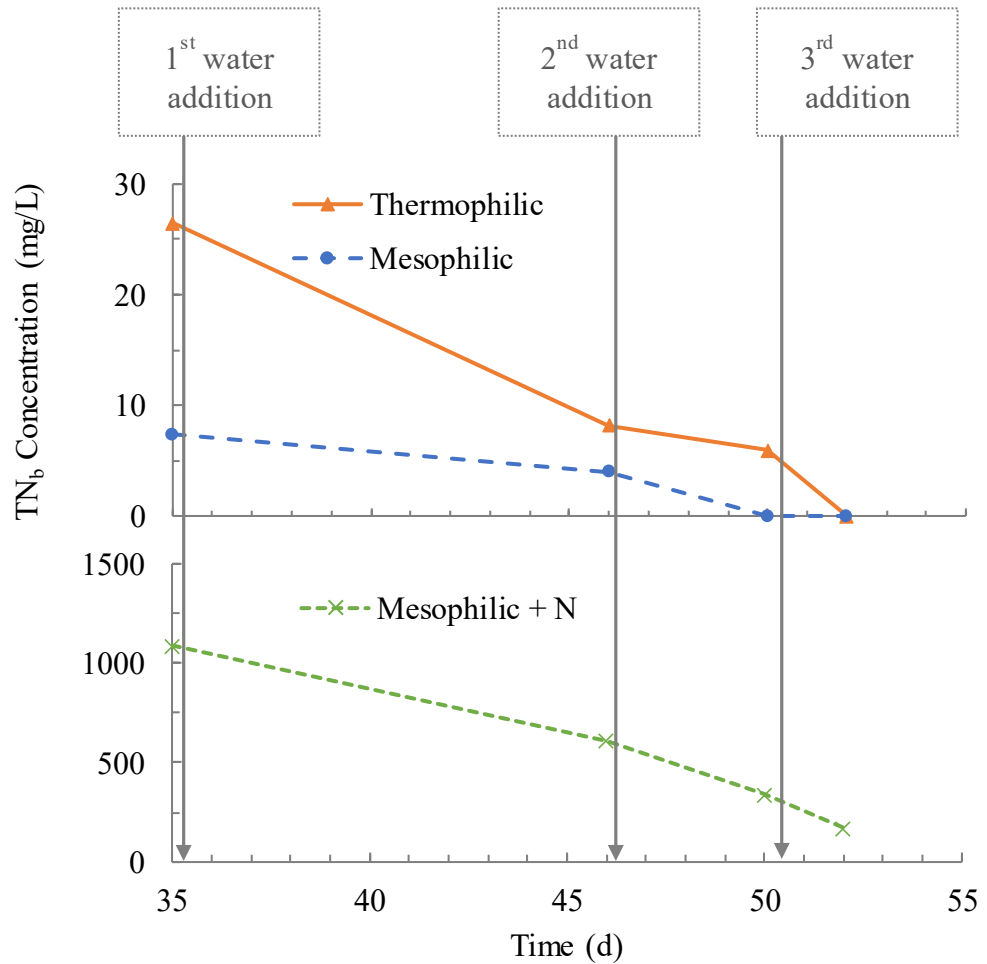


Figure 26. Total nitrogen (TN_b) concentrations of the LBR leachates. The last sample for the thermophilic LBR and the 2 last samples for the mesophilic without nitrogen supplementation were below the level of detection (< 5 mg/L).

6. DISCUSSION

There was high rate of hydrolysis, yet instability in methanogenesis in the AD of sedimented fiber. Hydrolysis in LBRs was highest in thermophilic temperature as well as in mesophilic temperature, when inoculation and nitrogen supplementation was carried out.

6.1 AD of sedimented fiber in a CSTR

The AD of sedimented fiber originating from pulp industry in a semi-continuously operated CSTR produced high concentrations of VFAs and SCOD, yet the methanogenesis was on long term failing. Overall, the cause of process failure was most likely due to an imbalance between the different microbial groups – namely hydrolyzing, acidogenic, acetogenic, and methanogenic microorganisms (Demirel & Yenigün, 2002). The imbalance was probably due to lack of buffering, possibly compared to too high OLR (Alvarez & Lidén, 2008). Inadequate buffering lead to decrease in pH that promoted hydrolysis over methanogenesis, leading to accumulation of VFAs as well as reinforcing the inhibitory effect of the VFAs.

An earlier AD research by Lahtinen (2017) on the samples from the same site showed mostly stable AD process. The TS content of the feed was higher (14.0 %) in this research compared to the earlier study (13.3 %) (Lahtinen, 2017). The OLR was as high (2.5 kg VS/(m³ d)), yet the HRT was lower: 60 d in the earlier study and 48 d in this one. Hence, also hydraulic overloading or washing out of the microorganisms could be the reason for the failure in AD (Alvarez & Lidén, 2008). Washing out would affect most severely the slowest growing microorganisms of the AD, the methanogens.

On days 43–47, the pH was 6.5–6.6 and decreased to 6.2–6.4 during days 50–54. Process failure was expected due to increase in SCOD and VFAs, and one day (day 53) was left unfed in order to provide more time for methanogens to recover. After that, the pH recovered to a peak of 6.7 on day 58, yet decreased again to 6.4 by day 60. Simultaneously, propionic acid concentration increased to 630 mg/L SCOD from below the level of detection. After that pH increased to ca. 7.0 until dropped to ca. 6.5 as the accumulation of VFAs, particularly propionic acid was observed after day 87 (2.7 g propionic acid/L). According to Wang et al. (2009), propionic acid inhibits methanogens significantly already at a concentration of 0.9 g/L. The momentary decrease in OLR decreased the total VFA concentrations back to below detection limit (on day 54) from 35 mg/L SCOD (on day 52). Since the decrease in OLR improved the process performance, the process seemed to be overloaded with the OLR of 2.5 kg VS/(m³ d) in these operating conditions. The methanogens are the most slowly growing microorganisms in AD (Gerardi, 2003).

