Co-digestion of meat-processing by-products, manure and residual glycerin

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Abstract

Until recent days, the customary treatment of animal by-products not intended for human consumption was directed primarily to the use of certain fractions as animal feed. However, current legislation affecting removing of these products restricts its use as animal feed because of health reasons, but enables the implementation of new technologies for their treatment, opening a wide field of work for the energy recovery using anaerobic digestion and the subsequent use of the digestate as a fertilizer source.

Under these circumstances, the LIFE VALPORC project (www.lifevalporc.eu) arises with the aim to demonstrate a sustainable alternative to the management of pig carcasses and manure, addressing current environmental problems derived from its management and valuing these waste streams through its transformation into biofuels and organic fertilizers, with the corresponding environmental and socio-economic added value.

Four streams and their mixtures have been considered for anaerobic co-digestion in this paper, all of them generated during pork meat processing or in related industrial activities: meat flour (MF), process water (PW), pig manure (PM) and glycerin (GL). The results show that the co-digestion of these products favors the anaerobic fermentation process when limiting the amount of flour in the mixture to co-digest, which should not exceed 10%. The proportion of other tested substrates is less critical, because different mixtures reach similar values of methane generation. The presence in the mixture of process water contributes to a quick start of the digester, something very interesting when operating an industrial reactor.

Keywords

Animal by-products , Anaerobic digestion , Biofertilizers , Biogas , Pig manure , Waste valorization.

Introduction

The production of pork is a significant part of meat production in Spain and Europe. More than 251 million head of pigs were slaughtered in 2014 in Europe [1], of which 43 million came from Spain [2]. It is a clear fact that the demand for meat has increased over time and as a consequence, the amount of organic by-products from slaughterhouses also did it. About 30% of the total weight of slaughtered pigs is not intended for human consumption. It is estimated that, annually, remains of pigs corpses in Europe and Spain is 5.4 and 0.9 million tons, respectively. Over the past 60 years, these remains of slaughterhouse, rich in proteins and lipids, have been treated and used for feed production. However, due to legal restrictions and consumers increasingly more aware with the environment, treatment of waste and animal by-products has become a major concern not only in the industry of pork, but also in the meat industry in general. For example, the outbreak of diseases such as bovine spongiform encephalopathy (BSE) in cattle and the dangerous Creutzfeldt-Jacob in humans in 2001, has resulted in an increased general awareness of the need for standards for the management of these products, greater control of processes, and the prohibition of the use of certain animal by-products.

According to current legislation [3, 4], slaughterhouse waste must be treated by different methods depending on the category of the animal by-product. Two Community regulations categorize animal by-products into three categories based on the risk: Category 1 is high-risk material (parts of infected animals, international catering, etc.) and it is not allowed to be composted or treated in biogas plants under no circumstance; Category 2 are by-products of animal origin medium risk (sick animals, manure, digestive tract content, etc.) that cannot be used as raw material in composting and biogas plants unless they first have been sterilized at least at 133 °C and 300 kPa for 20 minutes; and finally Category 3 material low risk (catering waste, meat, ready meals, etc.) approved for human consumption, to be treated at least at 70 °C for 1 hour in a closed system.

Anaerobic digestion is disclosed as a possible method for the treatment of animal subproducts, which in turn allows the production of energy as methane and the use of effluents of digestion as fertilizer for agricultural application (nutrients recovery) [5].

However, slaughterhouse wastes are generally considered as difficult substrates for anaerobic digestion, mainly because of their typically high protein and lipid content [6]. Protein degradation releases ammonia, which at high concentrations is an inhibitory compound for anaerobes [7-10]. It is generally considered that the unionized form of ammonia causes inhibition and concentrations of 0.1 to 1.1 kg m^{-3} turn out the process inhibitory [11]. Furthermore, the lipids can also cause problems in the anaerobic digestion because of its tendency to promote the presence of supernatants phases in the digesters and the possible accumulation of intermediate products of reaction, as long chain fatty acids (LCFA) [9, 12]. LCFA degradation can be the limiting step in the overall process of complex substrates (such as animal fats) degradation, requiring a gradual adaptation of the microorganisms to these substances and a careful dosing of lipid rich waste streams to avoid accumulation of LCFA. Even at very low concentrations (around 0.5 g L^{-1}), LCFA, especially unsaturated, are inhibitory for syntrophic acetogenic and methanogenic bacteria [13].

