

Anaerobic Co-digestion of steam pretreated wheat straw and sewage sludge

January 2021 – June 2021

Virginia Tosi



LUND
UNIVERSITY

Examiner: Ed Van Niel
Supervisor: Emma Kreuger
Co-supervisor: Åsa Davidsson

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Acknowledgment

This master thesis has been performed at the Department of Biotechnology at Lund University at the Faculty of Engineering, LTH, Lund University, under the supervision of Emma Kreuger. I would like to thank her for involving me in the project, which I got really passionate of, and for following every progress with a lot of enthusiasm, even for troubleshooting and despite of frequent miscalculations.

I want to thank Åsa Davidsson, the assistant supervisor, for her suggestions during the process start-up and thesis drafting, and the examiner, Ed Van Niel, for reviewing my work.

I want to acknowledge Agneta Thor Leander, Martin Hommel and Marika Murto at VA-Syd for providing information and material and the financing partners: The Swedish Energy Agency (project number 51445-1), Sweden Water Research, VA Syd, Sysav and Sysav utveckling AB.

My gratitude to the Department of Chemical Engineering, specifically to Christian Roslader who helped during substrates pretreatment, and the Department of Biotechnology, in particular Oliver Englund Örn for being always available and helpful, and Savina Leontakianakou for sharing her office with me.

A distinctive recognition to Tushar, that helped formatting this report.

Finally, I am thankful to my family, for the opportunity to come to Sweden, and to my partner for his patience and constant support. I want to thank my friends in Lund for the happiest years of my life, and my friends in Italy for being always such great ones.

Abbreviation

AD - anaerobic digestion
AMPTS - automatic methane potential test system
BMP - biomethane potential test
CSTR - continuous stirred-tank reactor
DM - dry matter
GHG - greenhouse gas
HAc - acetic acid
HF - formic acid
HisoVal - isovaleric acid
HLac - acetic lactic
HMF - hydroxymethylfurfural
HPLC - high-performance liquid chromatography
HPr - propionic acid
HSuc - succinic acid
HVal - valeric acid
MetOH - methanol
PS - primary sludge
SS - sewage sludge
SWS - steam pretreated wheat straw
TS - total solids
VFA - volatile fatty acids
VS - volatile solids
WAS - waste activated sludge
WIS - water insoluble substances
WS - wheat straw
WWTP- wastewater treatment plants

Abstract

Anaerobic digestion (AD) of wastes and residues is a widely applied and explored process. However, when the process is not optimized, inadequate concentrations of nutrients and inhibitory compounds are common and can lead to low maximum organic loading rates and increased risk of process problems, low organic dry solids degradation rate and reduced methane yields. To overcome process instability and low performance, the simultaneous digestion of two or more substrates, named co-digestion, has been implemented in the last 30 years. Nowadays, the expansion of co-digestion to the use of anaerobically slowly converted agro-waste, via pretreatments, is of major interest due to their availability and energy densities. Among the sustainable feedstocks, wheat straw is a good candidate for co-digestion with sewage sludge, since it has a high concentration of organic compounds, low water content and low concentration of many nutrient elements, which can be provided in higher amount by sewage sludge, being instead characterized by low organic content, high water content and high concentration of several nutrient elements in relation to carbon.

This study investigates co-digestion of sewage sludge (combined primary sludge and waste activated sludge) and steam pretreated wheat straw in continuous stirred-tank reactors. Wheat straw was pretreated through acetic acid catalyzed steam explosion at 190°C for 10 minutes, leading to 89% recovery of the total solids. A constant proportion of the two substrates was maintained in the inlet, where 35% of the feedstock volatile solids was provided by wheat straw, giving a C/N ratio of 12.6, and TS content of 10.7%. Digestions of the two single feedstocks were used as controls to compare mono- and co-digestion performance. Micro- and macronutrients were added in the wheat straw controls to reach the same concentrations in the feed as for co-digestion. Similar organic loading rate were applied 2.1 ± 0.1 gVS/Ld in all reactors, and the hydraulic retention time was maintained at 22 days.

All processes were stable most of the operational time, with methanogenesis inhibition occurring only at the beginning when wheat straw was introduced in the substrate, accompanied volatile fatty acids accumulation. Hence, the microbial population likely adapted to the new substrate. After 26 days the concentration of total volatile fatty acids was maximum only 0.30 g/L within 7 hours after feeding and less than 0.15 g/L at 7 hours after feeding, suggesting that higher organic loads might be tolerated by the system. The average methane yield from co-digestion was 0.27 LCH₄ / gVS. This was significantly different only from the methane yield obtained from wheat straw mono-digestion, and a synergistic effect was not demonstrated. Similar degradation degree of volatile solids were obtained from co-digestion and sludge mono-digestion, meaning a good accessibility to degradable compounds in wheat straw was gained from its pretreatment. More experiments need to be performed to assess the digestate quality and its impact on the economy and applicability of the process.

Finally, in a full-scale plant digesting sludge only, an increase in annual methane production of 43% was estimated through co-digestion of 35% wheat straw. Due to the high energy density (low water content) of wheat straw, the plant could operate at the same hydraulic retention time as today, with an increase in reactor volume of only 11%.

Please note the errata in chapter 7.

1. Introduction

The increasing demand of renewable energy is boosting research and optimization of well-established processes, such as AD of wastes and residues. The combination of different substrates in co-digestion processes can ensure an overall good content of micro- and macronutrients, dilution of inhibitory compounds, and balance between moisture and total solids content, improving the economic viability of AD plants. Wheat straw (WS) has been reported to be a promising substrate for anaerobic co-digestion with sewage sludge, in order to balance C/N ratios and other nutrient ratios as well as to make the residue easier to dewater. However, the influence of wheat straw pretreatment on the methane yield as well as co-digestion effects have been investigated mainly in batch reactors. The use of the two substrates in continuous experiments instead, gives the possibility to evaluate co-digestion effects without influence of the composition of inoculum as in batch test.

Please note the errata in chapter 7.

1.1. Aim of the study

This study aims to investigate the influence of co-digestion of sewage sludge (SS) and steam pretreated wheat straw (SWS), in comparison to mono-digestion of the two substrates, in continuous stirred tank reactors (CSTRs). The study aims at determining the theoretical potential of balancing nutrient composition in sewage sludge (combined primary and waste activated sludge) from Sjölanda sewage treatment plant and one sample of winter wheat straw, to fulfill the nutrient requirements of anaerobic digestion and improve the carbon to nitrogen ratio. The study further aims at determining if there are (aggregated) synergistic effects of co-digestion of the two substrates. The study will assess the influence of co-digestion, in comparison to mono-digestion, on specific methane yields and on process stability in terms of pH, acid to alkalinity ratio and events of foaming or other disturbances. The study further aims to explore the influence of co-digestion on dewaterability of the effluent. Finally, the study addresses co-digestion of SS and SWS in a full-scale plant.

2. Background

2.1. Anaerobic digestion

The first and still most widespread application of AD is sewage sludge (SS) treatment (Mata-Alvarez et al. 2007). Hence, for SS, AD is more advantageous compared to landfilling, since apart from reducing the organic content of primary and secondary sludges, biogas is produced and collected more efficiently, and nutrients can be returned to agricultural land if the digested residue keeps sufficient quality. Biogas transfers energy from waste handling to vehicle fuel, power and heat, while sludge stabilization provides a valuable biofertilizer (Siddiqui et al. 2011).

In Sweden, the first biogas plants was built in 1960 in wastewater treatment plants (WWTP) to reduce the volume of sludge produced. To respond to the oil crisis of 1970, the application of biogas as renewable energy source became of major interest, with the construction of anaerobic digesters in sugar refineries and pulp mills industries for wastewater

purification, and in farms for manure disposal. In the following 30 years biogas collection extended to landfills, food waste from industries and smaller producers (households and restaurants), slaughterhouses (European Biogas Association, 2021).

In early 2000 the use of energy crops increased largely in Europe until its use was restricted in the renewable energy act of EU and its amendment. WS and other cereal straws has a quantitatively large potential for biogas production and the use is not restricted by the aforementioned acts (Lantz, et al.,2017). However, the use is limited, largely due to the slow conversion rate of mature straws to methane, compared to that for green grasses such as maize and green full crop cereals (Kreuger et al., 2011; Croce et al., 2016).

Nowadays biogas applications are still expanding, with researchers reporting that biogas can potentially decrease the world's current emission by 10-13%. Specifically, emission reduction is achieved by collecting animal manure and digesting it to methane, otherwise released directly in the atmosphere, by generating digestate which substitutes energy demanding production of mineral fertilizers, and by decarbonization of the energy sector. Upgrade of biogas to biomethane is very much required in the transportation sector, since it is the only sector not reducing its greenhouse gas (GHG) emission but increasing it since 2014, currently accounting for 27% of the GHG emission in Europe. Furthermore, compressed natural gas and biomethane vehicles have the lowest carbon footprint, considering the entire life cycle assessment (LCA), among diesel, gasoline and electric vehicles (European Biogas Association, 2021).

In 2018, Europe registered 18 202 biogas plants producing about 6.5 TWh of electricity, corresponding to 14% of renewable energy available for final consumption. Biomethane, upgraded form of biogas, accounted for 660 plants producing 2.28 billion m³ of biomethane (bcm). Considering the availability and energy densities of sustainable feedstocks, defined by the EU Renewable Energy Directive 2009/28/EC as not competing for food and feed production, not leading to direct or indirect land use, and with a short carbon cycle, the amount of biomethane annually produced through AD is expected to increase to 62 bcm (660 TWh) (European Biogas Association, 2021).

With Europe aiming to cut to zero greenhouse gas emissions by 2050 (Weitzel et al., 2019), and the central role played by biogas in this scenario, research and optimization of AD is stringently required. Generally, the main limitation of AD are long retention time, low organic dry solids degradation for some lignocellulosic substrates, and low methane yields. Furthermore, the process is sensitive to hydraulic and organic overloads, pH and temperature fluctuation, lack of nutrients and presence of excess concentrations of nutrients and toxic compounds, which can make the process unstable (Elsayed et al., 2016). Process complexity results from the fine connection of the four steps constituting it, known as hydrolysis, acidogenesis, acetogenesis and methanogenesis. The process is unstable when the rate of an earlier step is higher or lower than the following one, as a result of insufficient microbial population or its inhibition. Low organic dry solids degradation usually results from low hydrolysis rate, determining limited substrate for the acetogenic step, and therefore low conversion to methane. Moreover, low methane yields are resulting from a weak connection of the last two stages: if methanogenesis is limited, the acids produced in the third step will accumulate, leading to pH drop and further inhibition of methanogens. Finally, long retention times derive from low hydrolysis rate and limited access to particulate substrates, and slow growth rate of methanogens. More specific constraints on the process are given by the substrate used. For instance, AD of organic compounds has acetate production as rate-limiting

step, whereas digestion of poorly biodegradable compounds is limited by the hydrolysis stage (Gerardi, 2003).

2.2. Co-digestion

Anaerobic co-digestion, the combination of two or more substrates with different composition has been reported to have synergic effects, leading to methane yields higher than the sum of the methane yields obtained in the mono-digestion of the same substrates, (Mata-Alvarez et al., 2007).

Among the substrate characteristics affecting the process efficiency, the carbon-to-nitrogen ratio (C/N) can be easily altered by co-digestion. Carbon is metabolized up to 30 times faster than nitrogen. Therefore, too high C/N ratios promote VFA accumulation, pH drop and process inhibition, whereas low C/N ratios are characterized by low conversion of substrate into biogas and process inhibition. The optimal C/N ratio for AD has been reported to be in the range 16-25 (Croce et al., 2016). Moreover, economically feasible digestion is characterized by Total Solids (TS) content around 10% in the reactors and a high degradation degree. Higher TS content hinders mixing and pumping and the use of solid substrates resulting in lower TS content in the reactors could limit the organic loading rate and the productivity of the reactors (Lantz et al., 2017).

Specifically, SS is characterized by C/N ratio around 10 and a TS around 4% and Volatile Solids (VS) content of 70-90% of the TS content (Asam et al., 2011, Biogas - Feedstocks, 2021). SS combination with a substrate with high carbon and TS, VS content - such as agro-waste - ensures optimal condition for the process, otherwise limited by the low organic loads of SS.