They may not have replenished after the drop of pH on days 50–54 causing methanogenesis to fall behind from the first steps of AD.

The accumulation of VFAs (up to 12 g/L SCOD, day 101) indicated fast hydrolysis, yet failure of the process in methanogenesis. VFAs are the intermediate products of early steps of AD and they were not consumed by the later steps producing methane. In the process, the production of VFAs was efficient, yet simultaneously the pH was low, 6.2 at the lowest. That is out of the optimum range for the methanogenic archaea (Gerardi, 2003), which probably slowed down methanogenesis leading to accumulation of VFAs. According to Jiang et al. (2013) that low pH promotes hydrolyzing activity in AD and leads to maximum VFAs yield (Jiang, et al., 2013). Particularly propionic acid was accumulating. The low pH of the reactor could have favored the production of propionate that is difficult to degrade once accumulated (Wang, et al., 2006).

Addition of nitrogen most likely improved the rate of hydrolysis compared to methanogenesis. The improvement was observed 15 d after nitrogen supplementation was began as SCOD content began to increase (from 2 g/L on day 31 up to over 13 g/L on day 105). The addition of nitrogen in this study was based on the target C:N ratio of 25:1 that was proven viable in a study by Lin et al. (2011) in mesophilic co-digestion of pulp and paper sludge with monosodium glutamate waste liquor. Lahtinen (2017) found that nitrogen supplementation enhanced hydrolysis of the sedimented fiber. In this case, hydrolysis was working well, yet the pH was out of the optimum of methanogenesis. Nitrogen was most likely not limiting AD. In the study by Lahtinen (2017), the target C:N had been lower than 25:1. In this case a commonly used, higher nitrogen concentration (C:N = 20:1 (Mao, et al., 2015)) was targeted. It is possible that nitrogen supplementation accelerated hydrolysis more as compared to methanogenesis that was struggling with inadequate buffering. The SCOD content was ever increasing over the semi-continuous operation indicating that the further steps of the AD process could not keep up with hydrolysis.

Increase in pH after supplementation of buffer (from day 54) improved the process performance, which was observed as improving methane production (from 135 m³ CH₄/t VS on day 49 to 176 m³ CH₄/t VS on days 60–75). The buffering may still have been inadequate, since pH was later (after day 90) decreasing (to 6.5) along with the accumulation of VFAs. As was found by Meng et al. (2018) and Alvarez & Lidén (2008), lack of buffering can lead to process failure. The decrease in pH to 6.2, may not have caused direct inhibition of methanogenesis (Al Seadi, et al., 2008). However, the low pH may have promoted hydrolysis and acidogenesis that have lower optimum pH than methanogenesis (Montañés, et al., 2014) causing instability between the process stages.

High VFA concentrations, especially propionic acid, inhibited methanogenesis (Wang, et al., 2009), which was observed as further decline in both biogas production and biogas methane content. The low pH further reinforced the inhibitory effect of VFAs (Al Seadi,

et al., 2008). The concentration of propionic acid reached 900 mg/L at the end of continuous operation, when gas production was very low (on days 87, 96–101, and 106) and throughout the batch operation (days 113–134). Methane content of biogas decreased, probably due to propionic acid inhibition of methanogenesis, from over 40 % (before day 87) to 25–30 % (days 90–106).

The feeding of the semi-continuous CSTR was stopped on day 105 and the process was began to be operated as batch in order to provide the methanogens more time to regain stability in the process. Recovery of the process was observed in terms of decreasing VFA (from 11 to 5 g/L SCOD) and SCOD (from 18 to 10 g/L) concentrations as well as improving biogas production (from 0.46 to 1.1 m³ CH₄/m³ reactor), methane content (from 30 to 80 %) and digestate pH (from 6.4 to 7.7). SCOD decreased less than VFAs, indicating that there were other solubilized organics than only VFAs present in the reactor and they were less readily transformable to biogas than VFAs. The decrease in VFAs was observed together with a steep increase of methane content in the biogas, indicating that the VFAs, the intermediate products of acidogenesis were consumed as methanogenesis took place more efficiently again along with increasing pH.

However, there were still considerable concentrations of VFAs and SCOD left in the digestate after a month of batch operation. Most of the improvement was observed at the end of batch operation, indicating slow recovery. Acetic acid was degraded to large extent (concentration after batch operation was ca. 2 g/L SCOD compared to the peak value of 9 g/L SCOD). Propionic acid concentrations were still increasing in the batch operation of CSTR as well as in batch assays. This indicates that production of propionic acid was favored probably by the low pH and its degradation was not functioning. Degradation of the accumulated propionic acid is considered as most difficult from the VFAs (Gallert & Winter, 2008).

The batch assays, conducted using the digestate from the CSTR experiments, showed that trace element deficiency was most likely not limiting methane production. The batch assays also showed that the methanogens had not yet recovered or they were still inhibited, since the SCOD concentration of the digestate had decreased only from the initial 11 g/L to 9 g/L after 43 d of batch assay following the 29 days of batch operation of CSTR. The total VFA concentrations decreased from 5.4 to 2.7–2.8 g/L SCOD.

6.2 LBR experiments for sedimented fiber hydrolysis

The screening experiments showed that the treatment of sedimented fiber from pulp industry in LBRs is technically viable. Leaching took place spontaneously. Total yield of leachate was ca. 10 % of the initial fiber volume. Higher column of material or larger mass increased the volume of leachate collected per sedimented fiber mass. Pressure from the mass of fibers presses the leachate out from the material. However, the results on the

effect of compaction on leachate volumes and the rate of leachate production were inconsistent. Compaction removed the void space in the material slowing down the flow of leachate in the reactor cylinder. Still, more forceful compaction increased the leachate yield and the rate of leaching compared to less compacted material.

