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# Counteracting ammonia inhibition in anaerobic digestion using wood residues

*Evaluating ammonium adsorption capacity of fibres from pulp and paper mills*

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## Abstract

One of the main interests in commercial methane production is to maximize the gas yield, and it is thus appealing to use material with relative high methane potential. However, such material often results in process instability whereas ammonia inhibition is common. Removal of ammonia through adsorption is a fairly unexplored method in the field of biogas production, and could prove to be cost-effective.

The adsorption capacity of pulp fibres from the paper making industry were investigated through batch adsorption experiments. Additionally, the fibres effect on small scale batch digesters in terms of methane production and cellulase activity was explored. Overall, the adsorption capacity of the pulp fibres was low, whereas Kraft hardwood had the highest adsorption capacity in both an aqueous ammonium solution and digester fluid at 11±3 and 60±20 mg $g^{-1}$, respectively. The initial total ammonium nitrogen concentration had the highest effect on the adsorption capacity with a positive correlation. The pulp fibres seemingly had no effect on the ammonia inhibited anaerobic digestion systems. However, the cellulase activity was higher after day 5 in the anaerobic digestion systems with a high ammonia concentration.

In essence, the overall results showed that the adsorption of the fibres was relatively low and most likely not suitable as a material to prevent ammonia inhibition in an AD.

# Table of content

## Abbreviations

| | |
|---|---|
| AD | Anaerobic digestion |
| BMP | Biochemical methane potential |
| C/N ratio | Carbon to nitrogen ratio |
| HRT | Hydraulic retention time |
| HW | Hardwood |
| LCFA | Long chain fatty acids |
| $NH_3$ | Ammonia |
| $NH_4^+$ | Ammonium |
| OLR | Organic loading rate |
| SBF | Scandinavian Biogas Fuels |
| SW | Softwood |
| TMP | Thermo-mechanical pulping |
| TAN | Total ammonia nitrogen |
| TN | Total nitrogen |
| TV | Tekniska Verken |
| VFA | Volatile fatty acids |
| VS | Volatile solids |

# 1. Introduction and aim

## 1.1 Introduction

The process of anaerobic digestion (AD) makes use of several microorganisms in an oxygen-free environment to convert organic matter into renewable biogas and digestate. Several sources of organic matter exist, e.g. municipal waste, slaughterhouse waste, agricultural waste, sludge from wastewater treatment, pig and cow manure (Schnürer et al., 2016), pulp waste (Ekstrand, 2019). One of the main interests for commercial biogas production is to maximize the methane yield, and it is therefore appealing to use substrate with relative high methane potential, such as protein-rich material (Moestedt et al., 2019). In addition, protein-rich substrates increase ammonium levels in the digestate, making it suitable as a bio-fertilizer (Westerholm et al., 2016). However, running an AD with high loads of protein-rich material could lead to process instability linked to ammonia inhibition (Chen et al., 2008). Ammonia is produced during the step of acidogenesis when amino acids are degraded and have been reported to be toxic for methanogens at concentrations above 3000 mg $L^{-1}$ (Chen et al., 2008; Appels et al., 2008). Ammonium ions ($NH_4^+$) and free ammonia ($NH_3$) are the two primary forms of inorganic nitrogen present in equilibrium. Free ammonia can pass through the cell membrane of the microorganisms, causing an intracellular imbalance (Chen et al., 2008; Appels et al., 2008). Ammonia concentrations above 4000 mg $L^{-1}$ have also shown to affect the degradation of cellulase (Sun et al., 2016; Liu et al., 2017) and could thus potentially also affect the pathway and activity of cellulase. The concentration at which ammonia is inhibitory to the microorganisms varies depending on several parameters, such as pH, temperature, acclimation, substrate composition, and the origin of inoculum. A total ammonia nitrogen concentration ranging between 1,7 to 14 g $L^{-1}$ have been reported to reduce the methane production by around 50% (Chen et al., 2008).

Several strategies to remove or counteract ammonia inhibition exist, for example pH and temperature adjustment, alteration of carbon to nitrogen (C/N) ratio through co-digestion, membrane distillation, ion exchange, and adsorption (Gupta et al., 2015; Huang et al., 2018; Mutegoa et al., 2020). However, most of these strategies have drawbacks (see Table 1; Mutegoa et al., 2020). Thus, it is still of great interest to find a method or technique that is viable for an AD process while still being efficient at removing ammonia as well as being cost-effective.

***Table 1:*** *Techniques and their appropriate conditions for the operation of AD system and disadvantages for ammonia removal. Table adapted from Mutegoa et al., 2020.*

| Technique | Appropriate conditions | Disadvantages |
|---|---|---|
| pH and temperature adjustment | • pH between 7.0-7.5<br>• Temperature between 35°C-45°C | • Low pH increases volatile fatty acids production rate.<br>• High pH induces ammonia. |
| Co-digestion | • C/N ratio between 15-30 | • High C/N ratio increases accumulation of volatile fatty acids. |
| Air stripping | • Temperature above 45°C<br>• pH between 8.8-10.2 | • High construction cost.<br>• Insufficient at ammonia concentrations above 100 mg $L^{-1}$.<br>• Process is time consuming. |
| Membrane distillation | • Temperature above 40°C | • Expensive.<br>• Short lifespan of membranes. |
| Ion exchange | • pH between 6.0-7.0 | • Quickly saturated.<br>• Low performance. |
| Adsorption | • pH between 3.0-8.0 | • Adsorbent needs to be replaced after a while. |

Adsorption of ammonia or ammonium onto an adsorbent is fairly new technique to be used to reduce the ammonia concentration in an AD (Mutegoa et al., 2020) and have earlier been applied on wastewater streams (Gupta et al., 2015; Huang et al., 2018). Agricultural residues and plant materials are a type of adsorbent which have been studied to remove ammonia from aqueous solutions and have shown promising results at a low cost. The cellulose fibres from these residues and materials are negatively charged (Horvath 2006) and can thus adsorb cations such as ammonium ions. However, the studies on cellulose fibres adsorption capacity have only been conducted at ammonia concentrations below 100 mg $L^{-1}$ (Mutegoa et al., 2020; Jellali et al., 2010, Wahab et al., 2011). Thus, more studies are necessary at ammonia concentrations closer to that in an AD system to understand the full potential and capacity of cellulose fibres' adsorption capacity.

Using cellulose fibres as an adsorbent in an AD process could prove beneficial in two ways: reduce the concentration of ammonium and subsequently ammonia through adsorption (Jellali et al., 2011; Mutegoa et al., 2020); and/or boost the methane production by being degraded into organic matter (Ekstrand et al., 2020). Furthermore, organic fibres for the use as an adsorbent could be easily accessible from the waste generated by pulp- and paper

making industry. Thus, using pulp- and paper fibres could potentially both stabilize an AD process affected by ammonia inhibition and make use of the waste generated in the pulp- and paper making process.

## 1.2 Aim and objectives

The aim of this project was to investigate and evaluate the adsorption capacity of fibres acquired from the pulp- and paper industry. By evaluating the adsorption capacity, it is possible to determine if pulp- and paper fibre is a feasible material for the purpose of counteracting ammonia inhibition in AD. Furthermore, it was investigated what effect pulp- and paper fibres had on the methane production and cellulase activity in a small-scale batch process (biochemical methane potential test) with a high ammonium concentration.

The research questions that were investigated were the following:

1. How does different parameters, e.g. initial ammonia concentration, fibre dosage, temperature, and contact time affect the adsorption capacity of the fibres?
2. What is the adsorption capacity of the different fibres in an ammonium solution compared to in digester fluid?
3. How does the different fibre types affect the How does a high ammonium concentration affect the activity of cellulase in anaerobic digestion?
4. process of anaerobic digestion with regards to methane production?

## 1.3 Boundary conditions and limitations

Fibres were only collected from two different pulping processes: thermo-mechanical pulping (TMP), and chemical kraft pulping. Two types of TMP fibres were investigated (unbleached softwood and bleached softwood) as well as two types of kraft fibres (bleached hardwood and bleached softwood). How these fibres differ from each other is explained in greater detail in Chapter 2.4, but these two pulping processes were mainly chosen due to them seemingly having the highest surface charge (Horvath 2006) and thus potentially a greater capacity for adsorption of ammonium ion.

One major boundary of this project was the restrictions followed by the Covid-19 pandemic which limited the laboratory-based activities. This impacted the original project plan and revised time schedule for the experimental work. This limited experiments with Kraft SW and unbleached TMP in digester fluid and the effect of initial pH for each type of fibre. In addition, the analysis of the fibres metal content was restricted to two fibre types.

# 2. Background

## 2.1 Anaerobic digestion

Anaerobic digestion is the process in which microorganisms, primarily bacteria and archaea, degrade and convert organic material in an oxygen-free environment into biogas and digestate. Biogas is the main product with a methane content generally between 50-70% (Angelidaki et al., 2011) which has an energy content of 21-25 MJ/$m^3$ (Appels et al., 2008). Methane can subsequently be used in several applications such as to generate electricity and heat through a combined heat and power unit, or be upgraded and used as vehicle fuel, or put in a gas grid for municipal use (Schnürer et al., 2018). Furthermore, the digestate contains nutrients such as carbon, nitrogen, and phosphorus and is therefore appealing when used as a bio-fertilizer to increase crop yields. Overall, the production of biogas via AD is highly sustainable since it provides an alternative to fossil fuel while at the same time contributing to the circular economy.

### 2.1.1 Anaerobic degradation pathways

AD is a complex process which is highly dependent on the microorganisms being in balance and working in unison to achieve process stability (Mosbæk et al., 2016). The process can generally be divided into four main stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (see Figure 1).

In the first step, hydrolysis, complex organic materials, such as proteins, lipids, and carbohydrates, are hydrolyzed into smaller soluble oligomers and monomers, such as monosaccharides, amino acids, and fatty acids (Schnürer et al., 2018). The hydrolyzation is done by extracellular hydrolytic enzymes which are secreted by hydrolytic bacteria (Merlin Christy, et al., 2014). Different materials are degraded at different rates during hydrolysis. The degradation of proteins depends on its solubility, whereas the more it is soluble the easier it is to degrade. The degradation rate of lipids is in contrast dependent of the particle size and environmental parameters such as pH (Angelidaki et al., 2011). Carbohydrates exist in various forms which are all degraded at different rates. Sugars and disaccharides are broken down easily and quick, while polysaccharides, such as cellulose and hemicellulose, are more difficult to degrade (Schnürer et al., 2018). Overall, degradation of proteins and lipids are faster than carbohydrates. Therefore, if substrates which contain a lot of cellulose (e.g. plant residues or manure) are used, the hydrolysis can be the rate-limiting step of AD (Schnürer et al., 2018). Therefore, substrates generally go through some sort of pre-treatment to accelerate the hydrolysis step (Karuppiah and Azariah 2019).

Acidogenesis, also known as fermentation, is the second step where the products from the hydrolysis are further degraded through different oxidative pathways. Several different intermediary products are produced, such as organic acids (butyric acid and other volatile fatty acids), alcohols, and ammonia, as well as hydrogen, carbon dioxide, and acetic acid (Merlin Christy et al., 2014; Angelidaki et al., 2011).

Acetogenesis is the third step of the AD process, where acetate is the main product of interest. Acetate can either be formed from organic acids and alcohols by hydrogen-producing acetogens, or through the reduction of carbon dioxide by hydrogen-utilizing acetogens (Merlin Christy et al., 2014; Angelidaki et al., 2011).

Methanogenesis is the last step where methane is produced primarily through two different pathways: the hydrogenotrophic and acetoclastic pathway. In the hydrogenotrophic pathway carbon dioxide is reduced using either hydrogen or formate as an electron donor to produce methane, water, and energy (Merlin Christy et al., 2014; Rönsch et al., 2016; Strobel et al., 2020). In the acetoclastic pathway, acetate is cleaved into methane, carbon dioxide, and energy (Merlin Christy et al., 2014; Ferry et al., 1992). The final product of the methanogenesis is biogas consisting of methane and carbon dioxide, as well as water, nitrogen, ammonia, and hydrogen sulfide. Methanogenesis could, like hydrolysis, be a rate-limiting step of the AD process due to methanogens having a slow growth rate and being sensitive to environmental changes (Angelidaki et al., 2011; Schnürer et al., 2016).

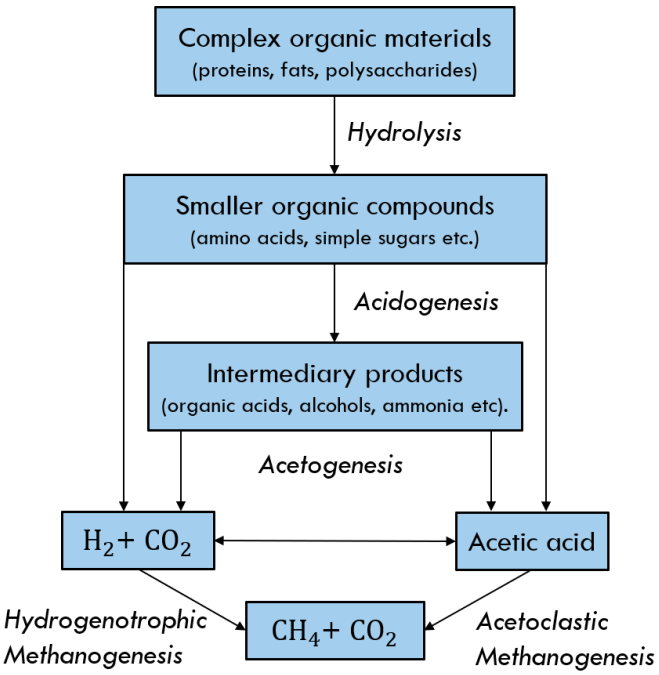


***Figure 1**: The degradation and conversion process of complex organic material into methane through the anaerobic digestion. The process is divided into the four main steps: Hydrolysis, acidogenesis, acetogenesis and methanogenesis (which are divided into the hydrogenotrophic and acetoclastic pathway). Flowchart adapted from Schnürer et al., 2018, with modifications.*

### 2.1.2 Substrates

Several sources of organic matter exist which can be used as substrate in AD: food waste from homes and restaurants; slaughterhouse waste; agricultural waste; sludge from wastewater treatment; energy crops etc. (Weiland et al., 2010; Appels et al., 2008). The composition and content of proteins, lipids and carbohydrates in the substrate used in the AD is an important

factor that can affect the process performance and stability (Schnürer et al., 2016). Lipids have the highest theoretical biogas potential of 1.014 L $g^{-1}$ $VS^{-1}$ with a methane content of 70% while proteins and carbohydrates have a theoretical biogas potential of 0.495 L $g^{-1}$ $VS^{-1}$ and 0.415 L $g^{-1}$ $VS^{-1}$ respectively with a methane content of 50% (Angelidaki et al., 2011). However, an abundance of either organic source can lead to different bottlenecks in the AD which can either inhibit or stop the process. An abundance of lipids has shown to induce foaming and are degraded into long-chain fatty acids (LCFA) which have inhibiting effects towards the microorganisms. When an abundance of protein is degraded, high levels of ammonia are normally formed which have deleterious effects on the methanogens. Similarly, an abundance of carbohydrates can lead to increased levels of VFAs which have inhibiting effects, such as acidification (Schnürer et al., 2016). The Carbon to nitrogen (C/N) ratio is a parameter which represents how much carbon that is present per mass unit nitrogen in the substrate (Mao et al., 2015). Different microorganisms have different performance optima (Mao et al., 2019). However, the general optimum of C/N ratio for an AD process has been shown to be between 20-30 (Mao et al., 2015). A too high C/N ratio can lead to the microorganisms having a nitrogen deficiency which reduces the biogas production. A low C/N ratio can instead lead to an accumulation of ammonia, inducing inhibition of the methanogens (Mao et al., 2015).

