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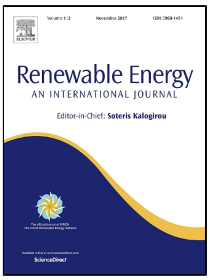

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PII:	S0960-1481(17)30662-6
DOI:	10.1016/j.renene.2017.07.047
Reference:	RENE 9022
To appear in:	*Renewable Energy*
Received Date:	06 October 2016
Revised Date:	12 March 2017
Accepted Date:	10 July 2017

Please cite this article as: M. Gottardo, F. Micolucci, David Bolzonella, Hinrich Uellendahl, Paolo Pavan, Pilot scale fermentation coupled with anaerobic digestion of food waste - Effect of dynamic digestate recirculation, *Renewable Energy* (2017), doi: 10.1016/j.renene.2017.07.047

PILOT SCALE FERMENTATION COUPLED WITH ANAEROBIC DIGESTION OF FOOD WASTE - EFFECT OF DYNAMIC DIGESTATE RECIRCULATION

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Abstract

The anaerobic digestion in double stage is a known and adopted system, but the process productivity and optimization still remain an aspect to investigate. The accumulation of organic acids (produced during fermentative metabolism) in the first stage generally decrease the pH below the optimal values (5.5). A pre-evaluation strategy by control charts for further pH control is proposed. The process combines in series the 1st Fermentation process and the 2nd Anaerobic Digestion process, using the recirculation of the anaerobic digestion effluent, rich in buffer agents, to control the pH in the 1st stage. The recycle ratio becomes a further operating parameter that should be properly managed. A proper management as dynamic recirculation flow allows to maintain the pH of the first phase to values higher than 5. Specific hydrogen production, specific methane production and volatile fatty acid production; 170 L/kgTVS at 40% H_2, 750 L at 67%

CH_4 and 14 gCOD/L VFA were obtained respectively.

Keywords

Hydrogen, methane, volatile fatty acids, process control strategy, control chart, multivariate analysis, anaerobic digestion, food waste

1. INTRODUCTION

Anaerobic digestion (AD) is a widespread and well known technology to treat organic waste of diverse stocks [1]. In the past it was considered as a system to manage the municipal waste. Nowadays the development of door-to-door separated waste collection makes the food waste an interesting source for energy and material production, and the AD becomes one of the main bioenergy processes able to answer to increasing energy demand. A further developing view of the AD process is to consider and manage it as a real production process [2]; therefore, the production should be maximized and its quality standardised.

AD involves different microorganism that through synergic way allow not only the production of methane but also other valuable products, hydrogen and volatile fatty acids [3]. In order to extract these different products, AD has to be split into two main phases [4][5][6].

The first phase of fermentation includes the step of hydrolysis, acidogenesis and part of the acetogenesis, instead the second phase substantially optimizes the last step, the methanogenesis. Therefore, through the optimization of the fermentation, hydrogen (gas), volatile fatty acid (liquid) and other low weight organic compounds such as alcohols and lactic acid [7] can be obtained. The VFAs can be used as external carbon source for biopolymers production, such as poly-hydroxyl-alkanoates [8][9].

Double stage AD is a known and adopted system, but the process productivity depends on HRT

distribution between the two phases and pH control in the fermentation (1st stage). In fact, HRT and pH control can affect (inhibit or promote) several metabolic pathways and consequently the production of volatile fatty acids and hydrogen.

At the first two stages AD process have been suggested to adjust the physiological conditions requirements by the respective microbes involved in the different process stages. The optimal pH values for the 1st and 2nd stage have, for example, been identified as pH 5.0-6.5 (for VFAs production), pH 5.5 (for Hydrogen production) and pH 7-8 (for Methane production), respectively [10][11].

The accumulation of organic acids (produced during fermentative metabolism) in the first stage generally decrease the pH [12] below the optimal values. Recently some authors [13][14] proposed a strategy for pH control, coupling in series the 1st fermentation process with a 2nd anaerobic digestion process and using the recirculation of the anaerobic digestion effluent, rich in buffer agents, to control the pH in the 1st stage.

The recycle ratio becomes a further operating parameter that should be properly managed. The literature points out how to work with an excessive recirculation may result in a gathering of ammonia in the system and consequently into an inhibition of methanogenic [15] and hydrogenogenic processes [12]. Conversely recirculation ratios too low may be insufficient to control the pH of the reaction medium where the hydrogenogenic process occurs. For instance, Gottardo et al. [16] investigated the influence of ammonia in the biological process of hydrogen production via dark fermentation focusing on the recirculation ratio that allows to reach a stable process. For this purpose, the authors tested four different recirculation ratio (0.33; 0.42; 0.66; 1) keeping constant the ammonia concentration in the recirculated flow (about 500 mg/L) through a separation process by evaporation. This study showed the impossibility of a stable hydrogenogenic process with constant recirculation ratio, even when ammonia removal system was used. Moreover the authors proved how the range of Recirculation Ratio to control the pH in fermentation reactor is from 0.42 to 0.66.

Many process variables to control the process are involved, hence the process monitoring and fault detection are very important tasks in this biological engineering systems since they aim to ensure the success of the planned operations and to improve the productivity [2]. Since the complexity of AD process, many highly correlated variables are measured and should be subject to considerable misleading in a non-statistical data mining. Further, Wise and Gallagher [17] stated that important information lies not only in any individual variable but also in how the variables change with respect to one another. On basis of these observations AD requires the application of analytical multivariate statistical methods. Multivariate analysis is a method to detect patterns and correlations in large datasets [18] such as the several parameters monitored in anaerobic process. This approach has been used for a long time in the chemical processing, but was only introduced into the industrial wastewater treatment plants in the late 1990s. However, our understanding of the multivariate statistical methods as evaluation to further control the AD processes is lacking in literature.

The aim of this work was the study of recirculation ratio effect by multivariate methods in order to further develop an optimized automatic control able to optimize the hydrogen and/or VFAs production in the first phase, and methane generation in the second methanogenic one. Multivariate analysis, focus on pH role, allowed to better understand the behaviour on recirculation ratio variance.

