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Anaerobic Co-Digestion of Cheese Whey and Industrial Hemp Residues Opens New Perspectives for the Valorization of Agri-Food Waste

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Abstract: Cheese whey (CW) and hemp hurds (HH) represent typically overabundant biowastes of food and agricultural production, and their circular management is crucial to improve both sustainability and profitability of the agri-food chain. By combining experimental biochemical methane potential (BMP) tests and literature data, the techno-economic aspects of a possible future bioenergy valorization of CW and HH through anaerobic digestion (AD) and co- digestion (coAD) were analyzed. Along the 42-days, BMP assays, CW, and HH alone rendered BMP values of 446 ± 66 and 242 ± 13 mL $CH_4 \cdot g\ VS^{-1}$, respectively. The application of coAD with CW and HH at a 70:30 ratio allowed to enhance the biomethane production by 10.7%, as compared to the corresponding calculated value. In terms of economic profitability, the valorization of HH as biomethane in a dual-purpose hemp cultivation could potentially enable net profits of up to 3929 €$\cdot ha^{-1}$, which could rise to 6124 €$\cdot ha^{-1}$ in case of coAD with CW. Finally, by projecting the biomethane potential from current and future available CW and HH residues in the national context of Italy, a total biomethane yield of up to 296 $MNm^3 \cdot y^{-1}$ could be attained, offering interesting perspectives for the sustainability of key sectors such as transportation.

Keywords: cheese whey; industrial hemp; residual feedstock; anaerobic digestion; methane; bioenergy perspectives; techno-economic assessment

1. Introduction

To satisfy the food demand of a steeply increasing human population, the agri-food sector has developed linear economy practices that generate high volumes of biowastes and disperse essential resources and elements (mainly C and N) in the environment. It is estimated that approximately 140 million tons of biodegradable wastes are yearly produced at the European Union (EU) level [1]. At the same time, EU encourages the member states towards the adoption of circular bioeconomy practices and the accomplishment of the Green Deal by 2050, which aims at making the EU resource efficient and completely climate-neutral [2]. However, major portions of organic waste are still improperly managed, mainly through landfilling, leading to the release of high CO_2 amounts having severe consequences in terms of global warming potential [3]. In this context, the implementation of new sustainable bio-based technologies is needed to produce clean, renewable energy and recover resources from organic waste. Despite the multitude of strategies that have been developed to valorize the energy content of organic feedstocks, anaerobic digestion (AD) still represents the most widely applied process due to its high efficiency, operational flexibility and overall environmental benefits [4,5] offered as compared to other biorefinery approaches.

Among the organic wastes generated by the agri-food chain, cheese whey (CW) is one of the most abundant. The dairy industry is widely spread all over the world, ensuring food security and economic incomes, especially for developing countries [6]. The processing of milk into dairy products results in the production of a highly carbon-rich liquid effluent, namely CW, characterized by chemical oxygen demand (COD) concentrations up to 100 g·L^{-1} [7]. Besides a limited reuse as animal feed additive, the main management practice for CW disposal has for years been the direct discharge in the sewage system [8]. However, owing to the ever increasing CW surplus, the application of AD to simultaneously recover organic carbon and bioenergy in the form of biomethane (CH_4) has gradually become a more suitable and sustainable alternative [9]. During the anaerobic biotransformation of CW, possible shortcomings are the recalcitrance to degradation of some proteins, the diversion of sugars towards H_2 and ethanol or the tendency to acidification due to its low alkalinity levels [8]. Notwithstanding, under proper process conditions, the AD of CW has enabled biomethane productions even higher than 0.5 L of CH_4 per gram of added volatile solid (VS) of substrate [6].

Besides dairy industry, new valorization opportunities are arising also from the emerging industrial hemp sector. Indeed, thanks to its multifaceted nature, hemp is being rediscovered as a precious source of bio-based materials, including fibers and seeds, and its cultivation is increasingly spreading at a global level [10]. Since in most cases the cultivation of each specific hemp variety targets the production of a single portion of the plant (e.g., seeds, fibers, and inflorescences), the remaining plant biomass is regarded as a waste [11]. In many countries such as Italy, the amount of such residual hemp biomass will inexorably increase in the next years as the commercialization of some plant components (mainly inflorescences) has stopped due to a contrasting legislation [12], prompting the accumulation of unused plant biomass directly on agricultural fields. Despite such increasingly large availability, the energy valorization of industrial hemp residues through AD has so far found a limited application. In the last decade, only a few studies have dealt with biomethane production from the whole hemp plant and hemp straw residues [13,14]. Thus, a new impulse should be given to the implementation of AD of hemp biomass, thereby limiting the unsustainable and still widely practiced open field burning of the residual hemp feedstock. However, a major bottleneck for the AD of hemp biomass residues, as lignocellulosic materials (LMs), is represented by their complex lignin- and carbohydrate-based structure, which significantly hinders the substrate bioaccessibility [15], as well as by the lack of crucial trace elements (TEs) for the biological process [16]. Given the above, an elegant way to improve the biomethane yields of the hemp residues is offered by co-digestion in the presence of a more easily degradable substrate, such as CW.