### 2.1.3 Operational parameters

There are several other important process parameters that are normally monitored to ensure a functioning AD process which includes pH, temperature, organic loading rate (OLR), and hydraulic retention time (HRT; Kleerebezem et al., 2015). The AD process generally operates at a pH between 6.8 and 8.5 since most methanogens function well in that interval (Schnürer et al., 2016, Mao et al., 2015). The pH of the digester mainly depends on the substrate composition as well as intermediary products such as VFA and ammonia (Schnürer et al., 2018). The OLR represents how much organic material, or volatile solids (VS), that is added per liter per day. The OLR is usually between 2-5 g VS $L^{-1}$ (Schnürer et al., 2016), and a too high OLR can lead to inhibition through an accumulation of VFA, ammonia or LCFA depending on the substrate. The temperature of the digester should be kept at a constant to ensure a stable process as high fluctuations can have deleterious effects on the microorganisms. AD is normally performed at a mesophilic temperature (37-42°C) or thermophilic temperature (50-55°C). A thermophilic system has faster reaction rates due to the substrates being more soluble at higher temperatures and can thus operate at a higher OLR and lower HRT. However, a high temperature and OLR still increase the risk of accumulation of ammonia which can inhibit the system. In addition, a thermophilic system generally has less diverse microflora than a mesophilic system (Schnürer et al., 2018), making it more sensitive to environmental fluctuations (Schnürer et al., 2018; Appels et al., 2008). Thus, a mesophilic system is more stable and less sensitive to temperature fluctuations but generally has a slower methane production (Mao et al., 2015). The HRT is the average time the organic material resides in the digester to be degraded and is typically between 15-30 days for a mesophilic system and 10-

20 days for a thermophilic system (Angelidaki et al., 2011). A too low HRT can lead to a washout of microorganisms and undigested organic material, resulting in loss of biogas.

Monitoring of these parameters is crucial for a stable and sufficient AD process, and a neglection of any could potentially lead to process instability and inhibition. The focus of this project will be on the inhibition which occurs due to an accumulation of ammonia.

## 2.2 Ammonia inhibition

Ammonia is one of the major inhibitors of AD and is naturally produced by the degradation of nitrogenous material, such as proteins, throughout the AD process. Organic wastes with high levels of nitrogenous matter and ammonia concentrations are for example animal manure, food wastes, and organic fractions originating from potato starch, dairy, and seafood processing industries (Calli et al., 2004). Substrates with a low C/N ratio increases the risk of ammonia inhibition.

Ammonia ($NH_3$) and ammonium ($NH_4^+$) are the two primary forms of inorganic nitrogen present in the digester, whereas ammonia is the most deleterious. Several mechanisms for ammonia inhibition have been suggested, such as ammonia causing intracellular changes in pH, proton imbalance, potassium deficiency, and inhibiting specific methane synthesizing enzymes (Chen et al., 2008; Westerholm et al., 2016; Appels et al., 2008). In addition, it has been noted in studies by Sun et al. (2016) and Liu et al. (2017) that the degradation of cellulose was significantly reduced, taking up to 83% longer for the microorganisms to degrade 50% of the cellulose substrate in digesters with a total ammonia nitrogen (TAN) concentration above 4.6 g $L^{-1}$. Similar results were noted in a study by Fischer et al. (2019) where it was also shown that hydrolytic bacteria responsible for the hydrolysis were reduced by up to 75% at a TAN concentration of 8 g $L^{-1}$. However, methanogenesis is thought to be the step primarily affected by ammonia inhibition due to the methanogens being the most vulnerable (Chen et al., 2008; Appels et al., 2008). The acetoclastic methanogens have shown to be more sensitive than hydrogenotrophic, but the concentration at which ammonia is inhibitory differs heavily on the type of system and different parameters. Several studies have reported an inhibiting concentration of TAN, i.e. the concentration of both free ammonia and ammonium ions, ranging between 1.7 to 14 g $L^{-1}$ which caused around a 50% reduction in methane production (Chen et al., 2008). The significant difference of inhibiting TAN concentration can be attributed to the differences in substrate compositions, pH, temperature, acclimation of the microorganisms, and the initial TAN concentration of the inoculum. However, several studies have also suggested that the process is completely inhibited at TAN concentrations above 3000 mg $L^{-1}$ (Rajagopal et al., 2013), and thus a general threshold of TAN is often stated as 2000-3000 mg $L^{-1}$ at 35-38°C and pH 7.6-7.9 (Westerholm et al., 2016).

### 2.2.1 Factors controlling ammonia inhibition

Ammonia and ammonium are in an equilibrium which is regulated by pH and temperature according to Equation 1 (Westerholm et al., 2016). Therefore, an increase in temperature and pH shifts the equilibrium towards ammonia, leading to an even greater ammonia inhibition

due to an increase in ammonia concentration. Thus, thermophilic systems are more prone to be inhibited due to ammonia compared to a mesophilic system. However, the resulting process instability often causes increased levels of VFA which lowers the pH, consequently shifting the equilibrium back towards ammonium, reducing the ammonia inhibition. The result is a stable process but with a reduced methane yield (Appels et al., 2008).

$$NH_3\text{-}N = \frac{TAN}{1+10^{0.09018+\frac{2729.92}{T}-pH}} \quad (1)$$

TAN = total ammonia nitrogen ($NH_4^+ + NH_3$)
T = temperature in kelvin

Acclimation, i.e. slowly adapting the microorganism to an increased ammonia concentration, also plays a role at which TAN concentration the process gets inhibited (Appels et al., 2008; Chen et al., 2008; Calli et al., 2004). Studies have shown that the methane production was completely inhibited at a TAN of 3000 mg $L^{-1}$ when using digester sludge typically below 1000 mg $L^{-1}$, while digester sludge that had been acclimated to 2400 mg $L^{-1}$ did not show any signs of inhibition at a TAN of 5000 mg $L^{-1}$ (Calli et al., 2004). This could be due to intracellular changes or a shift in the methanogenic population (Chen et al., 2008).

### 2.2.2 Methods counteracting ammonia inhibition

Various strategies which aim to counteract or alleviate ammonia inhibition exists, which can be categorized into three groups: optimization of substrate and operational parameters and properties, physical-chemical processes, and application of various additives (ion exchange, adsorption; Gupta et al., 2015; Huang et al., 2018; Mutegoa et al., 2020).

The most fundamental parameters to optimize for removal of ammonia are pH, temperature, and C/N ratio. As mentioned above, lowering the pH and temperature shifts the equilibrium of ammonia and ammonium towards ammonium, subsequently lowering the toxic concentration of ammonia. An increase of the C/N ratio also reduces excess nitrogen which reduces the risk of accumulation of ammonia (Chen et al., 2008). However, it is still important to operate the digester at appropriate conditions to ensure a stable process.

Air stripping and membrane distillation are two types of physical-chemical process which aims to reduce the TAN concentration of the substrate, i.e. a pre-treatment. Air stripping, most typically used on wastewater, is a technique that separates volatile ammonia from the wastewater by increasing the surface area of the wastewater exposed to air (Mutegoa et al., 2020). In contrast, the process of membrane distillation makes use of a hydrophobic microporous membrane to remove ammonia from either a liquid or gas phase (Mutegoa et al., 2020).

The method of using additives to remove ammonia are generally attributed to ion exchange and adsorption, in which they both aim to alleviate the ammonia inhibition by removing ammonium (Gupta et al., 2015; Huang et al., 2018). The addition of adsorbents or ion exchangers have shown to be simple, reliable, and economically feasible, however with a low performance (Mutegoa et al., 2020).

## 2.3 Adsorption

Adsorption is a process in which an atom, ion, or molecule, also called adsorbate, adheres to the surface of another material, also called an adsorbent (Kecili et al., 2018; Figure 2). The process is exothermic and depending on the interactions between the adsorbent and the adsorbate, two different processes can occur: physical adsorption (also called physisorption), or chemical adsorption (also called chemisorption). Electrostatic interactions and Van der Waals forces are involved in physical adsorption, while strong covalent bonds are formed in chemical adsorption (Kecili et al., 2018). Generally, physical adsorption is fast and reversible while not very specific, and usually forms a multilayer of the adsorbate on the surface. By contrast, chemical adsorption is slower, irreversible, more specific, and only forms a monolayer on the surface. Parameters that are believed to affect the adsorption efficiency includes initial ammonium concentration, adsorbent dosage, surface area of adsorbent, and pH and temperature of the solution (Huang et al., 2018).

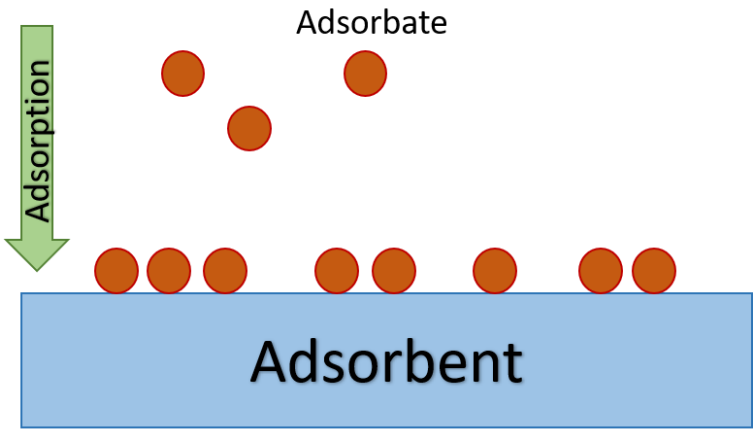


***Figure 2:*** *Schematic depiction of the adsorption process between an adsorbate and adsorbent. Adapted from Kecili et al., 2018.*

### 2.3.1 Adsorbents

The most studied and commonly used adsorbent for removal of ammonium is different types of zeolites, which are porous and have a large surface area (Gupta et al., 2015; Huang et al., 2018). Both natural and synthetic zeolites can be accessed in abundance, resulting in a low cost. However, they exhibit a fairly low adsorption in the range from 37 to 192 mg $g^{-1}$ ($NH_4$-N) (Mutegoa et al., 2020) and are thus mainly applicated in wastewater streams (Gupta et al., 2015; Huang et al., 2018). Consequently, surface modifications have been studied to improve

the adsorption capacity of adsorbents, and activated carbon showed improved adsorption after being modified with nitric acid (Mutegoa et al., 2020).

Agricultural residues and plant materials are other materials that have been studied for the purpose of removing ammonium from aqueous solutions. Cellulose fibres have an abundance of carboxyl and hydroxyl groups, making it negatively charged and thus able to form interactions with ammonium (Huang et al., 2018; Horvath 2006; Jansson 2015). Several studies have demonstrated that cellulose fibres are able to adsorb ammonium (Yusof et al., 2010; Jellali et al., 2011; Liu et al., 2010; Zhu et al., 2016), although with a low adsorption capacity ranging between 1.8 to 42.4 mg $g^{-1}$ (see Table 2).

***Table 2**: Adsorption capacity and other parameters for ammonium removal by plant residues.*

| Adsorbent | Adsorption capacity (mg/g) | TAN concentration (mg/L) | Contact time | pH | Temperature (°C) | Ref. |
|---|---|---|---|---|---|---|
| Synthesised zeolite from rice husk ash | 42.4 | 50 | 1.5 h | 4-6 | Room temperature | Yusof et al. (2010) |
| P. oceanica fibers | 1.8 | 10-50 | 30 min | 6-10 | Room temperature | Jellali et al. (2011) |
| Boston ivy leaves | 6.7 | 25, 50 | 18 h | - | 30 | Liu et al. (2010) |
| Phoenix tree leaves | 4.6 | 25, 50 | 18 h | - | 30 | Liu et al. (2010) |
| Acid activated avocado seeds | 5.4 | 50-450 | - | 5 | 25 | Zhu et al. (2016) |

## 2.4 Fibres from the pulp- and paper making process

The wood fibres used in the making of paper mainly consist of cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are different polysaccharides (i.e. carbohydrates) that are aligned together in sheets, while lignin is a hydrophobic polymer which acts as a glue, giving the structure stiffness (Horvath, 2006; Jansson, 2015). Wood fibres can be divided into two groups: Softwood (SW) and hardwood (HW). SW generally consists of around 44% cellulose, 25% hemicellulose, and 30% lignin, while HW consists of around 44% cellulose, 34% hemicellulose, and 20% lignin (Jansson, 2015). The carboxyl groups, which are mainly bound to xylan (type of hemicellulose), are what gives wood fibres its negative surface charge, and subsequently, HW has a higher surface charge density due to a higher hemicellulose content (Horvath 2006).

### 2.4.1 Pulping processes

The process of pulp- and papermaking can be divided into four main steps: debarking/wood chipping, pulping, bleaching, and papermaking (see Figure 3). In the first step, HW and SW are broken down mechanically into smaller pieces, either mixed or individually. The second step, pulping, can either be done mechanically or chemically. The most common mechanical pulping technique is thermo-mechanical pulping (TMP) where the wood chips are refined into pulp under elevated pressure and heat (Ekstrand, 2019). The most common chemical pulping technique is the kraft pulping process which involves cooking of the wood chips with sodium hydroxide and sodium sulphide at high temperature and pressure (Jansson, 2015; Ekstrand, 2019). Lignin and hemicellulose are degraded and dissolved from the fibre walls during the cooking process, resulting in a decrease of carboxylic groups and subsequently lowering the surface charge density (Horvath, 2006; Jansson, 2015). Mechanical pulping generally yields a higher ratio of wood to pulp (85-95%) than that of chemical (40-55%) due to the preservation of lignin and hemicellulose (Ekstrand, 2019). The remaining pulp from the mechanical or chemical process can then further be bleached to reduce the lignin content and brighten the fibres. Mechanical pulp is bleached using peroxide or sodium dithionite. In contrast, chemical pulp is either bleached with chlorine dioxide followed by alkaline extraction (elemental chlorine-free bleaching, ECF), or a mixture of oxygen, ozone and peroxide (total chlorine free bleaching, TCF) (Ekstrand, 2019). The remaining pulp is then diluted and fed to a paper machine which presses and form the paper sheets (Jansson, 2015).

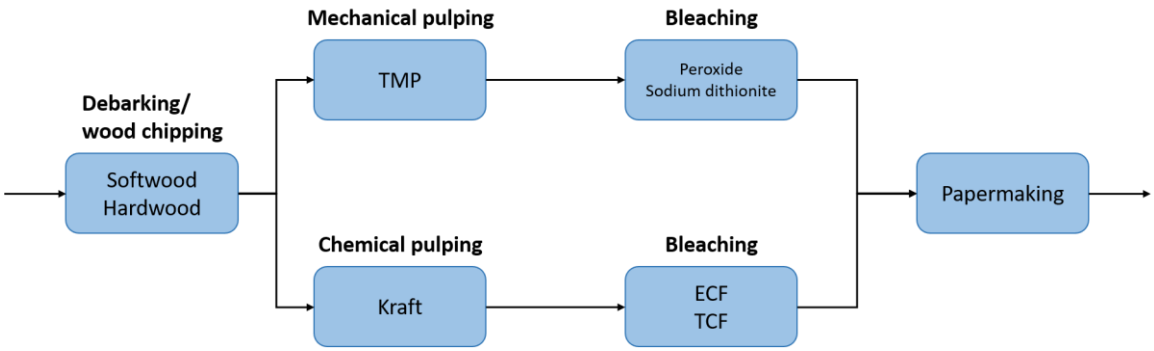


***Figure 3:*** *Schematic overview of the pulp- and papermaking process. The process is divided into four main steps: debarking/wood chipping, pulping, bleaching and papermaking. The pulping process is divided into mechanical and chemical pulping where thermo-mechanical pulping (TMP) and kraft pulping is the most common techniques for the pulping processes, respectively. These are followed by a bleaching process where mechanical pulping is bleached using peroxide and sodium dithionite, and for chemical either elemental chlorine-free bleaching (ECF) or total chlorine-free bleaching (TCF).*

### 2.4.2 Charge content

As mentioned, both wood type and pulping process affects the pulp fibre's total charge and surface charge. The charge of the fibres is typically expressed as the inverse of equivalent weight (eq/g), i.e. how many equivalent moles that can be taken up by a unit amount of a substance. For example, as the ammonium ion ($NH_4^+$) is univalent, i.e. has one charged group,

one mole of ammonium would be equal to 1 eq. The total charge and surface charge of unbleached SW and HW which has gone through either a kraft or TMP pulping process has been estimated in a previous study and is summarized in Table 3 (Horvath 2006). In this study, HW had a higher total and surface charge compared to SW when processed in a kraft pulping process. On the other hand, the TMP process yielded a fibre with a lower total charge but higher surface charge. This is because large amounts of fine material are released during mechanical pulping which has a higher specific surface area, resulting in a higher surface charge (Horvath, 2006).