2. MATERIAL AND METHODS

Initially, it has to be focused which region within the domain of possible values of the recirculation ratio to consider. This way can eliminate a variable from the system. In the case to operate in the region marked by high recirculation ratios, close to 1, the process control attention will be paid exclusively to the content of ammonia in the system, that accumulates persistently. Conversely, in the case to operate in the region characterized by low circulation ratio, next to 0.3, the goal will be to verify if this ratio is largely sufficient to ensure an effective and lasting control of the pH in the reaction medium of the fermentation process.

For this purpose, the experimental test was divided in three periods (RUNs): in RUN1 the recirculation ratio was kept on 0.4 during overall period while in RUN2 and RUN3 it was kept variable between 0.4 - 0.6 and 0.5 – 0.7 respectively, with a frequency of three weeks. In each trial of this study we wanted to understand the influence of each recirculation ratio choice has exercised alongside the fermentation process and the methanogenic process.

Table 1: Operational conditions applied during the experimental test

Parameters	units	RUN1	RUN2	RUN3
HRT 1 phase	d	3.3	3.3	3.3
HRT 2 phase	d	12.6	12.6	12.6
OLR 1 phase	KgTVS/(m^3.d)	17	17	17
OLR 2 phase	KgTVS/(m^3.d)	3.5	3.5	3.5
Recirculation Ratio	-	0.4	0.4 - 0.6	0.5 - 0.7

2.1. Analytical methods

Substrates and digestates of both reactors were monitored three times a week in terms of total and volatile solids (VS), soluble (sCOD) and total chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP). Process stability parameters, namely pH, VFAs, free ammonia (NH_3), total (T.ALK) and partial alkalinity (P.ALK) were checked daily. All the analyses, except for VFA and NH_3, were carried out in accordance with the Standard Methods [19].

NH_3 was determined from the equilibrium relationship with $N\text{-}NH_4^+$ (AMM) in soluble in the aqueous fraction as specified by Anthonisen et al. [20]. VFAs content was monitored using a gas chromatograph (GC) (Carlo Erba instruments) with hydrogen as gas carrier, equipped with a Fused Silica Capillary Column (Supelco Nukol TM, 15 m x 0.53 mm x 0.5μm film thickness) and with a flame ionization detector (200 ^oC). The temperature during the analysis started from 80 ^oC and

reaches 200 °C through two other steps at 140 °C and 160 °C, with a rate of 10 °C/min. Samples were centrifuged and filtrated on a 0.45 μm membrane prior analysis. Biogas production was measured with two flowmeters (Ritter Company, drum-type wet-test volumetric gas meters), fitted on the reactors. The specific methane production (SMP) was determined using the methane concentration in biogas which was measured by a GC equipped with a HP-Molesieve column (30 m x 0.3 mm x 0.25 μm film thickness) employing thermal conductivity detection (TCD).

2.2. Data analysis

According to Costa et al., [21] PCA is intended as a worthwhile chemometric technique when an effective reduction of the multidimensional space into few components is achieved, maintaining data variability. PCA provides an approximation of a dataset bringing back two matrices in reply: the matrix of scores and the matrix of loadings. In summary, these matrices capture the essential data patterns of the original dataset. Plotting the columns of the scores matrix gives a graph named score plot, where the relationship between observations is displayed and so clusters can be identified. Plotting the columns of the loading matrix returns another graph named loading plot, where the relationship between variables is showed. In this way, the power importance analysis of variables to identify clusters is accomplished.

2.3. Substrate and inoculum

The anaerobic digested sludge used as inoculum for the methanogenic reactors (single stage and second phase) was collected in the WWTP located in Treviso (northern Italy) where a 2000 m^3 anaerobic digester treats the source collected biowaste at 35 ºC. The sludge was acclimatized for one week to thermophilic temperature [22].

The substrate used in these experimental tests was the food waste from door-to-door collection of Treviso Municipality. The amount of total solids of biowaste used was 28% with a total volatile solids (TVS) on TS content of 92%. Regarding the content of nutrients, table 2 shows how food

150 waste used in this study was characterized by an adequate nutrients ratio, particularly COD:N ratio
151 with an average value of 41.
152 The fermentative reactor (first phase) was inoculated with food waste and water and then regularly
153 fed with separately collected food waste and water in order to reach the volume required.
154

155 *2.4. Reactor set-up*

156 The reactors used were made of stainless steel AISI-304 with a working volume of 230 L for one-
157 stage digester, and with reference to the two-stage process of 200 L for the fermentation unit and
158 380 L for the digester unit. Mechanical anchor agitators ensured the mixing in order to maximize
159 the degree of homogenization inside the reactor. The working temperature was set at 55°C ± 0.1
160 (thermophilic temperature range) and maintained by an external jacket for both reactors. The
161 reactors were slightly pressurised at 0.01 atm.
162

163 *2.5. First stage (Hydrolysis) batch tests*

164 Batch tests were carried out to determine the hydrolysis rate of food waste fermentation.
165 This part of the study was performed in order to investigate the hydrolysis in batch tests and the
166 effective amount of volatile acids (VA) produced in relation to the pH. Hydrolysis potential batch
167 tests (HPB) were carried out to determine the amount of VFAs and Lactic Acid (LA) production of
168 the food waste with tap water in thermophilic condition.
169 First batch test was set up in triplicate mimicking the fermenter using different food waste to water
170 ratio in order to determine the amount of VFAs and LA produced and observe the change in pH
171 while the hydrolysis proceeds. Afterwards all the vials were flushed with a mixture of N_2 and CO_2
172 (80% and 20% respectively). These batch tests were performed in 1L vials and were run for one
173 week. Vials were closed with a thick butyl rubber stopper which was hold in place by sealing with
174 an aluminium crimp. Everyday, samples were taken for pH, VFAs and LA analysis and hydrogen
175 production. The pH was measured using pH meter and VFAs analysis performed. As suspect of

lactic acid production, some representative samples were analysed with the HPLC. The procedure for lactic analysis, 2M H_2SO_4 was used during sample preparation and the analysis was conducted using a HPLC (Ultimate 3000 Dionex^{TM}); HPLC on a Dionex Ultimate 3000-LC system (Dionex Corporation, Sunnyvale, CA, USA) with an Aminex® HPX-87H column coupled to a refractive index detector. As mobile phase H_2SO_4 (4 mM) was used, with a flow rate of 0.6 ml/min at 60°C. All chromatograms were integrated using the Chromeleon software (Dionex Corporation).

