Investigation of food waste valorization through sequential lactic acid fermentative production and anaerobic digestion of fermentation residues

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INVESTIGATION OF FOOD WASTE VALORIZATION THROUGH SEQUENTIAL LACTIC ACID FERMENTATIVE PRODUCTION AND ANAEROBIC DIGESTION OF FERMENTATION RESIDUES. ~~PART I: TECHNICAL ASSESSMENT~~

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Abstract

This work concerns the investigation of the sequential production of lactic acid (LA) and biogas from food waste (FW). LA was produced from FW using a *Streptococcus sp*. strain via simultaneous saccharification and fermentation (SSF) and separate enzymatic hydrolysis and fermentation (SHF). Via SHF a yield of 0.33 g_{LA}/g_{FW} (productivity 3.38 $g_{LA}/L{\cdot}h$) and via SSF 0.29 g_{LA}/g_{FW} (productivity 2.08 $g_{LA}/L{\cdot}h$) was obtained. Fermentation residues and FW underwent anaerobic digestion (3 wt% TS). Biogas yields were 0.71, 0.74 and 0.90 Nm^3/kg_{VS} for FW and residues from SSF and SHF respectively. The innovation of the approach is considering the conversion of FW into two different products through a biorefinery concept, therefore making economically feasible LA production and valorising its fermentative residues. Finally, a mass balance of three different outlines with the aim to assess the amount of LA and biogas that may be generated within different scenarios is presented.

Keywords: biorefinery, fermentation, lactic acid, enzymatic hydrolysis, biogas

1. Introduction

Biowaste generation in EU was estimated at 94 Mt for 2015 and current treatment options include landfilling, incineration, mechanical-biological treatment (MBT), composting and anaerobic digestion (EU, 2010). Food waste (FW) from households, restaurants, caterers and retail premises represents an important fraction of biowaste. FW is globally one of the most severe environmental, social and economic problems of developed and developing countries, accounting for over one billion tonnes produced every year (Gustavsson, 2011).

Currently the main environmental threat from organic waste is methane production from such waste decomposing in landfills. Before the adoption of the Landfill Directive 1999/31/EC methane emissions from landfills accounted for 30% of the global anthropogenic emissions of methane into the atmosphere (COM, 1996). Landfill Directive 1999/31/EC obliges member states to reduce the amount of landfilled biodegradable municipal waste to 35% of 1995 levels by 2016, however it doesn't prescribe specific treatment options for the diverted waste. The response of EU member states since mid 1990s was the implementation of MBT, anaerobic digestion and composting processes. However 20 years later, it is mandatory to improve the management of biowaste by supporting technical solutions that are able to generate added value products.

The composition of FW is heterogeneous, being made of (w/w) 30-60 % starch, 5-10 % proteins and 10-40 % lipids (Pleissner et al., 2013). Hence, it represents an interesting feedstock for biorefinery processes. FW biological valorisation is not only an environmentally friendly waste treatment option, but it is also a benefit to the bio-based economy since valuable waste material can be employed instead of expensive raw substrates.

Lactic acid (LA) has many applications in the food and beverage sector as well as in the pharmaceutical and chemical industries, and its polymerisation gives origin to the biodegradable polymer poly(lactic acid) (PLA) (Abdel-Rahman et al, 2016). The main paths of fermentative production of LA from FW are separate enzymatic hydrolysis and fermentation (SHF), and simultaneous saccharification and fermentation (SSF) ~~(see Table 1)~~. While in SSF enzymatic hydrolysis and fermentation are performed in a single reactor (with uniform temperature and pH conditions), SHF foresees two separate phases, and thus allows the application of optimal temperature and pH conditions for each process and the use of an acid or enzyme in the hydrolysis step, which may be highly effective for complex substrates. SSF in comparison with SHF showed shorter processing time, reduced substrate/product inhibition and lower energy and plant costs (Castillo Martinez et al., 2013; Abdel-Rahman et al., 2013). SSF was either carried out by enzymes added with the inoculum (Wang et al., 2016), using a single microbial strain (Pleissner et al., 2017) or an indigenous microbial consortium (Kim et al., 2016; Tang et al., 2016).

The aim of this study is to investigate the technical, economic and environmental feasibility of the sequential fermentative production of LA and biogas from FW using either SHF or SSF. LA and biogas production can be carried out using mature technologies, however, the two options are usually considered separately. Fermentative production of LA from FW was already proven to be feasible (Pleissner et al., 2017; Pleissner et al., 2015a and 2015b; Kwan et al., 2015; Li et al., 2015); however its main drawbacks are the high process costs, necessary to achieve a marketable LA, and a relevant amount of fermentative residues to be managed. LA production costs include: sterilization, fermentation and downstream processes. The most expensive cost items are sterilization and downstream processes, which represent up to 41% of the conventional fermentation process (Wang et al., 2015) and 1.57-1.62 €/kg_{LA} (Joglekar et al, 2006). Sterilization and downstream processes are strictly recommended in order to achieve a LA quality that is commercially competitive, since food grade purity and pharmaceutical plastic grade purity are 80% and 90%, respectively (Vijayakumar, 2007). LA fermentation costs vary in the range of 0.72-1.13 €/kg_{LA} (Wang et al, 2015), while market value of LA is 1.36 €/ kg_{LA} (ICIS, 2016).

AD of FW has been widely explored ~~(see Table 2)~~, and it has been implemented on full scale for the last decades, in agreement with waste management hierarchy and EU policy about organic waste management. Biogas yields observed for FW were 0.26-0.63 m^3/kg_{VS} (Fantozzi et al., 2011; Pavi et al., 2017; Alibardi and Cossu, 2015; Kafle et al., 2013; Dinuccio et al., 2010; Gunaseelan et al., 2009), while methane yields were 0.15-0.25 m^3/kg_{VS} for fruit pulp (Gali et al., 2009) and 0.26-1.4 $m^3_{methane}/kg_{VS}$ for mixed FW (Labatut et al., 2011; Elbeshbishy et al., 2012; Maya-Altamira et al., 2008).

Plant size that makes AD profitable ranks at 50-100 kWe and investment costs vary between 3000-5000 € and 6000-7000 € for plant sizes of around 500-1000 kWe and 50-100 kWe, respectively (Insabato, 2013). Electric energy has a current value of 0.10 €/kWh and thermal energy of 0.105 €/kWh (Eurostat, 2016).

The novelty of the approach consists of taking into account the conversion of FW into two different high value products through a biorefinery concept, therefore making at the same time economically feasible LA production and solving the issue of fermentative residues valorization. This approach is consistent with EU strategy about circular economy; moreover, industrial biotechnology belongs to the Key Enabling Technologies (KET), whose development, exploitation and implementation into the development of marketable goods and services are among priority action lines of European industrial policy.

~~The present research, concerning the overall investigation of the feasibility of the proposed biorefinery chain, is structured in three parts. Part I (this study) covers the technical issues, while the economic and environmental assessments will be respectively discussed in parts II and III.~~ Considering LA fermentation, this study describes SHF while the details about SSF process are given elsewhere (Pleissner et al., 2017). Nevertheless, AD tests performed on SHF and SSF fermentation residues, as well as on FW, are here fully taken into account.

Finally, a mass balance was evaluated for three different process outlines, with the aim to assess the amount of LA and biogas that may be generated considering different scenarios. In detail, LA fermentation through SHF or SSF (Scenario 1), biogas production from FW anaerobic digestion (scenario 2) and sequential LA fermentation and AD of fermentation residues (scenario 3) were discussed and compared.