Anaerobic co-digestion (coAD) is commonly employed when using LMs to rebalance the too high carbon-to-nitrogen (C/N) ratio of the lignocellulosic matrix. This is normally performed by adding animal manure [17] or anaerobic sludge deriving from industrial processes [18] to the digesting mixture, in turn contributing to increase the buffer capacity as well. Furthermore, coAD can be beneficial for biogas production as the supplementation of a co-substrate procures essential TEs (e.g., Co, Ni, and Se). TEs enhance the enzymatic activity of the microbial consortia [19] and stabilize the digester by mitigating the buildup of volatile fatty acids (VFAs) [20], which is a negative outcome often present in CoAD of too rapidly biodegradable substrates. Experiences over the applications of coAD of CW and lignocellulosic residues are rather sporadic. Jung et al. [21] observed that adding CW to *Ulva* biomass increased the biomethane yield by 1.6 times and resulted in evolving bacterial and archaeal community structures. The authors concluded that the mixing ratio between the substrates is a crucial parameter for an efficient coAD. More recently, combining fish ensilage (15%) and CW (85%) allowed a C/N ratio ranging between 25% and 30% and a 12.5% higher biomethane production [22].

This study presents, for the first time, the application of coAD of CW and hemp hurds (HH) as a promising approach to synergistically enhance the biomethane yields of both substrates. Batch biochemical methane potential (BMP) assays were performed with four different substrate combinations, aimed at monitoring the production of biogas and the evolution of VFAs as AD intermediate compounds. Moreover, based on the experimental results obtained with the AD and coAD of CW and HH, a techno-economic assessment was conducted to estimate the profitability of combining dual-purpose hemp cultivation with HH and CW valorization as biomethane. Finally, as highly abundant residual feedstocks of the Italian agri-food sector, the waste stream volumes of CW and HH were used to estimate the present and future bioenergy valorization potential at the national level.

2. Materials and Methods

2.1. Sources of Cheese Whey and Raw Hemp

Fresh CW was collected from a cheese factory located near Salerno (Italy) mainly producing mozzarella cheese from both cow and buffalo milk. Once in the laboratory, CW was immediately poured in 500 mL plastic bottles and stored at −20 °C before being analyzed (Table 1) and used.

Table 1. Characterization of the cheese whey, retted hemp hurds, and inoculum used in this study. TS: total solids; VS: volatile solids: tCOD: total chemical oxygen demand; $N\text{-}NH_4^+$: ammonium nitrogen; N_{TOT}: total nitrogen. All the values are expressed as mean and standard deviation obtained using three replicates.

Parameter	Cheese Whey	Hemp Hurds	Inoculum
TS [%]	5.93 ± 0.63	89.66 ± 0.19	6.65 ± 0.08
VS [%]	5.00 ± 0.63	85.77 ± 0.50	4.51 ± 0.02
tCOD [$g\cdot L^{-1}$]	47.50 ± 0.46	n.a.	n.a.
$N\text{-}NH_4^+$ [$g\cdot L^{-1}$]	0.07 ± 0.01	n.a.	1.12 ± 0.05
N_{TOT} [$g\cdot L^{-1}$]	0.48 ± 0.05	n.a.	1.39 ± 0.04 [1]
pH	4.75 ± 0.08	n.a.	8.57 ± 0.02 [1]

[1] These values were calculated (N_{TOT}) and taken (pH) from Bianco et al. [23], who used the same digestate as inoculum in their study.

Hemp biomass belonged to the cultivar "Eletta campana" and was collected from an industrial hemp cultivation of *Cannabis sativa* L., located in the northern area of the metropolitan city of Naples (Italy). During harvesting, hemp stems were separately collected from leaves and inflorescences and then stored at room temperature prior to their use.

2.2. Processing of Raw Hemp

In the laboratory, hemp stems were processed with the aim to separate the hurds from fibers [24]. Stems were retted by soaking them into tap water for six days. Subsequently, fibers and hurds were manually separated and dried at 45 °C for 72 h in a TCN 115 laboratory oven (Argo Lab, Italy). Once dried, the HH were ground and sieved and only the fraction between 2 and 4 mm was considered. Table 1 reports the total solids (TS) and vs. of the hurds used in this study.

2.3. Biochemical Methane Potential Assays

The BMP tests were performed in 100 mL serum bottles (Wheaton, IL, USA), which were placed in a thermostatic water bath to maintain a controlled mesophilic temperature ranging between 36 and 38 °C. As anaerobic inoculum, a digestate from a full-scale AD digester treating buffalo manure, located in the area of Salerno (Italy), was used. Table 1 reports the physical-chemical characterization of the inoculum. In order to let methanogens acclimate to mesophilic conditions, the inoculum was maintained at 37 (± 1) °C for 2 days before starting the BMP assays. Afterwards, an amount of 40 g of inoculum, corresponding to 1.80 g of VS, was poured into each bottle. To guarantee an inoculum to substrate ratio of 2 g $VS\cdot g\ VS^{-1}$, 0.90 g of vs. of substrate was added to all the bottles. In particular, four different substrate combinations were used (on a vs. basis): 100% CW, 70% CW + 30% HH, 30% CW + 70% HH, and 100% HH. The final working volume in all experiments was adjusted to 50 mL by adding deionized water. Flushing with analytical grade nitrogen gas (Rivoira, Italy) for 2 min was performed to purge each bottle of residual oxygen. Finally, all the bottles were sealed with rubber septa and aluminum crimps.