The relatively high nitrogen content and high content of total solids (TS) of the animal subproducts rarely causes them to be treated in their original state, i.e., undiluted. It is for this reason that dilution is usually necessary. In this regard, a very attractive option is the co-digestion of these animal subproducts with other less concentrated organic waste, such as manure or wastewater generated in rendering processes. The presence of more dilute streams provides stability to the whole process and serves as a dilution medium while the residual stream itself is also treated [14].

In the present study, methanogenic yields in batch digesters of various subproducts proceeding from a rendering plant of pig carcasses, which operate in the mesophilic temperature range (35 °C), are analyzed when they are co-digest in different proportions with liquid manure, water process and glycerin generated as a by-product during the production of biodiesel from pig fat.

Materials and methods

The VALPORC concept proposes the comprehensive treatment and recovery of pig carcasses. This sustainable model (Fig. 1) includes, as an initial step, the stage of rendering of pig products of category 2.

The system is designed to optimize, from the energy point of view, the flour and fat production process and to encourage the recovery of these products. Generated fats will enter a process of biodiesel manufacturing while meat flour (with traces of fat), process water and glycerin generated during the manufacture of biodiesel will be co-digested with pig manure in an anaerobic digestion plant. The organic matter remaining fraction in the digestate, together with nutrients that have not been transformed in the process and other byproducts of the overall process will be utilized as fertilizer in areas close to the plant, which will result in important savings in chemical fertilizers.

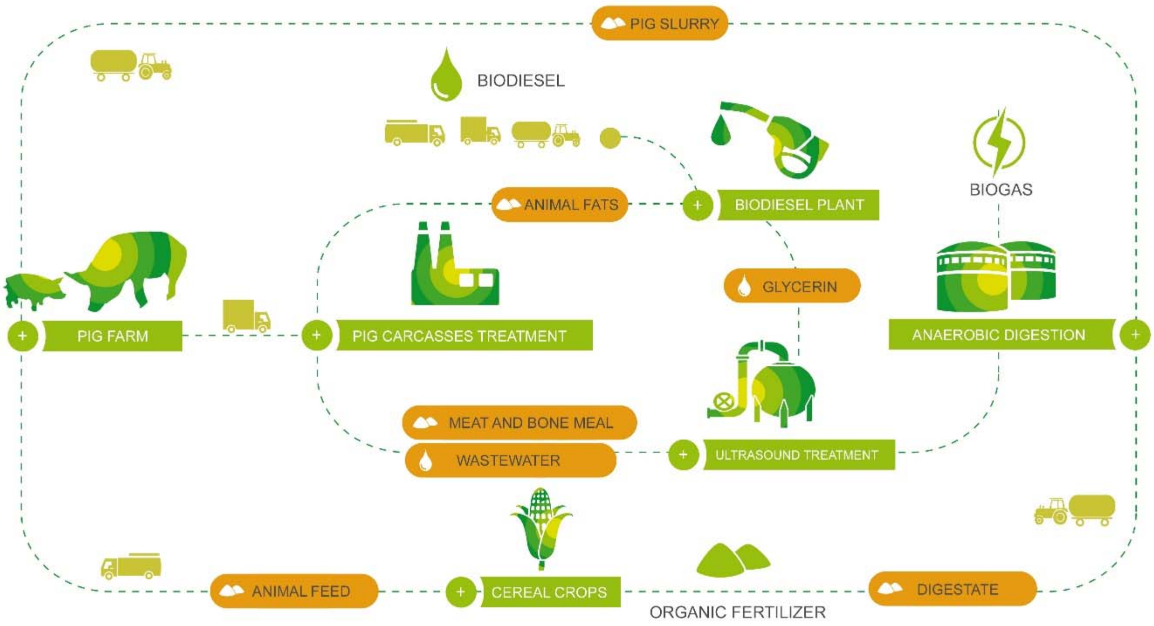

Fig. 1 VALPORC model for valorization of pig carcasses.

Substrates

Five streams generated in the pork industry have been the base of this study: fats (FA), meat flour (MF) and process water (PW) from a rendering plant in Soria (Spain); pig manure (PM) from a centralized livestock effluents treatment plant, also in Soria; and glycerin (GL), generated as a byproduct during the generation of biodiesel from fat pig in the laboratory. As inoculum for the mesophilic anaerobic digestion assays, sludge from an anaerobic reactor operating in a sugar factory was used.

Characterization analyzes and experimental tests were performed immediately after samples arrival at the laboratory.

