

Article

Primary Sludge from Dairy and Meat Processing Wastewater and Waste from Biomass Enzymatic Hydrolysis as Resources in Anaerobic Digestion and Co-Digestion Supplemented with Biodegradable Surfactants as Process Enhancers

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Abstract: Incorporation of various alternative resources as co-digestion substrates aids to reduce the consumption of agricultural crops for biogas production. However, the efficiency and limitations of these co-substrates is still not fully understood. Use of biomass waste remaining after enzymatic hydrolysis for high value chemical fermentation, meat processing and dairy wastewater primary sludge as co-substrates in an agricultural resource anaerobic digestion plant is tackled within this study. The results showed that anionic surfactants (<200 ppm) can be used to improve fat, oil and grease (FOG) solubility in water and, at the same time, enhance the biomethane potential of FOG-containing sludge by increasing it from 1374.5 to 1765 mLCH₄/gVS for meat processing wastewater primary sludge, and from 534 to 740 mLCH₄/gVS for dairy wastewater primary sludge, when agricultural digestate is used as a substrate and sludge loading is not more than 10% from the volatile solids loaded. At the same time, only 549.7 mLCH₄/gVS was produced as 30-day BMP when 5% biomass hydrolysis waste was used. Biomass hydrolysis waste co-digestion with primary sludge from dairy and meat processing wastewaters has an antigenic effect, and separate substrate anaerobic digestion gave a better results, thus, showing that excessive combination of various waste resources can be inhibitory for biogas production and the appropriate substrate selection and combination is a technical challenge for the biogas industry.

Keywords: anaerobic co-digestion; primary sludge; FOG; biodegradable surfactants; enzymatic hydrolysis waste



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1. Introduction

The limited reserves of fossil fuels, increased pricing and sustainability concerns have evolved into an intense surge for alternative resources and advanced technologies to produce energy. EU Green Deal targets [1] together with the European Union (EU) Renewable Energy Directive 2018/2001 [2] facilitate the use of inedible materials such as waste biomass, straw, sewage sludge, animal fats and used cooking oil. The regulation also supports the role of biogas and biomethane in providing renewable heat and power and recognizes the need to integrate “low-carbon gases”, including biomethane, in existing natural gas grids [3]. Anaerobic digestion (AD) is a proven technology for biomass reduction and recycling. One of the principal feedstocks is wastewater sludge and other organic and nutrient-rich sludge. One possible way of maximizing the prospects of anaerobic digestion of different secondary substrates is to employ anaerobic co-digestion: a process of adding supplemental high-strength organic substrates, such as food waste and fat, oil and grease (FOG)-containing waste to an anaerobic digestion system to augment biogas production. Anaerobic co-digestion has received significant attention in recent years [4,5]

because of the growing desire of wastewater treatment plants to become energy neutral, and private AD stations tending to search for degradable material to replace energy crop use, coupled with increasing concerns and strict regulations against organic waste land-fill [6,7]. This research is looking into the processual challenges of a co-digestion approach for secondary substrates, such as biomass enzymatic hydrolysis waste (BHW) and high-strength substrate—FOG from the food production industry, when biodegradable anionic surfactants are used as process enhancers. The findings of this study delve into probable future waste-to-resource challenges by outlining the potential technical boundaries for BWH anaerobic digestion and ways to boost the technological potential of this material by merging it with already available biomethane substrate flows that are derived from food production waste.

1.1. Meat and Dairy Wastewater Sludge

For every kilogram of dairy products, 2 to 4 L of water are consumed, and 0.5 to 20.5 L of wastewater are created, depending on the composition and variety of the final products [8,9]. The amount of wastewater generated in meat production is more dependent on the product type and factory specialization. Animal slaughtering, washing, and cutting meat, meat quality control, and processing meat into various products such as sausages or packed meat are all part of the meat processing process, and wastewater is generated in all these steps [10]. Poultry production units are reported to require, on average, 11.5 L of freshwater per animal, while the water requirement for beef production units is around 1325 L per animal and is mostly needed for washing [11].

Meat processing wastewater (MPWW) and dairy wastewater (DWW) mainly contains biogenic pollution in high concentrations, which increases their chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP) and total suspended solid (TSS) parameters (Table 1) [12]. To remove the dissolved pollution from the wastewater, prior to the activated sludge process, numerous pre-treatment technologies have been proposed.

Table 1. Indicative MPWW and DWW chemical parameters comparison before treatment. Adopted from Asgharnejad et al. [12].

Parameter	Meat Production Wastewater			Dairy Wastewater
	Beef	Poultry	Pork	
COD, mg/L	4220	950	4310	2131
BOD, mg/L	1209	400	-	1536
TN, mg/L	427	80	275	273
TP	-	-	-	60
TSS, mg/L	1164	240	1240	-
FOG, g/L	0.120	0.125		0.2–2.88 [13]

The dissolved air flotation (DAF) system can reduce chemical oxygen demand, nitrogen, phosphorus, and grease (FOG) by 70%, 55%, 70%, and 85%, respectively, in large-scale slaughterhouse wastewater applications [14]. Sedimentation with an energy consumption of 0.05–0.30 kWh/m³ is more economically favourable; however, it has been frequently noted that sedimentation in grease traps cannot achieve the required separation due to the high FOG concentration [14]. DAF systems are also used in the dairy industry, showing similar effectiveness [15]. FOG, along with the other contaminants, stays in the MPWW DAF sludge, which can be treated later in the AD process due to it is high volatile solid and fat content. Primary sludge from both industries contains a high quantity of dry solids (DS) and volatile solids (VS) which have to be extracted from the wastewater stream in the primary treatment process (Table 2). The volume of DAF sludge generated ranges from 1% to 3% of the overall volume of the wastewater effluent, with the actual volume of the DAF sludge dependent on the effectiveness of the DAF treatment system utilised in the specific meat processing plant [16]. In comparison to secondary sludge, primary sludge includes more biodegradable organic material, hence anaerobic digestion (AD) is

projected to be more efficient in terms of energy capacity and total methane output [17]. On the other hand, primary sludge and DAF sludge, which are rich with high fat content, can be used as an anaerobic co-substrate. Anaerobic digestion of high-lipid wastes has been linked to acetoclastic and methanogenic bacteria inhibition, substrate and product transport limitations, sludge floating, digester foaming, pipe and pump obstructions and clogging of gas collecting and handling systems [18]. Therefore, the use of this type of sludge as an AD co-substrate must be handled with precautions.

