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1 SCALE-UP EVALUATION OF THE ANAEROBIC DIGESTION OF

2 FOOD-PROCESSING INDUSTRIAL WASTES

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12 ABSTRACT

13 This work proposes a semi-pilot scale procedure for the evaluation of biogas production 14 potential and the employment of its results for the scale-up of the process. AD tests 15 were performed at 35°C in 6 L reactors, feeding 3-6% w/w TS in a fed-batch mode. 16 Several substrates, generated by food-processing industries, were considered in the 17 study. Assuming solubilization as the limiting step, a theoretical model was proposed 18 and the values of the disintegration kinetic constant (kdis) were calculated from the 19 experimental data. The obtained model was employed as a control tool during tests 20 afterward performed on pilot scale in a 300 L digester fed in a semi-continuous mode. 21 Biogas yields between 0.5 and 0.9 Nm³/kgvs, and methane contents of 55-63% v/v were 22 obtained on both scales. The model derived from the results of the proposed procedure 23 appeared adequate for a consistent evaluation of the scale-up of the AD process.

1 **KEYWORDS**: biogas, anaerobic digestion, food, waste, model

2

3 ABBREVIATIONS

4 AD: Anaerobic Digestion; Bexp: experimental biogas yield; Bth: theorethical biogas 5 yield; ANOVA: Analysis of Variance; BMP: Bio-Methane Potential; CCH4, CCO2, Csub: 6 Carbon amount in methane, carbon dioxide and substrate; CH4^{exp}: experimental methane vield; CH4th: % v/v methane calculated from stoichiometric equation; CHP: Combined 7 8 Heat and Power; COD: Chemical Oxygen Demand; CSTR: Completely Stirred Tank 9 Reactor; FOS/TAC: ratio between Organic Acids Concentration (Flüchitge Organische 10 Säuren, FOS) and Total Alkalinity (Totales Anorganisches Carbonat, TAC); HRT: 11 Hydraulic Retention Time; kdis: disgregation kinetic constant; NVS: non volatile solids; 12 OFMSW: Organic Fraction of Municipal Solid Waste; OLR: Organic Loading Rate; 13 PMMA: Poly Methyl Methacrylate; SS: Suspended Solids; TOC: Total Organic 14 Carbon; TS: Total Solids; VFA: Volatile Fatty Acid; VS: Volatile Solids; WWTP: 15 Wastewater Treatment Plant.

16

17 **1. INTRODUCTION**

18 Food-processing industrial wastes correspond to an interesting substrate for the 19 implementation of AD [1], being organic matrices characterized by properties that lay 20 between the high lignin and cellulose contents of crops and the high acidity and relevant 21 content of high soluble organics typical of food wastes. A summarized literature review 22 of biogas and methane yields obtained from agricultural and food wastes is reported in 23 Table 1. Wastes coming from rice, coffee, fruit and vegetables and food appear as the 24 most promising substrates, with biogas yields comparable to WWTP sludge and 25 OFMSW [2, 3]. Nevertheless AD of fruit and vegetable wastes is conventionally

affected by a lack of stability [4], therefore two-stage reactors [5], pre-treatments [6-8] 1