The bio-hydrogen produced was also measured with the GC abovementioned. Total alkalinity was measured during the trial. Methane (CH_4) production in the different vials was analysed by injecting gas samples from the headspace of each vial into the abovementioned GC for methane analysis and the batch vials were degasified whenever over-pressure of more than 1 bar was detected. Methane was analysed in order to understand when the hydrolysis in batch switched to a methanogenic activity.

3. RESULT AND DISCUSSION

3.1. First phase

The scope to find a suitable management of the process led to an accurate analysis the proper recirculation ratio to adopt. To control the pH in the first stage by means of the digestate recirculation is advantageous economically, however it must be operated appropriately, otherwise the process itself leads to instability. Micolucci et al. [14] have shown how working with a high recirculation ratio would lead to an accumulation of ammonia in the system, able to inhibit both the methanogen consortium that the hydrogenogenic process. For this aim, three runs were tested; in each run a different strategy for the controlling of the pH were applied. In RUN1 the recirculation ratio was maintained to 0.4 for overall period, instead the RUN2 and RUN3 were operated with a variable recirculation ratio between 0.4 – 0.6 and 0.5 – 0.7 respectively, by varying this parameter alternately with a three-week frequency.

In figure 1, the trend of pH and VFA for three RUNs is presented.

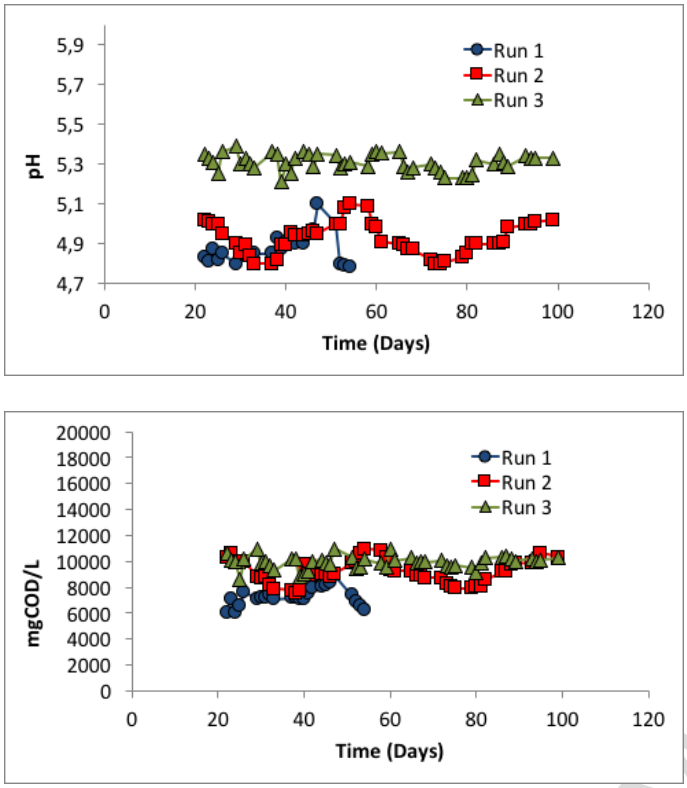
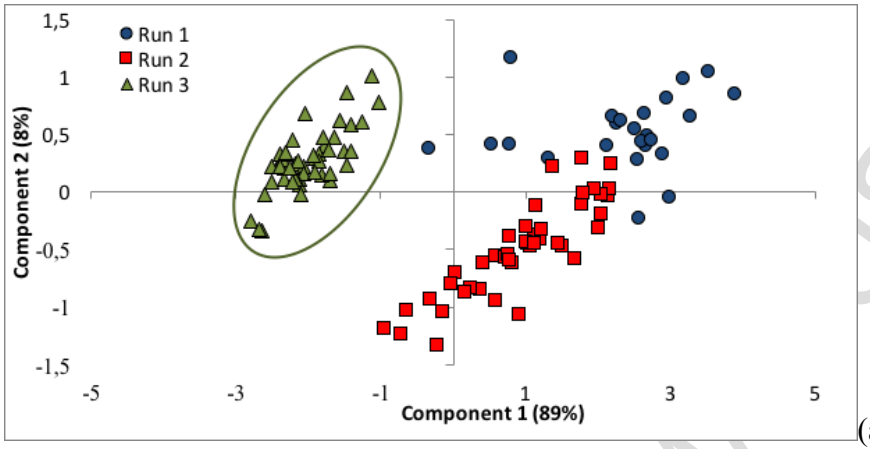


Figure 1: first phase pH and VFA trend during the three RUNs.

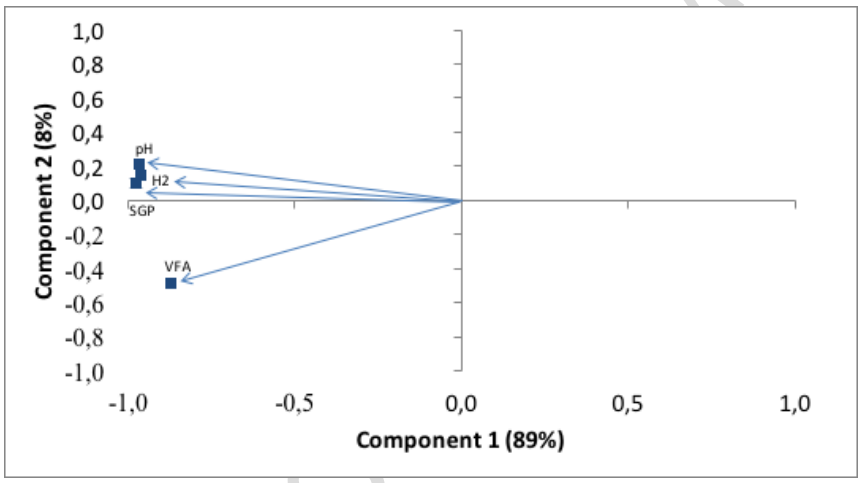
The figure 1 shows how the RUN3 was the sole run where the pH was kept above 5 for the overall experimental trial. During the RUN1 the pH of the reaction medium has exceeded the value 5 only towards the end of the trial and moreover it was able to remain in this condition for a very short time. The low pH value of the reaction medium has adversely affected the hydrogenogenic activity reporting a low production of hydrogen (27 LH_2/kgVS) and VFAs (7241 mg/L).