2. Material and Methods

2.1 Food waste

FW, made of noodles, potatoes, vegetables, rice, fruits, meat and sauce, was collected daily from the canteen of Leibniz Institute for Agricultural Engineering and Bioeconomy Potsdam for 15 days in July 2015 (141 kg in total). Immediately after collection, FW was blended through a kitchen blender and stored at -20°C. At the end of the sampling period, all FW blends were pooled and homogenised. FW amounts employed in all tests, as well as glassware, were autoclaved at 121°C for 15 minutes before use to exclude the presence of autochthon microorganisms competing with the ones specifically inoculated for the study.

2.2 Microorganisms

A mesophilic *Streptococcus* sp. strain A620 (internal label), isolated from tapioca starch, was employed in LA fermentations. The strain was classified by the German Collection of Microorganisms and Cell Cultures (DSMZ Braunschweig, Germany) and is available at the Leibniz Institute for Agricultural Engineering and Bioeconomy Potsdam. The strain was cultured in 300 mL flasks, containing 60 mL of MRS broth (Merck, Germany) and 0.67 g Everzit Dol (Evers, Germany) dolomite as buffer. Incubation occurred at 35°C for 24 hours.

The initial pH in all flasks was equal to 6.0. Flasks were shaken at 100 rpm in an orbital shaker.

~~The microbial consortium used as inoculum for anaerobic digestion tests was supplied from a mesophilic anaerobic digester at Leibniz Institute for Agricultural Engineering and Bioeconomy Potsdam. It consisted of 3.2 % (w/w) total solids (TS) and 54.4 % (w/w) volatile solids (VS). The pH was 7.8.~~

2.3 Enzymatic hydrolysis

Enzymatic hydrolysis tests were carried out without repetitions in presence of 1 L FW in a 2 L BIOSTAT bioreactor (Sartorius AG, Germany). Stargen and Fermgen (Genencor International, The Netherlands) were employed to hydrolyze starch and proteins at 59°C and pH 4.5 for one hour, respectively. Hydrolytic performance was investigated regarding different solid-to-liquid ratios (11, 12.5, 20 and 25%, w/w) and enzyme loading (see section 3.2.2). Enzyme loading investigations were carried out at a solid-to-liquid ratio of 20% (w/w). Mixing was set between 400 and 800 rpm depending on viscosity of the FW. Samples were withdrawn, then inactivated at 95°C for 20 minutes, centrifuged at 5000 RPM for 10 minutes and supernatant was stored at -20°C until used in analyses.

Yields of glucose and FAN per gram of dry food waste (Y, g/g) was calculated as follows:

$Y = P / FW$, where P [g] is the release in glucose or FAN and FW the amount of food waste applied [g].

2.4 Lactic acid fermentation

LA fermentation was carried out in duplicate using a 2 L BIOSTAT bioreactor (Sartorius AG, Germany) containing 1 L of FW with a 20% (w/w) solid-to-liquid ratio. After enzymatic hydrolysis (see section 2.3), the reaction conditions were changed to 35°C and pH 6.0. A 6%

(v/v) *Streptococcus* sp. strain A620 inoculum was used. Samples were analyzed for sugars (glucose, fructose and sucrose) and lactic acid concentrations. Results are presented as mean values of two replicates. After LA fermentation, solids and the oily phase were separated through centrifugation, and the supernatant was afterwards inactivated at 95°C for 20 minutes and stored at -20°C. The residual solids were mixed with the oily fraction floating on the supernatant and employed as feedstock for anaerobic digestion (AD) tests.

2.5 Anaerobic digestion

Three substrates (homogenized FW and fermentation residues from SHF and SSF processes) underwent AD. AD batch tests were carried out at 37°C using 3% (w/w) total solids (TS) in 2 L (1.5 L working volume) SCHOTT glass bottles. Substrate-to-inoculum ratio was 2:1. Digesters were manually shaken once a day. Each bottle was connected by 4/6 mm Teflon tubes (PTFE, Germany) to 3 L sampling tubes containing a saturated saline solution acidified with some drops of concentrate sulphuric acid. Biogas volume and composition were daily measured through water displacement and a gas analyzer (see section 2.6), respectively. Each AD test was carried out in triplicate. Furthermore, controls using inoculum and cellulose, and only inoculum (blanks) were carried out in triplicate. AD tests were finished when marginal biogas production was below 1%.

Solubilization (made of disintegration and hydrolysis*)* is assumed as the rate-limiting step during AD of complex substrates rich in suspended solids (Van Lier et al., 2008). The disintegration constant (k_d) values were calculated as follows (Angelidaki et al., 2009). Assuming a first order kinetic model, the disintegration rate may be achieved through the first part of the cumulative biogas curve obtained from AD tests, according to:

$$B(t) = B)_{exp}\left(1 - e^{-k_{dis}t}\right)$$

where:

B(t) represents the cumulative biogas/methane production at a given time

B_{exp} is the ultimate biogas/methane potential yield of the substrate

k_{dis} is the first order disintegration rate [1/d]

t is the time [day].

2.6 Analytics

Samples characterization was carried out in duplicate according to EPA reference methods (EPA, 2016) where not otherwise specified and mean values are presented. TS of FW and fermentation residues were analyzed after drying at 105°C until constant weight. Then dried FW and fermentative residues were weighted and combusted at 550°C for 5 hours in a muffle furnace for volatile solids (VS) analysis.

Fibers (ADF, NDF and lignin) were analyzed using an ANKOM^{2000} fiber analyser on FW pre-dried at 60°C for 48 hours.

Sugars determination was carried out by cold water extraction. 3-5 g of dried FW and 50 mL of demineralized water were shaken for 30 minutes; afterwards 2 mL of a 30% (w/w) $ZnSO_4$ solution and 2 mL of a 15% (w/w) $C_6N_6FeK_4$ solution were added. After shaking, the mixture was filtrated and the clear filtrate analyzed by HPLC.

LA and sugars concentrations in fermentation samples were analyzed by HPLC (DIONEX, USA): 10 µL of sample was injected in a Eurokat H column (300 mm × 8 mm × 10 µm, Knauer, Germany) and eluted isocratically with 0.8 mL/min of 5 mM H_2SO_4. Detection was carried out by a refractive index detector (RI-71, SHODEX, Japan). Each analysis was carried out in duplicate.

Cat- and anion concentrations in fermentation samples were analyzed by ion chromatography (DIONEX, USA). For quantification of cations, 25 µL of sample was injected in an IonPac

CS 16 column (250 mm × 4 µm, DIONEX, USA) and eluted isocratically with 1.0 mL/min of 30 mM CH_3SO_3H at 40ºC. For quantification of anions, 25 µL of sample was injected in on an IonPac AS9-HC column (250 mm × 4 µm, DIONEX, USA) and eluted isocratically with 1.2 mL/min of 9 mM Na_2CO_3 at room temperature. Detection of cat- and anions was carried out though a conductivity cell. Each analysis was carried in duplicate.

Lipids analysis was performed by means of ANKOM Technology (USA) according to the ANKOM Technology Method 2, 01-30-09: Determination of Oil/Fat Utilizing High Temperature Solvent Extraction (ANKOM, 2014). Kjeldahl-nitrogen content in FW was determined according to DIN-EN-25663 standard method using a Kjeldahl System K-370/37. Protein content was calculated by multiplying the Kjeldahl-N content by 5.7 (Leung et al., 2012). Free amino nitrogen (FAN) concentration was measured using the ninhydrin reaction method (Lie, 1973), employing glycine as standard.

