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Abstract

The organic fraction of municipal waste (OFMW), source-sorted (SS-OFMW) and non-sorted (NS-OFMW), was used as raw material for the sequential production of bioethanol and biogas. Non-isothermal and simultaneous saccharification and fermentation (NSSF) resulted in maximum ethanol concentrations of 51 g/L and 26 g/L for SS-OFMW and NS-OFMW samples, showing overall process yields of up to 80% and 59%, respectively, even without subjecting substrate to hydrothermal pretreatment. Subsequently, the solid residues resulting from the fermentation were further subjected to anaerobic digestion (AD), showing a methanogenic potential of 384±6 mL CH_4/g of volatile solids (VS_{in}) and 322±3 mL CH_4/g VS_{in}, respectively. These methane yields were similar or even higher to those obtained when using non-fermented OFMW substrates (SS-OFMW: 380±18 mL CH_4/g VS_{in} and NS-OFMW: 239±4 mL CH_4/g VS_{in}), highlighting NSSF as a beneficial step to enhance methane yields during AD. Overall, bioconversion of OFMW would benefit from coupling bioethanol and biogas production since the biogas produced might be further employed as bioenergy source to compensate operational costs.

Keywords: anaerobic digestion; bioethanol; fermentation; methane; organic fraction municipal waste

Abbreviation list

AD	Anaerobic digestion
BMP	Biochemical methane potential
EU	European Union

GC	Gas chromatography
GHG	Greenhouse gases
MSW	Municipal solid waste
NSSF	Non-isothermal and simultaneous saccharification and fermentation
OFMW	Organic fraction of municipal waste
NS-OFMW	Non-sorted organic fraction of municipal waste
SS-OFMW	Source-sorted organic fraction of municipal waste
TS	Total solids
VS	Volatile solids

1. Introduction

Although many countries are trying to limit production of municipal solid waste (MSW) through implementation of policies for waste reduction, increasingly large amounts of these residues are produced. Today in Europe, each person generates an average of 475 kg of MSW every year (Eurostat, 2018). According to the European Environment Agency (EEA), biowaste fraction accounts for more than 34% of the MSW generated in the European Union (EU), producing near 100 million tons (86 million tons in 2017) of biomass per year (European Environment Agency, 2020). This great amount of organic fraction in MSW (OFMW) can be regarded as useful feedstock for the generation of a wide range of biobased products instead of being discarded as waste (Kwan et al., 2019). This approach is in line with the nowadays mandate for promoting, developing and implementing a sustainable circular economy.

In order to achieve the objectives set by the EU related to greenhouse gases (GHGs) reduction and renewable energy share by 2030, at least 14% of the final consumption of energy in the transport sector of every EU country will need to come

74 from renewable sources, with a total share of at least 3.5% of advanced biofuels
75 (European Union, 2018). In this context, the production of biofuels from waste
76 feedstocks has become of great interest. Municipal biowaste includes biodegradable
77 garden waste, food and kitchen waste from households, offices, restaurants, wholesale,
78 canteens, caterers and retail premises and comparable waste from food processing plants
79 (European Commission, 2018). This OFMW mainly contains carbohydrates (30-40%),
80 proteins (5-15%) and lipids (10-15%) in terms of dry weight. Thus, bioethanol is an
81 interesting biofuel that can be produced from this raw material due to its high
82 carbohydrate content. This biofuel is the most globally used with a total production of
83 85 billion liters in 2017 (World Bioenergy Association, 2019). Bioethanol is mainly
84 obtained from sugar or starch containing products, such as sugar beet, corn and wheat
85 crops. However, the use of lignocellulosic sources for bioethanol production, including
86 the OFMW, would be preferred due to the competition with food and the concerns
87 about ecological systems (Moreno et al., 2017).

88 After bioethanol production from OFMW, a significant amount of organic
89 material from the feedstock still remains and can be transformed into biogas by
90 anaerobic digestion (AD). Since only cellulose, starch and some dissolved
91 carbohydrates are converted to bioethanol, a great fraction of the biowaste energy
92 content is still present in the effluent of the fermentation process (mainly non-fermented
93 carbohydrates, lipids and proteins). This makes the effluents from the fermentation
94 process a suitable substrate for biogas production.

95 Co-production of bioethanol and biogas from different biomass has been studied
96 previously (Karimi and Karimi, 2018; Park et al., 2012; Patel, 2017). These studies
97 showed that biogas production in the corresponding bioethanol production facilities can
98 contribute to the energy requirements of ethanol production as well as to increase the

99 energy yields from substrates, since biogas can be placed in local markets as biofuel or
100 electricity.