Control bioassays with only inoculum and deionized water were also prepared to evaluate the biomethane production of the sole inoculum. This was used to calculate the net biomethane production of the different substrate mixtures by subtracting it from the BMP values measured in the other tests. All the experimental conditions were investigated in triplicates.

2.4. Assessment of the Effect of Anaerobic Co-Digestion

In order to evaluate the synergistic effect of coAD on the BMP of CW and HH, the biomethane productions achieved with 70% CW + 30% HH and 30% CW + 70% HH were compared with those calculated (BMP_{calc}) by considering the 30% and 70% of the experimental BMP (BMP_{exp}) obtained with standalone CW and HH, as reported in Equations (1) and (2):

$$BMP_{calc}\ (70\%\ CW + 30\%\ HH) = 0.7 \cdot BMP_{exp}\ (100\%\ CW) + 0.3 \cdot BMP_{exp}\ (100\%\ HH) \quad (1)$$

$$BMP_{calc}\ (30\%\ CW + 70\%\ HH) = 0.3 \cdot BMP_{exp}\ (100\%\ CW) + 0.7 \cdot BMP_{exp}\ (100\%\ HH) \quad (2)$$

2.5. Analytical Procedures

Biomethane production was monitored along 42 days using a volumetric displacement system, consisting of a 12% NaOH carbon dioxide trap solution (400 mL) and a vessel (400 mL) containing deionized water to be displaced. The digestate in the bottles was sampled six times in the first two weeks for the measurement of VFAs. After sampling, the digestate was immediately frozen at −20 °C. Prior to VFA analysis, the samples were centrifuged with a Multispin 12 mini centrifuge (Argo Lab, Italy) at 10,000 rpm for 5 min. The supernatant was diluted with deionized water and filtered through 0.20 µm polypropylene membranes (VWR, Milano, Italy). The procedures used for TS and vs. determination as well as the VFA and lactic acid measurement protocol and equipment are reported by Bianco et al. [25]. Following the standard methods, total chemical oxygen demand (tCOD) of CW was determined spectrophotometrically after dichromate digestion [26]. Ammonium ($N\text{-}NH_4^+$) and total (N_{TOT}) nitrogen were measured through commercial kits (VWR, Milano, Italy).

2.6. Techno-Economic Assessment

The techno-economic feasibility of dual-purpose hemp cultivation for bioproducts and bioenergy generation was assessed by considering the production of fibers and the concomitant energetic valorization of HH as biomethane, with or without CW. The factors considered in the analysis included hemp biomass yields, growing and harvesting costs, fiber prices as well as biomethane yields and profitability. For reasons of simplicity, operational and capital costs relating to the AD or coAD processes were excluded from this study by considering the net profit of an average sized biomethane plant with a production capacity of 200 $Nm^3 \cdot h^{-1}$. In view of the economic incentives for biomethane production recently established in Italy, such plant size was identified as capable of generating net profits in the order of 0.23 $€ \cdot Nm^{-3}$ [27]. Average growing and harvesting costs of hemp were estimated to be 673 $€ \cdot ha^{-1}$ [28], while a reference market value of 1.13 $€ \cdot kg^{-1}$ was assumed for fibers [29].

Three different scenarios were identified based on minimum, average and maximum hemp biomass yields of 2, 10, and 18 $ton \cdot ha^{-1} \cdot y^{-1}$, respectively [30]. An average biomass composition of industrial hemp for fibers production was used to estimate the resulting biomass productivity in terms of fibers and HH [31]. Such composition considers a 20% fiber and a 50% HH content on a dry weight basis. The economic and energetic potentials of the remaining 30% biomass dry weight, composed by leaves, inflorescences, and seeds, were conservatively not considered in this study. The overall profits included the potential revenue from fibers and the net profits from the biomethane produced from AD or coAD of HH and CW. The biomethane profit in case of plain AD was obtained by using the BMP observed in this study to the different HH yields assumed. A constant and conservative vs. content of 90% was considered as characteristic of HH for all the biomass productivity scenarios. In case of coAD, the biomethane revenue was calculated by assuming that all the HH available could be co-digested with CW, yielding biomethane accordingly to the BMP observed in this study. Based on the above, three different scenarios of profitability from biomethane were foreseen. A first scenario (AD) considered only the AD of HH; a second scenario (coAD1) considered the coAD with 30% CW + 70% HH; and a third scenario (coAD2) considered the coAD with 70% CW + 30% HH. Finally, to account for potential price volatility, a price range for fibers and biomethane was defined by considering a ±20% variability from the reference values reported above. All conversions from US Dollar (USD) to Euro (€) were based on a 0.93 €·USD^{-1} factor.