Wheat straw (WS) is the second most used substrate in co-digestion (Mata-Alvarez et al., 2007); it does not compete with food production for land usage and it is one of the most abundant agro-waste produced worldwide according to the food and agriculture organization of the United Nations (The potential effects of climate change on world food production and security, 2021). Furthermore, alternative solutions for WS disposal need to be found, since nowadays it is mainly landfilled or incinerated with negative effects on the environment (Elsayed et al., 2016). The main hurdle in WS degradation is its recalcitrant content- cellulose (30%), hemicellulose (50%), and lignin (15%)- and structure (Kumaret al., 2009). In order to make it a good substrate for AD, mechanical, chemical or physical pre-treatments are applied on WS, resulting in increased enzyme accessibility into the substrate, higher hemicellulose solubility and lower amount of lignin residuals. Other than requiring a costly pretreatment, WS high content in polysaccharides (C/N ratio around 150), favors the acidogenic step of AD, leading to VFA accumulation, pH drop and inhibition of methanogens (Croce et al., 2016). When WS is mixed with SS, the overall C/N ratio can be decreased to a value close to the optimal range of 25, and VFAs produced are pH-compensated by generation of ammonia from protein hydrolysis in the sludge (Zhang et al., 2017).

Therefore, it is more advantageous, both for the economy and the efficiency of the process, to use WS in co-digestion processes

2.3. Pretreatment choice

The accessibility of degradable compounds in WS to microorganism and the degradation rate increases, when WS is mechanically, chemically or physically pre-treated (Croce et al., 2016). In this project, acid catalyzed steam explosion was chosen for WS pretreatment, based

on investigations of its potential in other studies, whose results are summarized in Table 1. The efficiencies of the pretreatments were assessed through Biomethane Potential (BMP) tests conducted at mesophilic condition for 30 to 60 days. The main difference between these studies were the particle size used in the pretreatment and inoculum used for the BMP test.

Different particle sizes are the result from the first step of the pretreatment, hence steam explosion is usually preceded with a mechanical pretreatment. The mechanical treatment ensures smaller particles in the digester that avoid clogging and facilitate mixing process. Following the mechanical treatment, temperatures between 140 and 200°C are applied, for different duration, and rapidly discharged to atmospheric pressure causing explosion of macromolecules, degradation of lignin and increased hydrolysis of hemicellulose. The application of the same temperature (150-210 °C) without explosion, has been associated with formation of recalcitrant compounds and lower methane yields instead (Mata-Alvarez et al. 2007). As an intermediate step, WS can be soaked in water with an acid catalyst, pressed, and subsequently steam exploded, as reported by Nkemka et al.

In the study conducted by Theuretzbacher et al., application of temperatures ranging from 140 to 170 °C did not result in considerable higher methane yields after 45 days compared to the one obtained with untreated WS ground to less than 5 cm. However, steam-exploded WS showed faster gas production rates in the first days than the ground one. Therefore, the experiment succeeded in increasing degradation speed of biomass which could allow operating at lower HRT. Improvement in methane yield through higher temperature pretreatment were instead recorded by Ferreira et al. Other than having an opposite correlation between temperature and methane yield compared to the work above mentioned, the author observed an unexpected behavior of smaller particles. Methane production with larger particles (3-5 cm) was 5–13% higher than for smaller particles (<1mm). The author explained these uncommon results with the different composition of smaller and bigger straw chips, since protein, carbohydrates and lipids could be not uniformly distributed. The methane yield obtained after 50 days from WS pretreated at 200°C for 5 minutes was 24-27% higher than the ground straw. The methane production rate was also higher, and it remained higher even after 50 days. From the comparison of the first study and this one, it can be concluded that other than increasing the size of the particles pretreated, operating at higher temperature for shorter time could possibly be advantageous. Similar particle sizes, pretreatment parameters and methane yields were obtained in the experiment performed by Bauer et al. The duration of the pretreatment seems to have a small influence on the methane yield. Pretreatment of straw for 10 min at 160°C or 15 min at 180°C increased the specific methane yield by 14% and 20% respectively, but pretreatment for 20 min at 180°C did not result in a big increase in methane yield. The negative effect of smaller particles investigated by Ferreira et al., is in line with the lower increase in the methane yield observed in Sapci et al., where particle size were at least 10 times smaller than the one used by Ferreira et al.

A parameter that should also be considered is the amount of water used in the pretreatment steps- pre-soaking in diluted acid and steam- that once evaporated, could lead to loss of organic and volatile matter (Nkemka et al.,2013). These could have happened in the first study, where the biomass treated at high temperature for long time could have lost volatile compounds, therefore not converted to biogas and resulting in low methane yield. Spraying of acid reduces the amount of water used for soaking but increases the cost of the pretreatment. In the same study, by steam pretreating WS in the presence of diluted acid, the methane yield increased by 39%, 90% of which was obtained in only 17 days, as result of enhanced

biodegradability of the substrate. It can be concluded that the methane yields obtained from grinding and from steam explosion are similar, where grinding is however more energy demanding. Energy consumption and cost can be reduced, using particle size in the range of 0.5-1 cm, since smaller particle size are not necessarily related with lower methane yields. Finally, pretreatment temperature has a greater effect on the biomass composition than the duration of the pretreatment, contributing again to lower energy use and costs. Water addition for soaking should preferably be limited, and pressing WS can help reducing the amount of air present in the fibers structure and favoring acid access into it. Moreover, the techno-economic analysis performed by Shafiei et al. can be used as validation of steam explosion profitability, since it reported that performing steam explosion on WS and paper at 180°C for 2 minutes requires 13% higher total capital investment, but the higher methane production decreases the manufacturing cost by 36%.

Table 1. Pretreatment conditions of WS and BMP results available in literature.

Pretreatment	BMP duration	Methane yield	BMP duration	Methane yield
Theuretzbacher et al. 2014				
Only grinding to <5 cm	20 days	216 L CH ₄ kg VS ⁻¹	45 days	276 LCH ₄ kg VS ⁻¹
5 cm 140°C -30 min		240 L CH ₄ kg VS ⁻¹		275 LCH ₄ kg VS ⁻¹
5 cm 140°C - 60 min		262 L CH ₄ kg VS ⁻¹		286 LCH ₄ kg VS ⁻¹
5 cm 160°C - 60 min		236 L CH ₄ kg VS ⁻¹		264 L CH ₄ kg VS ⁻¹
5 cm 178°C - 60 min		221 L CH ₄ kg VS ⁻¹		245 L CH ₄ kg VS ⁻¹
Ferreira et al.2014				
Only grinding to 3-5 cm			50 days	233 LCH ₄ kg VS ⁻¹
3-5 cm 200°C - 5 min				296 LCH ₄ kg VS ⁻¹
Bauer et al.2009				
Only grinding to 0.5–1.0 mm				276 L CH ₄ kg VS ⁻¹
5–10 cm 160°C-10 min				314 L CH ₄ kg VS ⁻¹
5–10 cm 180°C-10				311 L CH ₄ kg VS ⁻¹
5–10 cm 180°C-15 min				330 L CH ₄ kg VS ⁻¹
5–10 cm 180°C- 20 min				296 L CH ₄ kg VS ⁻¹
5–10 cm 200°C-10 min				305 L CH ₄ kg VS ⁻¹
Sapci et al.2013				
Only grinding to 5-0.25 mm			60 days	234 LCH ₄ kg VS ⁻¹
5-0.25 mm 210°C-10 min				281 LCH ₄ kg VS ⁻¹
Nkemka et al.2013				
Only grinding to powder			31 days	210 LCH ₄ kg VS ⁻¹
1-2 cm Soaking in 0.5% H ₃ PO ₄ 190°C-5 min			31 days	250 LCH ₄ kg VS ⁻¹

2.4. Digestate disposal

The most efficient application of the sludge obtained from AD is as fertilizer, allowing recycling of the nutrients present in it. Digestate quality depends on the feedstock, the digestion performance and the post-treatment applied (Alburquerque et al., 2016).

Generally, addition of agro-wastes as co-substrate can decrease the concentration of heavy metals, pharmaceuticals and/or pathogens present in SS (Iacovidou et al., 2012). Specifically, the ratio of heavy metals to phosphorus should comply with legislation limits, which have become more stringent in Sweden for zinc, lead, mercury, nickel, chromium, copper, silver and cadmium. The new limits proposed for 2030, are met for zinc, lead, mercury, nickel and chromium, through proper source management and reduction in the general levels. Massive reduction of silver and cadmium is instead required in many sludges in Sweden to comply with new limitations (Mattsson et al., 2017).

Regarding the degradation performance, organic compounds removed during co-digestion can be quantified by measuring the Biomethane potential (BMP) of the digestate. If the digestate is stored in open spaces for some time before its final disposal, the presence of residual carbon would influence the release of methane directly in the atmosphere, as well as production of N₂O. Hence, the importance of achieving high degradation of organic compounds through co-digestion.

Finally, WS addition could positively affect digestate post-pretreatment too, by improving its dewaterability. Sludge processing, and transport cost varies from 15 to 60% of the total operating cost of a WWTP, depending on the size of the treatment plant and the national regulations for the disposal of organic materials (Wang et al., 2018, Wendland et al., 2005). To decrease sludge management cost, its volume is decreased by removing water. The dewaterability of the sludge can be affected by the biomass present in the AD, since it is composed of extracellular polymers and inorganic minerals that can form flocs where water is tightly bound (Wang et al., 2018). As reported by Gu et al. year, co-digestion of rice straw with SS, followed by coagulants addition and mechanical fractionation, can increase sludge dewaterability. Specifically, Örmeci and Vesilind year showed that the addition of cellulose allows the formation of strong flocs, increasing the dewaterability and settleability of synthetic sludge. Therefore, co-digestion with agricultural-based lignocellulosic residues, such as WS, could improve dewatering characteristics of the sludge, allowing lower addition of polymers prior dewatering or higher degree of water removal.

3. Methodology

3.1. Substrates

The substrates used in the co-digestion experiment were SS, composed of 70% primary sludge (PS) and 30% waste activated sludge (WAS) based on volume, and WS. SS was provided by Sjölanda, a full-scale wastewater treatment plant located in Malmö, Sweden. Around 70 L of WAS was collected on the 4th of February (-28 days from experiment startup) and stored in batches of around 8 L in 10 L tanks at 8°C. Some of the tanks were hygienized the same day, while others were stored at 8°C and hygienized during the following days. The same procedure was applied for primary sludge, 70 L was collected on the 11th of February (-21 days). The WS was harvested in autumn 2020 in Skåne, Sweden.

3.1.1. Sludge hygienization and homogenization

Sludge hygienization was performed at 72°C for at least one hour in order to decrease the number of pathogens present in the sludge and ensure safer conditions for its handling. Generally, sludge hygienization takes place at 70°C for 60 min (for particle sizes <12mm), and is recommended for SS before mesophilic digestion, in order to be safely used as fertilizer (ENV.E.3/LM, 2000) (Bendixen et al., 1999). Of specific concern was the presence of SARS-CoV-2. Inactivation has been reported to occur after exposure at temperature above 65°C for more than 3 minutes (Abraham et al., 2020) and thereby it should be efficiently inactivated with standard hygienization methods. 70 % of the sludge tank was submerged in water baths at 83°C and the sludge temperature was measured with a thermometer. The hygienization time was recorded from when the sludge temperature reached at least 72°C, and it was stopped when the temperature was maintained or increased above 72°C for an hour. The maximum temperature recorded in any tank was 78°C for WAS and 80°C for PS. Measurements were made every 30 minutes, after shaking and in the middle of the tank, for representation of the whole sludge volume. The hygienized sludge was stored at 6°C in closed containers. On the 22th of February (-10) PS and WAS were mixed together in a ratio 0.7:0.3 volume base in a stirred-tank reactor, with continuous stirring. Two batches of hygienization were performed, and the tanks were stored again at 6°C. WAS and PS were sent for analysis of TS, VS and elemental composition.

