UV pretreatment of Alkaline Bleaching Wastewater from a Kraft Pulp and Paper Mill prior to Anaerobic Digestion in a Lab-scale UASB Reactor

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Abstract

The effects of UV pretreatment on alkaline bleaching (EOP) wastewater from a kraft pulp and paper mill were investigated prior to anaerobic digestion (AD) in an upflow anaerobic sludge blanket (UASB) reactor. The aim was to enhance the methane production, increase the reduction of total organic carbon (TOC) and determine the best UV exposure time. The exposure time of 2.6 minutes partially degraded the organic material in the EOP wastewater since it generated higher biogas and methane production than the reference period, while it also increased the reductions of solved chemical oxygen demand (COD<sub>sol</sub>) and TOC<sub>sol</sub>. The exposure time of 16 minutes, on the other hand, did not show any significant improvement regarding increased biogas and methane production nor did it increase the reduction of COD<sub>sol</sub>. However, it did increase the reduction of TOC<sub>sol</sub> but not to the same extent as the exposure time of 2.6 minutes. The presence of unwanted microbial growth in the system during the experiment might have affected the effectiveness of the UV pretreatment more during the exposure time of 16 minutes as the amount of growth was more substantial during this period of time. Furthermore, no optimal exposure time could be determined due to lack of time.

**Keywords:** Anaerobic digestion, biogas, UV pretreatment, UV/H₂O₂, UASB, EOP wastewater, lignin, kraft pulp and paper mill
Summary

The effects of UV pretreatment on alkaline bleaching (EOP) wastewater from a kraft pulp and paper mill were investigated prior to anaerobic digestion (AD) in a UASB reactor. The aim was to enhance the methane production, increase the reduction of TOC and determine the best UV exposure time. Two different exposure times were investigated, 2.6 and 16 minutes respectively. Another quest in the initial phase of the experiment was to achieve a stable reference period that generated a high methane (CH₄) and biogas production. During the experiment, a number of parameters were monitored to investigate and evaluate the effect of the UV-pretreatment on the EOP wastewater and consequently the biogas and methane production. The effluent of the reactor was, like the biogas samples, analyzed twice a week. The substrate was analyzed once upon arrival of a new batch from the kraft pulp and paper mill. The analyses performed during the experiment were TOC, COD, Volatile Fatty Acids (VFA), pH, SO₄²⁻ concentrations and determination of suspended solids. When the substrate was pretreated with UV light, lignin and H₂O₂ concentrations were also determined as well as UV/vis spectrophotometric wave scans were performed.

From the UV/vis spectra it could not be deduced what organic compounds that were present in the samples. When subtracting the absorbance spectra between the sampling points of the systems, it became apparent that there was an increase in absorbance around 220 nm with the UV pretreatment. The specific compound causing the increase could however not be determined. The lack of increase in lignin reduction indicated that native lignin was not the source of the increase in absorbance at 220 nm. Hydrogen peroxide (H₂O₂) is a chemical used in the bleaching process at the kraft pulp and paper mill, and was a vital constituent of the UV pretreatment in the experiment as it contributes to the production of hydroxyl radicals. The level of H₂O₂ in the samples were often undetectable or very low (i.e. ≤4 mg H₂O₂/L), indicating that merely direct UV photolysis were oxidizing the samples. The detection limit of the test strips used to determine the H₂O₂ concentration was 0.5 mg H₂O₂/L. With a functional UV/H₂O₂ process, the effects of the UV pretreatment might have resulted in a more positive effect in terms of enhanced biogas production and reduction of TOC.

The quest for a suitable reference period in the experiment resulted in a variety of process setups where the circulation in the system was manipulated; biogas circulation with dihydrogen sulphide (H₂S), biogas circulation without H₂S and no circulation in the system. The setup condition with H₂S stripped from the circulated biogas did not improve the efficiency of the reactor compared to the process setup without circulation in the system. It was also determined that the biogas circulation with H₂S present considerable decreased the biogas and CH₄ production as well as the reductions of TOC_sol and COD_sol in comparison to the other setup conditions. The process setup without circulation was chosen as reference period. The exposure time of 2.6 minutes partially degraded the organic material in the EOP wastewater since it generated higher biogas and CH₄ production than the reference period, while it also increased the reductions of COD_sol and TOC_sol. The exposure time of 16 minutes did not show any general significant improvement regarding increased biogas and CH₄ production nor did it increase the reduction of COD_sol compared to the reference period. However, it did increase the reduction of TOC_sol, but not to the same extent as the exposure time of 2.6 minutes. The presence of unwanted microbial growth in the system during the experiment might have affected the efficiency of the UV pretreatment more during the exposure time of 16 minutes as the amount of growth was more substantial during this period of time. Furthermore, no optimal exposure time could be determined due to lack of time.
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### Abbreviations

<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AD</td>
<td>anaerobic digestion</td>
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<tr>
<td>AIL</td>
<td>acid-insoluble lignin</td>
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<tr>
<td>AOP</td>
<td>advanced oxidative process</td>
</tr>
<tr>
<td>ASL</td>
<td>acid-soluble lignin</td>
</tr>
<tr>
<td>BC+H₂S</td>
<td>biogas circulation with H₂S present</td>
</tr>
<tr>
<td>BC-H₂S</td>
<td>biogas circulation without H₂S present</td>
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<tr>
<td>COD</td>
<td>chemical oxygen demand</td>
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<tr>
<td>E</td>
<td>alkaline extraction</td>
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<tr>
<td>ECF</td>
<td>elemental chlorine free</td>
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<tr>
<td>EOP</td>
<td>alkaline extraction with addition of oxygen and peroxide</td>
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<tr>
<td>G unit</td>
<td>guaiacyl unit</td>
</tr>
<tr>
<td>GC-FID</td>
<td>gas chromatograph with a flame ionizing detector</td>
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<td>H unit</td>
<td>p-hydroxyphenyl unit</td>
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<tr>
<td>HRT</td>
<td>hydraulic retention time</td>
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<td>NOM</td>
<td>natural organic material</td>
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<td>O</td>
<td>oxygen delignification</td>
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<td>P</td>
<td>hydrogen peroxide bleaching</td>
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<tr>
<td>REF</td>
<td>reference period</td>
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<tr>
<td>S unit</td>
<td>syringyl unit</td>
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<tr>
<td>SBF</td>
<td>Scandinavian Biogas Fuels AB</td>
</tr>
<tr>
<td>SRB</td>
<td>sulphate reducing bacteria</td>
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<tr>
<td>SS</td>
<td>suspended solids</td>
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<tr>
<td>TOC</td>
<td>total organic carbon</td>
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<tr>
<td>UASB</td>
<td>upflow anaerobic sludge blanket</td>
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<tr>
<td>UV light</td>
<td>ultra violet light</td>
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<tr>
<td>UV/vis spectra</td>
<td>ultra violet and visual spectra</td>
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<tr>
<td>VFA</td>
<td>volatile fatty acids</td>
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1 Introduction

The pulp and paper industry produces vast amounts of polluted wastewater every year and total wastewater streams are high in organic material. Through anaerobic treatment of the effluents, the amount of environmental pollution can be reduced during production of methane, an energy carrier that can be used as vehicle fuel or converted to electricity or heat. (Yang et al., 2010)

Today, most pulp and paper mills employ aerobic wastewater treatment in order to reduce environmental pollutions. However, an anaerobic treatment facility has several advantages compared to the aerobic treatment system such as no requirement of aeration, less generation of sludge and the production of the energy carrier methane. In the long run, the advantages can contribute to an increase in treatment capacity and reduction of operating costs at a paper and/or pulp mill. For example, an anaerobic treatment facility can treat larger volumes of wastewater per area of space the facility occupies and the produced methane can be used internally to reduce energy costs. These advantages have increased the interest in anaerobic treatments of wastewater. (Yang et al., 2010)

This master thesis is part of a project between the company Scandinavian Biogas Fuels AB (SBF), the department Water and Environmental Studies of the Tema Institute at Linköping University and the company Pöyry AB. In regard to the master’s thesis SBF acts as the commissioning body. The project group has earlier investigated the possibility to produce biogas from the effluents of an alkaline bleaching step (alkaline extraction, oxygen and peroxide (EOP)) at a kraft pulp and paper mill. It was discovered that the usage of hardwood as raw material in the pulping process generated higher methane yields from the EOP wastewater than when softwood was used (Ekstrand et al., 2013). According to Sierra-Alvarez et al. (1990), the main compounds that cause difficulties in the biogas process are wood resin, low molecular weight derivatives of lignin and chlorinated organic compounds. These can be toxic or inhibiting to the microbes producing methane and consequently lead to lower methane yields. Most of these compounds are present in softwood at higher concentrations than in hardwood (Rowell, 2005). This master’s thesis investigates the effects of the pretreatment to the EOP wastewater with ultra-violet light (UV), when using softwood as raw material in the pulping process, in an effort to enhance the methane yield of the anaerobic treatment. The anaerobic treatment was carried out in an Upflow Anaerobic Sludge Blanket (UASB) reactor.

1.1 Aim

The primary aim of the master’s thesis was to enhance the biogas and methane yield of EOP wastewater, derived from a kraft pulping process using softwood as raw material, in a lab-scale UASB reactor through utilization of UV pretreatment. An increased methane production will result in a decreased amount of effluent total organic carbon (TOC).
1.2 Questions at Issue
The primary aim of the project served as a basis for the questions at issue and they were:

- How efficient is UV-pretreatment of EOP in terms of enhanced biogas and methane production?
- What is the optimal exposure time of UV light to the EOP wastewater in terms of enhanced biogas and methane production?

1.3 Strategy
The strategy of the project was to operate an UASB reactor at mesophilic conditions (35 °C). When the reactor was determined as stable, an UV lamp was placed upstream from the reactor. The EOP substrate was then UV-irradiated with different exposure times in order to determine the conditions giving the highest biogas and methane production. To monitor the reactor’s state and in order to collect data to answer the questions at issue, the influent and the effluent of the reactor as well as the produced biogas were analyzed throughout the experiment. The performed analyses were biogas and methane production, chemical oxygen demand (COD), TOC, volatile fatty acids (VFA), pH as well as concentrations of sulphate, suspended solids, lignin and hydrogen peroxide, respectively. Spectrophotometric UV/vis wave scans were also performed.
2 Background

2.1 Pulp and Paper Industry
The pulp and paper industry produces a vast amount of wastewater every year. The pulping process alone produces approximately 200 m$^3$ wastewater per ton pulp produced. This wastewater is highly polluted and will, if not treated, affect the environment negatively. (Thompson et al., 2001) However, the total wastewater streams also contains high concentrations of COD since the pulp product only consist of 40-45 % of the original weight of the wood that is used as raw material (Chen et al., 2008).

The raw material in the pulp and paper industry is wood, which in turn consists of cellulose fibers, lignin and carbohydrates such as starch and sugars (Thompson et al., 2001). The trees that are used as the main raw materials for pulp production in Sweden are Norway spruce and Scots pine. (González-García et al., 2009).

Pulp can be produced through three different processes; mechanical, chemical or a combination of them. The first method involves mechanical forces where debarked wood is ground in a rotating grindstone in the presence of water. This results in stripped off fibers suspended in water. The chemical process, on the other hand, utilizes chemicals in combination with heat and pressure to break down the wood to fibers. The third method that is used to produce pulp is a combination of the two methods. The wood is first pre-treated by chemicals in order to soften the wood. In a second phase, the process enables mechanical forces to complete the pulping process. (Thompson et al., 2001)

The EOP wastewater used as substrate in the anaerobic digestion (AD) system in this master’s thesis originates from a sulphate pulp and paper mill, i.e. the chemical pulping process also called kraft pulping.

2.1.1 Traditional Treatment Methods for Polluted Wastewater in the Pulp Industry
The suspended solids that are present in the wastewater from pulp and paper industries primary consists of bark particles, fiber, fiber debris, filler and coating material (Pokhrel & Viraraghavan, 2004). A primary clarification step is usually carried out by either flotation or sedimentation in which high amounts of suspended solids are removed (Thompson et al., 2001).

