Acidification as a handicap in the anaerobic digestion of agro-industrial wastes: The role of co-substrate in volatile fatty acids mitigation

K. Aboudi*, X. Gómez-Quiroga, C.J. Álvarez-Gallego and L.I. Romero-García

Chemical Engineering and Food Technology Department, University of Cádiz, Campus Río San Pedro, 11510 Puerto Real, Cádiz, Spain
*kaoutar.aboudi@uca.es

Abstract

In this work, by-products from sugar beet plant after sugar extraction process were anaerobically co-digested with two different livestock manure in semi-continuous reactors. The aim of the research was to evaluate the effect of each manure as co-substrates, (pig manure and cow manure), on the volatile fatty acid mitigation. Similarly, the anaerobic process performance in terms of methane improvement and process stability was analyzed. In this context, different hydraulic retention times (HRT) and organic loading rates (OLR) were studied. The results demonstrated that both manures led to higher methane productions in comparison with individual digestion of sugar beet by-products. However, the beneficial effect of co-digestion was much more pronounced by the use of pig manure than for cow manure addition. Besides, higher OLR of 12.8 gVS/Lr*d (6 days-HRT) was achievable in co-digestion with PM, while OLR of 4.9 gVS/Lr*d (15 days-HRT) was feasible in co-digestion with CM. Only the OLR of 3.3 gVS/Lr*d corresponding to the HRT of 20 days was appropriate in the single digestion of SBB. In the second part of this study, the degree of biomethanation in semi-continuous assays was evaluated with respect of the maximum methane potential obtained in a previous batch study for similar mixtures of substrates. In most reactors, above 50% of methanation has been reached, indicating stable system operation at the corresponding conditions. It was deduced that critical operation conditions in all semi-continuous reactors has been related to acetoclastic methanogens inhibition.

Keywords: anaerobic co-digestion, sugar beet by-products, manure, semi-continuous reactors

Introduction

A large amount of manure produced in livestock facilities are a potential substrate for clean energy production such as biogas [1]. Hence, livestock production in the European Union is higher, being Spain and Germany the largest producers of pigs, while France held the largest number of bovines [2]. Similarly, agro-industrial wastes such as sugarcane bagasse, wheat straw, wheat bran, and many others are cheapest and abundantly available. Their valorization by anaerobic digestion has long been investigated and implemented for converting biomass into bioenergy [3,4]. By-products from sugar beet plant (SBB) after sugar extraction process may be bio-converted into energy through anaerobic digestion [5,6]. This process may be improved and optimized by co-digestion with a biomass with different/complementary characteristics such as manure [6-8]. Sugar beet by-products are rich in carbon while manures are potential substrates to compensate nitrogen deficiency in the anaerobic process. Moreover, manures provide buffer capacity which neutralize acidification of lignocellulosic wastes [9,10]. In this framework/given the background, individual digestion of SBB has shown some drawbacks mainly due to souring effect by higher volatile fatty acid accumulation in the reactor [11]. On the other hand, the manure type plays an important role in the anaerobic process. Hence, some manures resulted more effective in the co-digestion process with agro-industrial wastes than others. This effect returns to the animal digestive tract, the food given to animals in the course of animal husbandry and the synergy between the manure and agro-industrial wastes in co-digestion
[10-13].

Therefore, Evaluation of two manures, cow manure (CM) and pig manure (PM), in co-digestion process with sugar beet by-products was studied in semi-continuous assays. The effect of each manure in biogas production, process performance and mitigation of volatile fatty acids were analyzed.

2. Material and methods

2.2. Substrate

Sugar beet by-products were constituted by a mixture of dried exhausted pulp (85%) and molasses (15%) and were provided by the sugar processing company "Azucarera" (AB-sugar group) in southern of Spain. Cow and pig manures were collected from livestock facilities located in the same region.

2.1. Semi-continuous reactors

Three semi-continuous stirred tank reactors (SCSTR) were used for individual digestion of SBB, co-digestion of SBB with CM, and co-digestion of SBB with PM, at mesophilic temperature conditions (35ºC). Different hydraulic retention times (HRT) and organic loading rates (OLR) were studied as detailed in Table 1. Table 2 shows physic-chemical characteristics of substrates.