Table 2. Comparison of DWW and MPWW DAF sludge chemical content.

Parameter	DWW DAF Primary Sludge		MPWW DAF Primary Sludge	
	Literature Value [19]	This Study *	Literature Value [16]	This Study *
Dry mater (DM)% of Wt.	25.9	11.96	91.37	53.67 ± 0.81
OM (% of DM)	46.9	9.52	69	53.63 ± 0.93
pH	7.2	7.4	8.23	7.2 ± 0.1
TN (g/kg)	19.5	x	n/a	x
TP (g/kg)	65.9	x	n/a	x
TC (total carbon) (g/kg)	24.3	x	45.58 (dry basis)	x
Lipid content (wt% dry sludge)	x	x	12.98	x
n-Hexane extractable substances (wt% of DM)	x	14.21	x	82.32 ± 3.76

* The data represents the average values from dairy wastewater primary sludge (DWW) and meat processing wastewater primary sludge (MPWW) sample triplicates used in this study.

1.2. Biomass Waste after Enzymatic Hydrolysis

Lately, lignocellulosic biomass from various waste streams has become a highly valued feedstock for fuel production. Particularly in the EU, which has set targets for advanced biofuel and biogas production from alternative feedstocks to reach at least 3.5% in 2030 [2]. Despite the wide global abundance and reported 8–20 × 10⁹ tons of agricultural waste annually that is suitable for biofuel production, technological limitations and production costs still restrict the full use of this resource [20]. Biofuel production from lignocellulosic substrate typically includes pre-treatment, biological/chemical hydrolysis, fermentation of cellulose/hemicellulose monomers and product recovery from the fermentation broth (Figure 1). Each of these steps has been extensively researched. Similarly, AD of cellulose, hemicellulose and protein is widely investigated and understood. At the same time, aerobic and anaerobic digestion or separation of lignin compounds remains unclear.

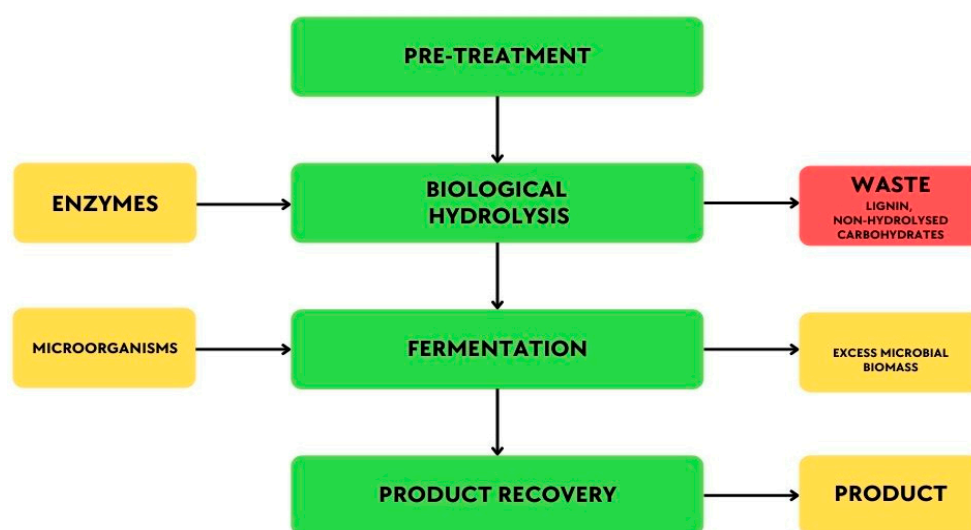


Figure 1. Biomass feedstock biological conversion process scheme with input and output of bio-based products.

Lignins have been generally classified into softwood lignin, hardwood lignin and grass lignin based on the chemical structure of their monomer units. The composition of guaiacyl (G), syringyl (S) and *p*-hydroxyphenylpropane (H) units produce cereal straw lignin or grass lignin (GSH-lignin or Gramineae lignin from grasses), which are known to be different from those of softwood (G-lignin), or hardwood (GS-lignin) and compression wood (GH-lignin) lignin [21]. Furthermore, GSH-lignins contain ester-linked ferulic and *p*-coumaric acids which also occur linked to polysaccharides and may act as cross-links between lignin and carbohydrate in the plant cell wall, thus, making them more acceptable for biological degradation. Aerobic degradation (also as a pre-treatment for AD) is mostly attributed to *Actinomycetes* and white rot fungi, where the first showed higher biodegradation properties of non-wood lignin than wood lignin [22]. Alternatively, some anaerobic bacterial species, for example, *Clostridium* sp. And *Methanoculleus* sp. [23,24], can break down lignin molecules under anaerobic conditions, thus, excluding the need for an individual pre-treatment system in AD technology. Examples of lignin degrading species *Acetoanaerobium* sp. [25], *Rhodococcus justice* [26], and *Sporomusa* sp. [27], have been reported but exact lignin AD mechanisms are still unclear.

To avoid the need for lengthy biological conversion, the AD process can be coupled with fuel production from biomass by using process waste (Figure 1) in anaerobic co-digestion. The waste material still contains non-hydrolysed carbohydrates and partially degraded lignin structures, especially if originating from biological hydrolysis with enzyme complexes from lignin-degrading fungi [28].

1.3. Fats, Oils and Grease Anaerobic Digestion

In recent years vast amount of research has been performed to study FOG-containing substrate co-digestion together with secondary wastewater treatment sludge in wastewater treatment facilities [29,30]. To some extent, this is related to the high amount of FOG in the primary sludge produced in the primary settling units [31]. At the same time, limited information is available on FOG-rich sludge and skimming co-digestion potential with agricultural substrates, such as corn and green mass silage and agricultural wastes that are regarded as alternative feedstocks for AD.