101 Despite the benefits of sequential production of biofuels, improving the
102 availability of the organic fraction in urban biowaste is one of the key elements for a
103 profitable use of this substrate. The composition of OFMW that ends in a treatment
104 plant is very diverse (Nielfa et al., 2015). The factors that influence this composition
105 mainly include the waste collection system that has been used, the region where the
106 MSW was generated and the climate variations during the year. The first factor
107 influences the amount of inert materials (glass, plastics, textiles, sand, etc.) that will be
108 contained in the biowaste fraction. This is a very important issue because the presence
109 of a high content of inert materials causes many technological problems in plant
110 facilities and reduces the efficiency of downstream valorization processes. In this
111 manner, pretreatments such as mechanical sorting of the OFMW are essential to achieve
112 the desired conversion efficiency of this complex feedstock into biofuels (bioethanol
113 and biogas) by means of fermentative processes.

114 This investigation was designed to evaluate an integrated biorefinery strategy for
115 the sequential production of bioethanol and biogas using OFMW as substrate (Figure 1).
116 For such a purpose, two set of samples (source-sorted (SS) and non-sorted (NS)
117 OFMW) collected at different seasons and locations were used as substrates with the
118 aim of minimizing the effects related to compositional variations. With this purpose,
119 bioethanol and biogas yields were determined for both types of samples. Special
120 attention was given to the hydrolysis required for making the carbohydrate fraction
121 available prior to the first fermentative process. At this point, it should be highlighted
122 that while other lignocellulosic feedstocks have been extensively studied for the

production of advanced biofuels, the use of OFMW is envisaged as a challenging substrate due to its complex and heterogeneous nature.

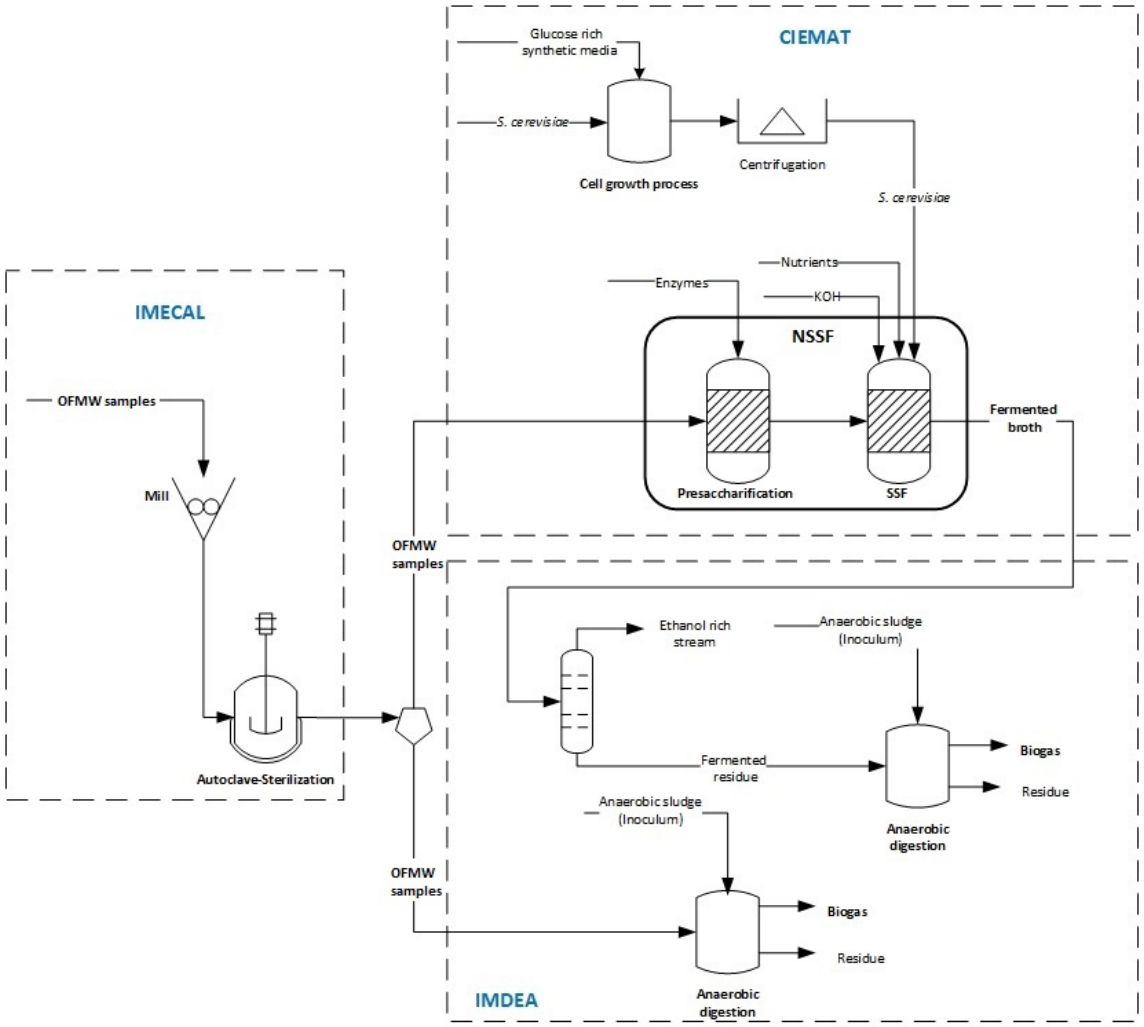

Figure 1. Experimental scheme followed in the present work for the sequential production of bioethanol and biogas from source-sorted (SS-OFMW) and non-sorted (NS-OFMW) biowaste. NSSF, non-isothermal and simultaneous saccharification and fermentation; SSF, simultaneous saccharification and fermentation.

