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Anaerobic digestion in wastewater reactors of separated organic fractions from wholesale markets waste. Compositional and batch characterization. Energy and environmental feasibility

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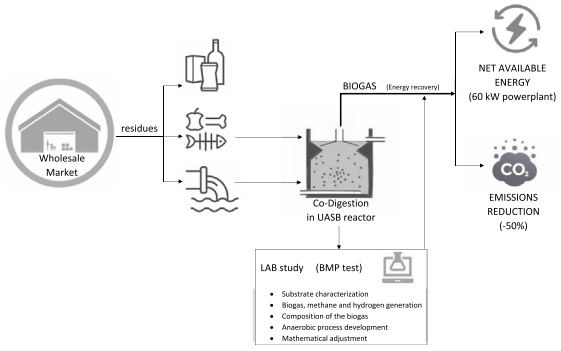
HIGHLIGHTS

- Wholesale markets residues can be adequately treated in UASB digesters of wastewater treatment plants.
- The biogas is generated in a stable process that lasts about 13 days.
- H_2 generation and transformation serves as a precise indicator of the process development.
- Wholesale markets can be transformed into power generation plants up to 600 kW
- The use of the biogas generated represents a 50% reduction in CO_2 equivalent emissions

GRAPHICAL ABSTRACT



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ABSTRACT

The food industry is one of the major industrial sectors in Europe and Spain, and therefore one of the major waste emitters, especially organic ones that can be classified into three different fractions (fruit and vegetables, meat and fish). One way to treat this waste environmentally responsible, energy-sustainable and economically cost-effective is anaerobic digestion. The generated biogas can be used as fuel and renewable energy source (providing a solution to the energy problem from an environmental point of view). As there must be a sewage treatment plant with anaerobic digesters in the wholesale markets, and if waste is treated on it, these facilities can be converted into power generators.

It has been studied that, when treated along with sludge from a UASB reactor, the residue of fruit and vegetables produces about 900 ml per 100 g of residue with a stable and robust process; the meat residue generates 1300 ml of biogas per 100 g with a process that is slightly affected by the accumulation of acidic elements, internally reversed by the buffer effect of ammonia released; and the fish residue generates 700 ml of biogas, but with very low novels of methane since the process is inhibited early by excessive accumulation of ammonia.

The proposed solution is positive, and the methods used to determine it are novel and robust, such as the use of hydrogen as an indicator of process stability. A deep characterization of the development of the process is provided, and feasibility for its application at the industrial level is studied. It is thus proven that wholesale markets

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can be converted into power generating plants up to 600 kW, assuming a reduction of up to 70 tons of CO_2 equivalent (50%) if the generated biogas is used, replacing a conventional source such as natural gas.

1. Introduction

The current context is characterized by a remarkable population growth, as well as an increase in economic, social and cultural growth. According to EUROSTAT data, in the European Union (EU), the demographic situation reflects upward growth; since 2008, the population has increased by 264% (Database - Eurostat, n.d.). Alongside this population growth, an increase in needs and consumption is associated, especially in the energy field. However, it is true that during the period 2010–2015, energy consumption declined slightly due to different causes such as the economic crisis and responsible consumption awareness, in 2017 (latest data provided by EUROSTAT) there was an uptick in the consumption by 1.30%, as shown in Figure A-SP from supplementary information.

It is to be hoped that this growth will continue over time. In fact, the International Energy Outlook (U.S. Energy Information, 2017) predicts a 28% increase in energy consumption for the period 2015–2040. Other agencies such as the International Energy Agency estimate this growth by 35% for the period 2010–2035 (Aliane et al., 2016).

Of all the energy consumed in Europe in 2017, approximately 80% came from fossil sources (International Energy Agency, 2017) such as coal, oil, natural gas and derivatives (Figure B-SP from supplementary information). This is mainly due to European energy dependence, so finding new forms of energy to reduce this dependence on foreign and fossil fuels is strategic. Moreover, given that fossil fuels are known emitters of greenhouse gases, the reduction in their use is not only strategic, but also necessary. In fact, of the 4.66 gigatons of CO_2 equivalent by the EU, 82.8% of emissions came from the energy sector (International Energy Agency, 2017). These two reasons mark the roadmap for a more renewable energy model (Ashraf et al., 2016).

Of all renewable energies, the one that takes advantage of local resources and also reduces the environmental impact by transforming certain waste management activities, is biomass (De Sanctis et al., 2019). Within it, the transformation of biomass into biogas and then using this as fuel is a very feasible solution (Zhang et al., 2014). It is especially interesting for waste with high humidity, which by direct combustion or incineration would be difficult to value (Caton et al., 2010). The benefits of using biogas as fuel instead of fossil fuels lie in the ease of obtaining it as the source of generation is from common substrates, such as local waste, crop debris, wastewater or the organic fraction of waste urban solids, among others (Yadvika et al., 2004) these provide renewable sources, and with the least environmental impact by reducing emissions, when compared to fossil fuels (Huttunen et al., 2014). Methane is the hydrocarbon with the lowest carbon content, and therefore the least likely to generate CO_2 (Abbasi et al., 2012), it does not release previous sequestered carbon as combustion of fossil fuels does (Lehmann et al., 2006), and furthermore, if it comes from the transformation of biomass, so its CO_2 emissions can be considered null (Leggett et al., 1992), as recognized by entities such as the IPCC.

If biogas is obtained from the transformation of waste, a further environmental benefit is added, since the way of processing those residues is changed (Girotto et al., 2015). At European level, the *Council Directive 1999/31/EC of 26 April 1999 on the landfill of waste* (*The Council of the European Union Council Directive 1999/31/EC of 26 April 1999 on the Landfill of Waste*, 1999) establishes a number of conditions when it comes to treating waste. Once their generation has been prevented, and the waste has been prepared for reuse, if there is no other way, it must be treated appropriately, prioritizing recycling first, secondly, valuing evaluating it, and as a last option, depositing it in landfill. Anaerobic digestion is a very suitable way for the treatment of any organic waste (Chiu and Lo, 2016), since it constitutes a form of recovery (energy in the form of biogas, agronomic or as a fertilizer of the digestate) and a way to reduce the disposal of waste in landfill, since if something is used to landfill, it would only be the digestate (which can be valued as fertilizer), which constitutes between 1: 3 and 1: 4 of the initial volume of all waste (Zhang et al., 2014).

Anaerobic digestion (AD) is then a process that brings environmental, economic and energy benefits (Morales-Polo et al., 2018). Precisely due to this last point in the energy report, the anaerobic digestion process must be self-sustaining. To promote the proper development of the process, it is necessary to maintain optimal temperature conditions, for which energy is necessary in the form of heat, and mechanical mixing conditions of addition of substances or other substrates, for which electrical energy is necessary (Bolzonella et al., 2006). A correct anaerobic digestion process will be able to, with the biogas generated, supply the needs and provide a remnant of energy for other external uses (Abbasi et al., 2012).

The agri-food industry comprises activities from all economic sectors (Eurostat Fooddrink Europe, 2019). A, the food supply chain (FSC) begins with stages of the primary sector (agriculture and livestock), which produces by-products (i.e., manure, waffle, cornstalk) and food waste (FW) and food loss (FL) in the form of low-quality products, damaged production, or products with no commercial value (Chiu and Lo, 2016; Parfitt et al., 2010). It continues with the stages of product transformation, characteristic of the secondary sector, where FL and FW are mainly generated within the entire process chain due to problems in storage, damages during transport, contamination along the process, or in separation stages that create by-products not intended for human consumption (i.e., feathers, skins, fruit peels…). The end of FSC comes with the sales and distribution stage, typical of the tertiary sector (Papargyropoulou et al., 2014). Losses and waste are generated in food markets and retail systems in association with problems in storage, conservation, or unsold perishable products. At the final stage of the product live life cycle (end consumer), the FW is generated by a purchasing excess, over preparation processes, bad storage conditions, and other consumption behaviour patterns (Bräutigam et al., 2014).

It is estimated that 33% of all agri-food production is lost in the form of waste (Buzby and Hyman, 2012). Particularly in the European Union (EU), 90 million tons of this waste are generated annually (Health and Food Safety, n.d.; Monier et al., 2010). This kind of waste production represents 12% of all the food entering a home, and a 25% of all the food of the FSC (Bräutigam et al., 2014). This implies that within the complete FSC, 40% of FW, and FL occurs during postharvest and processing stages and another 40% during the retail and consumer levels (Nellman et al., 2009).

In this context, the generation of waste and materials to be disposed of in the agri-food industry is one of the major environmental problems (Bernstad and Cour Jansen, 2012; Cossu, 2009), with an impact in the social, economic and political spheres (Girotto et al., 2015). Given the link to the growth of society, it is impossible to completely eliminate waste emissions. This is why they must be treated as appropriately as possible, achieving mutual and sustainable economic, energy and environmental benefit.

In Spain, wholesale sector is controlled by a public company of the State Administration. It promotes and manages, together with the respective municipalities, the Wholesale Markets Network (WSM) which, consisting of 23 food facilities for wholesale distribution and

logistics services, covers the entire Spanish geography. In these structures >3650 companies develop their activity, of which around 2200 are wholesalers installed in the Fruit and Vegetable, Fish, Flowers and Meat Markets, which transaction 7.8 million tons of food products annually. As for the influx of buyers - both retailers, wholesalers, hospitality and catering, or institutional demand - the average daily attendance is already around 90,000 users (MERCASA, n.d.).

Wholesale distribution is therefore one of the largest waste generators (Stenmarck et al., 2011). In particular, it can be estimated that the entire MSC Spanish network generates around 83,000 tons of waste per year, including both organic and inorganic matter. As indicated in the previous paragraph, these WSMs have fruit and vegetable markets, meat markets and fish markets. In the fruit and vegetable markets the organic composition of waste consists of organic vegetable-type remains such as leaves and fruits and vegetables in disrepair or unfit for sale; the inorganic fraction is basically made up of wooden and cardboard boxes, alveoli or fruit-protective grilles (paper or plastic) and exceptionally high-density polyethylene plastic boxes (Chalak et al., 2018). In meat markets, the organic faction consists of organic meat-type remains such as skins, bones, fats and shells, resulting from cutting and adequacy for sale; the inorganic faction is basically composed of plastic packaging (bags, packing film, porexpan trays), satin paper, cardboard boxes, egg-boxes and exceptionally high-density polyethylene boxes (Fehr et al., 2002). Finally, in the fish markets, the organic fraction of waste consists of organic remains such as skins, spines and shell casques resulting from cleaning, cutting and adaptation for sale, as well as blood and other liquid residual effluents with high organic load; the inorganic fraction is made up of meshes and exceptionally high-density polyethylene plastic boxes (Liu et al., 2016). Not only do these markets exist in WSMs, but they also serve buyers and sellers, in establishments such as cafes, restaurants and offices, which generate a fundamentally inorganic fraction consisting mainly of boxes of plastic packaging and packaging (bags, film and bottles), glass (non-returnable glass containers), canned goods and bricks (*Institut Cerdà Study and guide for waste management in municipal markets* (*Estudio y guía para la gestión de los residuos en mercados municipales*), 2005).

In this way, it has been estimated in studies carried out by the authors that, as shown, in general terms of the entire WSM network, 21% of the waste generated constitutes the organic fraction, while the rest of the residues are identified as much as possible with scraps of packaging (cardboard and paper, plastic, metal and glass). One way to take advantage of these organic wastes is AD, whose environmental, social and economic benefits have previously been presented (Uçkun Kiran et al., 2014), as it constitutes a form of energy recovery.

This type of facility generates a large amount of highly loaded organically charged wastewater, not only through organic effluents carried by the cleaning waters, but through the detergents and disinfectant elements of the facilities (Schneider, 2013). In accordance with Directive 91/271/EEC of the European Economic Community (*The Council of the European Communities Council Directive 91/271/EEC of 21 May 1991 Concerning Urban Waste-Water Treatment*, 1991), this organically charged wastewater should be treated in a wastewater treatment station (WWTP) preferably exclusive to the facility, or the industrial estate in which the WSM is framed. Because of the high organic load, these WWTPs have anaerobic digesters, both in the water line and in the sludge line (Morales-Polo and Cledera-Castro, 2016), so in the facilities there are already digesters available to treat the organic fraction of the waste, without the need to build new ones, or have an exclusive bio digestion plant.

In this sense, the proposed solution of a joint treatment of organic waste and sewage sludge brings with it clear environmental benefits indicated above, but also economic benefits. Several studies have been carried out, showing that this solution has benefits (Morales-Polo and Cledera-Castro, 2016), especially for the savings involved in terms of waste management and profit from the sale of biogas. Although the initial investment must be great in order to assemble the digestion plant, authors such as (Slorach et al., 2020) figure the return on investment in 9 years. The proposed solution entails a much greater saving, and a smaller investment by using the same sewage treatment facilities already in place in the plant. A return has been estimated in 5 years, which will be demonstrated in future publications.

There are several studies in the literature on anaerobic digestion of food residues, as can be seen in Table 1, of them most obtain good digestion data. However, it is difficult to find a previous characterization of the complete fraction as well as the same residue (Bolzonella et al., 2006). In addition, generic residue is usually treated at the level of the last stage of FSC, i.e. in final consumption (la Cour Jansen et al., 2004) or at domestic level. Wholesale market waste has been poorly treated, and as has been seen constitute a major research niche. Also, most of the residues that have been ratted are mixtures of different compositions (Banks et al., 2011), With this study it is determined the influence that each residue has separately, in its individual fraction, completing the information of other residues that treat the digestion of the mixture.

Owing to the above stated reasons, the objective of this manuscript is to analyze the feasibility of the organic waste found to be treated in the anaerobic digesters of the sewage treatment plants, along with the sludge contained in them. For this purpose, a study of the typology of waste generated in the WSM network will be carried out, these will be analysed and their suitability to be treated by AD will be studied from the point of view of their composition. They will then be laboratory tested to analyze biodegradability and to study how the anaerobic process develops when digesting the waste, determining the amount of biogas and methane that is generated, as well as the enrichment of it. Also, the development of the process, if there are synergies or inhibitions. Once the potentials of gas generation are determined, the viability of the solution is studied

Table 1
Literature review of food waste biodegradability through anaerobic mono-digestion.

Substrate	Operational conditions	CH_4 yield [ml_{CH4}/g_{VS}][a]	Reference
Food waste		28.4	(Iacovidou et al., 2012; Lesteur et al., 2010)
Food waste		23.4	(la Cour Jansen et al., 2004)
Food waste	Two stage	59.8	(Wang and Zhao, 2009)
Food waste	Full scale	48.7	(Banks et al., 2011)
Food waste	Batch	31	(Zhang et al., 2013)
Animal FW	Batch	45	(Kobayashi et al., 2012)
Vegetable FW		39	
Animal FW	Batch	25	(Naroznova et al., 2016)
Vegetable FW		19	

[a] Some of the results may vary from the literature review as they were originally expressed in Nml per gram of volatile soled degraded, instead of gram of volatile solid content in the residue (unit used in this manuscript).

from an energy point of view (if it is capable of generating excess or available energy for other uses) and from the environmental point of view (if the use of biogas generated as energy source means a reduction in CO_2 emissions equivalent to the atmosphere).

2. Materials and methods

2.1. Test samples

There is a very important variability in the composition of the residue vectors, and it depends on many external factors such as layout, season or level of sale. To avoid changes between test blocks, laboratory-prepared waste is used, (Fig. 1), which retains the same characteristics as the original residues, but avoids variation between blocks.