The most common primary clarification method in the pulp and paper industry is sedimentation. The method is based on separation of suspended solids and liquid in regard to gravimetric separation. Hence, the suspended matter must have a higher density than the liquid in order to settle. For separation to occur, the suspended solids have to reach the bottom of the basin before the wastewater exits the sedimentation plant. (UNEP, 1996)

The most common method of flotation is dissolved air flotation. The wastewater is collected in a basin where air is dissolved into the wastewater under pressure. The pressure is then released, resulting in small bubbles that adhere to suspended matter. Consequently, the suspended matter floats to the surface where it can be removed. (Edzwald, 1995)

The primary clarification is not suitable for the removal of soluble organic material, whereas a secondary treatment of biological nature often is employed. The most common biological treatment system is the activated sludge process. (Thompson et al., 2001) In this treatment method, the
wastewater and aerobic microorganisms are aerated in a tank, where the microorganisms biologically oxidize the organic material. The mixture of microorganisms, wastewater and waste sludge is then transferred to a clarifier where the biomass is separated from the liquid. The majority of the biomass is then recirculated to the aeration tank while the excess sludge is withdrawn. (UNEP, 1996; Gomes, 2009) The retention time for a facility like this is usually short, i.e. from a few hours to a couple of days. Even if the retention time is short the activated sludge process normally removes 70 to 80% of the oxygen-consuming material in the wastewater. (Kassberg et al., 1998) However, the activated sludge process is considered expensive mainly due to the intensive aeration of the wastewater (Menendez, 2013).

Another common biological treatment is the process of aerated lagoons. Instead of a fully aerated tank as in the activated sludge treatment, a surface turbine provides the necessary aeration and agitation. Another difference from the activated sludge treatment is the lack of biomass recirculation. (UNEP, 1996) These lagoons remove approximately 60 to 80% of the oxygen-consuming material and the retention time is between three to ten days. Due to the long retention time, the lagoons need to be quite large in order to achieve sufficient degradation of organic material. Thus, a facility like this requires a large land area in comparison to the activated sludge process. (Kassberg et al., 1998)

2.2 Kraft Pulping

Sulphate pulping, or kraft pulping as it is also called, is the most dominant process when producing pulp. It was accidentally discovered in 1879 by Dahl, a German chemist that added sodium sulphate to the soda process in an attempt to regenerate sodium hydroxide (NaOH). It was discovered that disodium sulphide (Na₂S) was formed instead of NaOH. The addition of sodium sulphate resulted in stronger pulp, faster delignification and consequently shorter cooking times. In the cooking process, the pH is held above 12 and the temperature between 160 and 180 °C for approximately 0.5 to 3 hours in order to dissolve as much lignin in the wood fibers as possible. (Biermann, 1996)

Even if the process of kraft pulping removes lignin from pulp, additional bleaching is often necessary in order to fulfill the customers’ specification of requirements regarding brightness. Hence, the brightness of unbleached kraft pulp is approximately 20%, which could be compared to a brightness of 75% in white tablet paper. (Biermann, 2006)

2.3 Elemental Chlorine Free Bleaching

As a result of stricter regulations from authorities regarding highly chlorinated environmental pollutions originating from bleaching facilities, the most common bleaching method is Elemental Chlorine Free (ECF). Chlorine dioxide, molecular oxygen, hydrogen peroxide and ozone are the compounds that act as the replacements of elemental chlorine in ECF bleaching sequences. Thereby, the amount of hazardous chlorinated compounds in the effluents is reduced. (Tarkpea et al., 1999)

The bleaching process consists of a bleaching sequence where letters represent different stages of the bleaching process. D stands for chlorine dioxide bleaching, E for alkaline extraction, O for oxygen delignification and P for hydrogen peroxide bleaching (Kukkola et al., 2006).

2.3.1 Chlorine Dioxide Bleaching (D)

In this bleaching stage, chlorine dioxide is utilized at a pH below 5 in water in order to oxidize lignin. Under these conditions, chlorine dioxide extracts electrons from the organic material in the pulp and
enters different states of oxidation. In the initial bleaching reaction, chlorine dioxide (ClO$_2$) transforms into chlorite (ClO$_2^-$) by extraction of hydrogen from phenolic groups and dissociation of the resulting chlorous acid (HClO$_2$). The attack on phenolic groups by chlorine dioxide leads to phenoxy radicals, which in turn also react with chlorine dioxide. The result of this reaction either leads to ring opening with the generation of muconic acid structures or side chain elimination with quinones as end product. (Suess, 2010)

A second reaction with chlorite generates chlorine dioxide derivatives that interact with the lignin and the rest of the pulp, i.e. hypochlorous acid (HOCl) and chlorine. The result is chlorinated organic compounds and chloride ions. The chlorination of organic compounds by hypochlorous acid is less prominent than with chlorine due to different reaction pathways. Regardless of the outcome of the chlorine dioxide bleaching process, it needs to be succeeded with an extraction stage. Hence, some of the lignin residues only become soluble in water at high pH and high temperatures. (Suess, 2010)

2.3.2 Alkaline Extraction (E)
The oxidized lignin is solubilized at this extraction stage with caustic soda where the pH is usually held between 9.5 and 11 and the temperature at 65°C. As a result of this bleaching stage, phenols and carboxylic acids dissolve as sodium salts that further enhance the delignification process. In addition to solubilizing oxidized lignin, the alkaline extraction step also contributes somewhat to removal of chlorinated organic compounds. Hydroxyl anions (OH$^-$) reacts with the halogenated compounds through nucleophile substitution and chlorine atoms are released. (Suess, 2010)

2.3.3 Oxygen Delignification (O)
The oxygen delignification stage oxidizes lignin under alkaline conditions, at a temperature between 90°C and 100°C, with molecular oxygen. The delignification process is initiated by the actions of diradical oxygen extracting hydrogen from phenolic hydroxyl groups or electrons from phenolate anions. As a result, new radicals such as phenoxy or quinone methide radicals are generated that subsequently undergo intramolecular nucleophile attacks. Thereafter, the lignin polymer is solubilized via carboxylates due to the alkaline conditions. (Suess, 2010)

However, it is not only the lignin component of the pulp that is affected by oxygen delignification. A variety of oxygen containing radical species exists during the process that each have different reaction mechanisms with the organic material in the pulp. (Suess, 2010)

2.3.4 Hydrogen Peroxide Bleaching (P)
This bleaching stage consists of alkaline bleaching with hydrogen peroxide (H$_2$O$_2$). Typically, the temperature is held between 60°C and 90°C and the pH between 10 and 11. The active compound in the bleaching reactions is the perhydroxyl anion (HOO$^-$), which is in equilibrium with hydrogen peroxide at alkaline conditions. Through nucleophile attacks, this bleaching agent adds to quinone structures and eliminates side chains of lignin. The reaction mechanisms are similar to those of the hypochlorite anions in the chlorine bleaching stage, but the solubility of the oxidized compounds is higher as a result of the alkaline conditions. (Suess, 2010)

2.3.5 Alkaline Extraction with Addition of Oxygen and Peroxide (EOP)
In this stage, the alkaline extraction is accompanied by the addition of oxygen and hydrogen peroxide without washing steps between the additions. Thus, it can be seen as a merged bleaching stage with steps of alkaline extraction, oxygen delignification and hydrogen peroxide bleaching. The additions of
oxygen and hydrogen peroxide have similar effects on lignin as in the individual bleaching stages. Though, the efficiencies are somewhat lower as a result of other operating conditions. (Suess, 2010)

The conditions of this bleaching stage are similar to the one in the oxygen delignification stage with the exception of a lower temperature. However, the temperature has to be around 75°C for the addition of oxygen to be effective. At a temperature of 65°C, which is the usual temperature for a conventional alkaline extraction, the addition of oxygen does not contribute to an enhanced delignification process. (Suess, 2010)

2.4 Organic Material in Bleaching Wastewater

The composition and characteristics of the organic material found in bleaching wastewater depend on a variety of factors, e.g. the type of pulping process, raw material and bleaching sequence utilized in the process. However, the main component is dissolved lignin, i.e. degraded lignin of different severity. The organic material found in the wastewater mostly consists of degradation products from lignin, wood extractives, cellulose and hemicellulose. The liquor created from one ton of pulp in the alkaline bleaching process of kraft pulp contains about 43 kg of degraded lignin, 16 kg of carbohydrates and about 1 kg of wood extractives. (Kettunen, 2006)

2.4.1 Dissolved Lignin

The majority of the organic material in the EOP effluent is dissolved lignin since the ultimate aim of a bleaching process is to increase the brightness of the pulp. The result of delignification during bleaching is a yellow coloration of the wastewater. Consequently, the EOP wastewater is yellow, see figure 1, which according to Thompson et al. (2001) is an indicator of dissolved lignin.

In nature, lignin functions as an adhesive in the cell wall between the cellulose and hemicellulose since it holds the cellulosic matrix together. It is a hydrophobic molecule that covalently binds to hemicellulose. (Hu & Ragauskas, 2012) There are three different monomeric structures of lignin that serves as model compounds for lignin, since there are many derivatives. The monomeric structures are p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. The structures are illustrated in figure 2 and natively the monomers form a strong phenolic polymer. (Zakzeski et al., 2010) The units of the monomeric structures that are a part of the polymeric lignin are called p-hydroxyphenyl (H), guaiacyl
(G) and syringyl (S). The unit H is derived from the monomeric structure of p-coumaryl alcohol, the unit G from coniferyl alcohol and the unit S from sinapyl alcohol. (Hu & Ragauskas, 2012)

![Chemical structures of lignin monomers](image.png)

*Figure 2: Three monomeric molecular structures of lignin; p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. Used with permission from Zakzeski et al. (2010).*

However, lignin is not just present as a complex polymer in the wastewater of a bleaching process as lignin is degraded into smaller fractions of different severity. Some of these degradation products result in chlorinated phenols as a result of the combination of lignin and chlorine dioxide. These molecules are potentially toxic to anaerobic bacteria and further decrease the biodegradability of bleaching wastewater. (Vidal & Diez, 2005)

Kukkola et al. (2006) investigated the composition of high molecular weight fractions in ECF bleaching wastewater (OD(EOP)DED) with softwood as raw material. They concluded that guaiacyl (G) derivatives from lignin were the most generated phenolic pyrolysis products in the EOP bleaching stage. Furthermore, the second most common phenolic pyrolysis product was 4-hydroxyphenyl (H) derivatives and least common derivatives of lignin in the EOP effluent originated from syringyl (S) units.

Sierra-Alvarez et al. 1990 evaluated the anaerobic biodegradability of paper mill wastewater constituents. They found that many of aromatic low molecular weight derivatives from lignin were non degradable in anaerobic systems. Among them were eugenol and benzene. They drew the conclusions that eugenol was non degradable due to the presence of alkyl side chains on the aromatic ring which increases its resistance to microbial attack. Regarding the benzene, they argue that the lack of polar functional groups is the main reason for it resisting microbial attack.

### 2.4.2 Degradation Products of Wood Extractives

Wood extractives are chemicals that can be extracted by using dissolvent agents and are located in the cell walls of the wood. The chemicals mainly consist of organic compounds such as fats, fatty acids, fatty alcohols, terpenes, phenols, resin acids, steroids, waxes and rosin. In nature, some of the extractives are produced in response to wounds and others are produced when the wood is under attack as a part of its defense system. (Rowell, 2005)

Resin and other extractives are removed from chemically produced pulp during the bleaching process (Bajpai, 2010). The majority of wood extractives that is present in the total wastewater stream of pulp and paper industries originate from the debarking facilities or the bleaching process (Leiviskä et al., 2009). According to Sierra-Alvarez et al. (1990), some of these wood extractives such as volatile terpenes and resin acids are persistent to AD.
2.4.3 Carbohydrates

Cellulose and hemicellulose are the main components of wood. Cellulose makes up approximately 45% of the wood’s dry weight, while the amount of hemicellulose constitutes about 25 to 30% (Pérez et al., 2002). As the pulp is bleached, hemicellulose and small amounts of cellulose are found in the effluents of the bleaching process in the form of carbohydrates (Biermann, 1996) even though cellulose is the desirable component of pulp.