2. Materials and Methods

2.1. Substrates

Taking into account the main factors that can affect OFMW composition, 16 representative OFMW samples were selected to be chemically characterized from South (Spain, 8 samples) and North (United Kingdom, 8 samples) of Europe. Out of the eight samples considered in each case, four came from separate collection systems

(considering both food waste and garden waste) and the other four from non-separate collection systems. Moreover, in order to address seasonal variability, samples were collected during spring, summer, autumn and winter. The collected samples (20 kg each) came from different industrial MSW treatment plants after mechanical pretreatments. Thereafter, the remaining inert materials content (glass, plastics, stones, textiles, etc.) in OFMW samples were removed manually at PERSEO Biorefinery Plant at IMECAL (L'Alcúdia, Spain) and then, samples were treated in a pilot plant mill in order to get a homogeneous feedstock. For this aim, an internal sampling protocol from IMECAL company was followed to avoid discrepancies in the OFMW samples collection. Samples were then sterilized at 121ºC for 1h in autoclave to avoid sugar loss during transportation and storage (-20ºC).

2.2. Fermentative microorganism

Saccharomyces cerevisiae (Lesaffre, France) was used as fermentative microorganism. Active cultures of this yeast were obtained by growing cells on glucose synthetic medium (20 g/L glucose, 2 g/L yeast extract, 1 g/L NH_4Cl, 1 g/L KH_2PO_4, and 0.3 g/L $SO_4Mg \cdot 7H_2O$) at 35ºC, pH 5 and 150 rpm. After 16 h, cells were harvested by centrifugation at 5000 *g* for 5 min, washed once with 0.9% NaCl and diluted with distilled water to obtain the desired inoculum concentration.

2.3. Non-isothermal and simultaneous saccharification and fermentation of the OFMW

Non-isothermal and simultaneous saccharification and fermentation (NSSF) processes were performed in triplicate in 250-mL shake flasks using 100 g of 20% (w/w) OFMW substrates. First, presaccharification process was performed at 50ºC and pH 5 for 24 h (during hydrolysability tests, this saccharification step was extended up to 48 h). A tailor-made preparation containing both cellulases and amylases (kindly provided by

Novozymes, Denmark) was used for saccharification of OFMW samples (López-Gómez et al., 2019). The enzyme dose was chosen according to the supplier instructions, using the same mg of enzyme per g of glucan for comparison purposes. After presaccharification, temperature was reduced to 35ºC, pH adjusted to 5.5 with 10 M KOH and the resulting hydrolysate was supplemented with the corresponding nutrients (the same components of the aforementioned rich medium without glucose; Section 2.2) and 1 g/L (dry weight) of *S. cerevisiae*. Flasks were then incubated in an orbital shaker at 150 rpm for 48-72 h.

2.4. Biochemical methane potential and sludge employed as seed inoculum

To evaluate the methane potential of the residues (raw biowaste and fermented biowaste), anaerobic batch tests were conducted. Batch tests were run in triplicate at 35ºC and 150 rpm in serum glass bottles of total volume of 120 mL and working volume of 70 mL. In all biochemical methane potentials (BMPs), substrate to inoculum ratio was $VS_{substrate}/VS_{inoculum} = 0.5$ (where VS refers to volatile solids). pH was adjusted to 7.5 at the beginning of the assay but not further controlled. 0.5 g $CaCO_3$/L was supplied to each bottle to buffer the system and prevent pH changes. Bottles were flushed with helium to ensure anaerobic conditions. The biogas volume was calculated by measuring the pressure of the bottle's headspace. For the determination of endogenous methane production, blanks containing only anaerobic sludge were run. The overall methane production was calculated by subtracting the blank productions measured in each sample.

The anaerobic sludge used as inoculum was kindly provided by the wastewater treatment plant of Valladolid (Spain). Total solids (TS) and volatile solids (VS) of the inoculum were 23.7 g/L and 17.1 g/L, respectively.