To perform the Biochemical Methane Potential (BMP) tests it is recommended to use a stable and easily accessible inoculum (Owens and Chynoweth, 1993). Fundamental standards such as UNE-EN ISO 11734 (AENOR UNE-EN ISO 11734, 1999) and VDI-4630 (VDI VDI 4630, 2016) recommend using sludge from WWTP. Several authors advise it because of its accessibility and permanence of biomass (Elbeshbishy et al., 2012; Li et al., 2019), including pioneers in conducting BMP test in 1979 (Owen et al., 1979), the following pioneers in the conduct of BMP assays (Chynoweth et al., 1993). As an inoculum, and therefore the source that provides methane-based and anaerobic biomass to trigger the biomethanization process, sewage sludge from a Wastewater Treatment Plant (WWTP) is used. In particular, in this study it is a granular sludge from a UASB reactor, from an agri-food industry sewage treatment plant. This type of sludge, along with its granule agglomeration characteristic makes it resistant to internal or process alterations (*Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactors - Schmidt*, 1996).

The residue of fruit and vegetables (V) is composed, mainly, of fruit and vegetables discarded on sale, spoiled product and peels. The variety is huge, depending on the tastes of the buyer and the season. To avoid these changes, a constant residue vector has been chosen. In particular, it will be used for the experimental phase, pumpkin, of the *carruecano* variety (*Cucurbita moschata*).

Meat residue (M) is mostly composed of skins, discards in the cutting, pieces of fat, bones, feathers and other inert material such as hooves or bristles. Seasonal variability is not as exaggerated as in fruit and vegetable residue, but it does greatly affect consumer behaviour. To avoid these changes, a constant residue vector has been chosen. In particular, it will be used for the experimental phase, a mixture of pork fat (*Sus Stropha domesticus*) and skins and bones of chicken wings (*Gallus Gallus domesticus*), in proportion 4:6. That is, a sample of 100 g of residue will consist of 40 g of pork fat and 60 g of skin discards and chicken wing bones.

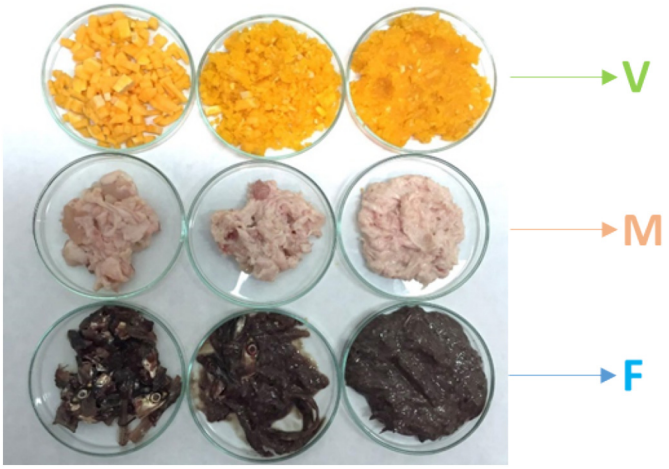

Fig. 1. Test samples of residues V, M and F.

Fish residue (F) is mainly composed of viscera, discards in the cutting of all kinds of fish, thorns, and blood. 70% is blue fish and large tuna. Again, to avoid these changes, a constant residue vector has been chosen. Specifically, it will be used for the experimental stage anchovy (*Engraulis encrasicolus*).

2.2. Analytical methods for sample and inoculum characterization

The objective of these tests is to know in depth the composition and characterization of the substrates and inoculum before and after being digested, in order to be able to make determinations about the development of the process. In addition, by analyzing the changes that occur in the composition after the digestion process is complete, you can determine the variables that have affected the process.

In particular, the following shall be characterized:

a) Physical-chemical composition of the substrate, for which the determination tests carried out are:
- Total Solids (TS): Total amount of solid matter.
- Volatile solids (VS): Amount of solid matter that can be removed by biomethanization.
- Humidity (Hum): Water content of the sample to be treated.
- Chemical Oxygen Demand (COD): Amount of organic matter that can be chemically degraded
  - o Total COD (COD_t): Total COD measurement.
  - o Soluble or filtered COD (COD_f): Measure of COD in the soluble fraction.
  - o Solubility coefficient (COD_f/COD_t): Provides information on the level of solubilization of the substrate.

b) The organic composition of the substrate is also determined by the Lipid, Protein and Carbohydrate (LPCH) content: It gives an idea of the macromolecular composition and what the degradation process will be developed, as studied in (Wagner et al., 2013).

c) Elemental analysis of substrates:
- Elemental analysis: Dry base content of C, N, O and S.
- C/N Ratio: Gives an idea of the buffer effect of the substrate and the probability of inhibition by ammonium accumulation.

d) Analysis and determination of pH, alkalinity and nitrogen content:
- pH
- Alkalinity
  - o Total alkalinity (TA)
  - o Partial alkalinity (PA): Alkalinity due to bicarbonates.
  - o Intermediate alkalinity (IA): Alkalinity due to VFAs.
- Nitrogen content:
  - o Total Nitrogen (TKN): Measured as Total Kjeldahl Nitrogen.
  - o Organic Nitrogen (ON): Nitrogen chemically linked to organic molecules such as proteins, amines and amino acids.
  - o Ammoniacal Nitrogen (AN): Total amount of nitrogen in the form of ammonia and ammonium.

Samples from each substrate and sludge are analysed, (a) in the natural state crushed, (b) in the natural state crushed and diluted, or (c) the liquid phase extracted by vacuum filtration of the substrates, previously centrifuged at 6000 rpm for 30 min.

The content in TS, VS and Hum is determined by gravimetric methods. To do this, a sample is subjected in a state (a) to drying and calcining. They are performed simultaneously following the APHA 2540-G method (APHA and AWWA, 2005).

The amount of solids provides information about the total amount of matter that is susceptible to methanization, especially the VS content. Humidity, on the other hand, provides information about the speed at which the process will develop, being faster the process in which the moisture content is higher, as the solubilization of the matter is facilitated.

The determination of COD, both COD_t and COD_f, has been carried out following the open reflux method APHA 5220-B (APHA and AWWA, 2005). For COD_t, substrates are used in shape (b) and for COD_f in form (c). The solubility coefficient is calculated as the proportion of COD_f contained in COD_t.

The COD is another measure of the amount of matter susceptible to methanize, so that high COD content is prone to generate large amounts of methane and therefore an abundant and enriched biogas. Especially important is CODf, the amount of COD directly accessible to microorganisms. High content in COD_f indicates that it is easier to transform that COD into methane. The solubility coefficient is the amount of directly accessible COD, of the total amount of COD.

Macro-nutritional analysis, also named as LPCH content, is determined by the methods described in UNE-EN 13804:2013 (AENOR UNE-EN ISO 13804:2013 roductos alimenticios, 2013).

The LPCH content provides information about the influence of the input substrate on the development of the digestion process (Wagner et al., 2013). Digestion of lipid-high substrates generates large amounts of methane because of the high carbon content, although the process is the slowest and they are susceptible to releasing acidic elements such as LCFA and VFA. Carbohydrate-rich substrates generate a low amount of methane with a fast process speed, the only drawback being in the process transforming simple sugars into VFA that can end up accumulating and causing inhibition. Protein-rich substrates, meanwhile, generate an average amount of methane, on average speed, which due to their high nitrogen content are susceptible to freeing up a large amount of AN that can end up inhibiting the process, or transform into peptides and amino acids that eventually become VFA.

As for the elemental analysis of the substrates, this has been performed by a CHNS "TruSpec Micro" equipment of LECO, having previously dehydrated the samples in state (a) at 65 °C for 24 h. The results measured on the dry base of the substrate are obtained. The C/N ratio is determined by a simple division of both contents.

This elemental analysis allows one to determine the nutrient content of the substrates. Particularly important is the C/N ratio, which provides information about process stability. A high C/N ratio indicates a high carbon content but nutrient defect, while low C/N ratios indicate excess nitrogen that may end up causing ammonium accumulation inhibition. An optimal C/N ratio is around 20 (Fernandez et al., 2008).

The pH of the substrates is determined by direct measurement on the sample in state (c). The alkalinities, both AT, AI and AP are analysed over the sample in state (c) following the APHA 2320-B method (APHA and AWWA, 2005).

The pH is a reliable indicator of process development, this should be kept in neutral values. An increase in pH is indicative of a process failure due to excessive accumulation of AN, while a decrease in pH indicates a build-up of acidic elements.

Alkalinity, on the other hand, measures resistance to sudden pH changes. Especially important is the IA. This will be used, together with the pH as an indicator of the accumulation of VFA, since, if reduced, it indicates that acidic elements have been released and accumulated.

The total nitrogen content TKN has been determined on samples in state (b) using method APHA 4500-Norg (APHA and AWWA, 2005). The AN is obtained using method APHA 4500-NH3 (APHA and AWWA, 2005) on samples in state (b). The ON is calculated as the TKN − AN subtraction.

Nitrogen content is important to know the development of the process. Especially important is the content in AN. A release of NA along with a decrease in pH indicates that AN has been released in excess and has accumulated, causing inhibition.

2.3. BMP test procedure

The method described in UNE-EN ISO 11734 (AENOR UNE-EN ISO 11734, 1999) has been used to determine anaerobic degradation. Which uses an indirect method through the pressure generated in the bottle or reactor, and the composition of the biogas is measured by gas chromatography by extracting a sample. Every day both the initial pressure and the final pressure are measured after the sample is removed to analyze the composition and release the necessary gas so as not to exceed the maximum pressure of the bottles. The daily pressure difference is transformed into a quantity of gas generated in accordance with VDI-4630 (VDI VDI 4630, 2016) procedures.

The volume of the reactor-bottles is 1 l and initially it is filled with substrate + inoculum mixture, leaving a headspace volume of 700 ml. The combination between substrate and inoculum in the mixture is performed in 1:3 ratio, as recommended by VDI-4630 (Huiru et al., 2019). Reactor bottles are equipped with a volume scale to measure headspace volume and its changes if needed.

At the start of testing, N_2 is used to displace the air contained in the bottles and to ensure anaerobic conditions inside the reactor.

To the standard procedure UNE-EN ISO 11734 (AENOR UNE-EN ISO 11734, 1999) is added an orbital incubator that keeps the temperature constant in the reactors, in addition to being kept permanently agitated, making the substrate always in contact with the inoculum and avoiding the stratification. In particular, the tests were carried out at a temperature of 37 ± 1 °C (mesophilic conditions), with a constant stirring velocity of 60 rpm.

Per each substrate 21 repetitions are made with the aim of obtaining reliable results with the least dispersion possible.

2.4. Measurement of biogas composition

A sample of the gas generated in the reactors is extracted daily and analysed in a gas chromatograph to determine the proportion of methane and hydrogen in the biogas. An Agilent 7820A gas chromatograph (GC) was used with a thermal conductivity detector (TCD), equipped with a Molsieve 5A-CP molecular sieve, and a PoraPLOT Q capillary column.

N_2 is used as a carrier gas to detect H_2 more easily. In addition, by using N_2 as a gas to ensure air displacement and anaerobic conditions inside the bottles. This ensures that there is no interference between the displacement gas and the carrier gas, and that the only measure is that of the gas composition.

The percentage of methane and hydrogen can be determined in 7.5 min. The hydrogen peak appears at 5 min, and the methane peak appears at 7 min. A combination of different flows (5 ml/min constants for 3 min + increase up to 10 ml/min for 2 min + 10 ml/min constants for 2.5 min) and temperatures (60 °C constants for 0.5 min + increment up to 100 °C for 0.75 min + decrement up to 60 °C for 5 min + 60 °C constant for 1.25 min).

2.5. Characterization of reactor contents before and after the BMP test

To fully characterize the digestion process, the mixture of the reactor inside (substrate + sludge) is analysed before and after the BMP test, following the same procedures as in Section 2.2.

2.6. Obtaining the curves of biodegradability, generation and content of methane and hydrogen

Biodegradability curves represent the amount of accumulated biogas generated, relative to time. Knowing also the % in methane and hydrogen of this gas, the accumulated generations of methane and hydrogen can be represented, and in the same way the content of methane and hydrogen of the biogas that has been generated.

2.7. Statistical analysis of results

In order to determine whether the results of the experimental trials are usable, and correlated with each other, a statistical analysis of them is carried out to check:

a) If there is a correlation between all the biodegradability curves of the same residue, and these can be assumed by a single:

To do this, an analysis of the ANOVA variance is carried out with its corresponding HSD Tukey and DMS contrast tests that allows one to confirm or reject the possible differences between curves, with a confidence interval of 95%.

The starting hypotheses are H_0 = Equality between curves, and H_1 = Inequality between curves.

Analyzing the *p*-value obtained during the analysis. if the p-value is less than the significance level (p-value < 0.05) the H_0 hypothesis is rejected, assuming the H_1 hypothesis of mean difference. Otherwise, the H_0 assumption of means equality is assumed.

b) The level of dispersion that exists between the biodegradation curves of the same residue: For this purpose, a visual method is used using box diagrams and a quantitative method through Analysis of the Coefficient of Variation (CV).

With box diagrams the dispersion of the variable can be estimated and compared with others, depending on the box width, which the higher, the more dispersed the variable will be.

Numerically it can be determined with the CV, since the lower its value, the more homogeneity is attributed to the sample.

c) If the biodegradability curves of a residue differ sufficiently from those of another residue, and it can therefore be assumed that the anaerobic process of one or the other is different and independent:

As with the first objective, an ANOVA analysis is performed with its corresponding HSD Tukey and DMS contrast tests, to confirm or reject the equality or difference between all curves of each residue.

2.8. Mathematical determinations

By mathematically analyzing the results of degradation and characterization, important variables are obtained to know the development of the process.

The theoretical generation of methane that was to be expected after anaerobic degradation of the substrate can be obtained from the reduction of COD experienced before and after the BMP test.

$$V_{theoretical\ CH4_{accumulated}}[Nml] = \frac{(COD_0 - COD_f)\cdot V_{test}\cdot 340}{1000}\cdot\frac{1}{0.9869} \quad (1)$$

where COD_0 and COD_f represents the COD levels measured at baseline at the end of the BMP test expressed in mg/l; V_{test} the test volume occupied by the mixture residue + sludge expressed in liters; 340 the conversion factor of COD in methane; and 1/0.9869 the conversion factor from standard conditions (0 °C and 1 bar) to normal conditions (0 °C and 1 atm).

If the accumulated methane generation curves are treated as a first-order kinetics, according to the method described by Veeken and Hamelers, the disintegration constant (k_{dis}) and the maximum amount of actual methane obtained in the process (CH_{4max}) can be determined. To do this, the experimental data obtained, by means of least squares, by the function must be adjusted:

$$CH_4(t) = CH_4\ max\cdot\left[1 - e^{-k_{dis}\cdot t}\right] \quad (2)$$

where $CH_4(t)$ represents the production of methane on the *t*-day; CH_{4max} the maximum generation of methane recorded (which can be assumed by the latest generation); and k_{dis} the average disintegration constant in $days^{-1}$.

With the k_{dis} disintegration constant, the depth and speed of the hydrolysis process can be measured.

The level of degradation of the substrate or residue can be calculated analytically through the COD reduction degradation levels, and provides information about the level of scope of the degradation of the substrate, regardless of degradation inoculum.

$$BD_{residue}[\%] = \frac{(COD_{mixture_0} - COD_{sludge_0}) - \left(COD_{mixture_f} - COD_{sludge_f}\right)}{(COD_{mixture_0} - COD_{sludge_0})}\cdot 100 \quad (3)$$

2.9. Energy balance analysis

To analyze the feasibility and energetics of the solution, whether excess or available energy can be extracted from the process, for external use, an analysis or energy balance of the anaerobic digester has been carried out, taking into account the needs of the anaerobic process in both electricity and heat (which will be covered by the biogas generated).

The analysis design includes a power generator (such as an Combined Heat and Power (CHP)) and boiler fed with the biogas produced for covering the electricity and heat demand of the process. The remaining biogas that is not used by the power generator and boiler is considered as net energy produced by the process (De Sanctis et al., 2019), or available energy. In this way, the process would be considered energy-efficient.