Units of D-glucopyranose make up the molecule of cellulose, which in turn is a linear glucan polymer. However, the building block of the molecule is called cellobiose due to the repeating unit in cellulose that consists of two sugar units, (figure 3). (Rowell, 2005) The hydroxyl groups located on cellulose polymer can form intramolecular and intermolecular hydrogen bonds. The intramolecular linkages increase the stiffness of the single linear polymer while the intermolecular bonds give rise to supramolecular structures such as cellulose fibrils. (Wood: chemistry, ultrastructure, reactions, 2011)

![Figure 3: Molecular structure of cellobiose, i.e. the building block of the polymeric compound of cellulose. Modified from Rowell (2005).](image)

Hemicellulose is also a polysaccharide, but the degree of polymerization is less than in cellulose and the polymers are branched instead of linear. The polymeric structure mainly consists of the sugars D-xylopyranose, D-glucopyranose, D-galactopyranose, L-arabinofuranose, D-mannopyranose, D-glucopyranosyluronic acid and D-galactopyranosyluronic acid. The monomeric units of these sugars in hemicellulose are illustrated in figure 4. (Rowell, 2005) In nature, hemicellulose surrounds the cellulose fibrils, thus forming larger supramolecular structures than the cellulose chains alone. These supramolecular structures are further supported and linked together by complex structure of lignin creating mechanical strength for the wood. (Kettunen, 2006)

![Figure 4: The monomeric sugar units of hemicellulose in wood; β-D-Glucose, β-D-Mannose, β-D-Galactose, β-D-Xylose, 4-O-Methylgucuronic acid and α-L-Arabinose. Modified from Rowell (2005).](image)
2.5 Biogas

The quest to find alternatives to fossil fuel for production of electricity, heat and vehicle fuel is an important mission where biogas is a piece of the puzzle. Biogas can be utilized as vehicle fuel and generation of heat and electricity. Approximately 50 % of the produced biogas in Sweden is utilized for heating purposes, 25 % for vehicle fuel and 10 % for electricity generation. (Börjesson & Ahlgren, 2012) Biogas is an energy carrier that can be produced from renewable sources or from treatment of waste and organic residues through AD. It is a gaseous mixture of mainly carbon dioxide (CO$_2$) and methane (CH$_4$) where methane is the energy rich component of the mixture. The organic matter that serves as substrate often originates from different kinds of waste, which otherwise pose as a burden for the society and the environment in terms of financial expenses and hazardous pollutions. In the perspective of waste management, anaerobic treatment is considered the most cost-effective approach among biological treatments. The usage of waste is one of the reasons for the increase of interest for biogas as a partial substitution for fossil fuels over the last couple of years. (Gupta et al., 2012)

Another reason for the peak in interest for AD is the posing threat of global warming in the world (Tauseef et al., 2012). AD of waste occurs naturally and the emission of methane to the atmosphere contributes to the greenhouse effect. Actually, methane carries higher global warming potential than carbon dioxide. Today, methane has the second greatest effect on the global warming after carbon dioxide. However, the atmospheric concentration of methane has steadily increased during the 20$^{th}$ century and the trend seems to continue if actions are not taken. In order to reduce methane emission to the atmosphere, the anaerobic digestion of waste can be performed in a controlled manner and the produced methane gas can be captured. (Kumar & Imam, 2013)

2.6 Anaerobic Digestion

The process of AD is depicted in figure 5 and consists of four different steps; hydrolysis, acidogenesis, acetogenesis and methanogenesis. The overall aim of AD is the production of the energy carrier methane. However, as the name anaerobic indicates, the process must take place in an oxygen free environment in order to produce the desirable methane. (Appels et al., 2008)

![Figure 5: A schematic illustration of the biogas process, i.e. the degradation of organic matter by the four steps of hydrolysis, acidogenesis, acetogenesis and the methanogenesis. Modified from Appels et al. (2008) and Schink (1997).]
Throughout the biogas process there is a consortium of microbes involved that interact and affect each other in a highly complex manner. The microbes active in the different degradation steps are highly dependent on each other and if one group of microbes are missing or failing to execute its task, the production of biogas as end-product is no longer performed. The operational collaboration among the microbes is called a syntrophic relationship. (Schink, 1997)

In the hydrolysis (figure 5), suspended organic matter is degraded to soluble organic matter by the action of extracellular hydrolytic enzymes. These enzymes are produced by the microbes that are active in the acidogenesis step of the AD process. (Appels et al., 2008; Schink, 1997)

The second step of the AD is the acidogenesis where the products from the hydrolysis are further degraded into volatile fatty acids (VFA) (figure 5). Besides VFA, ammonia (NH₃), carbon dioxide (CO₂), hydrogen (H₂) and hydrogen sulfide (H₂S) are also produced in this step (Appels et al., 2008). Some of these compounds, such as acetic acid, H₂, CO₂ and other one-carbon compounds can enter the step of methanogenesis without passing the acetogenesis (Schink, 1997). Remaining organic compounds are further degraded in the step of acetogenesis before the end-products are produced.

The step of the acetogenesis utilizes the remaining VFAs that are produced in the previous stage. The compounds are mainly converted to acetic acid, hydrogen (H₂) and CO₂, which acts as substrates in the final conversion step (figure 5). (Appels et al., 2008)

In the final step of the AD process, the methanogens are the active microorganisms that produce CH₄ and CO₂ utilizing products from previous stages. This mixture of gas is also known as biogas with a content of approximately 50-75 % CH₄ and 25-45 % CO₂ depending on the substrate (Ziemiński & Frąc, 2012).

2.6.1 TOC and COD
The measurement of TOC determines the amount of carbon by oxidizing organic compounds to carbon dioxide (Bourgeois et al., 2001). The available organic carbon is vital in the biogas process since organic matter is a fundamental element in the production process of biogas. It also serves as a measurement of environmental pollutions in wastewaters. However, all TOC might not be easily or not at all biodegradable without some sort of pretreatment (Puyuelo et al., 2011). COD is a measurement of the amount of oxidizable compounds in a media and is also often used to evaluate environmental pollutions in lakes or wastewater streams. The foundation of the analysis is that almost all organic compounds can be oxidized under acid conditions by strong oxidizing agents. More specific, COD is a measurement of the amount of oxygen that is required to oxidize a compound to carbon dioxide and water. (Bourgeois et al., 2001)

2.6.2 VFA and pH
Accumulation of VFA in the reactor can lead to inhibition of the methanogens, which in turn leads to lower biogas production. As a result of VFA accumulation, the pH in the reactor is lowered. (Appels et al., 2008) The microorganisms that are active in the biogas process are highly dependent on the pH as different groups of microorganisms in the biogas process, i.e. the active microorganisms in the hydrolytic step, the fermentation step and the methanogens thrive in different pH ranges. The most sensitive group of organisms, in regard to pH changes, is the methanogens. (Appels et al., 2008)
2.6.3 Sulphate Concentration
Among others, the microbe consortia consist of sulphate-reducing bacteria (SRB) which compete with the hydrogen-oxidizing methanogens. With excess sulphate in the reactor, the sulphate-reducing bacteria can utilize the hydrogen at lower partial pressure than the methanogens, i.e. the Ks value for hydrogen of the sulphate-reducing bacteria is more favorable than the one of the methanogens. In the end, the methanogens will be outcompeted and consequently produce less methane. (Appels et al., 2008)

2.6.4 H2O2 and Lignin Concentration
The UV absorption spectra of lignin exhibit two peaks. The first peak, which is the highest, is located around 279-280 nm. At this wavelength, the absorption originates from non-conjugated phenolic groups of the lignin molecule. These non-conjugated phenolic groups that are present in lignin are rich in G and S units. At a wavelength of 316-320 nm there is a second peak in the UV spectra, which originates from conjugated phenolic groups that are rich in H units and ferulic acid. (Rowell, 2005; Yang et al., 2013)

The adsorption of lignin at 280 nm enables a spectrophotometric method to monitor changes in lignin concentration. However, the method is rough since other phenolic groups which are not lignin molecules also absorb at this wavelength and there is nothing differentiating them. Another factor that brings uncertainty to the method is the adsorption coefficient for lignin used in the calculations. This coefficient varies significantly depending on the origin of the lignin and the processes used in the pulp and paper industry that affect the structure and composition of the lignin. (Lin & Dence, 1992)

The H2O2 concentration is an indirect measurement of the performance of the UV/H2O2 process (section 2.8). Hence, no H2O2 in the system results in merely direct photolytic effects of the applied UV light and reduces the effectiveness of the pretreatment (Jamil et al., 2011).

2.7 UASB
The UASB reactor was developed by Gatze Lettinga and coworkers at Wageningen University in the 1970s when experimenting with an anaerobic filter. They observed that the biomass had developed into free granular aggregates and eventually compact granules. These granules are the formation of the microorganisms in the UASB reactor, which fundamentally hinders them from leaving the system through the effluent. (Tauseef et al., 2013)

The UASB reactor is a high-rate anaerobic digester. This type of reactor operates in a continuous mode, where the wastewater enters the reactor in the bottom and flows upward through a blanket of granular sludge as illustrated in figure 6. The upward flow enables efficient mixing and contact between the consortia of microbes involved in the anaerobic digestion and the substrate. The production of biogas also enables some agitation when the gas rises to the top of the reactor where the gas-liquid-solid separation device is placed. This device separates the biogas from the liquid while retaining the solid matter, i.e. the granular blanket, before the liquid exits the system. Hence, the UASB reactor has a hydraulic- and a solid retention time. (Tauseef et al., 2013)
Figure 6: Schematic illustration of a UASB reactor. Modified from Tauseef et al. (2013).

2.8 Photochemical Degradation Process

In terms of degrading refractory organic compounds, advanced oxidation processes (AOPs) like UV and H$_2$O$_2$, have the ability to alter the composition of functional groups, molecular structure, molecular weight distributions and physical-chemical and biological characteristics of natural organic matter (NOM). The two foremost effects on NOM are minor alterations of functional groups without breakdown of macromolecule structures and breakdown of aromatic moieties into smaller compounds such as aliphatic organic acids. (Song et al. 2004)

Radicals are intermediates that possess unpaired electrons. In order to generate radicals, energy must be supplied for the hemolysis of covalent bonds. This can be achieved in two ways, i.e. by heating or irradiation of light. (Solomons et al., 2008)

UV radiation and hydrogen peroxide (H$_2$O$_2$), which are one of the chemicals added in the bleaching process and therefore already present in the EOP effluent, are well-known oxidizing agents. When the EOP effluent is irradiated with the UV light a photochemical degradation process (UV/H$_2$O$_2$) takes place where hydroxyl radicals (OH•) are produced, see equation 1. (Catalkaya & Kargi, 2007)

\[
H_2O_2 + h\nu \rightarrow 2OH •
\]  

(1)

The generation of hydroxyl radicals takes place due to the weak nature of the oxygen-oxygen bond in peroxides. In general, a collision between a radical and another molecule tend to result in pairing of the unpaired electron in the radical. The outcome of a collision like this either leads to extraction of an atom from the other molecule or the radical combines itself with the compound if it contains multiple bonds. However, either way, the reaction results in a new larger radical. (Solomons et al., 2008)

The OH• attacks the aromatic rings in the organic matter due to the double bond in the aromatic structures of lignin. (Jamil et al., 2011) A schematic illustration of reactions with lignin structural
elements and the hydroxyl radical can be seen in figure 7. The OH• have the ability to degrade refractory organic compounds efficiently (Catalkaya & Kargi, 2007).

UV light on its own also has the ability to degrade lignin. However, the efficiency in regard to COD removal is lower in comparison to the combined UV/H$_2$O$_2$ process. The direct UV photolytic process is based on supplying energy to reactant molecules that absorb the energy of the UV light. Consequently, the molecules enter excited states. These molecules then have the ability to promote reactions leading to degradation of organic material. (Jamil et al., 2011) Some chromophoric compounds that are formed from lignin as a result of UV irradiation are depicted in figure 8.

The aim of the UV pretreatment was to decompose large lignin complexes and to reduce the amount of aromatic structures in the organic material since they often are inhibitory and persistent to microorganisms in the AD process. Therefore, the second reaction pathway illustrated in figure 7 and the quinone structure in figure 8 were desirable in this thesis in order to enhance the CH$_4$ production and increase the reduction of TOC in the EOP wastewater. (Mudhoo, 2012; Sierra-Alvarez et al., 1990) Another desirable structure is the radical that is produced in the direct UV photolysis of lignin since radicals often undergo further reactions with other molecules.
3 Material and Methods

3.1 Process Setups

During the experiment, different process setups were used due to problems such as failing equipment and inhibitory reasons. The setups are illustrated as flow charts in figure 9. The first setup (a) consisted of a substrate tank and a peristaltic pump pumping substrate into the reactor. From the reactor, liquid was circulated back into the reactor while the excess liquid left the system as effluent. The produced biogas exited the reactor at the top and passed a condensation flask and a gas meter before it entered the gas balloon where it was collected. The second setup (b) consisted of biogas circulation instead of liquid circulation where the produced biogas was circulated back into the reactor in order to create agitation. In the third setup (c) no circulation was applied. In the fourth setup (d), a drying agent flask and a zinc oxide flask was mounted into the biogas system with the intention to strip hydrogen sulphide from the biogas before it reentered the reactor. The final setup (e) consisted of no circulation and with the addition of a UV lamp mounted prior to the reactor.

Figure 9: Schematic illustrations of the process setups during the experiment; anaerobic digestion with a) liquid circulation, b) biogas circulation (H₂S present), c) no circulation, d) biogas circulation (H₂S stripped) and e) final setup with UV lamp mounted prior to the reactor with no circulation.
3.2 The UASB Reactor

The reactor that was used in the experiment (*figure 10*) had a working volume of approximately four liters and a hydraulic retention time (HRT) of 12-15 hours. A temperature of 35°C was maintained in the reactor by circulating water in the heating jacket that was adjusted to the right temperature by an external water bath (Lauda Alpha A12, Lauda-Brinkmann, Germany). The substrate was continuously pumped into the reactor by a peristaltic pump (Watson Marlow Sci 323, Watson-Marlows Pumps Group, United Kingdom) at a speed of 3 rounds per minute.