Observing the pH trend of RUN2 (figure 1) it is possible to note the average pH was lower than 5, values above 5 were detected only for a few days at the end of the 0.6 recycle period ratio. In other words, during the RUN2 and RUN3 the alkalinity contribution provided by the digestate was not able to buffer acids produced during the fermentation phase. Also in this case the performance of the VFA and the hydrogen production were affected by the variability of the pH, 37 LH_2/kgVS and 9,185 mg/L respectively.

To understand the different strategies effects of recirculation ratio applied on the production variables, principal component analysis (PCA) was used. PCA allows to reduce the multidimensional space into few components and therefore to study the relationship among variables and objects in the modelled space formed by principal components (PCs), saving data variability. Figure 2 shows the score and loading plots formed by the first and second PC (explained variances were designated in parenthesis).



(a)



(b)

Figure 2. Score (a) and loading (b) plot

Observing the loading plot (figure 2b) we can note how the pH was directly correlated with volatile fatty acids and hydrogen production. These evidences are in according with Cavinato [13] and Baronofsky [23]. The latter authors showed how the acetic acid can inhibit the metabolic activity of *Clostridium thermoaceticum* when the pH of reaction medium was lower than 5. Thus, the lower

production of VFAs could been related to a detoxification mechanism of the cell to avoid the inhibitory effects.

From the Score plot (figure 2a) we can just identify the cluster associated to the RUN3 (green ellipse) while a portion of the cases associated to the RUN1 and RUN2 showed themselves not distinguishable. Higher pH, VFAs yields, specific gas production (SGP) and $\%H_2$ characterize RUN3 than the other RUNs. Moreover, in RUN1 and RUN2 we note a higher and non-random variability than RUN3.

For the study of the variability of the RUN1 and RUN2, the multivariate control chart approach was adopted [24].

RUN1 the control chart (figure 3) shows that in the period in which the reaction medium has exceeded value 5, which is returned to the desired range, the process has highlighted very different characteristics compared to the previous condition. In particular, in the production of volatile fatty acids and the specific biogas production.

To understand the direction these variables have been taking in order to determine the shift of the process, a reduction of the dimensionality of the problem was performed, through the use of the principal components and the application of the Shewhart control chart.

The first principal component extracted 77% of the total information and it was sufficient to describe the problem based on Rank Analysis criteria.

The x-bar chart RUN1 (x bar chart figure 3) confirmed an outside control signal which is much above the Control Limit (3σ). Whereas the loadings of the first component we can underline as the out of control signal was due to high values of all the variables considered: pH 0.90 (first PC), VFA 0.88, 0.83 SGP, H_2 0.85.

On reaching the pH value of the first phase to values greater than 5, the fermentation process has highlighted an important change of condition. The system switched from a purely solvatogenic condition, characterized by a low production of VFA and hydrogen, to an acidogenic one, vice

versa characterized by an increased production of volatile fatty acids and hydrogen. Several studies have shown that when too acid pH conditions inhibit the production of VFA [25], Zhang et al. have highlighted a pH range for the production of VFA between 5 and 11, with a value of 7 as optimum. This could be explained by the decouple capacity of the VFA demonstrated at pH values < 5. It is finally noted that the increase of the hydrogen production is mostly due to the increase of the SGP, instead of the hydrogen percentage in the biogas produced. In general, there was a positive correlation between the pH, the specific hydrogen production and VFAs production, which confirmed what we wanted to demonstrate.

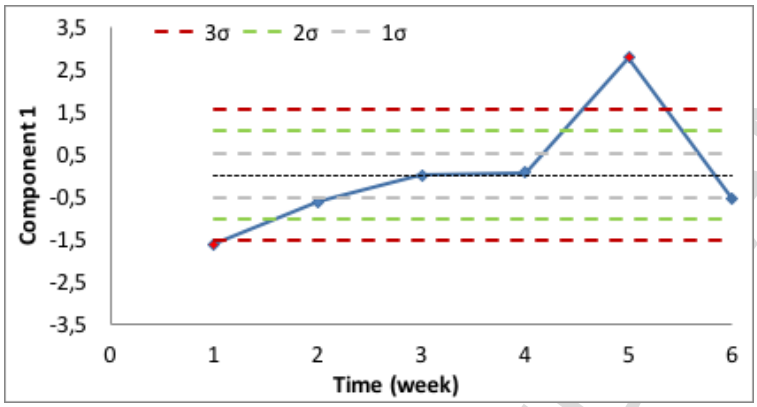


Figure 3. X bar chart of the RUN1

As a result of the accumulation of volatile fatty acids in the reaction medium, the alkalinity fed with the recirculated digestate was not able to maintain a condition of pH to a value greater than 5. A consequence of this the process switched back to the previous condition. In conclusion of this first RUN1 it was not possible to maintain the pH of the fermentation process above 5 through the use of a constant recirculation ratio equal to 0.4.

Through the RUN2 the first principal component describes the data to 90% and is therefore also in this case sufficient to describe the process.

The x bar chart (figure 4) confirmed the hypothesis expressed in the previous RUN1. The oscillatory trend of the principal component in the x-bar shows how the process does not respond to

a single distribution but two partially overlapping. On the basis of the considerations in the RUN1, also in the RUN2 is possible to consider that the recirculation ratio strategy adopted in RUN2 swung the process in two different conditions, one acidogenic and one solvatogenic. Also in this trial is decisive the pH contribution to promote the two processes.