2.9.1. Heat demand

Temperature is one of operating parameters that has more influence in the hydrolysis and anaerobic digestion process. A heat-shaped energy input is needed to maintain the process. The Head Demand (Qin) has been calculated as:

$$Qin = Qt + Qp + Qpre \quad (4)$$

where Qt (kJ/d) is the amount of heat needed to raise the temperature of the influent substrate when it is introduced by peristaltic pumps in the digester (Metcalf et al., 1979).

$$Qt = Fp\cdot Cp\cdot\rho\cdot(1 - Rre)\cdot(Tt - Te) \quad (5)$$

- Fp = Substrate flow rate (m^3/d)
- Cp = specific heat of the substrate (kJ/g °C) if it is liquid it will assume as water Cp.
- ρ = substrate density (kg/m^3) if it is liquid it will assume as water density.
- Rre = the heat recovery coefficient assuming to recover the 85%

according to (Lu et al., 2008).
- Tt = temperature of the reactor.
- Te = temperature of the substrate.

Qp (kJ/d) represents the heat loss to ambient to maintain reactor temperature.

$$Qp = \sum_{reactor}^{air,soil\ldots} U \cdot A \cdot (Tt - Ta) \cdot 86.5 \quad (6)$$

- U = heat transfer coefficient of the digester (W/m^2 °C)
- A = surface area of the digester (m^2)
- Tt = temperature inside the reactor
- Ta = ambient temperature
- 86.5 unit conversion from W to kJ/d

Qpre is the necessary heat in the substrate preheating if the system would have one

2.9.2. Electricity demand

The total energy demand, in form of electricity, for the correct development and digester function (Ein) is calculated with the next expression (Lu et al., 2008):

$$Ein = Eb + Em + Ee + Epre \quad (7)$$

Eb represents the pumping energy demand of substrate to the reactor, Em the stirring energy demand, Ee other equipment energy demand and Epre pre-treatment energy demand in case that one is needed. All units in kJ/m^3, and calculated according to (Lu et al., 2008):

$$Eb = 2 \cdot 1.8 \cdot 1000 \cdot Fp \quad (8)$$

$$Em = 3 \cdot 100 \cdot Vdigester \quad (9)$$

2.9.3. Generated biogas

The biogas generated by each residue is estimated through the BMP tests carried out in the laboratory, and the biodegradability curves obtained from it.

$$B = biogas\ generated\ in\ the\ process\ (laboratory\ graphics) \quad (10)$$

2.9.4. Biogas need for covering heat and electricity demand

The installation must have:

- Power generator of η_g efficiency (in this case a CHP generator has been considered)
- Boiler generator of η_b efficiency

The biogas request to cover heat and power demand:

$$Bin = B_{Qin} + B_{Ein} \quad (11)$$

$$B_{Qin} = \frac{Qin}{\eta_b \cdot LCV_{biogas}} \; B_{Ein} = \frac{Ein}{\eta_g \cdot LCV_{biogas}} \quad (12)$$

where B is the biogas flow request (Nm^3/day) and y LCV is the lower heating value of methane (kJ/Nm^3)

2.9.5. Global energy balance

The net Biogas left over from the process, after the heat and electricity needs are satisfied, is calculated as follows

$$Bn = B - Bin \quad (13)$$

In all the biogas generated, there is an energy available, which is estimated through the calorific power of biogas

$$K = B \cdot LCV_{biogas} \quad (14)$$

The energy required to meet the needs can also be calculated by the expression:

$$Kin = Bin \cdot LCV_{biogas} \quad (15)$$

Finally, the net energy available, obtained from the process, once all the needs have been met, results from the difference between the energy available in the biogas and the energy needed to meet the demand for heat and electricity.

$$Kn = K - Kin \quad (16)$$

Energy Efficiency of the AD process was calculated by the ratio between the net available energy and the energy produced by the process according to the following equation

$$Kef = \frac{Kn}{K} \quad (17)$$

3. Results and discussion

3.1. Characterization of substrates

The results of the characterization of substrates V, M and F are shown in Table 1, and are detailed below.

3.1.1. Physical parameters: humidity and solids

All results are expressed in $\%_{hb}$, i.e. in grams per 100 g of substrate or residue in its natural state.

In terms of humidity there is a big difference between residue M and residues V and F. While V and F have a high humidity content (87.90% and 77.50% respectively), residue M has a significantly lower moisture content. This is easily explained by the nature of the substrates, since vegetable residues have a high content in water, while the residue of fish is mainly composed of blood and is therefore fluid. On the other hand, the residue M, being mainly composed of fat and remains of skin and bones, has hardly any water content. Precisely, this low moisture content makes the biodegradation of residue M, at first glance, much slower than that of wastes V and P, because it has a lower solubilization capacity and slows down the onset of hydrolysis.

With respect to solids analysis, there is also a large difference between V and F residues versus residue M. Because residue M is low in moisture, its content in solids is much higher than that of V and F residues. Therefore, this greater content in solids will produce a higher content in gas and methane, as there is more load to digest. However, this may vary depending on the chemical and organic composition of the substrate.

In all three substrates, virtually all solid content is volatile solids, i.e. of all the solid present, almost all of it is directly accessible for methanization. For residue V, 10.91 of the 12.10 g of solids are volatile, or 90.10%. The same occurs with residues M and F, with a percentage in VS compared to the total solids of 97.60% and 86.62%, respectively. That is, the three residues are quite suitable to undergo anaerobic digestion (depending on their content in volatile matter), with residue M having the most material available to be digested, and therefore being

the candidate to generate more gas. However, its degradation will be slower than that of the rest considering its moisture content.

3.1.2. Macro-nutritional analysis: LPCH content

Analyzing the results of the macro-nutritional analysis, or LPCH content, another series of determinations are collected that complete the analysis performed with the results of physical parameters.

All results are expressed in $\%_{bh}$, i.e. in grams per 100 g of substrate or residue in its natural state.

As for lipid content, there is a clear difference between residue M, with a high lipid content, and residues V and F. The V residue barely contains lipids, just like the residue F which has a slightly higher content, but far from the composition of M. This makes M a residue that can be virtually assimilated as a lipid residue. It has the highest carbon content, which will degrade slowly, but will generate a large amount of biogas with high methane content. Excessive load of residue M can lead to acidification by the accumulation of LCFA, and sponging phenomena are likely to occur inside the reactor.

Analyzing the level of proteins, residue V barely contains them, residue M has an important content by its nature, but residue F is, compared to the other LPCH levels, a virtually pure residue in proteins. Precisely because of this high protein content, it will be the one with the highest content in nitrogen and sulfur. This makes F a residue relatively easy to metabolize, with a medium rate of degradation, gas generation and methane The only drawback is its high nitrogen content, which makes it susceptible to accumulating the AN released in degradation, and can therefore lead to inhibition in the process, along with a pH above 8.

Carbohydrates are the most easily soluble compounds and those that therefore methanize faster. The CH content of V (8.90%) is much larger than that of M and F, which is practically nonexistent. This makes V a residue assimilated as a residue of CH type, whose processing speed into biogas will be very fast. However, because of the low carbon content, its level of methane will be more moderate than that of residues M and F. Especially if it contains simple carbohydrates so it will be lighter and if there is a large content in cellulosic compound between them, digestion will be more stable.

3.1.3. Organic content analysis: COD

The amount of COD is a measure to indicate the amount of organic matter present. With the results of COD_{total}, i.e. all the organic matter present, the substrate with the best composition to methanize, and therefore that will generate more biogas with higher content in methane, can be inferred.

As expected with the macro-nutritional analysis, the compound with the highest COD_{total} and therefore with a higher content of organic matter, is residue M, which due to its high fat content (with large amounts of carbon) is the substrate that should generate more gas with higher methane content. It is followed by residue F, which because of its protein content the COD is high, and therefore generates an intermediate amount of gas with medium methane content. Finally, residue V, having a high moisture content, and a majority of carbohydrates, has the lowest COD and therefore its gas generation is expected to be less efficient, with lower content of methane.

The measure of $COD_{filtered}$ (COD_f) or $COD_{soluble}$ indicates how much COD is solubilized. Higher soluble COD content makes the disintegration + hydrolysis stage develop faster and therefore the gas generation process also faster.

In terms of solubility, it is observed that the substrate that presents the best, that is, the one that is most easily accessible by microorganisms, is the Residue V for its high content in CH, especially simple CH, which makes it the fastest residue transforming them into methane. Followed by residue F, and last of residue M, which as anticipated, will be the residue with less solubility, and therefore the one that has a slower conversion to methane.

All the results are expressed in mg_O_2/g_{waste} or mg_O_2/ml_{sludge}, the first referring to the substrate and the second, to the inoculum.

3.1.4. Nitrogen content analysis

With respect to the content in nitrogen, the F residue stands out for its composition of mostly protein. The AN of residue F is much higher than that of the other residues, and therefore is the most susceptible to the release and accumulation of ammonium. The substrate with lower nitrogen content, both protein and ammonium, is by far the V residue, which will be the least susceptible to release and accumulate ammonium, which also relates to its low pH.

3.1.5. pH and alkalinity analysis

In terms of pH, the most acidic residue is residue V. Residues M and F have a similar pH, with the V residue being slightly more acidic. By themselves, these three substrates have a slightly lower pH than recommended for a good development of the process (6.7–7.2). V residue with such a low pH will be prone to VFA accumulation and therefore to the acidification of the reactor, which is why it seems more convenient to treat it as a co-substrate of a main substrate with a more neutral pH. Precisely this ease of accumulation of VFAs is one of the characteristics of CH-rich substrates, which must be treated with higher pH substrates and with higher organic matter content to balance an excessive development of acidogenesis.

As for alkalinity, the residue with less alkalinity is residue M, being slightly higher the IA versus the PA. It is precisely the residue with less IA, and therefore more susceptible to change by the accumulation of VFAs, which being a fatty substrate is likely to occur. V residue also has low alkalinity, and being an acidic residue, it only has IA, also relatively low because it is susceptible to the accumulation of VFAs by its nature of CH-rich substrate. The residue P is the one that presents the highest TA being especially high the IA. Being F a protein-rich residue, it is not prone to the accumulation and presence of VFAs.

All the results of alkalinity are expressed in mg_CaCO_3/g_{waste} or mg_CaCO_3/ml_{sludge}, the first referring to the substrate and the second, to the inoculum.

3.1.6. Elemental analysis

The elemental analysis determines the C, H, N, and S content of the substrates. As expected by its fatty nature, residue M is greatly different from residues V and F because of their high M content, which favors higher COD and a larger generation of biogas enriched in methane. The residue with the highest level of N and S is residue F, of protein nature, and the most susceptible to release and accumulate ammonium during its degradation.

All results are expressed in $\%_{bs}$, i.e. in grams for every 100 g of dried substrate or dried residue.

The C/N ratio can be deducted from the elemental analysis. This should be as high as possible to compensate for a high generation of gas with high methane content, with a low accumulation of ammonium due to low nitrogen content. The C/N ratio of residue V is within the limits of the permissible ($C/N \approx 20$), residue M has a very high C/N ratio, because of its high C and low N content, while residue F has a very low C/N ratio as it is of protein nature. According to this analysis, digestion will be more stable with residue V, uncontrolled in residue M due to its high C content and low nutrient level, and probably unstable due to the release of ammonium with residue F (low C/N ratio).

3.1.7. Summary

From the above, it can be concluded that the three types of residue V, M and P are very different from each other in composition. It is therefore to be expected that the degradation of each of them will be very different.

V residue is a carbohydrate-rich residue, especially simple CH, with high solubility and moisture, which makes it a residue of rapid degradation, with gas generation and content in methane inferior to the rest for

its content in C and lower COD. However, its degradation process will be stable due to the cellulose content and the optimal C/N ratio around 20. It is also susceptible to the accumulation of VFAs due to its acidic pH and low alkalinity.

Residue M is a substrate with a high lipid content and therefore high carbon and COD content, as well as low nitrogen. It is capable of generating large amounts of biogas with high methane content but can lead to the generation of foams and sponging of the digestate. Its low N content does not make it susceptible to inhibition by accumulation of NA although the AI indicates that it is prone to the accumulation of VFAs and therefore to acidification. However, low solubility and humidity make its degradation slow.

Residue F is a protein-rich residue, with high N content compared to the C content. It therefore has a low C/N ratio, with low COD. It will generate less gas and methane than residue M and because of its high N content is susceptible to NA accumulation and causes pH rise and process inhibition. The solubility is intermediate, and because of the high moisture content the rate of degradation will be intermediate.

3.2. Characterization of the inoculum: sludge UASB (S)

The characterization results are presented in Table 2, and the conclusions of the analysis are drawn in a similar way than when analyzing the results for the residues.

It is an inoculum with high moisture content, logical by its nature as UASB sludge to treat wastewater, which makes it a substrate of rapid degradation, ideal for treating FW that usually presents a lower moisture content. The solids content is not very high, which makes it suspicious that there is not a large amount of matter ready to biodegrade, so that the results obtained by treating FW together with the sludge will be mostly due to the elimination of volatiles in the residue. Therefore, the sludge will act solely as a support and way to provide the biomass of anaerobic bacteria.

Table 2
Characterization results for residues V, M and F; and the inoculum S.

	Substrate			Inoculum
	Residue			UASB sludge
	V	M	F	S
Physical parameters				
Hum [$\%_{hb}$]	87.90	38.20	77.50	94.30
TS [$\%_{hb}$]	12.10	61.80	22.50	5.70
VS [$\%_{hb}$]	10.91	60.32	19.49	4.92
Macronutritional analysis (LPCH content)				
Lipids (L) [$\%_{hb}$]	0.48	64.51	1.30	0.47
Proteins (P) [$\%_{hb}$]	1.52	11.21	18.60	0.53
Carbohydrates (CH) [$\%_{hb}$]	8.90	0.10	0.25	0.56
Organic content analysis (COD)				
COD_t [mg $O_2/g_{residue}$-ml_{sludge}]	173.64	842.15	239.30	101.65
COD_f [mg $O_2/g_{residue}$-ml_{sludge}]	41.83	16.74	35.69	37.08
Solubility [%]	24.09	1.99	14.91	36.48
Nitrogen content analysis				
TKN [mg $N/g_{residue}$-ml_{sludge}]	2.46	18.91	34.42	2.00
AN [mg $N/g_{residue}$-ml_{sludge}]	0.03	0.96	4.65	1.15
ON [mg $N/g_{residue}$-ml_{sludge}]	2.43	17.93	29.76	0.85
pH and alkalinity analysis				
pH	4.96	6.84	6.12	7.46
TA [mg $CaCO_3/g_{residue}$-ml_{sludge}]	5.83	5.46	25.41	8.88
PA [mg $CaCO_3/g_{residue}$-ml_{sludge}]	–	2.44	8.12	5.22
IA [mg $CaCO_3/g_{residue}$-ml_{sludge}]	5.83	3.02	17.26	3.65
Elemental analysis				
C [$\%_{db}$]	34.52	63.79	24.61	11.19
H [$\%_{db}$]	6.43	11.03	8.08	9.00
N [$\%_{db}$]	1.69	0.86	6.94	2.22
S [$\%_{db}$]	0.09	0.05	0.59	0.18
C/N ratio	20.43	74.17	3.55	5.04

The macro-nutritional analysis shows that the sludge is quite inert because of its low LPCH content. Once again it can be inferred that it will not cause too much interference in the degradation of the substrate by the degradation of the sludge itself and will serve only as a support of bacterial biomass.

The only drawback that this inoculum can present is its high content of COD, so it is necessary to analyze the biodegradability of the residue to avoid interference of the high content of COD present in the sludge, versus the COD of the residue actually eliminated.

The nitrogen content is not very high, neither the total nor the ammoniacal or organic types. That is, if inhibition by accumulation of ammonium is caused, it will be due to the nitrogen present in the substrate and not to the nitrogen from the sludge.