*Figure 10: A photograph of the UASB reactor utilized in the experiment where the cylindrically shaped glass construction comprised four liters of liquid.*

In order to aid the release of the produced biogas from the granules, additional movement in the bed was applied. This was accomplished by recirculation of produced biogas (*figure 9a, b and c*). A secondary effect of the recirculation of biogas is an increase in contact between the granules and the substrate, with the objective to increase the biogas production. However, the produced biogas contains hydrogen sulphide (H$_2$S). In order to strip the biogas of this compound before recirculation to avoid inhibition, the biogas was led through a flask containing zinc oxide (ZnO) that bind hydrogen sulphide. The ZnO was mixed with water to generate a paste which was then dried in the form of beads in order to prevent the ZnO from leaving the flask. A flask containing drying agent was placed prior to the ZnO flask to prevent the beads from sticking together and harden.

3.2.1 Substrate and Additives

As mentioned before, the reactor was run on wastewater from an alkaline bleaching step at a kraft pulp and paper mill. The complete bleaching sequence at the kraft pulp and paper mill where the EOP wastewater was collected was D0(EOP)D1P. Due to the alkaline nature of the substrate (approximately pH 10.5), the pH was adjusted to roughly 7.5 in order to accommodate the well-being of the microorganisms in the reactor.
Nutrients such as phosphorus (P) and nitrogen (N) were also added to the substrate in order to create a more favorable environment for the microorganisms to thrive in. The pH adjustments and the addition of nutrients were performed prior to feeding. Once a week, trace elements were added to the UASB reactor in order to stimulate the activities of the microorganisms. According to Bayr et al. (2012), the microorganisms require trace elements as building blocks in order to grow and to other purposes as well, such as enzymatic activities. The added elements were iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), nickel (Ni), molybdenum (Mo), selenium (Se) and tungsten (W). The added concentrations were based on the level of COD in the substrate. The concentrations of the nutrients and the trace elements are presented in table 1.

Table 1: The concentrations of the nutrients added to the substrate prior to feeding and the trace elements added once a week. The concentrations are based on the level of COD in the substrate.

<table>
<thead>
<tr>
<th>Origin of Nutrient/Trace Element</th>
<th>Nutrient/Trace Element</th>
<th>Concentration (mg/g COD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na₂HPO₄·2H₂O</td>
<td>P</td>
<td>2.86</td>
</tr>
<tr>
<td>(NH₄)₂CO</td>
<td>N</td>
<td>14.3</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>Fe</td>
<td>2.70·10⁻²</td>
</tr>
<tr>
<td>CoCl₂-6H₂O</td>
<td>Co</td>
<td>2.85·10⁻³</td>
</tr>
<tr>
<td>CuCl₂·2H₂O</td>
<td>Cu</td>
<td>3.07·10⁻³</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>Zn</td>
<td>3.16·10⁻³</td>
</tr>
<tr>
<td>NiCl₂·6H₂O</td>
<td>Ni</td>
<td>2.84·10⁻³</td>
</tr>
<tr>
<td>(NH₄)₆Mo₇O₂₄·4H₂O</td>
<td>Mo</td>
<td>4.64·10⁻³</td>
</tr>
<tr>
<td>Na₂SeO₃·5H₂O</td>
<td>Se</td>
<td>3.82·10⁻³</td>
</tr>
<tr>
<td>Na₂WO₄·2H₂O</td>
<td>W</td>
<td>8.89·10⁻³</td>
</tr>
</tbody>
</table>

3.3 Source of UV Light

The UV light is provided by a medium pressure mercury (Hg) lamp of the brand Heraeus (Germany). It was mounted upstream from the UASB reactor in an online manner to accommodate a continuous flow through the system. The exposure time was adjusted by varying the number of quartz tubes in which the substrate was flowing adjacent to the UV lamp. See figure 11 for a schematic illustration of the setup of the UV lamp and figure 12 for a photograph of the UV device in the dark where one quartz tube was connected. The quartz tubes were, according to the supplier, susceptible to UV light. From 260 nm to longer wavelengths of the UV spectra the tubes let through 95 % of the UV light. Between 200 and 260 nm the tubes allow about 80 % of the UV light to pass the glass. The UV lamp was enclosed in another quartz tube; however, the UV transmission for this tube was unknown.

Figure 11: A schematic illustration of the UV pretreatment device. The substrate was lead through quartz tubes which were positioned adjacent to the UV lamp.
3.3.1 UV Exposure Times
The exposure times of UV to the substrate were calculated, see appendix A, and the results were 2.6, 5.2, 7.4, 10, 13 and 16 minutes respectively. The length of the exposed quartz tubes was determined for the calculation the exposure times. The length was defined as the length of the crackled surface on the outer enclosing tube of the UV device. Hence, the crackled surface was caused by the irradiated UV light.

3.4 Design of the UV Device
For the duration of the experiment, two different types of designs of the UV device were applied to the system. The first design of the UV device applied in the system was rejected due to practical issues. However, in this design, the substrate was led directly in the outer enclosing tube (figure 11).

A second design of the UV device was then developed, see figure 11, where the issues of the first design were eliminated. This design allowed a constant flow of substrate adjacent to the UV lamp and it also allowed the exposure time to be varied in a simple manner than in the first design. This design was used when the substrate was exposed to 2.6 and 16 minutes of UV light, respectively.

3.5 Unwanted Microbial Growth
Throughout the experiment, unwanted microbial growth was observed in the system. The affected equipment was frequently cleaned with ethanol and irradiated with UV light in an effort to remove the unwanted growth in the system. Though, one tube could not be disconnected without emptying the entire reactor content.

3.6 Parameters and Analyses
During the experiment, a number of parameters were monitored to investigate and evaluate the effect of the UV-pretreatment on the biogas and methane production from the EOP wastewater. The effluent of the reactor was, like the biogas samples, analyzed twice a week. The substrate was analyzed upon arrival of a new batch every other week from the kraft pulp and paper mill. The analyses performed during the experiment were TOC, COD, VFA, pH, concentrations of $\text{SO}_4^{2-}$ and
suspended solids. When the substrate was pretreated with UV light, concentrations of lignin and $\text{H}_2\text{O}_2$ were determined as well as UV/vis spectrophotometric wave scans were performed.

3.6.1 Biogas Analyses
In order to monitor the methane production, biogas samples were collected from the condensation flask and analyzed twice a week in a Gas Chromatograph with a Flame Ionizing Detector (GC-FID; Hewlett Packard, 5880A Series Gas Chromatograph, USA) with nitrogen as carrier gas. The injector temperature was set to 150 °C, the detection temperature to 250 °C and the oven temperature to 80 °C. The obtained result of the analysis in the GC-FID is the percentage of methane in the produced biogas. The total biogas production was monitored on a daily basis utilizing a gas meter (Ritter MilliGas Counter type MGC-1 PMMA, Germany), which utilizes liquid displacement to measure the biogas production. The data of biogas and methane production were transformed into normalized volumetric units (NmL), i.e. the volumetric transformation of the produced biogas and methane to theoretical gas volumes at 0 °C.

3.6.2 TOC and COD
The TOC and COD analyses were conducted with kits from Hach-Lange (Hach-Lange, Germany) in order to evaluate the amount of organic material in the reactor that was transformed into biogas. The kits used were LCK387, LCK014 and LCK514. The samples were filtrated (grade MGA; Munktell Filter AB, Sweden) prior to analysis in order to determine the amount of solved TOC and COD in the specimen, i.e. $\text{TOC}_{\text{sol}}$ and $\text{COD}_{\text{sol}}$. The executions of the analyses were performed in triplicates and according to the manufacturer’s instructions.

3.6.3 VFA and pH
The level of VFA in the reactor was another important parameter to observe during the experiment since accumulation of VFA indicates whether or not the biogas reactor was stable. The VFA measurements were conducted in a Hewlett Packard 6890 Series GC System (USA) that has a detection limit of 0.2 mM and quantification limit of 0.6 mM. The pH meters utilized for measurements were PHM 93 (Radiometer, Copenhagen) and inoLab pH 7310 (WTW, Germany). The pH was measured in triplicates succeeding measurements of a control solution.

3.6.4 Sulphate Concentration
Since the source of the substrate was a kraft pulp and paper mill, the level of sulphate in the reactor could have affected the methane production. The concentration of sulphate in the influent and the effluent was measured using kits from Hach-Lange (Hach-Lange, Germany), i.e. LCK153 and LCK353. The samples were filtrated (grade MGA; Munktell Filter AB, Sweden) prior to measurements in order to determine the solved amount of sulphate in the specimens, that is $\text{SO}_4^{2-}_{\text{sol}}$. The measurements were conducted in triplicates and according to the manufacturer’s instructions.

3.6.5 Determination of Suspended Solids
The determination of suspended solids (SS) in the effluent of the reactor was conducted to monitor whether or not the granular sludge was leaving the system. The samples were filtrated through a glass fiber filter of the grade MGA (Munktell Filter AB, Sweden) and a glass microfiber disc with a pore size of 1.6 µm (Munktell Filter AB, Sweden). The glass fiber filter was then dried at 105 °C between 8 and 16 hours. The filter was weighed before and after the filtration in order to determine the amount of SS in relation to the volume of the filtrated sample. The method was performed in triplicates and according to the standard SE-EN 872:2005 (Swedish Standards Institute, 2005).
3.6.6 Lignin Content

Lignin was one of the most resilient molecules in the substrate at high concentrations, which is why it was of interest to investigate the effects of UV irradiation on lignin. Since the lignin in the EOP wastewater originates from softwood, the wavelength of 280 nm was of interest in the experiment. After a discussion with Svenska Cellulosa Aktiebolaget the most fitting adsorption coefficient in regard to the EOP wastewater was selected (21.5 L/(g·cm)). The method was used in the experiment to monitor the effect of the UV-pretreatment on the EOP wastewater in regard to the lignin content. There were three sampling points; where sampling point I was the substrate tank, sampling point II after the UV pretreatment and sampling point III after the reactor. In order to investigate changes in the substrate, spectroscopic wave scans (samples collected at the three sampling points) between 190 and 700 nm were also performed.

Prior to analysis, the samples were diluted 1:15 or 1:16 depending on the characteristics of the substrate batch used in the process. In order to avoid interference to the greatest extent possible, the samples were filtrated through a glass fiber filter of the grade MGA (Munktell Filter AB, Sweden) prior to the analyses. The UV/vis spectrophotometer utilized to perform the UV/vis analyses was an Ultrospec 2100 pro (Amersham Pharmacia Biotech, Sweden). The lignin content was measured in triplicates.

3.6.7 Hydrogen Peroxide Concentration

The H₂O₂ parameter was part of the experiment in order to evaluate the performance of the UV/H₂O₂ process. The concentration of hydrogen peroxide was determined in sampling point I, II, and III during the experiment using colorimetric test strips (Merck, Germany). The test strips were a fast method to roughly estimate the concentration of peroxide in the samples. Three intervals were used in the experiment; 0.5-25, 1-100 and 100-1000 mg/L H₂O₂. The samples were filtrated through a glass fiber paper of the grade MGA (Munktell Filter AB, Sweden) prior to analysis in order to eliminate possible interferences to the tests. The tests were conducted according to the manufacturer’s instructions.

3.6.8 Statistical Analyses

For the statistical evaluation of the data sets regarding biogas and methane production as well as reduction of CODₜ₉₀, TOCₜ₉₀ and SO₄²⁻₉₀, Student’s paired t-test was applied (Montgomery et al., 2007). The data sets in each period were assumed to have different variance and a normal distribution. The tests were two sided with α = 0.05, i.e. a confidence level of 95 %. The hypotheses are formulated below, where H₀ is accepted if the p-value is > 0.05 and H₀ is rejected and H₁ accepted if the p-value < 0.05.

H₀: There is no significant difference between the compared data sets
H₁: There is significant difference between the compared data sets
4 Results

4.1 Substrate Characteristics
The different batches of substrate were designated SB1 through SB8 and their characteristics are compiled in table C.1 in appendix C.1.

4.2 Time Periods
The collected data of the lab-scale UASB was divided into five different time periods designated BC+H$_2$S (biogas circulation with H$_2$S present), REF (reference), BC-H$_2$S (biogas circulation without H$_2$S present), UV2.6 (2.6 minutes of UV exposure time) and UV16 (16 minutes of UV exposure time). Information of the time periods is compiled in table 2. Time periods BC+H$_2$S, REF and BC-H$_2$S were all run without UV pretreatment.