Table 1. Semi-continuous stirred tank reactors operation conditions (HRT and OLR)

		HRT (Days)						
Reactors		20	18	15	12	8	6	5
SBB	OLR (gVS/Lr*d)	3.3	3.6					
SBB + PM		4.2	4.7	5.9	7.4	8.5	11.2	12.8
SBB + CM		3.7	4.2	4.9	6.2			

Table 2. Characteristics of the three substrates used in the study

Parameters (Units)	**SBB***	**PM***	**CM***
pH	5.8±0.7	6.4±0.2	6.3±0.6
TS (g/kg)	873.3±1.0	221.6±20.8	211.1±32.8
VS (%TS)	89.5±0.7	71.7±11.8	76.8±11.3
$_{soluble}$COD (gO_2/kg)	61.9±12.0	15.7±3.8	17.2±1.0
$_{total}$COD (gO_2/kg)	146.0±8	44.8±0.2	75.0±4.0
TVFA (gHAc/kg)	1.8±1.2	6.4±12.2	4.6±0.5
Alkalinity ($gCaCO_3$/kg)	3.1±1.2	50.2±0.2	38.3±11.1
$N\text{-}NH4^+$ (gN/kg)	0.2±0.1	3.2±0.7	2.4±0.6
TN (gTN/kg)	12.8±1.3	35.7	32.5±2.8
Ratio C/N	40.8±1.0	13.2	13.8

*Data from [14,15]

2.4. Analytical methods

TS, VS and alkalinity were measured according to APHA Methods [16] 2540B, 2540E and 2320B, respectively.

The COD (total and soluble) was determined by colorimetric techniques using a HACH spectrophotometer (Model: DR/4000 U), according to the method 5220C. Total nitrogen (TN) and ammonium ($N\text{-}NH4^+$) were analysed by distillation (Selecta, Model PronitroII) according to 4500-NH3E method. For soluble constituents measurement, samples were lixiviated and filtered through 0.47 μm filter. Samples for the volatile fatty acids (VFA) analysis were filtered again through a 0.22 μm Teflon® filter and analysed with a gas chromatograph (Shimadzu®, Model GC-2010). The biogas was collected in a 10 L gas-bag (Tedlar®, Model SKC). The biogas volume was daily measured by a high precision drum-type gas meter (Ritter®, Model TG5). Composition of biogas was analysed by a gas chromatograph (Shimadzu®, Model GC-2014).

3. Results and discussion

The Fig.1 depicts the obtained daily methane productions in the three SCSTR at the different HRT studied. Each HRT has been maintained at least for three times the corresponding HRT, to ensure stable conditions.

As shown in the Fig. 1 (a), individual digestion of SBB at the HRT of 20 days was stable for a long period operation with values around 0.84 LCH4/Lr*d. The HRT of 18 days showed similar productions for about one period of this HRT, to then decrease by 44 %, in comparison with the previous HRT.

For SBB+CM reactor, the increase of HRT from 20 days to 18 days and 15 days consecutively, led to improve methane productions by 19 % and 28 %, respectively for each HRT. However, operation at the HRT of 12 days induced methane production decline by 61 %.

In the case of SBB+PM, daily methane productions increased by 24 %, 35 %, 46 %, 55%, 60 %, respectively for HRTs of 18 days, 15 days, 12 days, 8 days and 6 days, indicating a high system performance at higher OLR. Nevertheless, operation at the HRT of 5 days was critical leading to a pronounced decrease on methane generation with only 0.8 LCH4/Lr*d (drop of 28%).