3.1.2. WS pretreatment

Wheat straw was pretreated through acid catalyzed steam explosion. The pretreatment consisted of 4 parts: milling, soaking, pressing and steam explosion, as depicted in Figure 2. A fast-moving coarse mill was used to mill 6 kg of WS to 0.5-1cm. Afterwards, 1 part straw and 10 parts dilute acetic acid (1% in cold tap water) was mixed together in a bucket, covered with a weight and left overnight. The following day, the soaking solution was drained, and the solid part pressed in a High Pressure Tincture Press of 25 Liter volume. Pressing time was 3 min when the pressure was reached; 300 bar on the hydraulic piston and roughly 20 bar on the chamber pressure. The resulting cake was steam pretreated at 190°C for 10 minutes in a 10 Liter reactor. The WS was stored in the freezer in plastic bags, in portions of 130 g. Each bag was thawed, kept at 6°C and used for maximum 4 days from its opening. The low pH ensures low microbial growth. Air and room temperature exposure were limited to avoid spoilage.

3.2. Experimental set up

The experiments were conducted in six, 3L- jacketed glass CSTRs with 2.6 L active volume, depicted in Figure 1. The reactor content was mixed at 80 ± 5 rpm by a pitched bladed paddle impeller with two blades. The temperature was maintained at 37°C by circulating warm water inside the reactor water jacket with a recirculating water bath (Lauda Ecoline E 100 Immersion Bath). Biogas was collected through Tygon tubing (name here), connected on one side to the automatic methane potential test system II (AMPTS II), Bioprocess control AB, Lund, Sweden. The AMPTS was used for continuous gas volume measurements, and on the other side to an air-tight gas collection balloons, used during feeding. Carbon dioxide was absorbed in glass bottles placed before the AMPTS, containing 800 mL of 3 M NaOH and thymolphthalein pH indicator.



Figure 1. CSTR used for the experiment.

The reactors were loaded with 2.3 L of inoculum two by two, meaning the first couple of reactors was filled with inoculum three days before the last two reactors. The inoculum was run alone for three days after the last couple of reactors were filled. Thereafter SS was gradually added, to reach a final volume of 2.6 L, while maintaining an OLR of 2.05 gVS/Ld. In order to verify that the reactors all operate equally, they were fed for 12 days with SS only. Afterwards two of them were left as SS controls (R1 and R2), and four reactors were fed with combined pretreated WS and SS (R3, R4, R5, R6). The WS controls (R3 and R6) were started on day 35, since it was chosen to let the reactors adapt first to WS and SS together, and then switch to WS only. The share of WS in the co-digestion reactors was set to 30% based on VS and the OLR increased to 2.7 gVS/Ld during the first 4 days. On the 4th day the share was increased to 50% and the OLR decreased to 2.03 gVS/Ld, while adding water to maintain the same HRT. Since with this setup, the amount of SS treated was significantly reduced, it was chosen to decrease again the share of WS to 35% and operate the same OLR of the SS controls. However, due to miscalculations and variation in the feedstock's composition, the OLR applied in SS control was between 2 and 2.05 gVS/Ld, between 2.01 and 2.12 gVS/Ld

for the co-digestion reactors, and 2.13 and 2.24 gVS/Ld for the WS control. The OLRs applied throughout the experiment are reported in the appendix in Table I.

Substrate feeding and withdrawal were conducted manually once every day, through a 100 mL plastic catheter tip syringe, from a port on the side of the reactor. The inlet was always 118.18 g, and the amount of WS, water and supplementary medium (in the WS controls) varied until day 40. Afterwards, the feedstock for co-digestion reactors consisted of 30.98 g of DI water, 10.38 g of WS, and 76.82 g of SS. The supplementary medium solution was 2% less concentrated than planned until day 48 (corresponding to day 13 for WS controls). Initially, the WS controls were fed with 44.95 g of solution A ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CO}(\text{NH}_2)_2$, Na_2HPO_4 , and $(\text{NH}_4)_2\text{SO}_4$), 9.34 g of solution B ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, CuCl_2 , ZnCl_2 , $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{MoNa}_2\text{O}_4 \cdot 6\text{H}_2\text{O}$, $\text{Na}_2\text{O}_3\text{Se}$ $\text{NaWO}_4 \cdot 2\text{H}_2\text{O}$, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$), 34.24 g of DI water and 29.66 g of WS. From day 58 (23 days for WS control), solution B was fed every third day two hours before feeding to avoid precipitation of phosphate. The composition of the feedstock was 27.97 g of solution A, 44.95 g of solution B, and 15.6 g of water. On the days when solution B was not fed, the amount of water was increased to 43.56 g. The amount of effluent withdrawn daily was calculated assuming 60% conversion of VS to methane, and resulted to be 114.81 g. This value was corrected to 110 g after a month, when the volume loss from the plug was measured, ranging from correspondingly 1.5 to 3.5 g wet reactor material per day.

Normally, alkalinity pH and VFA were measured every third day on samples collected before feeding, and more frequently when the process showed instability. TS and VS were measured on the inlet regularly, and on the outlet at the end of the experimentation. The HRT was repeated 3 times for a total operation time of 66 days. Since the WS controls start was delayed, the HRT has been repeated twice only so far.

3.3. Inoculum

The inoculum used in the experiments was collected on the 3rd of March (-5) from the mesophilic anaerobic digester at Sjölanda municipal wastewater treatment plant, Malmö, Sweden, operating at 37°C, with an HRT of 21.6 days. It was kept at room temperature for two to five days in plastic buckets connected to balloons before being transferred into the reactors. The inoculum had TS value 3.11 % (± 0.01) relative to its dry weight, VS equal to 67.68 % (± 0.07) of its TS.

3.4. Nutrient additions

A supplementary medium was fed daily to the WS controls, to provide the same concentrations of nutrients and trace elements present in the co-digestion reactors.

Twelve trace elements were supplied in the following form: 1.28 g/l of Fe as $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 0.341 g/L of Ca as $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.051 g/L of Mg as $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.049 g/L Cu as CuCl_2 , 0.048 g/L Zn as ZnCl_2 , 1.104 mg/L of Ni as $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 0.248 mg/L of Co as $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.03 mg/L of Mo as $\text{MoNa}_2\text{O}_4 \cdot 6\text{H}_2\text{O}$, 0.042 mg/l of Se as $\text{Na}_2\text{O}_3\text{Se}$, 0.371 mg/L of W as $\text{NaWO}_4 \cdot 2\text{H}_2\text{O}$, and 7.447 mg/L of Mn as $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$. Macronutrients were added as follow: 22.5 g/L of N as $\text{CO}(\text{NH}_2)_2$, 0.536 g/l of P as Na_2HPO_4 , and 0.3652 g/L of S as $(\text{NH}_4)_2\text{SO}_4$.

3.5. Analyses

3.5.1. pH, alkalinity, ammonium, phosphate, total solids and volatile solids

Alkalinity and pH were measured with a tim800 Titralab instrument and Abu901 autoburette, on supernatants obtained after centrifugation at 5000 rpm for 10 minutes (Thermo Scientific™ Labofuge™ 200 Centrifuge). Partial alkalinity (titration to 5.75) and total alkalinity (titration to 4.3) were determined according to Jenkins et al. Measurements of the total solids (TS) and volatile solids (VS) were performed following the standard methods (Greenberg., 1992). Triplicate samples of 30 g of steam-pretreated WS were steeped in 150 g deionized water for 18 h at 8°C in a 500 ml flask with a lid. The steeping solution was separated from the solid fraction by filtering through a vacuum pump and Whatman filter paper (pore size 2.7 µm), prior analysis of acids and alcohols. The TS and VS content of WS was corrected for volatile compounds, according to correction factors developed for grass silage (Porter and Murray 2011).

3.5.2. Acids and alcohols

Samples were acidified to pH 1-3 with 20% sulphuric acid and filtered through polyether sulfone 0.45 µm syringe filter (Chromacol, Welwyn Garden City, UK). Samples were analyzed with HPLC equipped with column AminexHPX-87H coupled with a guard column (Biorad, Richmond, CA, USA), RI detector and intelligent JASCO autosampler equipped with column AminexHPX-87H coupled with a guard column (Biorad, Richmond, CA, USA). The temperature applied was 50°C and the flow rate was 0.5 mL/min, with 5 mM sulphuric acid as eluent. Reactors effluents were withdrawn a couple of hours before feeding, centrifuged for 10 minutes at 5000 rpm in a Beckman Coulter Spinchron centrifuge (Beckman Coulter inc., Brea, US). The supernatant was stored in the fridge before being acidified and filtered for HPLC analysis.

3.5.3. Gas

Gas volume was measured continuously with an automatic methane potential test system II (AMPTS II), (Bioprocess Control AB, Lund, Sweden). The gas volume is expressed as dry gas at 0 °C and 1 atm. The biogas was stripped from carbon dioxide with 800 mL of 3 M NaOH and thymolphthalein pH indicator, placed before the AMPTS, as described in the AMPTS protocol (Bioprocess control AB). The gas after carbon dioxide stripping was assumed to be methane gas and the potential content of hydrogen, ammonia and hydrogen sulphide was ignored.

3.5.4. Elemental composition

Elemental analysis was performed by an external lab on samples dried at 40°C. The elements B, Ca, Cu, Fe, K, Mg, Mn, Na, P and S were analysed with inductively coupled plasma optical emission spectroscopy (ICP-OES) (Optima 8300, PerkinElmer Inc., Waltham, MA, USA), while Co, Cr, Mo, Ni, Se, Zn and W were analysed with inductively coupled plasma mass spectroscopy (ICP-MS) (Aurora Elite, Bruker Corporation, Bremen, Germany). A total organic carbon analyser coupled to a total nitrogen measurement unit (Shimadzu Corp., Kyoto, Japan) were used for measuring total nitrogen (TN) and total carbon (TC) in liquid samples, whereas solid sample were analysed through Vario MAX CN element analyser (Elementar Analysensysteme GmbH, Langenselbold, Germany). Phosphate was analysed on an 861 Advanced Compact IC (Metrohm AG, Herisau, Schweiz). Ammonium was analysed with a flow injection analyser (Foss A/S, Hilleroed, Denmark).

3.6. Statistical Analysis

All statistical analyses were performed using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test using the statistical software Prism (Prism 5 for Mac OS X, version 5.0b; GraphPad Software Inc., La Jolla, CA, USA). The term 'significant' is only used where a statistical analysis of significance has been performed. The significance level of 5% was used throughout all statistical analyses. Values are given \pm 1 SD. For linear combinations (Equation 3) the SDs were combined according to Equation 4. For multiplicative expression (Equation 5) the SDs were combined according to Equation 6:

$$y = k + k_a a + k_b b + k_c c + \dots \quad (3)$$

$$\sigma_y = \sqrt{(k_a \sigma_a)^2 + (k_b \sigma_b)^2 + (k_c \sigma_c)^2 + \dots} \quad (4)$$

$$y = k a b / c d \quad (5)$$

$$\sigma_y / y = \sqrt{(\sigma_a / a)^2 + (\sigma_b / b)^2 + (\sigma_c / c)^2 + (\sigma_d / d)^2 + \dots} \quad (6)$$

Where s = standard deviation; a , b , c , d = independent measured quantities; and k = constant.

4. Results and discussion

4.1. Mass balance during wheat straw pretreatment

Mass balances over pretreatment steps are shown in Figure 2. 89 % of the TS of the initial WS was recovered. The steam pretreated WS (SWS) had water insoluble substances (WIS) content of $14.35\% \pm 0.16$ and had particles size of 0.2 -1 cm. The TS content of the paste collected after steam pretreatment had a TS content of 20 %. After correction for volatile fatty acids lost during drying (Kreuger et al. 2011), and using correction factors presented by Porter and Murray, the TS content was 20.71%. SWS characteristics are summarized in Table .

Even though homogenous portions of WS were divided in plastic bags before freezing, variation of TS content in these was recorded to be up to 8% based on 5 measurements. The composition of the liquid fraction of WS obtained from the steeping procedure is reported in Table 2. Glucose is mainly available as disaccharide, while other monomeric sugars- xylose, mannose, galactose and arabinose, are present in higher concentrations. Hydroxymethylfurfural (HMF) and furfural are below the range of inhibition for methanogens, reported to be 2.4 - 3.0 kg/m³ (Raj, 2009), considering substrate dilution in the reactor.