Elemental analysis was performed with a VARIO EL III elemental analyzer according to the manufacturers' protocol (Elementar Analysensysteme GmbH, Germany).

Quantification of methane, carbon dioxide, oxygen and hydrogen sulfide produced during AD was carried out using a GA 2000 (Ansyco, Germany) gas analyzer.

2.7 Statistical analysis

One way analysis of variance was carried out in SigmaPlot and used to measure the statistical difference of LA formation between repetitions. Statistically significant difference in median values was accepted for $P < 0.05$.

3. Results and discussion

3.1. Food Waste characterization

FW consisted of (w/w): 18.1% TS, 93.2% VS/TS, 33.5% starch, 14.8% protein, 12.9% fat , 8.5% free sugars, 8% NDF, 3.2% ADF and 0.1% lignin. Elemental analysis showed (values referred to dry weight): 47.9% C, 7.67% H, 2.56% N and 0.09% S. FW composition is in agreement with literature (Alibardi and Cossu, 2015; Campuzano and Gozalez-Martinez, 2016) and FW proved to be a suitable substrate for the proposed biorefinery concept.

3.2. Enzymatic hydrolysis

The efficient recovery of nutrients from FW strongly depends on the activity of enzymes added. Rosgaard et al. (2007) reported that the efficiency of an enzyme based hydrolysis of pretreated barley straw decreases when the viscosity of the slurry gets too high. To investigate this effect on food waste and to reduce the amount of enzyme needed to effectively hydrolysis food waste and to recover glucose and FAN different solid-to-liquid ratio and enzyme loadings were investigated.

3.2.1 Solid-to-liquid ratio

Glucose recovery was strongly dependent on the solid-to-liquid ratio (see Figure 1A). After 5-10 hours glucose concentration leveled off and 54.2 g/L was obtained when 11% (w/w) was applied. Glucose concentration steadily increased to 80.9 g/L when 25% (w/w) was used. A 33.5% (w/w) starch content and a 25% (w/w) solid-to-liquid ratio accounts to a starch loading of 83.8 g. The theoretical conversion of starch into glucose is 0.9 (Wymann et al., 2004), and thus 94.4 g/L can be theoretically recovered. The obtained glucose concentration (80.9 g/L) implies a recovery of 85%. Theoretically, 41.8 g/L of glucose can be obtained at a solid-to-liquid ratio of 11% (w/w). The obtained glucose concentration of 54.2 g/L, however, indicates the presence of a remarkable amount of free glucose. Table 1 ~~3~~ shows that the yield of glucose per gram of FW decreases with increasing solid-to-liquid ratio. It is assumed that better mixing conditions achieved at 11% (w/w) contributed to a better hydrolytic performance, and thus to a higher yield (0.49 g/g_{FW}), while at 25% (w/w) a yield equal to 0.33 g/g_{FW} was obtained.

Contrarily, even when the solid-to-liquid ratio was increased, the amount of recovered FAN remained relatively constant (see Figure 1B). Even though the concentration increased from 0.23 g/L to 0.29 g/L within 24 hours with increasing solid-to-liquid ratio, this trend is not comparable to the results shown in Figure 1A. The complete digestion of 14.3% (w/w) proteins in FW would certainly have an effect on FAN concentration. However, it might be concluded that proteases used are not appropriate for the digestion of proteins in FW. The yield of FAN (see Table 1 ~~3~~) decreased by increasing solid-to-liquid ratio. While 2.04 mg/g of dry FW was obtained at 11% (w/w), only 1.15 mg/g was obtained at 25% (w/w).

3.2.2 Enzyme concentration

In order to determine the lowest specific enzyme loading for glucose and FAN recovery different specific enzyme loadings as shown in Table 2 4 were tested. Contrarily to the solid-to-liquid ratio, the specific enzyme loading had no remarkable effect on glucose and FAN recovery (see Figures 1C and D). Yields were between 0.33 and 0.39 g glucose and between 1.82 and 1.92 g FAN per gram of dry FW (see Table 4).

3.3 Lactic acid fermentation

Due to the previously mentioned viscosity problems, 20% (w/w) solid-to-liquid ratio was chosen for LA fermentation. FW hydrolysis with Stargen was kept short for only one hour as it was found that the release of glucose occurs quickly (see Figure 1). After one hour 67.3 g/L of glucose was obtained which is in agreement with Figure 1. The hydrolyzed substrate was then inoculated with *Streptococcus* sp. strain A620 and the fermentation was carried out for

29 hours. Immediately after inoculation, LA concentration increased exponentially, reaching 39.2 g/L after 11 hours. Afterwards, it further increased linearly to 66.5 g/L until fermentation was stopped (see Figure 2). Glucose was completely consumed, but traces of sucrose and fructose, available as additional carbon sources, were still present. The first 11 hours was also the period of time where most of the FAN was consumed (see Figure 2B). Fermentation was carried out in duplicate and no statistical difference (P=0.637) was found for LA formation between repetitions.

The overall yield obtained in this study using SHF, considering the LA concentration after 29 hours, was 0.33 g_{LA} per gram of dry FW with a productivity of 3.38 $g_{LA}/L \cdot h$. SSF performed on same FW resulted in a yield of 0.29 g_{LA}/g_{FW} and a productivity of 2.08 $g_{LA}/L \cdot h$ after 28 hours (Pleissner et al., 2017), thus SHF resulted in higher yield and productivity. ~~Higher~~ Yields ($g_{LA}/g_{dry\ FW}$) ~~were~~ found in literature are 0.27 (Kwan et al., 2016) and 0.99 (Kitpreechavanich et al., 2016) for SHF processes; 0.85 (Kim et al., 2016) and 0.46 (Tang et al., 2016) are accounted for SSF processes. ~~(see Table 1),~~ However the yield strongly depends on substrate composition and on the strain. Productivity, defined as mass of LA generated per volume of fermentation broth in a time unit, is therefore a more reliable criterion to assess the performance of a fermentation process. During exponential phase 3.38 $g_{LA}/L \cdot h$ was produced in the present study, which is remarkably higher than productivity values in literature ~~(see Table 1)~~. It is known that *Streptococcus* sp. strain A620 (Pleissner et al., 2017) is able to degrade food waste, and thus this capability may additionally contribute to the release of glucose. Lowest productivity of 0.28 $g_{LA}/L \cdot h$ was found when FW was converted with an indigenous microbial consortium (Tang et al., 2016). This is not surprising, as the microbial consortium is not specialized to form only LA, but a mixture of different organic acids. The study of Kim et al. (2016) is of particular relevance for FW utilization approaches as it illustrates how FW can be utilized in repeated batch cultures over a long period of time. Even though a higher productivity was obtained in the present study and by Kwan et al. (2016) when FW was first enzymatically pretreated, the simplicity of processes presented by Pleissner et al. (2017), Kim et al. (2016) and Tang et al. (2016) clearly shows that the process steps can be reduced to a minimum.