<table>
<thead>
<tr>
<th>Time period (days)</th>
<th>BC+H$_2$S</th>
<th>REF</th>
<th>BC-H$_2$S</th>
<th>UV2.6</th>
<th>UV16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biogas circulation (H$_2$S)</td>
<td>12-21</td>
<td>37-41 and 50-57</td>
<td>69-75 and 82-90</td>
<td>108-115 and 117-127</td>
<td>131-137 and 139-142</td>
</tr>
<tr>
<td>No circulation (no H$_2$S)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>UV exposure time</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.6 min</td>
<td>16 min</td>
</tr>
</tbody>
</table>

BC+H$_2$S ranged from day 12 to day 21, while REF ranged from day 37 to day 57 with day 42 through 49 excluded due to practical issues with the reactor which resulted in unreliable values. BC-H$_2$S lasted from day 69 to day 90 with day 76 through 81 excluded due to lowered biogas production caused by unwanted microbial growth in the system.

In time periods UV2.6 and UV16 (figure 9e), the substrate was pretreated with UV light. Both of these periods were run without any circulation due to practical issues with the biogas circulation and process performance. Consequently, REF was designated the reference period in the evaluation of UV light as a pretreatment method. The time period UV2.6 lasted from day 108 to day 127 with day 116 excluded from the data set due to a power outage, thereby resulting in a failure to record data for this day. The UV exposure time for this period was 2.6 minutes. In UV16, the exposure time was 16 minutes and it ranged from day 131 to day 142 where day 138 was excluded due to issues with the peristaltic pump.

4.3 Parameters and Analyses

4.3.1 General Performance and Process Stability
Diagrams of parameters VFA, pH and SS can be found in appendix C.2, C.3 and C.4; figure C.I, C.II and C.III. During the experiment, the level of VFA in the reactor was almost undetectable indicating a stable anaerobic process. However, some levels of acetic acid were detected on day 15 through 22. The highest concentration of acetic acid during this period was 1.4 mM. On day 61 and 68, acetic acid concentrations were once again, 0.9 and 4.0 mM respectively. The pH was determined to 7.9±0.2 during the run of the reactor, i.e. a mean value of the measurements during the experiment. The
concentration of SS was initially high with the highest concentration of 112 mg/L, but stabilized at a lower level when the biogas circulation with H₂S present ended.

4.3.2 Methane and Biogas Production

The normalized methane and biogas production per grams of solved ingoing COD and TOC are depicted in figure 13. In general, the biogas and methane production fluctuated during the experiment even though the general performance and stability parameters indicated a fairly stable process in the reactor. The produced biogas per grams of TOC_{(sol)} varied between 43 NmL and 190 NmL, while the produced biogas per grams of COD_{(sol)} varied between 18 NmL and 77 NmL (day 22, 59 and 61 excluded due to unreliable data). The methane production, on the other hand, varied between 30-135 NmL and 12-69 NmL per grams of TOC_{(sol)} and COD_{(sol)}, respectively (day 22 and 61 excluded due to unreliable data). Furthermore, it was apparent that the methane production followed the biogas production (figure 13), which was expected since the methane contents of the biogas samples were rather stable over time.

Most of the unstable data seen in figure 13 was typically caused by practical issues with the equipment and not the process in the reactor. An elaborate compilation of laboratory notes is found in appendix B.1, table B.I. Worth mentioning is that the biogas and methane production decreased a bit during the experiment when the amount of unwanted microbial growth increased. However, after
extensive cleaning of the affected equipment, the biogas and methane production returned to the same level as before the decease.

Calculations were performed on the collected data in the selected periods BC+H₂S, REF, BC-H₂S, UV2.6 and UV16, see table 3. The statistical analyses regarding the biogas and methane production are presented in table 4.

Table 3: Compilation of results in the time periods BC+H₂S, REF, BC-H₂S, UV2.6 and UV16 in regard to the produced biogas and methane per gram of COD(\text{sol}) and TOC(\text{sol}) at 0 °C respectively. The results are presented as a mean value of the data points in each time period followed by the standard deviations. The number of data points used in the calculations is shown in brackets.

<table>
<thead>
<tr>
<th>Compared time periods</th>
<th>NmL biogas/g COD(\text{sol})</th>
<th>NmL biogas/g TOC(\text{sol})</th>
<th>NmL CH₄/g COD(\text{sol})</th>
<th>NmL CH₄/g TOC(\text{sol})</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV2.6 and UV16</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 4: Compilation of the results from paired Student’s t-tests when comparing relevant time periods. H₀ is rejected when p < 0.05, i.e. there is a significant difference between the compared data sets.

<table>
<thead>
<tr>
<th>Compared time periods</th>
<th>NmL biogas/g COD(\text{sol})</th>
<th>NmL biogas/g TOC(\text{sol})</th>
<th>NmL CH₄/g COD(\text{sol})</th>
<th>NmL CH₄/g TOC(\text{sol})</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC+H₂S and REF</td>
<td>p = 0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>BC+H₂S and BC-H₂S</td>
<td>p = 0.00</td>
<td>0.00</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>REF and BC-H₂S</td>
<td>p = 0.23</td>
<td>0.46</td>
<td>0.62</td>
<td>0.47</td>
</tr>
<tr>
<td>REF and UV2.6</td>
<td>p = 0.04</td>
<td>0.12</td>
<td>0.70</td>
<td>0.82</td>
</tr>
<tr>
<td>UV2.6 and UV16</td>
<td>p = 0.00</td>
<td>0.00</td>
<td>0.12</td>
<td>0.10</td>
</tr>
</tbody>
</table>

A few days after the process setup with H₂S present in the circulated biogas (figure 9b), the biogas and methane production decreased over time until day 33 when the biogas circulation was stopped. The biogas and methane production slowly recovered and increased to a stable level. It was during this period of time that the acetic acid concentration increased slightly to 1.4 mM in the effluent of the reactor. The succeeding period REF without circulation (figure 9c) in the system clearly generated a higher biogas and methane production (55±4 NmL biogas/g COD(\text{sol}) 131±18 NmL biogas/g TOC(\text{sol}), 39±3 NmL CH₄/g COD(\text{sol}) and 94±8 NmL CH₄/g TOC(\text{sol})) than in BC+H₂S (figure 9b) (38±3 NmL biogas/g COD(\text{sol}), 98±9 NmL biogas/g TOC(\text{sol}), 26±1 NmL CH₄/g COD(\text{sol}) and 67±2 NmL CH₄/g TOC(\text{sol}) (table 3). This difference between BC+H₂S and REF was statistically significant since all p-values in the statistical analyses were less than 0.05 (table 4). When comparing REF and BC-H₂S (figure 9b and d), there were no statistically significant differences between the two data sets regarding the monitored parameters. Hence, the p-values in the statistical analyses were all greater than 0.05 (table 4).

The last two periods were the ones where the substrate was pretreated with UV light (figure 9e). The first of these periods, UV2.6, exhibited an increase in biogas and methane production (68±5 NmL biogas/g COD(\text{sol}), 166±14 NmL biogas/g TOC(\text{sol}), 47±5 NmL CH₄/g COD(\text{sol}) and 116±13 NmL CH₄/g TOC(\text{sol}) when compared to REF (table 3). This difference was proven to be statistically significant since all of the parameters regarding produced methane and biogas exhibited p-values less than 0.05 (table 4). There was no distinct increase or decrease when the exposure time was changed from 2.6
minutes to 16 minutes (UV16). However, there was a slight decrease in biogas and methane production over time (figure 13). In the statistical comparisons between UV2.6 and UV16, a statistically significant difference could be established in regard to produced biogas (p-values < 0.05) but not in produced methane (p-values > 0.05) (table 4). Moreover, regarding UV16 and REF, only the produced biogas per grams of COD\textsubscript{sol}in exhibited a statistically significant difference with a p-value of 0.04 (table 4), where the biogas production was greater in UV16 than in REF (table 3).

### 4.3.3 COD\textsubscript{sol}, TOC\textsubscript{sol} and SO\textsubscript{4}^{2-}\textsubscript{sol} Reduction

The TOC\textsubscript{sol} and COD\textsubscript{sol} reductions were relatively stable during the experiment. However, the reductions seemed to correlate with the different process setups (figure 9 and 14). For instance, in BC+H\textsubscript{2}S, all of the reduction parameters slightly decreased over time (figure 14). However, no distinct difference between REF and BC-H\textsubscript{2}S could be deduced. Two other distinctive trends related to changes in process setups are found in period UV2.6 and UV16. In UV2.6 the COD\textsubscript{sol} and TOC\textsubscript{sol} reductions elevated slightly compared to REF and in UV16 it decreased a bit compared to UV2.6 (figure 14). The highest COD\textsubscript{sol} reduction was determined to 41% on day 112 while the highest TOC\textsubscript{sol} reduction to 51% on day 112 and 120.

![COD\textsubscript{sol}/TOC\textsubscript{sol}/SO\textsubscript{4}^{2-}\textsubscript{sol} reduction](image)

**Figure 14:** Diagrams depicting the reduction of COD\textsubscript{sol}, TOC\textsubscript{sol} and SO\textsubscript{4}^{2-}\textsubscript{sol}. *The data points represent the minimum reductions since the measured results were below the range of the lowest test kit available, the calculations were based on the test kit’s lowest limit.

One change that cannot be explained by a change in process setup was the sudden drop in SO\textsubscript{4}^{2-}\textsubscript{sol} on day 29 to 4% and the following rapid increase until day 50 (77%), where the sulphate reduction stabilized. However, from day 50 the sulphate concentration was too low to measure (<40 mg/L) and
thus showing a higher reduction. The reduction of $\text{SO}_4^{2-}$ remained at this level until day 124 when it decreased from ≥77% to 58% on day 145 (figure 14).

Throughout the experiment, the reductions of COD$_{sol}$ and TOC$_{sol}$ were almost inseparable from each other. However, during UV2.6 and UV16, the TOC$_{sol}$ reduction was slightly higher than the COD$_{sol}$ reduction resulting in a noticeable gap between the two parameters (figure 14).

As with the biogas and methane production in the previous section, the reductions of COD$_{sol}$, TOC$_{sol}$ and $\text{SO}_4^{2-}$ were calculated for the different time periods (table 5). The results from the statistical analysis performed on the COD$_{sol}$, TOC$_{sol}$ and $\text{SO}_4^{2-}$ reductions are presented in table 6.

Table 5: Compilation of results in the time periods BC+$\text{H}_2$S, REF, BC-$\text{H}_2$S, UV2.6 and UV16 in regard to the reductions of COD$_{sol}$, TOC$_{sol}$ and $\text{SO}_4^{2-}$$_{sol}$. The results are presented as a mean value of the data points in each time period followed by the standard deviations. The number of data points used in the calculations is shown in brackets.

<table>
<thead>
<tr>
<th></th>
<th>BC+$\text{H}_2$S</th>
<th>REF</th>
<th>BC-$\text{H}_2$S</th>
<th>UV2.6</th>
<th>UV16</th>
</tr>
</thead>
</table>

*dp = data points

Table 6: Compilation of the results from paired Student’s t-tests when comparing relevant time periods. H$_0$ is rejected when p < 0.05, i.e. there is a significant difference between the compared data sets.

<table>
<thead>
<tr>
<th>Compared time periods</th>
<th>COD$_{sol}$ reduction</th>
<th>TOC$_{sol}$ reduction</th>
<th>SO$<em>4^{2-}$$</em>{sol}$ reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC+$\text{H}_2$S and REF</td>
<td>p = 0.04</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>BC+$\text{H}_2$S and BC-$\text{H}_2$S</td>
<td>p = 0.09</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>REF and BC-$\text{H}_2$S</td>
<td>p = 0.11</td>
<td>0.16</td>
<td>-</td>
</tr>
<tr>
<td>REF and UV2.6</td>
<td>p = 0.22</td>
<td>0.01</td>
<td>0.47</td>
</tr>
<tr>
<td>REF and UV16</td>
<td>p = 0.03</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>UV2.6 and UV16</td>
<td>p =</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results from BC+$\text{H}_2$S were, as indicated in figure 14, overall lower (25±3% COD$_{sol}$ reduction, 25±3% TOC$_{sol}$ reduction and 45±3% SO$_4^{2-}$$_{sol}$ reduction) compared to REF and BC-$\text{H}_2$S (table 5). This was further supported by the statistical analysis, since all comparisons with BC+$\text{H}_2$S resulted in p-values <0.05 except for the comparison with BC-$\text{H}_2$S where the reduction of COD$_{sol}$ exhibited a p-value of 0.09 (table 6). When comparing REF and BC-$\text{H}_2$S, no statistically significant difference could be proven. Hence, all p-values were greater than 0.05. No comparison regarding the reduction of solved sulphate in BC-$\text{H}_2$S could be performed since only two data points were available.

