Effects of Co-substrates’ Mixing Ratios and Loading Rate Variations on Food and Agricultural Wastes’ Anaerobic co-digestion Performance

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Research

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Abstract

Tunisia is one of the developing countries which faces crucial challenges, the most prominent of which are the production of organic waste, the need for an appropriate waste treatment, and the demand for water and energy conservation. To this end, the present research was designed to develop a technical concept on closed cycle ‘biowaste to bioenergy’ treating food waste (FW) through combined biological processes. In this approach, semi-continuous anaerobic co-digestion (ACoD) of FW, wheat straw (WS), and cattle manure (CM) was tested to investigate the relationship between the effect of the feedstock mixtures and C:N ratio on biogas and digestate generation at different organic loading rates (OLRs) ranging from 2 to 3.6 kg VS/m3.d. Results showed that the mono-digested FW was optimal and reached 565.5 LN/kg VSin at an OLR of 2.4 kg VS/m3.d, and then a drop of biogas production was recorded. However, for co-digested substrates, the optimum mixture ratio was FW:CM 75:25, where 62%, 39.89%, 91.26%, 130.9% and 119.97% of the biogas yield improved for OLRs ranging from 2 to 3.6 kg VS/m3. d, respectively. Admittedly, the target of this work was to enhance the ACoD process, but it also examined the exploitation of different AD-effluents. Therefore, special attention was paid to the generated digestates to decide how it can be efficiently upcycled later. Thus, the closed cycle ‘biowaste to bioenergy’ treatment met two of the major Tunisian concerns: efficient organic waste management as well as sustainable bioenergy production.

1. Introduction

In recent decades, the increased amounts of solid organic waste has become a worldwide challenge as it creates alarming environmental concerns (Slorach et al. 2019). Particular attention has to be paid to this matter in Tunisia, where the stream of biowastes is incrementally rising, requiring specific consideration (Ben Mbarek et al. 2018). Indeed, up to 95% of solid waste is collected and sent to landfills without any pretreatments, which aggravates the situation in the country with regards to waste management, and environmental as well as socio-economic features (Mahjoub et al. 2020).

The lack of appropriate organic waste management in Tunisia not only poses problems in the landfill areas, but it also leads to a dead-end track with regards to the loss of the valuable organic residues which constitutes 68% of the total solid stream (Chaabane 2019). Since organic wastes contain high moisture content (MC), volatile solids (VS) and salinity (EC), any improper disposal practices will result in leachate and greenhouse gases (GHG) emissions and, in turn, to alarming environmental concerns (Lin et al. 2018). Thus, effective biowaste exploitation must receive adequate attention as it is the most appropriate technology to take advantage of the significant volume of biomass produced in the study area (Chaher et al. 2020a).

In this regard, anaerobic digestion (AD) is recognized as a well-established engineering concept ensuring the reduction of various streams of organics and reducing the GHG while producing sustainable energy (Bhatia et al. 2018). However, several parameters should be considered as basic criteria to ensure the process such as substrate structure, moisture content (MC), carbon to nitrogen (C:N) ratio, soluble/insoluble solids, and macro and micro nutrients concentrations (Pardo et al. 2017, Wang et al. 2018). Therefore, the selection of the appropriate biomass is challenging and differs from one country to other based on the availability of the organic residues as well as the nature of the biowaste collected (Barco et al. 2019, Awasthi et al. 2020). A particular divergence in terms of food waste (FW) characteristics is marked globally, as it depends on several cultural trends, norms, practices and behaviors that create the difference in terms of FW quantity and quality (Phasha et al. 2020). In this matter, Tunisia generates a relatively significant amount of FW from different sectors of activity, which promotes its selection as a main substrate for feeding anaerobic reactors (Wafi et al. 2019) (Chaher et al. 2020b).

When it comes to economic viability, treatment performance as well as process capacity to manage different kinds of biowaste, anaerobic co-digestion (ACoD) seems to be the most advantageous solution relieving several environmental and energic concerns. As FW mono-digestion is often prone to acidification, ammonia and long chain fatty acids inhibition, the selection of suitable co-substrates is considered a key factor enhancing the stability of the process (Kuruti et al. 2017, Sindhu et al. 2019). In general, the choice of co-substrates for FW anaerobic treatment is done referring to their high buffering capacity, capacity on C:N ratio regulation and the broad range of nutrients required by the methanogens, etc. (Zhao et al. 2018, Zhang et al. 2019). In this light, agricultural residues are considered as attractive co-substrates to be exploited for biological treatments, and aerobic and anaerobic processes. Furthermore, based on the availability of agricultural streams in Tunisia as well as the appropriateness of biowastes to enhance FW anaerobic digestion, cattle manure (CM) and wheat straw (WS) are the most pertinent residues to be exploited (Chaher et al. 2020b).

On one hand, most of the studies focusing on FW ACoD have been carried out using CM or WS separately (Zahan et al. 2018, Kainthola et al. 2019). On the other hand, the co-digestion of manures and straws has been widely studied (Zhao et al. 2018, Hassan et al. 2017); however, few studies have considered the mixtures of the three substrates simultaneously by varying the mixture ratios, which is the first target of the current research work. Moreover, most of the previous work has been carried out under batch conditions (Zhang et al. 2019, Menon et al. 2016, Di Maria et al. 2017); while under such conditions, it is hard to determine the reasons causing the inhibition or failure of the process (Zahan et al. 2017). To this end, continuous mode is widely recommended to be able to evaluate the strength of the AD process by monitoring several parameters including the organic loading rate (OLR) (Zahan et al. 2017). As the latter is a pre-eminent factor in precisely controlling the efficiency of the process, the evaluation of OLR variation effects on the AD's progress is the second target of the experimental work.

Numerous papers consider the ACoD of different kinds of biomass as a booster of methane yields, while few have focused on its effect on the digestate quality as well as its significant impact on the selection of the suitable post-treatment of the AD-residue (Zhao et al. 2018, Zhang et al. 2019, Zahan et al. 2018). So, to unlock the full sustainability potential of anaerobic treatment, both AD-effluents and biogas and digestate have to be efficiently exploited (Corrado et al. 2019). Therefore, at the end of the experimental works, special attention is paid to the effects of substrates mixtures on the digestate characteristics collected from each reactor.

To sum-up, the present research work aims to bridge the gap in knowledge related to the feasibility of biological treatment of different types of organic residues generated particularly in Tunisia as well as the anaerobic process stability. Thus, it critically evaluates the potential of ACoD of the dominant streams of biowastes generated in the studied area (i.e., FW, WS and CM) by focusing on the impact of different mixing ratios, C:N ratio adjustment as well as loading rates variations on key downstream AD-effluents: biogas and digestate.

2. Overall Concept

The research work was launched in the framework of a 'RenewValue project' aiming to optimize the exploitation of different types of biowastes: FW, WS and CM. The overall concept followed in the project is illustrated in Fig. 1. To this end, the experimental work was fundamentally divided into two phases. During the first, the input materials (FW, WS, CM) were subjected to AD, while the second phase was assigned to the recovery of the by-products.

In this approach, the main target was the selection of the most effective mixing ratios of food and agricultural wastes as feedstocks of semi-continuous anaerobic digesters. The selection of the exploited biowaste was based on their availability, their efficiency to adjust the initial C:N ratio and improve the process performance, as well as their influence on the by-product's characteristics: biogas and digestate (Giovanna Guarino et al. 2016). Over the experimental work, pH was held without any rectification to evaluate its combined effect with the OLR variations as this might be the best way of roughly estimating real conditions on a large scale.

3. Materials And Methods

3.1 Substrates and inoculum selection

The main target of the experimental work was to treat biologically different kinds of organic residues which are generated abundantly in the study area. Therefore, the selection of biowastes had to be rigorous, particularly with regard to the quality of AD-effluents such as biogas and digestate. To this end, two criteria were considered:

- Biowastes availability in the selected study area: Tunisia;
- "Positive list" illustrating the end-of-waste characteristics for biodegradable waste subjected to biological treatment (compost & digestate) (Saveyn et al. 2014);

Accordingly, three kinds of organic residues were chosen as feedstock materials; FW was used as the main substrate, while CM and WS were exploited as potential co-substrates for semi-continuous ACoD.