Analytical methods

In the case of fats used in the biodiesel production process, entering directly, although in small proportion, in the anaerobic digester as a part of the process water, the characterization analysis includes: acid index (expressed as a percentage of oleic acid and using the reference method Regulation (EC) No 2568/91. Annex II) and fatty acid composition (in gas chromatograph coupled to flame ionization detector (GC-FID) CP model 3800 GC equipped with capillary column CP-Sil 88 and CP-8410 autoinjector, all Varian Inc. brand).

In the case of the other substrates to be introduced into the anaerobic digester (pig manure, flour, process water and glycerin) the characterization analyzes performed include: solid (total and volatile) (TS, VS), pH, total phosphorus (TP), Total Kjeldahl Nitrogen (TKN) and chemical oxygen demand (COD) performed according to Standard Methods [15]. Elemental analysis of the samples (containing C, H and N) was determined by a UNE-CEN/TS 15104 EX equipment with an elemental analyzer LECO CHN TruSpec (S). The oxygen content was not directly measured but estimated assuming that no element other than C, H, N and P was present in the samples. All parameters were analyzed in triplicate.

Method for determining the methanogenic potential

In order to study the biomethane potential and biodegradability of different waste streams and their mixtures, batch experiments were run in glass serum bottles with a liquid volume of 300 mL (1000 mL of total volume). All the experiments were carried out at 37±1 ºC in a thermostatic room, at an initial pH 7.2±0.1 and continuously stirred on a shaking-table, at a speed of 125 rpm. As already mentioned, anaerobic sludge from an anaerobic digester operating in a sugar factory, with a VS concentration of 34±1 g L^{-1}, was used as inoculum for the anaerobic test. Final inoculum concentration in the tests medium was 5.0±0.5 g L^{-1}. The substrate/inoculum (S/X) ratio was maintained for all samples at 0.50±0.05 gSVsubstrate / gSVinoculum.

Table 1 shows the content of each stream in the selected mixtures. The results are presented in percentage of volatile solids (with 5%´of maximum standard deviation (SD) in all the cases). The proportions have been designed based on the actual availability of each stream, with the manure being the majority element in all the mixtures.

Table 1. Waste mixture composition

	Mixture 1 (%)	**Mixture 2 (%)**	**Mixture 3 (%)**	**Mixture 4 (%)**	**Mixture 5 (%)**
PM	60	50	40	60	80
MF	10	10	10	20	5
PW	20	30	40	10	13
GL	10	10	10	10	2

A set of blank tests (without substrate, only inoculum) was also performed in triplicate to determine the endogenous methanogenic production. To prevent assay acidification, $NaHCO_3$ (6 g L^{-1}) was added as buffer. Experiments were finalized when the rate of biogas production in assays with substrate decreased to the levels of the blank assay.

Biogas production was manually measured by using a pressure transmitter (Druck, PTX 1400, range 1 bar) located in the head space of each reactor. To avoid overpressure inside the reaction bottles, biogas was periodically released. The ideal gas Law was used to convert pressure differences into biogas volume, by using standard conditions (P=1 bar and T=0ºC). Biogas composition was measured before each release (using a Varian CP-4900 Micro-GC with a Thermal Conductivity Detector). Methane production was calculated by subtracting the amount of the methane produced by the blank assay from the methane production of each assay.

Results

Characterization of subproducts and waste streams

The analytical characteristics of the samples analyzed are shown in Table 2. Analyzing the ratios VS/TS, values of 0.64 are obtained for pig manure, 0.83 for meat flour, 0.98 for process water and 0.96 for glycerin. All of them are suitable values, keeping in mind an anaerobic digestion process, since they reveal a high VS content. With respect to the C/N ratio, a value of around 12 is found for PM, 5 for MF and PW samples and more than 350 for GL. The first three streams are considered rich in nitrogen, and as the optimal C/N ratio for anaerobic digestion is in the range of 0-25 [16], their co-digestion with a rich-carbon stream as the glycerin is appropriate to balance the composition of the global stream entering the reactor.

From the results shown in Table 2, a high organic matter content is observed for the four streams analyzed, with different protein proportions depending on the substrate considered.