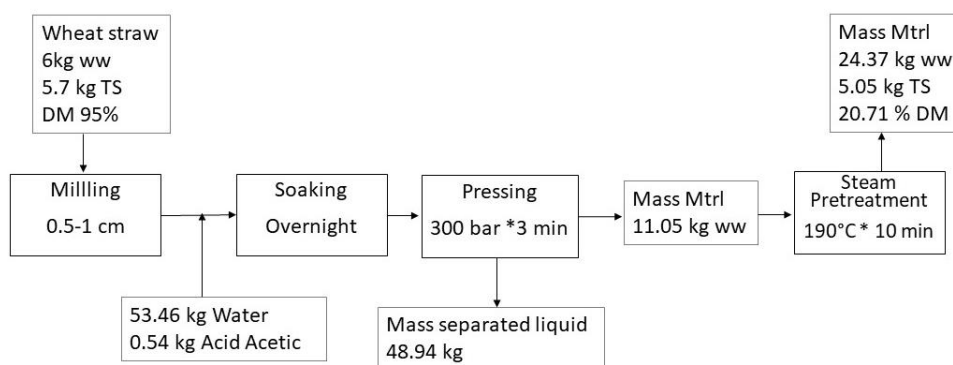


Figure 2. Schematic diagram of WS pretreatment units and material flow.

Table 2. Composition of WS steeping liquid.

g/L	Cellobiose	Glucose	XyGaMa	Arabinose	HMF	Furfural	Glycerol	Lactic acid	Formic acid	Acetic acid
Steam-pretreated WS	7.75±0.007	1.29 ± 0.001	9.68 ± 0.009	4.2 ± 0.007	0.205 ± 0.018	2.48 ± 0.008	0.218 ± 0.021	0.485 ± 0.003	1.699 ±0.011	7.79± 0.019

The elemental composition of WS and SWS is reported in Table II in the appendix. As reported by Nkemka et al., dilution of WS with water during soaking and steaming, are responsible for loss of nutrients and organic matter. In the aforementioned study, WS particles (1-2 cm) were soaked in water and 0.5% H₃PO₄ for an hour, pressed in the same condition as

in this study, and steam pretreated at 190°C for 5 minutes. In this last step, 6% loss of organic matter was reported.

In the current study, WS was diluted 10 times, 91% of the water was extracted in the pressing step, and loss of 11% of organic matter was recorded. Large amount of Ca, K, Mg, Na, P, S and Cl were lost during pre-soaking of WS with acetic acid, which can be avoided in full-scale process though re-circulation. Like-wise organic losses can be avoided in full-scale. Measure uncertainty adds up to this consideration.

Finally, in the SWS the amount of C increased from 43.5 to 47.2 %, probably as a result of higher hemicellulose solubilization obtained from the pretreatment, while the N remained almost the same, 0.63 in the WS and 0.6 in the SWS. The resulting C/N ratio is 77.3, extremely higher than the range recommended for AD of 16-25 (Croce et al.,2016).

4.2. Comparison of substrates composition and medium adjustments

Substrate characterization is summarized in Table . The TS content of SS was 5.5 % and the VS content 4.48%. The pH was 5.98, and total alkalinity 6922.8 mg CaCO₃ L⁻¹, ensuring high buffer capacity. The C/N ratio was determined to be 8.4. Co-digestion of 65% SS and 35% SWS results in a C/N ratio of 12.6, and a TS content of 10.8 %.

Table 3. Characteristics of the substrates used in co-digestion experiments.

Characteristics	Sewage sludge	Steam-pretreated WS
VS (% of TS)	81.41 ± 0.04	94.99 ± 0.37
TS (% of WW)	5.5 % ± 0.04	20.71 ± 0.7
TC (dry wt.%)	42.9	47.2
TN (dry wt.%)	5.1	0.6
C/N	8.39	77.34

Regarding the elemental composition, all the nutrients in SWS, except for Boron resulted to be present at concentration below the level suggested for AD by Zehnder et al. The nutrients expressed as mass ratio to carbon, was calculated for each feedstock based on gram of TS fed daily, and reported in Table III of the appendix.

Co-digestion of SWS with SS sludge can compensate the lack of nutrients such as Mg and Ca which combined with carbonate provide buffer capacity (Nkemka et al.,2013). However, only N, Fe, Zn and Cu were provided by the sludge in sufficient amount to fulfill the microbial requirement, in the range of concentrations defined by Romero et al. Nutrients addition was calculated in order to operate with the same concentrations in the WS controls and in the co-digestion reactors. Therefore, N, Fe, Zn and Cu were added in amounts above the recommended limit in the supplementary medium, while B and K were present in higher concentration in the WS controls than in the co-digestion reactors, due to their abundance in the SWS.

4.3. Alkalinity variation and effect on methane production

The pH of all reactors was throughout the experiment kept above levels that are known to make VFAs inhibiting for several methanogens and bacteria. Specifically, the SS controls operated in the range 7.8- 8.3, while by feeding WS the pH decreased to the range 7.3-8.3.

The optimal pH range for anaerobic digestion is in the range of 6.7 and 7.3. Acid-forming microorganism are tolerant to pH above 5, while methanogens require a higher pH of 6.2. In order to offset pH variation, high alkalinity has to be maintained in the reactors. The ratio Intermediate alkalinity/partial alkalinity, where the Intermediate alkalinity is an indirect measure of VFAs, can be used as indicator for process failure (Ripley et al., 1989). Indeed, pH drops were registered along with Intermediate alkalinity/partial alkalinity values above 0.3. To release the system from acid inhibition, the feeding was suspended on the same day, and the buffer effect of alkalinity quickly recovered.

Alkalinity variation over time is displayed in Figure 3. The first alkalinity measure was taken 5 days after switching from SS only to WS and SS, where the feedstock consisted of 30 % WS for the first 3 days, 50% WS on the 4th day, and 35% WS from the 5th day onwards. The alkalinity trend substantially differs in the SS control reactors and in the co-digestion ones. Specifically, in the co-digestion reactors the intermediate/partial alkalinity significantly increased above 0.3, in the first 10 days. This could be related to the lower amount of nitrogen provided when the reactors ran on mixed feedstock. Hence, SS has a greater protein content compared to WS, and their degradation into amino groups leads to ammonia release in the system. Ammonia is the primary alkalinity system present in the reactors, which is in equilibrium with protons, ammonium ions and hydroxide ions. Another important alkalinity system results from organic matter degradation to carbon dioxide producing carbonic acid, bicarbonate alkalinity, and carbonate alkalinity. The equilibrium between these species, as well as ammonia and ammonium ions, are functions of the pH. The pH values in all the reactors ensure that both species were partly present in the dissociated form, able to react and form ammonium bicarbonate, providing buffer capacity.

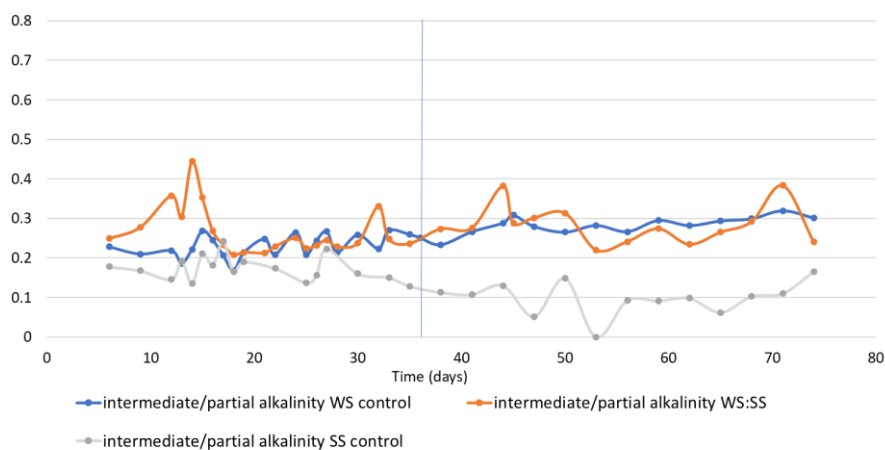
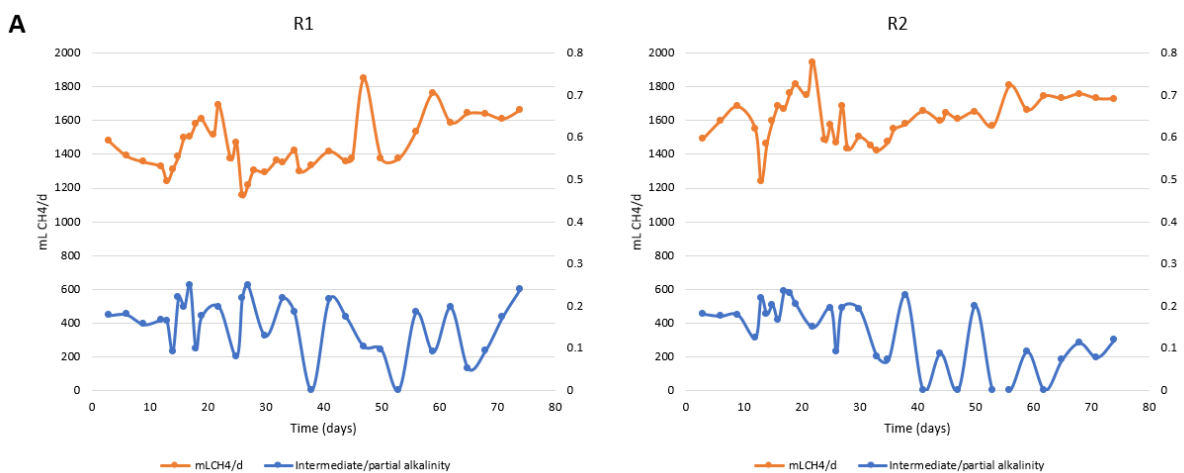


Figure 3. Intermediate/partial alkalinity variation over time, the line on day 35 represents the starting day for WS controls.

Acetate and other acids destroy ammonium bicarbonate, lowering the alkalinity in the system, until acetate is converted to methane (Gerardi, 2003). Figure 4 reports alkalinity variation and the effect on methane production, while VFA production and pH variation are displayed in Figure 5. The presence of 0.03 g/L of acetic acid throughout the experiment did not influence the pH stability in the SS control due to high system alkalinity provided by ammonia. The greatest disruption in alkalinity was recorded in reactor 5 (WS:SS) on day 12,

after accumulation of 2.6 g/L Acetic and 0.06 g/L propionic acid, preceded by decrease in methane production. The system took 5 days to recover from instability, during which feeding was halved for 1 day and suspended for the following 4 days, until acetic acid was efficiently consumed to 0.4 g/L. Lower accumulation occurred in reactor 4 (WS:SS), where 0.5 g/L of acetic and 0.02 g/L of propionic acid were detected on day 12. The day after the reactor was not fed and the system was relieved from instability, as can be observed from the increase in methane production and alkalinity. A slightly different trend was observed in reactor 6 (WS:SS), where 0.99 g/L of acetic and 0.04 g/L of propionic acid were detected on day 12th and the methane production dropped. The day after the reactor was not fed, but stability was maintained for one day only, since on day 15th the alkalinity decreased, and the feeding was suspended again. The detection of 0.25 g/L of methanol on day 12 could be a measurement bias. Methanol accumulation in the AD process results from low acidogenesis rate, meaning different steps in the process are inhibited. Moreover, overflowing from the reactor plug was observed on day 13. Possibly clogging in the gas tubing led to pressure build-up in the reactor, which resulted in overflowing. The stirring was decreased to 50 rpm and a 40 cm water column was connected to the reactor head space for 4 days, which would allow release of gas to the surrounding in case of overpressure. Carbon dioxide and VFAs accumulation in the reactor can decrease the surface tension of the liquid and be one of several reasons for foam formation (Gerardi, 2003). 100 microliters of antifoam were added on day 15th. Decrease in alkalinity in reactor 3 (WS:SS) occurred later on day 24 when 0.03 g/L of acetic and 0.02 g/L of propionic acid were detected, and the feeding was suspended.