In the three reactors, the daily methane productions drop at lower HRT indicated an overload of the reactor. The release of VFA, as the main intermediates inhibitors, was observed in each of these cases. At higher organic loads, higher volatile fatty acids accumulated in reactors leading to the pH drop, a souring effect, and therefore the inhibition of methanogenic archaea. Furthermore, it was observed that troubleshooting were mainly accompanied by propionic acid accumulation in reactors. Previous studies have reported that SBB is a rich carbonaceous material, suitable to be converted into biogas. However, limitations due to the high acidification resulted as a handicap for the process efficiency. Alkaya et al. [11] reported the increase of VFAs in the AD of sugar beet pulp at higher OLR and lower HRT. However, the use of an external source of alkalinity, decreased acidification by neutralizing the acid pH of reactors. Similarly, in a study of Demirel and Scherrer [17], the individual AD of sugar SBB (sugar beet silage) in SCSTR at six different HRT ranged from 95 to 15 days, chowed better performance at the HRT of 25 days, with higher biogas production. Authors reported that SBB are poor substrates in terms of nutrients such as nitrogen, phosphorus and buffering capacity. In their study, authors regularly used an external source of nutrient to avoid system failure. In the present research, the use of manures as co-substrate has been chosen.

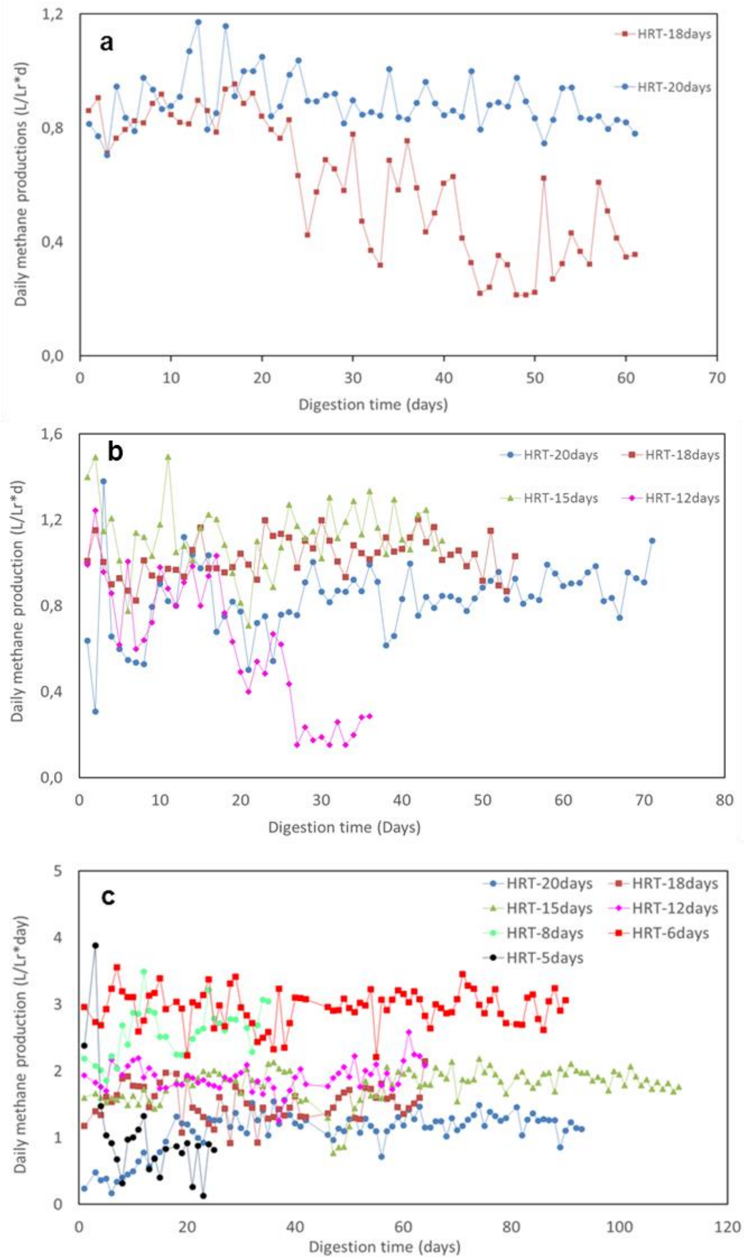

Fig. 1 Daily methane productions in the three semi-continuous reactors at different hydraulic retention times **a**: SBB, **b**: SBB+CM, **c**; SBB+PM