184 *2.5. Analytical methods*

185 The chemical composition of OFMW samples after mechanical pretreatment and
186 homogenization was analyzed in terms of carbohydrates (total glucans, starch, xylans,
187 and other carbohydrates), protein content, ash content, and moisture using the
188 Laboratory Analytical Procedures (LAP) for biomass analysis provided by the National
189 Renewable Energies Laboratory (NREL, Golden, CO, USA) (Sluiter et al., 2008). In
190 brief, samples were air-dried at 40ºC until constant moisture (ca. 10%), and milled using
191 a centrifugal mill (Retsch ZM200, Retsch, Ins., Haan, Germany) to 1 mm particle size.
192 Total glucans, xylans and other cabohydrates were determined after a two-step acid
193 hydrolysis using 1) 72% (w/w) sulfuric acid at 30ºC for 60 minutes and 2) 4% (w/w)
194 sulfuric acid at 121ºC for 60 min. The resulting liquid fraction was analyzed by high
195 performance liquid chromatography (HPLC) to determine sugar concentrations.
196 Glucose, xylose, galactose, arabinose, and mannose were analyzed by a Waters HPLC
197 system (Milford, MA, USA) equipped with a refractive index detector (model 2414)
198 and a Transgenomic CARBOSep CHO-782 column (Omaha, NE, USA) working at
199 70°C with ultrapure water (0.6 mL/min) as mobile phase.

200 To determine starch content, samples were milled to 0.5 mm in a Foss Cyclotec
201 1093. This carbohydrate was measured using the Total Starch Assay Kit (Megazyme,
202 Ireland) based on the use of thermostable α-amylase and amyloglucosidase.

203 Total carbohydrate content was determined by the phenol sulphate method
204 (Dubois et al., 1956). Nitrogen and protein content were estimated by the Kjeldahl
205 method using a Tecator digestor and Foss Tecator Kjeltec 8200 Auto Distillation Unit,
206 considering a nitrogen-protein conversion factor of 6.25.

For TS measurements, the samples were placed on a crucible, weighted in a balance (Sartorius TE64, Germany) and subsequently introduced in an oven in accordance with APHA standard methods (Eaton et al., 2005). To determine the VS content, the sample resulting from the TS procedure was incinerated at 550ºC for 5 h. The decrease in crucible weight represents the VS contained in the sample.

Ethanol was analyzed by gas chromatography (GC) using a HP 5890 Series II with an Agilent 6890 series injector. The system was equipped with a flame ionization detector and a Carbowax 20 M column, operating at 85ºC. Injector and detector temperature were kept constant at 150ºC.

Methane content in the biogas was determined by GC coupled with a thermal conductivity detector (Clarus 580 GC, PerkinElmer, USA) and equipped with an HSN6-60/80 Sulfinert P packed column (7' x 1/8" O.D.) and a MS13X4-09SF2 40/60 P packed column (9'x 1/8" O.D.) (PerkinElmer).

3. Results and discussion

3.1. Characterization of the OFMW

To minimize the effects promoted by substrate variability, this investigation evaluated 8 separately collected (SS-OFMW) and 8 non-separately collected (NS-OFMW) samples during different seasons from South (Spain) and North (United Kingdom) Europe. Table 1 shows the chemical composition of OFMW substrates in terms of average and range values.

Regardless of the collection system, country or season, glucan was the main component of biowaste and makes this substrate an attractive alternative for fermentation-based processes. The main carbohydrates contained in this glucan fraction

were starch and other glucans (including cellulose). These components were shown to be highly influenced by the collection system when compared to any other macromolecule (Table 1). The average glucan content in SS-OFMW substrates was 50% higher than NS-OFMW, with maximum glucan content of about 60% and 35% of the total dry matter for SS-OFMW and NS-OFMW, respectively. In addition to the higher glucan content, SS-OFMW also exhibited a higher starch content when compared to other glucans. Starch content in SS-OFMW ranged 23-43%, while NS-OFMW substrates showed a starch content of 6-17%. This might be attributed to a higher percentage of food-derived residues in SS-OFMW. Biowaste considers food waste (starch-based biomass) and garden waste (cellulose-based biomass) for selective sorting. Additionally, different collection systems can be used (i.e. garden waste and food waste can be collected either separately or together) (Seyring et al., 2015), which ultimately influences the proportions of these two wastes. In this work, systems collecting both food waste and garden waste were used for the recovery of SS-OFMW substrates. Nonetheless, the high ratio of starch vs. other glucans and the high starch content in the studied SS-OFMW samples suggest a higher proportion of food-derived waste in comparison to garden waste. In general, the amount of food waste discarded yearly is higher than the garden waste. According to EEA, about 60% of the total production of biowaste is food waste, while 30% is garden waste (European Environment Agency, 2020).

Table 1. Minimum and maximum content for each component of the separately (SS-OFMW) and non-separately (SS-OFMW) collected substrates.