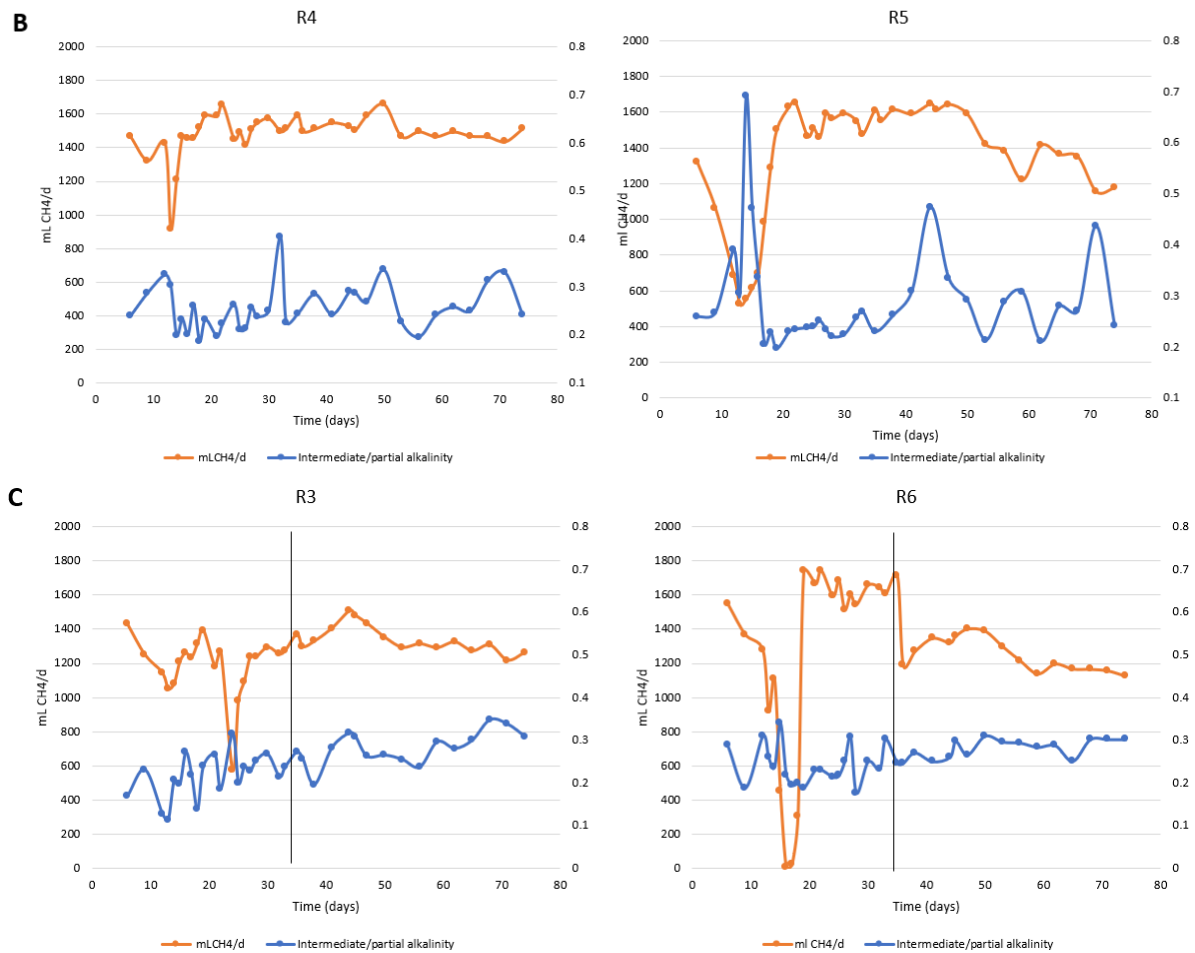


Figure 4. Alkalinity variation and the effect on methane production. A- SS control, B- Co-digestion reactors, C- WS control. The line on day 35 represents the starting day for WS controls.

Throughout the experiment the intermediate/partial alkalinity in the co-digestion reactors was below 0.3, except for few days, on which however propionic acid was not present and acetic acid was at low concentrations (see Reactor 4 on day 32). It can be concluded that in the co-digestion reactors, alkalinity drop due to VFA accumulation occurred only at the beginning of the process. The reason could be that the microbial communities' composition and activity was not adjusted to the new substrate. Furthermore, overloading, and often start-up periods, are associated with acid production rate being higher than the rate of methane production, and both acid accumulation and decreased methane production was recorded (Gerardi, 2003). Starting with a lower share of WS, might have avoided process instability, since the system would have gradually adjusted to the new substrate.

Regarding the WS control, ammonia supplementation in the form of urea was chosen to ensure sufficient alkalinity and a good pH range for AD. Generally, the alkalinity trend was more stable compared to the co-digestion reactors during the first HRT. This could be due to the microbial community being already acclimatized to WS when only WS was added as the only substrate in reactor 3 and reactor 6 from day 35. However, alkalinity decreased 18 days after switching to WS only, when acetic acid started to be detected above 0.03 g/L in both WS control reactors. Similarly to what was observed 10 days after switching from SS to WS:SS

for reactors 4 and 5, when much of the previous substrate in the reactors was rinsed out, alkalinity variation was observed.

4.4. Volatile Fatty Acids

VFA production over time and pH variation are depicted in Figure 5. As stated above, pH drops were related to VFA accumulation, and suspension of feeding helped in alkalinity recovery, whose buffer activity led to pH increase. Acetic acid was detected at concentration of 0.03 g/L throughout most of the experimental period in all the reactors, and at higher concentration on few days in the co-digestion reactors along with propionic acid. Hence, propionic accumulation is triggered by induced-feedback inhibition, when acetic acid, hydrogen or formic acid resulting from propionic degradation accumulate. Furthermore, only a narrow thermodynamic window allows syntrophic degradation of propionic acid to acetic, making propionic accumulation particularly problematic, considering toxicity is reported already at concentration below 5 mg/L (Gerardi, 2003).



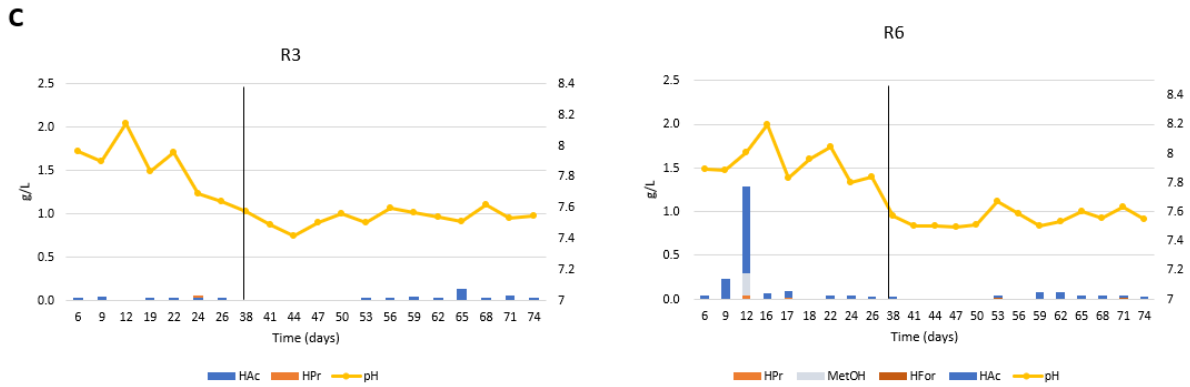


Figure 5. pH variation and VFA production over time. A- SS control, B- Co-digestion reactors, C- WS control. The line on day 35 represents the starting day for WS controls.

As reported by Sreerishnan et al., during anaerobic digestion microorganisms utilize carbon 25–30 times faster than nitrogen. Propionic accumulation during the first period of co-digestion might have occurred due to a sudden increase in the C/N ratio, from 8.3 to 12.3. When SS was fed, metabolism was slower due to the high amount of proteins, whereas when WS was introduced in the reactor, the higher amount of readily available carbon sped up metabolism, which led to acetic acid accumulation, and thereafter propionic accumulation. If the acid accumulation was caused by the hypothesized reason, the methanogenic population adjusted well by increased number of cells or increased conversion per cell to the higher availability of substrate after feeding, after some time. During the period 30-53 days VFA were not detected in the co-digestion reactors and in the WS controls, and as a result the pH stabilized around 7.5 (the pH variation in reactor 4 on day 47 was not related to VFA accumulation). This stable phase partially overlaps with the period where the highest and more stable methane production was recorded (day 35-50, Figure 7).

VFA concentration was usually measured 22 hours after feeding. On day 26, samples were withdrawn 2 hours before feeding, for 7 hours after feeding, with an hour interval, and 2 hours before the next feeding. VFA concentrations over 24 hours is depicted in Figure . The amount of VFA already present in the SWS and SS was estimated from the HPLC analysis and it is reported in Table 4. The two feedstocks have almost the same amount of acetic acid, and other acids are slightly lower in the mixed feedstock, except for formic acid. As can be observed in Figure 4, the mix feedstock and SS have similar VFA accumulation and reduction profile. The maximum amount of acetic acid was detected in 2 hours and got over in the SS control after 6 hours, while returned to the values recorded before feeding in the co-digestion reactors, except in reactor 5 where its consumption was delayed. Propionic acid got over after 6 or 7 hours in both reactors, suggesting that a major part of acidogenesis occurs in the first 6 hours. A higher amount of feedstock could probably be introduced without leading to VFA accumulation, since only 7 hours are required for its almost complete consumption.

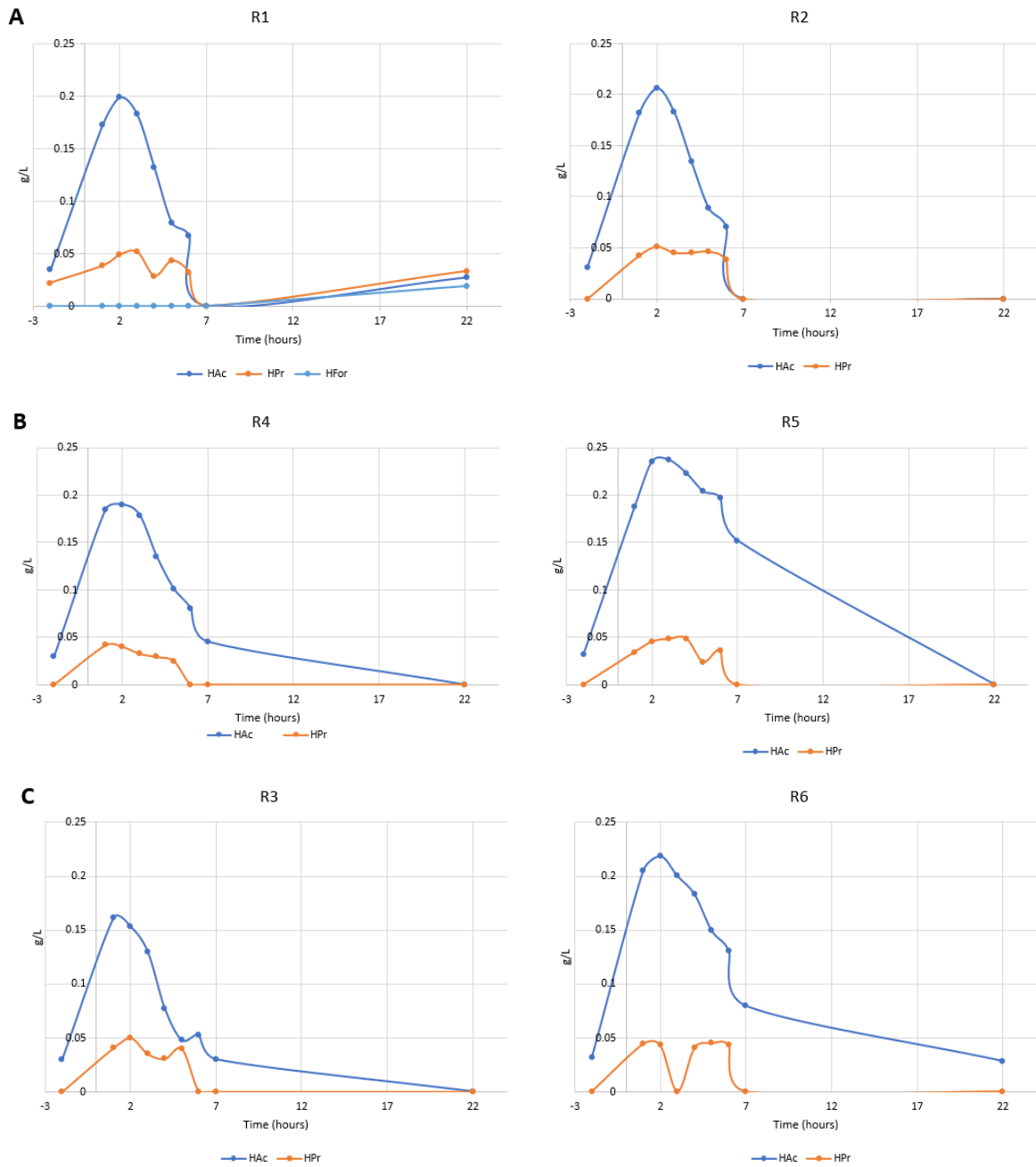


Figure 6. VFA accumulation after feeding. A- SS control, B- Co-digestion reactors, C- WS control.