3.2 Samples preparation

Food waste, which is mainly composed of rice, noodles, salads and bread, was firstly gathered from the canteen of the University of Rostock, Germany. However, WS and CM were provided by a cattle farm in the vicinity of Rostock. To improve the mixture aspect, the size of WS and FW was reduced using a lab blender type GRINDOMIX (Retsch GmbH, Germany) and then stored in plastic airtight buckets. FW and CM were kept at -20˚C to stop any biological reaction.

The start-up of an anaerobic digester is significantly influenced by the quality of the inoculum used as it plays a crucial role in supplying the reactors with acclimatized microorganisms as well as the required trace elements (TEs) (Parra-Orobio et al. 2018). Therefore, to set a desired anaerobic start-up condition, the inoculum was collected from a biogas plant treating FW under mesophilic conditions. At the beginning of the process, the inoculum was held anaerobically at 37˚C for several days to minimize background biogas production.

3.3 Experimental setup

The experiments were carried out in mesophilic lab digesters with a nominal volume of 20 L. The digesters were heated by warm air and kept at a constant temperature of 38 ± 1 °C. An internal stirrer (anchor-type) was installed in each digester. Each was stirred for 5 minutes every 30 minutes at an approximate speed of 80 ppm. Reactors were equipped with inlet and outlet valves for feeding and digestate withdrawal. Except on weekends, the reactors received different mixtures of organic wastes twice per day. The hydraulic retention time (HRT) was maintained for 30 days (Kuruti et al. 2017) and the OLR ranged between 2.0 and 3.6 kg VS/m^3.d with a stepwise of 0.4. Each tested set of parameters was performed in duplicate.

The biogas volume was monitored online using drum-type gas meters (type TG05, RITTER Mess Technik GmbH, Germany), and gas volume was logged continuously. To analyze the biogas composition, the headspace of each digester was analyzed every other day using a gas analyzer type EHEIM VISIT 30 (Eheim Mess Technik GmbH, Germany). CH_4, CO_2, H_2S and O_2 components were then determined. For total solid (TS), volatile solid (VS), volatile fatty acids (VFAs), alkaline buffer capacity (TAC), VFA: TAC and pH measurements, samples from the digester content were taken once per week via the feeding port. Regarding the liquid AD-effluent, digestates were fully analyzed to examine the physical-chemical properties of each and, therefore, decide how it could be efficiently recovered.

3.4 Analytical methods

The substrates FW, WS, CM and D (i = 1,2,3,4) were characterized by measuring different physical-chemical parameters. As such, MC, TS and VS contents were determined gravimetrically following CENT/TS 14744-1 (2009). In addition, C:N ratio, and major and minor mineral content analysis of the whole substrates were determined in an external laboratory following the methods described in EN ISO 16967 (2015) and 15297 (2011), respectively (Table 1).

Table 1
Physical and chemical parameter measurement of biowastes opted for biological treatments.

Parameters	Units	Method of Analysis	Reference
pH	-	(1:10 *w/v* sample: water extract)	ISO 10390 (1994)
Moisture content (MC)	% of FM[1]	Using electronic oven by drying at 105 °C for 24 h	NF ISO 11465 (1994)
Total solids (TS)	% of FM[1]		
Total Carbon (TC)	% of FM[1]	TOC (%) = ((100 − Ash%) ÷ 1/8)	**[31]**
Total Nitrogen (TN)	% of FM[1]	Titrimetric methods	NF ISO 11265 (1995)
Phosphorus (P)	% of TS[2]	Atomic absorption spectrometric methods	ISO 11885 (2007)
Potassium (K)	% of TS[2]		
Magnesium (Mg)	% of TS[2]	Spectrometer, Thermo-Elemental ICP MS-X Series	ISO 11885 (2007)
Lead (Pb)	mg/kg TS		
Copper (Cu)	mg/kg TS		
Zinc (Zn)	mg/kg TS		
Nickel (Ni)	mg/kg TS		
Cadmium (Cd)	mg/kg TS		
Arsenic (As)	mg/kg TS		

[1] FM: Fresh Matter; [2] TS: Total Solids

Regarding the operational parameters assessing the AD stability, VFAs, alkalinity and pH were determined using an automated titration unit type Titra-Lab 1000 (Hach instruments), centrifuging a digestate sample at 4000 rpm for 30 min to obtain a supernatant. Then, 5 mL of the latter was used for a titration with 0.1 mol/L sulfuric acid until they reached pH 5 and pH 4.4, respectively, in accordance with USEPA (1983). The volume of biogas was normalized to standard conditions comprising dry gas, standard temperature and pressure (0ºC and 1 bar) according to the method described by Somashekar et al., (2014), the results of which are presented as norm-liters (L_N).

4. Results And Discussion

4.1 Physical and chemical characteristics of the feedstock material

The physical and chemical characteristics of the residues are summarized in Table 2. The moisture content was found to be approximately 74.0%, 88.5% and 8.9% of the fresh matter, leaving behind dry matter contents of 26.0%, 11.5% and 91.1%, with volatile solid contents of 94.2%, 77.4% and 95.3% for FW, CM and WS, respectively. Because microorganisms as well as AD systems have a certain demand for carbon and nitrogen in any growth environment, C:N ratios were evaluated for each substrate. The C:N ratio of each substrate was initially determined to be 17.10 for FW, 25.64 for CM and 78.08 for WS; this latter was marginally higher than the acceptable upper limit for AD (Zheng et al. 2015). As minor minerals as micronutrients or essential supplements for the methanogenic bacteria, mieral concentrations were also examined (Akturk and Demirer 2020). Therefore, certain minor elements such as Copper (Cu), Cadmium (Ca), Lead (Pb) and Zinc (Zn), as well as some major constituents such as Phosphorus (P), Potassium (K) and Magnesium (Mg), were monitored. The characteristics of the various substrates analyzed compare closely with the reported literature (Ariunbaatar et al. 2016).

Table 2
Physio-chemical characteristics of the raw materials.

Parameters	Units	FW	CM	WS
Total solids	% of FM	26.00	11.50	91.10
Volatile solids	% of FM	24.50	8.90	86.80
Crude Ash (550 °C)	% of FM	5.80	22.60	4.70
Moisture content	% of FM	74.00	88.50	8.90
Carbon (C)	% of FM	20.52	42.61	47.63
Nitrogen (N)	% of FM	1.20	1.70	0.61
C:N ratio		17.10	25.64	78.08
Sulfur (S)	% of TS	0.33	0.50	0.16
Phosphors (P)	% of TS	0.48	0.60	0.06
Potassium (K)	% of TS	0.91	2.95	1.74
Magnesium (Mg)	% of TS	0.09	2.82	0.25
Calcium (Ca)	% of TS	0.06	0.61	0.07
Lead (Pb)	mg/kg TS	0.91	0.85	0.21
Copper (Cu)	mg/kg TS	6.82	18.20	1.78
Zinc (Zn)	mg/kg TS	16.33	131.00	16.6
Nickel (Ni)	mg/kg TS	0.95	6.91	5.78
Cadmium (Cd)	mg/kg TS	0.07	0.19	0.08
Chrome (Cr)	mg/kg TS	2.31	-	10.50
Arsenic (As)	mg/kg TS	0.57	0.28	0.07
Mercury (Hg)	mg/kg TS	< 0.01	< 0.01	< 0.01

[1] FM: Fresh Matter; [2] TS: Total Solids.

4.2 Effect of mixing substrates and C:N ratio regulation on process performance

In the current work, FW, WS and CM were initially analyzed to determine carbon and nitrogen concentrations of each substrate (Table 2). In fact, the identified C:N ratio of different organic material proved that the abundance of nitrogen contents, particularly for FW and CM or the carbonaceous aspect of WS, makes those residues unsuitable for anaerobic mono-digestion (Zahan and Othman 2019).