Fig. 2 shows the alkalinity, the total volatile fatty acidity and the propionic concentrations at each operation conditions. In co-digestion reactors, alkalinity was significantly higher than in the single digestion of SBB. Moreover, it was observed that co-digestion with PM showed the highest values of alkalinity, indicating that the PM brings high buffer capacity, able to neutralize VFAs at higher organic loads, which allowed to increase the OLR and decrease the HRT until 6 days. At the critical HRT in each operation condition, reactors were overloaded and VFAs concentrations increased with predominance of propionic acid. Propionic acid is well reported to be a strong inhibitor in the anaerobic process [18,19]. The characteristics of each manure in terms of alkalinity, ammonia, and the variety of microorganisms provided from the tracts animal digestives may be the main reasons for the observed behaviors. Thus, PM had a higher alkalinity value in comparison with CM (Table 2).

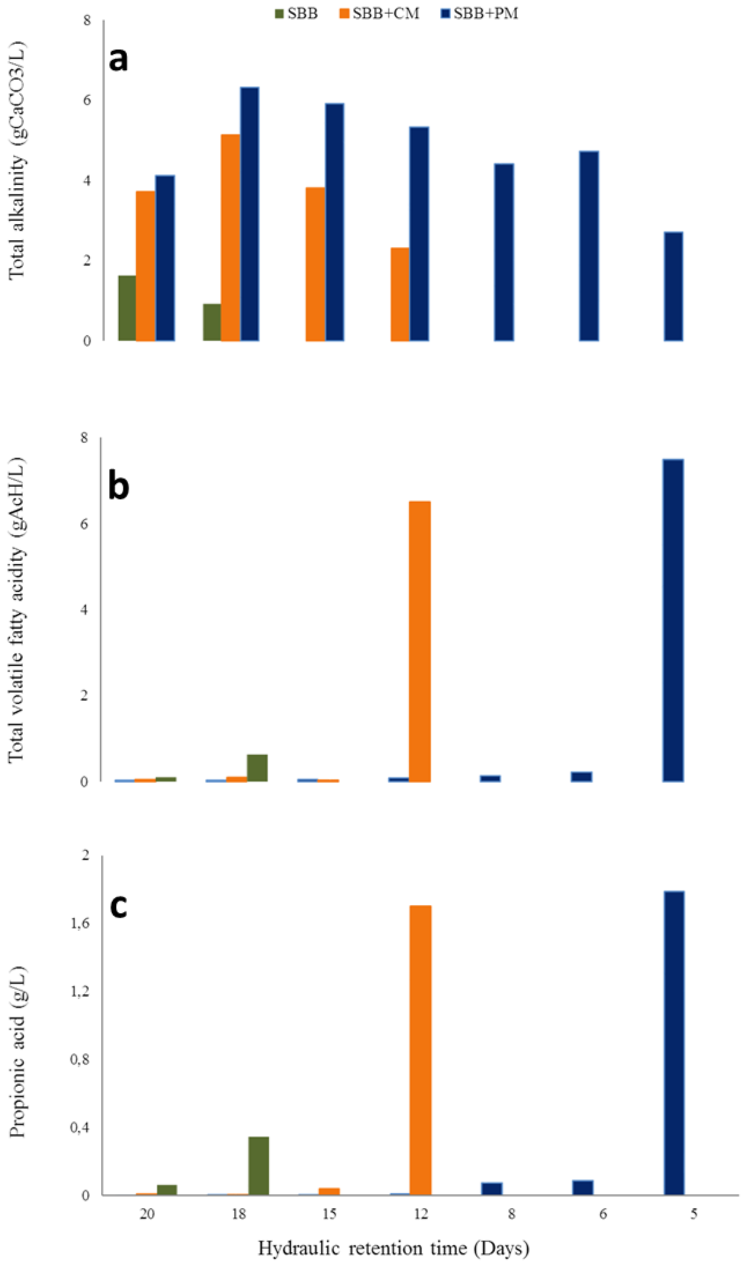

Fig. 2 Comparison of analytical parameters of semi-continuous reactors at the different hydraulic retention times