2.7. Assumptions for the Estimation of the Biomethane Potential from Cheese Whey and Hemp Hurds in Italy

The total biomethane potential from CW and HH in Italy was estimated based on their current (for CW) and perspective (for HH) availability within the Italian national context. The low (CW-L) and high (CW-H) scenarios for CW were calculated by considering an availability of 20% and 100%, respectively, of the 4.5 million tons of CW annually disposed in Italy [32]. The total biomethane potential was calculated by considering the same vs. content (i.e., 5%) and BMP measured for CW in this study. The low (HH-L), average (HH-A) and high (HH-H) biomethane potential scenarios from HH were calculated by assuming biomass productivities of 2, 10, and 18 ton·ha^{-1}·y^{-1}, respectively, and applying those together with the BMP values observed in this study for HH to a potential land surface of 100,000 ha.

3. Results and Discussion

3.1. Anaerobic Digestion of Individual Cheese Whey and Hemp Hurds

The individual anaerobic biodegradability of CW and HH towards the production of methane was initially investigated in this study. The specific cumulative biomethane profiles obtained from standalone CW and HH are shown in Figure 1:

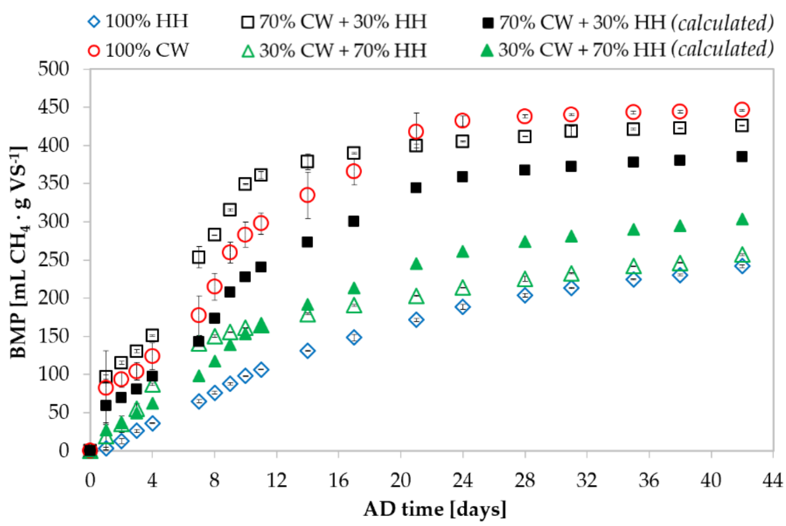

Figure 1. Cumulative, specific biomethane production profiles obtained along the 42 days of mesophilic anaerobic digestion and co-digestion with the four different substrate combinations tested. The filled squares and triangles indicate the calculated biomethane productions.

CW showed the highest AD performance, with the biomethane production reaching a final value of 446 ± 66 mL CH_4·g VS^{-1} (Table 2). This result well averages the interval of 300 to 600 mL CH_4·g VS^{-1} reported for CW in the scientific literature [6,22] A fast CW biodegradation was observed as indicated by the almost 50% of the final BMP achieved after only nine days. This was also due to the high initial

VFA concentration (see Section 3.3), immediately available to methanogens for the conversion into methane. An imbalance between the activity rates of acidogenic and methanogenic microorganisms has been reported as one of the most frequent causes of failure of anaerobic digesters fed with CW [8]. However, methanogenesis was favored in this study by the high alkalinity of the inoculum used [23], which prevented the acidification of the experiments. As a result, hydrogen gas (H_2) was not detected in the biogas obtained. In addition, the use of an anaerobic digestate as inoculum allowed to rebalance the C/N ratio within an appropriate range for the AD process.

Table 2. Final specific biochemical methane potential (BMP) obtained after 42 days under the four different substrate combinations used. The biomethane yields calculated (BMP_{calc}) considering the 30% and 70% of the BMP experimentally obtained (BMP_{exp}) with 100% cheese whey (CW) and 100% hemp hurds (HH) are also reported. Δ is the variation between BMP_{exp} and the corresponding BMP_{calc} owing to co-digestion. VS: volatile solids.

Experimental Condition	**Initial vs. [g]**		**BMP_{exp} [mL CH_4·g VS^{-1}]**	**BMP_{calc} [mL CH_4·g VS^{-1}]**	**Δ [%]**
	CW	**HH**			
100% CW	0.90	-	446 ± 66		
70% CW + 30% HH	0.63	0.27	426 ± 31	385	+10.7%
30% CW + 70% HH	0.27	0.63	257 ± 34	303	−15.2%
100% HH	-	0.90	242 ± 13		