or co-digestion processes [9-13] are often adopted. 2

3

Substrate	Yield	% solids/mode/scale/T	k _{dis}	Reference	
rape	0.25 m ³ methane/kgvs		0.24		
sunflower	0.20 m ³ methane/kgvs		0.23		
glycerol	0.30 m3methane/kgvs	0.50	F1 41		
orange pulp	0.25 m ³ methane/kgvs	BMP/0.25 L/35°C	0.29	[14]	
pear pulp	0.15 m ³ methane/kgvs		0.18		
apple pulp	0.18 m ³ methane/kgvs		0.15		
trilicate	0.76 m ³ biogas/kgvs		0.21		
maize silage	0.73 m ³ biogas/kgvs	n s / BMD /1 I /37°C	0.21	[15]	
onion	0.92 m ³ biogas/kgvs	11.5./ DIVIT / 1 L/37 C	0.34		
potato	0.83 m3biogas/kgvs		0.26		
rice husk and straw	0.22 m ³ biogas/kgvs	n.s./batch/190 L/35°C	n.a.	[16]	
rice straw	0.24 m ³ biogas/kgvs	7.5% TS/batch/2.5 L/35°C	n.a.	[17]	
rice chaff	$0.67 \ m^3_{biogas}\!/kg_{VS}$		n.a.		
wheat straw	0.57 m ³ biogas/kgvs	BMP/2 L/40.0 °C	n.a.	[18]	
dry bread	$0.65 \ m^3_{biogas}/kg_{VS}$		n.a.		
rice straw	0.42 m ³ biogas/kgvs				
tomato skins and seeds	0.42 m ³ biogas/kgvs			[10]	
grape stalk	0.22 m ³ biogas/kgvs	n.s./batch/2 L/40°C		[19]	
pomace	0.25 m ³ biogas/kgvs				
aoffaa nuln and husk	0.65-0.73	0.65-0.73		[20]	
conee puip and nusk	m ³ methane/kgvs	11.5.	n.a.	[20]	
fruit and vegetable wastes	0.32-0.63 m ³ biogas/kgvs	n.s./batch/n.d/35-40°C	n.a.	[21]	
olive mill and winery	0.18-0.21	n s /batch/1 I /35°C		[22]	
residues	m ³ CH4/kgCOD	n.s./batch/1 L/33 C	n.a.		
brewery waste	$0.51 \ m^{3}_{biogas}/kgvs$		n.a.	50.03	
bread waste	$0.58 \ m^3_{biogas}/kg_{VS}$	BMP/1-2 L/36.5°C	n.a.	[23]	
vegetable wastes	0.36 m ³ methane /kg _{COD}		n.a.		
-			na		
vegetable fats and oils	0.23 m ³ methane /kg _{COD}	BMP/0.12 L/35°C	n a	[24]	
1 1. 1	0.13-0.26		11.0.		
slaughterhouse wastes	m ³ methane/kgCOD		n.a.		
plain pasta	$0.33 \ m^3$ methane/kgvs		n.a.		
cabbage	$0.26 \ m^3$ methane/kgvs		n.a.		
used vegetable oil	$0.65\ m^3_{methane}/kg_{VS}$	BMP/0.25 L/35°C	n.a.	[25]	
potatoes	$0.33\ m^3\ {}_{methane}/kgvs$		n.a.		
cheese whey	0.42 m ³ methane/kgvs		n.a.		
food waste	0.4-1.4 m ³ methane/kgvs	BMP/0.2 L/35°C	n.a.	[26]	
tomato processing waste	0.33 m ³ methane/kgvs	BMP/1.1 L/35°C	n.a.	[27]	

4

n.s.: not specified; n.a.: not available

5

6 Table 1. Biogas and methane yields and kdis values obtained from AD of some 7 agricultural and food wastes considering batch/fed-batch feeding, BMP/laboratory scale, mesophilic conditions. 8

1 This research is focused on the evaluation of the feasibility of the AD of food-2 processing industrial wastes (coffee, rice, hazelnut, wine, sweets/snacks) in mono-3 digestion processes. The aim of this work is the assessment of a semi-pilot scale 4 procedure for a reliable and easy to manage evaluation of biogas production potential of 5 complex substrates with a high SS/COD ratio in mono-digestion processes. Several 6 substrates were taken into account as homogeneous mixtures of wastes generated by 7 different food-processing industries. Assuming solubilization as the limiting step for 8 AD of the considered wastes, a theoretical model was proposed and the values of the 9 disintegration kinetic constant (kdis) were calculated from the experimental data gathered 10 for each of the mixtures. AD tests were then repeated on pilot scale, and the previously 11 obtained model was employed as a control tool during the digestion process.

The proposed semi-pilot scale procedure and the model derived from its results have the purpose to overcome the frequent limitations of conventional BMP tests about heterogeneous substrates. The here-presented data descend from tests performed on a higher scale than of traditional BMP/batch tests (see Table 1) and employing a different feeding mode (*fed-batch*), which is more oriented to the scale-up of the process.

17 2. MATERIALS AND METHODS

18 **2.1. Substrates origin and characterization**

19 The following materials, gathered from producers of Piedmont region within Ecofood20 project, were considered as substrates in semi-pilot scale tests:

coffee husk (CH, removed with coffee bean shell) and *coffee dust* (CD, grinded after
roasting process);

23 - raw hazelnut skin (RHS, removed with hazelnut shell), fine hazelnut skin (FHS,

removed after roasting process), *large hazelnut skin* (LHS, removed after roasting
 process);

- rice husk (RH, removed in de-husking process), *rice bran* (RB, removed in whitening
process):

cookie by-products (C, from cookies production), *tea leaves* (TL, from tea beverage
production), *snack-cake without cocoa* (SC, from snack cakes production), *cocoa cream by-products* (CC, from cocoa cream production), *cocoa husk* (CH, removed during
cocoa beans de-husking);

9 - *pomace* (P, removed after grapes pressing), *lees* (L, removed after each fermentation
10 step in wine production).