Therefore, different substrates were combined at different ratios to balance the C:N ratio greater than or equal to 20 in compliance with the results of Zahan et al. (2017). Digesters fed only with FW (i.e. FW_{100}) were characterized by a C:N ratio of 17.10. However, C:N ratios of co-digested substrates increased from 17.10 to 20.03 for $FW_{75}CM_{25}$, 33.28 for $FW_{75}WS_{25}$ and 31.64 for $FW_{60}CM_{20}WS_{20}$. Despite the various types of the added substrates, it is visible that the diversity of the raw materials in terms of carbon and nitrogen contents guaranteed balanced C:N ratios for all the trials within the requested range of 20–40 (Dioha et al. 2013). Therefore, the co-digestion of FW with different agricultural residues ensured one of the fundamental criteria to overcoming the inhibition of the process caused by unequilibrated C:N ratios (Zheng et al. 2015, Chatterjee and Mazumder 2019).

When it comes to the effects of the substrate mixtures on the process performance, the volume of biogas and methane yields produced from different feedstocks, including the mono-digested FW, were monitored weekly. Starting with a stable OLR of 2 kg VS/m^3.d, the specific biogas yield (SBY) of FW_{100} reached 414.2 L_N/kg VS_{in}, while $FW_{75}CM_{25}$, $FW_{75}WS_{25}$ and $FW_{60}CM_{20}WS_{20}$ accomplished 62.07%, 27.16% and 25.45% of improvement, respectively. This progress signified that the initial C:N ratio exerted an influence on biogas production. However, in relation to the productivity of different digesters fed with different feedstocks, Fig. 3 shows that the poorest improvement of biogas production was marked in the reactors characterized by a C:N ratio higher than 30, and more particularly contained WS, which is in accordance with the findings of several previous works treating almost food and agricultural residues (Zahan et al. 2018, Hassan et al. 2017), Kainthola et al. 2020). Indeed, Hassan et al. 2017 reported that the optimal range of C:N ratio for ACoD of WS should not exceed 30 to ensure a good progress of the biodegradation (Hassan et al. 2017). Since the carbon is assumed as a source of energy, while nitrogen is required for the growth of microorganisms, a balanced utilization of nitrogenous and carboneous components is needed by the microbial community (Rabii et al. 2019). Therefore, because of the complicated lignocellulosic structure of WS, which makes it hardly degradable by the anaerobes, lower SBY improvement marked both $FW_{75}WS_{25}$ and $FW_{60}CM_{20}WS_{20}$ at an early stage. Similar findings were identified by Zahan et al. (2018a), highlighting the effects of the initial balancing of C:N ratio on the ACoD process of manure, straw and food waste.

Under the same experimental conditions, $FW_{75}CM_{25,}$ which was characterized by a C:N ratio of 20.03, generated the highest SBY compared to the other feedstocks. Although the latter was slightly high compared to the C:N ratio of mono-digested FW (17.10), an enhancement of the process performance in terms of biogas production was significant (around 62% of SBY improvement). Thus, the results reveal that even if a relatively acceptable C:N ratio is valid, which is the case of FW_{100}, a performant process is ensured by mixing different types of biowastes, which supplies the deficit of further components needed by the microbial community (Akturk and Demirer 2020). Several researchers have reported that micronutrients and TEs play an important role in the startup and operation of anaerobic reactors (Akturk and Demirer 2020, Fermoso et al. 2019). Therefore, the supply of certain nutrients, ensured by CM addition, promotes the process performance as the exploited manure is characterized by a relatively high concentration of Calcium (Ca) of around 0.61 g/kg TS, which enhances the buffering capacity of AD

(Arelli et al. 2018). Furthermore, some macronutrients such as Potassium (K), Magnesium (Mg) and Phosphorus (P) as well as some TEs: Copper (Cu), Nickel (Ni), Cadmium (Cd) and Zinc (Zn), were identified in wide range for different substrates, particularly CM, which boost microorganisms' development (Jansson et al. 2019). The same findings were reported by Nordell et al. (2016) revealing that manure is one of the most eminent substrates for biogas production as it stabilizes FW anaerobic processes by contributing the nutrients and TEs needed by the microbial community, particularly methanogens.

4.3 Effect of OLR on semi-continuous ACoD of FW

To evaluate the OLRs variation effects on FW ACoD processes, the average weekly SBY was identified and reported as L_N/kg VS_{in}. The variation of the OLR was achieved every two weeks with a pace of a 0.4, ranging from 2.0 to 3.6 kg VS/m^3.d. These increments were divided into five categories to assess the impact of OLR fluctuations on the process evolution. Starting with an OLR of 2 kg VS/m^3.d, mono-digested FW (FW_{100}) produced 414.2 L_N/kg VS_{in} to reach 561.05 L_N/kg VS_{in} (35.45% of improvement), once the OLR was increased to 2.4 kg VS/m^3.d. However, with a continuous rise of OLRs, a decline of SBY was logged to fall to 384.95 and then to 310.55 L_N/kg VS_{in} for 2.8 and 3.2 kg VS/m^3.d, respectively. Those results were in conformity with several reports' findings, that for FW mono-digestion, 2 to 2.5 kg VS/m^3.d was deemed the optimal OLR range to improve the overall system performance in terms of stability, productivity and efficiency (Akturk and Demirer 2020, Owamah and Izinyon 2015). However, with the continuous feeding of the digesters, the last category was marked by a failure causing a decline in terms of biogas production compared to the previous OLRs (Fig. 4). This was explained by the instable conditions for anaerobes caused by the high FW soluble organics and a relatively low C:N ratio (Rabii et al. 2019, Zahan et al. 2018). Therefore, focusing on the OLR limitations, particularly for large-scales, mono-digestion of FW is more resistant with moderately low and constant OLRs (Song et al. 2020, Logan et al. 2019). Accordingly, 2.4 kg VS/m^3.d was the optimal OLR ensuring a performant development of the process for FW_{100}.

With regards to the ACoD of FWs' effects on the process progress, a better enhancement in biogas production was registered throughout the treatment period for the digesters including food and agricultural wastes. For the first category, OLR of 2 kg VS/m^3.d, the increase of SBY was slightly considerable for the reactor comprising WS, as the improvement ranged between 25.45% and 62.07% compared to FW_{100}; in the order of $FW_{75}WS_{25}$ (25.45%) < $FW_{60}CM_{20}WS_{20}$ (27.16%) < $FW_{75}CM_{25}$ (62.07%). The second category was marked by more significant biogas yields compared to the mono-digested FW with associated SBY of 768.7, 676.85 and 608.5 L_N/kg VS_{in}, for $FW_{75}CM_{25}$, $FW_{60}CM_{20}WS_{20}$ and $FW_{75}WS_{25}$, respectively. It is visible that the addition of CM to $FW_{60}CM_{20}WS_{20}$ and $FW_{75}CM_{25}$ created a suitable environment for the microorganisms to act as the main consumer of the available feedstock material (Rajput and Sheikh 2019). Hence, for an increased OLR to 2.8 kg VS/m^3.d, the biogas yielded from each mixture reached an enhancement of 51.67%, 66.75% and 91.26% for $FW_{75}WS_{25}$, $FW_{60}CM_{20}WS_{20}$ and $FW_{75}CM_{25}$, respectively compared to FW_{100}. Furthermore, the fourth category, 3.2 kg

VS/m^3.d, was considered as optimal for some mixtures compared to the third one: $FW_{75}WS_{25}$, $FW_{60}CM_{20}WS_{20}$ and $FW_{75}CM_{25}$ represented peaks in terms of SBY with associated biogas productions of 564.1, 638 and 709.8 L_N/kg VS_{in} and efficiencies of 83.5%, 103.38%, and 130.9%, respectively. Thus, even with an increased OLR higher or equal to 3 kg VS/m^3.d, $FW_{60}CM_{20}WS_{20}$ and $FW_{75}CM_{25}$ generated very similar biogas volumes, whereas digesters comprising only WS as co-substrate did not. Indeed, although $FW_{60}CM_{20}WS_{20}$ included a certain fraction of WS, which was comparable to the amount added to $FW_{75}WS_{25}$, improved process progress marked the digesters included a combination of FW, WS and CM, which evidenced the efficiency of CM to boost the microbial community activity. Moreover, similar trends were identified by Zahan et al. 2018 who demonstrated that the combination of FW with chicken litter guaranteed higher process stability and biogas production than FW and lignocellulosic residues mixtures. However, for the last variation of OLR, some indicators of reactor inhibition appeared, and almost all the reactors dropped biogas production at an OLR of 3.6 kg VS/m^3.d. However, it should be mentioned that the decline in terms of biogas produced from co-digested substrates was not considerable, as it ranged between 10.9-22.42% compared to the previous yield (OLR of 3.2 kg VS/m^3.d). However, it was significant with regards to mono-digested FW, as it varied from 40.41–75.22% in the same conditions (Sembera et al. 2019). In fact, the drop of SBY was expected as it was a direct effect of the overloaded digesters followed by an accumulation of volatile acids, and then a subsequent process failure (Sect. 4.5).