The use of retted HH as the sole substrate for AD resulted in a final biomethane yield of 242 ± 13 mL CH_4·g VS^{-1}. The BMP observed in this work is in line with those reported by the few existing studies, which all investigated the AD of the whole hemp plant biomass. Adamovičs et al. [13] used finely ground (1–5 mm) hemp biomass from USO 31 and Futura 75 cultivars and obtained BMP values of 216 and 246 mL CH_4·g VS^{-1}, respectively. A BMP value of 234 mL CH_4·g VS^{-1} was reported for Futura 75 biomass digested under thermophilic conditions (50 °C) [14]. Higher biomethane yields up to 301 mL CH_4·g VS^{-1} were observed by Heiermann et al. [33], who used a hemp plant belonging to the Fedora 19 cultivar. Being most often wasted together with leaves as not suitable for the production of high value bioproducts, HH represents an optimal feedstock for AD. This consideration further justifies the choice of using it as the sole hemp biomass component in the BMP tests performed in this study. Moreover, considering that HH makes up to 52% (*w/w*) of the whole plant dry biomass, even in the case of dual-purpose production of hemp for seeds or fibers, AD would always benefit from a large availability of such residual substrate. On the other hand, the high lignin content (i.e., 21–24%) in HH [34] might pose challenges of slow rates for microbial degradation towards methane. In this sense, the coAD with CW, as a more easily degradable substrate, offers interesting perspectives.

3.2. Effect of Co-Digestion on the Biomethane Production of Cheese Whey and Hemp Hurds

Figure 1 shows the profiles of the specific biomethane production obtained during the coAD of 70% CW + 30% HH and 30% CW + 70% HH over 42 days. The addition of CW to HH at a 30:70 ratio (on a vs. basis) led to a 6% higher BMP, i.e., 257 ± 34 mL CH_4·g VS^{-1}, than that achieved with the HH alone. A further increase up to 426 ± 31 mL CH_4·g VS^{-1} was observed when using CW as main substrate at a 70:30 ratio with HH (Table 2). This BMP value was only 4% lower than that produced from the sole CW.

In this study, the use of two substrate combinations for coAD was considered mainly for two reasons. First, the selection and the mixing ratio between different substrates are highly dependent on the seasonal availability of feedstocks. Investigating multiple substrate mixtures has positive practical implications, as it allows to assess the efficiency of the coAD process at different feed dosages and to better deal with the limitations associated with the lack or deficiency of a specific feedstock in some periods of the year [35]. Second, the supplementation of different feed mixtures can reveal possible synergistic effects, resulting in an improvement of the process kinetics or final biomethane yield [36].

With the aim to evaluate the potential benefits of applying coAD, the biomethane productions here achieved with the two CW:HH blends were compared with those calculated considering the 30% and 70% of the BMP of the individual CW and HH. When using a feed mixture made up of 70% CW and 30% HH, a significant enhancement of the process kinetics, even faster than that achieved with the sole CW as substrate until day 17 (Figure 1), was observed. Moreover, the BMP of 426 ± 31 mL CH_4·g VS^{-1} obtained after 42 days was 10.7% higher than the calculated one (Table 2), revealing that coAD stimulated the anaerobic conversion to methane of the two substrates in comparison to the single feedstocks. Likely, the synergism obtained combining CW and HH under these operating conditions was not due to a more balanced C/N ratio or an increased presence of essential TEs. This benefit is, in fact, more typically observed when animal manures are chosen as co-substrates for CW [37] or lignocellulosic wastes [17], since both CW and HH are both rich in carbon and poor in nitrogen (Table 1) and TEs. Rather, the production and activity of hydrolytic enzymes in the presence of the readily-utilizable CW might have been stimulated, leading to an increased HH biodegradability [21].

On the contrary, the 257 ± 34 mL CH_4·g VS^{-1} experimentally obtained with 30% CW + 70% HH was 15.2% lower than the corresponding calculated value, i.e., 303 mL CH_4·g VS^{-1} (Table 2). During coAD of several substrates, antagonistic effects may arise from multiple factors including free ammonia toxicity, pH decrease, or an increased VFA concentration [38], and are often not easy to describe. As pH was not out of the optimal range for AD (see Section 3.3) and free ammonia concentrations did not likely rise beyond threshold values (given the low N amount in the substrates), the negative result could have been caused by local VFA accumulations. A more plausible reason could be that the amount of CW was not enough to significantly stimulate the enzymatic hydrolysis and, thus, the biomethane production from the HH-predominant mixture. In view of the above, future research should be addressed to shed light on the optimal CW:HH combination.

3.3. Evolution and Speciation of Volatile Fatty Acids

The evolution of AD of the single CW and HH and of coAD of the combined substrates was also assessed in terms of VFAs along the initial 14 days. The sum of acetic, propionic, isobutyric, butyric and isovaleric acids, all expressed as concentration of acetic acid equivalent (mg HAc·L^{-1}), was used to obtain the total VFA concentration, which is shown in Figure 2A. The percentage of the single acids (VFAs + lactic acid) over the total acid concentration was also monitored, as shown in Figure 2B.