3.4 Anaerobic digestion

AD tests lasted 20 days and resulted in following yields (see Figure 3): FW 0.710±0.02 Nm^3/kg_{VS} biogas, 0.398±0.035 Nm^3/kg_{VS} methane (56.35% v/v); fermentative residues from SSF: 0.743±0.01 Nm^3/kg_{VS} biogas, 0.499±0.008 Nm^3/kg_{VS} methane (67.19% v/v); fermentative residues from SHF: 0.90±0.016 Nm^3/kg_{VS} biogas, 0.62±0.013 Nm^3/kg_{VS} methane (68.8% v/v). Biogas and methane yields obtained from fermentation residues are higher than the ones achieved from FW, mostly likely because of the differences among the 3 substrates in relative abundance of carbohydrates, proteins and lipids. In detail, fermentative residues were rich in proteins and lipids, since their carbohydrate fraction was mostly already exploited in LA fermentation. Hence biogas and methane yields of fermentative residues were similar to pure proteins (0.7 Nm^3/kg_{VS} biogas, with an average methane content equal to 70%, v/v) and lipids (1.2 Nm^3/kg_{VS} biogas with an average methane content equal to 68%, v/v) (Weiland, 2010). FW was made of carbohydrates, proteins and lipids, but carbohydrates are the most abundant fractions, and thus biogas and methane trends were comparable to carbohydrates typical values (0.8 Nm^3/kg_{VS} biogas, with an average methane content of 50%, v/v) (Weiland, 2010).

Both SSF and SHF demonstrated two accomplishments: generation of a value added product (LA) and enhancement of biogas and methane yields. In a certain way, SSF and SHF had on AD the effect of a highly effective biological pre-treatment resulting in an improvement of methane production. In fact, the main purpose of AD pre-treatments is breaking the structure of substrate particles and transforming them in easily biodegradable liquefied products (Bracchitta, 2012). Considering the results achieved in the present research, it is possible to affirm that LA fermentation exploited carbohydrate (mainly) and protein (partly) fractions, leaving the lipids almost unaltered for the consequently carried out AD process (see Table 3 ~~5~~) and boosting the kinetics of methane production. This assumption was confirmed by the values of the disintegration constant (k_d), calculated according to Angelidaki (Angelidaki, 2009), which were equal to 0.43 1/d for FW, 0.35 1/d for SSF residues and 0.33 1/d for SHF residues. These values are of the same order of magnitude of the ones obtained in other studies (Fiore et al, 2016; Ruffino et al., 2015) using rice bran and husk (0.38 1/d), coffee dust and peel (0.31 1/d), mixed vegetable waste (0.38 1/d) and pesto sauce waste (0.25 1/d). Other Authors obtained 0.15-0.29 1/d for fruit pulp (Gali et al., 2009), 0.34 1/d and 0.26 1/d for onion and potato respectively (Giuliano et al., 2013), and 0.14-0.35 1/d for mixed food waste (Alibardi and Cossu, 2015). ~~Moreover~~, However, the trend of k_d values obtained in this study (FW>SSF>SHF) was expected because, as before mentioned, both fermentative residues were deprived from the readily digestible carbohydrate fraction, with a higher efficiency of enzymatic hydrolysis.

3.5. Mass balance

A mass balance was evaluated (see Figures 4-6) for three different process outlines with the aim to assess the amount of LA and biogas that may be generated considering different scenarios. In detail, LA production by means of SHF or SSF (Scenario 1); biogas generation through anaerobic digestion (Scenario 2); sequential production of LA from FW and of biogas from fermentative residues (Scenario 3). The mass balance starts with a theoretical amount of 1000 kg dry FW made of 335 kg of starch, 148 kg proteins 129 kg fat and 85 kg free sugars. About LA production, downstream processes are considered according to the process scheme usually adopted at Leibniz Institute for Agricultural Engineering and Bioeconomy in Potsdam. In detail, a sequence of micro- and nanofiltration, softening, mono- and bipolar electro dialysis, decolorisation, anion and cation exchange and distillation was taken into account.

Considering Scenario 1, 148.2 kg of LA and 851.8 kg of wastes (residual solids plus LA lost in downstream process) and 149 kg of LA and 851.1 kg of wastes (residual solids plus LA lost in downstream process) were produced respectively through SSF and SHF. Using Scenario 2, 260.49 Nm^3 of CH_4 and consequentially 2604.9 KWh of primary energy could be produced. Taking into account Scenario 3, combined SSF and AD produced 148.2 kg LA and 236.5 Nm^3 of CH_4 and therefore 2365 KWh of primary energy and 417 kg of digestate; while coupling SHF and AD produced 149 kg LA and 269.64 Nm^3 of CH_4 and therefore 2696.4 KWh of primary energy and 408.52 kg of digestate. Wastes generated within the three scenarios, residual solids generated by Scenario 1, as well as digestate deriving from Scenarios 2 and 3 could be valorized in a composting process.

The mass balance of Scenario 1 (see Figure 4) underlines that the main bottleneck of LA fermentation is the huge amount of wastes produced after fermentation and downstream processes. In Scenario 3, this drawback is partially solved by the consecutive AD. Anyway, downstream processes are usually highly complex and expensive, and they require a careful optimization (Komesu et al, 2017).

4. Conclusions

This work investigated the technical feasibility of a sequential biorefinery process for the production of LA and biogas from FW via either SHF or SSF, which was proven. The main findings of the research are that SHF achieved higher yield and productivity than SSF, lasting one hour more than SSF. Sequential LA and biogas production moved forward from biomass conventional management and showed two profits: first, AD reduced and valorised the fermentative residues generated from LA fermentation; second, SSF and SHF determined an effective enhancement of biogas and methane yields with respect of FW.

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Figure captions

Figure 1. Solid-to-liquid ratio and enzyme loading. Recovery of glucose (A) and FAN (B) when enzymatic hydrolysis of blended food waste was carried out in presence of 350 µl Stargen and 700 µL Fermgen at different solid-to-liquid ratios (w/w): 11.1% (open circle), 12.5% (closed circle), 20% (open triangle) or 25% (closed triangle). Recovery of glucose (C) and FAN (D) when enzymatic hydrolysis was carried out at a solid-to-liquid ratio of 20% (w/w) at different specific enzyme loadings: 3.5 µL/g Stargen and 5 µL/g Fermgen (open circle), 1.75 µL/g Stargen and 2.5 µL/g Fermgen (closed circle), 0.88 µL/g Stargen and 1.25 µL/g Fermgen (open triangle), 0.44 µL/g Stargen and 0.63 µL/g Fermgen (closed triangle) or 0.11 µL/g Stargen and 0.32 µL/g Fermgen (open square). Results are based on single measurements.

Figure 2. Lactic acid fermentation. Change of glucose (closed circle), fructose (open triangle), sucrose (open square), FAN (closed triangle) and lactic acid (open circle) concentrations during enzymatic pretreatment of food waste with 700 µL Stargen and subsequently carried out lactic acid fermentation using *Streptococcus* sp. strain A620 (A and B). Fermentations were carried out in duplicate and mean values are shown. No statistical difference (P=0.637) was found between replicates.

Figure 3. Specific methane production from food waste (continuous line), SSF fermentative residues (triangle-dot line) and SHF fermentative residues (dotted line) through anaerobic digestion.