FOG is considered to be a desirable substrate to enhance biomethane production through co-digestion as it has been reported to increase the methane yield by 250–350% [32,33] in activated sludge AD. Furthermore, it has a larger theoretical biomethane production potential ($1 \text{ m}^3/\text{CH}_4 \text{ kg}^{-1}$) than carbohydrate ($0.42 \text{ m}^3/\text{CH}_4 \text{ kg}^{-1}$) or protein ($0.63 \text{ m}^3/\text{CH}_4 \text{ kg}^{-1}$) [7,34]. The physicochemical properties of FOG vary widely based on the types of fat, oil and grease used, as well as their source [35]. FOG mainly consists of triglycerides, which are first hydrolysed into glycerol, and long chain fatty acids (LCFA) that consist mostly of 14–24 carbon atoms. LCFA degradation into short chain fatty acids (SCFA), acetate, H_2 and biomethane occurs via a β oxidation process [36]. In anaerobic systems, the suppression of methanogens by LCFAs is a serious operational issue, especially when the digesters are fed with waste with a high lipid content. The accumulation of LCFAs alters the cellular shape, reduces cell permeability and has an impact on mass transfer [37]. Inoculum acclimatization with FOG can cause the microbiological consortia shift by mainly increasing the phyla *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Thermotogae* [38]. Furthermore, several syntrophic *Firmicutes* bacteria have been found to digest SCFAs such as acetic and butyric acids by hydrolysing diverse substrates. Acetic acid is a well-known main substrate for acetoclastic methanogenesis, which is then converted into methane [39].

FOG-rich substrate addition to an AD system with activated sludge is possible, showing no inhibitory effect at up to 60% of the VS loaded; although there are experiences when process inhibition starts when FOG is added at more than 40% of the total VS loaded. Meanwhile FOG biomethane potential varies from 280 to 680 mL CH_4/gVS added for the best performing FOG concentration used in these studies [18].

AD process stability might be harmed by a higher FOG concentration due to probable LCFA suppression, which could result in a digestion failure due to the digester acidifica-

tion [40]. One of the most abundant LCFAs in nature is oleic acid. It is the main component of vegetable oils and animal fat [41]. Oleic acid is characterized by its low density and non-solubility in water [42]. Thus, elevated FOG substrate quantities in the AD system can cause, not only the chemical inhibition aspects, but also physical aspects such as mass transfer [33], when the FOG substrate is not well mixed into the digestate matrix, by staying in clumps. As a result, disintegration time and volume become unknown, therefore it is much harder to sustain a precise co-substrate dosing into a continuous AD process. To enhance the FOG digestion, lipase-based enzymes [33] or digestive juices such as bovine bile [43] can be used as well as different surface-active substances (SAS) that can emulsify and disintegrate sludge and FOG flocs [33] to optimize the mass transfer.

1.4. Surfactant Influence on the AD Process

Surfactant, as an amphipathic material, can improve hydrolysis efficiency by separating big sludge and releasing the encapsulated hydrolase, allowing for more substance to be available for future acidogenesis [44]. Following that, the main product—SCFA—is created. Surfactant has been shown to impact SCFA transformation previously [45], where acidification products were altered by stimulating variations in microbial activity and the carbon-to-nitrogen (C/N) ratio, particularly the ratio of acetic and propionic acid, which was used for either nutrient removal, methane production or polyhydroxyalkanoate synthesis.

The processes of surfactant-induced facilitated hydrolysis may be summarized in two aspects: sludge component disintegration and enzyme activity enhancement. The sludge blocks are distributed and dispersed into the media matrix, and the hydrolase from the sludge is released, increasing the efficiency of the hydrolysis process [46]. However, since enzymes and extracellular polymer substances interact electrostatically, complexes of extracellular polymer substance–enzyme aggregates are formed, trapping enzymes in the substrate and, thus, increasing the enzymatic hydrolysis [47,48]. In this way, suitable surfactant addition can solve both mass transfer problems, along with the LCFA accumulation problems, by increasing hydrolytic enzyme activity.

2. Materials and Methods

2.1. Substrate and Inoculum Analyses

All substrates and inoculum used for AD tests were evaluated prior for total dissolved solids and volatile organic solids. These were determined using a gravimetric method with drying and burning the sample at 105 °C for 12 h (or until constant mass) and 550 °C for 5 h (or until constant mass), respectively [49].

Fat, oil and grease content was analysed using the n-hexane extraction and gravimetric method [50] since the method covers almost all type of nonpolar compounds found in primary sludge and it can be used both for MPWW-DAF- and DWW-DAF-type sludge without modification. By using this method, it is also possible to extract the unfavourable compounds, despite none of the extraction methods offering high selectivity for only the triglycerides found in FOG [51]; therefore, it can be said that the n-hexane extractable substance (HES) concentration in primary sludge is representing the FOG concentration.

DWW DAF samples have been collected from a dairy processing company (located in Latvia, turnover around 15,000,000 EUR/year) that is producing a wide range of products: skimmed milk, yogurts, ice-cream, different types of cheese and butter. The wastewater capacity is up to 250 m³/day and before entering into biological treatment, wastewaters are treated in a DAF unit with coagulant and polymer addition—polymer concentration in sludge can vary from 100 to 500 ppm.

MPWW primary sludge, generated both from primary grease trap sludge and a DAF sludge mixture (MPWW DAF), was collected from a meat processing company operating its own slaughterhouse and producing a wide range of raw meat products, as well as smoked and marinated products (located in Latvia, annual turnover of 20,000,000 EUR/year). The dominant component in MPWW DAF is grease trap sludge. The plant wastewater capacity is around 70 m³/day.

Biomass hydrolysis waste was collected from a pilot-scale enzymatic hydrolysis unit (30 L) after hay (collected from semi-natural grasslands in Latvia, dry weight (DW): $92.8\% \pm 1.3\%$; 6.02% ash, particle size < 0.5 cm) enzymatic hydrolysis and dissolved carbohydrate removal [52].

General chemical characteristics of used substrates are represented in Figure 2.