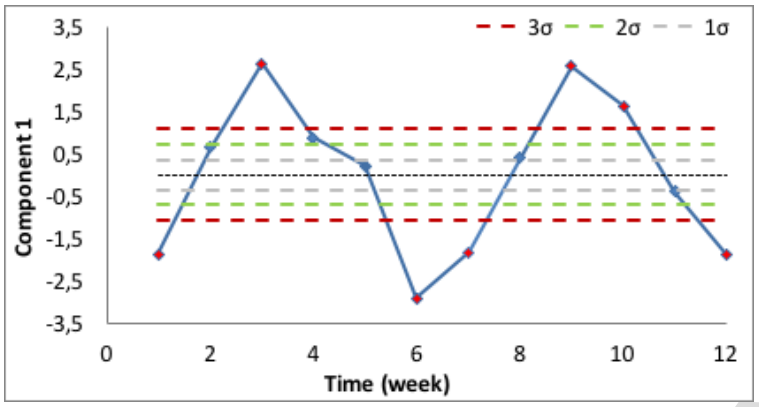


Figure 4. x bar chart of the RUN2

The fermentation process in RUN3 is most suitable for the production of VFA and hydrogen. The choice to operate with a variable recirculation ratio of 0.5 and 0.7 has allowed the accumulation of HCO_3^- in the reaction medium. It has favoured the establishment of a buffer capacity which ensured the process stability even in the period of 0.5 recirculation ratio.

The trial RUN3 never left its optimum fermentation environment (cluster analysis figure 3), one that is within the pH above 5. Moreover, in this case a control chart does not show points out of control due to metabolic switch toward solvatogenic neither methanogenic conditions. Unlike other approaches, the pH of the first stage is maintained for the entire experiment above 5 and it was not affected by the fluctuation of the recirculation ratio. Better performance on VFAs production (table 3 shows the main chemical - physical characteristics of the reaction medium, the stability parameters and production yields related to the fermentation process hydrogenogenic during RUN3), biogas composition and yields on the first stage.

The fermentation of RUN3 process is more suitable for the production of hydrogen and VFAs.

Table 3: stability parameters, chemical-physical characteristics and process yields for the Ist phase RUN3

	Parameter	M.U.	Average ± St.Dev.	Min	Max
I° PHASE	TS	gTS/Kg	53 ± 5	46	61
	TVS	gTVS/Kg	44 ± 4	39	46
	COD	gO_2/Kg	52 ± 9	41	63
	TKN	gN/Kg	1.6 ± 0.7	0.9	2.6
	P tot	gP/Kg	0.48 ± 0.10	0.45	0.50
	pH	-	5.3 ± 0.1	5.21	5.39
	VFA	mgO_2/L	13,920 ± 488	11,616	14,957
	Total Ammonia	$mgN\text{-}NH_4^+$/L	687 ± 5	678	696
	SGP	Nm^3/KgTVS	0.170 ± 0.010	0.165	0.172
	Gas Production Rate (GPR)	$Nm^3/(m^3.d)$	2.88 ± 0.04	2.72	2.95
	H_2	%	40 ± 2	36	44
	CO_2		52 ± 2	47	58
	CH_4		7 ± 1	5	10

3.2. Hydrolysis batch tests

Different amounts of water were added to mimic a higher water saturation of the waste. The results

in table 4 reveal that the VFA concentration increased with adding less water, but the total amount of VFA released from the waste decreased with lower water saturation. The highest VFA release (1.52 g) corresponded to a conversion efficiency of the total organic matter (with a VS content of 27%) into VFA of 24.8%.

Table 4. VFA release in batch hydrolysis setup with different amounts of water added

Set-up	OFMSW (g)	H_2O (mL)	Waste/percolate ratio	VFAs (g/L)	gVFA/gOFMSW (%)
A	23.4	300.0	7.8%	5.05	6.5
B	23.4	221.0	10.6%	6.17	5.8
C	23.4	158.0	14.8%	7.55	5.0
D	23.4	78.8	29.7%	11.30	3.8