Figure 4. Mass balance from food waste to lactic acid: Scenario1 represents the L(+)-lactic acid production through separate hydrolysis and fermentation (SHF) and simultaneous

saccharification and fermentation (SSF). Mass balance is based on dry weight. OFMSW: organic fraction of municipal solids wastes

Figure 5. Mass balance from food waste to biogas: Scenario 2 represents biogas and methane production through anaerobic digestion (AD). Mass balance is based on dry weight. OFMSW: organic fraction of municipal solids wastes

Figure 6: Mass balance from food waste to lactic acid and biogas Scenario 3 represents combined L(+)-lactic acid and biogas production. Mass balance is based on dry weight. OFMSW: organic fraction of municipal solids wastes

~~**Table 1.** Lactic acid productivity (Pr) and yields of lactic acid per gram of dry food waste (Y_{FW}) when fermentation was carried out after enzymatic hydrolysis or by simultaneous saccharification and fermentation.~~

~~Mode~~	~~Strain~~	~~Pr [g/L·h]~~	~~Y_{FW} [g/g]~~	~~Reference~~
~~SHF~~	~~*Streptococcus* sp. strain A620~~	~~3.38~~	~~0.33~~	~~this study~~
~~SHF~~[1]	~~*L. casei* Shirota~~	~~2.61~~	~~0.27~~	~~(Kwan et al., 2016)~~
~~SHF~~[2]	~~*Bacillus* sp. strain T27~~	~~0.44~~	~~0.99~~	~~(Kitpreechavanich et al., 2016)~~
~~SSF~~	~~*Streptococcus* sp. strain A620~~	~~2.08~~	~~0.29~~	~~(Pleissner et al. 2017)~~
~~SSF~~[3]	~~Indigenous microbial consortium~~	~~1.58~~	~~~0.85~~	~~(Kim et al., 2016)~~
~~SSF~~[4]	~~*L. casei*~~	~~0.70~~	-	~~(Wang et al., 2016)~~
~~SSF~~	~~Indigenous microbial consortium~~	~~0.28~~	~~0.46~~	~~(Tang et al., 2016)~~

[1]~~Food waste was pretreated with fungal enzymes~~

[2]~~Studies were carried out with model kitchen refuse pretreated with glucoamylase~~

[3]~~Fermentation was carried out as repeated batch culture~~

[4]~~Co-fermentation with sophoraflavescens residues in presence of cellulase and amylase~~

~~**Table 2.** Biogas and methane yields and disintegration constants (k_{dis}) from food waste through AD in mesophilic conditions. nd= not defined~~

~~Substrate~~	~~Yield~~	~~k_{dis} (1/d)~~	~~Reference~~
~~orange pulp~~	~~0.25 $m^3{}_{methane}/kg_{VS}$~~	~~0.29~~	~~Gali et al., 2009~~
~~pear pulp~~	~~0.15 $m^3{}_{methane}/kg_{VS}$~~	~~0.18~~	
~~apple pulp~~	~~0.18 $m^3{}_{methane}/kg_{VS}$~~	~~0.15~~	
~~onion~~	~~0.92 $m^3{}_{biogas}/kg_{VS}$~~	~~0.34~~	~~Giuliano et al., 2013~~
~~potato~~	~~0.83 $m3_{biogas}/kg_{VS}$~~	~~0.26~~	
~~tomato skins and seeds~~	~~0.42 $m^3{}_{biogas}/kg_{VS}$~~		~~Dinuccio et al., 2010~~
~~fruit and vegetable waste~~	~~0.32-0.63 $m^3{}_{biogas}/kg_{VS}$~~	~~nd~~	~~Gunaseelan, 2009~~
~~bread waste~~	~~0.58 $m^3{}_{biogas}/kg_{VS}$~~	~~nd~~	~~Kafle et al., 2013~~
~~vegetable waste~~	~~0.36 $m^3{}_{methane}/kg_{COD}$~~	~~nd~~	~~Maya-Altamira et al., 2008~~
~~plain pasta~~	~~0.33 $m^3{}_{methane}/kg_{VS}$~~	~~nd~~	~~Labatut et al., 2011~~
~~cabbage~~	~~0.26 $m^3{}_{methane}/kg_{VS}$~~	~~nd~~	
~~potatoes~~	~~0.33 $m^3{}_{methane}/kg_{VS}$~~	~~nd~~	
~~FW (50% bread, 20% vegetables, 10% fruit, 5% meat, 15 % nd)~~	~~0.43 $m^3{}_{biogas}/kg_{VS}$~~	~~0.35~~	~~Alibardi and Cossu, 2015~~
~~FW (50% meat, 20% vegetables, 10% fruit, 5% bread, 15 % nd)~~	~~0.59 $m^3{}_{biogas}/kg_{VS}$~~	~~0.14~~	
~~FW (36% bread, 20% vegetables, 10% fruit 19% meat, 15 % nd)~~	~~0.49 $m^3{}_{biogas}/kg_{VS}$~~	~~0.27~~	
~~OFMSW~~	~~0.26 $m^3{}_{biogas}/kg_{VS}$~~	~~nd~~	~~Fantozzi et al., 2011~~
	~~0.49 $m^3{}_{biogas}/kg_{VS}$~~	~~nd~~	~~Pavi et al., 2017~~
~~FW~~	~~0.4-1.4 $m^3{}_{methane}/kg_{VS}$~~	~~nd~~	~~Elbeshbishy et al., 2012~~

Table 1 ~~3~~. Yields of glucose ($Y_{Glc/FW}$) and FAN ($Y_{FAN/FW}$) per gram of dry food waste when enzymatic hydrolysis was carried out at different solid-to-liquid ratios.

Solid-to-liquid ratio [%, w/w]	$Y_{Glc/FW}$ [g/g]	$Y_{FAN/FW}$ [mg/g]
11.1	0.49	2.04
12.5	0.48	2.20
20	0.34	1.27
25	0.33	1.15

Table 2 4. Yields of glucose ($Y_{Glc/FW}$) and FAN ($Y_{FAN/FW}$) per gram of dry food waste when enzymatic hydrolysis was carried out at a solid-to-liquid ratio of 20% (w/w) and different enzyme concentrations of Stargen and Fermgen per gram of dry food waste (n. a. = not analyzed).

Enzyme concentration [µL/g]		$Y_{Glc/FW}$ [g/g]	$Y_{FAN/FW}$ [mg/g^{-}]
Stargen	Fermgen		
3.50	5.00	0.36	1.92
1.75	2.50	0.39	1.92
0.88	1.25	0.33	1.82
0.44	0.63	0.38	1.61
0.11	0.32	0.39	n. a.

Table 3 ~~5~~. Evaluation of anaerobic digestion performances in terms of biogas and methane yields, of methane content and of disintegration constant

Substrate	Biogas $[Nm^3/kg_{vs}]$	CH_4 $[Nm^3/kg_{vs}]$	CH_4 [%]	k_{dis} [1/d]
Food waste	0.71±0.020	0.39±0.035	56.35	0.43
Fermentative residues from SSF	0.74±0.01	0.499±0.008	67.19	0.35
Fermentative residues from SHF	0.90±0.016	0.62±0.013	68.80	0.33

Figure 1.