It is yet unknown, how the scale-up of the reactors to industrial scale would affect leaching. Probably, the high piles in the industrial scale operations would inflict more pressure on the material making the extraction of the leachate more effective. However, the compression would also more effectively remove the void spaces in the material, slowing down the initial rate of leaching compared to laboratory test conditions. This would increase the contact time of the recirculated leachate in the substrate bed, thus potentially increasing the degree of hydrolysis.

Experiments on the hydrolysis of sedimented fiber showed that SCOD and VFAs could be extracted from the sedimented fiber in LBRs. The highest concentrations of SCOD in the leachate (6.2–6.6 g/L) were observed as leachate recirculation was began. Recirculation of leachate most likely flushed the readily hydrolyzed products out of the reactor. Shortly after that, the leachate SCOD concentrations dropped in all of the reactors due to short-circuiting of the leachate flow. As the leachate recirculation was continued, the entire sediment bed was visibly wet from the recirculated leachate. Simultaneously, the leachate SCOD concentrations recovered and stabilized at 4–5 g/L. Leachate recirculation improved hydrolysis, yet there was a limit in the improvement, since the SCOD concentration stabilized in a matter of days. There was a minor decrease in the leachate SCOD concentrations over the following 20 d. This indicates that some of the extracted SCOD was bound again in the leach bed or the leachate storage container.

The specific SCOD production was higher in thermophilic reactor (LBR1, 43 g SCOD/kg VS) as suggested by Shi et al. (2013) and the mesophilic reactor with nitrogen supplementation and inoculation (LBR3, 42 g SCOD/kg VS) than in the other mesophilic reactor (LBR2, 37 g SCOD/kg TS). In a study by Jagadabhi et al. (2010), the maximum SCOD production of 510 g SCOD/kg VS was reached with leachate recirculation in treatment of grass silage. Majority of the total SCOD yield (thermophilic: 55 %, mesophilic: 60 %, and mesophilic with nitrogen and inoculum: 61 %) was extracted in the very beginning of operation of LBRs (day 3), when leachate recirculation was began. Further hydrolysis took place and SCOD could be extracted in the leachate, when the leachate was entirely replaced with fresh water.

The highest total VFA concentrations in the leachates from the thermophilic LBR (2.8 g/L SCOD) and mesophilic LBR (3.0 g/L SCOD) were obtained during the recirculation of leachate (days 21 and 11). Recirculation enhanced acidogenesis. The highest VFA concentration for the mesophilic reactor with inoculation and nitrogen supplementation (3.8 g/L SCOD) was achieved during the first day of operation.

The SCOD and VFA yields were highest in the thermophilic treatment and mesophilic treatment with inoculation and nitrogen supplementations, however, the difference to mesophilic treatment without nutrient supplementation was small. After inoculation, the total VFA of the inoculated mesophilic reactor were higher than in the other reactors indicating microbial acidogenic activity. Higher temperature, nutrient supplementation and inoculation improved hydrolysis of sedimented fiber. Optimization of inoculation could be used to further improve the VFA production (Degueurce, et al., 2016; Wilson, et al., 2016).

6.3 Practical implementations of the results

Sedimented fiber can be used in biogas production as long as adequate buffering and nitrogen supplementation is carried out. Process technology and the utilization of by-products, mainly digestate, play a crucial role in the practical viability of the treatment.

The VFA production was effective in the CSTR after the operation in low pH and buffering as well as nitrogen supplementation had favored hydrolysis over methanogenesis. Thus, CSTR could be used in two-stage AD configuration if the hydrolysis was similarly promoted in the first stage reactor as in the CSTR in this study. Then, in the second stage reactor, the conditions could be optimized for degradation of VFAs by increasing the pH with high buffering to promote methane production.

VFA concentrations in the leachates from LBRs were lower than in the digestate of the CSTR. However, LBRs are easier to operate with high-TS feedstocks than CSTRs, since LBRs do not include moving parts. However, the up-scaling of the LBR configuration to industrial scale based on these laboratory scale results is uncertain. Hydrolysis could be significantly improved by the higher compaction and longer contact times provided by taller piles of leach bed.

7. CONCLUSIONS

Sedimented fiber can be used in AD for the production of VFAs and biogas. The maximum methane production was 240 m³ CH₄/t VS in a CSTR when inoculation was still stabilizing the process. Mono-digestion of the sedimented fiber in a semi-continuous CSTR with an OLR of 2.5 kg VS/(m³ d), HRT of 48 d, buffering, and supplementation of nitrogen was not stable in the long term operation. Supplementation of nitrogen promoted hydrolysis, yet methanogenesis was falling behind.

In low pH conditions, high concentrations of VFAs (maximum total VFA concentration of 13 g/L), and particularly the slowly degradable propionic acid, were produced. Methanogenesis failed most likely due to decrease in pH in consequence of inadequate buffering. This caused imbalance between acidogenesis and methanogenesis, and consequent acidification and accumulation of VFAs. Finally, methanogenesis was inhibited by high concentrations of propionic acid. After failure, the process slowly recovered. The degradation of accumulated VFAs, particularly propionic acid, was slow. The process was not limited by trace element insufficiency.

Different reactor configurations may allow better optimization and efficiency of the treatment. Two-stage process with a dry digester for hydrolysis and acidogenesis and a high-rate wet digester for methanogenesis is one option that could decrease the retention times and reactor volumes required. LBR digesters are suitable for the treatment of sedimented fiber. Both thermophilic temperature and inoculation or nitrogen supplementation increase hydrolysis. Maximum SCOD concentration extracted in the leachates were 6.2–6.6 g/L, specific SCOD yields 37–43 g SCOD/kg VS and maximum total VFA concentrations 2.8–3.8 g/L SCOD. Thermophilic temperature as well as inoculation and nitrogen supplementation enhanced hydrolysis. The specific SCOD production was 43 g SCOD/kg VS for the thermophilic and 42 g SCOD/kg VS for the mesophilic reactor with nutrient supplementation. Leachate recirculation improved the hydrolysis for the reactors without nitrogen supplementation. In addition, the treatment of sedimented fiber in an LBR decreased the volume of the material.