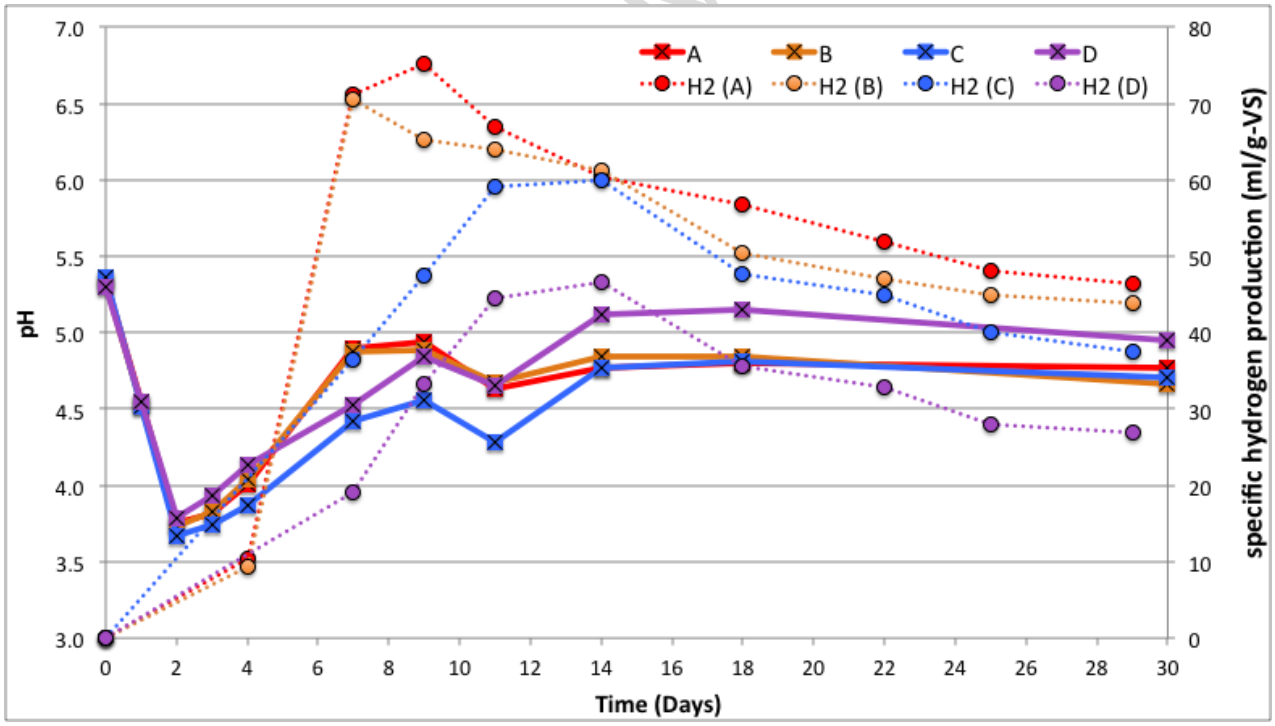


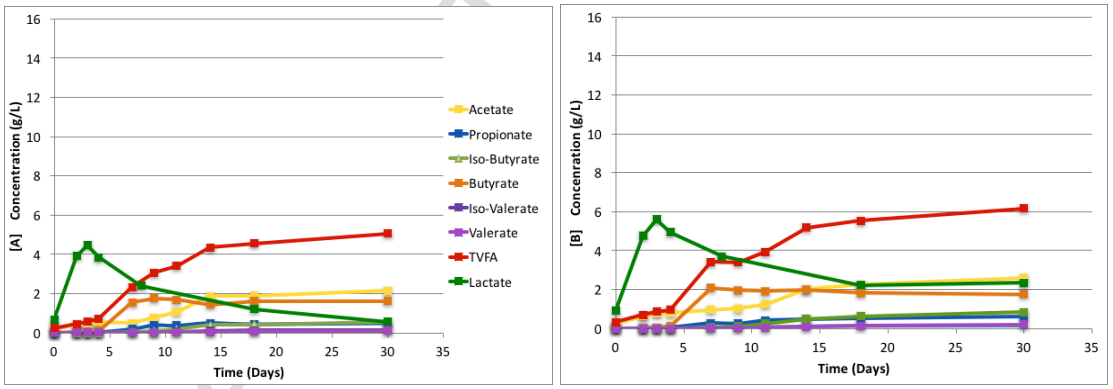
Figure 5. Hydrogen production during the second HPB trials, dotted lines refer to specific hydrogen production.

The production of hydrogen was detected and the data showed great variability amongst the batch

set-up. All samples showed an increase in hydrogen production over the first 10 days and eventually a decrease. Very low hydrogen production was detected when pH was below 4.5.

For all the samples, the pH fell rapidly in the first 2-3 days to around 3.70 before it rose again and reached a plateau (figure 5). The overall pH of each setting, after 30 days, has no significant differences; data after 30 days showed a standard deviation of 0.13. As expected, there is evidence of high production of lactic acid in all samples of the first 4 days that is responsible for the rapid drop in pH of all batch setup (figure 5). The maximum concentration of lactic acid ranges from 4.5 to 14 g/L. The higher waste/percolate ratio was, the higher the concentration of lactic acid was detected.

It is therefore important to not have too high the organic loading in the reactor setup as this would result in unwanted lactic acid production or to control the pH of the fermentation phase above pH 5. There is a high possibility that lactic acid is produced in the reactor setup below pH 5 [23]. However, it can be seen that the high lactic acid concentration for every batch setup correlate to a pH below 4.5 [26]. Therefore, lactic acid production could have already been avoided in the reactor as the pH will be keep strictly in the range of 5-5.5, by a dynamic digestate recirculation.



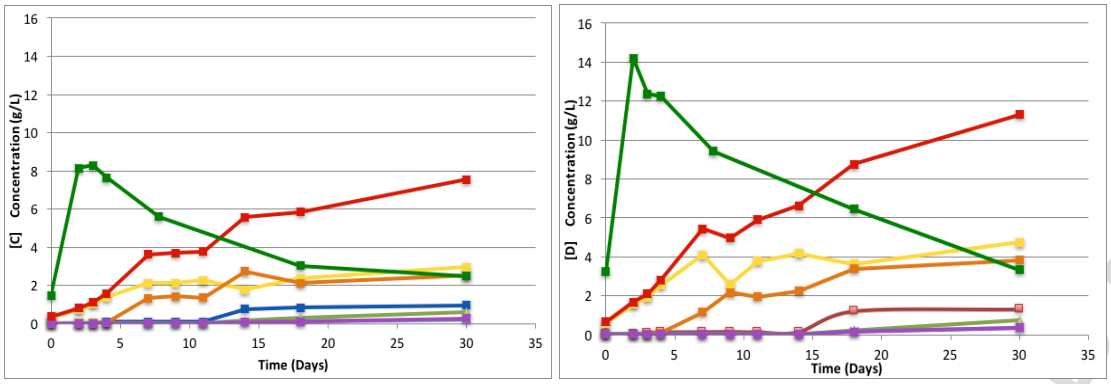


Figure 6. Lactic acid and VFAs production of the four hydrolysis batch tests.

Likewise, the VFA concentration increased as the water amount decreased. The concentration of VFA for A, B, C, D after 30 days were 5.05 g/L, 6.17 g/L, 7.55 g/L and 11.30 g/L respectively. However, the VFAs in grams for A, B, C and D were 1.51 g/L, 1.36 g/L, 1.19 g/L and 0.89 g/L.

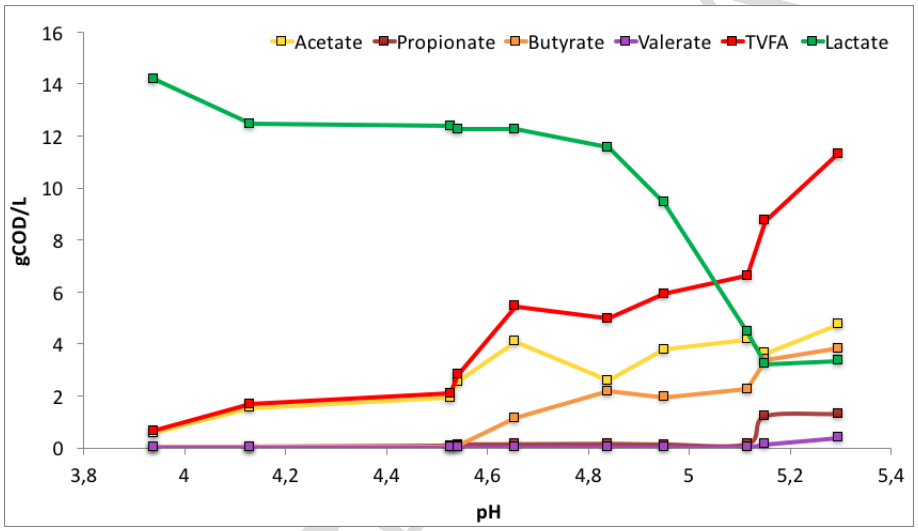


Figure 7. Lactic Acid and Volatile Fatty Acid production related to pH tendency on HBT.