The highest total VFA concentrations, i.e., approximately 7790 and 7235 mg HAc·L^{-1}, were observed in the first two initial days when using CW as the sole substrate or in combination with HH at a 70:30 ratio, respectively. This indicates a fast acidogenesis of lactose, as also confirmed by the 23% of lactic acid observed on day 1 (Figure 2B), and other organic compounds as well as the presence of already high VFA levels in CW. Despite VFAs rapidly increased, the pH values in the assays remained within the optimal range (i.e., 6.7–7.8) for AD, allowing to maintain VFAs in their non-protonated form. VFAs were then considerably depleted until day 10, particularly in presence of a CW:HH ratio of 70:30 (Figure 2A). The latter is in accordance with the quickly increasing biomethane production observed in all tests and especially for the coAD of 70% CW and 30% HH (Figure 1). The remaining VFA concentration on day 14 was 2210 and 60 mg HAc·L^{-1} with 100% CW and a CW:HH ratio of 70:30, respectively. In the case of 100% CW, propionic acid was the main residual VFA, as also reported by Atasoy et al. [39] who investigated the production of VFAs from CW in batch reactors at pH higher than 7.

In comparison with the high VFA levels obtained when CW was the sole or the main substrate in the digesting mixture, the use of HH alone or in combination with CW at a CW:HH ratio of 30:70 resulted in a significantly lower VFA buildup. The VFA concentrations observed with the sole retted HH did not exceed 90 mg HAc·L^{-1} (Figure 2A), whereas 1810 mg HAc·L^{-1} was obtained as VFA peak after two days during coAD of 30% CW and 70% HH. The low VFA accumulation with HH alone is typical of the AD of untreated LMs. The resistant cellulose and hemicellulose structures linked within a lignin network hampers the hydrolysis and the consequent acidogenesis of the material.

This often implies the need of pretreatment methods capable of delignification or cellulose dissolution to remove the kinetic limitations and eventually enhance biogas production [40]. After 14 days, the VFA concentration was below 25 mg HAc·L^{-1}, indicating the substantial completion of the AD process.

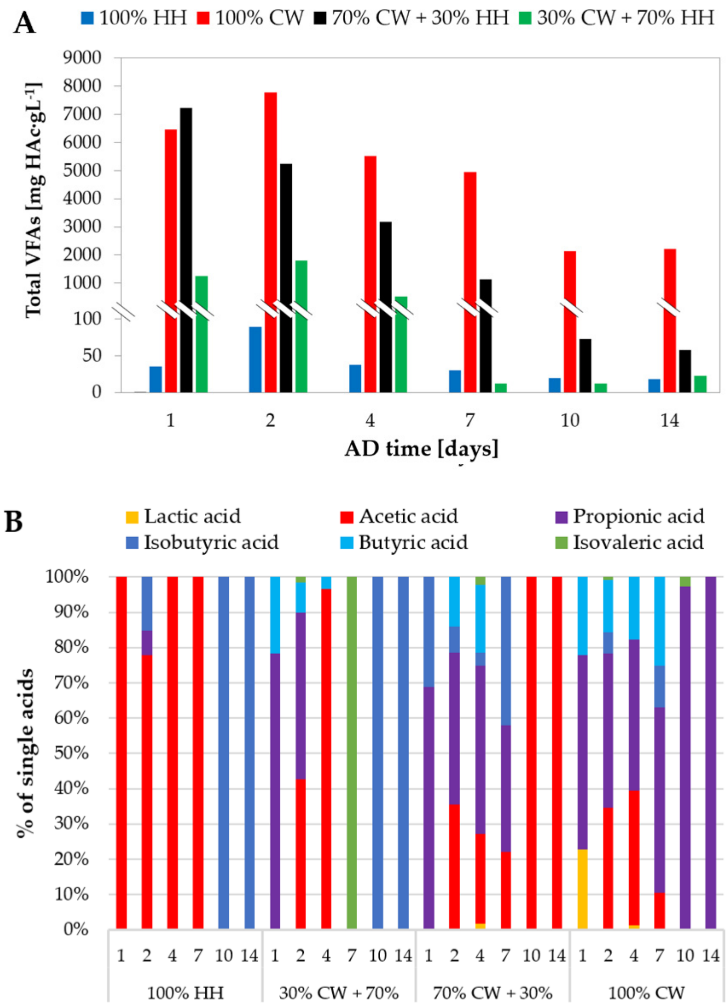

Figure 2. (**A**) Concentration of total volatile fatty acids (VFAs) as intermediates of anaerobic digestion (AD) of standalone cheese whey (CW) and hemp hurds (HH) as well as of co-digestion (coAD) of 70% CW + 30% HH and 30% CW + 70% HH. (**B**) Percentage of lactic acid and each VFA over the total acid concentration (calculated on an equivalent acetic acid basis) during the initial 14 days of AD and coAD.

3.4. Preliminary Techno-Economic Assessment of Bioenergy Valorization of Hemp Hurds with and without Cheese Whey

The energetic valorization of HH enables the cultivation of hemp as a dual-purpose crop for biomass and fibers. Figure 3 shows the potential revenues and profits (panel A) and overall profit (panel B) that could be attained by such a dual-purpose industrial hemp cultivation, with or without the availability of CW for coAD with HH. The BMP values observed in this study were used to estimate the total biomethane productivity based on a wide range of possible yields for hemp biomass and the corresponding ones for fibers and HH.