Table 4. Approximative VFA content in the feedstocks.

Feedstock	Formic acid (g/L)	Acetic acid (g/L)	Propionic acid (g/L)	Isobutyric acid (g/L)	Isovaleric acid (g/L)
SS	0.0002	0.0347	0.0070	0.0035	0.0053
35% WS: 65% SS	0.0020	0.0381	0.0050	0.0023	0.0035

4.5. Specific methane yields

The OLRs applied throughout the experiment and specific methane production (SMP) are displayed in Figure 6. The SMP was calculated dividing the volume of methane produced on each day into the amount of VS fed on the same day (mL CH₄/gVS). In this way variation in the VS content of SS, variation in the amount of WS fed, and suspension of feeding were taken into account. In this last case, the volume of methane produced during the days without feeding was summed up to the methane produced on the day the feeding started again, divided into the amount of VS fed on the same day and the number of days that contributed to the total methane production.

As can be noticed from Figure 7, the reactors running as duplicates performed differently, the standard deviation varied throughout the experiment, which could be a result of variation in the substrates or microbial degradation or because of small losses of reactor content via the stirrer tube, gas leakages and difference in reactors components (stirrer length and speed, plugs etc.).



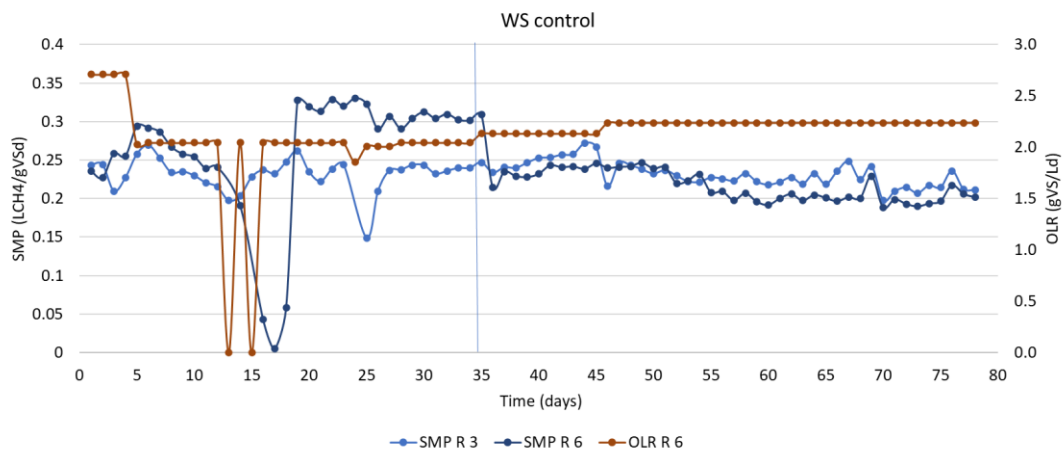


Figure 7. SMP and OLR applied throughout the study. A- SS control, B- Co-digestion reactors, C- WS control. The line on day 35 represents the starting day for WS controls.

Table reports the methane production for each HRT. Statistical analysis of the specific methane yields (SMY) is displayed in Figure . No significant difference was observed between SMYs of WS:SS and SS, and between SMYs of different HRTs. However, significant difference was observed between WS SMY and other substrates' SMY. The third HRT reflects the material fed best of the three periods, since most of the previous substrate has been rinsed out during this time. In order to verify the presence of any co-digestion effect, the SMYs of mono-digestions in the third HRT are summed up, with their relative share, and compared to the SMY obtained in co-digestion.

The theoretical yield for co-digestion was 272 mLCH₄/gVS with SD 13.4 mLCH₄/gVS. This yield was not significantly different from the SMY of SS:WS of the 3rd HRT which was 270.4 with SD 9.4, therefore no positive or negative effect of co-digestion was demonstrated. The comparison of these three conditions has some limitation. Since it was chosen to let the system adapt to the mixed feedstock before switching to WS mono-digestion, this one started 35 days after the others, and only two HRT were experimented so far. The effect of sludge age, WS variation between portions, and changes in the microbial community are not taken into account.

Moreover, as pointed out in Mata-Alvarez et al., the improvement in methane yields is mainly a consequence of higher OLRs applied in co-digestion rather than synergistic effects, and for an industrial perspective, the choice of the co-substrate is based first on its transport cost to the AD plant. It remains to evaluate if the co-digestion can facilitate a higher OLR than mono-digestion of the two substrates.

Table 5. Reactor's performance and HRTs.

HRT		SS control R1	SS control R2	Average SS control	WS:SS R5	WS:SS R4	Average WS:SS	WS control R3	WS control R6	Average WS control
1st	SMY (mLCH ₄ /gVS)	269.4	299.8	284.6 ±21.5	269.3	270.6	270.0 ±0.94	239.7	231.0	235.4 ±6.2

2nd	SMY (mLCH ₄ /gVS)	262.1	299.6	280.8 ±26.5	296.0	286.6	291.3 ±6.6	221.9	200.7	211.3 ±15
3rd	SMY (mLCH ₄ /gVS)	291.2	318.0	304.6 ±19	263.8	277.0	270.4 ±9.4	-	-	
1st	VS fed (g)	117.3	117.3		99.9	118.5		125.3	125.3	
2nd	VS fed (g)	114.2	114.2		116.3	116.3		122.3	122.3	
3rd	VS fed (g)	116.2	116.2		120.2	120.2		-	-	
1st	CH ₄ (mL)	31598	35164		26896	32063		30050	28960	
2nd	CH ₄ (mL)	29926	34205		34440	33350		27129	24541	
3rd	CH ₄ (mL)	33829	36946		31715	33314		-	-	

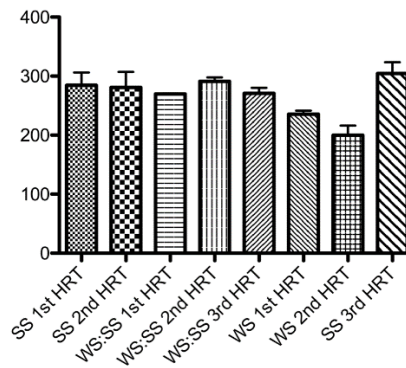


Figure 8. Statistical analysis of the specific methane yields (SMY).

The effluent obtained after 3 HRT from the SS control and the co-digestion reactors was used for BMP test. The TS and VS content of the effluents are summarized in Table . Similar degradation of SS and the mixed feedstock was measured, while WS degradation could only be estimated, since data from the WS controls are not available yet. WS degradation yield was estimated to be 60.9%, calculations are reported in the appendix.

Based on the low amount of organic matter left in the effluents a high gas production in the batch tests is not expected. However, no gas production has been recorded at all during the first days, which is less than expected. So far only one batch of effluent, and one bottle per reactors is started for batch digestion, and no positive controls are included. More replicates and positive controls need to be included prior conclusions can be made regarding methane potential of the effluent.

Table 6. TS and VS content at the end of the experimentation.

Reactor	TS inlet	TS outlet	VS inlet	VS outlet	TS degradation	VS degradation
SS control R1	6.26	3±0.255%	5.28	1.96 ±0.2%	48 %	62.9%
SS control R2	6.26	3.12±0.009%	5.28	2.09±0.01%	49.8%	60.5%
WS:SS R4	6.08	2.89±0.003%	5.47	2.11±0.002%	47.5%	61.4%
WS:SS R5	6.08	2.97±0.001%	5.47	2.17±0.002%	48.8%	60.4%

4.6. Full scale study

In order to evaluate the outcomes of this study in large scale, the full-scale plant providing the inoculum and sludge used in this experiment was taken as example. The plant

operates at an HRT of 21.6 days and an average OLR of 1.87 kg VS per reactor volume and day. The flow rate of PS and WAS is 31.4 m³/h, for a total of 275 228 m³ of sludge annually treated. The VS value of SS has a larger variation than the one measured during the experiment, and the average value was 4%. Table 7 reports the operational parameters applied now in the plant, and the ones resulting from its implementation with 35% WS. Since the amount of SS treated needs to be kept constant, WS addition leads either to shorter HRT or larger volumes.

In the first case the larger flow rate for a constant volume, would shorten the HRT to 19.4 days, which might not be enough for efficient degradation giving low methane yields and larger amount of digestate to be disposed. In the second scenario instead, the HRT is kept constant, and the volume increases. Specifically, the treatment of 1769.6 m³ should be integrated in the plant. The WWTP has a total volume of 16000 m³ distributed in 6 reactors, operated in pairs, meaning the construction of a new smaller reactor would be required.

Table 7. Operational parameters in the full-scale plant

OLR kgVS/m ³ d	WS share	Reactor volume (m ³)	kgVS/d WS	kgVS/d SS	WW (kg/d) WS	WW (kg/d) SS	total WW	Feedstock [kg/(m ³ d)]	F (m ³ /d)	HRT for co- dig.
1.87	0	15975	0	29873.3	0	739726	739726	46.3	739.7	
1.87	35%	15975	16085.6	29873.3	81939.4	739726	821665.4	51.4	821.7	19.4
OLR kgVS/m ³ d	WS share	Reactor volume (m ³)	kgVS/d WS	kgVS/d SS	WW (kg/d) WS	WW (kg/d) SS	total WW	Feedstock [kg/(m ³ d)]	F (m ³ /d)	V for co-dig. (m ³)
1.87	35%	15975	16085.6	29873.3	81939.4	739726	821665	51.4	821.7	17744.6

The methane yields obtained in the lab experiments can be used to estimate the plant productivity for co-digestion of 35% WS. The average methane yields for SS was 0.290 LCH₄/gVS. Considering the plant treats 270 000 ton of sludge per year, with VS content of 4%, the annual production of methane is 3162084 m³. The average methane yields for co-digestion was 0.270 LCH₄/gVS. The co-digestion plant would treat 270 000 ton of sludge and 29907 ton of WS per year, for a final VS load of 16774 ton VS per year. The annual production of methane would then be 4535954 m³.

Through co-digestion, annual biogas production of the plant would increase by 43%, with a volume increase of only 11%. The main costs for this implementation would be wheat straw, pretreatment, the construction of a new reactor, and perhaps of a feeding system and stirrers for WS addition into the reactors. The cost of digestate handling would change due to the larger volume produced (11% increase), and possibly the higher dewaterability and/or an improved quality of the sludge. In the small scale, chemicals were not added to the process, such as limestone for alkalinity maintenance, or nutrients to fulfil minimal concentration for microbial activity. Therefore, the demand of resources and the environmental impact would likely be low for the biogas produced.