The pH is neutral, not causing interference, and total alkalinity makes it resistant to sudden pH changes, being slightly more sensitive to changes by accumulation of VFAs since its IA in slightly lower than the PA.

The elementary analysis and the C/N ratio highlights its low C/N ratio, so this sludge should be treated with substrates with higher C/N ratio such as residues V or M. On the other hand, when the high nitrogen content extracted from the F residue is treated, it can be a cause of inhibition.

In short, the sludge (S) becomes a perfect biomass support for the joint treatment with FW. The results obtained will be mostly due to the degradation of the substrate, the effects of the degradation of the sludge itself being negligible.

3.3. Approaches to the anaerobic digestion process according to the changes in substrates after anaerobic degradation

Table 3 shows how the composition of the digestion mixture (waste + sludge) has changed when treating residues V, M and F by anaerobic digestion with inoculum S, just before starting the BMP test, and at 20 days, that is, when the test finishes.

3.3.1. V + S mixture

As can be seen, after 20 days of testing the moisture of the substrate + sludge mixture has been reduced slightly, only 2%, which is logical when they are airtight reactors to ensure anaerobic conditions. The VS reduction of almost 40% and TS of 21.59% is noteworthy. This gives a first indication that the digestion process has developed correctly since the OM associated with The VS and TS has degraded, causing the amount of these to drop.

As for the analysis of COD, the reduction has been low, of the COD_f, directly accessible by microorganisms, has been degraded by 70%, that is, most of the soluble components, in this case monosaccharides and cellulose have been eliminated after digestion. The digestion of these makes the process, in principle, more stable. Only 1.96% has been reduced from the COD_t. That is, of the whole OM has been degraded only 1.96%, and within that percentage, 70% corresponds to the OM directly accessible without hydrolyzing. This indicates that most of the directly accessible OM has been digested during the anaerobic digestion process. However, the encapsulated OM has not been digested as it is not directly accessible to microorganisms. This makes the process incomplete and indicates a failure of the disintegration + hydrolysis stage, and a low methane content is expected n the generated biogas.

Based on the nitrogen content, TKN has likely increased by the release of some of the nitrogen encapsulated in proteins (ON). Similarly, in AN it has increased by 31.8% to the value of 1.15 mg/ml of mixture, placing in the buffer effect zone (<2 g/l), providing resistance against sudden changes in pH, making the process stable. On the other hand, ON has decreased by about 9%, confirming the hypothesis that the nitrogen encapsulated in proteins released has become part of the AN.

The initial and final pH is very similar, although it may have changed during the process. The most stable of this analysis is the large increase in alkalinity, 136% TA, 145% PA and 128.3% IA. This demonstrates once

Table 3
Characterization results for BMP tests of residue V, M and F; at the start and after completion of the test.

	Mix before anaerobic process				Mix after anaerobic process		
	V + S	M + S	F + S		V + S	M + S	F + S
Physical parameters							
Hum [$\%_{hb}$]	92.10	80.31	89.8	→	90.25	78.14	81.56
TS [$\%_{hb}$]	7.41	19.73	10.01		5.81	15.73	7.85
VS [$\%_{hb}$]	6.52	18.64	8.76		3.99	13.12	5.36
Macronutritional analysis (LPCH content)							
Lipids (L) [$\%_{hb}$]	0.47	16.51	0.68	→			
Proteins (P) [$\%_{hb}$]	0.78	3.21	5.05				
Carbohydrates (CH) [$\%_{hb}$]	2.65	0.44	0.46				
Organic content analysis (COD)							
COD_t [mg $O_2/g_{residue}$-ml_{sludge}]	120.33	286.72	136.12	→	117.97	284.71	125.36
COD_f [mg $O_2/g_{residue}$-ml_{sludge}]	40.52	32.04	25.83		12.05	12.06	7.36
Solubility [%]	33.67	11.17	18.97		10.21	4.23	6.10
Nitrogen content analysis							
TKN [mg $N/g_{residue}$-ml_{sludge}]	2.11	6.24	10.10	→	2.28	6.26	10.61
AN [mg $N/g_{residue}$-ml_{sludge}]	0.87	1.10	2.01		1.15	1.20	6.13
ON [mg $N/g_{residue}$-ml_{sludge}]	1.23	5.13	8.09		1.12	5.05	4.48
pH and alkalinity analysis							
pH	7.02	7.31	7.06	→	7.12	7.02	8.14
TA [mg $CaCO_3/g_{residue}$-ml_{sludge}]	8.32	8.05	13.01		19.70	9.64	11.80
PA [mg $CaCO_3/g_{residue}$-ml_{sludge}]	4.22	4.53	5.96		10.34	8.12	8.24
IA [mg $CaCO_3/g_{residue}$-ml_{sludge}]	4.10	3.52	7.05		9.36	1.52	2.56
Elemental analysis							
C [$\%_{db}$]	17.43	24.35	14.54	→			
H [$\%_{db}$]	8.41	9.51	8.76				
N [$\%_{db}$]	2.09	1.88	3.31				
S [$\%_{db}$]	0.14	0.15	0.28				
C/N ratio	8.34	12.95	4.39				

again the stability of the anaerobic digestion process, by becoming more resistant to sudden changes in pH, mainly motivated by the increase in AN that exerts, in this case, buffer effect in the accumulation of VFAs and bicarbonates.

3.3.2. M + S mixture

The humidity level, initially 80.31%, is reduced by 2.70%, which is a first indication that some kind of degradation has taken place. TS content is 20.27% and VS content, i.e. associated with anaerobic degradation, is 29.61%. This shows that there has been a process of degradation of OM associated with these solids, mainly the volatile solids, were susceptible to biodegradation.

COD levels are decreased after the AD process, i.e. the OM present has been reduced, and therefore the COD is assumed as an indicator of degradation. Total COD has been reduced by just 0.70%. However, soluble COD, i.e. COD directly accessible to microorganisms and with less need to be hydrolyzed, has decreased by 62.36%. This indicates that the degradation process has occurred, however most of it is due to the degradation of soluble OM. Non-soluble, particulate or encapsulated OM has barely been degraded. Further analysis is needed but it could be stated that the process of disintegration + hydrolysis has been limited and has not developed properly. If this is true, residue M would be a good candidate for pretreatment before being introduced into an anaerobic reactor, in order to facilitate the access of microorganisms to the encapsulated OM.

In terms of nitrogen content, already high, TKN has suffered a slight increase of 0.39%, increasing the AN from 1.10 mg/ml to 1.20 mg/ml, an increase of 9.04%. In any case, the AN is always kept below the limit of inhibition by accumulation (<2 g/l), acting as a buffer against possible drastic changes in pH, for example by acidification or accumulation of VFAs or LCFAs. The increase in the AN, meanwhile, is associated with the destruction of ON from proteins, reducing by 1.47%, and releasing nitrogen that shifts the NH_3/NH_4^+ balance towards ammonium.

This buffer effect associated with the AN is vitally important, as residue M (as previously studied) is considered a residue of a fatty nature, and is sensitive to the accumulation of acids during its degradation, especially LCFAs.

The pH of the mixture, initially neutral with a value of 7.31, drops slightly (by 3.96%) to a pH of 6.74. It is to be expected that the pH will be reduced, since by the effect of the accumulation of LCFAs and VFAs the acidification of the reactor is likely. However, the content in AN exerts buffer or buffer effect, being able to counteract the inhibitory effects of accumulation.

Alkalinity due to VFAs (IA) is reduced by 56.81%, implying a loss of resistance to pH changes due to acid accumulation, and is therefore a sign of an obvious presence of VFAs in the reactor. However, since the pH has not been reduced to inhibition levels, it is likely that the buffer effect of the AN has counteracted the loss of IA. The PA increases by 79.44%, a net effect on total alkalinity of an increase of about 20%.

The high C/N ratio is noteworthy in terms of elemental analysis, implying high carbon presence and sufficient but not excessive nitrogen. However, it still falls below the optimal C/N ratio of 20, so residue C is a good candidate to be treated in co-digestion with other substrates to try to increase the C/N ratio and provide stability to the process.

3.3.3. F + S mixture

After 20 days of testing, the moisture content, already high, is reduced by 9.17%. TS, on the other hand, have declined by 21.58% and VS reduce by 38.81%. This large reduction, especially of volatiles, is a first indication that there has been a great degradation of OM associated with solids, both particulate and soluble.

The change in the COD is another indicative parameter of the development of the process. Total COD has been reduced by 7.90% and filtered COD decreases by 70.34%. The reduction in soluble or filtered COD is quite high, indicating that much of the directly solubilized OM has degraded, while the total COD only degrades a small part (7.90%). This denotes that much of the OM is encapsulated or directly accessible,

and since the decrease in filtered COD and total COD is so different, it can be assumed that virtually the entire reduction is due to the biodegradation of the soluble fraction, leaving much of OM undigested, and thus showing a failure of the process of disintegration and hydrolysis. Pretreatments are one way to solve this limitation of the hydrolysis.

In view of the changes in nitrogen content, an increase of 5.04% of TKN is observed, with the large increase in AN (203.8%) being particularly noteworthy, which has a decrease in ON of 44.54%. This is interpreted as a degradation of ON encapsulated in proteins, which was present in large quantities when the F substrate was considered a residue of a protein nature. The ON is released and by degradation it becomes ammonium, causing the AN to increase.

The accumulation of AN can lead to inhibition, and in this case it is very likely to occur since, initially the content in AN is at the limit of behaviour as buffer (<2 g/l), but once digestion is over, the AN greatly exceeds this limit when reaching values of 6.13 g/l. Ammonium accumulation can lead to inhibition, and this is detected by a failure in the process, a halt in methane formation or pH increments when the NH_3/NH_4^+ is moved towards ammonium.

As for pH, it is initially in AD capable values (7.06), however, once the digestion is complete dysing the pH is relatively high (8.14) which clearly confirms the possibility of accumulation of AN and the subsequent inhibition of the process, which should be tested with further analysis. TA drops by 9.33% making the biomass more sensitive to pH changes, such as when AN accumulates. This alkalinity drop is another indicator of possible Inhibition by accumulation of AN, as the buffer effect is not taken advantage of. The IA is greatly decreased by 63.38%, indicative of sensitivity to changes by LCFAs and possible accumulation (which would be counteracted in terms of pH with the accumulation of AN). The PA on the other hand, grows by 54.90% by increasing its resistance to changes due to bicarbonates.

Something that can be seen regarding the initial elemental analysis is not only the high nitrogen content (logical due to the high level of proteins), but the high sulfur content. This causes the appearance of sulfates that can affect the generation of methane, either by competition between sulfate-reducing bacteria with methanogenic microorganisms or by poor adaptation to the inoculum.

3.4. Biogas production

Fig. 2 shows the biogas production curves obtained during testing, and Table 4 shows the most relevant descriptive statistics. In the supplementary information, all biogas curves obtained during the BMP tests are available in Figure C-SP.

The initial part of the curves is very different for each substrate. The first growth phase is much faster for V and F residues, which slow and stabilize generation around 9–10 days. On the other hand, residue M stabilizes biogas production at day 13–14, being the slowest generation process.

In the average production curves of biogas, steep slope changes are observed at the V curve. This may be due either to inhibitions in the digestion process or due to digestion in two phases. It will be checked later when analyzing all process variables together. This change in slope is also seen in the degradation of substrate M, however, in this case it is likely due to an inhibition resulting from the accumulation of acidic elements (given the fatty nature of the residue), which is subsequently reversed thanks to the buffer effect of the accumulated AN in an adequate proportion. On the other hand, the residue F curve has no slope changes and it is stabilized early, indicating that biogas generation stops and inhibits. It is probably due to excessive accumulation of AN above the permissible values to act as a buffer. Both assumptions for the three residuals are confirmed or discarded in later sections.

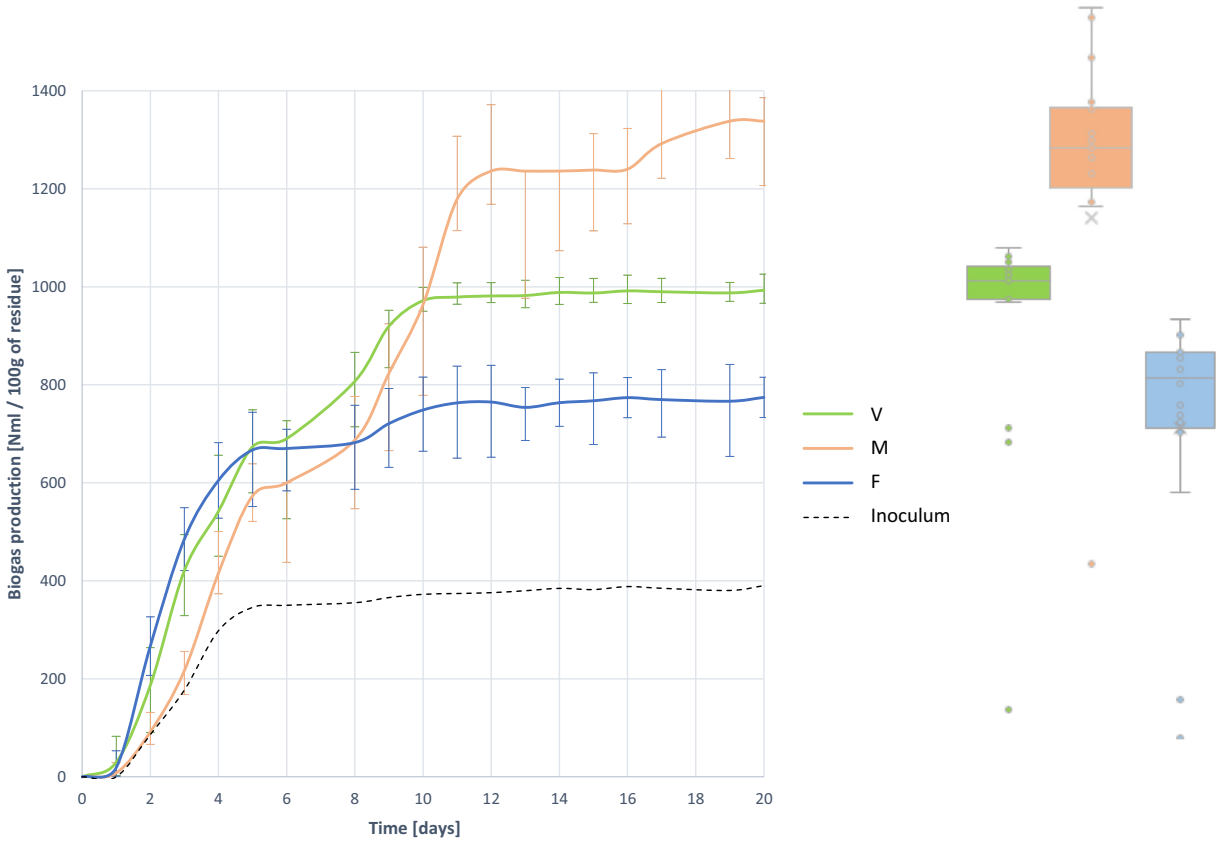

Fig. 2. Gross biogas production curves from the anaerobic digestion od 100 g of residue V, M and F.

Table 4
Numerical results of the BMP tests. Gross and specific production of biogas, methane and hydrogen, methane and hydrogen content of the produced biogas and descriptive statistics.