Nonetheless, at different OLRs, mixed feedstocks, which were marked by enhanced SBY, did not inevitably produce better specific methane yields (SMY). At the beginning of the process, SMY improvements were 22.09%, 30.29% and 51.65% for $FW_{75}WS_{25}$, $FW_{60}CM_{20}WS_{20}$ and $FW_{75}CM_{25}$, respectively. Therefore, for the second category, a slight decline was noted for $FW_{60}CM_{20}WS_{20}$ and $FW_{75}WS_{25}$, which generated 17.13% and 18.01% lower methane production comparing to FW_{100}. This was explained by the higher accessibility of the FW to be consumed by anaerobes and converted into methane compared to lignocellulosic materials which necessitate more time. A significant enhancement in terms of SMY was subsequently found for $FW_{75}CM_{25}$, highlighting again the effects of C:N ratio, and particularly the contribution in terms of nitrogen on the fulfilment of the biowaste degradation influencing the methane rate. For the tertiary class, the SMYs attained were 103.03%, 33.96% and 24.83% for $FW_{75}CM_{25}$, $FW_{60}CM_{20}WS_{20}$ and $FW_{75}WS_{25}$, respectively, becoming 161.34%, 90.83% and 73.92% at an OLR of 3.2 kg VS/m^3.d and falling to 93.83%, 30.23% and 50.20% at the end of the process. As the straw is known as a hardly digestible substrate, $FW_{60}CM_{20}WS_{20}$ and $FW_{75}WS_{25}$ showed lower SMY improvement compared to $FW_{75}CM_{25}$, which was explained by the rise of TS contents linked to the decline of VS contents, which then caused limited methane yields (Pohl et al. 2013). In the same context, during the OLRs fluctuation, $FW_{75}CM_{25}$ showed optimal rendering, which was a result of the buffering capacity of manure as well as the presence of some TEs required to stimulate the activity of enzyme and co-enzymes for better biogas production (Akturk and Demirer 2020, Bong et al. 2018). Ultimately, SBY and SMY recorded during the OLR variations indicated clearly a synergistic relationship between FW and agricultural residues, specifically cattle manure, which is in agreement with several previous works (Chuenchart et al. 2020, Guilford et al. 2019).

4.4 OLRs and substrates mixtures ratio effects on process stability

As is well known, OLR is one of the most influential parameters of anaerobic reactors as well as one of the most important design factors in full-scale AD plants (Owamah and Izinyon 2015). Hence, the current experimental work mainly consisted of identifying the optimum OLR value able to guarantee a performant ACoD of food and agricultural wastes at different mixing ratios. Therefore, several parameters which are useful to check the process progress such as VFA: TAC ratio and pH were, in addition, continuously monitored. The VFA: TAC is the ratio between VFAs, measured in mg/L, and TAC measured in mg/L of $CaCO_3$. Indeed, it is usually used as a parameter for the practical monitoring of anaerobic processes. As shown in Table 3, at an OLR of 2 kg VS/m^3.d, VFA: TAC ratios were found to be in a steady range for all the digesters. They ranged between 0.19 and 0.28, which was considered a suitable ratio for stable biogas yields (Di Maria et al. 2017). Indeed, during the start-up phase, VFA formation was directly affected by the different chemical composition of the feeding mixtures (Sembera et al. 2019). Unexpectedly, FW_{100} was characterized, initially, by a significant VFA concentration as well as a relatively high alkalinity, which might be linked to the nature and the primary characteristics of the residue (Bong et al. 2018).

Once the OLR rose from 2 to 2.8 kg VS/m^3.d, VFA concentration varied slightly from 4913 to 3671 mg/L for $FW_{75}CM_{25}$ and 4564 to 4202 mg/L for $FW_{60}CM_{20}WS_{20}$. However, from an OLR of 2.4 kg VS/m^3.d, a considerable increase to 5182 mg/L was identified for $FW_{75}WS_{25}$; then, a significant drop to 3758 mg/L was followed by a notable VFA accumulation that persisted until the end of the process. As the productivity of methanogens is straightly related to VFA production, which is essentially a result of initial polymers availability and decomposition, biogas and methane yields were greatly influenced by both substrates' mixtures, from a qualitative angle, and the organic loading of the reactors from a quantitative angle (Figs. 4 and 5). Hence, an overloading of the system, especially from 3.2 kg VS/m^3.d, entailed an accumulation of VFAs which marked almost all the digesters and caused a process inhibition.

As an additional indicator of stability, alkalinity was also monitored to prevent acid build-up in the digester. By increasing the OLR, a continuous decrease of TAC marked all the mixtures, particularly FW_{100} as well as $FW_{75}WS_{25}$. It was explained by the nature of the feedstock mixtures used, which were characterized by a low rate of nitrogen source. Accordingly, John Wiley (2003) reported that the alkalinity is the effect of the proteinaceous substance's degradation, then the high alkalinity recorded for the digesters comprising manure (2003). Moreover, mixtures comprising CM and WS separately, added as co-substrates to FW anaerobic reactors, were characterized by a significant divergence in terms of alkalinity tendencies ranging from 21470 to 15532 mg $CaCO_3$/L for $FW_{75}CM_{25}$ and 23419 to 9353 mg $CaCO_3$/L for $FW_{75}WS_{25}$, which further ascertained the potential buffering capacity of CM.

Table 3
Anaerobic process performance during OLR variation.

Feedstock	OLR (kg VS/m^3.d)	pH	VFA (mg/L)	TAC (mg $CaCO_3$/L)	VFA: TAC
FW_{100}	2.0	7.20	6010.00	21815.00	0.28
	2.4	7.03	4133.00	16566.00	0.25
	2.8	6.83	3732.00	12051.00	0.31
	3.2	6.13	3502.00	9273.00	0.38
	3.6	5.67	3797.00	7959.00	0.48
FW_{75} CM_{25}	2.0	8.12	4913.00	21470.00	0.23
	2.4	7.60	3533.00	18802.00	0.19
	2.8	8.10	3671.00	19721.00	0.19
	3.2	7.81	4845.00	17974.00	0.27
	3.6	7.46	5637.89	15532.79	0.37
FW_{75} WS_{25}	2.0	8.20	4382.00	23419.00	0.19
	2.4	7.81	5182.00	18138.00	0.29
	2.8	7.21	3758.00	13431.00	0.28
	3.2	6.80	4265.00	13084.00	0.33
	3.6	7.04	5705.00	9353.00	0.61
FW_{60} CM_{20} WS_{20}	2.0	8.16	4564.00	22615.00	0.20
	2.4	7.40	4823.00	17925.00	0.27
	2.8	8.10	4202.00	16467.00	0.26
	3.2	8.30	4702.88	12376.00	0.38
	3.6	7.62	5488.49	11201.00	0.49

Despite the divergence in terms of VFA and alkalinity fluctuations, the VFA: TAC ratio seemed to be stable during the start-up phase, affirming the steadiness of the process for OLR of 2 kg VS/m^3.d and 2.4 kg VS/m^3.d., and the efficient conversion of the organic matter into biogas for those experimental conditions. Once the OLR exceeded 2.8 kg VS/m^3.d, a rise of VFA: TAC above 0.3 was identified, indicating that an over loading of the digesters had occurred, which caused a process failure (Ghinea and Leahu 2020).