5. Future perspective

From the data obtained during this experimentation, possible conditions to optimize the process can be inferred, as summarized in Table . The highest OLR allowed in the SS controls for a constant HRT of 22 days is 2.04 gVS/Ld, above which the HRT decreases. However, the WS:SS reactors can operate maintaining the same HRT at higher OLR, up to 2.8 gVS/Ld when the WS share is 35%, and 3.3 gVS/Ld for 50% WS. By increasing the OLR to 2.8 gVS/Ld no water addition is required to dilute the feedstock consisting of 35% WS (scenario a) to maintain the same HRT as the sludge control. However, as the share of WS increases to 50%, water needs to be added to compensate for sludge loss (scenario b), or alternatively, the HRT would increase (scenario c). Water addition leads to larger volumes of digestate to be dewatered and disposed and is therefore not desirable in full-scale. To study the effect of increased share of WS without increasing neither the OLR or the HRT, water would need to be added (scenario b). A longer HRT could lead to benefits such as higher digestion of sludge and WS but might not give neither pros or cons. The batch tests indicate so far that after 22 days not much degradable organic matter was left unconverted.

Maintaining the WS share to 35% and increasing the OLR to 2.8 g_{vs}/Ld, by exchanging water in the feed with SS, would allow treatment of 36% more sludge per reactor volume, compared to the case experimented in the lab, and the resulting feedstock would have a high potential bioenergy content, as reflected in the VS content. This last one can be further increased by operating at OLR of 3.3 g_{vs}/Ld and 50% SS, which is the maximum share of sludge which maintains the HRT of 22 days. Hence, operating with 35% WS at this OLR, would decrease the HRT to 18 days, which might be too short for efficient conversion of VS to biogas. However, increasing the OLR and WS share at the same time, could increase the risk of organic overload compared to changing only one of them, therefore it seems safer to increase the OLR to 2.8 gVS/Ld and keep the WS share to 35% in next step of this study.

Table 8. Possible operational parameters for future experiments.

Co-digestion condition	HRT (d)	OLR (g _{vs} /Ld)	Inlet SS (g)	Inlet WS (g)	Water (g)	Tot VS/d	Flow rate (L/d)
(a) 35% WS	22	2.79	104.81	13.37	-	7.28	0.12
(b) 50% WS	22	2.79	81.3	18.54	18.3	7.28	0.12
(c) 50% WS	26	2.79	81.3	18.54	-	7.28	0.1
(d) 50% WS	22	3.31	96.22	21.95	-	8.62	0.12

6. Conclusions

It was demonstrated that WS could be co-digested with SS, with a share of 35% of WS, without addition of any external nutrient elements except from what was provided by the SS.

Theoretical estimations showed that the mass of N, Fe, Cu and Zn were provided by SS in the co-digestion reactors in amounts, in relation to carbon, reported to be required for optimal digestion. Even though 12 nutrient elements were present in SS at lower concentration than optimal, the nutrients resulted sufficient to support stable degradation of the substrates.

Hence, continuous co-digestion in CSTRs at an OLR of $2.1 \pm 0.1 \text{ g/(L*d)}$, for longer than three times the HRT, was a rather stable process, where VFA accumulation occurred only at the beginning. After an adaptation period, VFA did not accumulate to inhibitory concentration in the reactors. Specifically, low VFA concentration was detected 7 hours after feeding, suggesting that the microbial community established in the reactors might tolerate higher OLRs.

The VS and TS content of the effluent indicated that the mixed feedstock was degraded as efficiently as SS only, meaning good accessibility to degradable compounds in WS was gained from its pretreatment, which might allow higher WS share in the inlet.

So far, the OLR and WS share applied did not lead to an increase (nor decrease) in the methane yield.

The evaluation of the digestate composition and characteristics, such as concentration of heavy metals, pathogens and dewaterability, is still missing for assessment of the feasibility of the application of this co-digestion process in full scale.

7. Corrections / Errata

Unfortunately, the elements concentration expressed as mass ratio to carbon was miscalculated. The concentration of nutrients per wet weight was used in one place instead of the concentration per TS for all elements except carbon and nitrogen, leading to 5-20 times lower concentrations than the actual ones. Thereby the supplementary medium provided insufficient amount of all nutrients in the WS controls, except for N. The data in Table III and conclusions based on them are incorrect.

7.1. Comparison of substrates composition and medium adjustments

The actual nutrient concentrations expressed in mg/L and as a ratio to carbon mass were calculated for each feedstock based on gram of TS fed daily, and reported in Table 9.

Excess or lack of nutrients in each feedstock can be determined by comparing nutrients concentrations with recommended levels for anaerobic digestion found in literature. Romero et al., reported stimulatory concentrations for 15 elements, and inhibitory levels for 7 of them, as displayed in Table 9.

According to Romero et al., the feedstock consisting of 100% SWS provided stimulatory concentrations of Fe, Zn, Mn, Cu, Ni, Cr, and the feedstock consisting of 100% SS supplied Ca, Fe, Na, Mn, Mo, Co, W, Se, Ni, Cr at stimulatory level, while Zn and Cu were at inhibitory concentrations. The mixed feedstock (35% SWS and 65% SS) retained 10 elements at stimulatory concentrations, and only Zn remained in the inhibitory range. It can be concluded that co-digestion of SS with SWS compensated for the limiting concentrations of Ca, Na, Mo, Co and W running low in this last one, and reduced Cu concentration below inhibiting levels.

Sufficient ratios of 16 elements (for AD) expressed per carbon were described by Zehnder et al., according to which all the nutrients in the feedstock consisting of 100% SP-WS except for B resulted to be insufficient, while the feedstock consisting of 100% SS met the N, Ca, Fe, Zn, Mn, Cu, Se, Ni requirement. Consequently, SS provided sufficient amount of 6 elements in the mixed feedstock.

Table 9. Nutrient concentrations in the feedstock, bold and underlined values meet the suggested level, wavyly underlined values are in excess.

	Romero et al.,2016		Zehnder et al.,						
	Stimulatory concentration (mg/L)	Inhibitory concentration (mg/L)	Suggested concentration (C-ratio)	SWS (mg/L)	SS (mg/L)	SWS:SS (mg/L)	SWS (C-ratio)	SS (C-ratio)	SWS:SS (C-ratio)
N	N.A.		0.0494	306.13	2825.14	1943.580	0.0129	<u>0.1192</u>	<u>0.0820</u>
K	400	400-28934	0.0697	114.72	130.50	124.98	0.0048	0.0055	0.0053
Ca	100-1035	300-8000	0.0178	79.84	<u>921.32</u>	<u>626.83</u>	0.0034	<u>0.0389</u>	<u>0.0265</u>
P	N.A.		0.1105	11.78	1089.46	712.31	0.0005	0.0460	0.0301
S	N.A.		0.0381	30.44	812.44	538.76	0.0013	0.0343	0.0227
Mg	720		0.0071	12.22	119.13	81.72	0.0005	0.0050	0.0034
Na	100-350	3500-8000	1.3973	2.49	<u>171.01</u>	<u>112.04</u>	0.0001	0.0072	0.0047
Fe	0.3		0.0003	<u>2.00</u>	<u>1903.17</u>	<u>1237.83</u>	8.44E-05	<u>0.0803</u>	<u>0.0522</u>
Zn	0.02-2	7.5-1500	1.43E-05	<u>0.30</u>	18.53	12.15	1.27E-05	<u>0.0008</u>	<u>0.0005</u>
Mn	0.027		8.25E-05	<u>0.50</u>	<u>7.27</u>	<u>4.90</u>	2.12E-05	<u>0.0003</u>	<u>0.0002</u>
B	N.A.		5.20E-06	0.90	N.A.	0.317	<u>3.82E-05</u>	N.A.	<u>1.34E-05</u>

Cu	0.03-2.4	12.5-350	8.43E-06	<u>0.1714</u>	18.47	<u>12.06</u>	7.24E-06	<u>0.0008</u>	<u>0.0005</u>
Mo	0.05		1.62E-05	0.0204	<u>0.0958</u>	<u>0.0694</u>	8.59E-07	4.04E-06	2.93E-06
Co	0.03-19	35-950	9.95E-06	0.0022	<u>0.1892</u>	<u>0.1237</u>	9.28E-08	7.99E-06	5.22E-06
W	0.04		N.A.	0.0032	<u>0.1476</u>	<u>0.0971</u>			
Se	0.04		2.71E-05	0.0046	<u>0.0476</u>	0.0325	1.93E-07	2.01E-06	1.37E-06
Ni	0.003-27	35-1600	1.35E-05	<u>0.0126</u>	<u>0.4500</u>	<u>0.2969</u>	5.30E-07	<u>1.90E-05</u>	1.25E-05
Cd	1.6		N.A.	0.0030	0.4449	0.2902	1.28E-07	1.88E-05	1.23E-05
Cr	0.01-15		N.A.	<u>0.0297</u>	<u>0.3675</u>	<u>0.2492</u>	1.25E-06	1.55E-05	1.05E-05

In contradiction with the initial plan, the WS controls were run with the same nitrogen content of the co-digestion reactors, but a lower concentration of all the other elements.

Eventually, different performance in co-digestion and mono-digestion reactors could be attributed to nutrient depletion in the WS controls. Therefore, to evaluate synergistic effects of co-digestion of the two substrates, mono-digestion of WS needs to be run again with the same nutrient balance as co-digestion.

Contrary to the conclusions drawn above, co-digestion can balance nutrient composition in SS and WS, leading to stable methanogenesis without external addition of chemicals, being therefore a resource-saving process for conversion of waste into energy.

8. References

- Abraham, J.P., Plourde, B.D. and Cheng, L., 2020. Using heat to kill SARS-CoV-2. *Reviews in Medical Virology*, 30(5), p.e2115.
- Alburquerque, J.A., de la Fuente, C. and Bernal, M.P., 2012. Chemical properties of anaerobic digestates affecting C and N dynamics in amended soils. *Agriculture, Ecosystems & Environment*, 160, pp.15-22.
- Bauer, A., Bösch, P., Friedl, A. and Amon, T., 2009. Analysis of methane potentials of steam-exploded WS and estimation of energy yields of combined ethanol and methane production. *Journal of Biotechnology*, 142(1), pp.50-55.
- Bendixen, H.J., 1999, March. Hygienic Safety—Results of scientific investigations in Denmark (Sanitation requirements in Danish biogas plants). In *IEA BioEnergy Workshop: Hygienic and environmental aspects of AD: Legislation and experiences in Europe*.
- Ferreira, L.C., Nilsen, P.J., Fdz-Polanco, F. and Pérez-Elvira, S.I., 2014. Biomethane potential of WS: influence of particle size, water impregnation and thermal hydrolysis. *Chemical Engineering Journal*, 242, pp.254-259.
- Greenberg, A.E., Clesceri, L.S. and Eaton, A.D., 1992. *Standard Methods for the Examination of Water and Wastewater*, Washington, DC: Am. Public Health Assoc.
- Gu, T., Yamamoto-Ikemoto, R., Tsuchiya-Nakakihara, E., Watanabe, H., Suetsugu, Y. and Yanai, A., 2016. Improvement of dewatering characteristics by co-digestion of rice straw with SS. *Environmental technology*, 37(23), pp.3024-3029.
- Iacovidou, E., Ohandja, D.G. and Voulvoulis, N., 2012. Food waste co-digestion with SS—realising its potential in the UK. *Journal of environmental management*, 112, pp.267-274.
- Jenkins, S.R., Morgan, J.M. and Zhang, X., 1991. Measuring the usable carbonate alkalinity of operating anaerobic digesters. *Research Journal of the Water Pollution Control Federation*, pp.28-34.
- Kreuger, E., Nges, I.A. and Björnsson, L., 2011. Ensiling of crops for biogas production: effects on methane yield and total solids determination. *Biotechnology for biofuels*, 4(1), pp.1-8.
- Kumar, P., Barrett, D.M., Delwiche, M.J. and Stroeve, P., 2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial & engineering chemistry research*, 48(8), pp.3713-3729.
- Lantz, M., Kreuger, E. and Björnsson, L., 2017. An economic comparison of dedicated crops vs agricultural residues as feedstock for biogas of vehicle fuel quality. *Aims Energy*, 5(5), pp.838-863.