Two different *lees* samples (L1 and L2), showing different physic-chemical features, underwent the tests: L1, collected in October at the end of harvest period, was employed for semi-pilot scale tests; L2, collected in April, was employed for the pilot scale test. L2 sample exhibited detectable sulfur content, due to the use of sulfur dioxide, which produces sulfites, as anti-oxidant in intermediate phases of wine production

16 Pilot scale tests involved the following substrates:

17 - *lees* (L₂ sample): fed at an average of 4.3% TS, taking into account a HRT equal to 30

18 days and a resulting average OLR equal to 1.45 g_{TS}/L^*d ;

19 - rice mixture (same composition as in the semi-pilot scale tests) was considered in two

20 tests, performed in sequence (a complete degassing was executed after each test):

- in test 1 the substrate was fed at an average of 3% TS, considering a HRT equal to 20

22 days and a resulting average OLR equal to 1.50 g_{TS}/L*d;

- in test 2 the amount of the substrate was enhanced at 6% TS, considering a HRT equal

to 20 days and a resulting average OLR equal to 3.00 grs/L*d.

The considered substrates underwent the analysis of pH, TS and VS according to
 standard methods [28]. An Orion 420A pH-meter and a Kern MLS-N thermo-balance
 were employed to analyze pH and TS content. The elemental analysis was performed
 through a CHNS-O Thermo Fisher Flash 2000 Analyzer EA 1112.



Figure 1. Experimental apparatus employed for (A) semi-pilot scale tests and (B) pilot scale tests

assuming Oxygen content as the complementary fraction towards C, H, N, S amounts.
 COD was analyzed according to Raposo method [29]. All the analyses were conducted
 in five replicates.

4

5

2.2. AD tests (semi-pilot scale)

6 The tests were performed in mesophilic conditions (35°C) employing six reactors (6 L 7 PMMA digesters, 3 L working volume) for each mixture, made of unaltered samples 8 (see Figure 1A). The inoculum was prepared employing fresh digestate provided by 9 local WWTP, performing a complete degassing procedure [30]. The same inoculum was employed for all the semi-pilot scale tests that were executed consecutively. Between 10 11 the digestion of two consequent substrates a transition protocol was established: 150 mL 12 of fresh primary sludge from local WWTP was fed to the digesters as a single addition 13 and a complete degassing procedure was performed.

14 The feeding was performed in a *fed-batch* mode: 3% TS, content was reached after six 15 0.5% TS supplements (one every two days) during 11 days; these percentage are referred to the total mass of solids present inside each digester. 6% TS content (only 16 17 considering CC and CH materials) was achieved after six 1% TS additions. The 18 substrates were added as unaltered materials. The reactors were manually mixed once a 19 day. The tests were considered concluded when the observed variation in the cumulative 20 production was below 1%. TS and VS were analyzed in the digestate before and after 21 each cycle of digestion. Biogas volume (by water displacement) and components 22 (through a Biogas Check analyzer, Geotechnical Instruments Ltd) were determined 23 daily in each digester, as well as pH. Biogas was characterized in terms of CH₄, CO₂, O₂ 24 and "balance" (i.e. all the gases that are different from the first three).

1 The identification of the single reactors was randomized in each test to avoid any 2 memory effect of previous digestions. Furthermore, with the aim to evaluate the 3 influence of the sequence of the feedstocks in the AD tests, the first substrate (a mix of 4 CH and CD) was again digested at the end of the sequence.

5

6 **2.3. AD tests (pilot scale)**

7 The tests were performed at 35°C in a 300 L reactor (240 L working volume), equipped 8 with an 80 L gasometer and a system for on-line monitoring of biogas volume and 9 composition (see Figure 1B). Mixing inside the reactor was achieved through biogas re-10 circulating for 15 minutes at every hour. Digestate was daily analyzed for pH, TS, VS. 11 The inoculum was prepared from digestate provided by local WWTP and properly 12 degassed [30]. The start-up procedure was performed before the pilot-scale tests on the different considered substrates. FOS/TAC, that is the ratio between Organic Acids 13 14 Concentration (FOS, expressed as mg/L of equivalents of acetic acid) and Total 15 Alkalinity (TAC, expressed as mg/L of CaCO₃), was monitored daily in the digestate 16 according to a reference procedure [31].