			σ	CV	ε
Biogas					
Production	V	913.282 Nml	222.904	0.244	14.436%
[Nml/100 g of residue]	M	1337.585 Nml	499.593	0.448	29.011%
	F	769.239 Nml	279.144	0.420	24.722%
Specific production	V	82.862 Nml/g_{VS}	20.432	0.246	15.269%
[Nml/g of VS of residue]	M	22.220 Nml/g_{VS}	8.282	0.373	21.065%
	F	34.069 Nml/g_{VS}	14.322	0.420	29.450%
Methane					
Production	V	289.333 Nml	94.723	0.327	21.421%
[Nml/100 g of residue]	M	232.317 Nml	118.031	0.508	33.312%
	F	102.741 Nml	36.248	0.405	67.911%
Specific production	V	26.351 Nml/g_{VS}	8.654	0.328	21.376%
[Nml/g of VS of residue]	M	4.710 Nml/g_{VS}	1.956	0.415	23.211%
	F	4.590 Nml/g_{VS}	1.859	0.405	27.567%
Methane content					
Production	V	32.252%	7.906	0.245	12.051%
[%vol of CH_4 in biogas]	M	21.021%	6.555	0.311	15.305%
	F	13.679%	7.906	0.577	130.209%
Hydrogen (maximum production)					
Production	V	0.456 Nml + 0.200 Nml	0.298 + 0.200	0.655 + 1.000	57.583% + 90.609%
[Nml/100 g of residue]	M	0.539 Nml	0.266	0.493	36.425%
	F	0.032 Nml	0.043	1.330	99.985%
Specific production	V	0.041 Nml/g_{VS}	0.027 + 0.199	0.655 + 0.999	57.705% + 92.615%
[Nml/g of VS of residue]	M	0.011 Nml/g_{VS}	0.012	1.115	55.038%
	F	0.001 Nml/g_{VS}	0.002	1.487	127.624%
Hydrogen content (maximum production)					
Production	V	0.256% + 0.017%	0.128 + 0.019	0.484 + 1.124	42.311% + 92.375%
[%vol of H_2 in biogas]	M	0.359%	0.170	0.474	35.095%
	F	0.006%	0.008	1.373	112.548%

The highest biogas production is that of residue M, and it was to be expected by the fatty nature of the residue and its high carbon and COD values. Secondly, it is followed by the biogas production of residue V, which as a CH-rich residue should generate a smaller amount of biogas, but since the AD of residue F is inhibited, it comes second.

Speed in generation can be studied, not only with the disintegration constant (that will be detailed in Table 5), but through the shape of the curves. The slowest production is undoubtedly that of residue M, understandable by the fatty nature of the residue. The degradation rate of the V and F curves seems similar but cannot be compared as the generation of F is inhibited.

As for the variability between the curves, it is studied using the box diagrams represented and the value of the CV. The most stable biogas generation is undoubtedly that of residue V. It is clearly seen how the curves converge more homogeneously in the average biogas production value of 913,282 Mml per 100 g of residue V. The size of the box in the diagram is smaller, indicating greater homogeneity, as well as the CV value that is considerably lower than that of residues M and F. It is then assumed that the most stable digestion, in terms of biogas generation, is that of residue V.

As for the stability in the generation of biogas for residues M and F, the size of whiskers seems more dispersed for the generation of biogas for M. Analyzing the CVs effectively, is slightly greater the variability for M that for F. Thus the most stable generation of biogas is found when residue V is digested, and more unstable when residue M is digested, albeit with variation values similar to those of residue F, in which inhibition occurs.

Finally, it is necessary to analyze whether, statistically, there are differences between the biodegradation curves of residues V, M and F In this case, the difference and independence between them are clearly apparent. However, an ANOVA analysis is performed with DMS, Games-Howell and HS Tukey contrasts. In all cases the significance level is 0, so the null hypothesis of mean equality can be rejected, and therefore it is assumed that biogas generation is statistically different between V, M and F production.

Table 5
Results obtained in mathematical processing of the parameters of waste V, M and F biodegradation.

Mathematical determinations and adjustments			σ	ε
Theoretical methane generation	V	292.808 Nml	91.809	22.260%
[Nml/100 g of residue]	M	249.386 Nml	105.087	71.189%
	F	47.742 Nml	26.346	4.723%
Maximum methane generation	V	323.000 Nml	90.961	16.786%
[Nml/100 g of residue]	M	167.002 Nml	104.452	65.298%
	F	105.147 Nml	130.246	16.291%
Disintegration constant	V	0.200 d^{-1}	0.044	17.920%
[$days^{-1}$]	M	0.133 d^{-1}	0.028	0.028%
	F	0.194 d^{-1}	0.054	18.823%
Substrate biodegradation	V	16.045%	1.677	7.422%
[%]	M	3.527%	0.402	0.272%
	F	15.219%	1.824	10.430%

3.5. Specific biogas production

The shape of the specific biogas production curves for each residue resembles the shape of the gross production curves. However, there are differences in levels, because of the different VS content of each substrate.

The gross generation of biogas was higher for residue M, in this case it is the residue that has the lowest specific production, due to its high VS content. Residue V is the one with the highest biogas production per VS of residue V added, followed by the generation of residue F.

As for the variability in the specific generation, according to the size of the boxes and the value of the CVs. in residue V is again the most

stable, followed by residue M, and finally with greater dispersion the residue F.

The specific biogas production curves for V, M and F residues can be considered independent according to the ANOVA analysis performed.

- Residue F stops the generation of methane on day 3, due to the accumulation of AN due to its protein nature, and the consequent inhibition. The high sulfur content also affects the slowing and inhibition of the process.

3.6. Methane production

The generation of methane is very different for each residue. It depends not only on gas generation, but on methane content. It is therefore a better indicator of process development than biogas, providing information on developments and possible inhibitions.

As can be seen in Fig. 3 and Figure D-SP from supplementary information, the initial part of the curves is quite dispersed in all cases, which is logical since methane does not finish developing until the last moment, being a chain process. Residue V stabilizes its methane production on day 9, residue M on day 11–13, and residue F, when inhibition occurs, stops its methane development on day 3–4.

The curves show that the fastest generation is that of residue V, and the slowest, residue M. Both data are consistent with the nature of the residue, being V a residue rich in CH and therefore the one that presents the fastest conversion rate. Residue M, being fatty, produces methane at a slower rate.

The evolution and comparison of curves gives information about the development of the process:

- Residue V has a slope change on day 6, which likely indicates a two-phase digestion phenomenon (this should be later confirmed with the hydrogen production curves).
- Residue M has a slope change on day 6, keeping slope zero until day 8 in which methane generation resumes. This null generation permanence shows that on day 6 there is an inhibition of methanogenesis (accumulation of LCFA), resuming on day 8 at a slower rate (AN buffer effect).

As for the level of methane generated, residue V is the one that generates the most methane, around 290 Nml, followed by residue C, with about 230 NML, and lastly the residue P with a generation of just over 100 N_{ML} of CH_4. Low levels of F generation are due to inhibition, so no conclusions can be drawn. However, as for V and M, the generation of methane for V is higher, theoretically having to be greater than that of M because of its high fat content and COD.

This change is due to two fundamental factors. The good solubility of V makes it more accessible to microorganisms, and therefore more susceptible to methane generation, and in addition the V residue has no type of inhibition.

This is why residue M is a good candidate to be co-treated with another residue to limit the inhibition caused.

The variability between curves, which provides information about the stability of the methane generation process, is studied using the box diagrams shown in Fig. 3 and through the coefficient of variation detailed in Table 4. It is then concluded that the residue that provides greater stability when generating methane is residue V, the most uncontrolled being the residue M. Given the nature of the residue it was expected, since the CH provide a more stable AD by containing monosaccharides and cellulose. However, fats cause acid buildup and spongeing of the sludge, making methane generation more uncontrolled.

The generation of methane can be considered statistically different for each residue, as is inferred from the ANOVA analysis carried out, so the type of residue to be treated affects the gross production of methane.

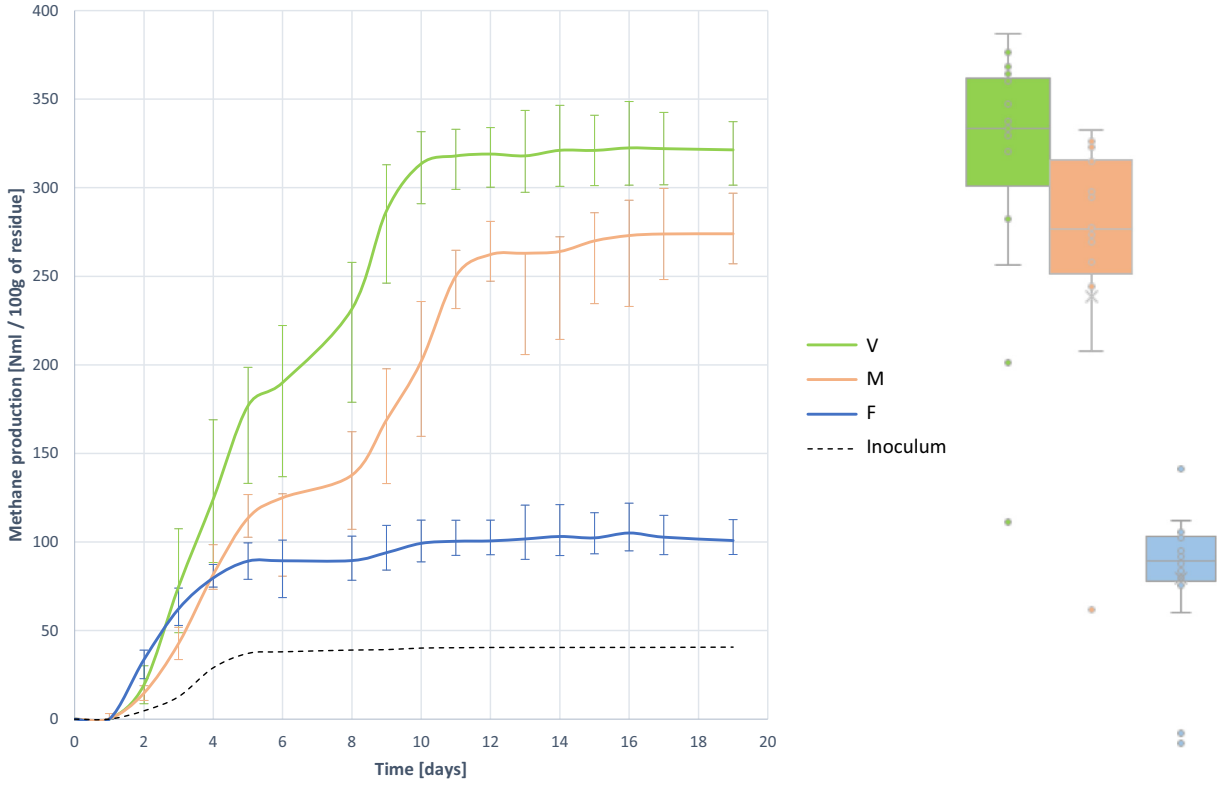

Fig. 3. Gross methane production mean curves from the anaerobic digestion od 100 g of residue V, M and F.

3.7. Specific production of methane

The shape of the specific production curves of methane for each residue, i.e. production for each gram of VS added, yields the same information as that developed in the gross generation of methane. However, as shown in Table 4, generation levels vary.

The V residue is the one that has the most specific generation, exceeding 25 Nml/g_{VS}, much higher than that of the M and F residues, which are similar, around 5 Nml/g_{VS}. The case of M is striking, as it is the one with the highest biogas yield, but with the lowest methane yield. This specific low generation responds to the high VS content of the substrate.

The stability of the process, measured through the CVs and visually by the box diagrams, again shows that the AD of residue V is the most stable of the three. The AD stability of M and F is similar, but lower than that of V.

The dependence of the specific generation of methane with each type of residue is analysed through the results of the ANOVA analysis. Once again it is shown that the curves can be understood as different from each other, i.e. the generation of residue V is different from that of residue F and residue M, and vice versa.

Table 1 shows methane generation data obtained by other authors in the literature. It is noted that the data from this study are quite close to those obtained in the literature, although it should be understood that they are slightly higher in the literature as they are residues from the final part of FSC, and are therefore somewhat richer in organic content and fat content. Batch results are quite similar and are superior in cases where waste has been treated continuously and on a large scale or in two-phase reactors.

3.8. Methane content of biogas produced

The methane content of biogas is a very important variable, not only to evaluate the effectiveness of the AD process, but also its development. High methane content denotes process stability, and low proportions of failure, inhibitions or poor development from low degradation.

Fig. 4, Figure E-SP from supplementary information and Table 4 show the evolution of methane content for each residue when the AD is developed, and the final values obtained together with the descriptive statistics required for analysis.

As for the evolution of the percentage of methane during the first few days, it is very similar. That is, the rate of growth is very similar.

However, residue F is the one that previously reaches a maximum value of approximately 13%, coinciding with the day on which the inhibition by accumulation of AN develops. Residue M reaches it a little later, on day 4, coinciding with the day on which LCFA accumulation inhibition begins, and re-grows on day 9–10, when generation resumes by AN buffer action, reaching a percentage of about 20%.

Residue V reaches a first maximum on day 5 and continues to increase the proportion of methane until day 10, as the phenomenon of digestion in two phases has occurred and a maximum proportion in methane of 32% is obtained.

The residue that provides the greatest content of methane is residue V, and is therefore assumed to be the one that produces the most stable development of AD. Residue M is the second most stable and with the highest proportion of methane, and lastly is residue F, which has the most unstable AD due to inhibition.

In either case, the proportion of methane obtained is below expected as calculated using the Buswell-Mueller and Boyle formulas (Buswell and Mueller, 1952).

- Residue V, considered a purely CH residue, would provide a methane content of 50%, however it is relatively minor. Since there are no inhibitions and taking into account digestion in two phases, it has been shown that it is due to poor biodegradability of the substrate, which would improve with pretreatment.
- Residue M is considered a fatty residue, and methane content is expected to be close to 70%. There is a big difference caused by inhibition

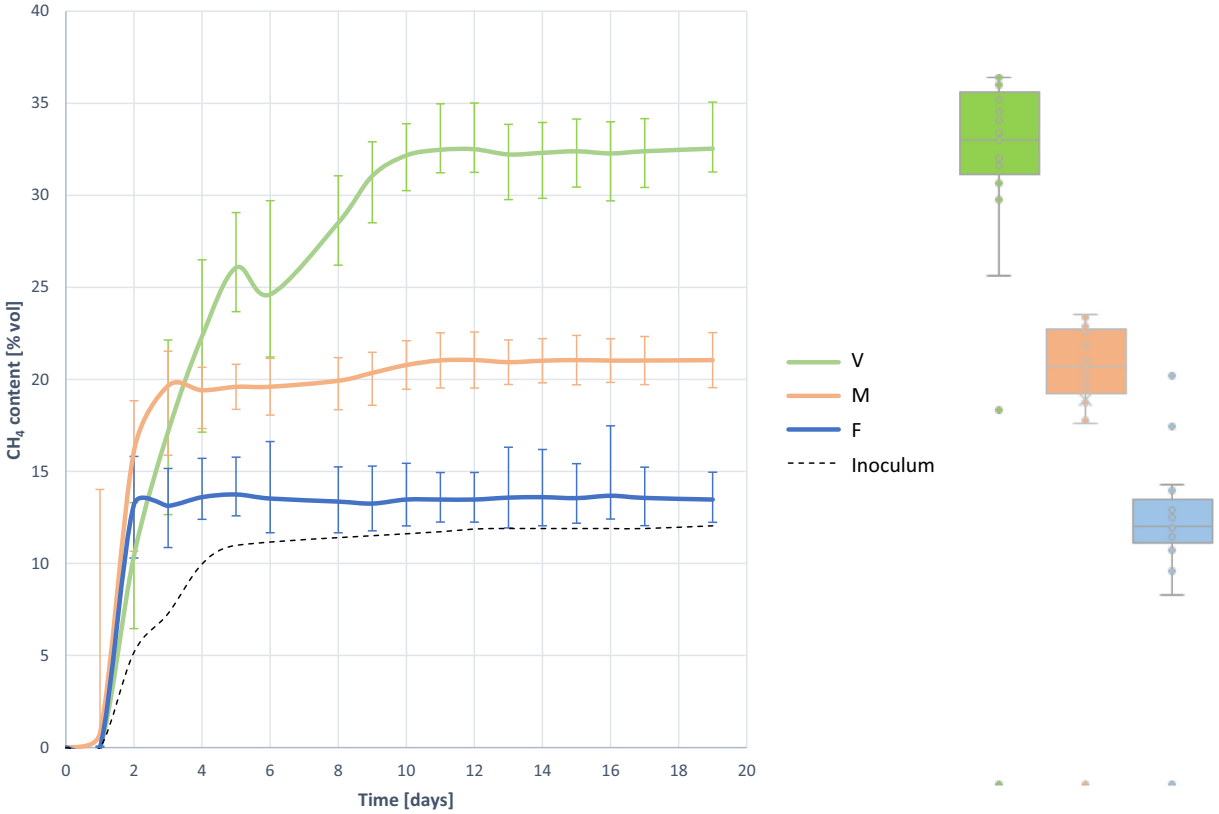

Fig. 4. Methane content mean curves from the biogas produced in the anaerobic digestion of 100 g of residue V, M and F.

due to accumulation of LCFA and by a very low biodegradability of the substrate. Better methane content would be achieved with pretreatment to increase the level of degradation, and with co-digestion to prevent the accumulation of acidic elements.