In the comparison between UV2.6 and REF, no apparent difference in COD$_{sol}$ reduction could be interpreted since the standard deviations overlapped. The statistical analyses showed that there were no significant differences between the data sets regarding this parameter (table 6). However, this was not the case when comparing the TOC$_{sol}$ reductions; the reduction of TOC$_{sol}$ was statistically greater in UV2.6 than in REF. Hence, the p-value was <0.05. The sulphate reduction in UV2.6 was difficult to compare since the calculated values only represent lower limits of the true values.

In UV16, the mean values of the parameters dropped in comparison to UV2.6 and all of the p-values were <0.05. Thus, there were statistically significant differences between the data sets regarding all reduction parameters, i.e. reductions of COD$_{sol}$, TOC$_{sol}$ and SO$_4^{2-}$$_{sol}$. Though, the values of sulphate...
reduction in UV2.6 were merely lower limits of the true values. Between UV16 and REF only the reduction of TOC\textsubscript{sol} was slightly higher in UV16 and exhibited a p-value <0.05. Thus, the reductions of COD\textsubscript{sol} and SO\textsubscript{4}\textsuperscript{2-\textsubscript{sol}} did not differ significantly from REF.

4.4 Effect of the UV pretreatment on Absorption Patterns
All of the wave scans exhibited absorbance at wavelengths ranging from 190 nm to approximately 330 nm with the highest absorbance intensities located in the areas of the shorter wavelengths (figure 15). Only one peak can be deduced from the diagrams, i.e. around 200 nm. Furthermore, a shoulder between 250 and 290 nm is visible.

The UV/vis absorption spectra for the three sampling points I, II and III are depicted in figure 15. The samples collected at sampling point I, the substrate tank, exhibited similar wave scans at day 127, 134 and 142. This indicates that substrate batch 7 and 8 have similar characteristics, which is further supported by the results in table C.1 (appendix C.1). SB5 absorbs slightly less than SB7 and SB8 between 190 and 210 nm, while SB6 deviates the most from SB7 and SB8.

The sampling point located after the UV pretreatment, sampling point II, generated similar results as sampling point I. However, when comparing the wave scan from sampling point I with the one from sampling point III, it can be noted that the absorption in the region between 210 and 250 nm was slightly higher for day 127, 134 and 142 in effluent of the reactor than in sampling point I.

In figure 16, the absorption spectra from succeeding sampling points have been subtracted from the ones located upstream in the process setup. The first diagram, figure 16A, illustrates the absorption difference between sampling point I and II. Some difference could be observed between 190 nm and
270 nm. However, the greatest deviation from zero was merely ±0.75 absorption units and no trends related to UV exposure times could be observed.

In figure 16B, the absorption difference is greater than in figure 16A. A trend around 220 nm can be deduced with a deviation of approximately -5.5 absorption units, which means that the substrate from sampling point II absorbed more around this wavelength than the effluent from sampling point III. When comparing the wave scans from day 127 and 134 that originates from the same substrate batch (SB7) and was exposed to different UV exposure times, a longer exposure time seems to result in a greater absorption difference. However, a contradiction arises when comparing day 140 and 142 that also originates from the same substrate batch (SB8) but with the same exposure time, since the absorption difference of these wave scans also differs significantly from each other.

The total absorption difference, i.e. between sampling point I and III, is illustrated in figure 16C and a similar trend with a decrease in absorption difference at 220 nm as in figure 16B can be seen. Figure 16C exhibits a wave scan that was not pretreated with UV light. However, the absorption difference for this spectrum was not as prominent as the ones that were exposed to UV with the exception of the wave scan from day 120.

The lignin and H₂O₂ concentrations were measured and the results are illustrated in figure 17A. Note that UV pretreatment with 2.6 minutes exposure time was applied on day 104 and that the exposure time of 16 minutes on day 128. The H₂O₂ concentration in sampling point I was generally zero (figure 17A). However, the concentration measured 200 mg/L at day 106 and 112, and 30 mg/L at day 127.
and 140. During the experiment it was observed that the H$_2$O$_2$ concentration decreased in sampling point I over time even when no microbial growth was observed in the system. As can be deduced from the figure, the concentration at sampling point II was also mostly undetectable if not close to zero (≤4 mg H$_2$O$_2$/L) with the exception of day 106 and 112 (200 mg H$_2$O$_2$/L) as well as day 127 and 140 (20 mg H$_2$O$_2$/L). The hydrogen peroxide concentration in sampling point III was also almost always undetectable (≤2 mg H$_2$O$_2$/L) which was expected since hydrogen peroxide is very reactive.

The lignin concentrations varied from one substrate batch to another (figure 17A). The lignin concentration in sampling point III was somewhat lower than the others. Between day 120 and 131, the lignin concentration in the effluent, sampling point III, seem to have decreased a bit in comparison to the other sampling points. However, the same trend is observed on day 100 and 103 where no pretreatment was applied. The greatest lignin reductions were found on days where no pretreatment was applied (figure 17B). The second greatest reduction of lignin was exhibited during the period between day 118 and 131 as deduced from figure 17A. In the succeeding period, day 134 to 142, the lignin reduction is the lowest. 2.6 minutes of UV exposure time was applied from day 104 to day 128 and 16 minutes from day 128 to day 145.

![Figure 17](image-url)

**Figure 17:** Diagrams depicting (A) the lignin and hydrogen peroxide concentrations and (B) the lignin reduction. Unwanted microbial growth was observed in the system on day 103, 120, 124, 131, 134, 138, 140, 142 and 145.

A mean value of the total lignin reduction, i.e. sampling point I – III, for the time periods UV2.6 and UV16 was calculated. In UV2.6, the total reduction was determined to 6.4±1.3 % while the total reduction in UV16 was determined to 3.6±2.1 %. A two-sided Student’s t-test between the two data sets resulted in a p-value of 0.10 which, as in the previously performed statistical analyses, means that H$_0$ is accepted at a confidence level of 95 %. Thus, there was no significant difference in the total lignin reduction between UV2.6 and UV16. Six data points of UV2.6 and four data points of UV16 were used in the calculations.
5 Discussion

5.1 Process Setups
During the experiment, the process setup was changed in order to enable suitable and functional conditions for anaerobic digestion in the UASB reactor. The theory was that circulation in the reactor creates agitation in the granular bed, which in turn increases the contact between the microorganisms and the substrate which is beneficial for the process performance (Quaff & Guha, 2011).

The first setup consisted of circulation where liquid from the reactor was recirculated instead of ending up in the effluent. However, the peristaltic pump used for this purpose in the project was not suitable since it malfunctioned due to the presence of biogas and solids in the liquid. Since the liquid circulation failed, biogas circulation was applied instead. The thought was to circulate the produced biogas back into the system. The biogas bubbles were released in the bottom of the reactor. On the way to the top of the reactor, the bubbles created movement in the granular bed achieving increased contact between microorganisms and substrate while at the same time releasing trapped biogas bubbles from the granular bed.

However, as can be seen in figure 13, the biogas production decreased over time when biogas circulation was applied. After a while, it was concluded that it most likely was the presence of hydrogen sulphide in the biogas that caused the drop in biogas production. This was concluded from the increase in biogas production after the biogas circulation with hydrogen sulphide present was terminated and that scientific literature has shown that hydrogen sulphide have negative effects on the AD process (Wiemann et al. 1998). According to Gustavsson (2012), Fe, Co, Ni Cu and Zn are precipitated as sulphide when the total level of H$_2$S, HS$^-$ and S$^{2-}$ exceeds the total level of the trace elements. Due to the low solubility of these sulphide metal precipitations, the bioavailability of the trace metals decreases causing the biogas production to drop. Gustavsson (2012) also suggests that sulphides can form large metal complexes that hinder the microbial uptake of the trace elements. Also, hydrogen sulphide has the ability to diffuse into cell membranes, causing a direct toxic effect on the microorganisms as it denatures native proteins that are vital to the functions of the cell (Appels et al., 2008).

The stripping of H$_2$S in the recirculated biogas showed to be a significant better solution than having H$_2$S present since the biogas production did not decrease over time. Conclusively, it was shown that the biogas circulation with H$_2$S present did not have a positive effect on the biogas and methane production nor did it improve the reduction parameters compared to the process setup when no circulation was applied. However, a higher circulation rate of the biogas might have resulted in a more positive outcome of the usage of biogas circulation without H$_2$S present. Unfortunately, the UASB reactor with EOP as substrate did not produce enough biogas to facilitate such a rate. Therefore, the process system with no circulation was selected as a reference period (REF).

5.2 Design of UV Device
The first design of the UV device could not guarantee a constant flow of substrate when exposed to the UV light, which in turn complicated the determination of the exposure time of the substrate. Another issue with the construction was that the peristaltic pump pumping the substrate in and out of the UV device did not pump at the same speed. Since the outlet pumping speed was faster than
the inlet pumping speed, there was a risk of pumping air into the reactor. Therefore, a container was placed between the UV device and the reactor ensuring that only liquid entered the reactor. However, this resulted in the usage of another pump point in order to maintain the influent to the reactor. Consequently, the liquid level in the container did not stabilize and kept decreasing. Another disadvantage of this design was the difficulty to change the exposure time while still keeping the pretreatment at an online manner. The second design of the UV device eliminated the practical issues of the first design.

5.3 UV as Pretreatment Method

5.3.1 Unwanted Microbial Growth
During the majority of the experiment, unwanted fungi or bacterial growth was observed in the system and the effect of its presence in the system is purely speculative since no analyses were performed to investigate it further. However, it was noticed that the biogas production decreased quite rapidly when a substantial amount of growth was observed in the system. Therefore, the substrate tank and the tubes leading to the reactor were disinfected with ethanol and UV light. However, since the tube that was connected to the bottom of the reactor could not be removed and properly cleaned without emptying the content of the reactor, the unwanted growth kept coming back. Since the presence of the unwanted growth resulted in a cloudy appearance of the substrate, as seen in figure 18, it might have affected the effectiveness of the UV pretreatment. In a clear liquid, the UV rays can reach the entire content of the quartz tubes in the UV device. However, when it is cloudy it is difficult for the rays to pretreat the entire content since the UV rays will be blocked or absorbed by the bacterial cells.

![Figure 18: Photographs illustrating the unwanted microbial growth in the quartz tubes of the UV device (left) and the substrate tank (right). The substrate without the unwanted growth is a clear and yellow liquid.](image)

Another possible effect of the unwanted microbial growth in the system could be the production of the enzyme catalase. Catalase is an enzyme that is produced by almost all aerobic bacteria as a defense system against H₂O₂ which has damaging effects on the cells by causing oxidative stress (Madigan et al., 2008). The enzyme decomposes one H₂O₂ molecule into two water molecules, thereby protecting the cell from damage. The daily feeding of the reactor was performed after
samples had been collected. This means that the collected samples had been exposed to the unwanted organisms for almost a day before the analyses were conducted. The effects of the hydrogen peroxide concentration in the system are discussed in section 5.3.4.

5.3.2 Evaluation of Methane and Biogas Production as well as Reduction Parameters

According to the conducted statistical analysis, there was a significant difference in biogas and methane production between REF and UV2.6. This indicates that the UV pretreatment was effective in partially degrading the organic matter, i.e. making it more susceptible to the microbes in the reactor. However, the lack of lignin reduction indicates that it might not have been this particular molecule that was the main target of the hydroxyl radicals. Hence, a more aggressive pretreatment than the process of H$_2$O$_2$/UV might be needed in order to transform lignin to an easily biodegradable structure. However, a more aggressive method could result in the degradation of other less complex organic compounds to CO$_2$, thus losing part of the CH$_4$ potential. Since the radicals primarily attack aromatic structures, it is possible that the targeted molecules were degradation products of wood extractives or other more degradable compounds.

When comparing UV16 with REF, no significant difference could be proven for most of the parameters. Only the produced biogas per grams of COD$_{\text{sol,lin}}$ and the TOC$_{\text{sol}}$ reduction exhibited a significant increase in UV16. In addition, the parameter produced biogas per grams of COD$_{\text{sol,lin}}$ had a p-value of 0.4 and was close to accepting H$_0$, i.e. the hypothesis that there is no significant difference between the data sets. Therefore, it is possible that the organic matter, subjected to partial degradation in UV2.6, instead was fully degraded to CO$_2$ and water in UV16 since there was a significant increase in TOC$_{\text{sol}}$ reduction in UV16 but a weaker significant difference in the parameters of the produced biogas and methane in comparison to REF.