Table 2. Streams characterization

	PM	**MF**	**PW**	**GL**
pH	7.4	6.5	6.2	9.8
TS (mg L^{-1})	30985	93.8[1]	16795	792.3
VS (mg L^{-1})	19698	78.1[1]	16513	760.2
COD (mg L^{-1})	54163	1230[2]	67953	15787
TKN (mg/L^{-1})	5114	103[2]	4637	0.03
TP (mg kg^{-1})	3016	25.5	79.3	<25
C (% dry base)	34.7	49,4	48.3	35.8
H (% dry base)	4.8	7.3	9.7	11.4
N (% dry base)	2.8	9.5	9.4	0.1
Fat (% dry base)	-	19.3[3]	5.3[3]	-
Protein (g L^{-1})[4]	11	540[2]	24	-

[1]percentage; [2]g kg^{-1}; [3]Soxhlet Method
[4]Protein content has been estimated from the ratio 6.25 g protein per g Norg [17].

Fat, as such, is a substrate that will not go directly to the digester, as their destination will be the production of biodiesel, but it has been characterized since it is a part of the flour and process water entering the reactor.

The results indicate a high acid index for the analyzed fat (9.4%), which is indicative of a high free fatty acids content. The average fatty acid composition of the sample (Table 3) reveals that the major components of the pig fat are oleic acid, palmitic acid, stearic acid and linoleic acid, in this order, and together account for 92% of total fatty acid composition of the fat samples analyzed. Lalman and Bagley [18] showed that palmitic acid is the main product generated in the anaerobic degradation of oleic and linoleic acids, so its presence is expected both, in batch experiments and on a larger scale reactors.

Regarding the chain length of the fatty acids found in the analyzed fat, it can be seen that C18 and C16 compounds are the major compounds, and represent a 95.95% of the total.

Table 3 also reveals that the sample has 39.03% of saturated fatty acids and 60.97% of unsaturated fatty acids, most of which are monounsaturated fatty acids (49.41%). The fact that most of the fatty acids are saturated or with low unsaturation index will soften potential inhibitory effects of these compounds on acetogenic and methanogenic bacteria [13].

Table 3. Fatty acid composition of porcine fat samples

LCFA	Lipidic number	% in the sample
Myristic acid	C14:0	1.84
Palmitic acid	C16:0	22.18
Palmitoleic acid	C16:1	3.23
Margaric acid	C17:0	0.73
Margaric acid	C17:1	0.53
Stearic acid	C18:0	13.79
Oleic acid	C18:1	45.19
Linoleic acid	C18:2	10.84
Linolenic acid	C18:3	0.72
Arachic acid	C20:0	0.13
Gaddoleic acid	C20:1	0.46
Behenic acid	C22:0	0.22
Lignoceric acid	C24:0	0.08
Other LCFA	several	0.06

Anaerobic biodegradability of individual samples

Figure 2 shows the production of biogas generated by the individual substrates in the batch assays. It is shown for each tripled the average value.

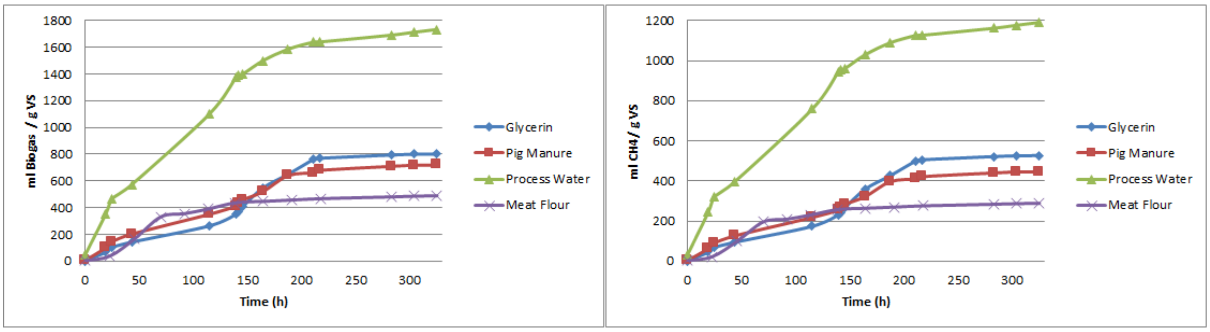

Fig. 2 Biogas production (left) and methane production (right) for individual substrates

Curves in Figure 2 clearly show the higher biodegradability of WP over the other streams analyzed. Biogas generation begins almost immediately after starting the test for process water, unlike other streams that show longer starting times.

