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Anaerobic Digestion of Steam-Exploded Wheat Straw and Co-Digestion Strategies for Enhanced Biogas Production

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Abstract: Wheat straw (WS) is considered a favourable substrate for biogas production. However, due to its rigid structure and high carbon to nitrogen (C/N ratio), its biodegradability during anaerobic digestion (AD) is usually low. In the present study, the effect of steam explosion pre-treatment on WS, combined with C/N adjustment with inorganic nitrogen, on biogas production was evaluated. Additionally, co-digestion of WS with protein-rich agri-industrial by-products (dried distillers' grains with solubles (DDGS) and rapeseed meal (RM)) was assessed. Steam explosion enhanced biogas production from WS, whereas the addition of NH_4Cl was beneficial ($p < 0.05$) for the digestion of steam-exploded wheat straw (SE). Furthermore, mono-digestion of the four different substrates seemed to be efficient in both inoculum to substrate ratios (I/S) tested (3.5 and 1.75 (*w/w*)). Finally, during co-digestion of WS and SE with DDGS and RM, an increase in the cumulative methane production was noted when higher amounts of DDGS and RM were co-digested. This study demonstrated that DDGS and RM can be used as an AD supplement to stimulate gas production and improve wheat straw biodegradability, while their addition at 10% on an AD system operating with WS can enhance gas yields at levels similar to those achieved by steam-exploded straw.

Keywords: anaerobic digestion; co-digestion; steam explosion; C/N; wheat straw; rapeseed meal; DDGS; biogas

1. Introduction

The transition towards a more sustainable economy, both from an economic and an environmental point of view, can be achieved through the replacement of fossil fuels (e.g., coal or petroleum) with renewable alternatives. Reliance on traditional fuels could be decreased through the development of efficient strategies for biomass-derived biofuels, such as biogas, which is the main product of anaerobic digestion (AD). Furthermore, biofuels are expected to play an important role in the reduction in carbon emissions, which is one of the most important causes of global warming. Biogas is a renewable gas, mainly comprising methane (CH_4) and carbon dioxide (CO_2), and can be used to produce heat and/or electricity. Biogas can also be utilised directly as a fuel for vehicles or, following upgrade to biomethane, can be injected into the natural gas grid. Energy crops are commonly used today for the production of biofuels, such as biogas and bioethanol [1–3].

Alternative materials that can be used as feedstock for AD systems include lignocellulosic residues, such as wheat straw (WS). WS is considered the most abundant source of biomass in Europe [4,5] and the second worldwide after rice straw [6]. Unfortunately, a high percentage of the annually produced straw worldwide still remains unexploited. As an example, only China produces between 180 and 280 million tons of rice straw annually and more than half of it is left unused [7]. WS is primarily used as feed and bedding material for ruminants [8]. Along with its high availability, its relatively low price is another factor which renders WS an attractive substrate for AD [9]. For example, in the UK, the market price for a premium-quality WS variety was within the range of GBP 10–15/t in 2020 [10]. However, the anaerobic biodegradability of WS is usually low due to its rigid structure and its chemical composition. Firstly, WS contains high amounts of non-anaerobically degradable lignin [11]. Lignin is a cross-linked polymer that creates bonds with cellulose and hemicellulose generating a structure that is not easily accessible by the microorganisms of AD. Additionally, its usually low protein content renders WS not favourable as a sole feedstock for AD systems where no supplementation of nitrogen takes place [12]. An important parameter that limits the anaerobic biodegradation of WS is the ratio of carbon to nitrogen content (C/N), which is sometimes higher than 90 (*w/w*) [13,14]. Previous studies have already stated that the microorganisms of AD consume carbon faster than nitrogen and the optimal C/N ratio for an AD system is usually within the range of 20 to 30 (*w/w*) [15]. C/N values lower than the optimal range can cause an increase in total ammonia nitrogen levels and/or high accumulation of volatile fatty acids (VFAs), which can, in turn, inhibit the methanogenesis stage and lead to failure of the whole AD process [16]. On the other hand, higher C/N values may inhibit the microbial growth and decrease the biodegradability of the feedstock. As a consequence, when the C/N ratio is not close to the optimal range for AD, biogas production and substrate biodegradability are negatively impacted.