Further research is required on long-term stability of AD of sedimented fiber, and the optimum operational parameters as well as nutrient and buffer supplementations. Another line of research would be the optimization of hydrolysis in two-stage configurations. One option is to promote hydrolysis of the material using pretreatments. Up-scaling of the LBRs should be examined in larger or higher test reactors, since a higher leach bed may influence the leaching as well as leachate properties. In addition, research on methanogenesis of the leachates from the LBRs or digestate from the CSTR with high concentrations of VFAs is required for the evaluation of viability of the two-stage configuration.

REFERENCES

- Abbassi-Guendouz, A., Brockmann, D., Trably, E., Dumas, C., Delgenès, J.-P., Steyer, J.-P. & Escudé, R., 2012. Total solids content drives high solid anaerobic digestion via mass transfer limitation. *Bioresource Technology*, 111, pp. 55-61.
- Abendroth, C., Vilanova, C., Günther, T., Luschnig, O. & Porcar, M., 2015. Eubacteria and archaea communities in seven mesophile anaerobic digester plants in Germany. *Biotechnology for Biofuels*, 87, pp. 1-10.
- Adney, W. S., Rivard, C. J., Shiang, M. & Himmel, M. E., 1991. Anaerobic digestion of lignocellulosic biomass and wastes - Cellulases and related enzymes. *Applied Biochemistry and Biotechnology*, 30, pp. 165-183.
- Al Seadi, T., Rutz, D., Prassl, H., Köttner, M., Finsterwalder, T., Volk, S. & Janssen, R., 2008. *Biogas Handbook*. Esbjerg, Denmark: University of Southern Denmark Esbjerg.
- Alvarez, R. & Lidén, G., 2008. Semi-continuous co-digestion of solid slaughterhouse waste, manure, and fruit and vegetable waste. *Renewable Energy*, 33, pp. 726-734.
- Autiola, M. & Holopainen, M., 2016. *0-kuidun pilaantuneisuustutkimus, Tampereen kaupunki, Hiedanranta*, Tampere: Ramboll Finland Ltd.
- Bayr, S. & Rintala, J., 2012. Thermophilic anaerobic digestion of pulp and paper mill primary sludge and co-digestion of primary and secondary sludge. *Water Research*, 46, pp. 4713-4720.
- Benner, R., Maccubbin, A. & Hodson, R., 1984. Anaerobic biodegradation of the lignin and polysaccharide components of lignocellulose and synthetic lignin by sediment microflora. *Applied and Environmental Microbiology*, 47, pp. 998-1004.
- Bougrier, C., Dognin, D., Laroche, C., Gonzalez, V., Benali-Raclot, D. & Rivero, J. A. C., 2018. Anaerobic digestion of brewery spent grains: Trace element addition requirement. *Bioresource Technology*, 247, pp. 1193-1196.
- Börjesson, P. & Mattiasson, B., 2008. Biogas as a resource-efficient vehicle fuel. *Trends in Biotechnology*, 26, pp. 7-13.
- Chan, G. Y. S., Chu, L. M. & Wong, M. H., 2002. Effects of leachate recirculation on biogas production from landfill co-digestion of municipal solid waste, sewage sludge and marine sediment. *Environmental Pollution*, 118, pp. 393-399.

- Chawla, S. P., Kanatt, S. R. & Sharma, A. K., 2014. Chitosan. In *Polysaccharides*: Springer International Publishing Switzerland, pp. 1-24.
- Chen, Y., Cheng, J. J. & Creamer, K. S., 2007. Inhibition of anaerobic digestion process: A review. *Bioresource Technology*, 99, pp. 4044-4064.
- Chen, Y.-d., Ho, S.-H., Nagarajan, D., Ren, N.-q. & Chang, J.-S. 2018. Waste biorefineries - integrating anaerobic digestion and microalgae cultivation for bioenergy production. *Current Opinion in Biotechnology*, 50, pp. 101-110.
- Cirne, D. G., Lehtomäki, A., Björnsson, L. & Blackall, L. L., 2007. Hydrolysis and microbial community analyses in two-stage anaerobic digestion of energy crops. *Journal of Applied Microbiology*, 103.
- Council of State of Finland, 2007. *Valtioneuvoston asetus maaperän pilaantuneisuuden ja puhdistustarpeen arvioinnista*. Helsinki: Finlex - open database, Ministry of Justice, Finland.
- Cysneiros, D., Banks, C. J., Heaven, S. & Karatzas, K.-A. G., 2012. The effect of pH control and 'hydraulic flush' on hydrolysis and volatile fatty acids (VFA) production and profile in anaerobic leach bed reactors digesting a high solids content substrate. *Bioresource Technology*, 123, pp. 263-271.
- Degueurce, A., Tomas, N., Le Roux, S., Martinez, J. & Peu, P., 2016. Biotic and abiotic roles of leachate recirculation in batch mode solid-state anaerobic digestion of cattle manure. *Bioresource Technology*, 200, pp. 388-394.
- Demirel, B. & Yenigün, O., 2002. Two-phase anaerobic digestion processes: a review. *Journal of Chemical Technology and Biotechnology*, 77, pp. 743-755.
- Ekman, J., Hakula, A., Lankia, S., Moisala, A., Ruutikainen, T., Ylä-Anttila, K., Lehtomaa, J., Salli, R., Vesanen, L., Vänskä, M. & Suolahti, J., 2016. *Lielahden rannan kehittämisvisio, Kantakaupungin yleiskaava 2040, Maankäytöltään muuttuvien alueiden selvitykset*, Tampere.
- Ekstrand, E.-M., Karlsson, M., Truong, X.-B., Björn, A., Karlsson, A., Svensson, B. H. & Ejlertsson, J. 2016. High-rate anaerobic co-digestion of kraft mill fibre sludge and activated sludge by CSTRs with sludge recirculation. *Waste Management*, 56, pp. 166-172.
- Evangelisti, S., Lettieri, P., Borello, D. & Clift, R., 2014. Life cycle assessment of energy from waste via anaerobic digestion: A UK case study. *Waste Management*, 34, pp. 226-237.