Net methane production is 1200 ml gVS^{-1} in the case of PW, 500 ml gVS^{-1} in the case of GL and 450 ml gVS^{-1} in the case of PM. The lower biodegradability is shown for MF that do not exceed 300 ml gVS^{-1}. This is probably related to its higher protein content and/or the presence of structural proteins (collagen or keratin type), considered highly resistant to anaerobic degradation [19].

After 250 h of assay all tests are practically exhausted. This is interesting when calculating residence times in an industrial reactor.

The maximum methane production rates (8.0 ml $gVSh^{-1}$) were reached by PW, while the minimum are again reached by MF (1.2 ml $gVSh^{-1}$). This result is consistent with expectations, because 90% of the organic matter in the PW is in solution and it is easily accessible to the anaerobic microorganisms. On the other hand, MF needs longer periods of operation to be accessible for microbiota. PM and GL have a methane production rate of 6.2 and 6.5 ml $gVSh^{-1}$, respectively.

The higher content of methane in the biogas generated corresponds to PW (68.7%), followed by GL (65.7%), PM (62.0%) and, finally, MF (61.0%).

The analysis of the fraction digested in the biodegradability tests reveals that the four analyzed streams can be, a priori, suitable for agronomic valorization once digested. As shown in Table 4, all digestates have a phytotoxicity lower than 25 Equitox m^{-3}, which means, if taken as reference the Spanish legislation of the Community of Madrid (Law 10/1993), or the Community of Murcia (Law 3/2000), in which discharges to the sewerage system are permitted with up to 25 Equitox m^{-3}, that these digestates are considered non-toxic to the environment. It is worth highlighting the total absence of *Salmonella* and *E. Coli* in the four digestates, low metal content in all of them and the presence of remaining organic matter and nutrients (N, K and P), which increases their potential value as fertilizer.

Table 4. Composition of digested samples

		PM	**MF**	**WP**	**GL**
Conductivity (mS cm^{-1})		8.4	7.9	31.2	6.3
pH (unit pH)		7.7	7.7	8.1	7.3
COD (mgO_2 L^{-1})		2613	2285	1733	1713
TOC (mg L^{-1})		238	137	582	450
TOC (mg L^{-1}) water-soluble		231	125	574	426
TKN (mg L^{-1})		255	434	516	88
TKN (mg L^{-1}) water-soluble		166	219	208	53
Nitrates (mg L^{-1})		< 2.00	< 2.00	< 2.00	< 2.00
Nitrites (mg L^{-1})		<0.04	<0.04	24.6	0
C/N		0.9	0.3	1.1	5.1
Macronutrients	K (mg L^{-1})	281.3	199.0	190.7	226.7
	Ca (mg L^{-1})	897.3	1.406.7	671.0	291.3
	Mg (mg/L)	30.8	15.1	16.7	11.4
Micronutrients	Fe (mg L^{-1})	16.9	22.7	12.2	6.3
	Co (mg L^{-1})	317.7	239.3	423.0	339.0
	Mn (mg L^{-1})	3.1	4.3	1.8	1.0
	Cu (mg L^{-1})	569.0	90.2	139.0	52.7
	Zn (mg L^{-1})	5.1	0.6	0.3	0.1
	Mo (mg L^{-1})	37.7	20.7	43.7	32.8
	Se (mg L^{-1})	43.7	28.1	63.4	38.2
Heavy metals	Ni (mg L^{-1})	43.1	45.3	52.9	77.3
	Cr (mg L^{-1})	24.9	24.5	19.2	19.5
	Cd (mg L^{-1})	2.2	0.4	0.3	0.7
	Pb (mg L^{-1})	8.4	9.3	9.3	3.0
Phytotoxicity (Equitox m^{-3})					
Pathogens	*Salmonella*	nd	nd	nd	nd
	Esterichia Coli	nd	nd	nd	nd

nd: not detected

Co-digestion

Figure 3 shows the production of biogas generated by the substrates mixtures listed in Table 1. Figure 3 gathers the average value for each triplicate sample.

The higher methane content in the biogas generated corresponds to Mixture 2 (73.2%), followed by Mixture 1, Mixture 3, Mixture 4 and Mixture 5, in this order (70.2%, 70.2%, 69, 8% and 63.3%, respectively). After 300 h of testing all tests are practically exhausted.