A commonly used strategy for AD efficiency improvement when utilising feedstocks with C/N values outside the optimal range is co-digestion. Different organic materials with high nitrogen content have been tested as co-substrates along with WS, including food processing by-products [17] and various animal wastes, such as chicken manure [18]. Furthermore, the addition of inorganic nitrogen (e.g., NH_4Cl) is another potential way to balance the C/N ratio in AD systems operating with WS as a sole substrate.

Examples of agri-industrial by-products with high nitrogen content that can be used as co-substrate in AD systems are dried distillers' grains with solubles (DDGS) and rapeseed meal (RM). DDGS is the main by-product of the dry-grind distillation process for the production of alcoholic drinks (whisky) or biofuels (bioethanol) [19]. On the other hand, RM is the main by-product of the rapeseed (*Brassica napus*) oil production process. The main use of these two food processing by-products (FBPs) is as livestock feed, while they also hold potential as raw materials for the production of high added-value compounds, such as biopolymers, platform chemicals and biofuels [20,21]. The expected increase in the production of biofuels due to the EU regulations [22] could potentially result in the subsequent increase in RM and DDGS production. The use of DDGS or RM in AD could provide a market value for their producers through their efficient reuse for energy production. In the past, RM and DDGS have been tested as sole substrates in different AD systems [21,23–26]. However, up to now, neither DDGS nor RM have been tested as co-substrates with WS in AD systems. This novel approach would be proved valuable to the AD industry as an efficient strategy to stimulate gas production and improve wheat straw biodegradability.

The main aim of the study was to evaluate the enhancement of biogas production from WS offered by the steam explosion pre-treatment, as well as by the balancing of the C/N ratio. To this end, untreated and steam explosion pre-treated WSs were used as the main feedstock, in combination with either an inorganic nitrogen source or RM and DDGS in different co-digestion scenarios, and their effect on biogas production was monitored and discussed.

2. Materials and Methods

2.1. Substrates and Inoculum

Three independent batch experiments were conducted with four different materials as feedstock. The four different feedstocks were untreated WS, steam-exploded wheat straw (SE), dried distillers' grains with solubles (DDGS) and rapeseed meal (RM). DDGS was supplied by a UK bioethanol plant (Vivergo, Yorkshire, UK), was ground into a fine powder using a coffee grinder (DeLonghi, Australia), sieved through sieve mesh No. 20 (particle size smaller than 0.85 mm) and stored at 4 °C prior to use. RM was kindly provided by Stainswick Farm (Oxfordshire, UK) and was generated via a cold pressing oil extraction process. RM samples were ground using a dry-grinder and sieved to obtain uniform sized particles (<0.85 mm). The remaining oil in the meal was removed using a supercritical CO_2 extraction rig (SciMed, UK) at 60 °C and 300 bar pressure for 1 h, with ethanol (10%, *v/v*) as a co-solvent. The residual defatted meal was kept at 4 °C prior to use. WS was collected from fields in the wider area of Norfolk, UK. Part of this residue was steam-exploded with the use of an economiser (Economizer SE, Biogas Systems, Austria). The economiser was fed with raw WS and operated at high temperature (155 °C) and high pressure (5 bar) for 3 min with subsequent release of generated pressure by a valve, causing rapid depressurization of the substrate. Subsequently, both the non-steam-exploded straw (stated as untreated WS from now on) and the steam-exploded straw (stated as SE from now on) were stored in plastic bags at −20 °C until further use.