The feeding was performed in a *semi-continuous* mode. The unaltered substrates were fed to the digester after a pre-mixing phase, in which a proper volume of water, necessary to achieve the desired TS content, was added. When a whole HRT passed the feeding was stopped and the tests were declared concluded when no significant biogas production was detected. Biogas was continuous characterized in terms of CH₄, CO₂, O₂ and "balance" (i.e. all the gases that are different from the first three) through a GA3000 Range Gas Analyzer, Geotechnical Instruments Ltd.

3. MODELLING APPROACH

2 Solubilization (made of disintegration and hydrolysis) is generally assumed as the 3 rate-limiting step during AD of complex substrates with a high SS/COD ratio [32]. It 4 can be proven that the hydrolysis is the rate-limiting step during the uninhibited 5 anaerobic digestion of complex particulate substrate [33]. Moreover, disintegration has 6 the slowest kinetic in the solubilization step [14, 34] and it may be considered as a 7 bottleneck. Disintegration may be considered a surface phenomenon, which is heavily 8 affected by the structure of the particulate matter and by the availability of free 9 accessible surface area.

10 Assuming a first order kinetic model, the disintegration rate may be achieved through 11 the first part of the cumulative biogas curve obtained from BMP tests [30], according to 12 Eq. (1).

13
$$B(t) = B)_{exp}(1 - e^{-k_{dist}})$$
 (1)

14 where:

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

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

17 k_{dis} is the first order disintegration rate [day⁻¹]

18 t is the time [day]

However the drawback of this approach is that k_{dis} value changes depending on the time used to estimate it [35]. In this framework appears licit to consider if it may be possible, in the analysis of AD of a complex substrate, to derive robust values of a first order k_{dis} from biogas cumulative curves obtained from fed-batch tests. With the aim to assess the robustness of the experimental parameters (k_{dis} and B_{exp}) gathered from semi-pilot scale tests, a model predicting daily biogas production in a semi-continuous CSTR reactor, 1 was designed. The daily biogas production at *t-th* day of experimentation was calculated
2 by means of Eq. (2).

$$B(t) = k_{dis} \times S_e(t) \times B_{exp} \times V_w$$
⁽²⁾

- 4 where the parameters represent:
- 5 \mathbf{k}_{dis} : first order disintegration rate [day⁻¹]
- 6 V_w : working volume of the digester (CSTR) [m³]
- 7 B_{exp} : ultimate biogas/methane potential yield of the substrate [Nm³/kgvs]

8 $S_e(t)$: apparent concentration of Volatile Solids into the digester [kgvs/m³]. This 9 parameter represents the amount of biodegradable VS. If B_{exp} was identical to the 10 theoretical value it means that not biodegradable VS content in the substrate is 11 negligible.

12 The $S_e(t)$ was calculated by the resolution of the following differential equation (3):

13
$$\begin{cases} \frac{dS_{e}(t)}{dt} = \frac{q(t) \times S_{o}(t)}{V_{e}} - \frac{q(t) \times S_{e}(t)}{V_{u}} - k_{dis} \times S_{e}(t) \\ S_{e}(0) = 0 \end{cases}$$
(3)

14 The parameters, not identified before, represent:

- 15 $S_{\alpha}(t)$: VS input concentration
- 16 **q(t)** : input and output volumetric flow rate of the anaerobic reactor

17 All calculations described in this section were performed by means of Matlab/Simulink..

18

19 4. RESULTS AND DISCUSSION

20 **4.1. Characterization of the substrates**

The results of the characterization of the studied substrates are schematically represented in Table 2: the single materials exhibited acidic pH values (apart from rice processing substrates, cookies by-products and tea leaves, which are neutral), high 1 VS/TS values, and carbon contents above 40-50%. Considering C/N, single materials' 2 values were sometimes quite high (particularly considering the substrates deriving from 3 hazelnuts and rice processing). Moisture content, pH value, C/N ratio and VS content 4 are the most important parameters to consider in planning an AD process. Typical 5 values of these parameters commonly reported for a correct anaerobic digestion are pH 6 values between 6.5 and 7.5 and C/N between 25 and 30 [1], while moisture content 7 influences the choice of the digester's technology (wet, semi-wet or dry) and the need of 8 a mechanical mixing equipment. VS amount is related to the organic substance content 9 available for biological degradation.

10 The semi-pilot scale tests considered six mixtures, which were designed gathering 11 substrates generated by single food-industry macro-categories and with the main 12 purpose to obtain an optimal C/N value (see Table 2). The molecular formula of the 13 single substrates, derived from elemental analysis, according to stoichiometric 14 assumptions enabled to calculate the theoretical production of biogas (see B_{th} and CH₄th 15 values in Table 3).