***Table 3*** *Charge profiles for different types of unbleached pulp from various mills in Sweden. Table and results from Horvath (2006).*

| Company/Mill | Wood type | Pulping process | Total charge (µeq/g) | Surface charge (µeq/g) |
|---|---|---|---|---|
| **Stora Enso/ Skoghall** | SW | Kraft | 107 | 3.4 |
| **M-real/ Husum** | HW | Kraft | 137 | 6.3 |
| **Holmen/ Hallsta** | SW | TMP | 83 | 11 |

# 3. Material and methods

## 3.1 Cellulosic fibres

ECF bleached kraft HW and SW fibre was acquired from BillerudKorsnäs, while the unbleached and bleached TMP SW fibre were acquired from Holmen paper. All samples were collected directly from the product line. The fibre was stored in a refrigerator at 5±1°C for three weeks during the preparation and was then stored in a freezer at -20±2°C until further sampling was necessary.

### 3.1.1 Washing of the fibres

Previous studies conducted by the Department of Thematic Studies at Linköping University have shown that similar woody material (kraft mill pulp) have had a high content of calcium (data not published). Therefore, the fibres were washed with Milli-Q followed by washing with ethylenediaminetetraacetic acid (EDTA; tetrasodium salt dihydrate 99%, Acros Organics) to remove metals that might be present (Ekstrand et al., 2020). A wet weight of 8 g for each fibre sample was suspended in 160 mL of Milli-Q and placed on an Infors HT platform shaker at 200 rpm for 1 h. Afterwards, the samples were centrifuged two times at 17000×g for 20 min at 20°C in an Avanti® J-E high-speed centrifuge using a JA-10 rotor (Beckman Coulter). The supernatant was removed after each centrifugation. After the centrifugation, the fibre

samples still had a high visible water content, and since drying the fibres has shown to cause irreversible changes to the surface structure (Taherzadeh et al., 2008), a belt press simulator with a force gauge (Crown press, Model SP-2689, Phipps & Bird, USA) was used to further increase the TS of the samples. The content of one centrifuge flask was pressed with a force of 100 kgF for 30 sec followed by 150 kgF for 45 sec. The resulting fibre samples were stored in a refrigerator at 10±1°C until further analysis or preparation.

As for the metal extraction with EDTA, a wet weight of 10 g of the fibres treated with Milli-Q was re-suspended in 150 mL EDTA at a concentration of 44-181 mg EDTA $mg^{-1}$ fibre $L^{-1}$ (1,68 mol EDTA $mol^{-1}$ metal complex) based on the metal content analysis of untreated fibre samples (see section 3.1.3) and placed on the platform shaker at 200 rpm for 1 h. The samples were centrifuged at 17000×g for 15 min at 20°C and the supernatant was removed. The pellets were re-suspended in 150 mL Milli-Q to wash off any remaining EDTA and centrifuged two additional times at 17000×g for 15 min at 20°C. The pellets were then pressed once more in the belt press as previously, before being stored in a refrigerator at 10±1°C until further analyses.

### 3.1.2 Total solids and volatile solids

The total solids (TS) and volatile solids (VS) of the samples were determined according to the Swedish standard method (SS-028113). The samples were weighed before ($m_{wet}$) and after being dried at 105°C for at least 20 hours ($m_{105°C}$). The TS was acquired through Equation 2. The samples were then weighed again after being combusted at 550°C for 2 hours ($m_{550°C}$) in an oven, after which the VS was acquired through Equation 3.

$$\text{TS}\ (\%) = \frac{m_{105°C}}{m_{wet}} * 100 \quad (2)$$

$$\text{VS}\ (\%\ \text{of TS}) = \frac{m_{105°C} - m_{550°C}}{m_{105°C}} * 100 \quad (3)$$

### 3.1.3 Metal content determination

The metal content was determined by ALS Scandinavia AB with an ICP-SFMS according to SS-EN ISO 17294-2:2016 and US EPA Method 200.8:1994. The data was acquired with a confidence interval of 95%. The analysis was performed on untreated samples, samples which had been washed with Milli-Q, and samples which had been treated with EDTA. However, due to the analysis being costly, only Kraft HW and unbleached TMP were analysed. In addition, no analysis on unbleached TMP washed with Milli-Q was performed. 1 g TS was sent for analysis, and the results were given in the unit of mg $kg^{-1}$ $TS^{-1}$ fibre.

## 3.2 Ammonium adsorption experiments

The adsorptions experiments consisted of analysing the adsorption capacity of the different fibres in an aqueous ammonium solution and digester fluid, respectively. The experiments

were conducted to investigate the effect ammonium concentration, fibre dosage, temperature, and contact time had on the adsorption capacity.

### 3.2.1 Preparation of ammonium solution

Synthetic ammonium solutions were used throughout the experiments. Stock solutions with a concentration of 1000 mg $L^{-1}$, 3000 mg $L^{-1}$, or 5000 mg $L^{-1}$, were prepared before each experiment by dissolving ammonium chloride ($NH_4Cl$, VWR chemicals) in Milli-Q. The experimental solutions were then prepared by diluting the stock solution with Milli-Q to the desired ammonium concentrations.

### 3.2.2 Digester fluid

Digester fluid was acquired from a full-scale co-digestion plant using food waste and slaughterhouse waste as substrate (Tekniska Verken (TV), Linköping, Sweden) and a lab-scale reactor operating on chicken and cow manure as substrate (Scandinavian Biogas Fuels (SBF), Linköping, Sweden). The digester fluid from TV and SBF had a TAN concentration of 2700 mg $L^{-1}$ and 3800 mg $L^{-1}$, respectively. Therefore, no additional ammonium was added. Two out of the three experiments involving digester fluid was conducted the same day as the digester fluid was extracted. The third experiment was conducted the following day, after which the digester fluid had been stored in a refrigerator at 10±1°C overnight.

### 3.2.3 Experimental setup of ammonium adsorption

The adsorption experiments were performed in two stages: Preliminary and Main experiment (Figure 4). The preliminary experiments were conducted to mainly test and evaluate the method of adsorption. These tests were done with fibres that had only been treated with Milli-Q. The main experiments were performed using the resulting method derived after the preliminary experiments, and was done with fibres that had been treated with EDTA.

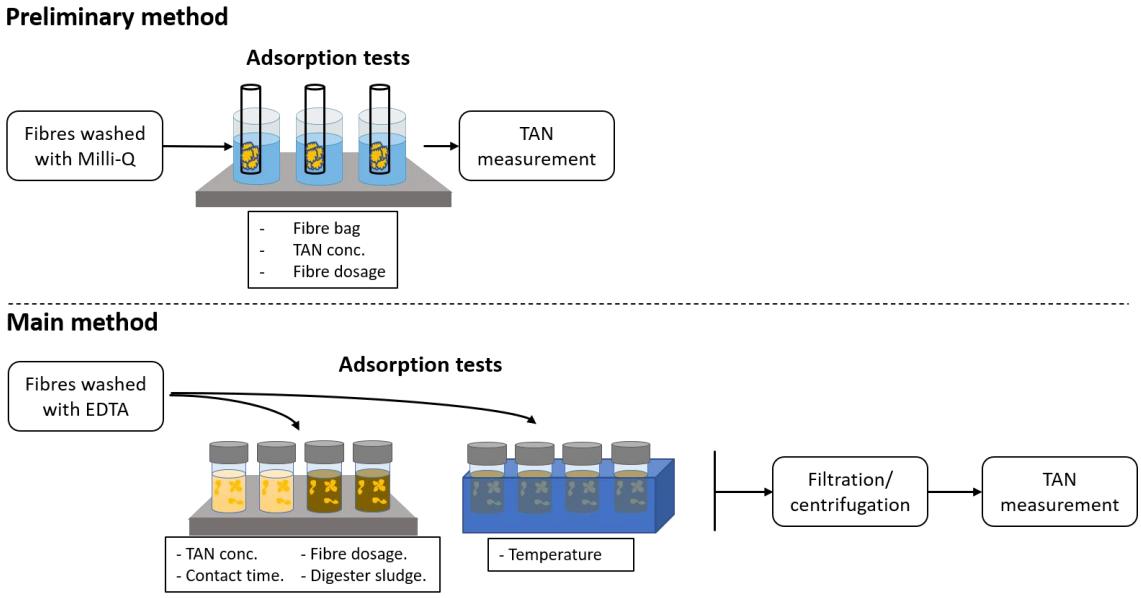


***Figure 4:*** *Schematic illustration of the preliminary and main method of the ammonium adsorption tests. The main method was derived from the preliminary method.*

#### 3.2.3.1 Preliminary experiment

The initial setup consisted of batch tests which were conducted in 250 mL beakers with a liquid volume of 100 mL. Polyester fibre bags with a pore size of 30 µm were used to submerge fibre dosages of 4, 5 and 6 g TS $L^{-1}$ in ammonium solutions with a concentration of 60, 100 and 150 mg $L^{-1}$. Each sample was stirred for 40 min at 200 rpm using a Variomag telemodul 20p magnetic stirrer at room temperature (21±1 °C). A blank control was carried out for each experiment, consisting of ammonium solutions without any fibres. The pH of each sample was measured at the start and end of the experiments with an inoLab 7310 pH meter with a HAM Polilyte Bridge Lab pH electrode which was calibrated once every second week with buffer solutions (pH 4, 7, and 8). The TAN content of each sample was measured at the end of the experiments according to section 3.2.4.

#### 3.2.3.2 Main experiment

These tests were all done as triplicates in 100 mL capped flask bottles with a liquid volume of 80 mL. The fibres were added to the bottles directly without the use of fibre bags, i.e. the fibres were homogeneously mixed with the solution. Each sample was stirred the same way for 40 min as the preliminary experiment, and a blank control was carried out for each test. In addition, a control was carried out in which each fibre type was added to Milli-Q to see if they contained any free nitrogen. pH and TAN content were measured the same way, but since the fibres were homogeneously mixed with the solution, the samples were either filtered or centrifuged before the TAN analysis (see section 3.2.4).

##### Ammonium concentration

The effect that the ammonium concentration had on the adsorption capacity was estimated using ammonium solutions with a TAN concentration of 100 mg $L^{-1}$, 1000 mg $L^{-1}$, and 3000 mg $L^{-1}$, as well as in digester fluid from SBF with a TAN concentration of 3800 mg $L^{-1}$. A set fibre dosage of 5 g $L^{-1}$ was added to respective solutions. The experiments in the ammonium solution were carried out for each fibre type, while for the digester fluid only kraft HW and bleached TMP was tested.

##### Fibre dosage

The effect that the fibre dosage had on the adsorption capacity was conducted using fibre dosages of 3, 4, 5, 6 and 7 g TS $L^{-1}$. Each fibre dosage was added to ammonium solutions with a TAN concentration of 3000 mg $L^{-1}$. The effect of fibre dosage was only conducted on kraft HW.

##### Temperature

The effect of an increased temperature was conducted using water baths (AQUAline AL 25, Lauda) at 37°C and 55°C. The tests were carried out for kraft HW and bleached TMP using a set fibre dosage of 5 g $L^{-1}$ together with digester fluid from TV which had a TAN of 2700 mg $L^{-1}$. The flasks were submerged in the water and stirred manually every 10 min for 40 min.

#### Contact time

The effect of contact time was conducted with kraft HW and bleached TMP in both an ammonium solution and digester fluid with a TAN concentration of 3000 mg $L^{-1}$ and 2700 mg $L^{-1}$, respectively. A set fibre dosage of 5 g $L^{-1}$ was used, and the TAN concentration was measured at six time points: 15, 30, 45, 60, 120, and 240 min. A volume of 1 mL was extracted from each sample at each time point and centrifuged once at 15300×g for 10 min in an Eppendorf 5417C Centrifuge before analysing the TAN content. The pH was only measured at the start and end (last time point) of the experiment due to the extracted volume of each time point being too small to measure of pH.

### 3.2.4 Total ammonium nitrogen content

The TAN concentration was determined using a Hach DR2800 spectrophotometer with a LCK 302 ammonium cuvette test kit (60-167 mg $L^{-1}$ $NH_4^+$, Hach Lange), which is based on the indophenol blue method. The TAN concentration of the control which was carried out to see if any of the fibres contained free nitrogen was determined using LCK 304 ammonium cuvette test kit (0.02-2.5 mg $L^{-1}$ $NH_4^+$, Hach Lange).

Each sample was either filtered or centrifuged before the analysis to remove the fibres from the mixture. For each test conducted in an ammonium solution, the mixture was filtered through a micro-glass fibre filter (pore size of 1.6 µm, Munktell) and adjusted to pH 7 using 0.1 M and 0.01 M NaOH solutions. The added volume of NaOH did not exceed 2% of the total sample volume. For each test conducted in digester fluid, the mixture was instead centrifuged once at 17881×g for 10 min at 20°C in an Avanti® J-E high-speed centrifuge using a JA-14.50 rotor (Beckman Coulter). Each sample was, if necessary, diluted with Milli-Q to be within the range of the test kit before analysis.

The Hach LCK 302 cuvette was tempered to 20°C before analysis. The protective aluminum foil on the cap was removed, and the cap was opened. 200 µL of the sample was added to the test kit and the cap was closed upside down, as intended. The cuvette was shaken 2-3 times and allowed to settle for 15 min at room temperature before being measured in the spectrophotometer.

### 3.2.5 Estimation of adsorbed ammonium

After each experiment, the adsorption capacity of the fibres was determined by the decrease of the ammonium concentration in the experimental solution using Equation 4,

$$\text{Adsorption capacity} = \frac{(C_c - C_t) \times V}{M} \quad (4)$$

where $C_c$ is the ammonium concentration (mg $L^{-1}$) of the control at time t, $C_t$ the ammonium concentration (mg $L^{-1}$) after the experiment at time t, V the sample volume (L) and M the mass (g) of the fibre dosage used.

## 3.3 Biochemical methane potential test

Biochemical methane potential (BMP) test is a technique used to determine the methane potential and degradability of different substrates, as well as to evaluate different operation parameters (Filer et al., 2019; Bioprocess Control., 2011). The BMP tests were more specifically used in this project to investigate how the addition of pulp fibre would affect the biogas production of a system with and without a high TAN concentration. The BMP tests were done in triplicates using an Automatic Methane Potential Test System II (AMPTS II, Bioprocess control). The experimental setup can be seen in Table 4. The tests were carried out in 500 mL bottles with a working volume of 230 mL. Digestate, with a TAN concentration of 1400 mg $L^{-1}$, acquired from TVs sewage treatment plant was used as inoculum. The inoculum was pre-incubated for 3 days prior to the initiation of the BMP tests to reduce the amount of biogas produced from the inoculum itself (Bioprocess control, 2011). 200 ml (4.2 g VS) of the inoculum was added to the bottles and an inoculum to substrate VS ratio of 2:1 was adopted. 6.9 mL of an ammonium chloride ($NH_4Cl$) solution with a TAN concentration of 65000 mg $L^{-1}$ was added to achieve a TAN concentration of 3100 mg $L^{-1}$. The tests were conducted using kraft HW and bleached TMP which was added with an OLR of 0.5 g VS (2.6 g VS $L^{-1}$). Household food waste acquired from TV's food waste collecting facility was used as the main organic source, and was stored in a freezer at -20±2°C until the initiation of the BMP tests. 1.6 g VS of the food waste was added to maintain the 2:1 inoculum to substrate VS ratio. Since pulp fibres are of organic material (i.e. can be degraded in an AD system) two controls were carried out with an OLR of 2.1 g VS (FW) and 1.6 g VS (FW2) consisting only of food waste, to be able to compare the results better. In addition, a blank control was carried out consisting entirely of inoculum (Y) to compensate for the methane produced by the inoculum, and a positive control (P) consisting of Whatman cellulose paper to ensure the microbial activity and quality of the inoculum (Bioprocess control, 2011).