As an inoculum source, the effluent of four lab-scale mesophilic (42 ± 1 °C) semi-continuous stirring tank reactors (CSTRs) with a working volume of 4 L, operating under a steady-state at an Organic Loading Rate (OLR) of 5 g volatile solids (VS)/L per day, was used. Two of the CSTRs were digesting untreated WS while the other pair of reactors were fed with steam-exploded WS. The reason for mixing two slightly different inocula was to minimise, as much as possible, the chances of having an inoculum acclimatised to one of the two substrates (untreated WS vs. SE). Equal amounts of the two effluents (2 L of each inoculum) were manually mixed and degassed at mesophilic conditions (42 ± 1 °C) for one week before the beginning of the AD experiments. The reason for adding the degassing step was to minimise the inoculum's endogenous microbial activity and gas production.

2.2. Analytical Techniques

Total solids (TS) were determined in triplicate for WS, SE, DDGS, RM and the inoculum, according to the protocol described in Standard Methods for examination of water and wastewater [27].

The crude protein content for SE, WS, DDGS and RM was also measured using the Kjeldahl method [28]. Lignin, hemicellulose and cellulose contents for the four substrates were measured using the NREL protocol as proposed earlier by Sluiter et al. [29]. Finally, carbon in WS, SE, DDGS and RM was calculated based on the composition of the four feedstocks in carbohydrates and proteins with the assumption of all carbohydrates as glucose ($C_6H_{12}O_6$) and all proteins as gluten ($C_{29}H_{45}N_5O_8$).

2.3. Biochemical Methane Potential Test

The biochemical methane potential test (BMP) is a commonly used method to determine the anaerobic biodegradability of different organic materials and their potential to produce biogas [30]. In this study, 150-mL serum glass vials with a working volume of 70 mL were utilised across three batches of experiments with an incubation period of 30 d for each experiment at mesophilic conditions (42 ± 1 °C). During the first experiment, inorganic nitrogen (NH_4Cl) was added in the system to adjust the C/N ratio to 30 (*w/w*) for both feedstocks, alongside bottles with no added nitrogen (controls), at an inoculum to substrate ratio (I/S) of 3.5 (*w/w*). The second experiment comprised two different I/S ratios (3.5 and 1.75 (*w/w*)) across separate WS, SE, DDGS and RM substrates. Finally, the two different straw samples (e.g., untreated and steam-exploded straw) were mixed on ratios of 50:50, 70:30 and 90:10 (*w/w*) with DDGS and RM, respectively. Based on the results of the second experiment, the I/S ratio for the third batch trial was also set at 1.75 (*w/w*). The BMP outcome from the mono-digestion experiment was

assumed to represent the average potential for the two agri-industrial by-products. It was also assumed that the microorganisms would selectively catabolise the more easily degradable available feedstock (either DDGS or RM) and then switch to WS or SE, which are less digestible. As such, the produced gas from WS or SE was presented as the remaining amount from the total yield minus the theoretical expected yield from DDGS or RM. For all three experiments, methane production was measured daily by the liquid displacement method, where NaOH (aq) was used for scrubbing the CO_2 from the produced biogas. All BMP variations were performed in triplicate, alongside blank and control trials. The blanks were used for measuring the endogenous methane production, while the obtained values were subtracted from those acquired from the vials with the substrate. In addition to the blank vials, during all experimental setups, control samples with microcrystalline cellulose (Avicel) along with the inoculum were used as a positive control, as suggested in previous publications [31]. All BMP vials were manually shaken every 12 h to ensure the homogenisation of the samples. Finally, all methane measurements were reported and presented after correction to normal conditions based on Equation (1):

$$CH_4\ \mathrm{n} = \frac{CH_4\ \mathrm{e} \times K}{(K + T)} \quad (1)$$

where CH_4 n, total methane production at normal conditions; CH_4 e, experimentally measured methane values; T, room temperature at the point of the measurement; and K equals 273.15 and is used to express the temperature in Kelvin.

2.4. Statistical Analyses

The statistical analyses were conducted on Excel software (Microsoft Office 365 ProPlus, version 1908) with a paired Student's *t*-test, and the statistical significance was assigned to $p < 0.05$.