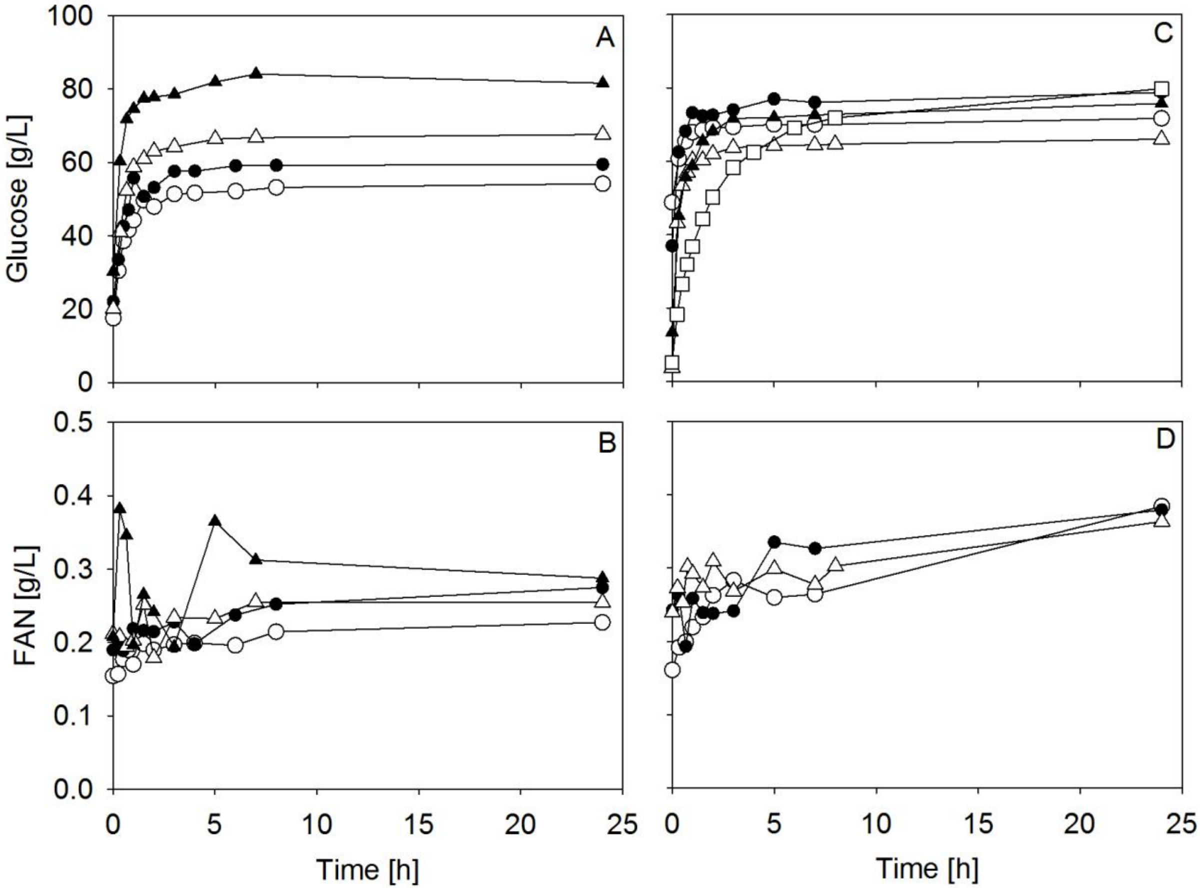

A
B
C
D
Glucose [g/L]
FAN [g/L]
Time [h]
Time [h]
100
80
60
40
20
0
0.5
0.4
0.3
0.2
0.1
0.0
0
5
10
15
20
25

Figure 2.

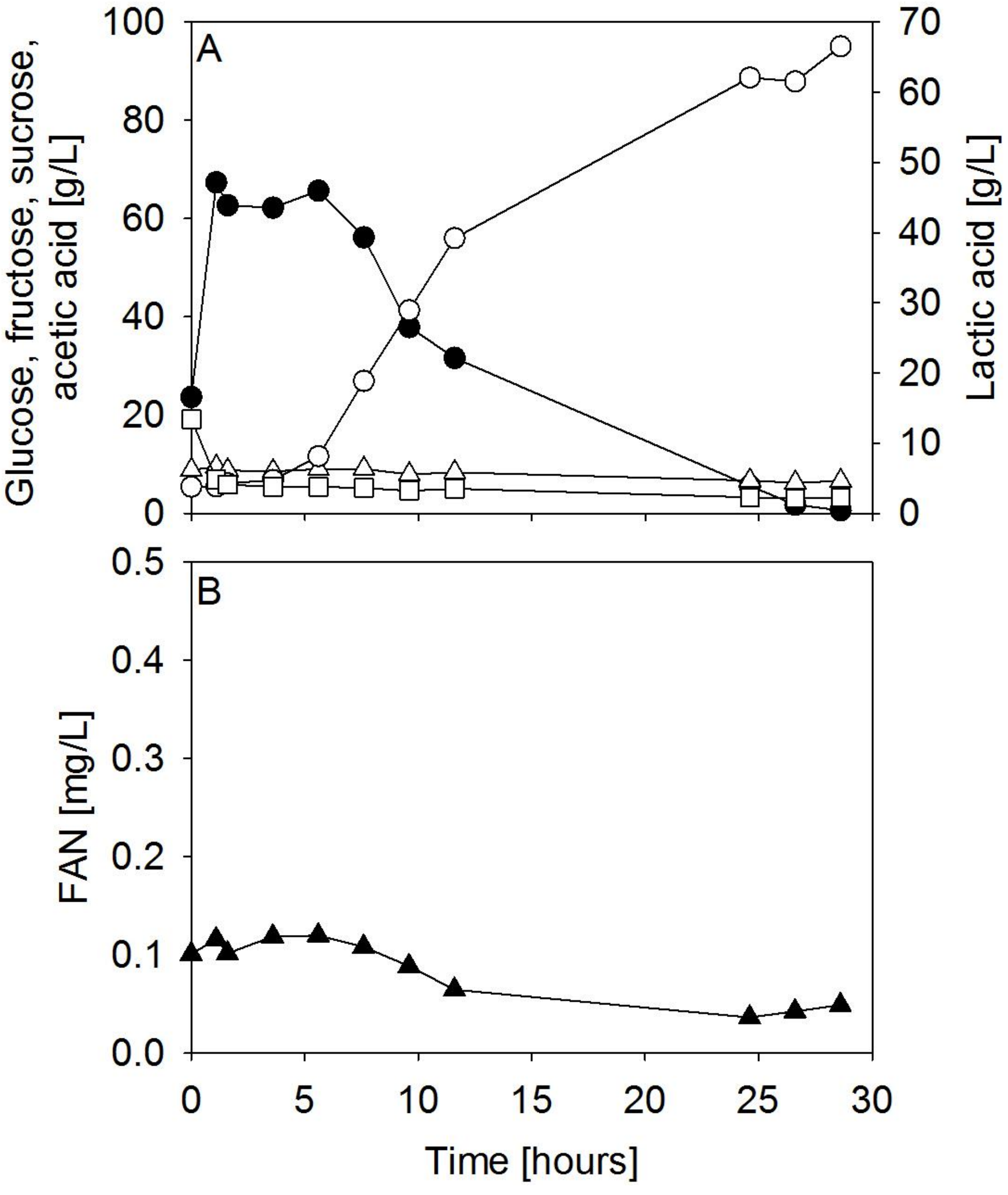
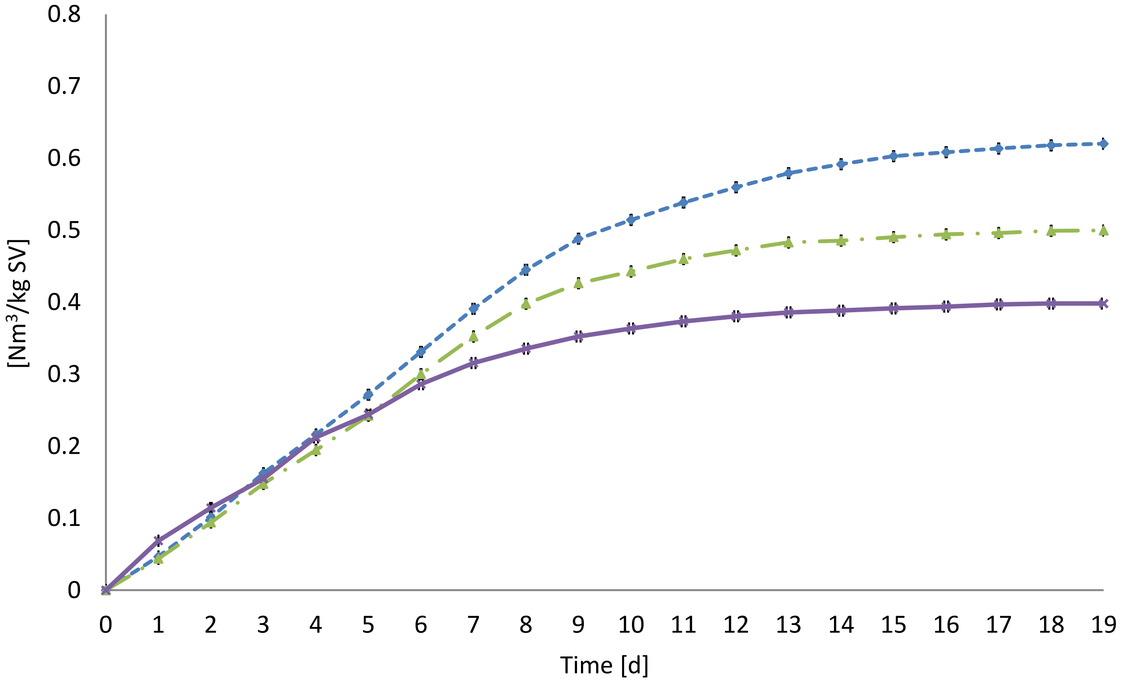