16

17 **4.2. AD tests (semi-pilot scale)**

On the grounds of the results of semi-pilot scale tests performed feeding a 3% TS (see Table 3), the substrates characterized by the highest biogas specific production are the mixtures of wine wastes (0.89 m³/kgvs) and of sweets without cocoa (0.80 m³/kgvs). The other substrates exhibit a rather homogenous trend (0.48-0.72 m³/kgvs), with coffee wastes placed at the bottom end. Methane content exceeded 55% in all cases, with hazelnut and wine mixtures reaching 62-63%. The significance of differences in average biogas yields and methane contents were determined by single factor analysis

Substrate	pН	TS (%)	VS/TS	С	Н	N	S	formula	mixture	Mixture formula	relative abundance (%)	TS (%)	VS/TS	C/N
СН	5.8	92.9	91.4	45.9	5.9	2.8	0.2	$C_{23}H_{34}O_{12}N$	coffee mix	CullerOuN	60	05.0	03 7	19
CD	5.1	96.4	95.3	51.7	6.7	2.7	0.1	$C_{19}H_{27}O_{13}N$	conee mix	$C_{21}II_{31}O_{11}IN$	40	95.0	95.1	10
RHS	5.7	89.3	96.5	45.7	5.4	1.1	< 0.1	$C_{49}H_{53}O_{31}N$			10			
FHS	5.2	95.7	97.5	56.8	6.8	1.2	0.1	C57H76O24N	hazelnut mix	$C_{29}H_{42}O_{11}N$	10	94.1	97.6	29
LHS	5.5	94.5	97.7	54.6	7.2	2.2	0.1	$C_{29}H_{42}O_{12}N$			80			
RH	7.2	92.0	83.2	38.5	5.1	0.5	< 0.1	$C_{100}H_{125}O_{93}N$	rico mix		15	02.0	97.9	20
RB	6.9	92.0	88.7	44.9	6.9	2.4	0.1	$C_{22}H_{34}O_{14}N$	fice mix	$C_{22}\Pi_{34}O_{101}N$	85	92.0	2.0 87.8	20
С	7.3	92.1	98,4	47.2	7.1	2.0	< 0.1	$C_{28}H_{45}O_{20}N$	awaata no		50			
TL	7.0	24.9	95.8	55.0	38.3	4.7	< 0.1	$C_{14}H_{14}O_7N$	sweets lio	$C_{30}H_{46}O_{17}N$	5	83.6	98.5	25
SC	6.1	80.5	98.7	54.7	9.6	1.9	< 0.1	C33H50O16N			45			
CC	6.7	99.7	98.2	56.6	8.6	1.3	< 0.1	$C_{51}H_{93}O_{22}N$	sweets cocoa	$C_{36}H_{61}O_{19}N$	50	05.2	04.5	21
CH	4.8	92.6	91.1	48.3	6.6	2.6	0.2	$C_{22}H_{36}O_{14}N$	mix		50	95.5	94.5	51
Р	3.2	16.3	91.5	42.6	2.5	2.8	< 0.1	$C_{18}H_{13}O_{16}N$	wing min		60	15 /	08.0	22
${ m L_1}^*$	3.4	14.3	80.5	52.4	5.7	1.6	< 0.1	$C_{38}H_{50}O_{22}N_1$	while mix	C261127O191N	40	13.4 90.0	90.0	55
L2**	4.2	12.3	85.4	45.4	5.6	3.7	0.2	C ₃₂ H ₅₄ O ₂₄ N	lees**	C ₃₂ H ₅₄ O ₂₄ N	100	12.3	85.4	20

*fed in semi-pilot scale tests ** fed in pilot scale tests

Table 2. Characterization of the considered substrates and mixture design.

of variance (ANOVA), choosing a level of significance equal to 0.05. ANOVA results
showed that the 6 substrates are different from a statistical point of view (F (22.84) >
F_{crit} (2.30)).

4 The comparison between the results of the first and the second test concerning coffee 5 mix shows no significant difference about biogas and methane production (respectively equal to 0.48 and 0.47 Nm³ biogas/kgvs and 55.1-55.7 CH₄%). The analysis of the 6 7 results of the two tests on coffee mix by means of the statistic inference of variance and 8 average values (test F and test t, both with 0.05 significance level), showed no 9 significant differences, hence the sequence of digestion of the different substrates may 10 be considered negligible from the point of view of the properties of the inoculum (F 11 $(3.01) < F_{crit}(7.15)$).