In previous studies of Aboudi et al. [8,20], authors studied mesophilic batch anaerobic digestion of SBB, SBB+PM and SBB+CM in BMP (biomethane potential) assays. The obtained results showed that the maximum biomethanation potential of SBB, SBB+PM and SBB+CM in the studied conditions were 308.8 $mlCH_4/gVS_{added}$, 464.5 $mlCH_4/gVS_{added}$ and 451.4 $mlCH_4/gVS_{added}$, respectively.

In order to understand the effect of each manure in the semi-continuous process, the biomethanation degree was calculated as the percentage of the achievable of biomethane obtained in semi-continuous assays with respect to the maximum biomethane potential achieved in BMP tests according to eq. 1.

$$Biomethanation\ degree\ (\%) = \frac{100 * SMPsemicontinuous}{MBMPbatch} \qquad \text{Eq.1}$$

$SMP_{semicontinuous}$ is the specific methane production in SCSTR, expressed as $mlCH4/gVS_{added}$. $MBMP_{batch}$ is the maximum biomethane potential obtained in batch assays at similar conditions, expressed as $mlCH4/gVS_{added}$.

Fig.3 shows the obtained biomethanation degree in each assay. For SBB single digestion and at the optimum HRT of 20 days, a higher biomethanation degree of 73% was obtained. On the other hand, in both co-digestion assays, above 50% of biomethanation degree was achievable at the different HRT studied, excepting at the critical HRT tested such as at 5 days in SBB+PM (only 13 %) and at 12 days in SBB+CM (only 14 %).

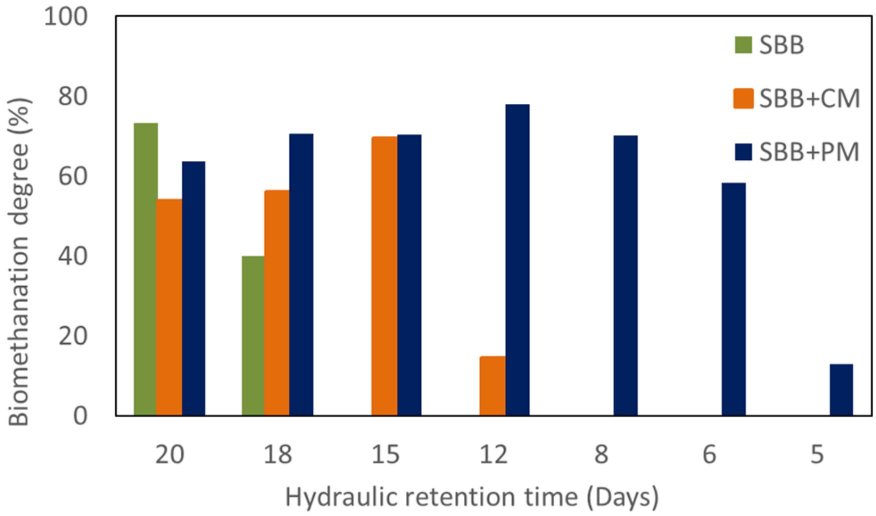

Fig. 3 Biodegradation degree in the three semi-continuous reactors with respect to the maximum biomethane potential in batch assays.

It is noteworthy that microorganisms have enough time for the organic matter degradation in batch system. Thus, the reactor is operating until the completion of the fermentation and microbes are in contact with substrates for a long period. Nevertheless, in semi-continuous assays, and at higher organic loads, microorganisms do not have enough time between every two feeds in a regular feeding interval (i.e. over 24 hours for daily feeding). In critical conditions, wash out of biomass occurs and inhibitory intermediate compounds accumulate. In this context, the behaviour of semi-continuous reactors should not only be considered in terms of methane yield or organic matter removal efficiency, but also in terms of kinetics.