Component	SS-OFMW **% (w/w)**		NS-OFMW **% (w/w)**	
Total glucans	33.9 - 61.3	(44.6 ± 9.7)	26.4 - 33.6	(29.8 ± 2.6)
Starch	23.5 - 43.1	(33.8 ± 7.5)	5.8 - 16.7	(10.3 ± 3.9)
Other glucans	4.8 - 18.2	(10.9 ± 5.2)	11.7 – 26.4	(19.5 ± 5.3)
Xylans	1.1 – 3.2	(1.7 ± 0.7)	2.6 - 4.4	(3.4 ± 0.7)
Other carbohydrates	2.4 - 5.3	(3.6 ± 1.2)	2.4 - 5.5	(3.3 ± 1.0)
Protein	12.4 - 17.3	(14.8 ± 1.7)	8.0 - 12.0	(9.7 ± 1.4)
Ash	6.2 - 9.7	(7.7 ± 1.3)	22.3 - 31.0	(27.1 ± 3.0)
Moisture	61.2 - 80.5	(69.7 ± 6.5)	49.3 - 59.7	(53.7 ± 3.8)

Lipids and acid insoluble solids were not determined. Mean values ± SD from n = 16 (8 substrates x 2 technical replicates for separately and 8 substrates x 2 technical replicates for non-separately collected) are listed in brackets.

253

254 Proteins and ash are also major components of OFMW substrates and were
255 influenced by the waste sorting system as well (Table 1). In this context, SS-OFMW
256 showed about 12-17% protein content and 6-10% ash content, while NS-OFMW
257 exhibited 8-12% protein content and 22-31% ash content. It should be noted the high
258 ash content in NS-OFMW (27.1 ± 3.0 g per 100 g of substrate). This result may be
259 attributed to the presence of higher inert materials in NS-OFMW, even after the sorting
260 processes (Alessi et al., 2020). The presence of a higher ash content can negatively
261 influence the total glucan content in these samples, which might be the reason for the
262 lower glucan concentrations in NS-OFMW.

263 Overall, the raw material was suitable to conduct the two-stage process. Given
264 the glucan-rich nature of the biowaste, ethanol production seemed to be an excellent
265 choice for valorizing this residual stream while the rest of the organic matter could be
266 further used for biogas production purposes given the high organic matter content.

267 *3.2. Non-isothermal and Simultaneous Saccharification and Fermentation processes*

268 The high glucan content of OFMW (Table 1) makes this substrate attractive for several
269 fermentation-based processes. Among them, the present work was focused on
270 bioethanol production, since this chemical is leading current biofuel production
271 worldwide (World Bioenergy Association, 2019) and has been recognized as an
272 important building block to obtain industrially relevant alternatives (e.g. ethylene, ethyl
273 acetate, acetaldehyde, etc.) (Posada et al., 2013). Bioethanol production from
274 lignocellulosic substrates (including OFMW) requires ethanol titers of about 40 g/L to
275 reduce the costs of the subsequent distillation step (Xiros et al., 2017). Higher ethanol

concentrations can be obtained by increasing substrate loadings. However, increased substrate concentrations usually lead to lower process yields mainly due to a significant reduction of the enzyme performance (Kristensen et al., 2009; Wang et al., 2011) and the mixing conditions (Demichelis et al., 2017). In this sense, with the aim of evaluating final saccharification yields, hydrolysability of OFMW samples was firstly assessed for 48 h at substrate concentrations as high as 20% (w/w). These tests showed average glucose yields of about 60% and 50% for SS-OFMW and SS-OFMW, respectively (Table 2).

Table 2. Glucose concentrations and saccharification yields obtained with separately (SS-OFMW) collected and non-separately (NS-OFMW) collected samples

Substrate	Glucose (g/L)	$Yield_{glucose}$ (%)[a]
SS-OFMW	46 – 98	45 – 82 (63.7 ± 13.7)
NS-OFMW	30 – 47	43 – 56 (50.1 ± 4.6)

[a]Yields have been estimated as the percentage of the glucose released form the potential glucose contained in each substrate, subtracting the free glucose contained in the enzymatic preparation and substrates. Values representing average ± SD from n = 16 (8 substrates x 2 technical replicates for separately and 8 substrates x 2 technical replicates for non-separately collected) are indicated in bracket.

In general, SS-OFMW showed higher saccharification yields, reaching values as high as 82%. This result is due to the fact that higher ratio of starch vs. other glucans were observed for SS-OFMW samples, thus highlighting the good hydrolysability potential of this substrate even when working at high substrate concentrations. Compared to cellulose, enzymatic hydrolysis of starch polymer is relatively simple and usually leads to higher hydrolysis yields (Salimi et al., 2019). In this context, samples with higher ratios of starch vs. other glucans would exhibit higher saccharification yields than other samples with similar total glucan content but lower ratios of these carbohydrates. The higher saccharification yields, besides the higher total glucan content in SS-OFMW, led to higher glucose concentrations when compared to NS-OFMW (46-98 g/L vs. 30-47 g/L). It is remarkable that saccharification processes resulted in a wide range of both glucose concentrations and yields. This is due to the

299 high variability found in OFMW substrates for the total glucan content and the ratio
300 between starch and other glucans (Table 1). Glucose concentrations within these ranges
301 have been reported previously at similar substrate loadings. López-Gómez et al. (2019)
302 reported glucose concentrations of 37-55 g/L when subjecting OFMW to hydrolysis at
303 20% (w/w) substrate loadings, while Demichelis et al. (2017) observed glucose
304 concentrations of about 70 g/L when using food-derived biowaste at the same substrate
305 loading.