12 These results in overall demonstrated that disintegration was the limiting step of the 13 process. Moreover the particulate nature of the tested substrates was a crucial factor, as 14 proven by other studies [18] and even if the microbial community could change over the 15 time, this phenomenon did not influence the results. The pH values measured during the performed tests (see Supplementary Material, Figure I), as well as biogas 16 17 composition (see Figure 2), reflected the evolution of the different phases of the AD 18 process, which is influenced by the adopted feeding procedure. In all tests the feeding 19 phase lasted 11 days, although the biogas production continued until 19-29 days 20 depending on the relative content of carbohydrates and lipids in the substrates. 21 Comparing the cumulative biogas production curves of the six tested mixtures (see 22 Figure 3), the mixtures may be divided in two groups characterized by analogous 23 production speed in the starting phase of the tests. The error bars in Figure 3 represent 24 the standard deviation of cumulative biogas production calculated on 6 replicates for







4 Figure 2. Mean Biogas composition during the semi-pilot scale tests (average values of
5 6 replicates) performed at 3% TS



Figure 3. Cumulative curves obtained from semi-pilot scale tests performed at 3%TS
(the arrow indicates the stop of the feeding phase).

each substrate. A higher-speed group, made of sweets without cocoa and wine mixtures (having a higher amount of highly biodegradable carbohydrates), and a lower-speed group made of hazelnut, coffee and sweets with cocoa mixtures (rich in less readily degradable substances). Rice mixture exhibited a behavior analogous to the first group in the first 6 days, and then switched to the second group, witnessing its complex nature. Considering the experimental biogas and methane yields (see Table 3), all values were lower than theoretical ones. Experimental yields were considerably higher if compared with literature values referred to BMP tests (see Table 1), with the exception of rice mix

1 that shows a behavior similar to what reported by Menardo and Balsari [18] (0.67 2 m³_{biogas}/kg_{VS} - 0.56 % CH₄). All mixtures exhibited a removal of VS above 80% (with 3 the exception of Coffee mixture), and methanation grade values are consistent with 4 carbon balance. Considering carbon balance (see Table 3) the difference between the 5 amount fed and the amount transferred in biogas is due to the carbon content of the 6 digestate, therefore a high transfer of carbon in biogas is connected with an enhanced 7 biodegradation of the substrate and a highly stabilized digestate in the considered 8 experimental conditions. The not complete agreement between the carbon balance and 9 VS balance (see Table 3), is due to the assumption of an equal distribution of the 10 different fractions of the substrates into VS and the digestate; moreover the C content in 11 the mixtures was calculated assuming its equal partitioning between NVS and VS.

12 The obtained k_{dis} values (see Table 3), slightly higher than the ones found in literature 13 and deriving from BMP tests (see Table 1), revealed that disintegration was a critical 14 phase particularly for hazelnut mixture, while the other substrates exhibited similar 15 values. The comparison of the experimental daily biogas curves with the ones calculated 16 from the gathered kdis values and Eq. (1) (see Figure 4) allowed some general 17 evaluations about the kinetic features of the AD process in the considered operative 18 conditions in the fed-batch system. The peaks in experimental curves didn't happen 19 straight after the feed, but in all cases they occurred about one day after, because of the 20 complex nature of the substrates. See Supplementary materials (Figure II) for details 21 about the model. Generally the deviations of the experimental curves towards the 22 calculated ones, which were higher in correspondence of the two days of the week in 23 which the feeding didn't happen, may be due to a scarce mixing of the systems and to 24 the obvious variability of a biological process performed in six replicates on

1 heterogeneous substrates. Taking into account hazelnut mix, the largest deviations were 2 observed from the twelfth day: the substrate is rich in lipids, which are characterized by 3 a slower kinetic if compared to carbohydrates and proteins, and the determined kdis may 4 be different from the one that could be achieved in absence of limiting factors (i.e. 5 scarceness of lipid degrading bacteria, that needed 12 days to be overtaken). It was not possible to obtain a kdis value for wine mixture: the involved materials were rich of 6 7 sugars and contained a certain amount of alcohols, therefore hydrolysis was probably 8 not a limiting step of their AD.

9 The stability of the system about the substrate amount was evaluated on a mixture of 10 CC and CH substrates, performing a semi-pilot scale test feeding 6% TS. The gathered 11 results (see Table 3) showed analogous biogas yields and methane contents if compared 12 with the results obtained feeding 3% TS, therefore the possibility to enhance the amount 13 of fed wastes may be positively evaluated (although a possible stress of the system may 14 be supposed considering the lower k_{dis} and that pH values were placed in a wider range 15 if compared with 3% TS) (see Supplementary Material).