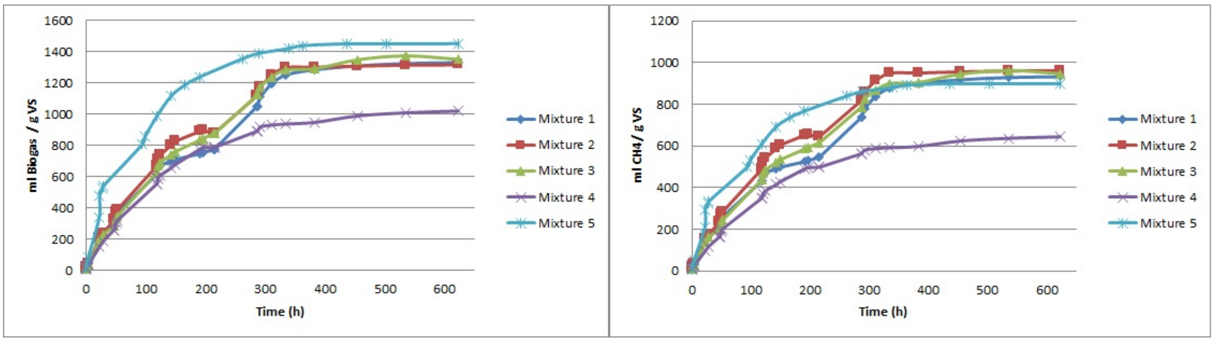

Fig. 3 Biogas production (left) and methane production (right) for mixtures

It is observed that the Mixture 4, the one with the higher MF content, reaches the lower net methane production. The remaining mixtures have similar behavior in terms of total generation of methane. It is noteworthy that, in all cases, including Mixture 4, co-digestion favors the methanization process. This effect results in a methane generation higher in the mixtures than which would be theoretically obtained if the contributions of each single substrate were added up, considering the stoichiometric composition of each mixture and the methane production of each substrate, as shown in Table 5. Here, the fact that the tests with mixtures have been active for longer than those with individual substrates influences. This has allowed a greater depletion of substrates and, therefore, an increased methane generation in absolute terms.

Table 5. Comparison of methane production (ml biogas VS^{-1})

Mixture	Direct experimental data	Data calculated from individual test
M1	932	561
M2	961	636
M3	947	711
M4	643	443
M5	899	525

The maximum rates for methane production are reached by the Mixture 5 (8.4 mL $gSVh^{-1}$), while the minimum corresponds to Mixture 4 (4.8 mL $gSVh^{-1}$). The performance of other mixtures is very similar in terms of methane production rate (5.3 mL $gSVh^{-1}$, 5.8 mL $gSVh^{-1}$and 5.5 mL $gSVh^{-1}$ for Mixture 1, Mixture 2 and Mixture 3, respectively).

Another remarkable fact is that the generation of biogas begins immediately after starting the test for the five tested mixtures, unlike what happened with most individual substrates. This may be due to the presence, in all mixtures, of PW, highly biodegradable, which contributes to the absence of latency periods during startups.

It is noteworthy that biodegradability curves for Mixture 1, Mixture 2 and Mixture 3 show a step at 250 h, approximately, which may correspond to the time when the degradation of any of the substrates forming the mixture ends and the degradation of another substrate starts. This is why two different sections are observed in these curves.

Conclusions

The study shows that animal subproducts are substrates to be considered in the biogas production process, but its high fat and protein content can cause inhibition problems by LCFA and ammonia in industrial digesters, which could paralyze the reaction, preventing to take advantage of all the methanogenic potential of these substrates.

The co-digestion of these products with waste streams such as pig manure and process water from rendering favors the anaerobic digestion process, with the limitation on the amount of flour in the mixture to co-digest, which should not exceed 10% of total. The proportion of other tested substrates is less critical, because with different mixtures similar values of methane generation are reached. The presence in the mixture of process water contributes to a quick start of the digester, a very interesting aspect when operating an industrial reactor. The analysis of the digested fraction reveals that this can be, a priori, suitable for agricultural recovery.

The system proposed in the VALPORC project for pig carcasses and manure valorization will be materialized in a processing plant of 1 t day^{-1} capacity, currently under construction, designed to optimize the energy consumption of the flour and fat production process and to promote the safe recovery of these elements.

Nomenclature

COD: Chemical Oxygen Demand
GL: Glycerin
LCFA: Long Chain Fatty Acids
MF: Meat Flour
PM: Pig Manure
PW: Process Water
SD: Standard Deviation
TKN: Total Kjedahl Nitrogen
TP: Total Phosphorus
TS: Total Solids
VS: Volatile Solids

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