***Table 4:*** *Experimental setup of the biochemical methane potential tests consisting of a positive control with Whatman cellulose paper (P), blank control (Y), control of inoculum and food waste (FW), control of inoculum and food waste at a TAN concentration of 3100 mg/L (FW-A), control of inoculum and food waste at a lower total organic loading rate (FW2), system with kraft HW fibres at a TAN concentration of 1400 mg/L (HW), system with kraft HW fibres at a TAN concentration of 3100 mg/L (HW-A), system with bleached TMP fibres at a TAN concentration of 1400 mg/L (TMP), and system with bleached TMP fibres at a TAN concentration of 3100 mg/L (TMP-A).*

| Test | Inoculum (g VS) | Food waste (g VS) | Fibre (g VS) | Final TAN conc. (mg/L) |
|---|---|---|---|---|
| **P** | 4.2 | - | 2.1 | 1400 |
| **Y** | 4.2 | - | - | 1400 |
| **FW** | 4.2 | 2.1 | - | 1400 |
| **FW-A** | 4.2 | 2.1 | - | 3100 |
| **FW2** | 4.2 | 1.6 | - | 1400 |
| **HW** | 4.2 | 1.6 | 0.5 | 1400 |
| **HW-A** | 4.2 | 1.6 | 0.5 | 3100 |
| **TMP** | 4.2 | 1.6 | 0.5 | 1400 |
| **TMP-A** | 4.2 | 1.6 | 0.5 | 3100 |

After each bottle was filled, the motor and stirrer were assembled, and the bottles were placed in a water bath (37°C) and flushed with nitrogen gas for approximately 30 sec to achieve an anaerobic condition. The system was initiated, and the stirrer was set to 200 rpm with an interval of 20 min on/off. The produced biogas passed through a carbon dioxide fixing unit and the production of the methane gas was recorded automatically using a wet gas flow measuring device. The accumulated methane gas was reported as per VS added in each sample in normalized units (Nml), i.e. at 1.0 standard atmospheric pressure, 0°C and zero moisture content to compensate for the actual pressure, temperature and moisture content registered in the device (Bioprocess control, 2011).

### 3.3.1 Enzymatic activity

The enzymatic activity during the BMP tests was analysed to see how a high TAN concentration affected the activity of cellulase and subsequently the degradation of cellulose. The activity was measured at the initiation of the BMP tests and after five days. 1 mL of digester sludge was extracted from a valve on the flasks using a syringe with a silicone tube. The extracted sample was centrifuged for 10 min at 15300×g in an Eppendorf 5417C Centrifuge. The supernatant was removed and stored in a freezer at -20±2°C until the analysis was performed. The analysis was performed by an external part using a MarkerGene™ Fluorescent Cellulase Assay Kit. The kit makes use of a cellobioside marked with the fluorescent marker resorufin, which can be measured spectrophotometrically after being cleaved by cellulase (MarkerGene™ protocol, MGT Inc.). The samples were thawed and 50 µL was added in triplicate to wells on a 96-well microtiter plate. A 0.5 mM substrate reagent solution was prepared by diluting 0.5 mM Resorufin Cellobioside (in Dimethyl sulfoxide) 1:10

in reaction buffer (100 mM sodium acetate, pH 6.0) and was added to each well (50 µL/well). Fluorescence was recorded using a BMG Clariostar plate reader, with 550 nm excitation filter and 595 nm emission filter, and readings of relative fluorescent units (RFU) were taken at 2-minute intervals for 120 minutes.

Furthermore, a blank control was carried out for each sample, as well as an autocatalysis control and positive control. The blank control consisted of 50 µl sample together with 50 µl of reaction buffer to record any background emission. The autocatalysis control instead consisted of 50 µl 0.5 mM Resorufin Cellobioside and 50 µl reaction buffer with the intent to record decomposition of the substrate into fluorescent resorufin. The positive control consisted of 50 µl cellulase (1 mg $L^{-1}$) from *Trichoderma reesei* mixed with 50 µl 0.5 mM Resorufin Cellobioside to ensure that the substrate, proportions, and equipment worked as intended.

# 4. Results and discussion

## 4.1 Preparation of fibres

The metal content analysis was carried out to determine the prevalence of metals, which could hinder the adsorption, in the fibres, and to evaluate the efficiency of the washing with Milli-Q and EDTA. The analysis conducted on Kraft HW and unbleached TMP (Figure 5) shows that the untreated fibres had a total metal content of 3400 mg $kg^{-1}$ $TS^{-1}$ and 6600 mg $kg^{-1}$ $TS^{-1}$, respectively. After being washed with Milli-Q, the metal content of Kraft SW dropped to 2700 mg $kg^{-1}$ $TS^{-1}$, a decrease of 21%. In contrast, after metal extraction with EDTA, the metal content was reduced to 700 mg $kg^{-1}$ $TS^{-1}$, a decrease of 74%. As for unbleached TMP, a metal analysis was not conducted for fibres washed with Milli-Q. However, after being washed with EDTA, the metal content was reduced to 1700 mg $kg^{-1}$ $TS^{-1}$, a reduction of 74%. Although the analysis was not conducted on unbleached TMP washed with Milli-Q, these results indicate that a metal extraction with EDTA is necessary to effectively reduce the metal content.

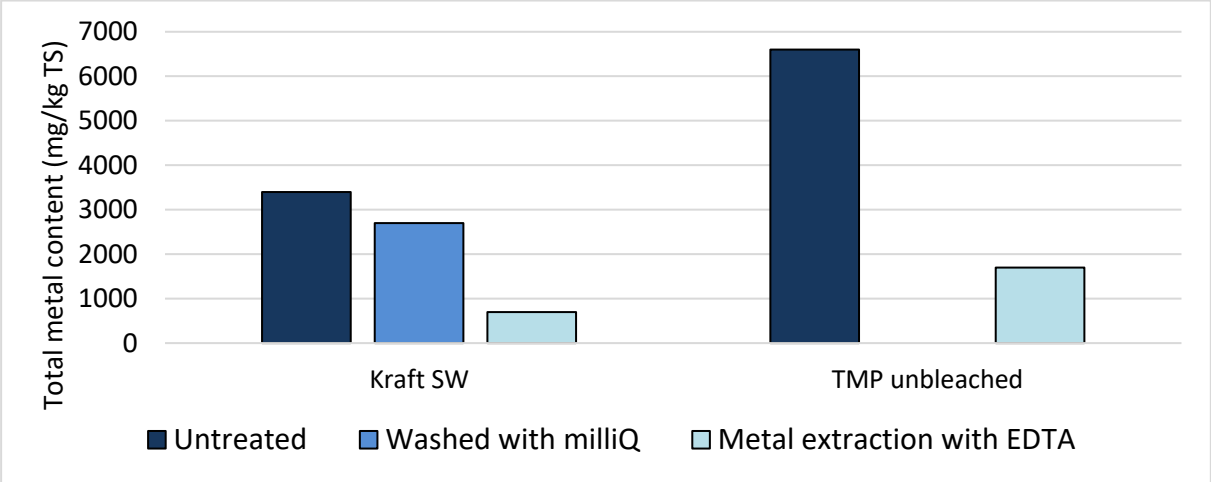


***Figure 5**: The total metal content (mg/kg TS) for Kraft SW and unbleached TMP being untreated, washed with Milli-Q, and after metal extraction with EDTA. No data available for unbleached TMP washed with Milli-Q.*

For untreated Kraft SW, the most abundant metal was sodium followed by potassium and calcium, accounting for 46%, 34% and 15% of the total metal content, respectively. For untreated unbleached TMP sodium accounted for 60%, potassium 8%, and calcium 21% (Table 5; see appendix A Table 6 for detailed metal content). Sodium's abundance in Kraft SW could be expected since sodium hydroxide and sodium sulphide are added during the pulping process, but no extra sodium is added during the pulping process of TMP (Ekstrand, 2019; Jansson, 2015). After the washing with EDTA, sodium was still the most abundant metal, accounting for 69% and 86% of the total amount of metal, respectively. However, the intention of the washing was to remove metal ions that would bind to the fibre with a greater affinity than ammonium and subsequently reduce the adsorption capacity. As ammonium ($NH_4^+$) is a monovalent cation, i.e. has a charge of 1+, metal ions which are divalent, such as $Ca^{2+}$, $Mg^{2+}$ etc., have a greater binding affinity. However, these metal ions are reduced after extraction with EDTA (Table 5). Relative binding affinities for the mentioned cations to a negative site are as follows: $Ca^{2+} > mg^{2+} > K^+ > NH_4^+ > Na^+$ (DuPont, 2019).

***Table 5:*** *The prevalence of Na, K, Ca, and Mg in Kraft HW and Unbleached TMP, before and after metal extraction with EDTA.*

| **Fibre** | **Kraft HW** | | | **Unbleached TMP** | |
|---|---|---|---|---|---|
| **Treatment** | **Untreated** | **Milli-Q** | **EDTA** | **Untreated** | **EDTA** |
| **Metal ion (mg/kg TS)** | | | | | |
| **Na** | 1540 | 818 | 450 | 4000 | 1500 |
| **K** | 1160 | 1100 | 50 | 547 | 60 |
| **Ca** | 490 | 516 | 80 | 1400 | 50 |
| **Mg** | 130 | 157 | 50 | 140 | 30 |

The acquired values for the average TS (%) and average VS of TS (%) can be seen in Figure 6. The untreated fibres had the highest TS ranging from 28% to 34% while the washed fibres had a TS of around 25%. Fibres that were washed with Milli-Q had a more similar TS while the fibres washed with EDTA had a larger variation and standard deviation. The TS of the washed fibres could most likely have been higher if they would have been dried after the centrifuge process of the preparation. Drying has however shown to irreversibly affect the surface structure of fibres (Taherzadeh et al., 2008), and was thus avoided. The standard deviation was still relatively high for Kraft SW (2.1%) and Kraft HW (2.0%) which had been treated with EDTA. Overall, the fibres were very adequate at absorbing water, making it hard to achieve completely homogeneous samples. Moreover, fibres were pressed manually in the last step of the dewatering process using a manual bench scale belt press simulator. The force applied to the fibres using the belt press was not kept stable and varied with ±5 kgF which most likely contributed to the variance in TS. This variation in TS indicates an uncertainty of exactly how much of the fibres (g TS) which was added to the adsorption experiments. The average VS of

TS (%) of the fibres was high, between 97.2%-99.8%. The VS did not change too much after each washing, with the exception of bleached TMP that went from 97.2% to 98.6% after the first wash with Milli-Q.

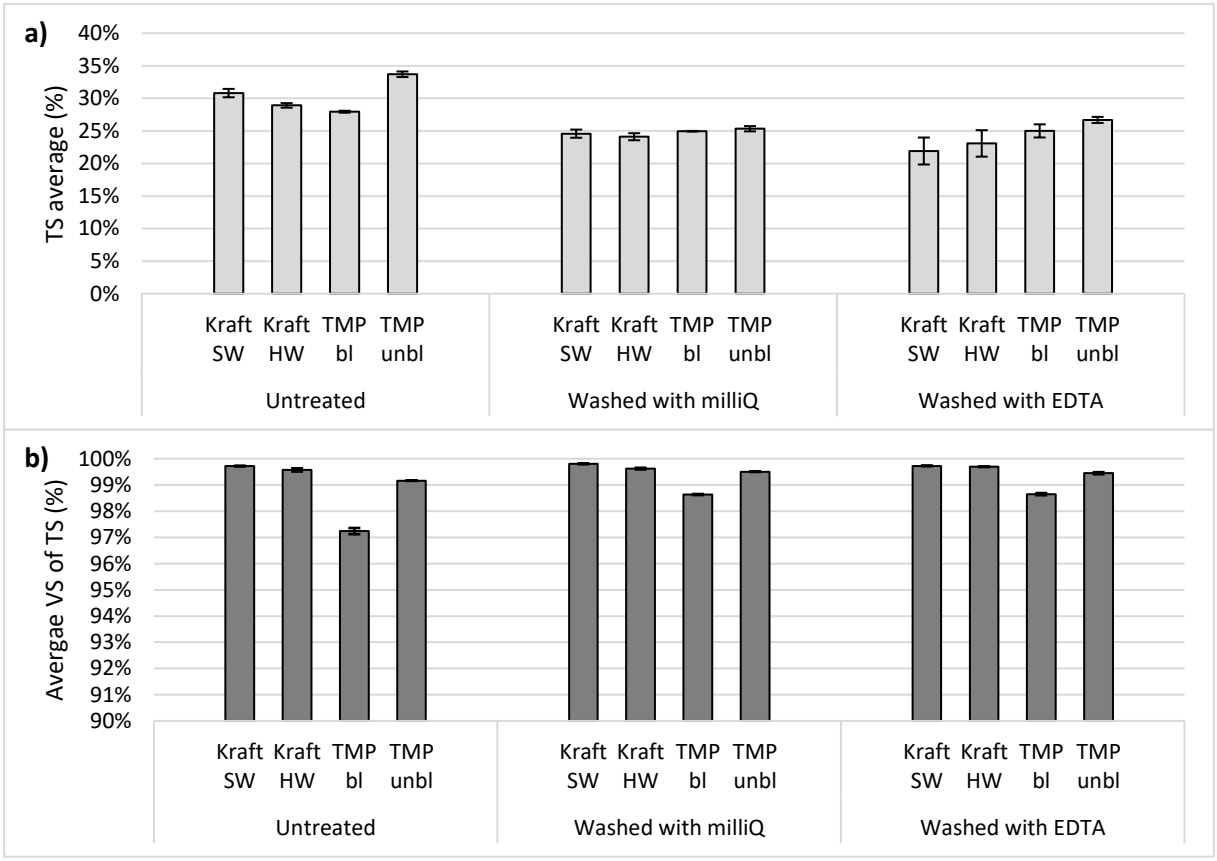


***Figure 6: a)*** *The average total solids (TS) (%) and* ***b)*** *average volatile solids (VS) of TS (%) for each fibre type (Kraft SW; Kraft HW; bleached TMP; unbleached TMP) for untreated samples, samples washed with Milli-Q, and samples washed with EDTA.*

## 4.2 Ammonium adsorption tests

### 4.2.1 Preliminary experiments

The preliminary experiments were mainly carried out as singlets with the main intent to evaluate the use of a fibre bag to submerge the fibre into the ammonium solution (Figure 7). The other tests aimed at evaluating the effect that different ammonium concentrations (Appendix B, Figure 20) had on the adsorption onto Kraft HW and the adsorption difference between Kraft HW which had been washed with Milli-Q and EDTA (Appendix B, Figure 22). However, these tests were as mentioned carried out as singlets, and they were not filtered nor centrifuged before the analysis of the TAN concentration and will thus not be presented in this section.

The adsorption capacity of Kraft HW (Figure 7.a) is clearly lower when using a fibre bag to submerge the fibres into the ammonium solution, compared to when they are added freely to the solution. A similar reduction in adsorption has been reported previously by Jellali et al.

(2011) where the adsorption capacity of *Posidonia oceanica* decreased with a higher fibre dosage. A higher concentration of the fibres enables them to tangle up with each other, which seems to lower the available contact area of the adsorption sites. Thus, having the fibres free in the solution increases its adsorption capacity.

While the adsorption capacity is higher for unbleached TMP when added freely compared to using a fibre bag, the adsorption acquired when using a fibre bag was negative (Figure 7.b), i.e. the ammonium concentration had increased after the addition of the fibre. The reason for this is unclear. The samples were not diluted nor filtered before the analysis of the TAN concentration, and thus fibre particles might have interfered with the test.