Fardim, P. (edit.), 2011. *Papermaking Science and Technology Book 6: Chemical Pulping Part 1 - Fiber Chemistry and Technology*. Porvoo: Paper Engineers' Association / Paperi ja Puu Oy.

Field, J., Leyendeckers, M., Sierra Alvarez, R., Lettinga, G. & Habets, L., 1988. The methanogenic toxicity of bark tannins and the anaerobic biodegradability of water soluble bark matter. *Water Science and Technology*, 20, pp. 219-240.

Finnish Forest Industries, 2018. *Pulp and Paper Industry Statistics 2016*. Available (2018) at: www.metsateollisuus.fi/tilastot/massa-ja-paperiteollisuus.

Gallert, C. & Winter, J., 2008. Propionic acid accumulation and degradation during restart of a full-scale anaerobic biowaste digester. *Bioresource Technology*, 99, pp. 170-178.

Gasum Oy, 2018. *Gasum: Finland's gas network and Gas fueling stations*. Available (2018) at: www.gasum.com/en/About-gas.

Ge, X., Xu, F. & Li, Y., 2016. Solid-state anaerobic digestion of lignocellulosic biomass: recent progress and perspectives. *Bioresource Technology*, 205, pp. 239-249.

Gerardi, M. H. (edit.), 2003. *The Microbiology of Anaerobic Digesters*. New Jersey: John Wiley & Sons.

Ghosh, S., 1986. Novel processes for high-efficiency biodigestion of particulate feeds. Teoksessa: M. M. El-Halwagi, toim. *Biogas Technology, Transfer and Diffusion*. Dordrecht: Springer, pp. 400-416.

Google Maps, Tampere. Available (2018) at: www.maps.google.com.

Grando, R. L., de Souza Antune, A. M., da Fonseca, F. V., Sánchez, A., Barrena, R. & Font, X., 2017. Technology overview of biogas production in anaerobic digestion plants: A European evaluation of research and development. *Renewable and Sustainable Energy Reviews*, 80, pp. 44-53.

Guo, J., Zhao, L., Lu, W., Jia, H. & Sun, Y., 2016. Bacterial communities in water and sediment shaped by paper mill pollution and indicated bacterial taxa in sediment in Daling River. *Ecological Indicators*, 60, pp. 766-773.

Hinken, L., Urban, I., Haun, E., Urban, I. & Weichgrebe, D., 2008. The valuation of malnutrition in the mono-digestion of maize silage by anaerobic batch tests. *Water Science & Technology*, 58, pp. 1453-1459.

Hoffman, E., Lyons, J., Boxall, J., Robertson, C., Lake, C. & Walker, T., 2017. Spatiotemporal assessment (quarter century) of pulp mill metal(loid) contaminated

- sediment to inform remediation decisions. *Environmental Monitoring and Assessment*, 189, pp. 257-274.
- Hofsten, B. & Edberg, N., 1972. Estimating the rate of degradation of cellulose fibers in water. *Oikos*, pp. 29-34.
- Hussain, A., Filiatrault, M. & Guiot, S. R., 2017. Acidogenic digestion of food waste in a thermophilic leach bed reactor: Effect of pH and leachate recirculation rate on hydrolysis and volatile fatty acid production. *Bioresource Technology*, 245, pp. 1-9.
- Huttunen, R., 2017. *Governmental report on the National Energy and Climate Strategy for 2030*, Ministry of Economical Affairs and Employment.
- Hynynen, J., Palomäki, A., Meriläinen, J., Witick, A. & Mäntykoski, K., 2004. Pollution history and recovery of a boreal lake exposed to a heavy bleached pulping effluent load. *Journal of Paleolimnology*, 32, pp. 351-374.
- Igoni, A. H., Ayotamuno, M. J., Eze, C. L., Ogaji, S. O. T. & Probert, S. D., 2008. Designs of anaerobic digesters for producing biogas from municipal solid-waste. *Applied Energy*, 85, pp. 430-438.
- Jackson, T., 2016. Historical variations in the stable isotope composition of mercury in a sediment core from riverine lake: Effects of dams, pulp and paper mill wastes, and mercury from a chlor-alkali plant. *Applied Geochemistry*, 71, pp. 86-98.
- Jagadabhi, P. S., Kaparaju, P. & Rintala, J., 2010. Effect of micro-aeration and leachate replacement on COD solubilization and VFA production during mono-digestion of grass-silage in one-stage leach-bed reactors. *Bioresource Technology*, 101, pp. 2818-2824.
- Jagadabhi, P. S., Kaparaju, P. & Rintala, J., 2011. Two-stage anaerobic digestion of tomato, cucumber, common reed and grass silage in leach-bed reactors and upflow anaerobic sludge blanket reactors. *Bioresource Technology*, 102, pp. 4726-4733.
- Jagadabhi, P. S., Kaparaju, P., Väisänen, A. & Rintala, J., 2017. Effect of macro- and micro-nutrients addition during anaerobic mono-digestion of grass silage in leach-bed reactors. *Environmental Technology*, Issue DOI: 10.1080/09593330.2017.1393462.
- Jiang, J., Zhang, Y., Li, K., Wang, Q., Gong, C. & Li, M., 2013. Volatile fatty acids production from food waste: Effects of pH, temperature, and organic loading rate. *Bioresource Technology*, 143, pp. 525-530.
- Jin, P., Bhattacharya, S. K., Williams, C. J. & Zhang, H., 1998. Effects of sulfide addition on copper inhibition in methanogenic systems. *Water Research*, 32, pp. 977-988.