306 Considering the results obtained during hydrolysability tests, bioethanol
307 production from OFMW was evaluated at 20% (w/w) substrate loadings. In addition, a
308 NSSF strategy with 24 h prehydrolysis was chosen for bioethanol production, since this
309 configuration allows better integration of the different process steps (Moreno et al.,
310 2017). NSSF of OFMW substrates led to ethanol concentrations of 24-51 g/L and 17-26
311 g/L for SS-OFMW and NS-OFMW, respectively (Table 3). High overall process yields
312 of up to 80% and 59% from the theoretical (and about 60% and 50% in average) were
313 reached for these substrates. Similar to hydrolysability tests, NSSF processes exhibited
314 high variation for ethanol concentrations and process yields within samples collected
315 with the same sorting system. This variability correlates to the different nature and
316 content of the glucan fraction as explained above (Table 1). Furthermore, it is important
317 to highlight the high heterogeneity and the different origin of samples, which might
318 have also affected both ethanol titers and yields.

319 In general, higher ethanol concentrations and yields were reached when using
320 biowaste separated at source. This result is supported by the high saccharification yields
321 observed for these samples and by the absence of microbial inhibition during NSSF
322 processes. The higher starch content and the higher ratio between starch and other
323 glucans of these substrates (Table 1) resulted in higher saccharification yields for SS-

OFMW (Table 2). On the other hand, no delays were found in ethanol production after yeast inoculation during NSSF assays of SS-OFMW or NS-OFMW (Figure 2). Also, final ethanol yields for both SS-OFMW and NS-OFMW were similar to the enzymatic hydrolysis yields for these substrates (Table 2 and 3). These results can therefore exclude any inhibitory effect of the microbial processes, evidencing enzymatic hydrolysis as the major limitation during conversion of OFMW substrates and highlighting the potential of OFMW as substrate for bioethanol production.

Table 3. Ethanol concentrations and saccharification yields obtained with separately (SS-OFMW) and non-separately (NS-OFMW) collected samples

Substrate	Ethanol (g/L)	$Yield_{etanol}$ (%)[a]
SS-OFMW	24 – 52	46 – 80 (61.6 ± 12.7)
NS-OFMW	17 – 26	42 – 59 (52.2 ± 5.6)

[a]Ethanol yields have been estimated considering the maximum ethanol concentrations reached during NSSF processes and the potential glucose in samples (including glucose from enzyme preparation). Values representing average ± SD from n = 16 (8 substrates x 2 technical replicates for separately and 8 substrates x 2 technical replicates for non-separately collected) are indicated in bracket.

Final process yields as high as 60% were reached in average for SS-OFMW substrates even though no thermochemical pretreatment process took place prior to NSSF assays. These yields are similar to those obtained after subjecting NS-OFMW substrates to thermal pretreatment. Ballesteros et al. (2010) reported final ethanol yields of about 60% of the theoretical after subjecting OFMW to thermal pretreatment (active hygienization). This pretreatment process requires to maintain 160°C for 30 min, which influences the energy requirements and thus, the overall process costs. Also, pretreatment processes generally release certain biomass degradations compounds that are inhibitory for the hydrolytic enzymes and the fermentative microorganisms. For this reason, pretreated materials usually require detoxification and/or highly tolerant fermentative strains to trigger fermentation (Mahmoodi et al., 2018a, b). In this work, the absence of a thermochemical pretreatment and the use of *S. cerevisiae* as fermentative microorganism, which usually shows high tolerance to lignocellulose-

derived inhibitors (Moreno et al. 2019), resulted in no inhibition of the bioethanol production process. Although further techno-economic assessments are needed, avoiding the pretreatment and detoxification steps offers huge advantages for reducing operational costs, since only pretreatment may represent up to 40% of the overall process costs (Moreno et al., 2017). It is also interesting to note that ethanol concentrations above 90% of the maximum observed were reached within the first 24 h from inoculation (Figure 2). This also offers the possibility of shortening overall process time and, consequently, reducing costs.