When it comes to ACoD effects, it was clear that substrates' mixtures enhanced the progress of the process. As CM and WS were the main co-substrates exploited to improve FW AD, the high bicarbonate and ammonia contents most likely contributed to the buffering of the system and prevention of reactor failure observed for FW_{100}. However, the results showed that for OLRs ranging from 2.4 kg VS/m^3.d. to 3.6 kg VS/m^3.d, digesters fed with WS presented a fluctuation in terms of VFA, which reached higher concentrations at an OLR of 2.4 kg VS/m^3.d'; a drop in terms of VFA concentration was then recorded until the end of the process. Indeed, VFAs' tendencies were predicted from the SBY as well as the SMY produced from $FW_{75}WS_{25}$ during the start-up period. The produced VFAs during the start-up period, for an OLR ranging from 2 to 2.4 kg VS/m^3.d, were efficiently consumed by methanogens to produce the highest amount of biogas and methane gathered from $FW_{75}WS_{25}$. However, a significant increase of VFA: TAC was identified to peak at 0.61 for an OLR of 3.6 VS/m^3.d for the same feedstock mixture, which entailed the inhibition of the process and the decline in biogas and methane yields. While the VFA: TAC remained lower for digesters containing CM, it reached 0.37 at the end of the process, which was not considered a limiting-value of inhibition; this is in reference to Brambilla et al. (2012) who demonstrated that with well-balanced combinations of biowastes, the AD process is sustained in terms of biogas production and methane content, even for VFA: TAC ratios up to 0.5 (Brambilla et al. 2012). Accordingly, the methane peaks were most likely owing to the degradation of easily biodegradable compounds such as FW and CM, and corresponded to VFA:TAC ratios ranging between 0.2 and 0.4, which is the recommended range ensuring a stabilized process (Li et al. 2014). It was explained by the considerable buffering capacity of CM, which also contributed, to a certain extent, to the maintenance of $FW_{60}CM_{20}WS_{20}$ progress compared to $FW_{75}WS_{25}$. As reactor acidification through reactor overload is one of the most common reasons for a fast process deterioration, this aspect notably arose in $FW_{75}WS_{25}$; thus, illustrating a considerable concentration of VFAs of about 5705 mg/L at an OLR of 3.6, which was a direct result of an imbalance between acid producers (mostly bacteria) and consumers to digest the accumulated fatty acids and ensure, therefore, their conversion into methane. Hence, VFA and TAC behaviors were also coherent with the trends in biogas production, where well-buffered digesters generated improved volumes of biogas and methane (Fig. 4). Furthermore, literature studies reported that, as a general rule, the VFA: TAC ratio should be in the range of 0.3–0.4 to guarantee biogas production, and that VFA: TAC ratios above 0.4 could result in unsustainable operating conditions for the microbial population, which is an accordance with the current findings.

The tendencies of VFAs and TAC in most situations had a significant impact on pH behavior. Indeed, alkalinity is required to maintain the pH within the desired range (6.8–7.8) for microbial growth to avoid VFA accumulation. Table 3 summarized the fluctuation of pH during the OLR variations for each substrates' combination. During the start-up period, pH was 7.20, 8.12, 8.20 and 8.16 for FW_{100}, $FW_{75}CM_{25}$, $FW_{75}WS_{25}$ and $FW_{60}CM_{20}WS_{20}$, respectively. However, for OLRs ranging from 2 to 2.8 kg VS/m^3.d, pH tended toward neutral values for FW_{100} and $FW_{75}WS_{25}$. The recorded pH values were suitable to promote the microbial growth for efficient activity at low OLRs for co-digested FW and WS. However, from an OLR of 3.2, an acidification of the reactors occurred causing a drop of pH to around 6. Indeed, optimized hydrolytic enzymes' activities maintained at a pH around 6 and, hence, contributed to VFA accumulation as well as a reduction of methanogens (Rabii et al. 2019). Therefore, the produced alkalinity was not sufficient for sustaining the required pH range and buffering the generated VFAs (Anukam et al. 2019). While for reactors comprising manure, higher pH ranges were recorded to fluctuate around the neutral range during the whole anaerobic process for both $FW_{75}WS_{25}$ and $FW_{60}CM_{20}WS_{20}$, which is ideal for stable methanogenesis. Thus, for highly buffered systems, pH variations, which would predict process inhibition, as the later did and not change significantly, even when the process was stressed. Moreover, stable methane production was sustained, especially for $FW_{75}CM_{25}$. To this end, pH adjustment was not always the most appropriate solution to guarantee a performant process, which validated the selected option (no pH regulation during the process), as well as to evaluate the process progress of different substrates' mixtures at different OLRs. Hence, the combination of different inhibition indicators was crucial for reliable process monitoring. Several researchers have revealed that the follow-up of different factors was more suitable for applications as early warning indicators, particularly for AD of FW (Li et al. 2014, Franke-Whittle et al. 2014, Jiang et al. 2013). As the ideal indicators were universally unpredictable, it is essential to supply the necessary information with regards to the initial feedstock mixtures and operational conditions, as well as the concerned study area, in order to guarantee a high-performing and sustainable process.

4.5 Process performance vs digestate quality

Apart from effective decomposition of the biowaste, it is also crucial to pay attention to AD-effluents: biogas and digestate. So, to unlock the full sustainability potential of the process, the by-products need to be efficiently exploited (Corrado et al. 2019). With regards to the digestate use, certain criteria are required to decide whether digestates can be spread out directly on soil after digestion or if further upcycling processes are needed before utilization (Teglia et al. 2011). Therefore, full analysis of each digestate collected from different digesters was achieved. Table 4 summarized the physio-chemical characteristics of D1, D2, D3 and D4, which were gathered from $FW_{75}WS_{25}$, $FW_{60}WS_{20}CM_{20}$, FW_{100} and $FW_{75}CM_{25}$, respectively. The definition of agronomic value is very challenging; there is not a specific quality-indicator of each digestate produced from different feedstock mixtures. Therefore, the evaluation of several parameters was needed. Initially, pH was identified for all the AD residues and it was clear that pH ranged around the neutral value, which was beneficial for digestate exploitation (Saveyn et al. 2014). Indeed, low pH values boost the heavy metal (HMs) solubility and then cause phytotoxicity issues, thus preventing a direct land application (Teglia et al. 2011). As one of the steering factors, MC was first identified where a high-water content of around 97% marked all the digesters. In practice, important moisture can cause certain concerns such as odors, cost-intensive transport and hard storage facilities (Hosseini Koupaie et al. 2019). Therefore, a pertinent selection of further criteria was selected as a guide to better understand how to deal with that liquid AD-effluent. Accordingly, pH, C:N ratio, and macro and micro-nutrients were additionally measured. As carbon and nitrogen are the most important constituents of organic matter, the carbon to nitrogen rate was evaluated (Giovanna Guarino et al. 2016). Indeed, FW_{100} and $FW_{75}CM_{25}$ were characterized by lower C:N ratios at around 8.53 and 8.45, respectively, which was related to the effect of the initial feedstock's characteristics. Both FW and CM were initially relatively rich in nitrogen; then, the total nitrogen was converted by the microbial community into soluble forms and conserved in the digestates D3 and D4. Nevertheless, higher values were considerable for reactors included WS to be about 9.51 for $FW_{60}WS_{20}CM_{20}$ and 12.97 for $FW_{75}WS_{25}$. As the later resulted from a biological treatment of hardly degradable biomass, it featured a residual organic element such as lignocellulosic compounds, which enhanced the tenor of carbon in both of D1 and D2. Even though, $FW_{60}WS_{20}CM_{20}$ and $FW_{75}WS_{25}$ were characterized by a relatively close initial C:N ratio, the generated digestates were qualified by significantly different C:N values as a consequence of the different chemical compositions of the mixtures as well as the impact of manure addition in terms of nitrogen conversion during the AD (Teglia et al. 2011). However, it should be mentioned that the rate of nitrogen ammonification has to be assessed, as it can lead to potentially phytotoxic digestates preventing a direct land application; then, digestates with higher nitrogen concentration might be phytotoxic and require a post-treatment. Moreover, the nitrogen rate is always associated with potassium (K) and phosphorus (P) concentrations, as the fertilizing effect is mostly influenced by the bioavailability of essential nutrients (N, P, K). Then, the concentrations of P and K were checked to be, on average, around 3 and 4% of TS for all the digestates. The same findings were reported by Beggio et al. (2019) who suggested that for such ranges of P, a digestate post-treatment is needed to increase the phosphorus concentration (Beggio et al. 2019).