- The residue F, of a protein nature, suffers an inhibition by accumulation of AN and therefore the expected methane levels of 60% are not reached. Co-digesting it with residues with higher carbon content and lower nitrogen would favor the process, reducing and even eliminating Inhibition by accumulation of AN. In addition, the high level of sulfur in the F residue improves the AD process, as the presence of sulfates slows methanogenesis by competition between methanogens and reducing sulfate bacteria.

As with the other results, a variability analysis has been carried out, resulting in residue V the one with least variation in the count in methane generated followed by residue M and residue F. The results of the ANOVA analysis show that the proportion of methane achieved with residue V is different than that achieved by residue F and by residue M, and can be considered different for each residue. That is, the type of residue affects the proportion of methane that is generated in biogas.

3.9. Hydrogen production

Fig. 5 and Figure F-SP from supplementary information shows the production curves of H_2 for every 100 g of residue added to the sludge, and in Table 4 the descriptive statistics for this H_2 production.

Hydrogen production curves are more out of control, and it is necessary to go to the average production curves, also shown in Fig. 5. Previous ANOVA analyses had shown the correspondence between curves for the same residue, so that all H_2 production curves for residue V can be approximated by the mean curve; the same is true for residues M and F.

It is shown that the fastest hydrogen generation is given for V residue. This indicates that the hydrolysis rate is faster, and therefore it is to be expected that the disintegration constant is higher. It also corresponds to the fastest degradation residue as seen in previous sections and confirms that digestion is more stable. The two peaks that appear on the V-curve (days 2 and 9) demonstrate the phenomenon of digestion in two phases.

Meanwhile, the peak of H_2 for residue M appears later, on day 4, showing that its degradation is slower, and a minor disintegration constant is expected for this residue. The peak takes longer to disappear, analogous to a slowing of hydrogenotrophic methanogenesis. In addition, while in the disappearing phase, a slope change occurs between days 5 and 6, to disappear again at a faster rate. This shows that there is inhibition, which is subsequently reversed. This is why the AD process is more unstable than that of residue V.

The maximum values obtained from H_2 for residue V and M are equal, however, the methane content is higher for residue V. This is due to the appearance of two peaks of H_2 in this residue, and to better degradation and stability in the AD process.

The F residue shows very low H_2 generation levels. In addition, the peak remains for a long time and takes 8 days to disappear. This means that an inhibition of methanogenesis has occurred and confirms the theory of inhibition by accumulation of AN.

Differences in the AD process created by the generation other than H_2 are demonstrated by concluding that the production curves of H_2 are dependent on the type of residue, and in no case can it be assumed that the production curve of H_2 of V is the same as that of M or F, or vice versa. To this end, an ANOVA analysis has been used, showing that the equality of the curves can't be assumed.

3.10. Specific hydrogen production

Hydrogen-specific generation values are shown in Table 4. The conclusions drawn are the same as for gross generation, with the only

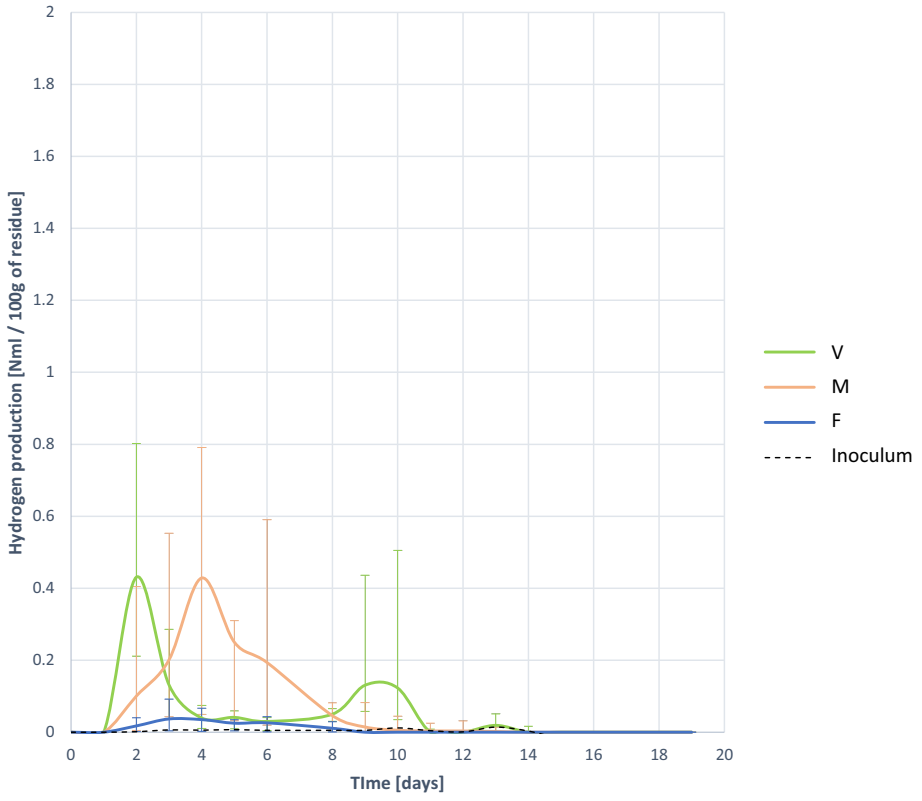

Fig. 5. Hydrogen production mean curves from the biogas produced in the anaerobic digestion of 100 g of residue V, M and F.

caveat that, because of the high VS content of residue M, the specific production drops greatly from that of residue V, when its gross production was similar.

3.11. Hydrogen content of biogas produced

The hydrogen content of the biogas produced is also an indicator of process stability and speed, as it must appear and then transform into methane. The earlier it appears, the earlier hydrogenotrophic methanogenesis will occur, and the more proportion of H_2 means that hydrolysis has been more efficient, and therefore more likely to generate more methane.

Fig. 6, Figure G-SP from supplementary information and Table 4 show the evolution of the ratio of hydrogen in biogas generated during the degradation of the residues, and the values it achieves.

As with the gross generation of hydrogen, it is necessary to go to the average proportion curves in which the percentage trend is better visualized, In addition, according to the ANOVA analyses developed previously, you can assume the average curve of each residue as the overall hydrogen ratio curve.

It can be seen that the proportion of hydrogen grows slightly faster in the case of V than in the case of M, also reaching a slightly higher value and disappearing at a higher rate. This is a sign of deeper and more stable hydrolysis, as well as of a faster process. That is why the AD process of residue V is much more stable.

In the case of residue M, growth is slightly slower, and the rate of disappearance is also lower, denoting that hydrolysis and methanogenesis are slowed, making the process slightly more unstable.

Residue F, when AN accumulation inhibition occurs, H_2 stays much longer as methanogenesis slows down, and the content in H_2 is much lower because of process failure.

3.12. Mathematical analysis and determinations

With mathematical analysis of the data obtained for the biodegradation of residue, information is obtained about the expected generation of methane, the level of biodegradation, the maximum expected generation and the constant disintegration.

Table 5 lists all the results obtained and are discussed below.

As for the theoretical generation of methane based on the removal of COD, that is, what is expected to be obtained, for residue V it is adjusted to the actual generation. This means that the process has been developed without inhibitions, and that given the level of biodegradation experienced, the process has developed correctly. For residue M, the theoretical generation is slightly higher than the real one, caused by inhibition and a low level of degradation. As for residue F, the expected generation is much higher than the real one, symptom of the inhibition by accumulation that the process undergoes.

The disintegration constant measures the rate at which hydrolysis occurs, and as expected, residue V is the one with the greatest disintegration constant and is therefore assumed to be the one that develops hydrolysis more quickly. The slowest is residue M, because of its fatty character. Residue F, despite inhibition, has an intermediate hydrolysis rate.

The maximum expected methane generation for biodegradation data is much higher for residue V. This indicates a greater stability in the process, and that it develops without inhibitions. Residue M, in terms of maximum generation, is in second place, caused by an inhibition that is then reversed. Thirdly, with a very low maximum generation, is residue F, due to its inhibition by the accumulation of AN.

The percentage of biodegradation of the substrate relies on the proportion of residue that has been degraded by biomass. Residue V is the one with the highest percentage, when most solubles and COD are

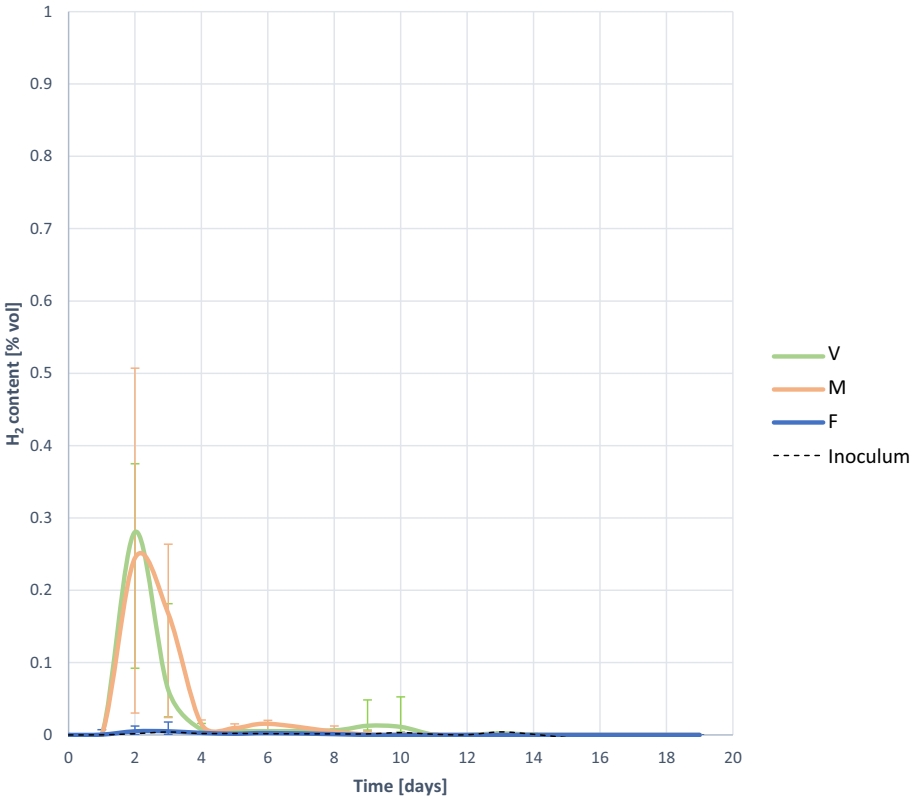

Fig. 6. Hydrogen content mean curves from the biogas produced in the anaerobic digestion of 100 g of residue V, M and F.

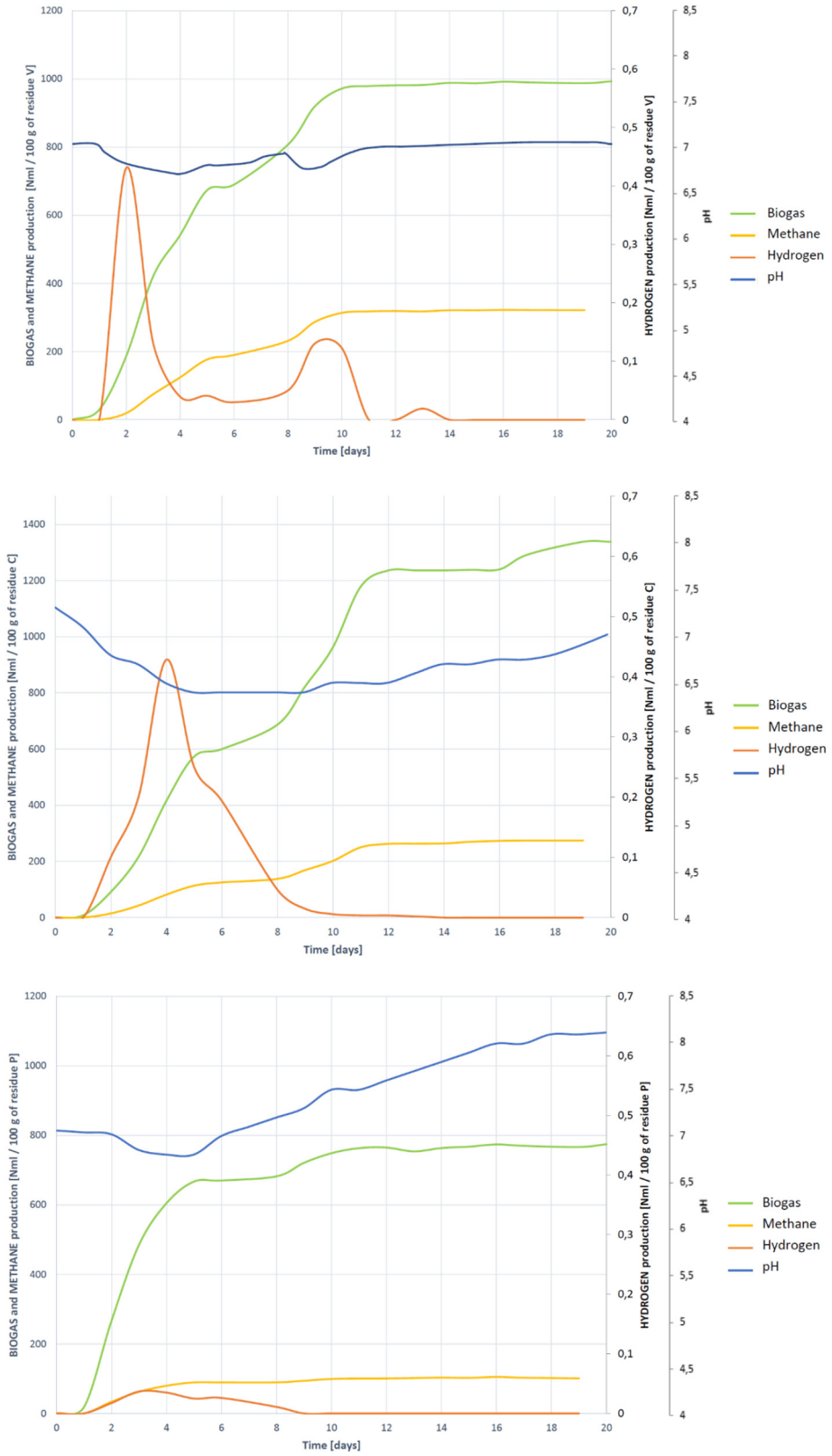

Fig. 7. Evolution of the digestion process of waste V, M and F respectively. Comparison of the generation of biogas, methane and hydrogen together with the evolution of pH

removed. However, it is a relatively low result, and it should be improved, for example, by applying pre-treatment.