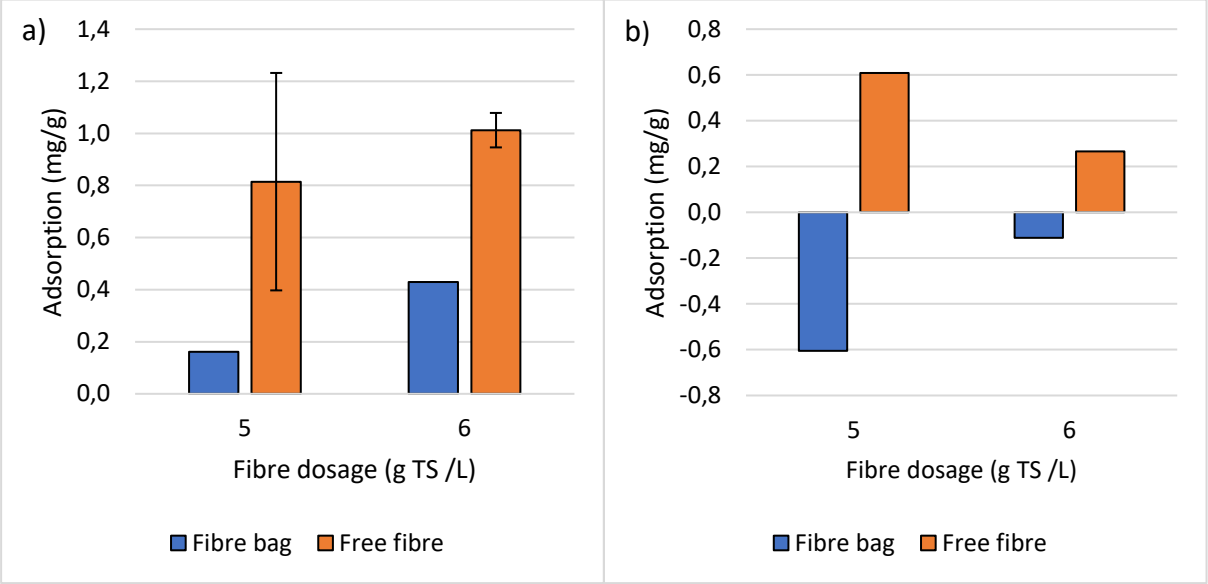


***Figure 7:*** *The adsorption (mg/g) of* ***a)*** *Kraft HW washed with Milli-Q and* ***b)*** *unbleached TMP washed with Milli-Q when using a fibre bag compared to having the fibre free in the solution (TAN concentration: 100 mg/L; Fibre dosages: 5 g TS/L and 6 g TS/L)*

### 4.2.2 Main adsorption results

#### Effect of ammonium concentrations

Figure 8 shows the adsorption capacity for each fibre type at three different TAN concentrations: 100 mg $L^{-1}$, 1000 mg $L^{-1}$, and 3000 mg $L^{-1}$. For the tests at a TAN concentration of 100 mg $L^{-1}$ and 1000 mg $L^{-1}$ the adsorption capacity was at or below 4 mg $g^{-1}$ for all fibre types. Bleached TMP fibres had the highest adsorption capacity at 100 mg $L^{-1}$ and 1000 mg $L^{-1}$ at 3±0.2 mg $g^{-1}$ and 4±1.2 mg $g^{-1}$, respectively. At a TAN concentration of 3000 mg $L^{-1}$ the highest adsorption capacity was acquired by Kraft HW at 11±3.2 mg $g^{-1}$ followed by bleached TMP with an adsorption at 9±1.5 mg $g^{-1}$. In contrast, there was not a large difference in adsorption capacity for Kraft SW at a TAN concentration of 1000 mg $L^{-1}$ and 3000 mg $L^{-1}$. No conclusive remarks can be drawn from the results from unbleached TMP as it yielded a

negative adsorption of -2±4 mg $g^{-1}$ at a TAN concentration of 1000 mg $L^{-1}$, meaning that the ammonium concentration increased after the addition of the fibres. Consequently, a blank control was carried out in order to see if any of the fibres released free nitrogen to a Milli-Q solution. However, this control indicated no significant increase in the TAN concentration (below 0.04 mg $L^{-1}$) for any of the fibres. The adsorption capacity for unbleached TMP was larger at a TAN concentration at 3000 mg $L^{-1}$ albeit with a relatively large standard deviation (7±5.5 mg $g^{-1}$). Thus, the adsorption tests at a TAN concentration at 1000 mg $L^{-1}$ and 3000 mg $L^{-1}$ for unbleached TMP should be redone in order to draw any conclusions about the effect of TAN concentration on the adsorption capacity for unbleached TMP.

Disregarding unbleached TMP, the results indicates that the adsorption capacity increases with the TAN concentration. Similar findings have been reported by Jellali et al. (2011) where the adsorption capacity of seagrass fibres increased from 0.6 to 1.7 mg $g^{-1}$ when the TAN concentration was raised from 5 to 50 mg $L^{-1}$. With higher TAN concentrations, there is a higher possibility for the ammonium to come in contact with the surface of the fibres which subsequently increases the uptake from the fibres (Jellali et al., 2011; Zhu et al., 2016). The difference in adsorption capacities between the fibres could potentially be explained by their charge content. Kraft HW have the largest total charge compared to Kraft SW and TMP fibres (Table 3; Horvath, 2006). The more negative, the stronger are the interactions formed between the ammonium and the functional carboxyl groups of the fibres (Jellali et al., 2011).

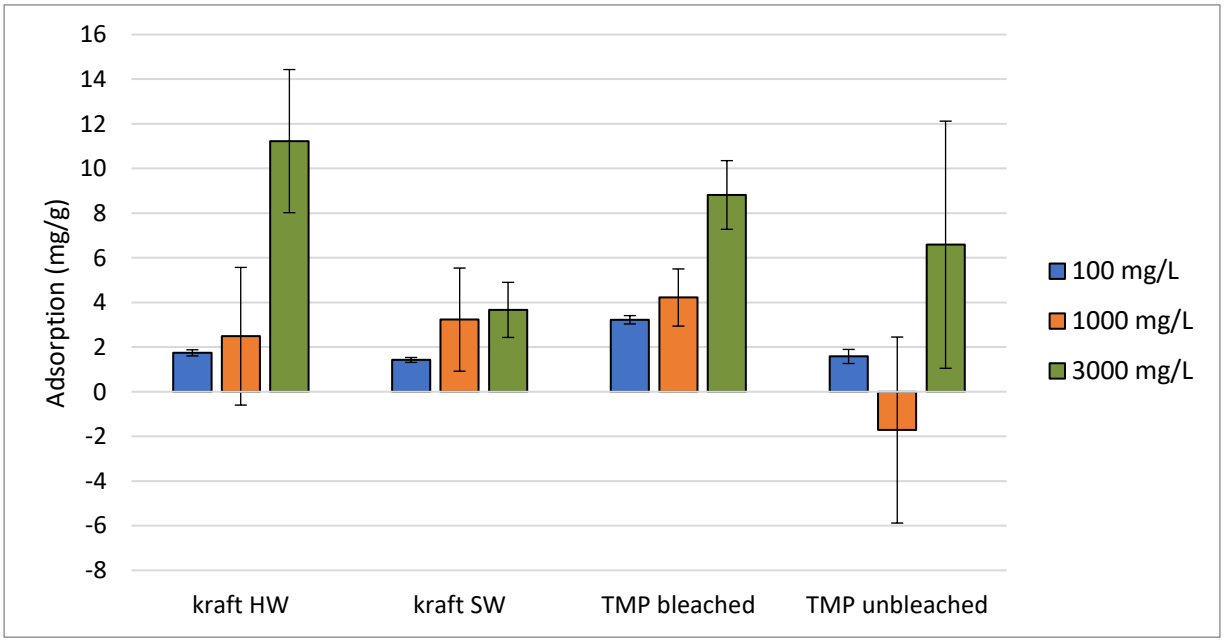


***Figure 8:*** *The adsorption (mg/g) of each fibre type at different TAN concentrations: 100 mg/L (pH 5.8), 1000 mg/L (pH 5.8), and 3000 mg/L (pH 5.2).*

### Effect of fibre dosages

When comparing different fibre dosages of Kraft HW, a fibre dosage of 4 and 6 g $L^{-1}$ seemingly had a larger mean adsorption compared to the rest. However, since the standard deviation

for those two fibre dosages are much larger compared to the others, and since there is no significant difference between the fibre dosages of 3, 5, and 7 g $L^{-1}$, the larger adsorption of 4 and 6 g $L^{-1}$ can most likely be ruled out as a measurement error, and thus be disregarded. Indeed, it is most unlikely that an adsorption pattern such as this would be true. Thus, the results indicates that the adsorption capacity of Kraft HW is not affected by the fibre dosage within the range of 3-7 g $L^{-1}$. However, other studies have shown that the fibre dosage have had an effect on the adsorption capacity. In a study by Riahi et al. (2009) it was shown that a fibre dosage greater than 6 g $L^{-1}$ reduced the adsorption capacity of date palm fibres. Similarly, in a study by Jellali et al. (2011), the adsorption capacity of seagrass fibres decreased at fibre dosages grater than 8 g $L^{-1}$. Both studies attribute the results to the fact that at significantly large dosages, the fibres tangle up in each other which hinders a good contact between ammonium and the adsorption site on the fibres (Jellali et al., 2011; Riahi et al., 2009). However, these studies were conducted at TAN concentrations in the range of 5-50 mg $L^{-1}$, indicating that the TAN concentration has a greater effect on the adsorption than that of fibre dosage, or that the entanglement phenomena occur at a higher fibre dosage than 7 g $L^{-1}$ for Kraft HW.

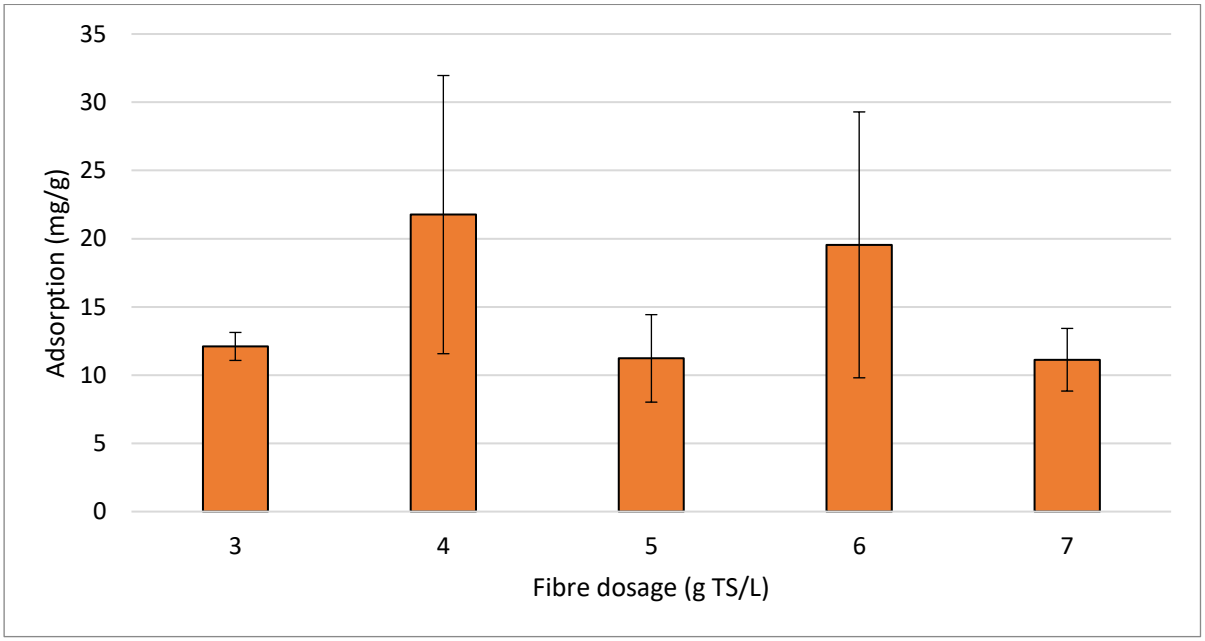


***Figure 9:*** *The adsorption of ammonium (mg/g) using Kraft HW at different fibre dosages: 3, 4, 5, 6, and 7 g TS/L (TAN concentration: 3000 mg/L).*

### Ammonium solution compared to digester fluid

Figure 10 shows how the adsorption of ammonium by Kraft HW and bleached TMP differed when in an ammonium solution with a TAN concentration of 3000 mg $L^{-1}$ compared to digester fluid with a TAN concentration of 2700 mg $L^{-1}$. The adsorption was significantly greater for both Kraft HW and bleached TMP when in digester fluid, with a mean adsorption of 60±20 mg $g^{-1}$ and 30±20 mg $g^{-1}$, respectively. Digester fluid contains metals and other compounds (Chen

et al., 2008; Appels et al., 2008), which was believed to hinder the adsorption of ammonium. This does not however seem to have a negative effect. However, one factor that most likely have had an effect on the adsorption are the difference in pH. Studies have shown that the adsorption capacity of organic adsorbents and zeolites increases with pH (Wahab et al., 2010; Jellali et al., 2011; Mutegoa et al., 2020), and for these tests, the ammonium solution and digester fluid had a pH of 5.2 and 7.8, respectively. An increase of pH enhances the negative charge of the functional carboxyl groups on the fibres (Horvath, 2006), increasing its electrostatic interactions with ammonium (Jellali et al., 2011).

These results show that bleached TMP has a lower adsorption than that of Kraft HW, which could be because TMP fibres have a higher metal content than Kraft fibre (Figure 5), or due to HW having a higher charge content than SW (Horvath, 2006). Additionally, it was noted that bleached TMP was less homogeneously mixed in digester fluid compared to Kraft HW, which could further explain its lower adsorption.

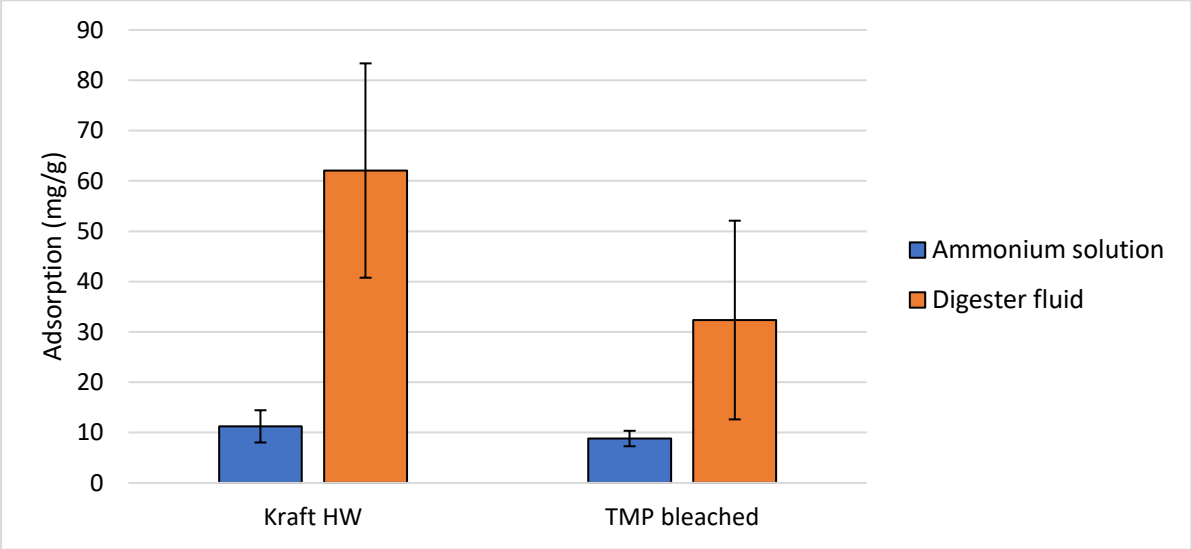


***Figure 10:*** *The adsorption of ammonium (mg/g) using Kraft HW and bleached TMP in an ammonium solution with a TAN concentration of 3000 mg/L (pH 5.2) and digester fluid with a TAN concentration of 2700 mg/L (pH 7.8).*

### Effect of temperature

The effect temperature (21°C, 37°C, and 55°C) had on the adsorption capacity differed between Kraft HW and bleached TMP (Figure 11). For Kraft HW, there was no significant difference in the adsorption at 21°C and 37°C, but the adsorption was reduced at 55°C (39±9 mg $g^{-1}$). Similarly, there was no significant difference for bleached TMP at 21°C and 37°C, but had on the other hand the highest adsorption at 55°C (50±10 mg $g^{-1}$). Although there is no significant difference for both types of fibres at 21°C and 37°C, a trend is still noticeable for them both. For Kraft HW, the adsorption capacity decreases with the temperature, as for bleached TMP the adsorption capacity increases with the temperature. In a study by Wahab et al. (2010) it was shown that the adsorption capacity of seagrass fibres increased with

temperature. The seagrass fibres in this study were not chemically or mechanically treated and thus resembles TMP fibres the most (both lignin and hemicellulose are present). Therefore, the decrease in adsorption for Kraft HW could be due to less structural stability at a higher temperature. Moreover, the experiment was conducted in digester fluid consisting of microbes which degrades organic material, such as pulp fibres. Degradation rates increase at higher temperatures (Schnürer et al., 2018), and comparably, Kraft fibres are generally easier to degrade than TMP fibres, since they contain less lignin and hemicellulose due to their pulping process (Ekstrand, 2019; Jansson, 2015). Therefore, Kraft HW could have been degraded at a rate which counteracted the adsorption. However, experiments in ammonium solution are needed to say for certain if the degradation rates have a significant effect at these temperatures.