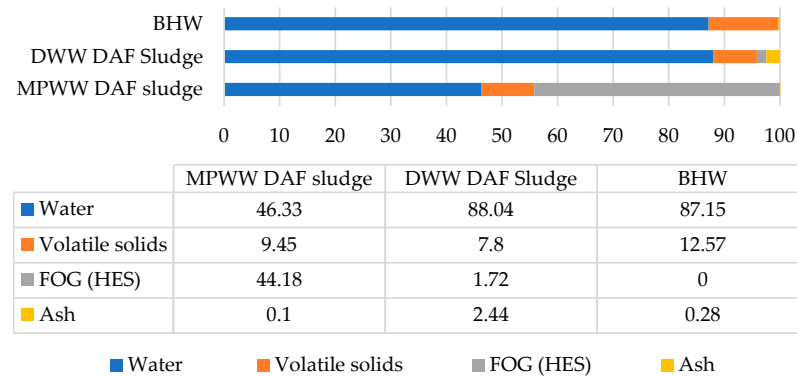


Figure 2. Chemical characteristics of substrates used in AD experiments (concentration represented in $w/w\%$).

2.2. Biomethane Potential Determination

Since no standardized biomethane potential (BMP) determination technique is available [53], the protocol was adjusted by selecting the most suitable measurement and incubation equipment, as well as substrate inoculum loading rate and inoculum type. The BMP test was carried out in a batch-type BMP test system constructed within a laboratory (Figure 3). An Anaero Technology 15 Channel automated gas flowmeter and a RITTER MilliGascounter single channel gas flow meter were used to measure the biogas output from each batch reactor separately. To neutralize any existing CO_2 and measure just the methane content, the biogas was cleaned with 3M NaOH before measurements [54].



Figure 3. Batch BMP test system with an incubator capable of maintaining temperatures between 25 and 60 °C, orbital shaker (Ohaus-HEAVY DUTY ORBITAL SHAKERS) that provides a rotating orbital of 2 cm and a maximum rotation speed of 500 RPM.

A pre-filtered (2 mm aluminium screen) digestate from an agricultural biogas plant that is using corn silage as a main substrate was used as an inoculum for all samples prepared. Before each fermentation, new digestate was taken from the biogas station and post-fermented in a closed jar for 10 days at room temperature. TS and VS, as well as FOS/TAC values, were determined on a regular basis. TS and VS content varied between 46.6–52.8 g/kg and 30.3–32.9 g/kg, respectively, but FOS/TAC values were between 0.12 and 0.18, which is an optimal value for the discarded digestate [54].

Each sample was prepared in a 1 L tight-sealed glass reactor with a maximum loading coefficient of 0.5 in order to maintain better mixing in the reactor. During the experimental phase, 6 replicates for AD were performed; therefore, the inoculum BMP is changing for each experiment performed and the BMP value is represented in Table 3 along with the substrate loading.

Table 3. AD sample loading and sample ID for all experiments performed. DWW DAF—dairy wastewater dissolved air flotation; MPWW DAF—meat processing wastewater dissolved air flotation; BHW—biomass hydrolysis waste; SAS—surface active substance. Table shows the reference BMP values for the inoculum used in each repetition.

Sample ID	VS Total Loaded, (g/kg)	Inoculum VS, (gVS/kg)	GEHL, (gVS/kg)	DWW DAF Sludge, (gVS/kg)	MPWW DAF Sludge, (gVS/kg)	SAS, ppm	Substrate BMP, mLCH ₄ /gVS
BHW 2.5%	34.9	34	0.9	X	X	X	39.8
BHW 5%	35.7	34	1.7	X	X	X	39.8
BHW 7%	34.6	32.3	2.3	X	X	X	106.1
BHW 13%	36.5	32.3	4.2	X	X	X	106.1
DWW DAF 10%	34.0	32.4	X	1.6	X	X	94.8
DWW DAF 20%	35.6	32.4	X	3.2	X	X	94.8
DWW DAF 30%	37.3	32.4	X	4.9	X	X	94.8
DWW DAF 40%	37.9	32.4	X	5.5	X	X	94.8
MPWW DAF 10%	30.3	27.5	X	X	2.75	X	52.4
MPWW DAF 20%	33.0	27.5	X	X	5.5	X	52.4
MPWW DAF 30%	35.8	27.5	X	X	8.3	X	52.4
DWW DAF 10%/0.1% SAS	33.4	30.3	X	3.03	X	100	80.5
DWW DAF 10%/0.2% SAS	33.5	30.3	X	3.03	X	200	80.5
DWW DAF 10%	33.3	30.3	X	3.03	X	X	80.5
MPWW DAF 10%/0.2% SAS	44.8	40.6	X	4.1	X	100	61.5
MPWW DAF 10%/0.4% SAS	44.9	40.6	X	4.1	X	200	61.5
MPWW DAF 10%	44.7	40.6	X	4.1	X	X	61.5
DWW DAF 10%/BHW 2.5%/SAS 0.2%	33.1	29.3	0.7	2.9	X	200	106.3
DWW DAF 10%/BHW 5%/SAS 0.2%	33.9	29.3	1.5	2.9	X	200	106.3
MPWW DAF 10%/BHW 2.5%/SAS 0.2%	38.5	34.0	0.9	X	3.4	200	39.8
MPWW DAF 10%/BHW 5.0%/SAS 0.2%	39.3	34.0	1.7	X	3.4	200	39.8

The samples with added FOG were homogenized by adding proteo-lipid with an alkali chain-based anionic surfactant (Happyfish Ltd., Riga, Latvia). According to the surfactant manufacturer, this active substance easily degrades SAS with LC₅₀ value of 350 mg/L (*Daphnia Magna*). Before adding the FOG substrate to the full media volume, it

was mixed with SAS and an amount of inoculum and homogenized for 1 min using a hand blender in order to form a stable emulsion.

2.3. Statistical Analyses

For data analysis and statistical comparison MS 365 Excel *t*-test (two tailed distribution) and ANOVA single parameter tools were used.