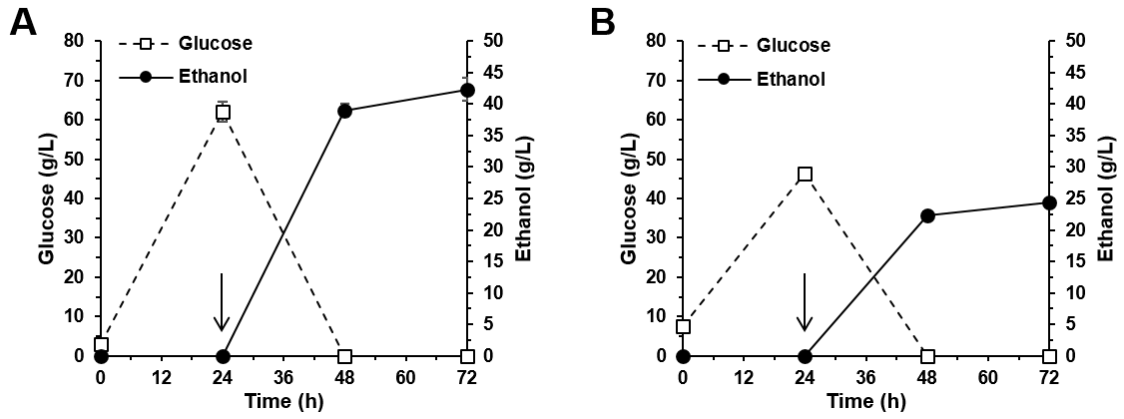

Figure 2. Time-course fermentations during non-isothermal and simultaneous saccharification and fermentation (NSSF) of representative (**A**) separately (SS-OFMW) and (**B**) non-separately (NS-OFMW) collected samples (Spain, spring). Arrows are indicative of the inoculation timing. Macromolecular characterization of these particular samples can be found in Supplementary information.

3.3. Methane yields attained via anaerobic digestion of the raw and fermented substrates

The remaining residue after ethanol distillation (stillage) is still a source of unconsumed organic matter (mainly carbohydrates, proteins and lipids). Whereas glucose is the main monomer employed for alcoholic fermentation, the rest of the carbohydrate fraction (*e.g*. hemicelluloses), as well as proteins and lipids can be further digested for bioenergy production purposes through AD. After ethanol production (fermented residue), the characterization of samples revealed a slight increase of the rest of the macromolecules

with respect to carbohydrates. This was attributed to glucose consumption. Macromolecular composition of the fermented residues is presented in Table 4.

The lower ash content in SS-OFMW and the percentages for the rest of the macromolecules were in accordance with values obtained in previous ethanol production assays as well as found in literature (Barampouti et al., 2019; Morales-Polo et al., 2018).

Table 4. Macromolecular composition of the fermented selective (SS-OFMW) and non-selective (NS-OFMW) collections employed in anaerobic digestion for methane production.

	SS-OFMW		NS-OFMW	
Component	**Raw**	**Fermented**	**Raw**	**Fermented**
Carbohydrates (%DW)	44 ± 4	24 ± 4	37 ± 3	24 ± 3
Proteins (%DW)	20 ± 3	25 ± 3	8 ± 2	16 ± 4
Lipids (%DW)	30 ± 9	43 ± 8	30 ± 8	33 ± 9
Ash (%DW)	5 ± 2	8 ± 1	25 ± 3	27 ± 2

Values represent average ± SD.

In order to compare whether the AD process could contribute to further exploiting the unconsumed organic matter available in the fermented residue, non-fermented and fermented SS-OFMW and NS-OFMW collections were anaerobically digested for assessing their methanogenic potential.

The accumulated methane yields obtained can be observed in Figure 3. Methane potential attained by SS-OFMW were 380.9±18.4 mL CH_4/g VS_{in} and 384.6±6.5 mL CH_4/g VS_{in} for the raw and fermented residues, respectively, whereas NS-OFMW resulted in 239.9±4.2 mL CH_4/g VS_{in} and 321.7±2.9 mL CH_4/g VS_{in} (non-fermented and fermented residues, respectively). Residues coming from a selective collection (SS-OFMW) achieved higher methane yields than those attained in non-selective (NS-OFMW) batches (Figure 3). It seems that the SS-OFMW presented better properties than NS-OFMW when it comes to their anaerobic digestibility. Most probably, the high sorting efficiency decreased the presence of most recalcitrant fractions and improved

the organic fraction bioavailability, which was beneficial for methane yields. Likewise, methane content in the raw NS-OFMW was 50% v/v CH_4 whereas higher values (60% v/v CH_4) were attained for the rest of the samples. With regard to the methane yield of the non-fermented residues, the values obtained were in accordance to the values available in literature (200-500 mL CH_4/g VS_{in} (Abudi et al., 2016; Allegue et al., 2020; Bala et al., 2019; Nielfa et al., 2015b)). The wide range obtained in the different studies in literature was attributed to the heterogeneity of the OFMW samples.