- Jones, W. J., Nagle, D. P. J. & Whitman, W. B., 1987. Methanogens and the diversity of archaeobacteria. *Microbiological Reviews*, 51, pp. 135-177.
- Jönsson, L. J., Ariksson, B. & Nilvebrant, N.-O., 2013. Bioconversion of lignocellulose: inhibitors and detoxification. *Biotechnology for Biofuels*, 6, pp. 1-10.
- Kamali, M., Gameiro, T., Costa, M. & Capela, I., 2016. Anaerobic digestion of pulp and paper mill wastes - An overview of the developments and improvement opportunities. *Chemical Engineering Journal*, 298, pp. 162-182.
- Kinnunen, V., Ylä-Outinen, A. & Rintala, J., 2015. Mesophilic anaerobic digestion of pulp and paper industry biosludge - long-term reactor performance and effects of thermal pretreatment. *Water Research* 87, pp. 105-111.
- Kokko, M., Koskue, V. & Rintala, J., 2018. Anaerobic digestion of 30-100-year-old boreal lake sedimented fibre from the pulp industry: Extrapolating methane production potential to practical scale. *Water Research*, 133, pp. 218-226.
- Kostamo, A., Holmbom, B. & Kukkonen, J., 2004. Fate of wood extractives in wastewater treatment plants at kraft pulp mills and mechanical pulp mills. *Water Research*, 38, pp. 972-982.
- Kuisma, M., 1993. *Metsäteollisuuden maa: Suomi, Metsät ja Kansainvälinen Järjestelmä 1620-1920 (1st ed.)*. Jyväskylä: Gummerus Kirjapaino Oy.
- Kymäläinen, M. & Pakarinen, O. (edit.), 2015. *Biokaasuteknologia - Raaka-aineet, prosessointi ja lopputuotteiden hyödyntäminen*. Hämeenlinna: Häme University of Applied Sciences.
- Kähkönen, M., Suominen, K., Manninen, P. & Salkinoja-Salonen, M., 1998. 100 years of sediment accumulation history of organic halogens and heavy metals in recipient and nonrecipient lakes of pulping industry in Finland. *Environmental Science & Technology*, 32, pp. 1741-1746.
- Lahtinen, L., 2017. *Selluteollisuuden nollakuitusedimentin hyödyntäminen biokaasuprosessissa (Master of Science Thesis)*, Tampere: Tampere University of Technology.
- Lai, T. E., Nopharatana, A., Pullammanappallil, P. C. & Clarke, W. P., 2001. Cellulolytic activity in leachate during leach-bed anaerobic digestion of municipal solid waste. *Bioresource Technology*, 80, pp. 205-210.
- Lee, M., Hidaka, T., Hagiwara, W. & Tsuno, H., 2009. Comparative performance and microbial diversity of hyperthermophilic and thermophilic co-digestion of kitchen garbage and excess sludge. *Bioresource Technology*, 100, pp. 578-585.

- Lehtomäki, A., Huttunen, S., Lehtinen, T. M. & Rintala, J. A., 2008. Anaerobic digestion of grass silage in batch leach bed processes for methane production. *Bioresource Technology*, 99, pp. 3267-3278.
- Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, 2015. 144. *Thermoanaerobacter sp. medium*.
- Liew, L. N., Shi, J. & Li, Y., 2012. Methane production from solid-state anaerobic digestion of lignocellulosic biomass. *Biomass and Bioenergy*, 46, pp. 125-132.
- Lindholm-Lehto, P., Knuutinen, J., Ahkola, H. & Herve, S., 2015. Refractory organic pollutants and toxicity in pulp and paper mill wastewaters. *Environmental Science and Pollution Research*, 22, pp. 6473-6499.
- Lin, Y., Wang, D., Li, Q. & Xiao, M., 2011. Mesophilic batch anaerobic co-digestion of pulp and paper sludge and monosodium glutamate waste liquor for methane production in a bench-scale digester. *Bioresource Technology*, 102, pp. 3673-3678.
- Li, Y., Park, S. Y. & Zhu, J., 2011. Solid-state anaerobic digestion for methane production from organic waste. *Renewable and Sustainable Energy Reviews*, 15, pp. 821-826.
- Luonsi, A., Junna, J. & Nevalainen, I., 1988. The development of waste water treatment in the Finnish pulp and paper industry. *Water Science & Technology*, 20, pp. 25-36.
- Madigan, M. T. (edit.), 1999. *Brock Biology of Microorganisms*. Pearson Education.
- Mahmood-Khan, Z. & Hall, E. R., 2003. Occurrence and removal of plant sterols in pulp and paper mill effluents. *Journal of Environmental Engineering and Science*, 2, pp. 17-26.
- Mao, C., Feng, Y., Wang, X. & Ren, G., 2015. Review on research achievements of biogas from anaerobic digestion. *Renewable and Sustainable Energy Reviews*, 45, pp. 540-555.
- Mattson, K., Lehtinen, K.-J., Tana, J., Härding, J., Kukkonen, J., Nakari, T. & Engström, C., 2001. Effect of pulp mill effluents and restricted diet on growth and physiology of rainbow trout (*Oncorhynchus mykiss*). *Ecotoxicology and Environmental Safety*, 49, pp. 144-154.
- Meng, X., Yu, D., Wei, Y., Zhang, Y., Zhang, Q., Wang, Z., Liu, J. & Wang, Y., 2018. Endogenous ternary pH buffer system with ammonia-carbonates-VFAs in high solid anaerobic digestion of swine manure: An alternative for alleviating ammonia inhibition? *Process Biochemistry*, 69, pp. 144-152.