3. Results and Discussion

3.1. Selected Substrate BMP and Organic Loading

In order to understand the substrate co-digestion potential, first it is important to evaluate the substrate loading rates and biomethane potential separately. The study with BHW anaerobic digestion using activated sludge digestate as the inoculum showed that the BHW substrate becomes toxic if the loading is higher than 2.5% from the VS inoculum loaded, or 0.4 gVS/kg [55]. This is almost two times lower than the unprocessed agricultural substrate loading due to the lower activated sludge digestate inoculum VS content. More BHW substrate can be loaded in AD using agricultural digestate as the inoculum, but the substrate toxicity effect is present when substrate loading concentration are increasing to 5% from the loaded inoculum VS (Figure 4). Digestion with a 2.5% load has a higher AD velocity and, on average, 5–15% more BMP is obtained from day 3 to day 25 in comparison to the 5% sample. Although the 30-day average BMP value for both samples reach the same value of 549 mLCH₄/gVS, studies with activated sludge digestate as the inoculum and 2.5% BHW demonstrated significantly lower biomethane potential-378 mLCH₄/gVS [55]. It is probably the more adapted bacterial consortia in the agricultural digestate containing a higher amount of *Acetoanaerobium* spp. [25], *Rhodococcus justice* [26], *Sporomusa* sp. [27], *Clostridium* sp. and *Methanoculleus* sp. [23,24], which are able to use the lignin biomass leftovers in the BHW more efficiently than consortia from the activated sludge digestate. Thus, 2.5% was selected for further co-digestion tests.

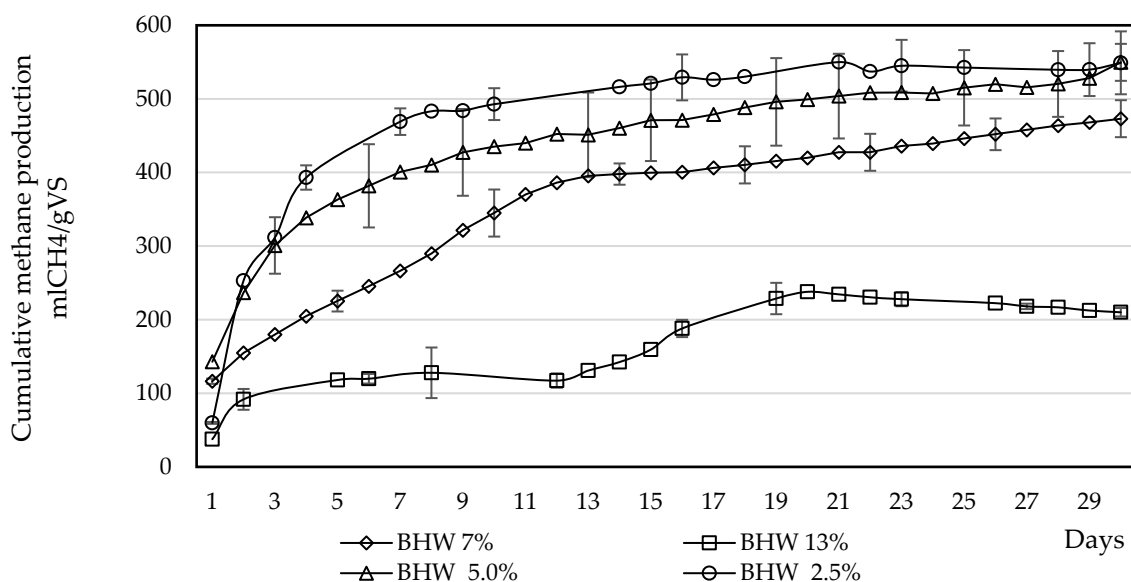


Figure 4. Cumulative methane outcome in 30 days for biomass hydrolysis waste digestion in different VS load from inoculum VS.

The increase in BHW substrate concentration in the AD resulted in the decrease in biogas potential to 473 and 210 mLCH₄/gVS for 7% and 13% BHW, respectively.

Similar inhibition is seen with the sample that has 30% FOG loaded from the inoculum VS. In the beginning of the fermentation, the BMP value drops below zero, showing that batches with only the inoculum loaded had generated more biomethane than the 30% FOG sample (Figure 5). The AD with 30% FOG is highly inhibited until day 23 and, afterwards,

a rapid increase in the methane production occurs and continues until day 30, when the experiment was stopped. At 845 mLCH₄/gVS BMP, the stationary phase was still not achieved; however, it should be noted that a decrease would follow in the later days. In general, the biogas industry is interested in producing as much biomethane from the biomass unit as possible but, at the same time, the hydraulic retention time is crucial to achieve a stable and economical production; therefore, co-substrates with a high retention time are not favourable for the industrial AD process.