It has been reported that co-digestion with manures features a nutrient balance and C/N ratio in the reactor as well as creating a synergistic effect in the medium [21]. Chen and Zhong [21], reported that despite of the high VFAs concentration in co-digestion reactors of cotton stalk with swine manure in comparison with single digestion of cotton stalk, the anaerobic co-digestion assay showed higher stability with high biogas generation, indicating the role of swine manure on neutralizing acid pH and VFAs in the medium. In the same way, Pagéz-Díaz et al. [22] studied semi-continuous anaerobic digestion of solid cattle slaughterhouse (SB) and SB co-digestion with manure and various crops. They reported a failure of individual digestion of SB. However, stable performance with higher loadings was observed for co-digestion with different manure sources. Authors indicated that this was due to the established synergy effect obtained by mixing substrates with different characteristics.Hence, the extent of biodegradation of complex substrates in co-digestion, explain the observed synergistic effects between substrates in specific operation conditions [23].

Aiming to a comprehensive comparative of the obtained results in the present research, specific methane productions for the three semi-continuous reactors are depicted in Fig.4, The highest SMP from SBB+PM (from 8 to 18 days of

HRT) and the highest SMP from SBB+CM (HRT of 15 days) as representative of the optimal operation area (the blue box). The average value of the five data of SMP is 331 mLCH4/kgSV$_{added}$. According to Smith and Mah [24] and from results of their research using C^{14} tracers in an AD study of sludge, authors observed that the 73 % of the total methane produced in an anaerobic process comes from acetoclastic bacteria pathway, while the 27 % usually comes from the hydrogen-utilizing archaea pathway. In Fig. 4, the black discontinuous line indicate the SMP value corresponding to a 27% of the average SMP obtained (89.5 mLCH4/kgSV$_{added}$).

Using this criteria, SMP from assays carried out at lower and critical HRT in each assay, are slightly below this limit value, indicating that the process was mainly affected by inhibition of the acetoclastic methanogens. On the other hand, and considering the SMP obtained from SBB individually at the HRT of 20 days (green discontinuous line in the graph), the area in which the co-digestion assays have achieved a more efficient operation can be clearly defined indicating a good operation behavior in reactors at these conditions

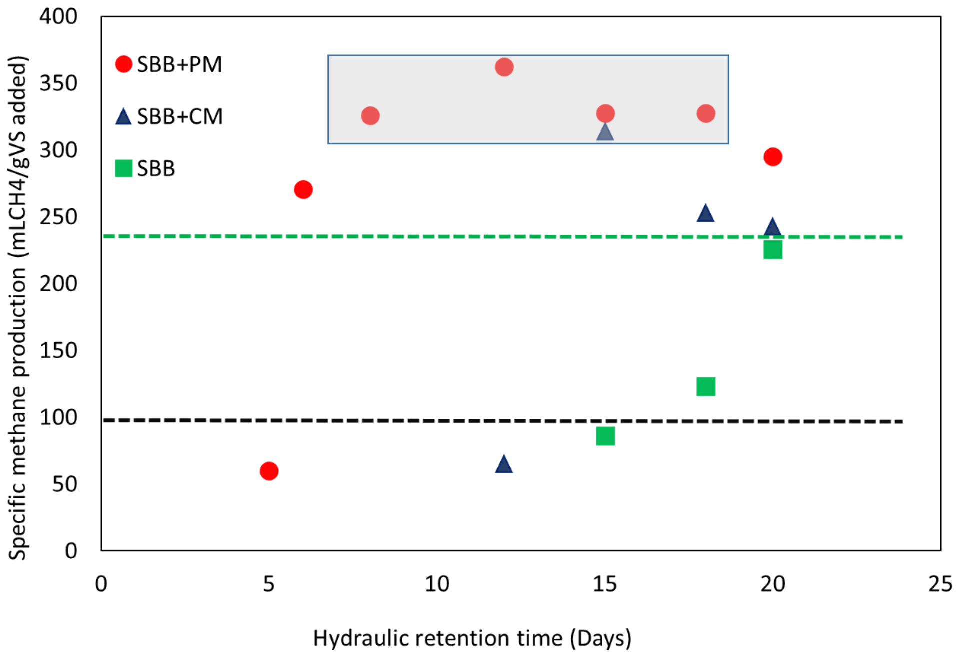

Fig. 4 Specific methane productions at the different hydraulic retention time.