Table 4
Characteristics of the gathered digestates.

Parameters	Units	D1	D2	D3	D4
pH	-	7.49	7.51	7.02	8.13
Moisture content (MC)	% of FM	96.70	95.90	97.50	97.30
Carbon (C)	% of FM	37.60	35.20	40.10	37.20
Nitrogen (N)	% of FM	2.90	3.70	4.70	4.40
C:N ratio	-	12.97	9.51	8.53	8.45
Phosphorus (P)	% of TS	3.02	3.17	2.87	2.91
Potassium (K)	% of TS	4.16	4.04	4.21	4.86
Magnesium (Mg)	% of TS	0.4	0.65	0.38	0.81
Calcium carbonate (CaCO3)	% of TS	5.68	8.31	5.35	11.10

Further factors can be harmful to the environment. The pH level is important to determine innocuousness as it controls the behaviors of metals which are detrimental for soil (Al Seadi et al. 2013). Therefore, guidelines are usually established on total content of heavy metals. Table 5 shows the digestates' characteristics in terms of heavy metals contents as well as some guidelines proposed by the European commission for digestates designed for agricultural use. Indeed, in order to sustain metabolic activity of the cell, some TEs such as Cd, Cu, Hg, Ni, Pb, As and Zn are required.

Table 5
The limits of total metal contents with reference to the European commission *[64] [65]*.

HMs (mg/ kg TS)	**Digestate**				**Standards of digestate (EU recommendations)**	
	D1	**D2**	**D3**	**D4**	**2015**	**2025**
Lead (Pb)	2.33	2.46	2.29	2.54	500	300
Copper (Cu)	38.86	46.02	44.07	60.02	800	600
Zinc (Zn)	165.64	185.07	167.65	223.41	2000	1500
Nickel (Ni)	8.08	7.24	6.48	9.00	200	100
Cadmium (Cd)	0.32	0.40	0.35	0.38	5	2
Arsenic (As)	1.40	1.95	1.70	1.76	-	-
Mercury (Hg)	0.02	0.05	0.07	0.09	5	2

Table 5 illustrates that zinc (Zn) was the most abundantly trace element, followed by copper (Cu) and nickel (Ni). However, D2 and D4 were characterized by a relatively high rate of TEs, which was due to the initial contribution of CM, particularly in terms of Zn, Cu and Ni. As methanogenesis is one of the most trace-element enriched enzymatic pathways in biology, the intensive concentrations of certain TEs in digesters, including manure such as $FW_{75}CM_{25}$ and $FW_{60}CM_{20}WS_{20,}$ was predictable from the SMY recorded (Fig. 5). Some researchers have reported that the trace-element requirements depend on the used substrates as well as the methanogenesis demands (Akturk and Demirer 2020)(Fermoso et al. 2019). Moreover, special attention was paid to cadmium (Cd), as it is relatively soluble on soils, readily consumed by crops, and then it is toxic to humans (Aydi 2015). However, the collected digestates were characterized by acceptable Cd concentrations, which were below the requested ranges. As the treated biomasses were initially characterized by low contents in terms of heavy metals, the examined digestates were also outlined by heavy metals concentrations below the limits imposed by the European Standards.

5. Perspective

This research work aimed, essentially, to take advantage of biowastes abundantly generated in Tunisia using biological treatments. Therefore, the aerobic and AD were carried out simultaneously (Fig. 1). However, during the biological experiments, two issues arose:

- A significant quantity of digestate was gathered from the anaerobic reactors, requiring a huge consumption of energy to achieve an efficient post-treatment such as sanitization or nutrients recovery.

- A significant quantity of fresh water was required to supply the composters treating FW and WS, which might cause a further issue for a semi-arid area such as Tunisia, suffering from water shortage.

Therefore, to unlock the sustainability of aerobic and anaerobic processes, digestate was upcycled in a cost-effective way. As AD-residues were characterized by high MC, relatively balanced C:N ratios and significant TEs contents (Table 4), digestates were chosen to be exploited as a moisturizing agent as well as aerobic digestion booster feeding in-vessel FW-composting.

6. Conclusion

To efficiently manage organic residues and unlock the full sustainability of AD potential, this research work aimed to develop quantitative relationships between the physical properties of the different types of organic residues abundantly generated in Tunisia. Therefore, a closed cycle ‘biowaste to bioenergy’ treatment, mainly of food waste, was examined. To this end, ACoD of food and agricultural residues was examined. To this end, ACoD of FW, CM and WS were examined under semi-continuous conditions. Steering parameters were monitored such as variable substrates’ mixture ratios, adjusted C:N and increased OLRs. Results showed that the most appropriate operational scenario was a feedstock ratio of FW:CM = 75:25 and operating at an OLR of 3.4 kg VS/m^3.d. However, with regards to FW_{100}, $FW_{60}CM_{20}WS_{20}$ and $FW_{75}WS_{25}$, lower biogas and methane yields were recorded. This might be due to the relatively high initial C:N ratio for digesters comprising WS and the use of untreated raw materials. However, the latter suited the operational conditions required by the project. For sustainable biological treatments, special attention had to be paid to the efficient recovery of both AD-effluents: biogas and digestate. Therefore, digestates were collected from different anaerobic reactors to be characterized in order to determine how AD-residues might be effectively upcycled. Therefore, additional works were achieved to evaluate the post-treatment of the generated effluents.

Abbreviations

FW: food waste

AD: anaerobic digestion

ACoD : anaerobic co-digestion

WS : wheat straw

CM : cattle manure

C :N : carbon to nitrogen

OLRs: organic loading rates

MC: moisture content

VS: volatile solids

EC: salinity

GHG: greenhouse gases

D: digestate

TEs: trace elements

HRT: hydraulic retention time

TS: total solids

VS: volatile solids

VFAs : volatile fatty acids

TAC: alkaline buffer capacity

(100% FW): FW_{100}

(FW:WS= 75:25): $FW_{75}WS_{25}$

(FW:CM= 75:25): $FW_{75}CM_{25}$

(FW:WS:CM= 60:20:20): $FW_{60}WS_{20}$ CM_{20}

SBY: Specific biogas yield

SMY: Specific methane yield

Declarations

Ethics declarations

- ***Ethics approval and consent to participate***

Not applicable.

- ***Consent for publication***

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Conflict of interest

The authors declare no conflict of interest.

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Authors’ contributions

Conceptualization, N.E.H.C.; methodology, N.E.H.C.; formal analysis, N.E.H.C., N.E.; investigation, N.E.H.C., data curation, N.E.H.C., N.E.; writing—original draft preparation, N.E.H.C.; writing—review and editing, N.E.H.C.; supervision, A.N. and M.N.