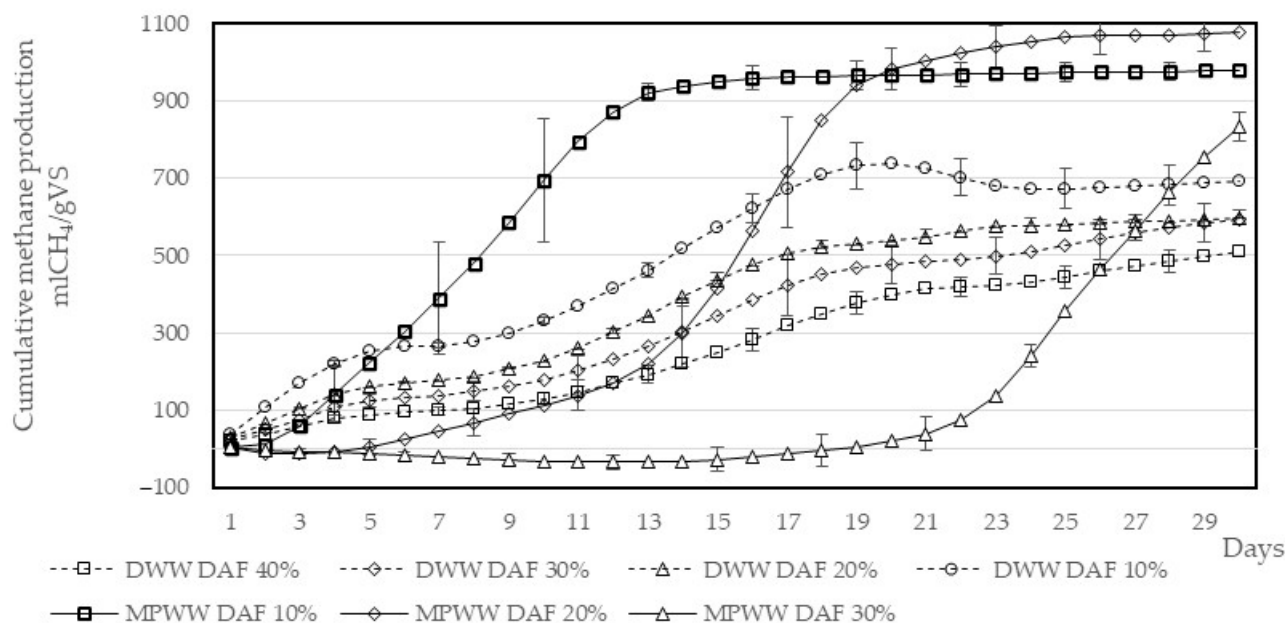


Figure 5. Meat and dairy processing wastewater FOG cumulative biomethane outcome in 30 days with different sludge loads from inoculum VS.

FOG-containing substrates such as grease trap waste from wastewater treatment plants have been added to the AD process in different concentrations, from 30 to 60% from the VS total, and have shown different BMP potential 344–681 mLCH₄/gVS [18], respectively. However, there is a lack of information about FOG-containing grease trap waste co-digestion in agricultural biogas stations or the maximum possible FOG loading rate under these conditions. The FOG substrate loading rate for the un-adopted inoculum is a very important factor due to the fact that it can cause the LCFA and SCFA concentration to increase and inhibit the methanogenesis process [56]. The AD process inhibition with agricultural digestate and different FOG-containing substrate load levels was also observed in this study (Figure 5).

The assessment of DWW DAF as a co-digestate showed that the inoculum substrate's toxic effect appears when the total inoculum VS exceeds 10%. The 30-day BMP value of 691 mLCH₄/gVS was obtained. At the same time, the DWW DAF substrate load of 20 and 30% from the inoculum VS is showing almost the same BMP value of 597 and 593 mLCH₄/gVS, respectively. This is, on average, 14% lower than for the 10% loading. The BMP value is decreasing even more when DWW DAF is increased to 40%, producing only 510 mLCH₄/gVS (26% less than the best performing concentration) (Figure 5).

The same inhibition pattern is also seen with MPWW DAF anaerobic digestion when the substrate concentration is more than 10% from the inoculum VS loaded, using the same type of inoculum. Although, in this case the total BMP outcome from two-times higher FOG load is showing 1077 mLCH₄/gVS, but the sample with a 10% load is reaching only 978 mLCH₄/gVS. Even though the BMP value for sample with a 10% FOG load is lower, the AD velocity for this sample is higher and the cumulative BMP curve is reaching the plateau after 12 days, while the sample with 20% loading reached it after 20 days, indicating

that the bacterial consortia present in the reactor had difficulty turning all the hydrolysis products into biomethane.

3.2. Surface Active Substance Influence on Selected Substrate BMP

Agricultural biogas stations can benefit from the FOG-containing substrates in co-digestion due to both an increase in the biomethane outcome, but also having no negative effect on the digestate dewaterability [57]. To act towards the minimization of the inhibitory effects on BMP, an anionic surfactant (100 and 200 ppm from the final mass) was added to the FOG anaerobic digestion samples using agricultural digestate as the inoculum. These surfactant concentrations have been chosen from the preliminary experiment results with FOG emulsification efficiency in water, as well as the results from the experiments with biodegradable surfactant influence on BHW [55].

Anionic, linear, readily biodegradable surfactants, such as sodium dodecyl sulphate, maintains the inhibitory effect on the AD process [58], but biologically derived surfactants, such as those found in bovine bile, show a favourable effect for the FOG substrate AD [43]. Within this study, FOG pre-treatment was performed with a biodegradable anionic surfactant, where the active substance is a proteo-lipid with alkali chains based on natural fatty acids. The results showed an increase in the BMP potential of MPWW DAF sludge from 1374.5 to 1765 mLCH₄/gVS (with 10% FOG and 200 ppm surfactants), as well as the surfactant showing a favourable effect already, at 100 ppm concentration (Figure 6), if compared to the non-treated sample. The BMP potential increase after 200 ppm or 100 ppm SAS addition reached a 10% and 22% increase, respectively. At the same time, the increase in BMP for DWW DAF sludge is even higher and, for the same used concentrations, reached 27% and 30% enrichment, respectively. Simultaneously, surfactant addition is not increasing the AD velocity, leaving it approximately the same for both sludge types used.