A
B
Glucose, fructose, sucrose, acetic acid [g/L]
Lactic acid [g/L]
FAN [mg/L]
Time [hours]
100
80
60
40
20
0
70
60
50
40
30
20
10
0
0.5
0.4
0.3
0.2
0.1
0.0
0
5
10
15
20
25
30

Figure 3.



0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
[Nm³/kg SV]
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Time [d]

Figure 4.

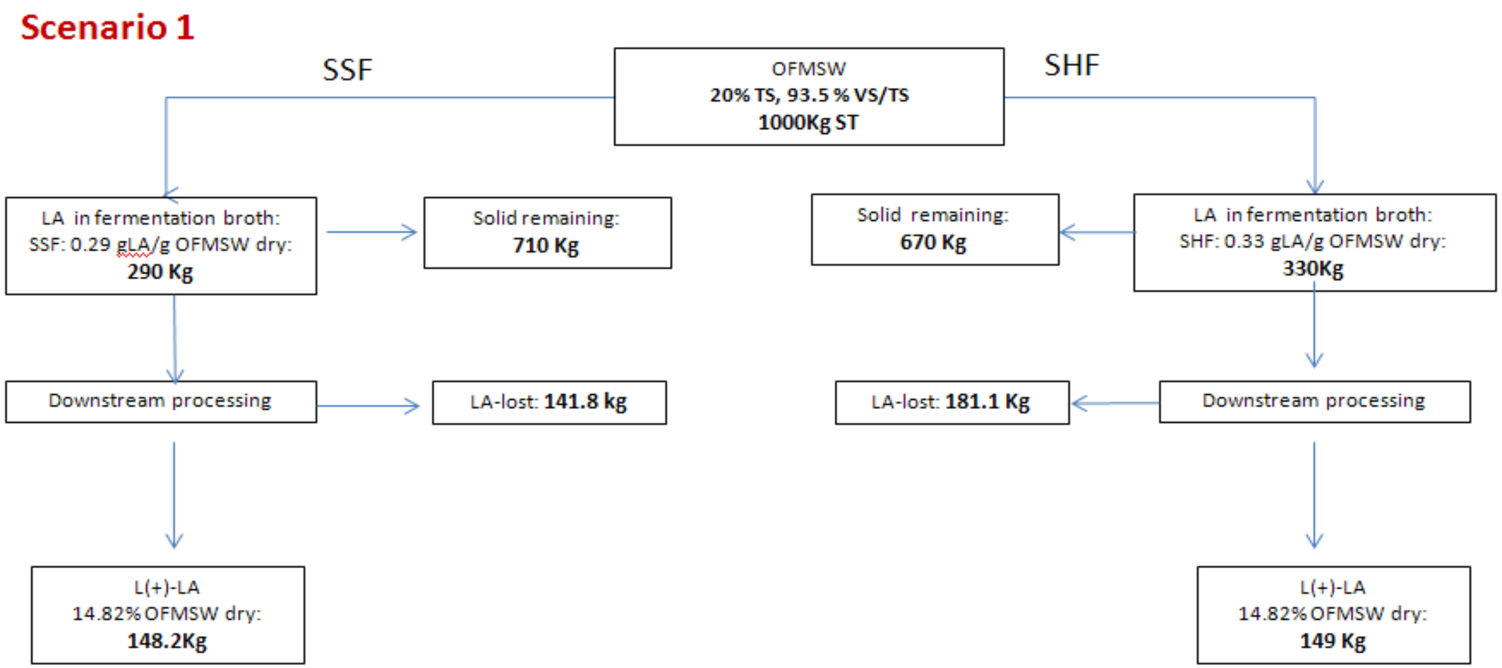

Scenario 1
SSF
SHF
OFMSW
20% TS, 93.5 % VS/TS
1000Kg ST
LA in fermentation broth:
SSF: 0.29 gLA/g OFMSW dry:
290 Kg
Solid remaining:
710 Kg
Solid remaining:
670 Kg
LA in fermentation broth:
SHF: 0.33 gLA/g OFMSW dry:
330Kg
Downstream processing
LA-lost: 141.8 kg
LA-lost: 181.1 Kg
Downstream processing
L(+)-LA
14.82% OFMSW dry:
148.2Kg
L(+)-LA
14.82% OFMSW dry:
149 Kg

Figure 5.