3. Results and Discussion

3.1. Physicochemical Characterisation of the Feedstock

The total solids analysis showed that the steam explosion pre-treatment affected the solids concentration in WS. More specifically, the TS content decreased from 36% in WS to 21.5% (*w/w*) in SE (Table 1). Similarly, SE had a volatile solids (VS) content of 19.37% (*w/w*), while in WS, VS accounted for 33.38% (*w/w*) of the total biomass. Prior to the initiation of steam explosion, water was added to the pre-treatment vessel to allow the feedstock to be pumped out after the steam explosion process. It is likely that the increased temperature during SE pre-treatment (155 °C) allowed the pores of the substrate to open and absorb moisture. Similar results have been reported in previous studies examining the effect of steam explosion pre-treatment on WS [32]. DDGS and RM were found to have similar VS contents of 86.1% (*w/w*) and 86.36% (*w/w*), respectively, whereas the inoculum contained 2.53% VS (*w/w*).

Furthermore, the steam explosion pre-treatment seemed to increase the percentage of cellulose content in the WS from 37.41% to 47.9% (*w/w*) of the total dry biomass. These results are not in agreement with previous studies where it was pointed out that cellulose content in lignocellulosic residues does not follow a specific trend after steam explosion pre-treatment [32,33]. However, differences in the configuration of cellulose (e.g., crystalline and amorphous structures) and the crystallinity index (the relative amount of crystalline structures in cellulose) within different lignocellulosic residues might affect the response of this material to steam explosion pre-treatment [34]. Unlike cellulose, the percentage of hemicellulose in WS samples did not seem to be affected by steam explosion. This result can be attributed to the fact that the applied steam explosion temperature (155 °C) was relatively low for hemicellulose hydrolysis to occur; this is reported to happen in temperature treatments between 150 and 230 °C [35]. However, a total breakdown of hemicelluloses of WS into oligosaccharides is not necessary for biogas production, since microbial clusters in AD, such as *Caldicoprobacter* sp. and *Clostridium* sp., inherently possess hemicellulose-degrading enzymes [34,35].

Table 1. Physicochemical characteristics of the feedstock and the inoculum used in the present study.

%, *w/w* (db)	Wheat Straw (WS)	Steam-Exploded Straw (SE)	DDGS	RM	Inoculum
Total solids (TS)	36.06 ± 0.52	21.04 ± 0.57	91.46 ± 0.29	92.42 ± 0.65	3.37 ± 0.13
Volatile solids (VS)	33.38 ± 0.63	19.37 ± 0.53	86.1 ± 0.34	86.39 ± 0.68	2.53 ± 0.11
Crude protein	0.29 ± 0.01	0.47 ± 0.02	28.3 ± 0.5	25.28 ± 0.15	n.d.
Cellulose (glucose)	37.41 ± 0.77	47.92 ± 0.50	11.1 ± 0.4	20.17 ± 2.32	n.d.
Hemicellulose	27.56 ± 0.75	28.23 ± 0.12	20.3 ± 1.7	14.03 ± 2.03	n.d.
Acid insoluble lignin	28.09 ± 2.6	25.28 ± 1.68	n.d.	16.08 ± 0.15	n.d.
Acid soluble lignin	2.38 ± 0.02	2.05 ± 0.11	2.9 ± 0.1	1.9 ± 0.1	n.d.

All data were produced in duplicate and the standard deviations are presented. n.d: not detected.

The analytical measurements also confirmed the high protein content of DDGS and RM (28% and 25%, *w/w*, respectively). At the same time, crude protein content was lower than 1% (*w/w*) for both straw samples. As expected, both DDGS and RM contained significantly lower concentrations of lignin than WS samples (Table 1).