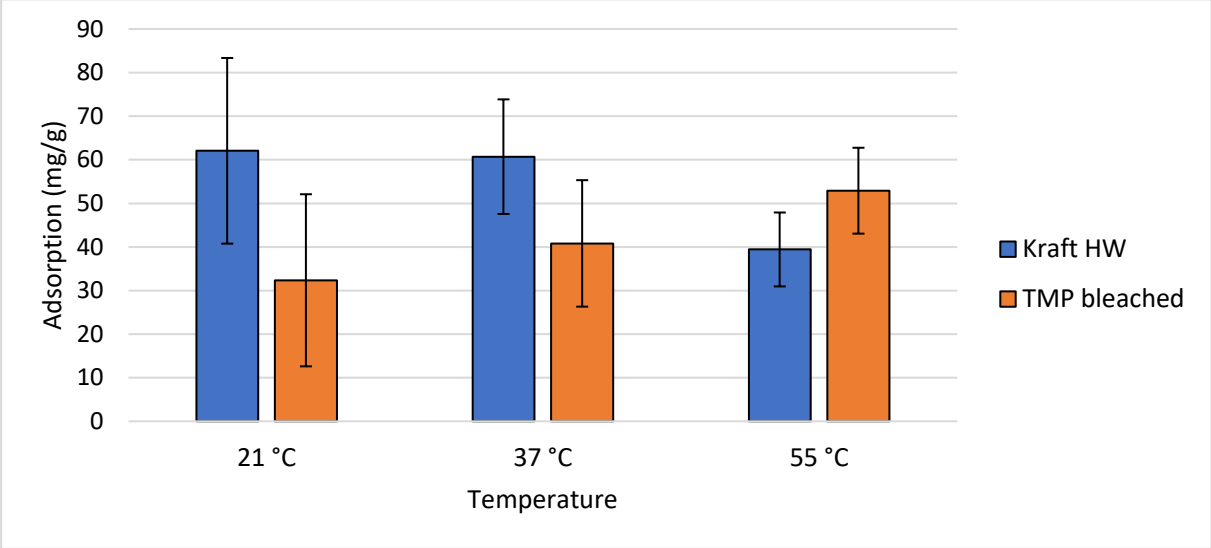


***Figure 11:*** *The adsorption of ammonium (mg/g) using Kraft HW and bleached TMP at different temperatures (21°C, 37°C, and 55°C) in digester fluid (TAN concentration: 2700 mg/L; pH 7.8).*

### Effect of contact time

The effect that contact time had on the adsorption was investigated for Kraft HW and bleached TMP in both an ammonium solution (Figure 12) and digester fluid (Figure 13). For the experiment in ammonium solution, both Kraft HW and bleached TMP follow the same pattern, which is quite peculiar. The expected result would be an adsorption which increases and reaches a steady-state after a while (Jellali et al., 2011; Wahab et al., 2010; Kecili et al., 2018). As both Kraft HW and bleached TMP has a low adsorption at 120 min compared to 60 min and 240 min, an error might have occurred during the dilution of the samples or during the TAN measurement. However, it is recommended to redo this experiment to be able to draw any conclusions and to see at what time the adsorption reaches a steady-state.

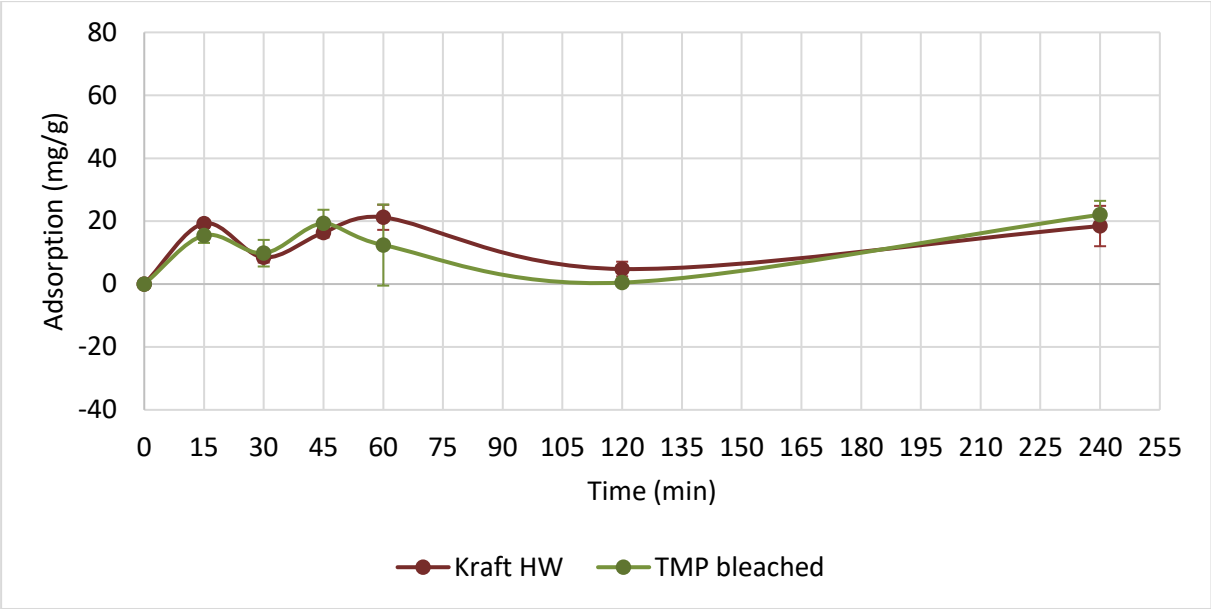


**Figure 12:** *The effect of contact time on the adsorption of ammonium (mg/g) using Kraft HW and bleached TMP in an ammonium solution with a TAN concentration of 3000 mg/L.*

The effect contact time had on the adsorption of ammonium using Kraft HW and bleached TMP in digester fluid (Figure 13) was even more curious than that of ammonium solution. For Kraft HW, there is no significant difference in adsorption between the time point 15, 30 and 45, and after 60 min the adsorption goes below 2 mg $g^{-1}$. As for bleached TMP, the time point at 30 min is significant with an adsorption of 3±3 mg $g^{-1}$. However, at 120 min, TMP bleached showed a negative adsorption of -15±18 mg $g^{-1}$, indicating that the results are perhaps not the most reliable. Although, as mentioned above, these fibres are prone to degradation, which consequently could affect the adsorption. If the fibres would be fully degraded the adsorption capacity would be lost, which Kraft HW shows signs of. However, this experiment is also recommended to redo to be able to draw better conclusions regarding at which time the adsorption capacity reaches steady-state, and if degradation of the fibres have a significant effect.

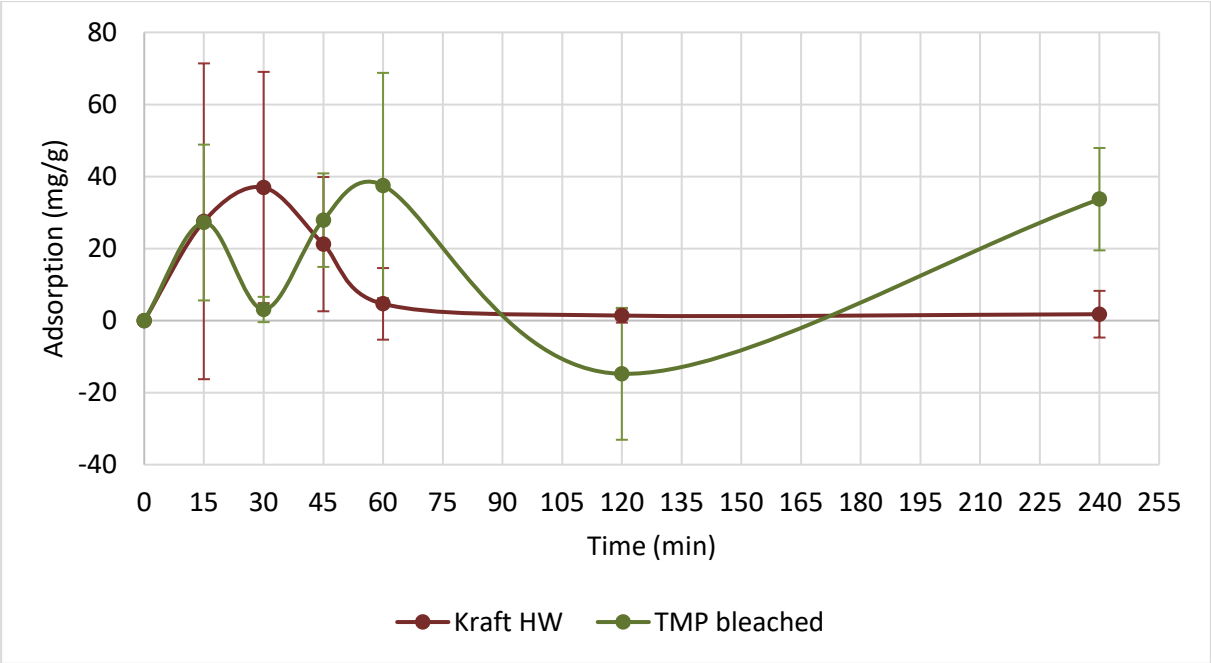


**Figure 13:** *The effect of contact time on the adsorption of ammonium (mg/g) using Kraft HW and bleached TMP in digester fluid with a TAN concentration of 2700 mg/L.*

## 4.3 Biochemical methane potential tests

### 4.3.1 Biogas potential and system evaluation

The average methane production and the standard deviation of each BMP test can be seen in Figure 14 and 15. The tests are divided into two figures to present the results and compare the degradability of the fibres with the respective control of OLR more easily. The methane potential of Kraft HW was normalized by 2.1 g VS (Figure 14) while the methane potential of bleached TMP were normalized by 1.6 g VS (Figure 15). This is due to the lignin content of TMP fibres, making it unlikely to have been degraded during the BMP test, and thus not contributing the methane production. The BMP tests went on for a total of 36 days but reached a steady-state after day 12 and is thus the last day that is shown in the graphs (see appendix C Figure 21 for full BMP data).

The BMP tests showed that there was a slight difference in the methane potential that was reached at steady state at day 12 (Figure 14). FW, FW-A, HW, and HW-A showed methane potentials of 456±15, 430±19, 398±19, and 420±5 Nml $g^{-1}$ $VS^{-1}$, respectively. The lower methane potential and initial methane production rates of FW-A compared to FW indicates that the system was inhibited at a TAN concentration of 3100 mg $L^{-1}$. Ammonia inhibition mainly affects the methane production rates and is thus the main parameter of interest when comparing the different systems. However, since Kraft HW fibres have had its lignin content reduced, they are prone to be degraded and converted into methane by the microorganisms (Ekstrand et al., 2020). Wood fibres does however have a lower methane potential than food waste, and since the systems all have the same OLR it is hard to make an objective comparison between the systems without a control where it is shown how much methane that is produced from Kraft HW. Nonetheless, if trying to compare HW-A with FW-A, we can see that the initial

methane production rates are similar, indicating that the addition of Kraft HW had no to little effect on the ammonia inhibited system. Looking at HW, we can see that between day 0 and 3, it has about the same production rates as FW, but does however reach a slightly lower methane potential than HW-A.

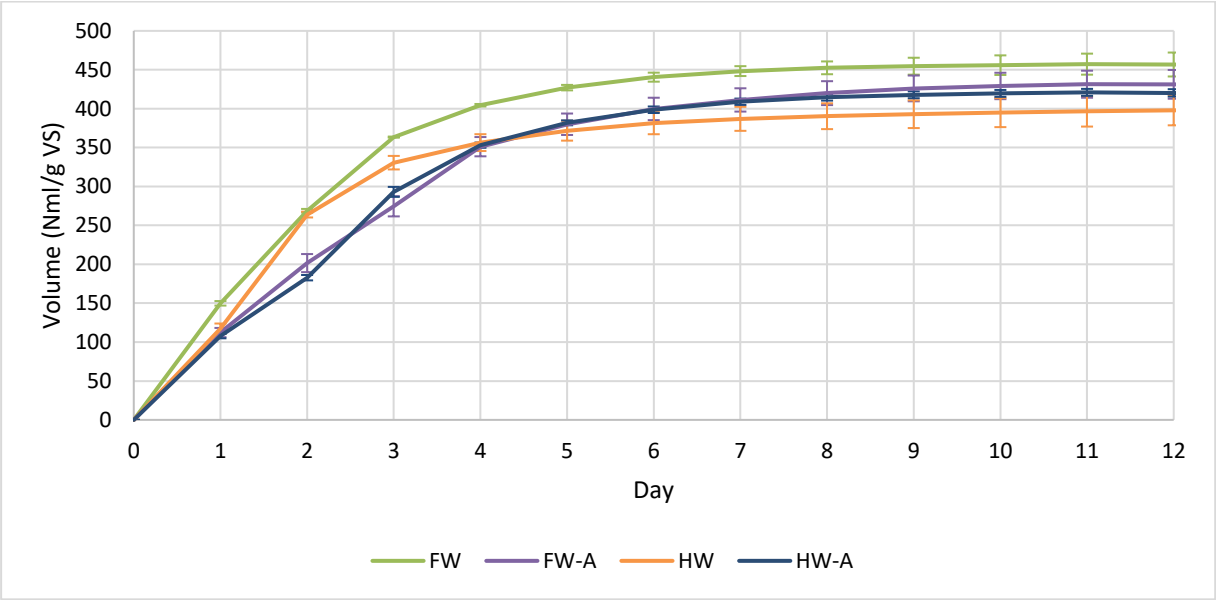


***Figure 14**: The methane potential (Nml/g VS) of control with inoculum and food waste (FW), control with inoculum and food waste at a TAN concentration of 3100 mg/L (FW-A), system with kraft HW fibres at a TAN concentration of 1400 mg/L (HW), and system with kraft HW fibres at a TAN concentration of 3100 mg/L (HW-A), from the BMP tests during a period of 12 days. Methane pontential was normalized with 2.1 g VS.*

In Figure 15, we can see the BMP tests for FW2, FW-A, TMP, and TMP-A which showed methane potentials of 432±32, 430±19, 467±22, and 450±35 Nml $g^{-1}$ $VS^{-1}$, respectively. TMP and TMP-A are in this case normalized with 1.6 g VS with the assumption that TMP fibres was not degraded and converted into methane by the microorganisms. TMP fibres still have high fractions of lignin remaining in the fibres after its pulping process and is thus hard for the microorganisms to degrade (Ekstrand et al., 2020). However, both TMP and TMP-A reaches a higher methane potential than FW2 and FW-A, indicating that some conversion most likely still have occurred. This makes it hard to objectively compare the different systems, but it is clear that the two inhibited systems (FW-A and TMP-A) are inhibited compared to their counterpart by looking at their reduced methane production rates. However, it is hard to see what effect the addition of TMP fibres had to the inhibited system. There might be an indication that there has been a slight positive effect, but a control which shows how much methane that is produced from the TMP fibres are necessary to say for certain.

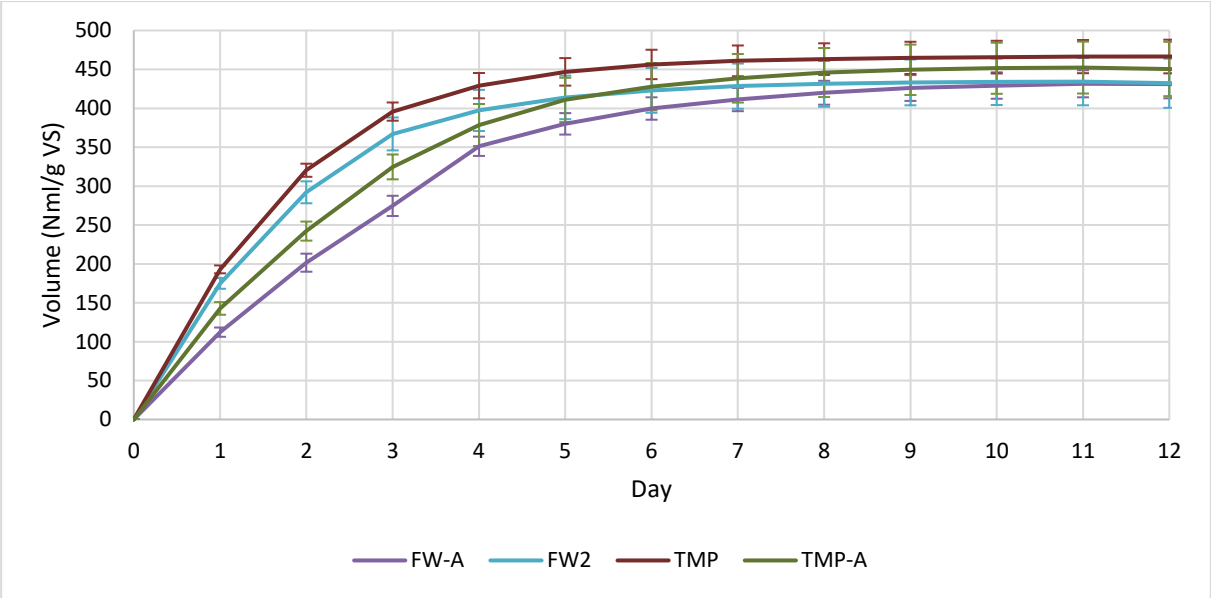


***Figure 15**: The methane potential (Nml/g VS) of control with inoculum and food waste at a TAN concentration of 3100 mg/L (FW-A), control with inoculum and food waste at a lower total organic loading rate (FW2), system with bleached TMP fibres at a TAN concentration of 1400 mg/L (TMP), and system with bleached TMP fibres at a TAN concentration of 3100 mg/L (TMP-A) from the BMP tests during a period of 12 days. Methane pontential of FW-A normalized with 2.1 g VS while FW2, TMP, and TMP-A are normalized with 1.6 g VS.*

### 4.3.2 Enzymatic activity

The blank control for each BMP sample (not plotted) was stable and gave under 1000 RFU. Therefore, the background emission was insignificant, but was nonetheless extracted from each sample. The autocatalysis control and positive control of the enzymatic assay can be seen in Appendix D Figure 22, which indicated no complications with the substrate and instrument.