- Luste, S. and Luostarinen, S., 2010. Anaerobic co-digestion of meat-processing by-products and SS—Effect of hygienization and organic loading rate. *Bioresource Technology*, 101(8), pp.2657-2664.
- Mata-Alvarez, J., Macé, S. and Llabres, P., 2000. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. *Bioresource technology*, 74(1), pp.3-16.
- Mattsson, A., Finnson, A. and l'Ons, D., 2017. Heavy metal content of Swedish municipal wastewater sludge—status and goals. *Water Science and Technology*, 76(4), pp.869-876.
- Michael H.Gerardi, 2004. *The Microbiology of Anaerobic Digesters*. Wiley, Somerset, NJ. pp.94.
- Nkemka, V.N. and Murto, M., 2013. Biogas production from WS in batch and UASB reactors: the roles of pretreatment and seaweed hydrolysate as a co-substrate. *Bioresource technology*, 128, pp.164-172.).
- Örmeci, B. and Vesilind, P.A., 2000. Development of an improved synthetic sludge: a possible surrogate for studying activated sludge dewatering characteristics. *Water Research*, 34(4), pp.1069-1078.
- Porter, M.G. and Murray, R.S., 2001. The volatility of components of grass silage on oven drying and the inter-relationship between dry-matter content estimated by different analytical methods. *Grass and Forage Science*, 56(4), pp.405-411.
- Poulsen, T.G., Nizami, A.S., Rafique, R., Kiely, G. and Murphy, J.D., 2011. How can we improve biomethane production per unit of feedstock in biogas plants?. *Applied Energy*, 88(6), pp.2013-2018.
- Rico, C., Diego, R., Valcarce, A. and Rico, J.L., 2014. Biogas production from various typical organic wastes generated in the region of cantabria (Spain): methane yields and co-digestion tests. *Smart Grid and Renewable Energy*, 5(06), p.128.
- Ripley L.E., Boyle W.C. and Converse J.C. 1986. Improved alkalimetric monitoring for anaerobic digestion of high-strength wastes. *Journal of WPC*, 58:5, 406–410. 2. APHA Standard methods for examination of water & wastewater.
- Romero-Güiza, M.S., Vila, J., Mata-Alvarez, J., Chimenos, J.M. and Astals, S., 2016. The role of additives on anaerobic digestion: a review. *Renewable and Sustainable Energy Reviews*, 58, pp.1486-1499.
- Sapci, Z., Morken, J. and Linjordet, R., 2013. An investigation of the enhancement of biogas yields from lignocellulosic material using two pretreatment methods: Microwave irradiation and steam explosion.
- Shafiei, M., Kabir, M.M., Zilouei, H., Horváth, I.S. and Karimi, K., 2013. Techno-economical study of biogas production improved by steam explosion pretreatment. *Bioresource technology*, 148, pp.53-60.

Siddiqui, Z., Horan, N.J. and Anaman, K., 2011. Optimisation of C: N ratio for co-digested processed industrial food waste and SS using the BMP test. *International journal of chemical reactor engineering*, 9(1).

Theuretzbacher, F., Lizasoain, J., Lefever, C., Saylor, M.K., Enguidanos, R., Weran, N., Gronauer, A. and Bauer, A., 2015. Steam explosion pretreatment of WS to improve methane yields: investigation of the degradation kinetics of structural compounds during anaerobic digestion. *Bioresource technology*, 179, pp.299-305.

Wang, Q., Zhang, W., Yang, Z., Xu, Q., Yang, P. and Wang, D., 2018. Enhancement of anaerobic digestion sludge dewatering performance using in-situ crystallization in combination with cationic organic polymers flocculation. *Water research*, 146, pp.19-29.

Wei, L., Qin, K., Ding, J., Xue, M., Yang, C., Jiang, J. and Zhao, Q., 2019. Optimization of the co-digestion of SS, maize straw and cow manure: microbial responses and effect of fractional organic characteristics. *Scientific reports*, 9(1), pp.1-10.

Weitzel, M., Vandyck, T., Keramidas, K., Amann, M., Capros, P., den Elzen, M., Frank, S., Tchung-Ming, S., Vázquez, A.D. and Saveyn, B., 2019. Model-based assessments for long-term climate strategies. *Nature Climate Change*, 9(5), pp.345-347.

Wendland, A. and Ozoguz, Y., 2005. Operation costs of wastewater treatment plants. Ahrensburg, Germany.

Websites

Biogas.ifas.ufl.edu. 2021. Biogas - Feedstocks. [online] Available at: <<https://biogas.ifas.ufl.edu/feedstocks.asp>> [Accessed 10 June 2021].

Europeanbiogas.eu. 2021. European Biogas Association. [online] Available at: <<https://www.europeanbiogas.eu/>> [Accessed 17 January 2021].

Fao.org. 2021. 9. The potential effects of climate change on world food production and security. [online] Available at: <<http://www.fao.org/3/W5183E/w5183e0b.htm>> [Accessed 10 February 2021].

9. Appendix

Table I. Different OLRs applied throughout the experiment.

SS control	OLR (gVS/Ld)	g_{vs}/d	TS	VS		
17.03.21-11.04	2.05	5.33	5.52%	4.51%		
12.04.21	1.77	4.59	4.78%	3.89%		
13.04.21-15.04.21	2.00	5.19	5.41%	4.40%		
16.04.21-27.04.21	2.00	5.21	5.43%	4.41%		
28.04-08.05	2.01	5.23	5.45%	4.43%		
09.05-11.05	2.03	5.27	5.48%	4.46%		
11.05-19.05	2.043	5.31	5.51%	4.50%		
19.05.21-23.05.21	2.036	5.29	5.49%	4.48%		
24.05.21-25.05.21	2.05	5.32	5.52%	4.51%		
27.05.21-06.06.21	2.06	5.36	5.55%	4.53%		
WS:SS	OLR (gVS/Ld)	g_{vs}/d	TS	VS	TS	VS
20.03-23.03	2.71	7.04	5.52%	4.51%	20.71%	19.63%
24.03.21	2.03	5.27	5.52%	4.51%	20.71%	19.63%
25.03.21- 11.04.21	2.04	5.31	5.52%	4.51%	20.71%	19.63%
12.04.21	1.86	4.83	4.78%	3.89%	20.71%	19.63%
13.04.21-15.04.21	2.01	5.23	5.41%	4.40%	20.71%	19.63%
16.04.21-27.04.21	2.05	5.32	5.43%	4.41%	20.71%	19.63%
28.04-03.05	2.05	5.34	5.45%	4.43%	20.71%	19.63%
4.05-06.05	2.09	5.44	5.45%	4.43%	20.71%	19.63%
07.05-23.05	2.09	5.44	5.45%	4.43%	20.71%	19.63%
09.05-11.05	2.10	5.46	5.48%	4.46%	20.71%	19.63%
11.05-19.05	2.11	5.49	5.51%	4.50%	20.71%	19.63%
19.05.21-23.05.21	2.11	5.48	5.49%	4.48%	20.71%	19.63%
24.05-25.05.21	2.11	5.50	5.52%	4.51%	20.71%	19.63%
27.05.21-06.06.21	2.12	5.52	5.55%	4.53%	20.71%	19.63%
WS control	OLR (g_{vs}/Ld)	g_{vs}/d			TS	VS
23.04 - 3.05	2.13	5.55			20.71%	19.63%
06.05.21-	2.24	5.82			20.71%	19.63%

Table II. Compositions of WS and SWS ($\mu\text{g/g}$, TS) and water for soaking, liquid fraction after soaking (mg for total mass)

	WS $\mu\text{g/g}$, TS	SWS $\mu\text{g/g}$, TS	WS mg	SWS mg	Water mg	Liquid a.soaking mg
B	8.31	18.02	47.37	91.01	2.54	8.86
Ca	2892.16	1590.91	16485.31	8034.97	1210.84	12113.63
Cu	3.11	3.42	17.73	17.27	0.16	6.17
Fe	28.28	39.82	161.20	201.11	0.54	13.75
K	10660.67	2285.91	60765.82	11545.10	72.95	53241.19
Mg	845.59	243.53	4819.86	1229.96	85.97	5026.72
Mn	25.37	10.01	144.61	50.56	0.22	80.26
Na	126.89	49.69	723.27	250.96	453.33	1041.10
P	489.72	234.71	2791.40	1185.41	BDL	2984.51
S	1493.77	606.56	8514.49	3063.46	118.96	7450.48
Cr	4.41	0.59	25.14	2.98	0.023	0.39
Co	0.05	0.04	0.29	0.20	0.001	0.00
Ni	2.66	0.25	15.16	1.26	0.011	0.12
Zn	8.17	6.01	46.57	30.35	0.38	33.03
Se	0.01	0.09	0.06	0.45	0.005	0.51
Mo	0.34	0.41	1.94	2.07	0.011	0.27
Ag	0.01	0.04	0.06	0.20	0.001	0.00
Cd	0.11	0.06	0.63	0.30	BDL	0.28
W	0.01	0.06	0.06	0.30	BDL	BDL
Hg	0.00100	0.00019	0.0057	0.001	0.0005	0.00
Pb	0.05	0.10	0.285	0.51	BDL	0.10
Cl	749.80	147.54	4273.86	745.16	140.48	22308.66

a.- after

Table III.

Nutrient concentrations are expressed as mass ratio to carbon, and the concentration in the reactors was based on gram of TS of substrate added daily.

Table III. Composition of feedstocks for AD, concentration expressed as mass ratio to carbon.

	100% SWS	100% SS	35% WS: 65% SS	AD requirement
C	1	1	1	1
N	0.0129	0.1192	0.0820	0.0494
K	0.0009	0.0003	0.0005	0.0697
Ca	0.0006	0.0022	0.0017	0.0178
P	0.0001	0.0026	0.0017	0.1105
S	0.0002	0.0020	0.0014	0.0381
Mg	9.75E-05	0.0003	0.0002	0.0071
Na	1.99E-05	0.0004	0.0003	1.3973
Fe	1.59E-05	0.0046	0.0030	0.0003
Zn	2.41E-06	4.49E-05	3.00E-05	1.43E-05

Mn	4.00E-06	1.76E-05	1.28E-05	8.25E-05
B	7.21E-06	0	2.52E-06	5.20E-06
Cu	1.37E-06	4.47E-05	2.96E-05	8.43E-06
Mo	1.62E-07	2.32E-07	2.08E-07	1.62E-05
Co	1.75E-08	4.58E-07	3.04E-07	9.95E-06
W	2.56E-08	3.58E-07	2.41E-07	0.00E+00
Se	3.64E-08	1.15E-07	8.77E-08	2.71E-05
Ni	1.00E-07	1.09E-06	7.44E-07	1.35E-05
Cd	2.42E-08	1.08E-06	7.09E-07	-
Cr	2.36E-07	8.90E-07	6.61E-07	-

Estimation of WS degradation in co-digestion reactors

Mono-digestion of SS had an average degradation yield of 61.7%. If in the co-digestion reactors the only component being degraded was SS, and with the same extent as in the mono-digestion, the effluent would have a content of: $5.47 - 0.617 \times 3.55 = 3.27$ gVS. Assuming the effluent is 35% WS, which corresponds to 0.75 gVS, the amount of WS degraded can be estimated to be: $1.91 - 0.75 = 1.16$ gVS – where 1.91 gVS is the amount of WS in the inlet- giving a degradation yield of $1.16 / 1.91 = 60.9\%$.

The VS content of the co-digestion effluent can be back calculated with the estimated degradation yield for WS. The amount of SS degraded is 2.19 gVS and the amount of WS is 1.16 gVS, therefore the VS left in the outlet would be $5.47 - 2.19 + 1.16 = 2.1$ gVS which is really close to the measured values.

Calculation of the correction factor for TS, considering VFA loss

The concentration of VFAs were calculated for the steeping liquid using equations from Kreuger et al 2011.,

Concentration in steeping liquid (g/kg) = $(m_1 + m_2 - m_3) \times c_1 / m_1$ (1)

Concentration after drying related to SWS (g/kg) = $c_1 \times D \times m_3 / m_1$ (2)

Where m_1 = original wet weight related to TS added, g; m_2 = water added, g; m_3 = substrate TS added, g; c_1 = concentration of analyzed compound, g/kg; and D = dilution factor = 5

The TS and VS were corrected in two ways: (1) according to the volatilization coefficients for grass silage dried at 100°C presented by Porter and Murray: lactic acid 0.375, formic acid and acetic acid 0.892; and (2) the measured losses of lactic, formic and acetic acid during drying (the difference between Equations 1 and 2) were added to the TS and VS values measured using standard method