Mixture	$CH_{i_{th}}\left[\frac{m^3}{kg_{VS}}\right]$	CH_4^{th} [%]	$B_{exp}\left[rac{Nm^3}{kg_{VS}} ight]$	СН _{4 ехр} [%]	СН _{1 е.ср} /СН _{1 сh} [%]	removed VS [%]	$\frac{C_{(CH_4+CO_2)}/C_{sub}}{[\%]}$	$k_{disexp}[d^{-1}]$
coffee mix (3% TS)	0.54	54	0.48 ± 0.05	55 ± 0.65	49	57	53	0.31
hazelnut mix (3% TS)	0.63	57	0.64 ± 0.01	63 ± 0.58	64	79	65	0.15
rice mix (3% TS)	0.57	56	0.69 ± 0.02	56 ± 0.61	67	79	82	0.38
sweets no cocoa mix (3% TS)	0.51	53	0.82 ± 0.03	56 ±0.41	88	94	85	0.56
sweets cocoa mix (3% TS)	0.54	57	0.72 ±0.01	57 ±0.87	75	80	73	0.34
sweets cocoa mix (6% TS)	0.54	57	0.72 ±0.02	58 ±0.83	76	73	75	0.29
wine mix (3% TS)	0.48	48	0.89 ±0.02	62 ±1.61	1.16	81	c	с
3 6 1 1 1 1 1	1							

^a referred only to LHS ^b referred only to RB ^c data not available

Table 3. Biogas and methane production: theoretical and experimental values gathered from semi-pilot scale tests, mass balance referred to

VS and carbon, disintegration kinetic constant values (k_{dis})



Figure 4. Daily biogas curves (calculated and experimental) gathered from semi-pilot scale tests: a) sweets no cocoa mix; b) sweets cocoa
 mix; c) rice mix; d) coffee mix; e) hazelnut mix



Figure 5. Cumulative curves obtained from pilot-scale tests on rice mixture 3% TS (*test*1) and 6% TS (*test* 2)

1

5 **4.3. AD** tests (pilot scale)

6 The results of pilot scale tests showed that, feeding a 3-4% TS, lees produced a higher 7 biogas yield (1.13 $Nm^3/kgvs$, with an average CH₄ content above 55%), if compared 8 with rice mixture, which generated 0.69 $Nm^3/kgvs$ of biogas (average CH₄ content 9 47%). Probably the high content of sugars and alcohols of lees was a crucial factor.

Taking into account rice mixture, Tests 1 and 2 had the aim to explore the stability of the system towards the doubling of the amount of the fed substrate. Moreover Test 1 was employed to evaluate the implementation of the proposed model to the scale-up of the process from semi-pilot to pilot scale. The test performed feeding 6%TS of rice mixture (Test 2) produced a biogas yield (0.58 Nm³/kgvs, with an average CH₄ content around 54%) which was analogous to the one registered with 3% TS (Tests 1). The cumulative biogas production curves are reported in Figure 5.



3 4

Figure 6. Biogas composition during the pilot scale tests: (A) lees (4.3% TS), (B) rice 5 mixture (3%TS, Test 1)

Biogas composition (see Figure 6) reflected the periodical trend of the feed, which happened 5 days/week: the registered minimum values correspond to the two days in which the feed didn't happen. Hydrogen sulfide could be potentially a critical issue during the pilot scale tests: an average concentration of 150 ppm, with a maximum of 280 ppm around the middle of the digestion period, was measured for lees although methane production was not inhibited. Hydrogen sulfide content remained generally below 100 ppm within rice mixture digestion, with a maximum around 180 ppm.

9 During the pilot scale tests FOS/TAC value was monitored daily in the digestate: it is 10 one of the most significant operative parameters in continuous/semi-continuous fed AD 11 processes [28], allowing a well-timed intervention in case of stress of the system due to 12 an accumulation of organic acids when a high organic load is applied. In general, it is 13 assumed that total alkalinity should be above 2000-3000 mg/L CaCO₃ to buffer pH 14 decreasing and to prevent the consequent inhibition of methanogenesis, and that the 15 FOS/TAC value should be around 0.3 to have a stable process [37, 38]. Considering the 16 FOS/TAC trend at the beginning of the test on lees (see Figure 7), sodium bicarbonate 17 was added to the substrate since the fourth day of digestion (a stoichiometric amount of 18 0.42 g NaHCO₃/g_{TS} was calculated to achieve a TAC equal to 3000 mg/L of CaCO₃) in 19 order to increase the buffer capacity of the system. The evolution of pH and FOS/TAC 20 trends outlined the efficacy of the correction. The addition of sodium bicarbonate 21 probably had a positive effect also in preventing hydrogen sulfide over-production.