The positive control P of the BMP test (Figure 16) shows a strong induction of cellulase activity from day 0 to day 5 during the BMP test. The microbes have a deficiency of easily degradable sugar, but an abundance of hard to access sugar in the form of cellulose (Whatman cellulose paper). Thus, the microbes start to produce cellulase in order to degrade the cellulose.

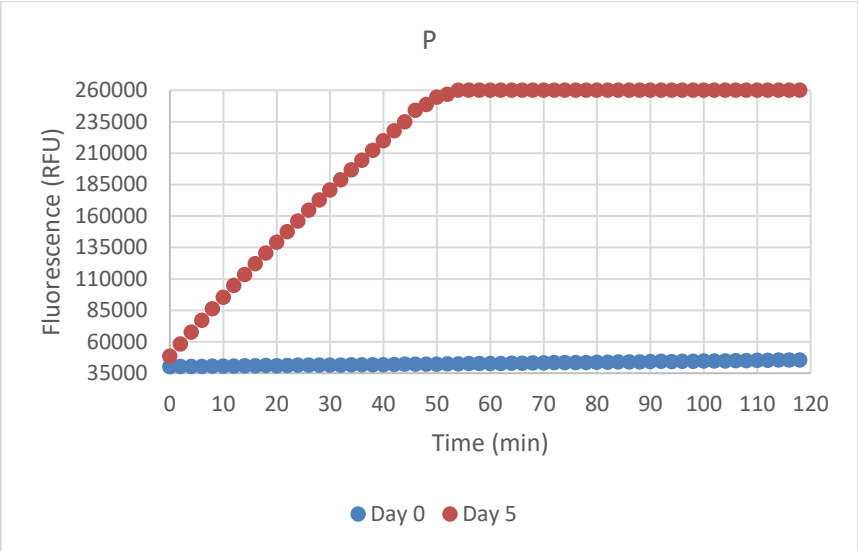


***Figure 16**: The cellulase activity of the positive control (P) with Whatman cellulose paper at day 0 and day 5 of the BMP test. Fluorescent emission recorded at a 2-minute interval over a period of 120 min.*

The different controls only show small or no difference in cellulase activity from day 0 to day 5 (Figure 17). The test containing only inoculum (Y) shows a lower cellulase activity after day 5 (Figure 17.a) which is reasonable since no additional sugars or cellulose are added. Thus, the activity that was there from the start decreases. As for FW-A, FW2, and FW there is only small alterations. FW (Figure 17.d) have a slightly higher activity than FW2 (Figure 17.c) which is fitting since FW has a higher OLR than FW2 (2.1 g VS and 1.6 g VS, respectively), meaning that FW has more sugars which could be degraded. As for FW-A (Figure 17.b) the initial cellulase activity is slightly higher (2000 RFU) than that of the others, indicating that the production of cellulase might have started earlier compared to the others. This could be due to the fact that there is a relative deficiency of carbon compared to nitrogen, i.e. the C/N ratio is low. This could in turn trigger the production of cellulase, subsequently increasing its activity (Speda et al., 2016).

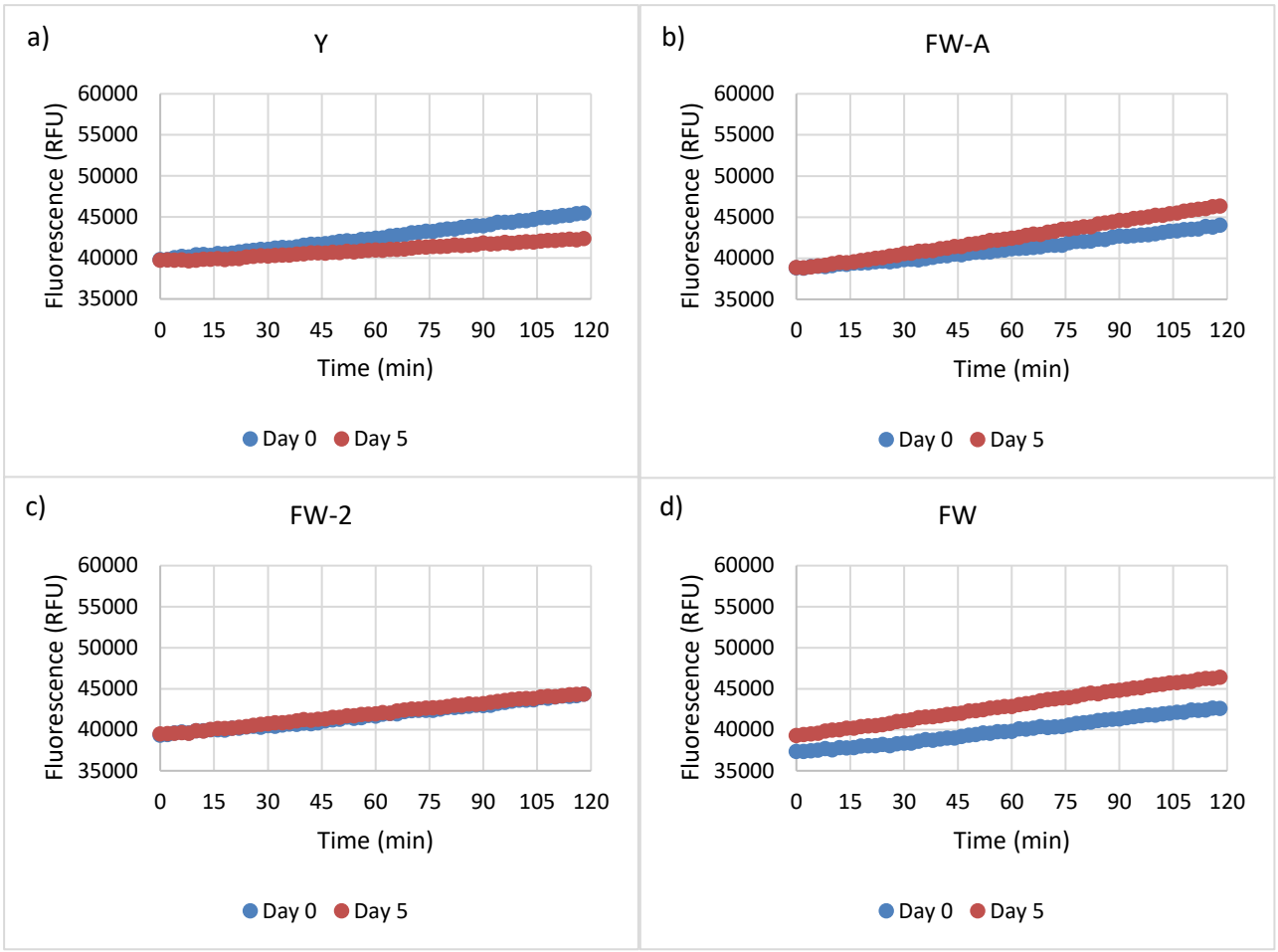


***Figure 17**: The cellulase activity of **a)** blank control (Y), **b)** control of inoculum and food waste at a TAN concentration of 3100 mg/L (FW-A), **c)** control of inoculum and food waste at a lower total organic loading rate (FW2), and **d)** control of inoculum and food waste (FW), at day 0 and day 5 of the BMP test. Fluorescent emission recorded at a 2-minute interval over a period of 120 min.*

All tests with pulp fibre show an increase of cellulase activity from day 0 to day 5 (Figure 18). Both TMP-A (Figure 18.b) and HW-A (Figure 19.d) shows a higher cellulase activity than TMP (Figure 18.a) and HW (Figure 18.c), respectively. This is for the same reasons as mentioned above. With an increased TAN concentration, the relative C/N ratio becomes low, which induces the microbes to start producing cellulase to break down more cellulose into sugar. Although, the increase over time is notably lower than that of the positive control (Figure 16), which is because the microbes still have sugars available from the food waste. However, both HW and HW-A has a higher activity than that of respective TMP and TMP-A. This is unclear since naturally TMP fibres should induce a higher cellulase activity due to TMP being harder to degrade because of its lignin content. Overall, the results indicates that the production of cellulase has a positive correlation with an increased TAN concentration. However, these results are contradicting to those proposed by Fischer et al. (2019) where it was shown that the transcription of cellulase had a negative correlation with the TAN concentration. Additionally, other studies have also shown that the degradation of cellulase decreased with

the TAN concentration (Liu et al., 2016; Sun et al., 2016). The reason for this is unclear, but acclimation of the microbes might have an effect. No analysis of the microbial community was performed in this study which would have given a more complete insight on how the composition of the hydrolytic bacteria and subsequently cellulase activity was affected by the TAN concentration. Furthermore, more than two time points are necessary to conclude the behaviour of the microbe cellulase production in these BMP systems. It would be of great interest to see the cellulase activity during the initial days 1-4 since most of the cellulase activity could have been lost after 5 days as we do not know if this is the peak or not.

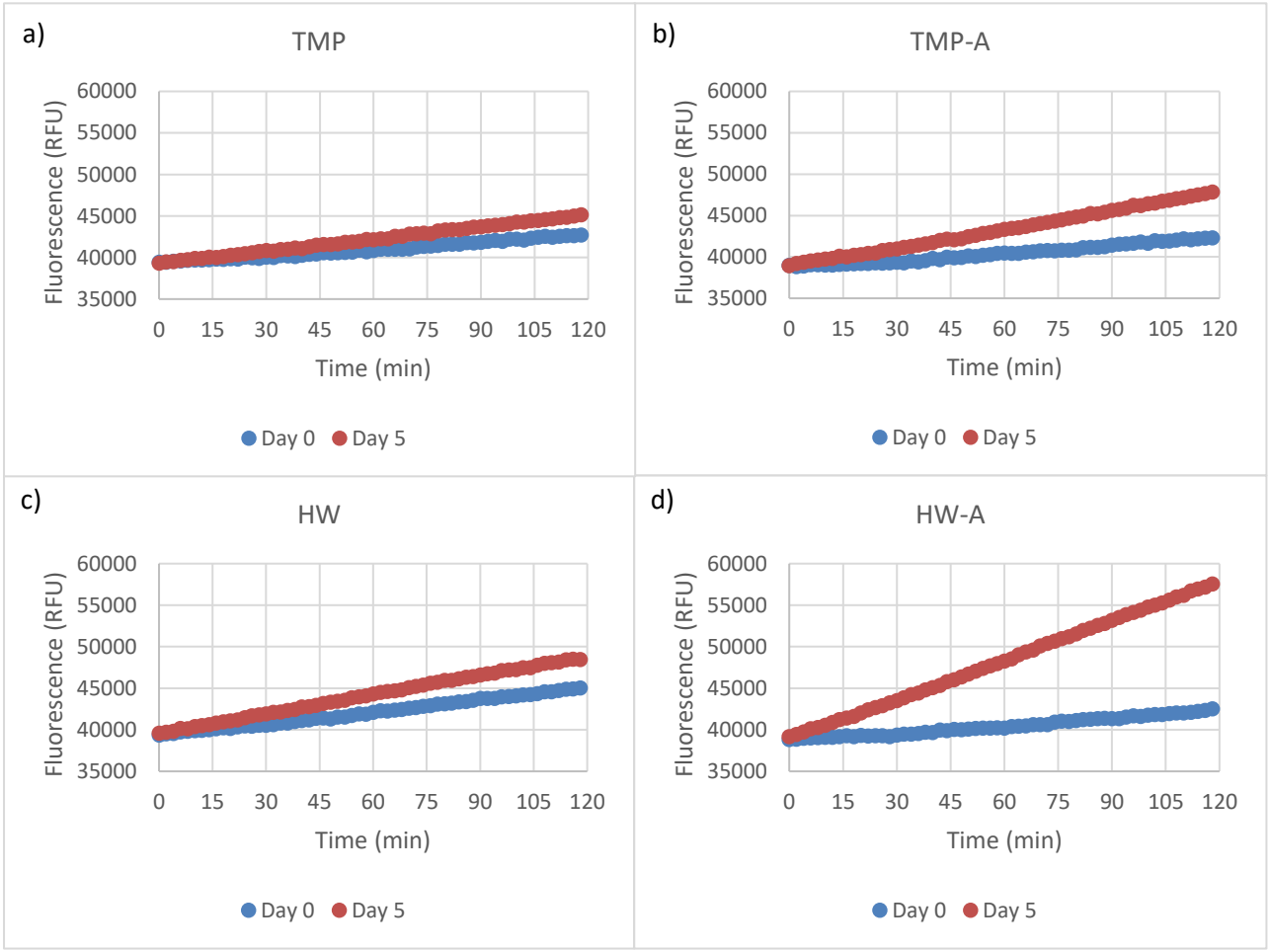


***Figure 18**: The cellulase activity of **a)** system with bleached TMP fibres at a TAN concentration of 1400 mg/L (TMP), **b)** system with bleached TMP fibres at a TAN concentration of 3100 mg/L (TMP-A), **c)** system with kraft HW fibres at a TAN concentration of 1400 mg/L (HW), and **d)** system with kraft HW fibres at a TAN concentration of 3100 mg/L (HW-A). Fluorescent emission recorded at a 2-minute interval over a period of 120 min.*

# 5. Conclusions

The main aim of this study was to investigate and evaluate the adsorption capacity of Kraft HW, Kraft SW, unbleached TMP SW, and bleached TMP SW to see if it would be feasible to use as a means to counteract ammonia inhibition in AD. More specifically it aimed to answer the follow research questions:

1. ***How does different parameters, e.g. initial ammonia concentration, fibre dosage, temperature, and contact time affect the adsorption capacity of the fibres?***
    - TAN concentration had the highest effect with a positive correlation, although with an increased variance as well.
    - Different fibre dosages within the range of 3-7 g TS $L^{-1}$ did not seem to affect the adsorption capacity. However, the fibre dosages were only evaluated for Kraft HW and can thus not be concluded for all fibre types.
    - For Kraft HW, the adsorption had a negative correlation with temperature. On the contrary, for bleached TMP, the adsorption had a positive correlation with temperature. However, tests in aqueous ammonium solutions are necessary to confirm this.
    - No conclusions can be drawn from the experiments on contact time due to inconclusive results.

2. ***What is the adsorption capacity of the different fibres in an ammonium solution compared to in digester fluid?***
    - Overall, the adsorption capacity was higher in digester fluid compared to in an ammonium solution. Kraft HW had the highest adsorption in digester fluid (60±20 mg $g^{-1}$) at 21°C followed by bleached TMP (53±10 mg $g^{-1}$) at 55°C. The highest achievable adsorption in an ammonium solution for each fibre was at a TAN concentration of 3000 mg $L^{-1}$: Kraft HW 11±3 mg $g^{-1}$; Kraft SW 4±1 mg $g^{-1}$; bleached TMP 9±2 mg $g^{-1}$; unbleached TMP 7±6 mg $g^{-1}$.

3. ***How does the different fibre types affect the process of anaerobic digestion with regards to methane production?***
    - The BMP tests showed indications that the addition of pulp fibres to an AD system inhibited with high ammonia concentration had little to no effect. It is uncertain how much the degradation of the fibres affected the methane potential.