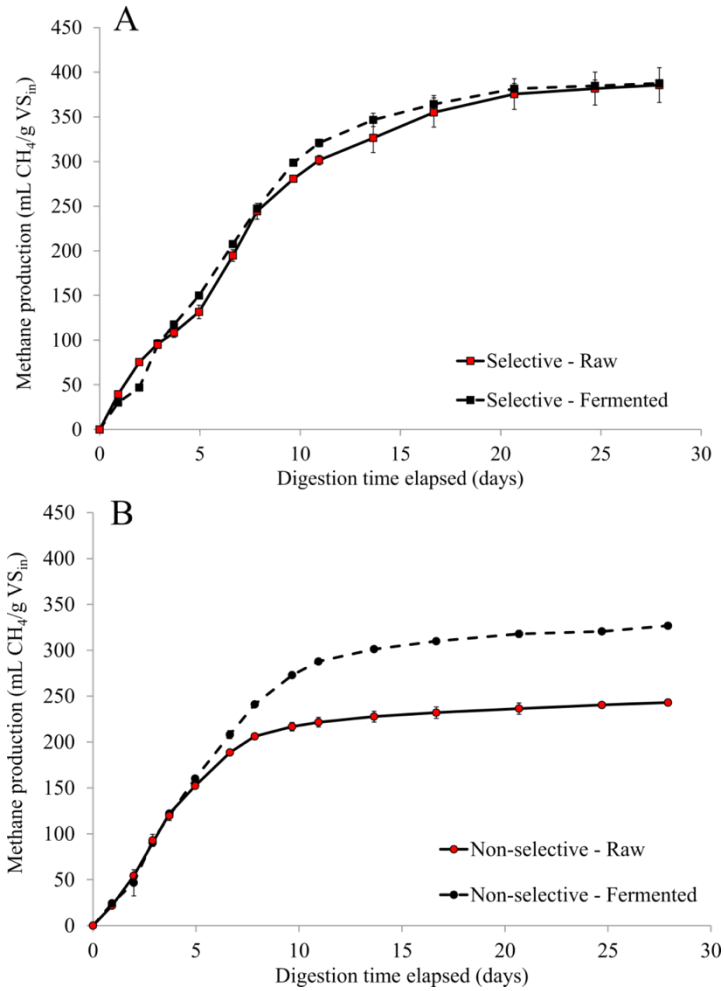

Figure 3. Methane yields of the fermented and raw fractions from (**A**) the selective collection (SS-OFMW) and (**B**) the non-selective (NS-OFMW) collection.

In this work, biowaste was collected with two different sorting systems from different locations, countries and seasons, leading to differences in the macromolecular

composition of OFMW. This wide variability of the chemical components of biowaste might results in variable methane yields. Alibardi and Cossu (2015) compared the methane production of three different fractions of OFMW with different macromolecular compositions (carbohydrate-rich, protein-rich and lipid-rich fractions). In that study, the methane production ranged from 400 mL to 600 mL CH_4/g VS_{in} depending on the assessed fraction. It should be highlighted that the methane yields obtained in the present study using the fermented residue as substrate were slightly higher when compared to other investigated substrates such as sugarcane bagasse, kitchen and garden wastes or algae (157-283 mL CH_4 / g VS_{in}) (Karimi and Karimi, 2018; Liang and McDonald, 2015; Park et al., 2012; Tian et al., 2013). This result showed the particular suitability of OFMW for the sequential production of bioethanol and biogas.

Compared to non-fermented residues, fermented residues resulted in similar or even higher methane yields (Figure 3). The increase in methane yields when using fermented residues has been also reported for other substrates. For instance, the fermented residue obtained from oat straw yielded 245 mL CH_4/g VS_{in}, while AD of the non-fermented raw material resulted in 201 mL CH_4/g VS_{in} (Dererie et al., 2011). This result might indicate that bioethanol production can influence substrate availability during the enzymatic saccharification and fermentation steps, which could make the organic matter more easily available for the anaerobic microbiome (i.e. the bioethanol production process may act as a pretreatment step in the digestion of the fermented residues). The high lipid content determined in the selective fermented residues might have prevented methane yield to increase more than the raw waste due to the toxicity exerted by these macromolecules. Lipids hydrolysis results in high amounts of long

chain fatty acids, which are detrimental for methanogens under concentrations higher than 40% in TS basis (Hu et al., 2018)."

Overall, the use of fermented OFMW and the raw residue gave as a result similar or even higher methane yields in batch mode. Therefore, coupling alcoholic fermentation and AD processes for bioethanol and energy generation might be regarded as a promising strategy to increase the cost-effectiveness of the process, even though these results still require confirmation in semi-continuous operation.

4. Conclusion

SS-OFMW is a more preferable substrate for integrated biorefineries when compared to NS-OFMW due to i) its lower amount of inert materials, ii) the higher glucan content, and iii) the higher ratio between starch and other glucans. This raw material positively influences the bioethanol production by increasing the glucose available in this process. On the other hand, AD of fermentation residues results in similar and even higher methane yields than their raw counter partners regardless of biowaste collection type. This sequential strategy offers a more complete use of OFMW increased the carbon conversion yield of this substrate into energy.

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