In figure 7, the volatile acids on pH function are reported. It is possible to underline in what way below pH 5 the lactic acid is predominant, on the contrary the VFAs production, particularly acetic acid and butyric acid is noticeably incremented at pH values higher than 5. It is demonstrated how above pH 5 volatile fatty acid production is enhanced, this is well correlated with literature data of Valdez-Vazquez and Poggi-Varaldo [27].

1.1. Second phase

As regards the second phase, in the next figure 8 the stability parameters trends are reported.

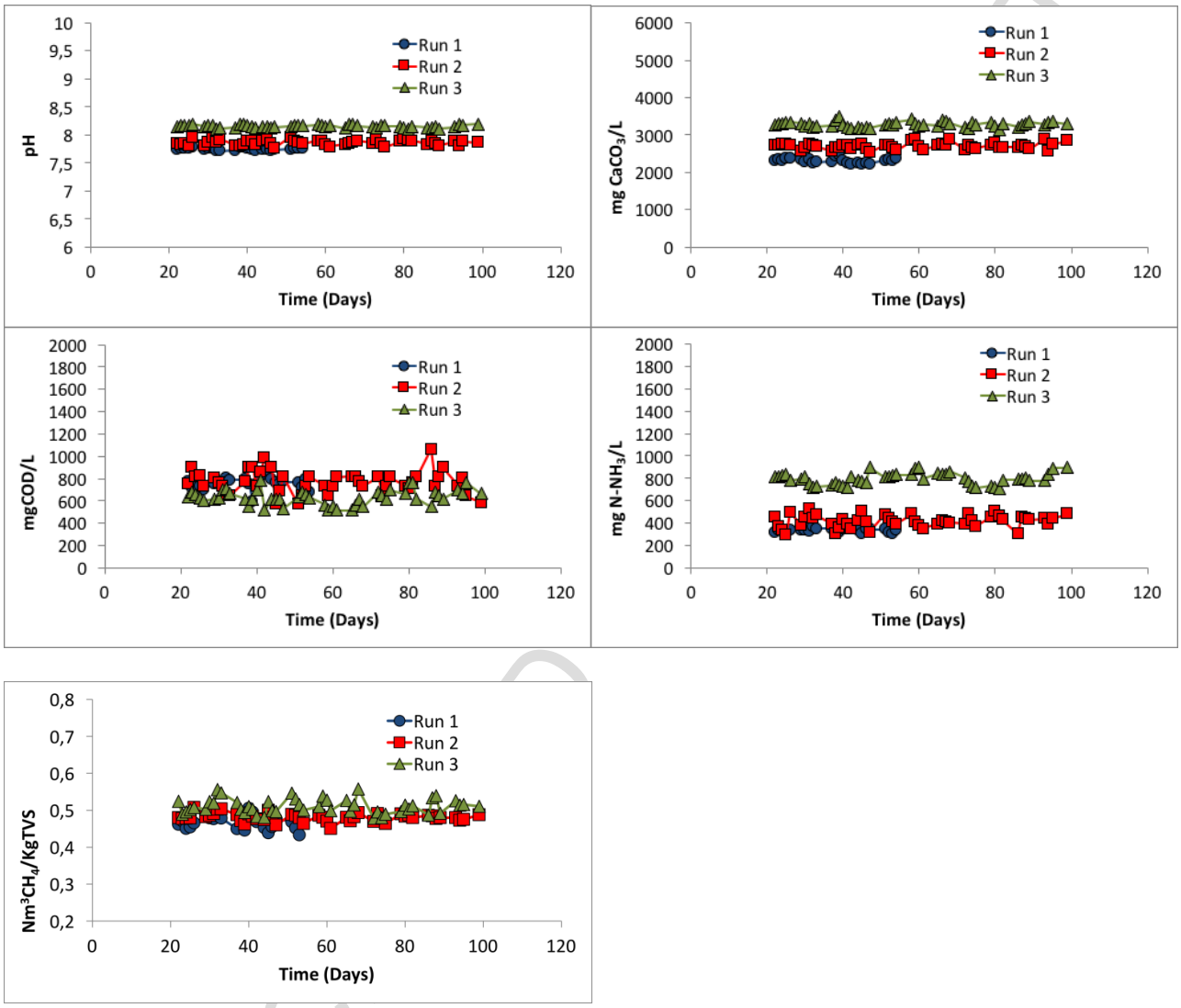
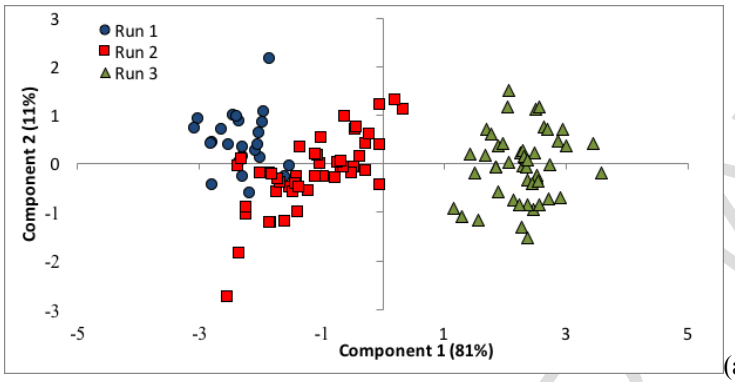


Figure 8. Stability parameters during the three RUNs in the second methanogen phase.