During test 1 (rice mixture fed at 3% TS), the experimental daily biogas production values were plotted together with the curves calculated as specified in section 3 (the losses of substrate connected to semi-continuous feeding mode were taken into account) (see Figure 8 and Figure 9). See Supplementary materials for the values of the

1 parameters employed in the model. A good agreement between experimental and 2 expected data was observed, although some differences in their trends may be noticed. 3 First of all, the deviations in correspondence of the two days of the week in which the 4 feeding didn't happen (already observed at a semi-pilot scale), which may be due to a 5 scarce mixing of the reactor. As the test proceeded, after about 16 days, the variance of 6 the two trends became consistent: it may be hypothesized that the semi-continuous 7 feeding mode of the tested mixture determined a loss of substrate, that couldn't be 8 digested in the considered experimental conditions. Nevertheless, at a semi-pilot scale 9 the maximum difference between the biogas cumulative production given by the model and the experimental value (recorded at 25th day) is equal to 8.5 %, instead at the end of 10 11 the pilot-scale test this difference drop down at the 7.1 %. The comparison between the 12 experimental and calculated cumulated biogas curves didn't take into account test 2 13 (rice mix 6% TS) because the parameters considered in the proposed model were 14 derived for a lower OLR.

15 A comparison of the results obtained from rice mixture fed at 3% TS at the two 16 different scales may be performed on the grounds of methane production. Pilot scale 17 supplied a value (0.312 methane $Nm^3/kgvs$) that is equal to 81% of the one obtained on semi-pilot scale (0.386 methane Nm³/kgvs, see Table 3). A study performed in the same 18 19 apparatus and operating conditions, on a mix of vegetable wastes, returned a ratio of 20 approximately 0.76 between the methane specific production obtained on a semi-21 continuous mode (0.223 Nm³/kgvs added) and the methane specific production obtained 22 on a fed-batch mode (0.294 Nm³/kgvs added) [39].

Hypothesizing the valorization of biogas generated by AD fed with 3% TS in a CHP
unit, the potential specific energy production of the single mixtures was broadly

calculated (see Table 4). On the grounds of the evaluated scale effect, a precautionary
 conversion factor equal to 0.75 was applied to the results gathered from the semi-pilot
 scale tests (see Table 3). CHP electric and thermal efficiency values were considered as
 in Ruffino et al. [39].



5

Figure 7. pH and FOS/TAC trends measured during the pilot scale test performed on
 lees (4.3%TS) (the arrow shows the starting of sodium bicarbonate addition to the feed)

8

waste	primary energy production	gross electric energy production	gross thermal energy production
coffee mix	1.98	0.69	0.83
hazelnut mix	3.02	1.06	1.27
rice mix	2.90	1.01	1.22
sweets no cocoa mix	3.44	1.21	1.45
sweets cocoa mix	3.08	1.08	1.29
wine mix	4.14	1.45	1.74

9

10 **Table 4.** Preliminary evaluation of the potential energetic valorization of the biogas

11 generated by the considered wastes (AD fed with 3% TS). Data are expressed in

12 kWh/kgvs.





Figure 8. Daily biogas production gathered from pilot scale tests (test 1, 3% TS) on rice mixture Vs expected daily biogas production



4 Figure 9. Cumulative biogas production gathered from pilot scale tests (test 1, 3% TS)
5 on rice mixture Vs expected daily biogas production

6 5. CONCLUSIONS

7 The employed test procedure allowed the comparison of the implementation of AD on 8 different homogeneous mixtures of industrial food wastes without any pre-treatment. 9 The proposed semi-pilot scale procedure was easy to manage, reliable with 10 heterogeneous substrates, likely to prevent inhibition of methanogenesis. Fed-batch 11 mode revealed itself as a valuable tool to avoid an overload of the system, and to 12 achieve biogas yields higher than literature values obtained from BMP tests. Despite the 13 differences about the scale of the reactors and the feeding mode, the results gathered from semi-pilot and pilot scale tests exhibited a good consistency (0.81 coefficient about methane production). The proposed model, based on the values of k_{dis}, was employed as a control tool during the pilot scale tests and it appeared adequate for the evaluation of the scale-up of the AD process. The observed differences between experimental and calculated values at the two scales were around 7-8%.

6 The obtained results in terms of biogas production and VS/TS consumption are the 7 consequence of a preliminary investigation towards mixtures of homogeneous wastes, 8 however the performed tests demonstrated that the studied substrates may be considered 9 interesting matrices to be degraded in mono-digestion processes.

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