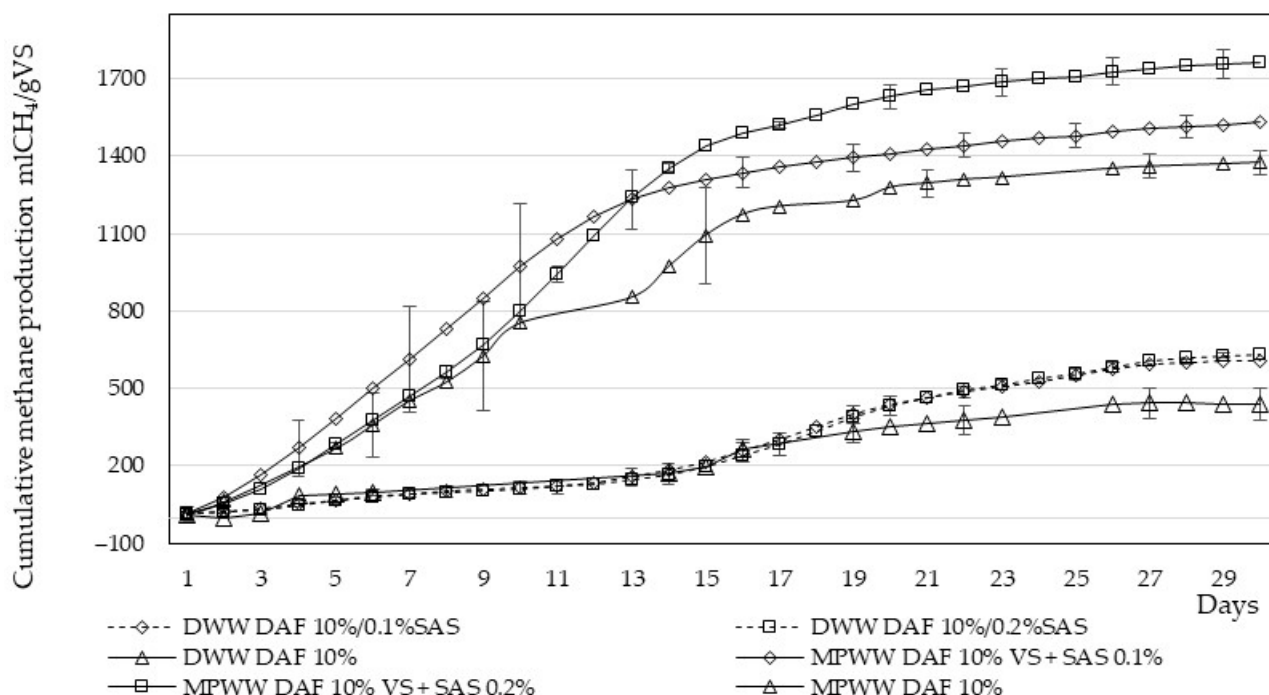


Figure 6. MPWW DAF sludge substrate and DWW DAF sludge substrate 30-day cumulative BMP value with anionic surfactant effect.

Anionic surfactant positive effect on the AD process cannot be directly linked to the HES amount added to the system, since MPWW DAF sludge contains around 82% HES from the total VS, while DWW DAF sludge only contains around 18% of (Figure 2). This positive effect has also been detected in the preliminary studies where the same surfactant showed a favourable effect for the BHW substrate AD [55]. Although the BMP values

were detected in different experimental set-ups, there is a clear beneficial influence on the addition of a biodegradable anionic surfactant to the AD. Still, studies related to in-depth understanding of the mechanisms are needed.

3.3. BHW and FOG-Containing Primary Sludge Co-Digestion with SAS Addition

BHW co-digestion with a FOG-containing substrate has an antigenic effect in the AD system with agricultural digestate inoculum. Cumulative BMP development (Figure 7) shows an optimal fermentation pattern without an extended AD lag phase for both the AD system with MPWW DAF sludge and BHW, and DWW DAF sludge and BHW with 200 ppm SAS addition, and it is reaching their BMP curve plateau starting from day 13 and day 19, respectively. This is similar to what has been observed in FOG digestion in the presence of SAS (Figure 6). Traces of the BHW substrate toxic effect is also present in the sludge and co-digestion system, because the 30-day BMP value for 2.5% BHW addition from the inoculum VS is 12% higher and reaches 534 mLCH₄/gVS for DWW DAF sludge co-digestion, and 22% higher (reaching 740 mLCH₄/gVS) for MPWW DAF substrate co-digestion, compared to the 5% BHW addition.

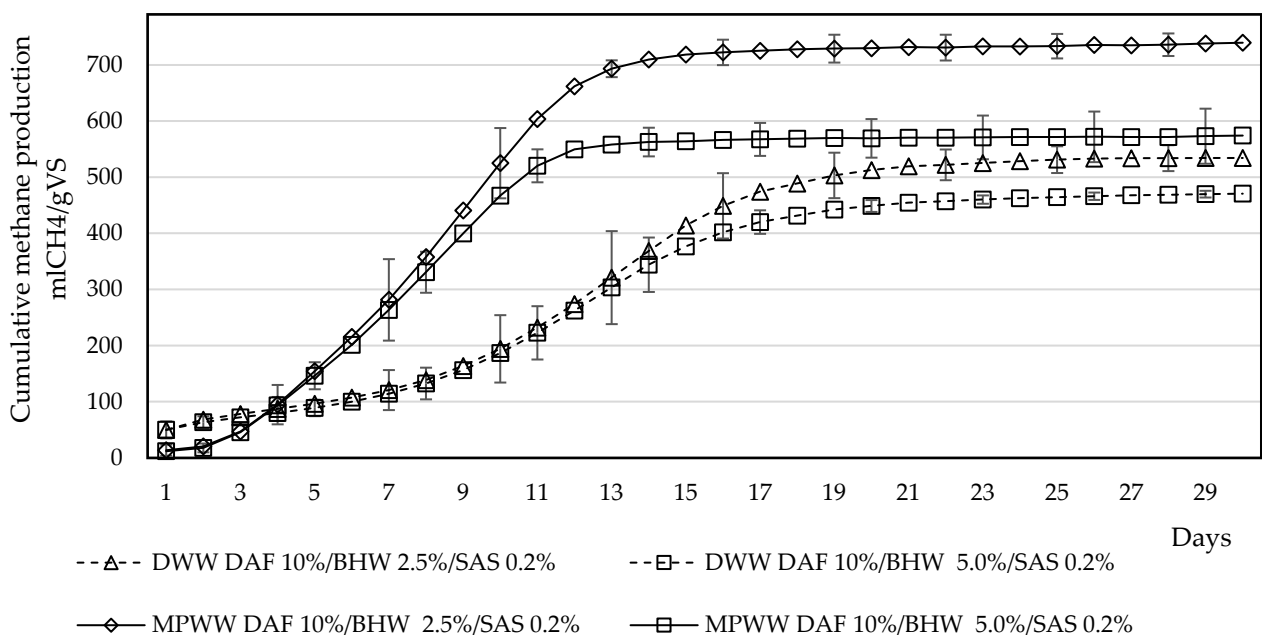


Figure 7. Meat production wastewater (MPWW) DAF sludge together with biomass hydrolysis waste (BHW) co-digestion with anionic surfactant, and dairy wastewater (DWW) DAF sludge with biomass hydrolysis waste (BHW) co-digestion with anionic surfactant 30-day cumulative BMP.

A combination of MPWW DAF and DWW DAF with BHW substrate compositions are, in general, showing lower BMP values when compared to other experimental setups. To some extent, this can be explained by the fact that it is hard to directly compare different experimental sets (new inoculum was taken every time) and the exact bacterial consortia changes have not been determined, as well as the inoculum BMP varying from 39 to 103 mLCH₄/gVS. Nevertheless, it cannot be rejected that FOG-containing sludge and BHW substrate co-digestion has a more or less alginic effect on the AD process than its use as a separate co-substrate. One of the reasons might be the unfavourable C/N ration or the lignin and lignocellulose hydrolysis product accumulation that disturbs further acetogenesis and methanogenesis processes.