The level of degradation of residue M is very low, and considering that it is the one that produces the highest generation of biogas, a pretreatment would help not only to increase the percentage degraded, but to increase the production of gas and methane to grow at a higher rate. The percentage of degradation of P remains low, but can be understood, as the process is inhibited in the first few days.

3.13. Joint evaluation of the digestion process

By analyzing the production curves of biogas, methane and hydrogen, together with the evolution of pH, a more thorough study of the residue AD process can be carried out. Since it has been assumed, through the results of the ANOVA analyses, that all the production curves of biogas, methane and hydrogen can be joined in the average curve, in Fig. 7 (residues V, M and F respectively) both CH_4 and H_2 productions are represented against with pH development.

3.13.1. Residue V

During the first day (day 0–day 1) there is a delay, the formation of methane or hydrogen not being yet appreciated, so it is assumed that the disintegration + hydrolysis stage occurs throughout the first day.

Between days 1 and 2, hydrogen formation begins, peaking on day 2. That is, during day 2 the acidogenic and acetogenic phase takes place. At this moment biogas and methane begin to appear. Methane growth is slower as it occurs only by acetoclastic methanogenesis. In addition, acidification occurs when the pH is lowered by the formation of acetic acid and other VFAs.

There is no inhibition by acidification or accumulation of VFA as hydrogen evolves disappearing and transforming into methane.

From day 2 to day 4 hydrogen transformation occurs, and methane growth is more pronounced as it is produced by acetoclastic and hydrogenotrophic methanogenesis.

From day 5 nitrogen levels remain constant and virtually zero. The generation of biogas and methane stops, maintaining constant levels, with the disappearance of acetic acid and VFAs to transform into methane, pH is increased to stable neutral values.

On day 8 a second peak of hydrogen appears, so it is assumed that a new stage of digestion takes place when the generation of methane resumes and acidification (indicative of generation of acetic and other acids) occurs.

The process ends on day 11 with the disappearance of H_2 and acids (pH increases to neutral values) and stop methane generation. Therefore, the time required to complete the digestion process does not have to be 21 days and can be shortened to 11–12 days.

In conclusion, the process has been developed correctly without inhibitions by acid accumulation.

There is a two-phase digestion, clearly identified by the resumption of hydrogen generation, methane and pH changes. It is to be assumed that in the first stage of digestion the OM is digested directly accessible, and in the second stage part of the non-soluble OM, which begins to hydrolyse.

3.13.2. Residue M

As can be seen, the low level of methane is striking compared to the average production of biogas, a clear indication of failure in the process.

There is a first phase of delay during the first day (day 0 to day 1) in which there is no evidence of biogas, methane or hydrogen generation. From day 1 the three curves begin to grow, so it is assumed that, during the first day, the disintegration + hydrolysis phase occurs.

Biogas begins to grow at a very high rate, much more than CH_4. On day 5 it reaches a maximum and growth slows down (coinciding with the total halt in methane generation), and until day 8 the generation is much slower. Rapid gas generation is subsequently resumed, coinciding again with a resumption in methane production. It finally stabilizes around day 12–18.

The methane present in biogas increases at a moderate rate from day 1 to day 5 when it ceases, coinciding with a deceleration in biogas generation. From day 8 to day 11 it grows again, stopping again on the 11th, without resuming in the 20 days of test time.

In either case, the shutdown and resumption of production corresponds to a deceleration and acceleration of biogas production.

As for the hydrogen present, it begins its growth on day 1, peaking on day 4. They begin to lower the levels at an appropriate speed until day 5, when the elimination speed decreases, to disappear completely on day 8.

The achievement between the curves of biogas and methane indicate that, due to internal factors, the process of methane generation (methanogenesis) is inhibited or slowed. While biogas generation does not stop, but continues at a slower pace, the generation of methane paralyzes. Then, between days 5 and 8, there has been an inhibition of methanogenesis. That is, intermediate elements such as hydrogen (which maintains its growth) and acetic acid are formed, but not final elements such as methane. This causes a buildup of acetic acid and other VFAs, with consequent acidification and inhibition.

Methanogenesis resumes again on day 8 and day 11, so it seems that inhibition is reversed, to stabilize on day 12. Therefore, the time required to complete the digestion process does not have to be 21 days and can be shortened to 12–13 days.

With this it can be inferred that, until day 5, methanogenesis has occurred by the two pathways (hydrogenotrophic and acetoclastic) and that from that day on inhibition takes place, coinciding with the total disappearance of the content in H_2. When methanogenesis is resumed and no H_2 content is found, it must be done acetoclastly, so it is assumed that acid elements have been accumulated during the inhibition phase.

As for the analysis of the pH curve, it begins to descend from the initial pH 7.31, to a minimum on day 7, coinciding with the days on which inhibition is assumed to exist. Therefore, inhibition by accumulation of acids (characteristic of the substrate type) between days 5 and 8, is shown by pH levels and by the results obtained from IA tests.

As the generation of biogas and methane resumes, the pH begins to increase, thus reducing the level of accumulated VAs, as these become methane via acetoclastic methanogenesis. This resistance and recovery to the abrupt change of pH is the effect of alkalinity and, fundamentally, of the buffer effect caused by the accumulation of AN resulting from the degradation of the protein content of the substrate, a characteristic that is studied at the beginning of the Heading. The buffer effect of AN accumulation is then tested.

3.13.3. Residue F

As can be observed, the first feature is the low content of methane, and the rapid stop in its generation, is a clear indicator of poor biodegradation or inhibition of the process.

There is a delay phase during the first day (day 0–day 1) not appreciating generation of biogas or evidence of H_2 or CH_4 in this, it is assumed that the previous phases of disintegration and hydrolysis occur on the first day.

Biogas begins its growth at a faster rate than H_2 and CH_4, peaking at day 3, and remaining constant thereafter until the end of the test.

Methane increases from day 1 to day 4–5, maintaining its constant levels until the final day of the test.

The generation of H_2 starts on day 1 and reaches the maximum on day 3, remaining without disappearing until day 8–9. This indicates that the previous phases of acidogenesis and methanogenesis have been maintained for several days and hydrogenotrophic methanogenesis has slowed down causing the H_2 to stay longer in the reactor.

On day 5, the moment when methane ceases its growth, there is a stop in the removal of H_2, maintaining a state of pause in the process, which is subsequently resumed in terms of elimination of H_2, but not in terms of gas and methane generation.

It is appreciated that methane and hydrogen increase at the same rate, descending the hydrogen level on day 3, but increasing the level of methane until day 4. From day 4 the methane content stabilizes, does not increase, while the hydrogen content slowly drops.

The same applies to biogas generation, growing until the moment methane stops increasing. It shows that there is some form of inhibition of methanogenesis.

Monitoring H_2 allows one to detect inhibition, and the pH curve is key to understanding the type of inhibition that occurs:

Table 6
Quantity and distribution of the residues generated in the wholesale markets studied.

	Generated residue [ton/year]	Properly separated organic residue [ton/year]						
		V		M		F		Total
wsm - A	25.5		0%		0%	1.8	100%	1.8
wsm - B	2774.7	138.7	100%		0%		0%	138.7
wsm - C	859.8	129.0	100%		0%		0%	129.0
wsm - D	54.97	6.0	61%		0%	3.8	39%	9.9
wsm - E	23,551.45	5770.1	62%	1059.8	11%	2472.9	27%	9302.8
wsm - F	3039	372.3	64%		0%	212.7	36%	585.0
wsm - G	738.92	73.9	50%		0%	73.9	50%	147.8
wsm - H	345.6	57.0	100%		0%		0%	57.0
wsm - I	1597.2	119.8	60%		0%	79.9	40%	199.7
wsm - J	379.5	47.4	68%		0%	22.8	32%	70.2
wsm - K	451.7	49.7	65%		0%	27.1	35%	76.8
wsm - L	2190.9	536.8	62%	98.6	11%	230.0	27%	865.4
wsm - M	245.7	51.6	71%		0%	20.9	29%	72.5
wsm - N	29,553.5	7388.4	57%	1182.1	9%	4433.0	34%	13,003.5
wsm - O	2705	324.6	65%		0%	175.8	35%	500.4
wsm - P	1271	311.4	62%	57.2	11%	133.5	27%	502.0
wsm - Q	2702.1	553.9	89%	67.6	11%		0%	621.5
wsm - R	504.3	105.9	66%		0%	55.5	34%	161.4
wsm - S	348	76.6	100%		0%		0%	76.6
wsm - T	3524.75	863.6	62%	158.6	11%	370.1	27%	1392.3
wsm - U	1182	183.2	100%		0%		0%	183.2
wsm - V	3330	549.5	100%		0%		0%	549.5
wsm - W	2039	499.6	62%	91.8	0%	214.1	27%	805.4

The pH during the first day remains constant at stable neutral values around 7, specifically 7.06 at the start of the test.

After the second day, and coinciding with the start of the hydrogen acidification, reaching a minimum of pH on day 5. This is to be expected since the appearance of H_2 involves the development of acids such as acetic acid in the phases of acidogenesis and methanogenesis.

The level of H_2 continues to decrease with a pH drop until on day 5, when the destruction of H_2 stops, and the pH begins to increase.

The moment the pH begins to increase coincides with the point at which methane stops its generation and remaining at stable values. This, coupled with the slow degradation of H_2 implies inhibition. Not because of VFA accumulation, but because of AN accumulation. Proof of this is the uncontrolled increase in pH.

AN accumulation inhibition is proven with increased pH, H_2 content evolution, reduced CH_4 levels that stop generation at the time of pH increase, and excessive AN growth evidenced by the characterization of substrates at the end of BMP tests.

In addition, it should be remembered that the high sulfur content of the F substrate causes the appearance of sulfates that can affect the generation of methane by competition between sulfate-reducing and methanogenic bacteria, thus stopping the generation of methane.

3.14. Energy balance

Once the laboratory study was completed, the results obtained were used to see where its implementation would be optimal and cost-

Table 7
Biogas (and methane) at each wholesale market annually if properly separated residues are treated by anaerobic digestion.

	Biogas production [Nm^3/year]				Methane production [Nm^3/year]			
	Total	V	M	F	Total	V	M	F
wsm - A	13.73			13.73	1.87			1.87
wsm - B	1267.04	1267.04			408.64	408.64		
wsm - C	1177.86	1177.86			379.88	379.88		
wsm - D	84.82	55.22		29.60	21.86	17.81		4.04
wsm - E	79,872.37	52,697.33	8152.51	19,022.52	21,311.77	16,995.94	1713.74	2602.09
wsm - F	5036.34	3399.94		1636.40	1320.39	1096.55		223.84
wsm - G	1243.24	674.84		568.40	295.40	217.65		77.75
wsm - H	520.79	520.79			167.96	167.96		
wsm - I	1708.33	1094.02		614.31	436.87	352.84		84.03
wsm - J	608.39	433.23		175.15	163.68	139.72		23.96
wsm - K	662.26	453.78		208.47	174.87	146.35		28.51
wsm - L	7430.21	4902.28	758.39	1769.59	1982.55	1581.06	159.42	242.06
wsm - M	631.87	471.22		160.65	173.95	151.98		21.97
wsm - N	110,670.73	67,476.69	9093.48	34,100.55	28,338.74	21,762.58	1911.54	4664.61
wsm - O	4317.02	2964.51		1352.51	1141.12	956.11		185.01
wsm - P	4310.46	2843.94	439.96	1026.58	1150.13	917.21	92.48	140.42
wsm - Q	5578.58	5058.94	519.64		1740.84	1631.61	109.23	
wsm - R	1393.91	967.19		426.72	370.31	311.93		58.37
wsm - S	699.20	699.20			225.50	225.50		
wsm - T	11,953.83	7886.77	1220.11	2846.94	3189.55	2543.64	256.48	389.43
wsm - U	1673.22	1673.22			539.64	539.64		
wsm - V	5018.02	5018.02			1618.41	1618.41		
wsm - W	6915.06	4562.34	705.81	1646.90	1845.09	1471.44	148.36	225.28

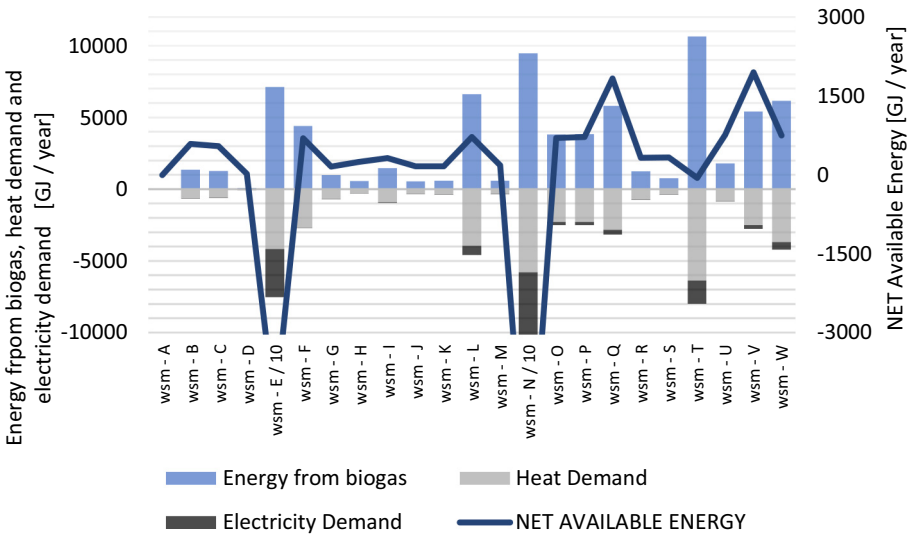

Fig. 8. Energy balance of the digesters of each wholesale markets, in case the waste is treated by anaerobic digestion.

effective in the Spanish network of wholesale. Which comprises the main markets with a total of 23 large areas.

The type of waste that is extracted and correctly separated in the wholesale markets network has been studied. Table 6 shows the total residue generated, and the amount that is properly separated from each of the organic fractions V, M and F. For example, the wholesale market wsm-L produces 2190 tons of waste annually, of which 865.4 tons of organic waste are properly separated, corresponding 62% of those tons to V residue, 11% to M and 27% to F. This is especially important as the amount of gas generated, and the development of the process depends on it, and consequently the suitability of the proposed solution.

From there, the energy balance of the digesters has been determined to know how much energy is available for use once the energy needs of the process have been satisfied, and the energy efficiency and suitability of the process, as studied in Sections 2.9.1 to 2.9.4. All these results are shown in Table 7.

The wholesale markets, E, N, T are the ones that handle the most volume of waste, but it will be seen in the following tables and in Fig. 8, which, precisely for that reason, are not profitable, together with wsm-A, because the latter is a market that correctly separates only the fish fraction.

Table 7 shows both biogas and methane that would occur for each of the fractions in so far as an anaerobic digestion is performed expressed on Nm^3 per year. In order to represent the results in the same graph, the data obtained for surfaces E and N have been divided by 10.

Table 8 lists the energy obtained from the biogas, but also the thermal and electric demand is collected and in the last column the available energy that would remain for uses.

As can be seen, there are four cases in which thermal and electric demands exceed the energy produced, therefore in such cases it is not cost-effective to carry out the co-digestion of the waste. These cases are the wholesale markets that receive more E, N and T waste and the wholesale market that only correctly separates the fish fraction.

Table 8
Energy balance of the digesters of each wholesale market.