UV2.6 was significantly better than UV16 regarding biogas production. However, the normalized methane production per grams of COD$_{\text{sol,lin}}$ and TOC$_{\text{sol,lin}}$ did not exhibit a statistically significant difference. This indicates that some of the organic material was fully degraded to CO$_2$ and water in UV2.6 but not in UV16. Further supporting this is the higher reduction of TOC$_{\text{sol}}$ and COD$_{\text{sol}}$ in UV2.6. Conclusively, this means that the UV pretreatment in UV16 was less effective than in UV2.6 and that a longer UV exposure time did not cause more organic material to fully degrade. However, another explanation to the lower amount of fully degraded organic material in UV16 could be the presence of unwanted growth in the system. Hence, during UV16 it was warmer in the laboratory than during UV2.6 which led to an increased growth rate of the unwanted microbial growth.

The cause of the small gap between COD$_{\text{sol}}$ and TOC$_{\text{sol}}$ reduction (figure 14) when the UV pretreatment was applied is unknown. According to the instructions of the manufacturer regarding the COD Hach Lange kit LCK 514 used in the experiment, all oxidizable compounds are measured as COD. In the manufacturer’s instructions, it also says that chloride concentrations greater than 1500 mg/L can interfere with the accuracy of the test kit. Though, these interferences on COD measurements results in higher COD values and the reduction of TOC$_{\text{sol}}$ was greater than the one of COD$_{\text{sol}}$. Thus, the measurements of TOC are more likely to be inaccurate. According to the instructions of the manufacturer regarding the TOC test kit LCK 387, exposure of the cuvettes containing the indicating dye to air can cause the TOC levels to increase due to CO$_2$ contamination. However, all measurements were performed the same way during the experiment. Another possible cause is the variation in characteristics regarding the substrate batches. An increase in TOC$_{\text{sol}}$
reduction but no difference in COD_{sol} reduction is possible. Hence, one batch might contain more but larger oxidizable compounds resulting in higher TOC but the same COD. However, this does not seem to be the case since feeding of SB6 was initiated on day 103 and SB7 on day 120. Since all possible scenarios have been eliminated as the cause to the small gap in TOC_{sol} and COD_{sol} reductions, no explanation can be presented.

Regarding the sulphate reduction it is difficult to draw any conclusions, since the concentration of SO_4^{2-} in sampling point III was too low to measure in REF, BC-H_2S and UV2.6. However, on day 29, the reduction of this parameter dropped considerably and thereafter increased to a higher level than before the decrease. The drop in sulphate reduction occurred during the process setup with H_2S present in the biogas circulation at the same time as the decline of biogas and methane production took place. Hydrogen sulphide is toxic to both SRBs and methanogens, but different species within the groups of SRBs and methanogens responds differently to hydrogen sulphide (O'Flaherty et al., 1998). The micro flora in the reactor was not determined. Thus, it is difficult to draw any conclusions regarding the potential toxicity of hydrogen sulphide on specific microorganisms. However, the results suggest that the methanogens were more sensitive than the SRBs since the biogas and methane production started to decrease before the sulphate reduction. The biogas circulation with H_2S present ended on day 33 after sampling. Hence, the rapid increase in sulphate reduction started after the change in process setup. However, no explanation as to why the reduction of sulphate increased to a higher level than before the decrease can be presented.

5.3.3 Evaluation of UV/vis Spectra

The highest absorption was observed in the region around 200 nm (figure 15). According to Solomons et al. (2008), the wavelength at which compounds with conjugated multiple bonds absorb increases with the number of multiple bonds. Thereby, indicating that the majority of the organic materials in the samples were of less complex structures. Though, the structures are not simple hydrocarbons since they absorb at shorter wavelengths than 190 nm (Hesse et al., 2008). Conjugated trienes and dienes absorb at wavelengths between approximately 220 and 300 nm. Benzene is one of the possible degradation products of lignin and it absorbs at 189, 208 and 262 nm. However, monosubstituted bezenes also absorb in the area between 200 and 250 nm and parasubstituted benzenes absorb in the region between 250 and 280 nm. Enones and saturated carbonyl compounds are another group of organic compounds that absorb in this area, i.e. 220-250 nm and 200-300 nm respectively. The quinones belong to the group of enones but they absorb at longer wavelengths, with the first absorption peak at 240 nm, second peak at 280 nm and then furthermore in the visible spectra. (Hesse et al., 2008)

Conclusively, it is difficult to differentiate which structural elements or specific compounds that gave rise to the UV/vis spectra in figure 15 since the wavelengths of these structures overlap and due to the diverse nature of the organic material present in the samples. Hence, the structures are most likely degradation products of lignin and other wood constituents. However, it can be concluded that the structures in the samples can be benzene, mono- or parasubstituted benzenes, conjugated trienes and dienes, enones and/or quinones. The majority of the organic material in the samples is most likely not native lignin, since the highly complex structure of lignin absorbs around 280 nm and 316-320 nm (Rowell, 2005). Though, some absorption was present in these regions where native lignin absorbs.
The UV/vis spectra showed absorption differences in the region of short wavelengths. The most prominent trend in figure 16C, depicting the total absorption difference, is an increase in absorption around 220 nm since the absorption difference is negative. It is difficult to identify the compound that was responsible for this particular change in absorption for the same reasons as discussed earlier. The increase in absorption did most likely not come from degradation of native lignin, since its reduction did not increase with increasing UV exposure time. However, it is possible that some of the degradation products, e.g. benzene, interfered with the lignin measurements since they also absorb at 280 nm. There is also a slight decrease in absorbance around 190 nm which indicates a decrease in the simplest structures absorbing in the UV/vis spectra. One explanation to this could be that these compounds have been transformed into gaseous molecules such as CO₂ or CH₄ in the process. However, the difference in absorbance between sampling point I and II does not exhibit the same trends. The UV lamp had some effect on the substrate between 190 and 290 nm. Though, no coherent trend can be deduced and the greatest absorption difference is merely about 0.75 absorption units.

The hypothesis that some of the organic matter was fully degraded into CO₂ and water when the substrate was subjected to an UV exposure time of 16 minutes is not likely when considering the increase in absorbance at 220 nm and the slight decrease around 190 nm, see figure 16C. Hence, the increase in absorption at 220 nm is somewhat greater for UV16 than UV2.6 while the minor decrease around 190 nm is somewhat less in UV16 compared to UV2.6 for day 127 and 134 where substrate from the same batch was used. In some degree, this suggests that UV16 should result in a higher biogas production since there are more degradable organic compounds available. A more plausible explanation is that degradation products inhibitory to the AD process were formed, which consequently led to a less efficient degradation process in the reactor. However, another explanation can be that the increase in the amount of unwanted microbial growth during UV16 affected the efficiency of the UV pretreatment more than in UV2.6.

### 5.3.4 The Effect of Hydrogen Peroxide

As discussed earlier, the concentration of H₂O₂ in the substrate might have been affected by the presence of unwanted microbial growth. However, there is another possible explanation for the low concentrations found in the sampling points which is the handling and reactivity of hydrogen peroxide. During the experiment, it was observed that the concentration of H₂O₂ decreased over time in the substrate tank even if no unwanted growth was visually present (section 4.4). Though, the decrease in concentration was considerably less in comparison to when unwanted growth was observed in the system. Hence, the effects of the UV/H₂O₂ process might be more successful at the site of the kraft pulp and paper mill since the H₂O₂ would not be lost in the handling of the substrate. It would also lead to a more stable concentration of H₂O₂ as the anaerobic treatment facility would be placed in proximity to the EOP wastewater stream.

Ghaly et al. (2001) investigated the effect of H₂O₂ concentration on the degradation of p-chlorophenol when utilizing the UV/H₂O₂ process. It was concluded that UV irradiation on its own is a poor advanced oxidation process and that its efficiency radically improves with the addition of H₂O₂. They found that the optimal concentration of H₂O₂ for degradation of p-chlorophenol was 0.02 mol/L (680 mg/L). They also concluded that the degradation rate was even slower than direct photolysis at H₂O₂ concentrations above this optimum. The H₂O₂ molecules started to react with the generated hydroxyl radicals and thus acted as an inhibiting agent. The highest concentration of H₂O₂
in the substrate tank of the present investigation was determined to 200 mg/L, but this only occurred twice during the experiment. Most commonly, the level of H$_2$O$_2$ was undetectable. The lowest concentration of H$_2$O$_2$ that Ghaly et al. (2001) used in their investigation was 0.005 mol/L (170 mg/L). Even if there were some positive effects with the addition of H$_2$O$_2$ at this concentration, it did not improve the degradation rate of p-chlorophenol that much.

Jamil et al. (2011) performed a similar investigative study as Ghaly et al. (2001) but with wastewater obtained from a board paper mill industrial plant. They concluded that the optimal concentration of H$_2$O$_2$ was 5 g/L (pH 3) in order to achieve the fastest reaction time in regard to removal of COD. As Ghaly et al. (2001), they also discovered that a higher concentration than the optimum had a negative impact on the reaction rate. Catalkaya and Kargi (2007) investigated the effects of initial pH on the UV/H$_2$O$_2$ process treating pulp mill effluent and determined that the removal of TOC was 8 % at pH 3, 10 % at pH 7 and 11 % at pH 11. When testing the effects of pH, the concentration of H$_2$O$_2$ was 50 mM and the reaction time 30 minutes. In the experiment of this master’s thesis, the pH should not affect the UV/H$_2$O$_2$ process dramatically in regard to TOC reduction, since the pH in the effluent of reactor was determined to 7.9±0.2.

Overall, it can be concluded that the H$_2$O$_2$ concentration in the experiment was too low to achieve a good photolytic degradation of the organic material with the UV/H$_2$O$_2$ process. However, an optimal concentration is difficult to predict, since it seems to vary depending on the characteristics of the wastewater. It is also not desirable to fully degrade the organic material to carbon dioxide. Hence, a complete degradation leaves nothing for the anaerobic bacteria to produce methane from. Though, lignin is difficult to degrade and a higher concentration of H$_2$O$_2$ might generate a higher biogas production and a greater reduction of TOC.

5.3.5 Environmental Aspect
The kraft pulp and paper mill, at which the EOP wastewater was collected, has an environmental requirement that limits them to discharge more than 7.5 tons of TOC per day in total. The environmental requirement is calculated as a mean value of the yearly limit in TOC discharge.

As presented in section 4.3.3, the reduction of TOC in REF was determined to 35±2 % while UV2.6 resulted in a total reduction of 47±5 % and UV16 reduced the amount of TOC with 41±3 %. The reduction in TOC at the wastewater treatment facility treating the total waste stream of the kraft pulp and paper mill is approximately 63 %. Conclusively, the TOC reduction by the anaerobic treatment performed in this master’s thesis did not match the current TOC reduction of the total wastewater stream at the kraft pulp and paper mill. Though, an advantage of AD is the production of methane which can be used as an energy carrier within the mill. However, the comparison is difficult to perform since other waste streams affect the TOC reduction in the wastewater treatment facility and the EOP wastewater only comprises a part of the total wastewater stream. Another predicament is that the fate of the EOP wastewater in the existing wastewater treatment facility of the kraft pulp and paper mill is unknown. From the bleachery at the kraft pulp and paper mill approximately 5 tons of TOC per day is discharged into the total waste stream, while the total waste stream after sedimentation lets through approximately 8.3 tons of TOC per day. This means that about 60 % of the total dissolved TOC of the wastewater stream originates from the bleachery.

Even though anaerobic treatment probably would not be able to fully replace the aerobic treatment process that currently is applied at the kraft pulp and paper mill, there are still some advantages
when utilizing them in combination. An ECF bleaching process still produces some chlorinated organic compounds that are toxic to the environment and the usage of anaerobic treatment processes have proven successful in the reduction of these substances (Buzzini et al., 2005). According to Jamil et al. (2011), the utilization of UV pretreatment also has a positive impact of the degradation of chlorinated organic compounds since the mechanism of hydroxyl radicals is distinguished by direct dechlorination.
6 Conclusions
The ultimate purpose of this master’s thesis was to evaluate the effectiveness of UV pretreatment of alkaline bleaching wastewater from a kraft pulp and paper mill prior to anaerobic digestion in a UASB lab-scale reactor and to determine the optimal UV exposure time in regard to an enhanced biogas and methane production. The main conclusions are listed below.