BWH is a new substrate for the AD industry with the potential to grow in volume as a side product from upstream processes. Little is known about this substrate digestion and co-digestion possibilities to fully use its BMP; therefore, more research is needed to gain this knowledge and provide it to the industry to incorporate BHW into a circular economy.

Still, there is a need for research to evaluate potential co-digestion substrates, but also BWH pre-treatment and pre-digestion strategies and techniques. These findings may then be used to uncover concerns related to waste management and recycling.

3.4. BMP Outcome from Substrate Digestion and Co-Digestion and the Influence on Biomethane Release

Substrate BMP potential is one of the main parameters which describes the substrate industrial potential [53], and provides data about the biomethane release velocity and notes when 75% of the 30-day BMP is generated. FOG-containing wastewater treatment DAF sludge has shown that it has higher biomethane potential when compared to the BHW (Table 4). The highest BMP of 5% BHW loading from the total VS was 549.7 ± 25.1 mLCH₄/gVS. At the same time, the best FOG-containing wastewater treatment DAF sludge dosing produced 690.7 ± 54.8 and 979.9 ± 37.3 for 10% VS load from total VS for DWW DAF and MPWW DAF, respectively, without SAS addition. SAS addition further increased the wastewater treatment DAF sludge BMP value from 10–30%, depending on the wastewater treatment DAF sludge type used in the experiments.

Table 4. Sample BMP potential and biomethane release velocity and inoculum from agricultural biogas station BMP when supplemented with meat production wastewater (MPWW) DAF sludge, together with biomass hydrolysis waste (BHW) co-digestion with anionic surfactant and dairy wastewater (DWW) DAF sludge with BHW.

Sample ID	Inoculum BMP, mLCH ₄ /gVS	Sample BMP, mLCH ₄ /gVS	75% BMP Output, Days
BHW 13%	39.8 ± 1.8	473.2 ± 5.8	15
BHW 7%	39.8 ± 1.8	210.2 ± 25.1	10
BHW 5%	106.1 ± 5.3	549.7 ± 25.1	8
BHW 2.5%	106.1 ± 2.3	549 ± 42.7	4
DWW DAF 40%	94.8 ± 4.8	510.0 ± 30.2	19
DWW DAF 30%	94.8 ± 4.8	593.1 ± 48.5	18
DWW DAF 20%	94.8 ± 4.8	597.4 ± 19.2	15
DWW DAF 10%	94.8 ± 4.8	690.7 ± 54.8	14
MPWW DAF 30%	52.4 ± 5.3	834.7 ± 25.8	28
MPWW DAF 20%	52.4 ± 5.3	1077.1 ± 45.0	18
MPWW DAF 10%	52.4 ± 5.3	979.9 ± 37.3	10
DWW DAF 10% VS 0.1% SAS	80.5 ± 3.4	607.2 ± 12.4	21
DWW DAF 10% VS 0.2% SAS	80.5 ± 3.4	629.4 ± 10.2	21
DWW DAF 10%	80.5 ± 3.4	440.8 ± 61.7	19
MPWW DAF 10% VS + SAS 0.1%	61.5 ± 2.1	1531.1 ± 50.2	12
MPWW DAF 10% VS + SAS 0.2%	61.5 ± 2.1	1764.9 ± 55.4	14
MPWW DAF 10%	61.5 ± 2.1	1374.5 ± 47.6	15
DWW DAF 10%/BHW 2.5%/SAS 0.2%	106.3 ± 4.2	534.3 ± 25.8	15
DWW DAF 10%/BHW 5.0%/SAS 0.2%	106.3 ± 4.2	470.7 ± 6.2	16
MPWW DAF 10%/BHW 2.5%/SAS 0.2%	39.8 ± 1.8	739.5 ± 22.8	10
MPWW DAF 10%/BHW 5.0%/SAS 0.2%	39.8 ± 1.8	574.3 ± 50.2	9

The produced substrate co-digestion systems have not shown the expected increase in BMP, although the tested FOS/TAC values after 30 days of fermentation were not higher than 0.3, showing that the system has not been suffering from organic overload. The produced co-digestion systems demonstrated 70% and 15% lower BMP values when compared to the best performing MPWW DAF and DWW DAF biomethane potential values with SAS as process enhancers, respectively.

The fastest degrading substrate was BHW with the loading of 2.5% or less from the total loaded VS. In this case, 75% from 30-day BMP was generated within 4 days. Higher degradation times are shown by more complex substrates such as FOG-containing sludge, then 75% of 30-day BMP is produced in 10 or 14 days for DWW DAF and MPWW DAF, respectively (Table 4). In general, the outcome of this study demonstrates the possible use

and combination of various food production waste, with agricultural waste as substrates in co-digestion with agricultural digestate.

4. Conclusions

Anionic surfactant addition to the AD system, using agricultural digestate as the inoculum, is leaving a positive effect on meat production wastewater DAF sludge and dairy wastewater DAF sludge BMP values, increasing those by 22% and 30%, respectively. At the same time, the biodegradable anionic surface-active substance beneficial effect on different FOG-containing sludges is not linked to the HES concentration added to the AD.

Co-digestion with biomass hydrolysis waste was shown to be effective only at loadings below 5%. The 30-day average BMP value of 549 mLCH₄/gVS can be obtained for both 2.5% and 5% BHW. Supplementation with SAS and FOG increased the BMP for 25% and 5%, respectively. Nevertheless, BHW co-digestion with FOG-containing sludge produced an antigenic effect, and individual substrate AD at optimum loading will produce higher methane yields.

Substrates used for co-digestion must be strongly evaluated with respect to the available AD inoculum. All substrates used in this study demonstrated a decrease in biomethane velocity when the substrate concentration was increased, and it seems the SAS addition did not have a significant influence on this parameter. Thus, the evaluation of co-digestion on the biomethane generation velocity is complicated due to the changing impact of each selected substrate parameter.

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