Conclusion

The long-term semi-continuous operation systems allows to confirm that manures are potential co-substrates for sugar beet by-products anaerobic degradation. Pig manure resulted as best co-substrate in comparison with cow manure. In overloaded anaerobic systems of sugar beet by-products as a sole substrate or in co-digestion, acetoclastic methanogens are inhibited.

Acknowledgments

This research was financially supported by the Spanish Ministry of Economy and Competitiveness (CTM2013-43938-R and CTM2016-79071-R projects) and co-funded by the European Regional Development Fund (ERDF).

References

[1] K.E. Ileleji, C. Martin, D. Jones, Basics of Energy Production through Anaerobic Digestion of Livestock Manure, in: Bioenergy, Elsevier, 2015: pp. 287–295. doi:10.1016/B978-0-12-407909-0.00017-1.

[2] Eurostat, No Title, (2017). http://ec.europa.eu/eurostat/statistics-explained/index.php/Agricultural_production_-_animals#Livestock_population.

[3] J. Vasco-Correa, S. Khanal, A. Manandhar, A. Shah, Anaerobic digestion for bioenergy production: Global status, environmental and techno-economic implications, and government policies, Bioresource Technology. 247 (2018) 1015–1026. doi:10.1016/J.BIORTECH.2017.09.004.

[4] U. De Corato, I. De Bari, E. Viola, M. Pugliese, Assessing the main opportunities of integrated biorefining from agro-bioenergy co/by-products and agroindustrial residues into high-value added products associated to some emerging markets: A review, Renewable and Sustainable Energy Reviews. 88 (2018) 326–346. doi:10.1016/J.RSER.2018.02.041.

[5] B. Joanna, B. Michal, D. Piotr, W. Agnieszka, K. Dorota, W. Izabela, Sugar Beet Pulp as a Source of Valuable Biotechnological Products, in: Advances in Biotechnology for Food Industry, Elsevier, 2018: pp. 359–392. doi:10.1016/B978-0-12-811443-8.00013-X.

[6] A. Boldrin, K.R. Baral, T. Fitamo, A.H. Vazifehkhoran, I.G. Jensen, I. Kjærgaard, K.-A. Lyng, Q. van Nguyen, L.S. Nielsen, J.M. Triolo, Optimised biogas production from the co-digestion of sugar beet with pig slurry: Integrating energy, GHG and economic accounting, Energy. 112 (2016) 606–617. doi:10.1016/j.energy.2016.06.068.

[7] S. Borowski, M. Kucner, A. Czyżowska, J. Berłowska, Co-digestion of poultry manure and residues from enzymatic saccharification and dewatering of sugar beet pulp, Renewable Energy. 99 (2016) 492–500. doi:10.1016/j.renene.2016.07.046.

[8] K. Aboudi, C.J. Álvarez-Gallego, L.I. Romero-García, Evaluation of methane generation and process stability from anaerobic co-digestion of sugar beet by-product and cow manure, Journal of Bioscience and Bioengineering. 121 (2016) 566–572. doi:10.1016/j.jbiosc.2015.10.005.

[9] S.A. Neshat, M. Mohammadi, G.D. Najafpour, P. Lahijani, Anaerobic co-digestion of animal manures and lignocellulosic residues as a potent approach for sustainable biogas production, Renewable and Sustainable Energy Reviews. 79 (2017) 308–322. doi:10.1016/J.RSER.2017.05.137.

[10] V. Vivekanand, D.G. Mulat, V.G.H. Eijsink, S.J. Horn, Synergistic effects of anaerobic co-digestion of whey, manure and fish ensilage, Bioresource Technology. 249 (2018) 35–41. doi:10.1016/J.BIORTECH.2017.09.169.