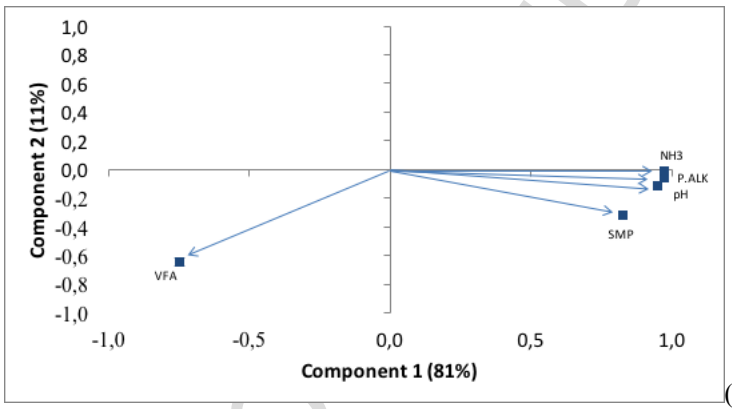
Based on univariate analysis reported in Figure 8 it seems difficult to differentiate the various RUNs characteristics except for few parameters. This is why it becomes necessary to proceed through a cluster analysis. As for the study of the first phase, in the second phase we have proceeded with a cluster analysis.

Figure 9 shows the score and loading plots formed by the first and second PC.

Like the previous results, the Score Plot allows the first principal component, which extracts 78% of the information overall, to show a clear separation among the observations on the RUN3 among the other RUNs. This analytical methodology highlights how recirculation ratio 0.4 and ratio 0.4 – 0.6 (variable) did not produced a visible change in the characterization of the methanogenic process. On the other hand, the recirculation ratio of RUN 3 allowed to obtain a distinguishable process among the previous RUNs, based on the 5 variables considered (pH, NH_3, alkalinity, SMP, VFA). The obtained result underlined that it was necessary to analyse the role of these variables that helped to distinguish the methanogenic process RUN 3.



(a)



(b)

Figure 9. Score plot (a) and Loading plot (b) of the second methanogenic phase RUNs.

The analysis of the Score plot jointly the Loading Plot highlights the role of the five variables. In particular, the loading plot shows how the RUN3 is distinct from the other two RUN tests for a higher specific production of methane as well as for a higher partial alkalinity and pH. Whereas RUN3 is the only test where the pH in the fermentation reactor was maintained at pH > 5 this

makes reasonable to think the pH in the fermentation stage enables not only the management of the operation of the fermentation process (aimed at production of hydrogen and VFA) but also allows a greater efficiency of substrate/product conversion, demonstrated by higher values of SMP and lower concentrations of VFA observed in the RUN3, at the same time a better resilience to the stability of the methanogenic phase, demonstrated by a better buffering capacity observed in the RUN3. Moreover, in the RUN3 a higher partial alkalinity can be explained by the better efficiency of VFA conversion, in fact, it is clear how the two parameters are inversely related, but also by the pH in the fermentation phase. As also reported by Cavinato et al. [28], a higher value of pH in the first stage of the reactor favours the accumulation of HCO_3^- which therefore is subsequently fed into the reactor of the second stage. This could help to support a higher concentration of this ion in solution and therefore a better buffering capacity. The RUN3 is also characterized by a higher accumulation of free ammonia. This is due both to the higher recirculation ratio and the higher pH value. However, it was not observed inhibition borne by the methanogenic component.

Table 5 shows the main chemical - physical characteristics of the reaction medium, the stability parameters and the production yields related to the methanogenic process during RUN3.

Table 5. Stability parameters, chemical-physical characteristics and process yields for the IInd phase RUN3

	Parameter	M.U.	Average ± St.Dev.	Min	Max
II° PHASE	TS	gTS/Kg	23.2 ± 4	26	30
	TVS	gTVS/Kg	16 ± 3	10	21
	COD	gO_2/Kg	20 ± 2	19	23
	TKN	gN/Kg	1.5 ± 0.2	1.0	1.8

P tot	gP/Kg	0.21 ± 0.01	0.10	0.25
pH	-	8.15 ± 0.10	8.10	8.20
P. Alkalinity	$mgCaCO_3/L$	3,283 ± 73	3,145	3,498
T. Alkalinity		5,256 ± 50	5,157	5,376
VFA	mgO_2/L	631 ± 72	449	781
Total Ammonia	$mgN\text{-}NH_4^+/L$	1,539 ± 148	1,290	1,885
Free Ammonia	$mgN\text{-}NH_3/L$	794 ± 52	706	898
SGP	$Nm^3/KgTVS$	0.75 ± 0.02	0.71	0.79
GPR	$Nm^3/(m^3.d)$	2.50 ± 0.10	2.37	2.77
CH_4	%	67 ± 2	64	70
CO_2		32 ± 2	29	35

2. CONCLUSIONS

In conclusion, Cluster analysis allowed to understand how among the three processes studied only the RUN3 has shown a different condition, in the direction of a better efficiency of the process, both from yields point of view and through stability process parameters, in particular the higher alkalinity amount in the reaction medium.

A proper management of the recirculation allows to maintain the pH of the first phase to values higher than 5. It allows to foster metabolic hydrogenogenic processes and it seems also to improve the environmental conditions occurring the methanogenic processes, in particular by increasing the alkalinity of the reaction medium.

Acknowledgments

The work was carried out by the financial support of the project PRIN 2012 WISE "Advanced process to sustainable useful innovative products from organic waste".

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- Two-stage anaerobic digestion treating food waste at pilot scale
- Hydrogen, methane and volatile fatty acids production optimization
- Evaluation of recirculation ratio effect by multivariate methods
- Multivariate statistical approach as new tool for development of a control strategy
- Fermentation pH control by control charts is proposed

Composition and characterization of the food waste used in this study

Table 2. Food Waste Characterization

Parameters	units	average ± S.D
TS	g/Kgww*	281.4 ± 40.1
VS	g/Kgww*	259.8 ± 22.9
VS/TS	%	90.1 ± 3
COD	g O_2 /KgTS	1,111 ± 399
TKN	g N /KgTS	29 ± 5
P	g P /KgTS	4.3 ± 0.3

*w.w. wet weight