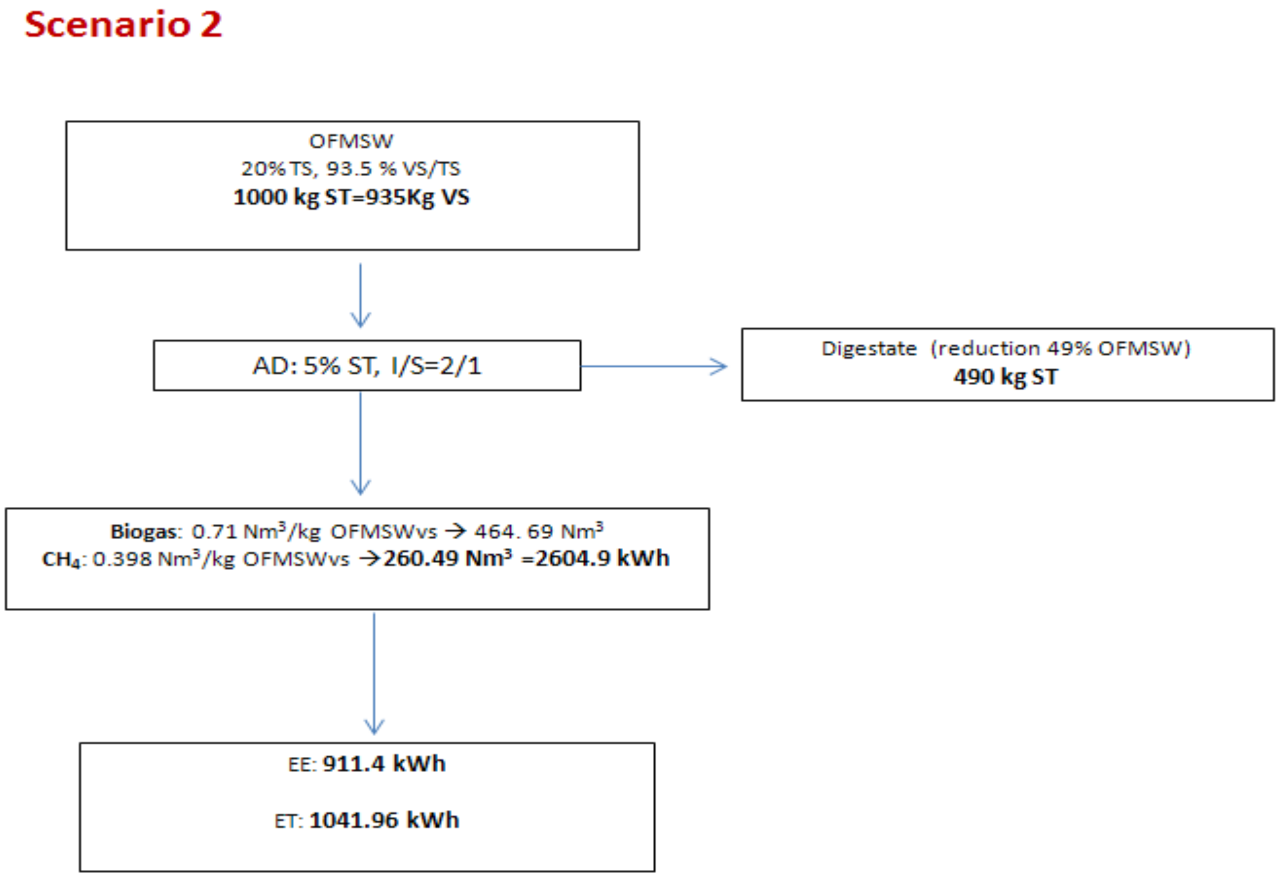
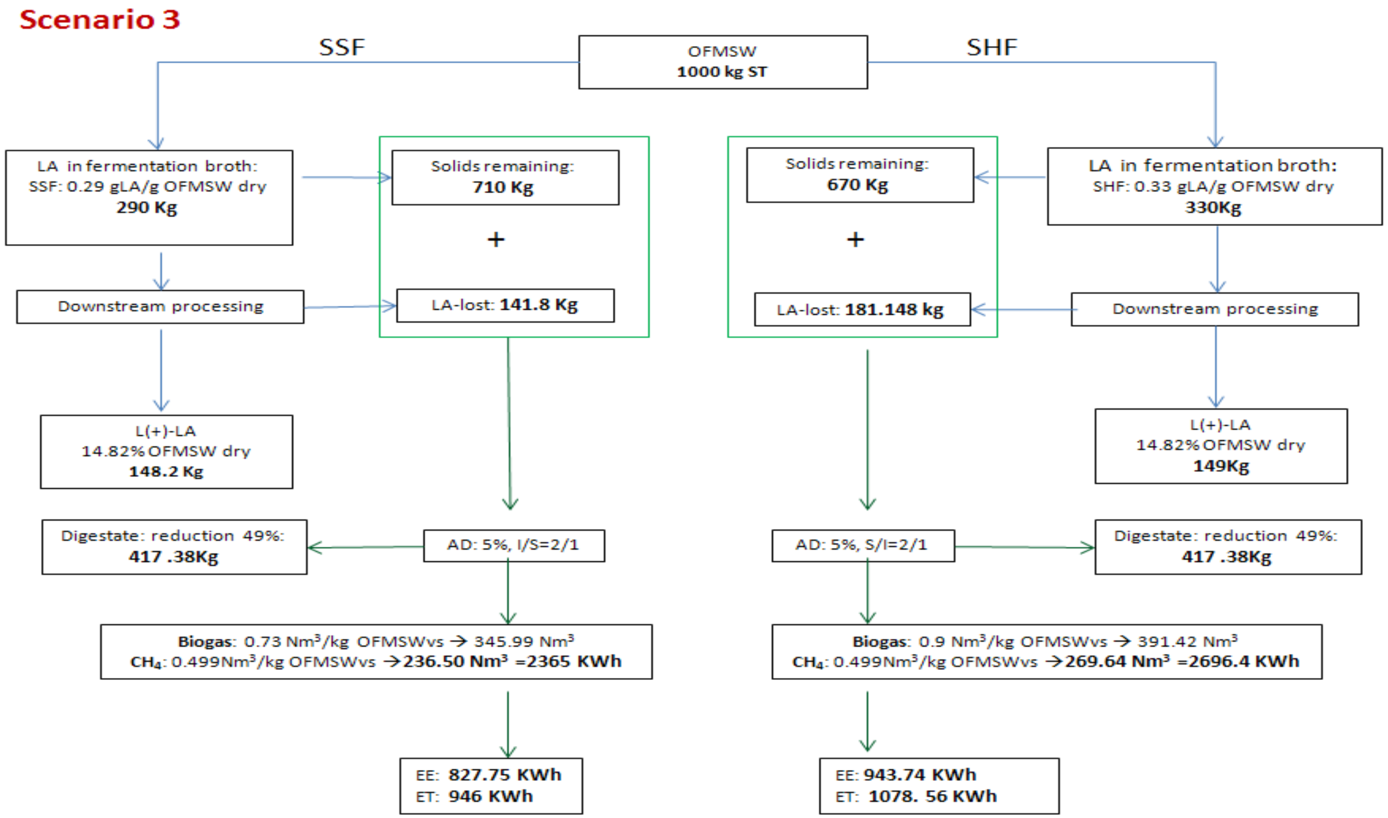

Scenario 2
OFMSW
20% TS, 93.5 % VS/TS
1000 kg ST=935Kg VS
AD: 5% ST, I/S=2/1
Digestate (reduction 49% OFMSW)
490 kg ST
Biogas: 0.71 Nm3/kg OFMSWvs → 464. 69 Nm3
CH4: 0.398 Nm3/kg OFMSWvs → 260.49 Nm3 =2604.9 kWh
EE: 911.4 kWh
ET: 1041.96 kWh

Figure 6.


Scenario 3
SSF
OFMSW
1000 kg ST
SHF
LA in fermentation broth:
SSF: 0.29 gLA/g OFMSW dry
290 Kg
Solids remaining:
710 Kg
+
LA-lost: 141.8 Kg
Downstream processing
L(+)-LA
14.82% OFMSW dry
148.2 Kg
Solids remaining:
670 Kg
+
LA-lost: 181.148 kg
LA in fermentation broth:
SHF: 0.33 gLA/g OFMSW dry
330Kg
Downstream processing
L(+)-LA
14.82% OFMSW dry
149Kg
Digestate: reduction 49%:
417 .38Kg
AD: 5%, I/S=2/1
AD: 5%, S/I=2/1
Digestate: reduction 49%:
417 .38Kg
Biogas: 0.73 Nm³/kg OFMSWvs → 345.99 Nm³
CH₄: 0.499Nm³/kg OFMSWvs → 236.50 Nm³ =2365 KWh
Biogas: 0.9 Nm³/kg OFMSWvs → 391.42 Nm³
CH₄: 0.499Nm³/kg OFMSWvs → 269.64 Nm³ =2696.4 KWh
EE: 827.75 KWh
ET: 946 KWh
EE: 943.74 KWh
ET: 1078. 56 KWh