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Figures

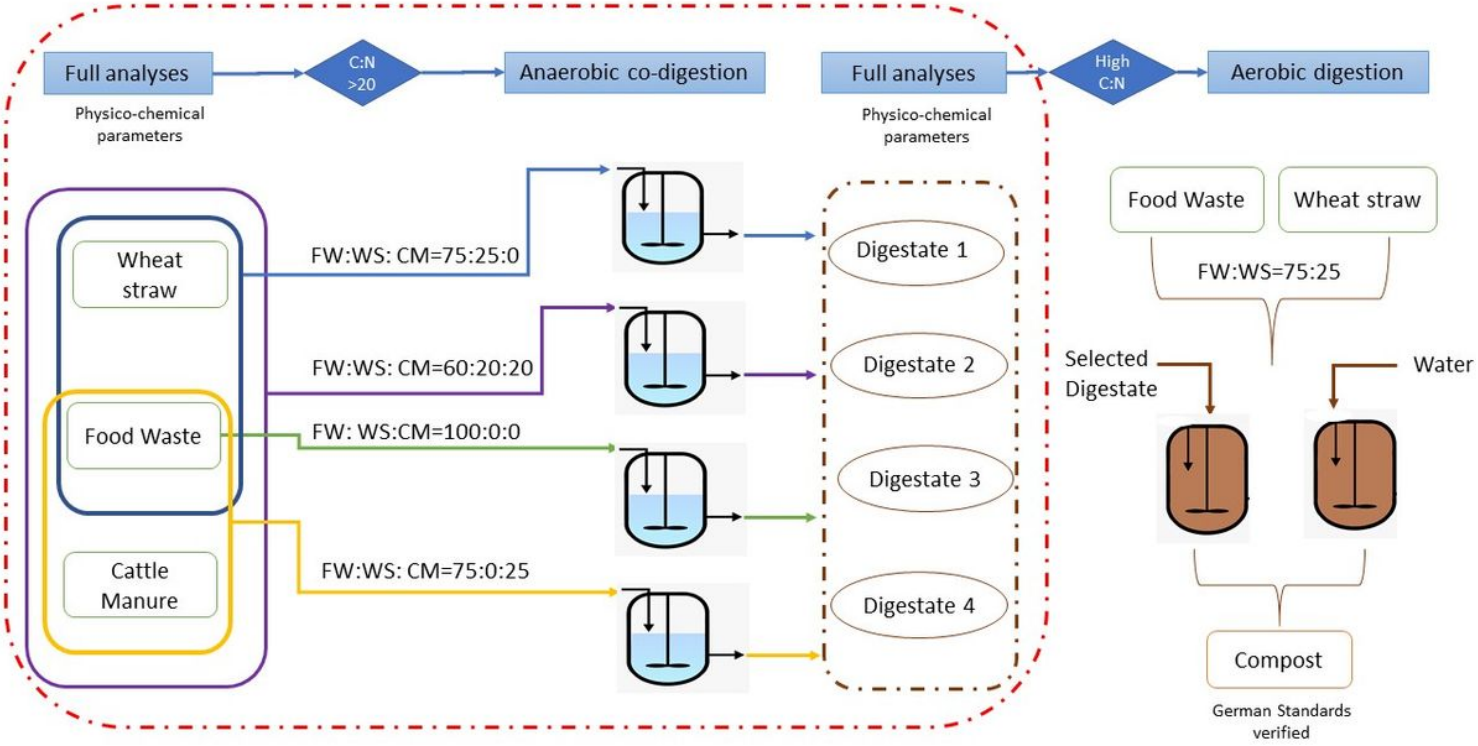

Figure 1

Conceptualization of the overall « RenewValue » approach.

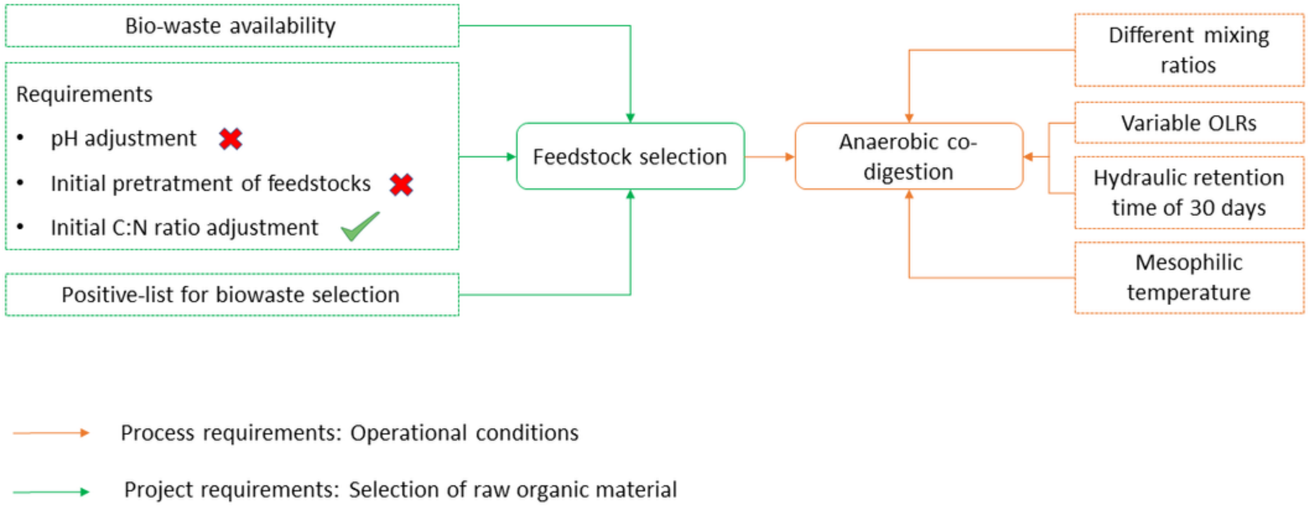

Figure 2

Experimental scheme of "Renew-Value" approach.

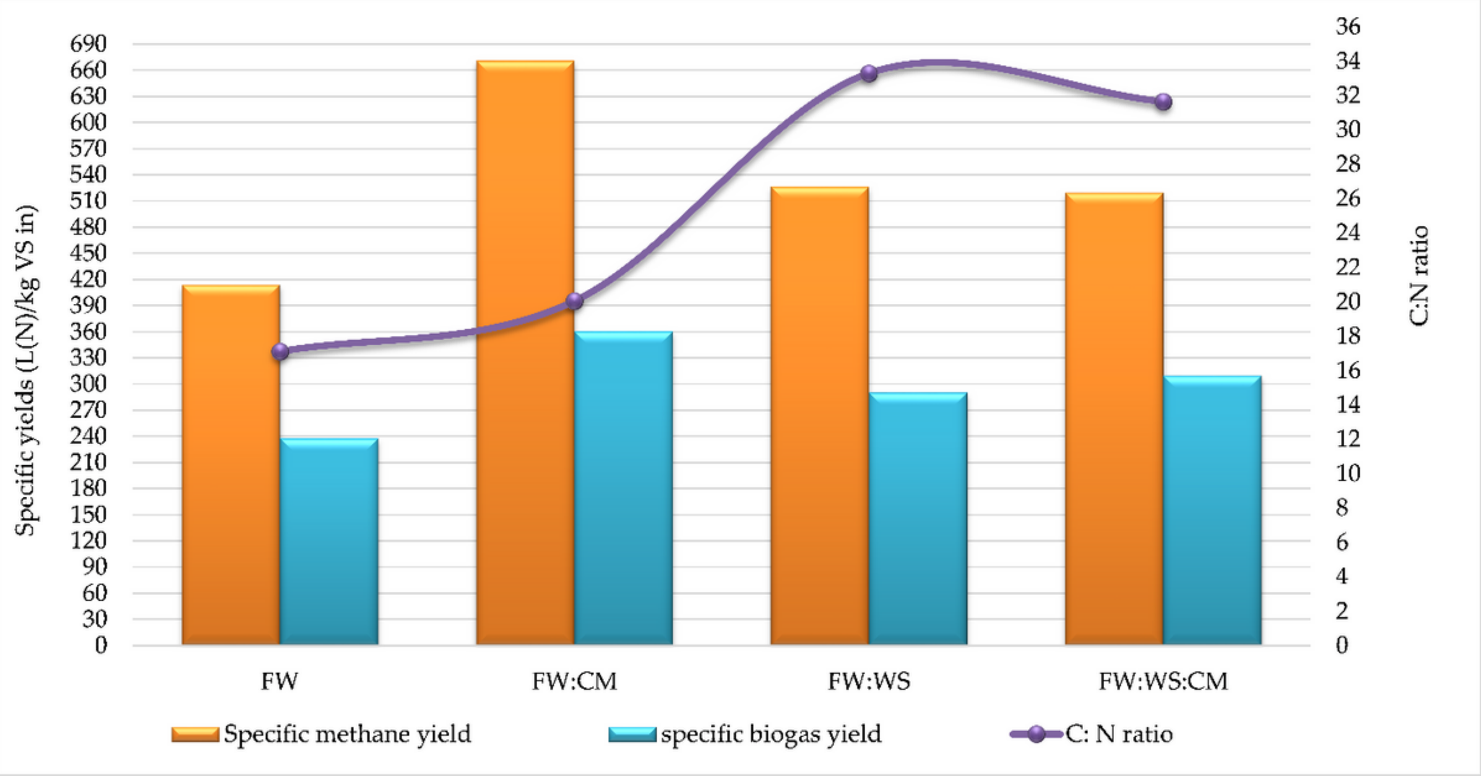

Figure 3

Effect of mixing substrates and C:N ratio regulation on process performance.