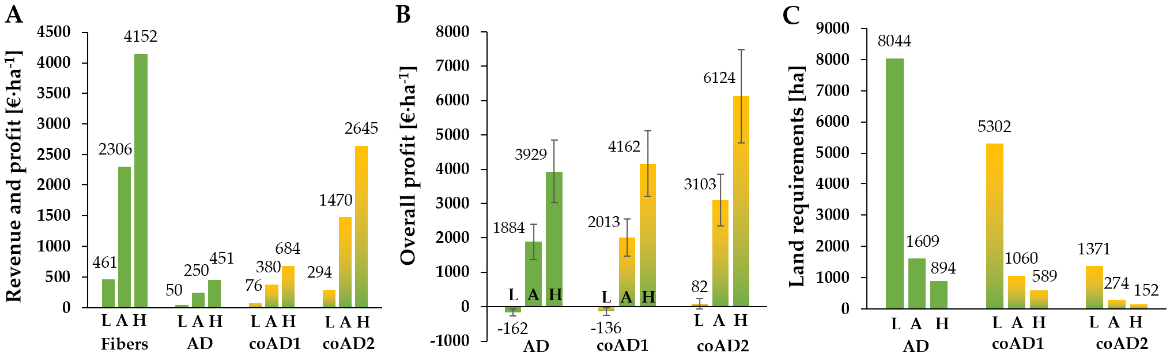

Figure 3. (**A**)Potential revenue per hectare from hemp fibers and net profits of biomethane from hemp hurds (HH) with and without cheese whey (CW), (**B**) overall profit per hectare, (**C**) and land required to sustain the production of a 200 Nm^3·h^{-1} biomethane plant. Bars in panel B indicate the range of overall profit obtained by considering a low (−20%) and a high (+20%) price range for fibers and biomethane. L, A, and H relate to low, average and high hemp biomass productivities of 2, 10 and 18 ton·ha^{-1}·y $^{-1}$, respectively. AD: AD of single HH; coAD1: coAD with 30% CW + 70% HH; coAD2: coAD with 70% CW + 30% HH.

With an average market value of 1.13 €·kg^{-1}, a potential revenue of 461, 2306 and 4152 €·ha^{-1} could be attained from fibers alone in case of low, average, and high fibers yields of 0.4, 2.0 and 3.6 ton·ha^{-1}, respectively. The potential for bioenergy valorization of HH as biomethane considered instead low, average and high HH yields of 1.0, 5.0, and 9.0 ton·ha^{-1}, respectively. Based on an average biomethane net profit of 0.23 €·Nm^{-3} calculated from a 200 Nm^3·h^{-1} plant (see Section 2.6), the AD of HH could profit from a minimum of 50 to a maximum of 451 €·ha^{-1}. In case of CW availability for coAD, the same amounts of HH could sustain a much higher biomethane productivity, which would provide profits ranging between 76 and 684 €·ha^{-1} with 30% CW + 70% HH, and from 294 to 2645 €·ha^{-1} with 70% CW + 30% HH.

When the revenue from fibers and the profits from biomethane are considered together with an average cost for biomass production and processing of 673 €·ha^{-1}, an overall profitability estimate can be made (Figure 3B). In the scenario with a biomass productivity as low as 2 ton·ha^{-1}, the corresponding low yields of fibers and HH would not be able to offset the production and processing costs, resulting in a negative profit of 162 and 135 €·ha^{-1} in case of AD with 100% HH and of coAD with 30% CW + 70% HH, respectively. Only a coAD with 70% CW + 30% HH would generate a positive profit of 82 €·ha^{-1}. When average and high biomass yields are considered, the overall potential profits would instead be markedly positive, going from a minimum of 1884 €·ha^{-1} for AD with 100% HH to a maximum of 6124 €·ha^{-1} for coAD with 70% CW + 30% HH. Such increased profitability per hectare could be attained only if CW would be available from dairy activities in the surroundings of the hemp cultivation and biomethane plant. The availability of CW to perform coAD with 30% HH would also offer the great benefit of lowering by more than 80% the land required to sustain the production of a 200 Nm^3·h^{-1} biomethane plant, which would decrease, for example, from 1609 to 274 ha in case of an average hemp biomass productivity of 10 ton·ha^{-1} (Figure 3C).

While few attempts of techno-economic assessment have been recently made to estimate the overall profits of dual-purpose industrial hemp production, the main focus of these studies was the valorization of hemp biomass as bioethanol [28,30]. Indeed, to the authors' best knowledge, this is the first study that attempts to estimate the profitability potential of dual-purpose hemp cultivation for fibers and biomethane production. The overall profit of 1884 €·ha^{-1} calculated for an average biomass productivity and AD of 100% HH is comparable with the highest profit of 2132 €·ha^{-1} calculated by Das et al. [30], who considered a production of both seeds and bioethanol from NWG 331 hemp cultivar having a yearly productivity of 7.7 ton·ha^{-1}. In a previous study, the same authors calculated an overall profitability of 2448 €·ha^{-1} from bioethanol and seeds from Futura 75 cultivar produced at

5.3 ton·ha^{-1} [28]. Although the bioethanol route could be more profitable at the average 10 ton·ha^{-1} biomass yield here considered, the fact that HH can be converted into biomethane without the need of physical-chemical or enzymatic pretreatments required for ethanol fermentation, makes the biomethane route a more scalable and flexible bioenergy platform.