4. ***How does a high ammonium concentration affect the activity of cellulase in anaerobic digestion?***
    - The cellulase activity was higher in the BMP systems inhibited with ammonia, indicating a delayed or prolonged cellulase activity due to ammonia.

Of all the pulp fibres tested in this study, Kraft HW had the highest adsorption capacity, both in an aqueous ammonium solution and digester fluid. However, the overall adsorption capacity were low with large variations, and is most likely too small to adequately counteract ammonia inhibition in an AD system.

## 5.1 Future work

Despite this study implicating that pulp fibre might not be able to counteract ammonia inhibition, a more thorough study should be conducted with a method that is able to yield more consistent results, with a lower variation. However, while it might not be applicable for AD systems, pulp fibre could still be used for its adsorption properties in other areas, such as reducing the ammonium or phosphate concentration in wastewater streams. Additionally, it would be interesting to see if the more inert TMP fibres could be utilized in AD as a micro carrier, and in this way protect the microbes from an ammonia inhibition.

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Tack!

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# Appendix A –Metal content for kraft HW and unbleached TMP

***Table 6:*** *The metal concentration in Kraft SW (Untreated, washed with Milli-Q, treated with EDTA) and unbleached TMP (untreated, treated with EDTA) for all metals investigated in this study.*

| | | **Kraft SW** | | | **TMP unbleached** | |
|---|---|---|---|---|---|---|
| ELEMENT | SAMPLE | Untreated | Milli-Q | EDTA | Untreated | EDTA |
| Sampling Date | | 2021-02-03 | 2021-02-03 | 2021-02-03 | 2021-02-03 | 2021-02-03 |
| Decomposed | | Yes | Yes | Yes | Yes | Yes |
| Al | mg/kg TS | 13,8 | 7,74 | 2,11 | 414 | 86,8 |
| As | mg/kg TS | <0.08 | <0.09 | <0.1 | <0.08 | <0.10 |
| Ba | mg/kg TS | 2,27 | 2,1 | 1,02 | 17,2 | 7,5 |
| Ca | mg/kg TS | 490 | 516 | 84,2 | 1380 | 50,3 |
| Cd | mg/kg TS | 0,00588 | 0,00796 | <0.007 | 0,0118 | <0.006 |
| Co | mg/kg TS | 0,0066 | 0,00715 | <0.007 | 0,0141 | <0.006 |
| Cr | mg/kg TS | <0.03 | 0,0845 | <0.04 | 0,155 | 0,0521 |
| Cu | mg/kg TS | 0,126 | 1,61 | 0,414 | 0,387 | 0,236 |
| Fe | mg/kg TS | 7,63 | 9,76 | 4,22 | 17,5 | 3,1 |
| K | mg/kg TS | 1160 | 1100 | 53,2 | 547 | 63,6 |
| Mg | mg/kg TS | 133 | 157 | 49,5 | 139 | 30,6 |
| Mn | mg/kg TS | 1,85 | 2,08 | <0.06 | 18,4 | <0.05 |
| Mo | mg/kg TS | <0.004 | <0.004 | <0.006 | 0,0421 | 0,0122 |
| Na | mg/kg TS | 1540 | 818 | 452 | 4000 | 1490 |
| Ni | mg/kg TS | 0,0945 | 0,262 | 0,0673 | 0,0703 | 0,0584 |
| P | mg/kg TS | 10,5 | 57,3 | 4,34 | 71,7 | 7,88 |
| Pb | mg/kg TS | 0,068 | 0,142 | <0.06 | 0,129 | <0.05 |
| Ti | mg/kg TS | 0,0919 | 0,318 | 0,123 | 4,97 | 0,736 |
| V | mg/kg TS | <0.02 | <0.02 | <0.03 | 0,128 | <0.02 |
| Zn | mg/kg TS | 0,73 | 2,45 | 0,442 | 3,44 | 0,628 |
| Total solids (TS) at 105°C | % | 30,4% | 24,2% | 23,0% | 32,8% | 23,7% |

# Appendix B – Initial adsorptions tests

Tests consisting of Kraft HW with different ammonium concentrations of 60, 100 and 150 mg $L^{-1}$ was conducted together with a fixed fibre dosage of 4 g TS $L^{-1}$ being submerged using a polyester fibre bag. The results (see Figure 19) showed that a TAN concentration of 100 mg $L^{-1}$ gave the highest adsorption of 0.4 mg $g^{-1}$. However, these tests were conducted as singlets and thus no variance are reported.

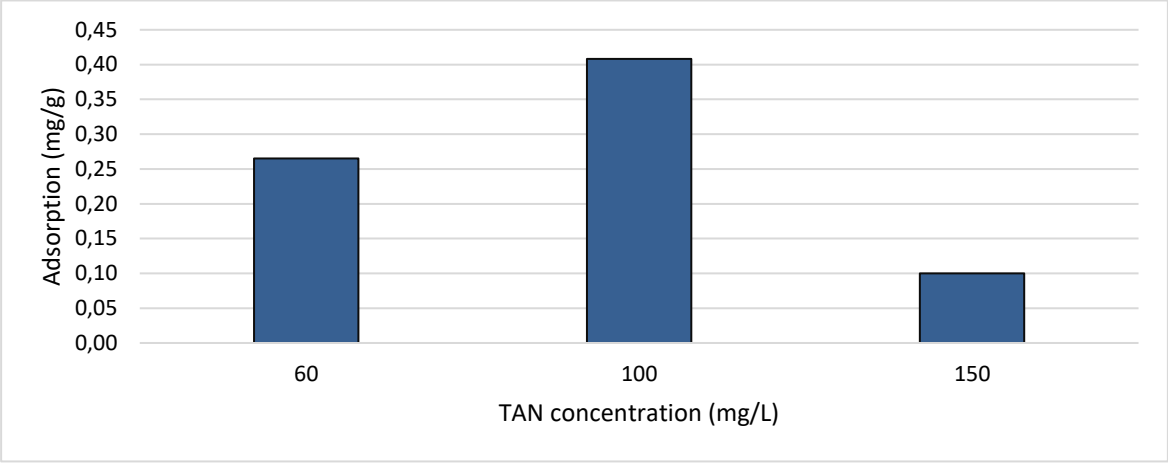


***Figure 19**: The adsorption of ammonium (mg $g^{-1}$) onto kraft HW (4 g TS $L^{-1}$) in ammonium solutions with concentrations of 60, 100 and 150 mg $L^{-1}$.*

The last initial test was conducted between kraft HW which had been washed with Milli-Q and washed with EDTA to determine if a change in adsorption capacity was noticeable (see Figure 20). These tests were conducted without the use of fibre bags to submerge the fibres, i.e. they were added freely to the solution, at a TAN of 100 mg $L^{-1}$. For the fibre dosage of 5 g TS $L^{-1}$ the adsorption of fibres washed with EDTA was 153% greater than that of the fibre washed with Milli-Q, while for 6 g TS $L^{-1}$ the increase was 21%. However, the samples were not centrifuged nor filtered prior to the TAN analysis.

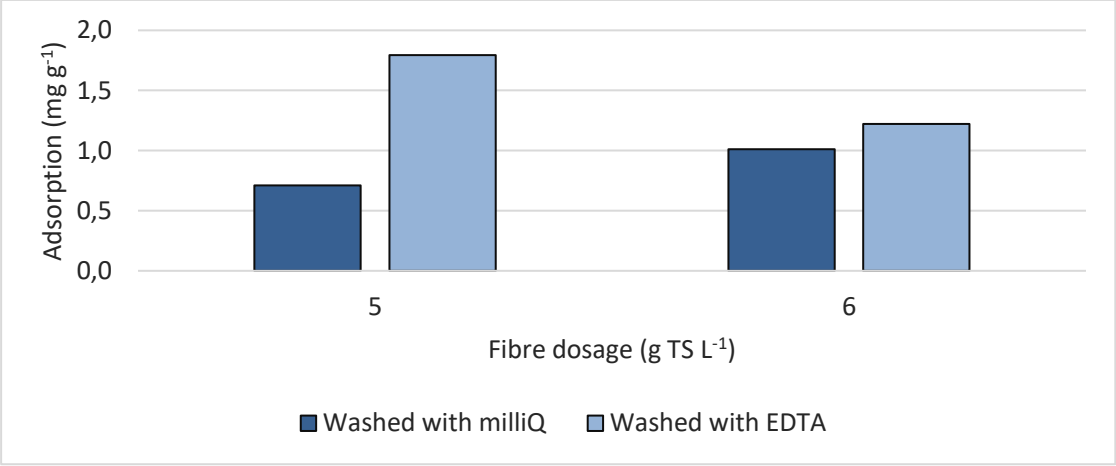


***Figure 20**: Comparison of adsorption (mg $g^{-1}$) between fibre dosages of 5 and 6 g TS $L^{-1}$ washed with either Milli-Q or EDTA.*

## Appendix C – Full BMP data

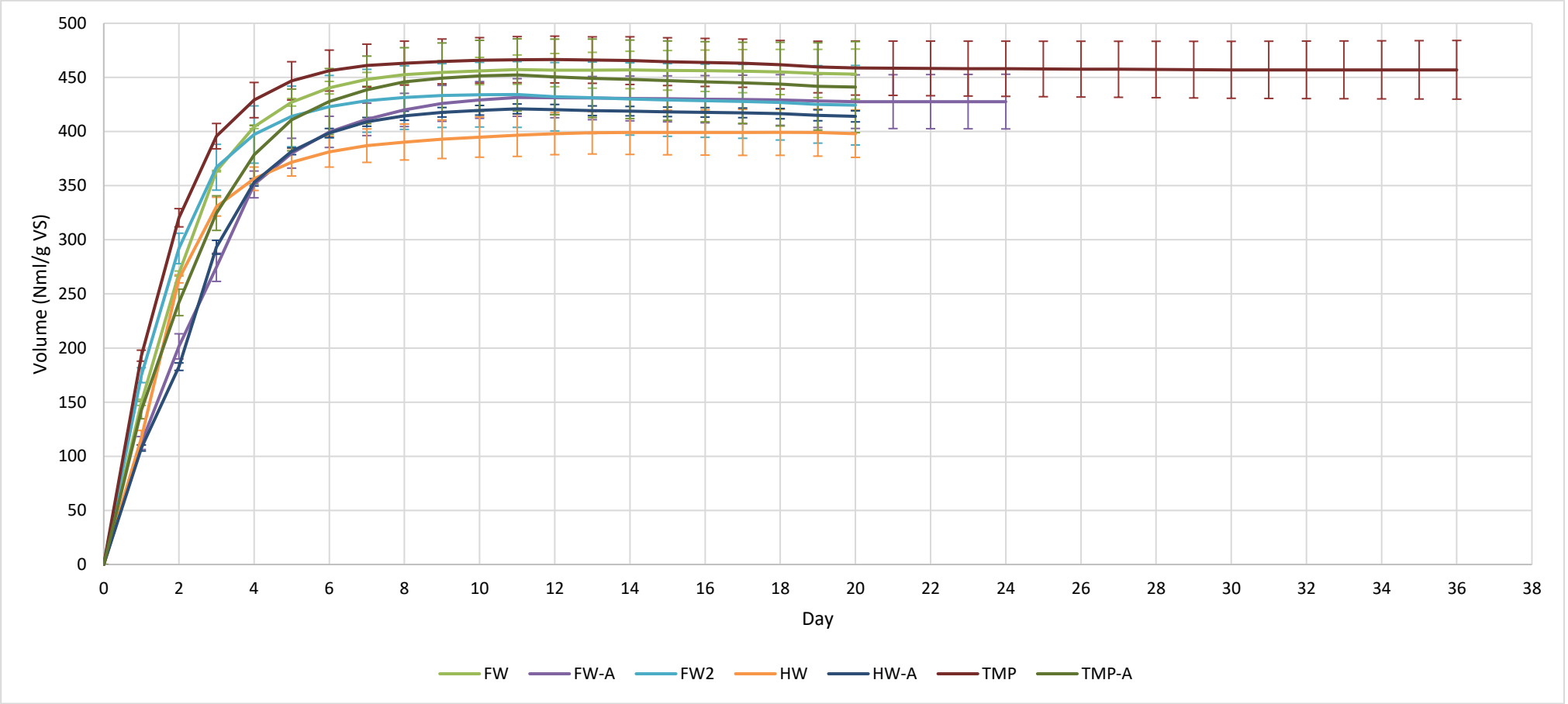


Figure 21: *The methane potential (Nml/g VS) of control with inoculum and food waste (FW), control with inoculum and food waste at a TAN concentration of 3100 mg/L (FW-A), control with inoculum and food waste at a lower total organic loading rate (FW2), system with kraft HW fibres at a TAN concentration of 1400 mg/L (HW), and system with kraft HW fibres at a TAN concentration of 3100 mg/L (HW-A), system with bleached TMP fibres at a TAN concentration of 1400 mg/L (TMP), and system with bleached TMP fibres at a TAN concentration of 3100 mg/L (TMP-A) from the BMP tests during a period of 36 days.*

## Appendix D – Autocatalysis and positive cellulase controls

The autocatalysis control nor the positive control showed any complications (Figure 22). The delta-fluorescence of the autocatalysis (Figure 22.a) only achieved an emission of 500 RFU over the period of 120 min, thus implicating no significant degradation of the substrate. In the positive control, the degradation of the substrate happened immediately (Figure 22.b), before the plate reader could capture it, resulting in a maximum 260 000 RFU. A cellulase concentration of 1 mg $L^{-1}$ was perhaps a little too high for a substrate concentration of 0.5 mM, but does nonetheless show that there were no implications with the substrate nor instrument.

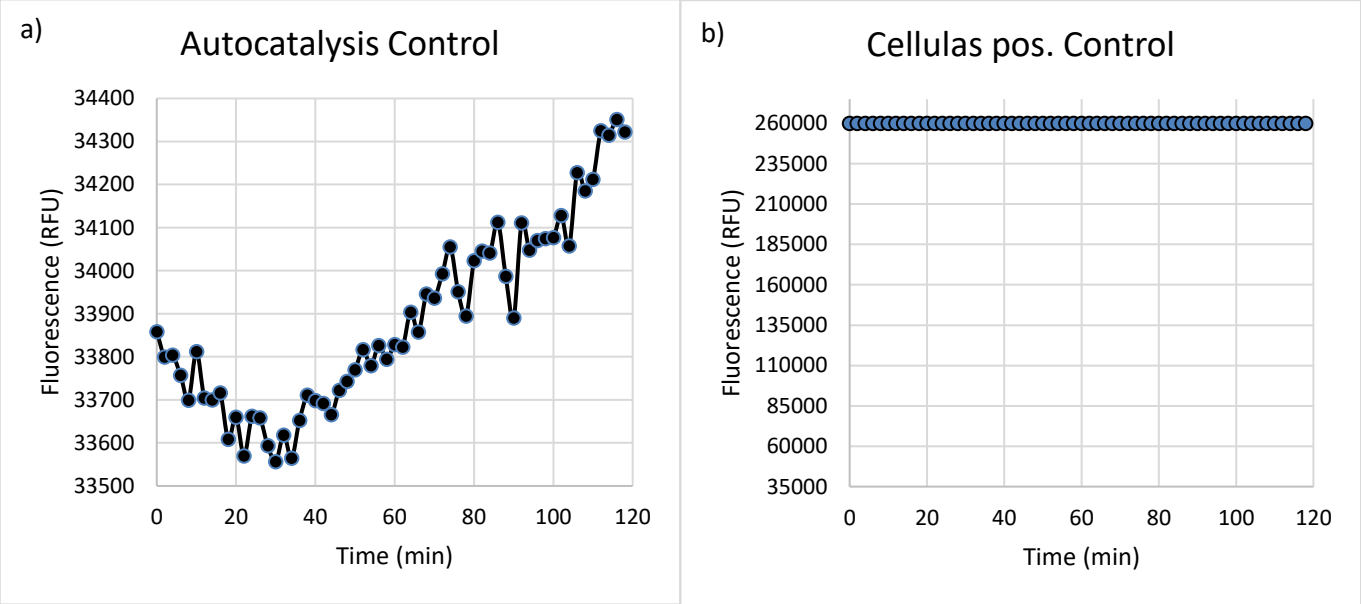


***Figure 22**: The cellulase activity of the **a)** autocatalysis and **b)** positive control in the cellulase activity assay. Fluorescent emission recorded at a 2-minute interval over a period of 120 min.*