[11] E. Alkaya, G.N. Demirer, Anaerobic acidification of sugar-beet processing wastes: Effect of operational parameters, Biomass and Bioenergy. 35 (2011) 32–39. doi:10.1016/j.biombioe.2010.08.002.

[12] J. Pagés-Díaz, I. Sárvári-Horváth, J. Pérez-Olmo, I. Pereda-Reyes, Co-digestion of bovine slaughterhouse wastes, cow manure, various crops and municipal solid waste at thermophilic conditions: A comparison with specific case running at mesophilic conditions, Water Science and Technology. 67 (2013) 989–995. doi:10.2166/wst.2013.647.

[13] A. Lehtomäki, S. Huttunen, J.A. Rintala, Laboratory investigations on co-digestion of energy crops and crop residues with cow manure for methane production: Effect of crop to manure ratio, Resources, Conservation and Recycling. 51 (2007) 591–609. doi:10.1016/j.resconrec.2006.11.004.

[14] K. Aboudi, C.J. Álvarez-Gallego, L.I. Romero-García, Semi-continuous anaerobic co-digestion of sugar beet byproduct and pig manure: Effect of the organic loading rate (OLR) on process performance, Bioresource Technology. 194 (2015) 283–290. doi:10.1016/j.biortech.2015.07.031.

[15] K. Aboudi, C.J. Álvarez-Gallego, L.I. Romero-García, Biomethanization of sugar beet byproduct by semi-continuous single digestion and co-digestion with cow manure, Bioresource Technology. 200 (2016) 311–319. doi:10.1016/j.biortech.2015.10.051.

[16] APHA-AWWA, Standards Methods for the Examination of Water and Wastewater, 20th editi, American Public Health Association Washington DC, USA, 2005.

[17] B. Demirel, P. Scherer, Production of methane from sugar beet silage without manure addition by a single-stage anaerobic digestion process, Biomass and Bioenergy. 32 (2008) 203–209. doi:10.1016/j.biombioe.2007.09.011.

[18] U. Marchaim, C. Krause, Propionic to acetic acid ratios in overloaded anaerobic digestion, Bioresource Technology. 43 (1993) 195–203. doi:10.1016/0960-8524(93)90031-6.

[19] P.C. Pullammanappallil, D.P. Chynoweth, G. Lyberatos, S.A. Svoronos, Stable performance of anaerobic digestion in the presence of a high concentration of propionic acid, Bioresource Technology. 78 (2001) 165–169. doi:http://dx.doi.org/10.1016/S0960-8524(00)00187-5.

[20] K. Aboudi, C.J. Álvarez-Gallego, L.I. Romero-García, Influence of total solids concentration on the anaerobic co-digestion of sugar beet by-products and livestock manures, Science of The Total Environment. 586 (2017) 438–445. doi:10.1016/j.scitotenv.2017.01.178.

[21] S.A. Neshat, M. Mohammadi, G.D. Najafpour, P. Lahijani, Anaerobic co-digestion of animal manures and lignocellulosic residues as a potent approach for sustainable biogas production, Renewable and Sustainable Energy Reviews. 79 (2017) 308–322. doi:10.1016/J.RSER.2017.05.137.

[22] J. Pagés-Díaz, J. Westman, M.J. Taherzadeh, I. Pereda-Reyes, I. Sárvári Horváth, Semi-continuous co-digestion of solid cattle slaughterhouse wastes with other waste streams: Interactions within the mixtures and methanogenic community structure, Chemical Engineering Journal. 273 (2015) 28–36. doi:10.1016/j.cej.2015.03.049.

[23] S. Astals, D.J. Batstone, J. Mata-Alvarez, P.D. Jensen, Identification of synergistic impacts during anaerobic co-digestion of organic wastes, Bioresource Technology. 169 (2014) 421–427. doi:10.1016/j.biortech.2014.07.024.

[24] P.H. Smith, R.A. Mah, Kinetics of Acetate Metabolism during Sludge Digestion, Applied Microbiology. 14 (1966) 368–371. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC546717/.