- Menon, V. & Rao, M., 2012. Trends in bioconversion of lignocellulose: Biofuels, platform chemicals & biorefinery concept. *Progress in Energy and Combustion Science*, 38, pp. 522-550.
- Meriläinen, J., Hynynen, J., Palomäki, A., Veijola, H., Witick, A., Mäntykoski, K., Granberg, K. & Lehtinen, K. 2001. Pulp and paper mill pollution and subsequent ecosystem recovery of a large boreal lake in Finland: a paleolimnological analysis. *Journal of Paleolimnology*, 26, pp. 11-35.
- Monlau, F., Sambusiti, C., Barakat, A., Guo, X. M., Latrille, E., Trably, E., Steyer, J.-P. & Carrere, H., 2012. Predictive models of biohydrogen and biomethane production based on the composition and structural features of lignocellulosic materials. *Environmental Science and Technology*, 46, pp. 12217-12225.
- Montañés, R., Pérez, M. & Solera, R., 2014. Anaerobic mesophilic co-digestion of sewage sludge and sugar beet pulp lixiviation in batch reactors: Effect of pH control. *Chemical Engineering Journal*, 255, pp. 492-499.
- Mulat, D. G., Dibdiakova, J. & Horn, S. J., 2018. Microbial biogas production from hydrolysis lignin: insight into lignin structural changes. *Biotechnology for Biofuels*, 11, pp. 1-16.
- Munawar, M., Munawar, I., Sergeant, D. & Wenghofer, C., 2000. A preliminary bioassessment of Lake Baikal sediment toxicity in the vicinity of a pulp and paper mill. *Aquatic Ecosystem Health and Management*, 3, pp. 249-257.
- Munkittrick, K., Servos, M., Carey, J. & Van Der Kraak, G., 1997. Environmental impacts of pulp and paper wastewater: Evidence for a reduction in environmental effects at North American pulp mills since 1992. *Water Science & Technology*, 35, pp. 329-338.
- Naess, B. & Sandvik, O., 1973. The in vitro effect of peptide-precipitating lignosulphonic acids on various biologically active substances produced by or with action on microorganisms. *Acta Veterinaria Scandinavica*, 14, pp. 129-146.
- National Land Survey of Finland. Available (2018) online at: www.retkikartta.fi.
- Nges, I. A., Björn, A. & Björnsson, L., 2012. Stable operation during pilot-scale anaerobic digestion of nutrient-supplemented maize-sugar beet silage. *Bioresource Technology*, 118, pp. 445-454.
- Nizami, A.-S., Korres, N. E. & Murphy, J. D., 2009. Review of the integrated process for the production of grass biomethane. *Environmental Science & Technology*, 43, pp. 8496-8508.

- Nizami, A.-S. & Murphy, J. D., 2011. Optimizing the operation of a two-phase anaerobic digestion system digesting grass silage. *Environmental Science & Technology*, 45, pp. 7562-7569.
- Nkemka, V. N. & Hao, X., 2018. Start-up of a sequential dry anaerobic digestion of paunch under psychrophilic and mesophilic temperatures. *Waste Management*, 74, pp. 144-149.
- Oikari, A., Holmbom, B. & Bister, H., 1982. Uptake of resin acids into tissues of trout (*Salmo gairdneri* Richardson). *Annales Zoologici Fennici*, 19, pp. 61-64.
- Pandelides, Z., Guchardi, J. & Holdway, D., 2014. Dehydroabietic acid (DHAA) alters metabolic enzyme activity and the effects of 17beta-estradiol in rainbow trout (*Oncorhynchus mykiss*). *Ecotoxicology and Environmental Safety*, 101, pp. 168-176.
- Pearson, T., 1980. Marine pollution effect of pulp and paper industry wastes. *Helgoländer Meeresuntersuchungen*, 33, pp. 340-365.
- Pirkanmaan ympäristökeskus, 2006. *Ympäristölupapäätös 1999Y02200-111 Ligno Tech Finland Oy*. Tampere: Pirkanmaan ympäristökeskus, Valvonta- ja ympäristölupaosasto.
- Pobeheim, H., Munk, B., Johansson, J. & Guebitz, G. M., 2010. Influence of trace elements on methane formation from a synthetic model substrate for maize silage. *Bioresource Technology*, 101, pp. 836-839.
- Pokhrel, D. & Viraraghavan, T., 2004. Treatment of pulp and paper mill wastewater - a review. *Science of the Total Environment*, 333, pp. 37-58.
- Poole, N., Wildish, D. & Kristmanson, D., 1978. The effects of pulp and paper industry on the aquatic environment. *Critical Reviews in Environmental Control*, 8, pp. 153-193.
- Pro Agria Pohjois-Karjala, 2018. *Liikennebiokaasu*. Available (2018) at: www.liikennebiokaasu.com.
- Procházka, J., Dolejš, P., Máca, J. & Dohányos, M., 2012. Stability and inhibition of anaerobic processes caused by insufficiency or excess of ammonia nitrogen. *Applied Microbiology and Biotechnology*, 93, pp. 439-447.
- Pyykkö, L. & Lehtovaara, O., 2011. *Lielahden kemihierretehtaan alue - Yhteenvedo ympäristöselvityksistä*, Tampere: Ramboll Finland Oy.
- Ramboll Finland Oy, 1984. *Lielahden vesistö tutkimus, pumppaamo, kuitupitoisen lietteen levinneisyys Näsijärvessä, Project no. 11774*, Ramboll Finland Oy.
- Ramboll Finland Oy, Infra & Liikenne, 2017. *0-kuidun näytteenotto - Hiedanranta, Tampere*, Tampere: Ramboll Finland Oy.

Riggio, S., Torrijos, M., Vives, G., Esposito, G., van Hullebusch, E. D., Steyer, J. P. & Escudié, R., 2017. Leachate flush strategies for managing volatile fatty acids accumulation in leach-bed reactors. *Bioresource Technology*, 232, pp. 93-102.