3.5. The Overall Biomethane Potential from Cheese Whey and Hemp Hurds in Italy: A Future Outlook for the Transportation Sector

The uniqueness of the Italian context allows to complete the present techno-economic analysis with further outlooks over the national-scale and future bioenergy potentials of CW and HH. Italy is the third cheese producer in Europe, and disposes about 4.5 million tons of CW each year [32,41]. Until the first half of the XX century, Italy was also the second global producer of hemp, with over 100,000 ha of cultivations [42]. At present, hemp cultivation is again ramping up with increasing speed in Italy, as the land dedicated to hemp has increased more than 10 times from 2013, achieving more than 4000 ha in 2018. Finally, Italy is the first European country for natural gas utilization as transportation fuel, with an annual demand of 90 and 1100 MNm^3 of methane for public and private transportation, respectively [43].

Figure 4A shows how, by valorizing only one-fifth or the total CW currently available, Italy could attain a biomethane production of approximately 20 and 100 MNm^3·y^{-1}, respectively.

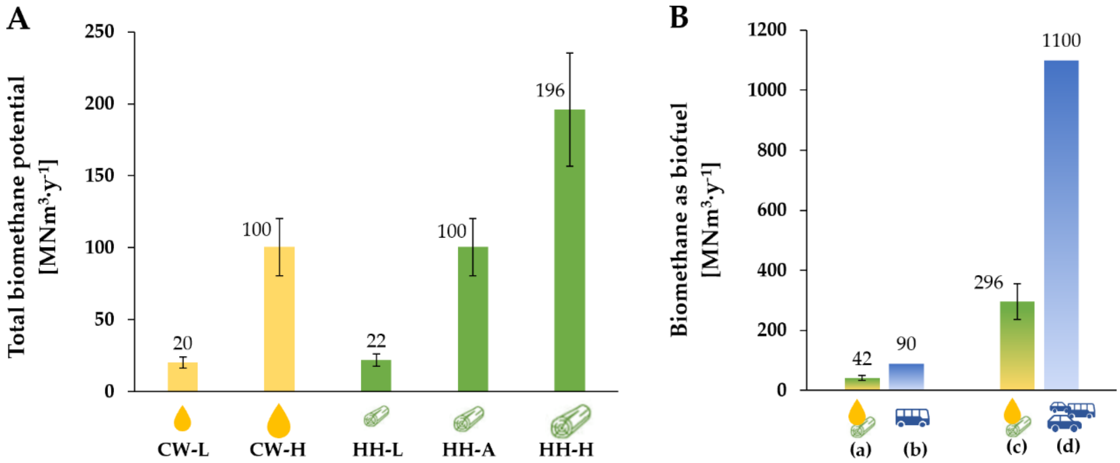

Figure 4. (**A**) Total biomethane potential from the current surplus cheese whey (CW) and the possible future hemp hurds (HH) available in Italy. (**B**) Comparison between the methane produced biologically from CW and HH and the current Italian natural gas demand as transportation fuel. Letters (a) and (c) in panel B indicate the low (CW-L + HH-L) and high (CW-H + HH-H) combined biomethane potential of CW and HH, conservatively calculated without considering the potential synergistic effect of coAD. Letters (b) and (d) indicate the annual natural gas demand in Italy for public transportation alone (b) and public and private transportation together (d). The bars in both panels indicate a range of total biomethane potential based on a ±20% variability of the CW and HH BMP values obtained in this study.

By assuming that Italy could recover its past hemp cultivation potential of 100,000 ha in the near future, the plain HH valorization through AD would enable the production of 22 to 196 MNm^3·y^{-1} (Figure 4A). In view of the above, Figure 4B demonstrates how, by combining the future biomethane production from CW and HH together, Italy could provide substantial shares of the current fossil gas demand from the transportation sector as renewable biomethane. Interestingly, the latter would also secure enough renewable energy to meet the 14% target set by EU for the transportation sector in 2030 [44].

4. Conclusions

The combined energetic valorization of CW and HH as biomethane showed both technical and economic advantages. The coAD experiments revealed potential synergistic effects in relation to the final cumulative biomethane production, leading to a 10.7% higher biomethane production as compared to the corresponding calculated value in presence of a CW and HH ratio of 70:30. Moreover, the latter coAD experimental condition showed also faster process kinetics, pointing towards the potential beneficial effects in relation to the enzymatic hydrolysis of the lignocellulosic structure of HH. These results offer useful insights for the technical implementation of the coAD of such biowastes. When the bioenergy outputs are converted into economic benefits, the high biomass yields of hemp combined with the large availability of CW would result in high profits per unit of land of up to 6124 €·ha^{-1}. In the framework of Italy, the future rise of hemp cultivation and the largely available volumes of CW could unlock unprecedented renewable biomethane potentials, which could achieve values as high as 296 $MNm^3 \cdot y^{-1}$. Such potential could replace almost one third of the natural gas currently used in the transportation sector, thus strongly contributing to the urgent biofuel revolution needed to ensure future environmental sustainability.

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