- The UV exposure time of 2.6 minutes partially degraded organic matter in the EOP wastewater generating approximately 26 % higher biogas production and 22% higher methane production compared to the reference period. It also increased the reduction of TOC$_{sol}$ by 34 % and the COD$_{sol}$ reduction by 12 %.
- The UV exposure time of 16 minutes did not show any general significant improvement regarding increased biogas and methane production compared to the reference period nor did it increase the COD$_{sol}$ reduction. However, it did increase the reduction of TOC$_{sol}$ by 17 %.
- No optimal exposure time could be determined, since only two different UV exposure times were investigated due to lack of time.
- No circulation in the UASB reactor was determined to be the most suitable process setup for the experiment. Hence, biogas circulation with H$_2$S present considerably decreased the performance of the reactor compared to no circulation. Furthermore, the biogas circulation without H$_2$S present did not improve the efficiency of the reactor compared to the process setup without circulation when EOP wastewater was treated.
7 Future Perspectives

The interest for the usage of anaerobic treatment processes in the pulp and paper industry has increased due to stricter regulations regarding environmental discharge. Conventional treatments methods such as aerobic treatments will soon be insufficient in meeting the environmental requirements set by the authorities. However, the anaerobic treatment in combination with the conventional treatment techniques holds a promising future. With the addition of UV pretreatment in the process, the environmental effect of the bleaching wastewater is decreased even more as the level of toxic chlorinated organic compounds is reduced (Jamil et al. 2011). The most prominent advantage of implementing an anaerobic treatment facility is the production of biogas. The energy trapped in the biogas can be utilized within the industry in order to reduce energy costs and reduce the usage of the fossil fuels. However, in order to optimize the anaerobic process and the UV pretreatment, some additional research is needed.

The optimal UV exposure time is a crucial parameter that directly influences the efficiency of the UV pretreatment and further experiments are needed in order to determine it. Even though some research has been performed in this area, the characteristics of wastewater streams within a paper or pulp industry vary significantly and consequently also the required exposure time. Thereby, a specific exposure time needs to be determined for each specific wastewater being treated.

Further experiments are needed at the site of the kraft pulp and paper mill. Hence, the conditions under which the experiment was conducted did not fully represent the conditions at the kraft pulp and paper mill, e.g. the concentration of hydrogen peroxide and the presence of unwanted microbial growth in the system.

The anaerobic digestion with UV pretreatment did not match the reduction of TOC in the total wastewater stream of the kraft pulp and paper mill when performing a direct comparison. Therefore, the combination of UV pretreatment, anaerobic digestion and aerobic treatment should be investigated as it could potentially be the future process constellation of EOP wastewater treatment.
8 References


Gustavsson, J. “Cobalt and Nickel Bioavailability for Biogas Formation”, Diss. 2012, Linköping University, pp. 11-12.


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Menendez, M.R. 2013, How We Use Energy at Wastewater Plants...And How We Can Use Less.


Appendices

Appendix A - Calculations

A.1 UV Exposure Times

Length of UV-exposed quartz tube (l): 25 cm

Inner diameter of quartz tube: 0.8 cm

Volume of substrate exposed to UV light (V):

\[ V = \pi \cdot r^2 \cdot l = \pi \cdot \left(\frac{0.8}{2}\right)^2 \cdot 25 = 12.5663706144 \, cm^3 \]

Flow through quartz tube: 289±11 mL/h

The mean value is based on the data points from the selected time periods which are considered stable.

One quartz tube connected:

\[ Exposure \ time = \frac{V}{Flow} \cdot 60 = \frac{12.5663706144}{289} \cdot 60 = 2.608935078 \approx 2.6 \, min \]

The calculated UV exposure times for the different number of connected tubes in the system are compiled in table A.I.

Table A.I: A table compiling the calculated UV exposure times based on the design of the reactor with 6 quartz tubes. The tubes connect one by one in order to facilitate variation in UV exposure. The exposure times are given in minutes and seconds.

<table>
<thead>
<tr>
<th>Number of connected quartz tubes</th>
<th>Exposure time (min)</th>
<th>Exposure time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.6</td>
<td>157</td>
</tr>
<tr>
<td>2</td>
<td>5.2</td>
<td>313</td>
</tr>
<tr>
<td>3</td>
<td>7.8</td>
<td>470</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>626</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>783</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>939</td>
</tr>
</tbody>
</table>
Appendix B

B.1 Compilation of Laboratory Notes

In table B.1, a compilation of significant laboratory notes during the run of the reactor is shown. These notes are helpful when interpreting the diagrams in the result section of the report. Note that the gas meter was zeroed after the action in the note was taken or observed and the biogas production belonging to a specific day was recorded after the daily reset.

Table B.1: A compilation of significant laboratory notes during the run of the reactor. Note that the gas meter was zeroed after the observation or the action described in the note was taken.

<table>
<thead>
<tr>
<th>Day</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Startup of reactor. Started feeding SB1. Liquid circulation was applied.</td>
</tr>
<tr>
<td>12</td>
<td>Stopped liquid circulation. Started biogas circulation with H₂S.</td>
</tr>
<tr>
<td>20</td>
<td>Started feeding SB2.</td>
</tr>
<tr>
<td>21</td>
<td>Increased the biogas circulation in terms of rpm.</td>
</tr>
<tr>
<td>22</td>
<td>Air was pumped into the reactor when adding trace elements.</td>
</tr>
<tr>
<td>33</td>
<td>Biogas circulation was stopped. No circulation in the system.</td>
</tr>
<tr>
<td>37</td>
<td>Cleaned the reactor and the substrate tank due to microbial growth.</td>
</tr>
<tr>
<td>41</td>
<td>Started feeding SB3.</td>
</tr>
<tr>
<td>45</td>
<td>The peristaltic pump squeezed the tube to tight which resulted in no influent or effluent of the reactor.</td>
</tr>
<tr>
<td>49</td>
<td>Cleaned the substrate tank due to microbial growth.</td>
</tr>
<tr>
<td>57</td>
<td>Cleaned the tubes leading from the substrate tank due to microbial growth.</td>
</tr>
<tr>
<td>58-65</td>
<td>Problems with the biogas circulation.</td>
</tr>
<tr>
<td>58</td>
<td>Started biogas circulation with a zinc oxide and drying agent flask connected to the system (1 rpm).</td>
</tr>
<tr>
<td>62</td>
<td>The biogas circulation was increased to 2 rpm. Cleaned the substrate tank and the tubes from the substrate tank due to microbial growth. Started feeding SB4.</td>
</tr>
<tr>
<td>63-64</td>
<td>Problem with negative pressure in the system resulting in no reliable data.</td>
</tr>
<tr>
<td>65</td>
<td>Blockage in the zinc oxide flak was detected and fixed. The biogas circulation was started again.</td>
</tr>
<tr>
<td>68, 75</td>
<td>Some air entered the system when the flask with drying agent was changed.</td>
</tr>
<tr>
<td>82</td>
<td>Cleaned the substrate tank and the tubes leading from the substrate tank due to microbial growth. Started feeding SB5.</td>
</tr>
<tr>
<td>89</td>
<td>Cleaned the substrate tank and the tubes leading from the substrate tank due to microbial growth.</td>
</tr>
<tr>
<td>91</td>
<td>Start of UV pretreatment (first design).</td>
</tr>
<tr>
<td>93</td>
<td>Stopped the UV pretreatment (first design).</td>
</tr>
<tr>
<td>96</td>
<td>Cleaned the substrate tank and the tubes leading from the substrate tank due to microbial growth.</td>
</tr>
<tr>
<td>103</td>
<td>Cleaned the substrate tank and the tubes leading from the substrate tank due to microbial growth. Started feeding SB6.</td>
</tr>
<tr>
<td>104-108</td>
<td>Problems with the biogas circulation resulted in no circulation during the nights.</td>
</tr>
<tr>
<td>104</td>
<td>Started UV pretreatment (second design; 2.6 min exposure time)</td>
</tr>
<tr>
<td>106</td>
<td>Cleaned the reactor and started the biogas circulation again (5 rpm).</td>
</tr>
<tr>
<td>107</td>
<td>Negative pressure in the system during the night resulted in no reliable data.</td>
</tr>
<tr>
<td>108</td>
<td>Stopped the biogas circulation. No circulation.</td>
</tr>
<tr>
<td>111</td>
<td>Cleaned the substrate tank and the tubes leading from the substrate tank due to microbial growth.</td>
</tr>
<tr>
<td>116</td>
<td>Power outage. The peristaltic pumps, stirrer and water bath were off. The water bath was cold. No reliable data.</td>
</tr>
<tr>
<td>Page</td>
<td>Description</td>
</tr>
<tr>
<td>------</td>
<td>-------------</td>
</tr>
<tr>
<td>120</td>
<td>Cleaned the substrate tank and the tubes leading from the substrate tank due to microbial growth. Started feeding SB7.</td>
</tr>
<tr>
<td>125</td>
<td>Cleaned the substrate tank and the tubes leading from the substrate tank due to microbial growth.</td>
</tr>
<tr>
<td>128</td>
<td>UV pretreatment (second design; 16 min exposure time)</td>
</tr>
<tr>
<td>131</td>
<td>Cleaned the substrate tank and the tubes leading from the substrate tank due to microbial growth.</td>
</tr>
<tr>
<td>135</td>
<td>Cleaned the substrate tank and the tubes leading from the substrate tank due to microbial growth.</td>
</tr>
<tr>
<td>139</td>
<td>The peristaltic pump squeezed the tube to tight which resulted in no influent or effluent of the reactor. Cleaned the substrate tank and the tubes leading from the substrate tank due to microbial growth.</td>
</tr>
</tbody>
</table>
### Appendix C – Diagrams and Data

#### C.1 Substrate Characteristics

*Table C.1: A compilation of the substrate characteristics where the batches are designated SB1 through SB8. Mean value and standard deviation are presented for each substrate batch.*

<table>
<thead>
<tr>
<th>Substrate batch</th>
<th>COD$_{\text{tot}}$ (mg/L)</th>
<th>COD$_{\text{sol}}$ (mg/L)</th>
<th>TOC$_{\text{tot}}$ (mg/L)</th>
<th>TOC$_{\text{sol}}$ (mg/L)</th>
<th>SO$<em>4^{2-}$$</em>{\text{tot}}$ (mg/L)</th>
<th>SO$<em>4^{2-}$$</em>{\text{sol}}$ (mg/L)</th>
<th>SS (mg/L)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB1</td>
<td>1552</td>
<td>1489</td>
<td>600</td>
<td>582</td>
<td>236</td>
<td>220</td>
<td>9</td>
<td>10.3</td>
</tr>
<tr>
<td>SB2</td>
<td>1577</td>
<td>1546</td>
<td>653</td>
<td>637</td>
<td>193</td>
<td>193</td>
<td>9</td>
<td>10.2</td>
</tr>
<tr>
<td>SB3</td>
<td>1280</td>
<td>1267</td>
<td>546</td>
<td>528</td>
<td>188</td>
<td>176</td>
<td>7</td>
<td>10.8</td>
</tr>
<tr>
<td>SB4</td>
<td>1384</td>
<td>1359</td>
<td>586</td>
<td>572</td>
<td>194</td>
<td>190</td>
<td>6</td>
<td>10.5</td>
</tr>
<tr>
<td>SB5</td>
<td>1342</td>
<td>1342</td>
<td>573</td>
<td>573</td>
<td>151</td>
<td>151</td>
<td>8</td>
<td>10.4</td>
</tr>
<tr>
<td>SB6</td>
<td>1174</td>
<td>1164</td>
<td>473</td>
<td>470</td>
<td>206</td>
<td>193</td>
<td>6</td>
<td>10.7</td>
</tr>
<tr>
<td>SB7</td>
<td>1610</td>
<td>1590</td>
<td>662</td>
<td>661</td>
<td>303</td>
<td>279</td>
<td>5</td>
<td>10.0</td>
</tr>
<tr>
<td>SB8</td>
<td>1619</td>
<td>1595</td>
<td>686</td>
<td>683</td>
<td>479</td>
<td>470</td>
<td>7</td>
<td>10.2</td>
</tr>
<tr>
<td><strong>Mean value</strong></td>
<td><strong>1442</strong></td>
<td><strong>1419</strong></td>
<td><strong>597</strong></td>
<td><strong>588</strong></td>
<td><strong>244</strong></td>
<td><strong>234</strong></td>
<td><strong>7</strong></td>
<td><strong>10.4</strong></td>
</tr>
<tr>
<td><strong>Standard deviation</strong></td>
<td><strong>170</strong></td>
<td><strong>160</strong></td>
<td><strong>70</strong></td>
<td><strong>71</strong></td>
<td><strong>105</strong></td>
<td><strong>102</strong></td>
<td><strong>2</strong></td>
<td><strong>0.27</strong></td>
</tr>
</tbody>
</table>

#### C.2 VFA

*Figure C.1: Diagrams comprising the levels of VFA in the effluent of the reactor; i.e. the concentration of acetic acid and propionic acid. Detection limit was 0.2 mM and quantification limit 0.6 mM.*
C.3 pH

Figure C.II: Diagrams comprising the pH of the reactor's effluent.

C.4 Suspended Solids

Figure C.III: Diagrams comprising the concentration of suspended solids in the substrate (influent) and the effluent of the reactor.