3.2. BMP—Inorganic Nitrogen Addition

In the first BMP series, the effect of steam explosion pre-treatment together with the addition of inorganic nitrogen was evaluated. Figure 1 depicts the cumulative methane production in BMPs with NH_4Cl addition to untreated and steam-exploded wheat straw samples. After 30 d of BMP digestion, untreated WS presented an average cumulative methane production of 280 mL CH_4/g VS. Slightly higher methane production values (304 mL CH_4/g VS) for untreated WS in BMPs have been reported elsewhere [14], attributed to higher culture temperatures (55 °C) compared to the mesophilic system (42 °C) used in the present study. The methane production from SE samples, without nitrogen addition, reached values of up to 332 mL CH_4/g VS after the end of the 30-day digestion period, which was significantly higher ($p < 0.05$) than the yields offered by the untreated WS without the addition of nitrogen (280 mL CH_4/g VS; Figure 1). This increase was equivalent to almost 15% (*v/v*) compared to WS. In addition, the biomethane yields offered by SE BMPs after NH_4Cl supplementation (SE + N) reached values of 387 mL CH_4/g VS, which were significantly higher (13% (*v/v*)) than those from SE without N_2 addition (332 mL CH_4/g VS; Figure 1). It is possible that higher gas yields were the result of an enhanced microbial activity due to the pre-treated straw offering increased accessibility to available carbon and with the concomitant increased availability of nitrogen in the culture.

3.3. BMP—Comparison of WS, RM and DDGS as AD Feedstock

In the second BMP experiment, four different lignocellulosic residues were examined solely as substrates for their anaerobic biodegradability at two different inoculum to substrate ratios (I/S) expressed as g of VS of the inoculum to g of VS of the substrate (3.5 and 1.75 (*w/w*)). The C/N ratios of WS and SE were 88 and 64, respectively, while for both DDGS and RM, it was ~9 (*w/w*). The digestion of all four feedstock types was efficient for both tested I/S ratios, while the higher feeding rate seemed to be slightly more favourable in terms of gas yields for all examined feedstocks (Figure 2A,B). DDGS and RM had a higher biomethane potential compared to WS and SE, while no inhibition phenomena occurred as a result of their high nitrogen content. However, it is expected that in long-term digestion of DDGS and RM without a prior balancing of the C/N ratio, the AD system would not be biologically sustainable due to ammonium accumulation [21]. In the present study, the highest produced gas yields for DDGS and RM were 445 and 405 mL CH_4/g VS, respectively, both at an I/S ratio of 1.75 (*w/w*) (Figure 2A,B). These results are in agreement with previous studies where DDGS and RM were evaluated as sole substrates for BMP systems [26,36]. Specifically, with regards to the digestion of RM, the methane production was close to the maximum theoretical value for this feedstock (450 mL CH_4/g VS) [21].

The significantly lower concentration of lignin and higher presence of hemicellulosic carbohydrates that are present in DDGS and RM (Table 1) can partially explain the enhanced methane production. RM and DDGS also contain other, more assimilable carbohydrates, such as pectin [37], as well as beta-glucans [20]. It is possible that due to the differences in the hemicellulosic carbohydrates of the feedstocks, the microorganisms of the system had increased affinity towards pectin or beta-glucans, as opposed to arabinoxylans in straw, leading into enhanced biomethanation.