Sawatdeenarunat, C., Surendra, K.C., Takara, D., Oechsner, H. & Khanal, S. K., 2015. Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. *Bioresource Technology*, 178, pp. 178-186.

Scherer, P., Neumann, L., Demirel, B., Schmidt, O. & Unbehauen, M., 2009. Long term fermentation studies about the nutritional requirements for biogasification of fodder beet silage as mono-substrate. *Biomass and Bioenergy*, pp. 33, 873-881.

Finnish Standards Association SFS, 2007. *Characterization of waste. Calculation of dry matter by determination of dry residue or water content*. Finnish Standards Association SFS.

Finnish Standards Association SFS, 1988. Determination of chemical oxygen demand (CODCr) in water with the closed tube method. Oxidation with dichromate. *Standard*, pp. 1-4 Finnish Standards Association SFS.

Shi, J., Wang, Z., Stiverson, J. A., Yu, Z. & Li, Y., 2013. Reactor performance and microbial community dynamics during solid-state anaerobic digestion of corn stover at mesophilic and thermophilic conditions. *Bioresource Technology*, 136, pp. 574-581.

Sierra-Alvarez, R. & Lettinga, G., 1991. The methanogenic toxicity of wastewater lignins and lignin related compounds. *Journal of Chemical Technology & Biotechnology*, 50.

Sponza, D., 2003. Application of toxicity test into discharges of the pulp-paper industry in Turkey. *Ecotoxicology and Environmental Safety*, 54, pp. 74-86.

Stahlschmidt-Allner, P., Allner, B., Römbke, J. & Knacker, T., 1997. Endocrine disrupters in the aquatic environment. *Environmental Science and Pollution Research*, 4, pp. 155-162.

Finnish Standards Association SFS, 2007. *Characterization of waste. Determination of loss on ignition in waste, sludge and sediments*. Finnish Standards Association SFS.

Walden, C., 1976. The toxicity of pulp and paper mill effluents and corresponding measurement procedures. *Water Research*, 10, pp. 639-664.

van Oel, P. R. & Hoekstra, A. Y., 2012. Towards Quantification of the Water Footprint of Paper: A First Estimate of its Consumptive Component. *Water Resource Management*, 26, pp. 733-749.

- Wang, L., Zhou, Q. & Li, F. T., 2006. Avoiding propionic acid accumulation in the anaerobic process for biohydrogen production. *Biomass and Bioenergy*, 30, pp. 177-182.
- Wang, Y., Zhang, Y., Wang, J. & Meng, L., 2009. Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. *Biomass and Bioenergy*, 33, pp. 848-853.
- Vepsäläinen, M., Kivisaari, H., Pulliainen, M., Oikari, A. & Sillanpää, M., 2011. Removal of toxic pollutants from pulp mill effluents by electrocoagulation. *Separation and Purification Technology*, 81, pp. 141-150.
- Whiting, A. & Azapagic, A., 2014. Life cycle environmental impacts of generating electricity and heat from biogas produced by anaerobic digestion. *Energy*, 70, pp. 181-193.
- Wilson, L. P., Sharvelle, S. E. & De Long, S. K., 2016. Enhanced anaerobic digestion performance via combined solids- and leachate-based hydrolysis reactor inoculation. *Bioresour. Technol.*, 220, pp. 94-103.
- Wood, N., Honghi, T. & Master, E., 2009. Pretreatment of pulp mill secondary sludge for high-rate anaerobic conversion to biogas. *Bioresour. Technol.*, 100, pp. 5729-5735.
- Väliharju, R. & Toivonen, K., 2017. *Hiedanranta, Tampere, Finland - Smart and sustainable city district of the future*, Tampere: Tampereen kaupunki.
- Xu, F., Wang, Z.-W., Tang, L. & Li, Y., 2014. A mass diffusion-based interpretation of the effect of total solids content on solid-state anaerobic digestion of cellulosic biomass. *Bioresour. Technol.*, 167, pp. 178-185.
- Xu, S. Y., Lam, H. P., Karthikeyan, P. & Wong, J. W. C., 2011. Optimization of food waste hydrolysis in leach bed coupled with methanogenic reactor: Effect of pH and bulking agent. *Bioresour. Technol.*, 102, pp. 3702-3708.
- Yang, L., Xu, F., Ge, X. & Li, Y., 2015. Challenges and strategies for solid-state anaerobic digestion of lignocellulosic biomass. *Renewable and Sustainable Energy Reviews*, 44, pp. 824-834.
- Yenigün, O. & Demirel, B., 2013. Ammonia inhibition in anaerobic digestion: A review. *Process Biochemistry*, 48, pp. 901-911.
- Ylä-Outinen, A., 2014. *Metsäteollisuuden lietteiden metaanintuotto ja sen tehostaminen*, *Diplomityö*, Tampere University of Technology.

Zandvoort, M. H., van Hullebusch, E. D., Feroso, F. G. & Lens, P. N. L., 2006. Trace metals in anaerobic granular sludge reactors: Bioavailability and dosing strategies. *Engineering in Life Sciences*, 6, pp. 293-301.

Zhang, Q., Hu, J. & Lee, D.-J., 2016. Biogas from anaerobic digestion processes: Research updates. *Renewable Energy*, 98, pp. 108-119.

Zhou, M., Yan, B., Wong, J. W. C. & Zhang, Y., 2018. Enhanced volatile fatty acids production from anaerobic fermentation of food waste: A mini-review focusing on acidogenic metabolic pathways. *Bioresource Technology*, 248, pp. 68-78.

Ziganshin, A. M., Liebetrau, J., Pröter, J. & Kleinstüber, S., 2013. Microbial community structure and dynamics during anaerobic digestion of various agricultural waste materials. *Applied Microbiology and Biotechnology*, 97, pp. 5161-5174.