	Energy from biogas [GJ/year]	Energy needs [GJ/year]		Net available energy [GJ/year]	Efficiency [%]	Power plant [kW]
		Heat demand	Electricity demand			
wsm - A	6,27	8,15	0,00	−3,33	−53,01%	−1,05
wsm - B	1364,88	634,50	15,98	586,45	42,97%	185,96
wsm - C	1268,81	589,88	13,84	547,17	43,12%	173,51
wsm - D	73,01	45,27	0,08	19,59	26,84%	6,21
wsm - E	71,181,33	41,661,01	33,490,16	−44,811,95	−62,95%	−14,209,78
wsm - F	4410,11	2675,55	284,31	693,78	15,73%	220,00
wsm - G	986,64	675,89	18,14	155,19	15,73%	49,21
wsm - H	561,00	260,83	2,71	248,72	44,34%	78,87
wsm - I	1459,16	913,13	33,13	318,63	21,84%	101,04
wsm - J	546,72	321,09	4,09	160,77	29,41%	50,98
wsm - K	584,07	351,18	4,89	161,13	27,59%	51,09
wsm - L	6621,72	3957,95	622,16	721,00	10,89%	228,63
wsm - M	581,01	331,48	4,36	182,32	31,38%	57,81
wsm - N	94,651,39	57,926,77	46,812,74	−67,123,24	−70,92%	−21,284,64
wsm - O	3811,36	2288,72	208,08	702,59	18,43%	222,79
wsm - P	3841,44	2296,15	209,47	721,14	18,77%	228,67
wsm - Q	5814,42	2842,35	320,83	1828,81	31,45%	579,91
wsm - R	1236,84	738,03	21,61	325,35	26,30%	103,17
wsm - S	753,20	350,17	4,88	331,47	44,01%	105,11
wsm - T	10,653,12	6367,62	1610,42	−59,03	−0,55%	−18,72
wsm - U	1802,42	837,93	27,90	760,82	42,21%	241,26
wsm - V	5405,50	2512,93	250,81	1947,49	36,03%	617,55
wsm - W	6162,62	3683,57	539,01	750,99	12,19%	238,14

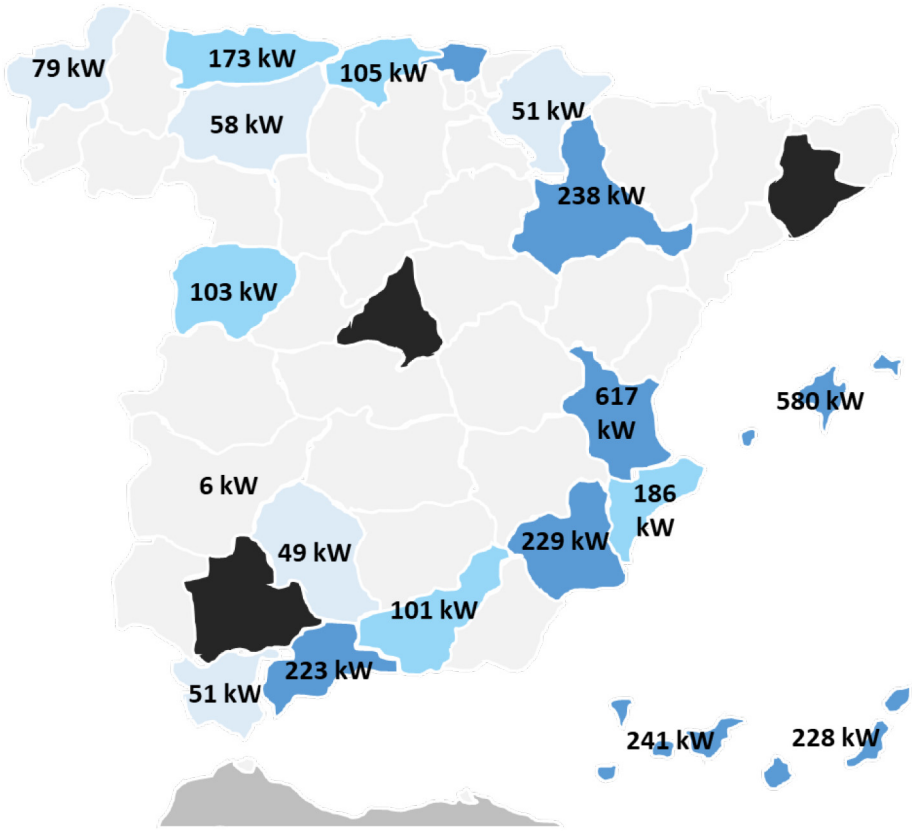

Fig. 9. Geographical distribution of available power in each wholesale market in case of treatment of waste by anaerobic digestion.

From the results obtained the generated power map has been drawn, as seen in Fig. 9. It is noted that this solution is especially useful in isolated areas such as the two archipelagoes, where this extra generation of energy could be used, for example, to support street lighting or public facilities. On the map, the provinces that are not profitable are shaded in black.

Table 9
Calculation of CO_2 equivalent emissions and estimation of their reduction for each wholesale market.

	Conventional energy source		Energy from the generated methane		Emissions reduction [tons CO_2eq/year]	
	Natural gas [Nm^3/year]	Emissions [tons CO_2eq/year]	Methane gas [Nm^3/year]	Emissions [tons CO_2eq/year]	Considering methane emissions	Considering zero emissions from methane (biomass)
wsm - A	–	–	–	–	–	–
wsm - B	17,969,97	38,64	19,245,16	17,25	−21,39	−38,64
wsm - C	16,766,28	36,05	17,956,05	16,09	−19,96	−36,05
wsm - D	600,37	1,29	642,97	0,58	−0,71	−1,29
wsm - E	–	–	–	–	–	–
wsm - F	21,258,98	45,71	22,767,57	20,40	−25,30	−45,71
wsm - G	4755,47	10,22	5092,93	4,56	−5,66	−10,22
wsm - H	7621,37	16,39	8162,20	7,31	−9,07	−16,39
wsm - I	9763,51	20,99	10,456,36	9,37	−11,62	−20,99
wsm - J	4926,29	10,59	5275,87	4,73	−5,86	−10,59
wsm - K	4937,41	10,61	5287,78	4,74	−5,88	−10,61
wsm - L	22,092,87	47,50	23,660,63	21,21	−26,30	−47,50
wsm - M	5586,80	12,01	5983,25	5,36	−6,65	−12,01
wsm - N	–	–	–	–	–	–
wsm - O	21,528,88	46,29	23,056,61	20,66	−25,62	−46,29
wsm - P	22,097,21	47,51	23,665,28	21,21	−26,30	−47,51
wsm - Q	56,038,43	120,49	60,015,04	53,79	−66,70	−120,49
wsm - R	9969,29	21,43	10,676,74	9,57	−11,87	−21,43
wsm - S	10,157,01	21,84	10,877,77	9,75	−12,09	−21,84
wsm - T	–	–	–	–	–	–
wsm - U	23,313,21	50,12	24,967,57	22,38	−27,75	−50,12
wsm - V	59,675,17	128,30	63,909,86	57,28	−71,03	−128,30
wsm - W	23,011,98	49,48	24,644,96	22,09	−27,39	−49,489
Total tot reduction of emissions					−407,13	−735,47
Total reduction of emissions (compared to the conventional energy source)					−55,36%	−100,00%

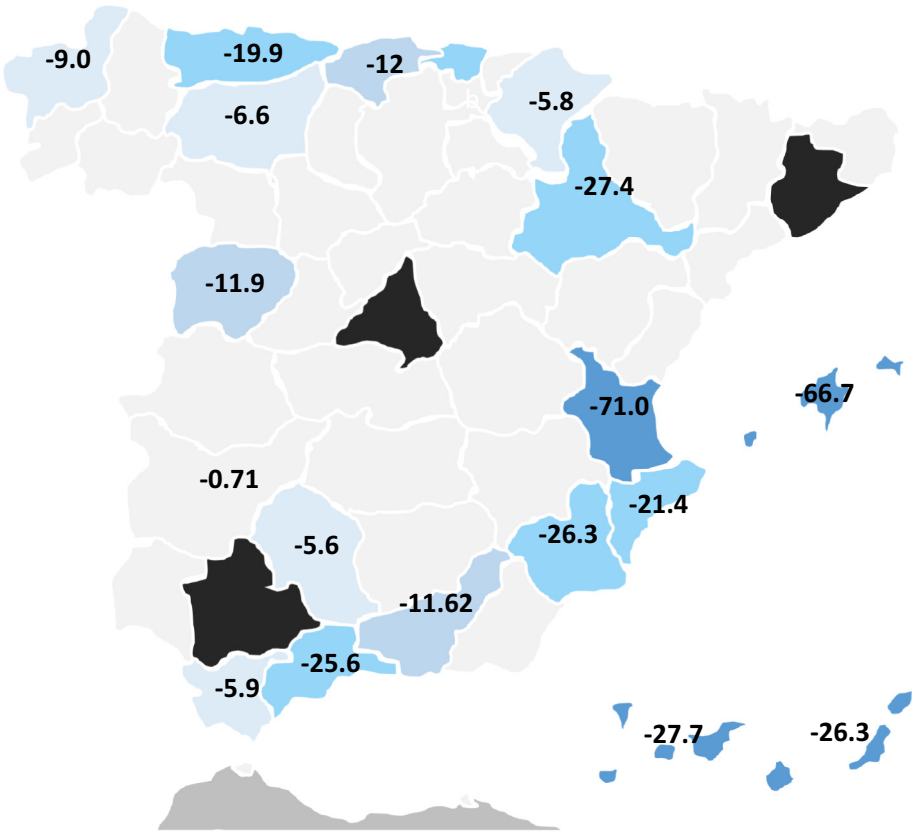

Fig. 10. Geographical distribution of CO_2 equivalent emissions reduction in the case of treating waste by anaerobic digestion and using biogas as an energy source replacing a conventional source.

3.15. Reduction of emissions to the atmosphere

Once the energy viability of the solution has been verified, we studied whether it is an environmentally sustainable or cost-effective solution. In order to do this, the reduction in emissions to the CO_2 equivalent atmosphere was studied. The result indicates the importance of the use of biogas as an energy source, instead of a conventional source such as natural gas.

In view of the results set out in Table 9, it is confirmed that there is a reduction of 55.4% of greenhouse gas emissions into the atmosphere by using methane as biogas from anaerobic digestion with respect to the use of natural gas, a reduction especially pronounced in the case of coastal populations as can be seen in Fig. 10.

It is important, in turn, to take into account that all these wastes are considered biomass according to the decision of the European Commission of 18 July 2007 establishing guidelines for the monitoring and reporting of GHG emissions, so that if they are issued in the combustion process would be zero, which represents a reduction in the impact of global warming in terms of the use of this fuel of 100%. It can be concluded that it represents an excellent economic and environmental solution for isolated and highly industrialized systems, as they could have energy independence and adequate reuse of their waste.

4. Conclusions

4.1. On the anaerobic digestion of residue V

As for its composition, residue V is formed, mostly, by carbohydrates. It has the best solubility and is also relatively resistant to sudden changes in pH.

During its digestion, as it has been determined with all the variables studied, comparing the gases generation and evolution with the composition and its changes during de BMP test, it is not affected by any type of inhibition and its digestion is the most stable from the point of view of biogas generation and methane content.

Compared to the three residues it is the second in biogas generation (913.282 Nml/100 g of residue V), and the first in methane generation (289.333 Nml/100 g of residue V) and methane content (32.252%).

Because of its high solubility, digestion occurs in two phases, a first one in which solubilized OM is digested, and a second, in a smaller proportion, in which the rest of the COD is digested. This is determined with the evolution of hydrogen during the degradation, that present two peaks of formation/transformation, compared with the evolution of pH that determines that two phases of acidification and methanogenesis occur. This provides a deep development of the process by digesting the solubilized organic matter and part of the encapsulated one.

In accordance with mathematical adjustments, it has a faster degradation, with a fast and stable hydrolysis.

However, the level of biodegradation can be improved through pretreatments, making POM more accessible and increasing solubilization.

4.2. On the anaerobic digestion of residue M

Residue M is mostly made of fats, with a moderate protein content. Because of it, during its digestion it is affected by inhibition due to the accumulation of LCFAs, but thanks to the released content in AN during proteins degradation, inhibition is reversed by the buffer effect of the AN in low concentration.

Compared to the three residues it is the first in biogas generation (1337.585 Nml/100 g of residue M), and the second in methane generation (232.317 Nml/100 g of residue M) and methane content.

Digestion occurs in a single phase, and despite having a high carbon and COD content, digestion is not complete. Partly due to inhibition, and partly due to a low level of biodegradation, so de POM is not digested, only the solubilized one, as can be stated when analyzing de H_2 evolution together with the pH analysis and alkalinity changes. Thus the level of biodegradation is low and could improve with pretreatment.

When treated together with sludge acidification occurs in the reactor as well as spongeing of the digestate.

In accordance with mathematical adjustments, it is the residue that has a minor disintegration count, indicating a slower degradation, with a less rapid and less complete hydrolysis.

It is a good candidate to be treated by co-digestion with another substrate in order to balance the C/N ratio and avoid inhibition by accumulation of LCFAs.

4.3. On the anaerobic digestion of residue F

Residue P is mostly made of proteins, with a high nitrogen content.

During its digestion it is affected by inhibition due to the accumulation of ammoniacal nitrogen released during the high protein content digestion and accumulating beyond the limit of 2 g/l, causing the process to fail in the first few days.

Compared to the three residues it is the last in biogas generation, in methane generation and methane content.

Digestion occurs in a single phase, and due to the low carbon content and high proportion of nitrogen, it releases an excess of AN that inhibits methanogenesis, as stated with the low hydrogen formation, and the rapid inhibition of the process.

In accordance with mathematical adjustments, it has a medium disintegration count, indicating a slow degradation, with a less rapid and less complete hydrolysis.

The level of biodegradation is low and could improve with pretreatment, however the pretreatment would increase the released AN and the inhibition could even be stronger. It is a good candidate to be treated by co-digestion with another substrate in order to balance the C/N ratio. Treated together with high carbon residue reduces the release and accumulation of AN.

4.4. Suitability of the solution

The solution of jointly digesting the organic waste of WSMs in the anaerobic digesters of the sewage treatment plants is a feasible solution, generating biogas in a stable process that lasts about 13 days for any of the waste. This feasibility not only comes from the point of view of the process, but also from an environmental point of view by providing a solution for two different types of waste, and also by creating a new energy source by turning the sewage treatment plant (a necessary and mandatory facility) into a power generator.

It is a very effective solution to generate energy, especially in markets with large quantities of sales, especially of fruit and vegetable residue, combined with average amounts of meat. As a general rule, the less fish residue is generated, the more beneficial it is, since the process of production of biogas and methane is inhibited, generating less gas than expected and necessary to meet the needs of the process.

This solution can be considered, at best, as a power station of approximately 600 kW whose use can reduce about 70 tons of CO_2 equivalent emitted to the atmosphere, in case that power was generated by conventional sources, which means a 50% reduction in emissions if biogas is used as an energy source rather than a conventional source such as natural gas.

Abbreviations

AD	anaerobic digestion
AN	ammoniacal nitrogen
BMP	biochemical methane potential
CHP	combined heat and power
COD	chemical oxygen demand
CV	coefficient of variation
EU	European Union
FL	food loss
FSC	food supply chain
FW	food waste
GC	gas chromatograph
Hum	humidity
IA	intermediate alkalinity
LCFA	long chain fatty acid
LPCH	lipids, proteins and carbohydrates content
OM	organic matter
ON	organic nitrogen
PA	partial alkalinity
TA	total alkalinity
TCD	thermal conductivity detector
TKN	total Kjeldahl nitrogen
TS	total solids
UASB	upflow anaerobic sludge blanket reactor
V, M F and S	vegetable, meat and fish residues; sludge
VFA	volatile fatty acid
VS	volatile solids
WSM	wholesale market
WWTP	wastewater treatment plant

CRediT authorship contribution statement

Carlos Morales-Polo: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **María del Mar Cledera-Castro:** Validation, Supervision, Writing - original draft. **Katia Hueso-Kortekaas:** Writing - review & editing, Visualization. **Marta Revuelta-Aramburu:** Resources, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.138567.

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