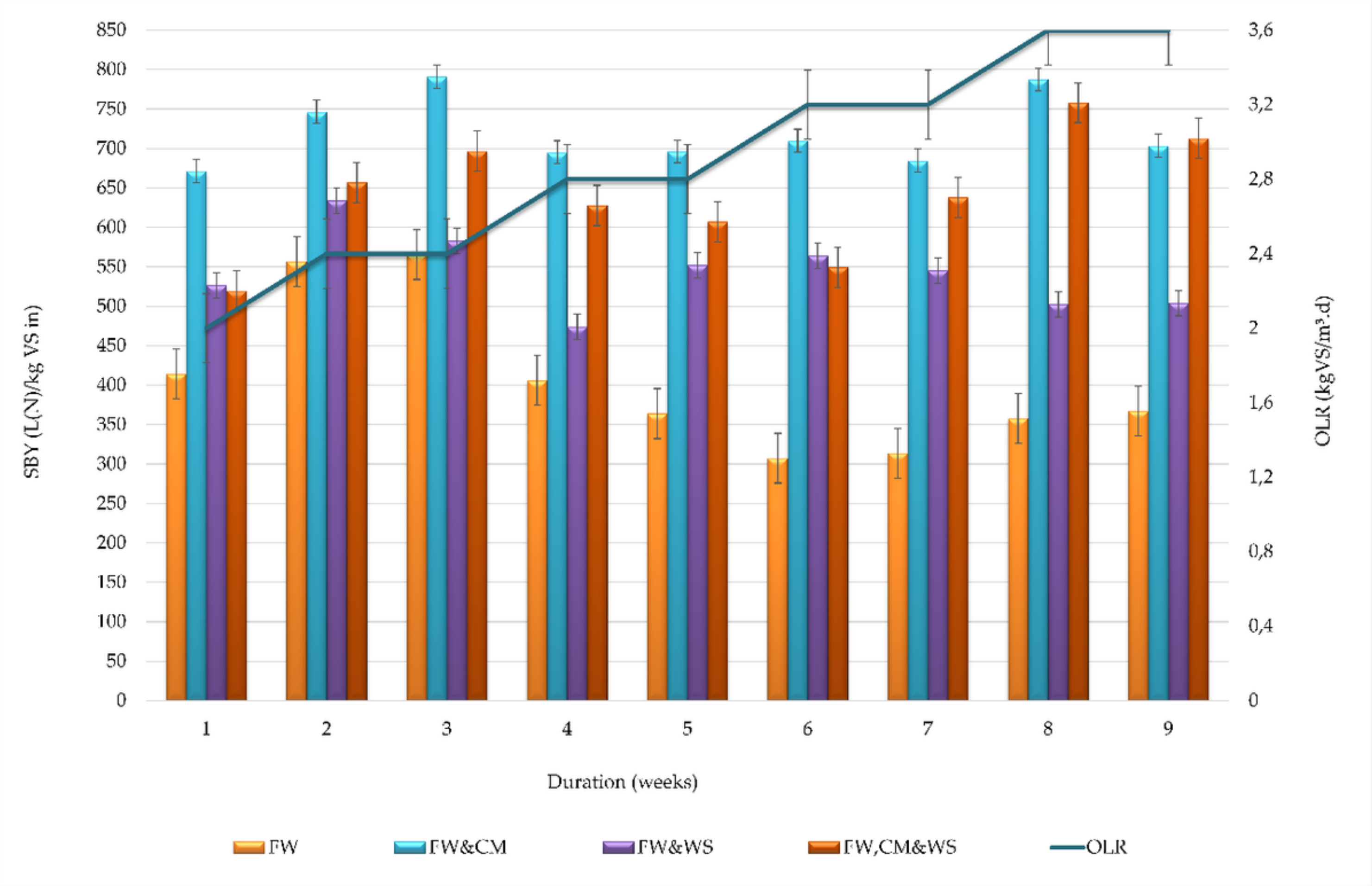

Figure 4

Specific biogas yields of different mixtures at different OLRs.

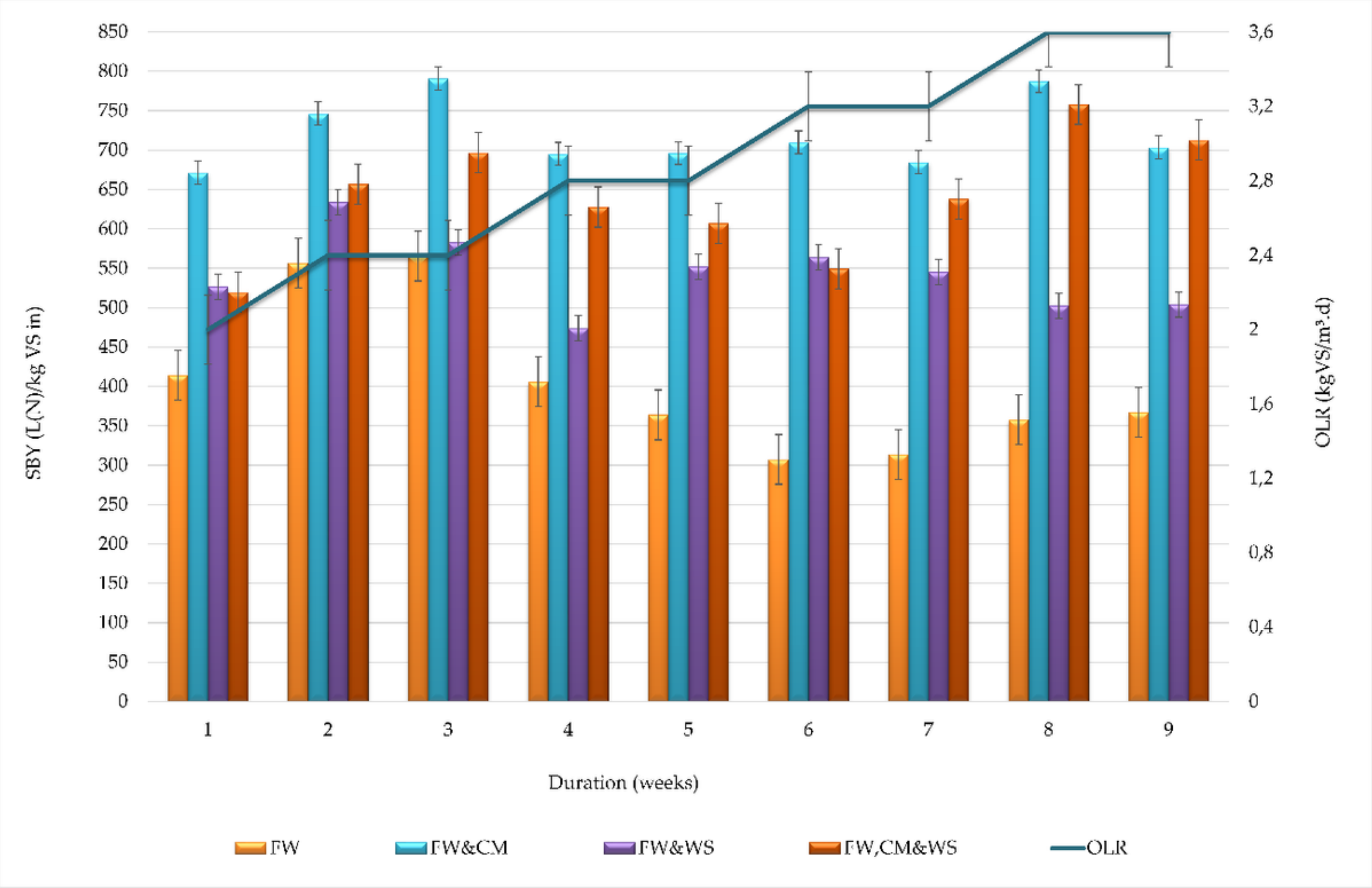

Figure 5

Specific methane yields of different mixtures at different OLRs.

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