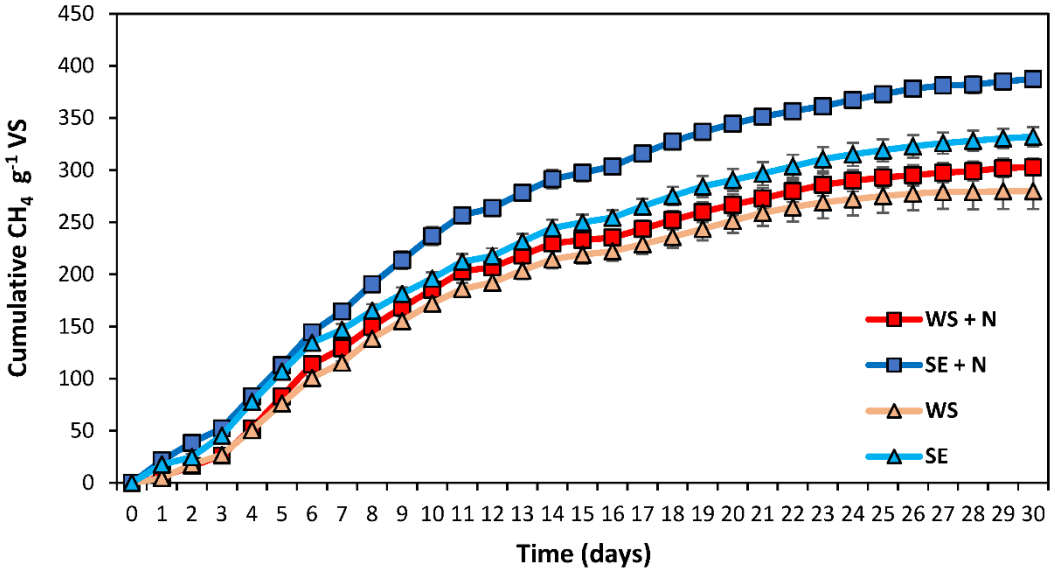

Figure 1. Cumulative methane production by untreated and steam-exploded wheat straw samples. WS + N: wheat straw with the addition of NH_4Cl; SE + N: steam-exploded wheat straw with the addition of NH_4Cl; WS: wheat straw; SE: steam-exploded wheat straw.

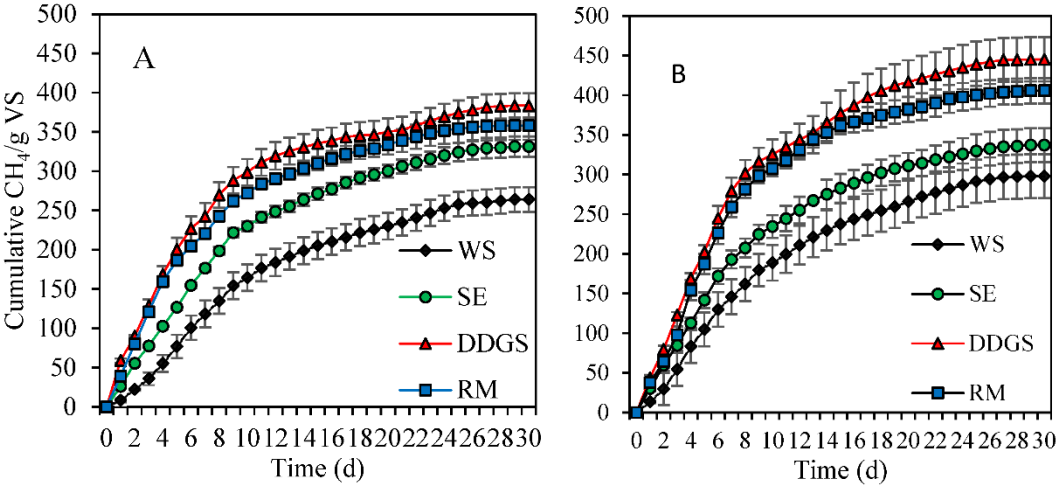

Figure 2. Cumulative methane production examining the anaerobic digestion of wheat straw (WS), steam-exploded wheat straw (SE), dried distillers' grains with solubles (DDGS) and rapeseed meal (RM) at (**A**) I/S ratios of 3.5 (*w/w*) and (**B**) 1.75 (*w/w*).

In addition to the above, the high protein content in DDGS and RM (Table 1) can also explain the improvement in methane production from these two materials. The presence of nitrogen in AD systems not only balances the C/N ratio but also offers higher amounts of biogas per g of dry matter. The methane potential at standard temperature and pressure conditions (STP) for proteins is higher (0.496 L CH_4/g VS) compared to carbohydrates (0.415 L CH_4/g VS). As a consequence, the increased

availability of nitrogen inside an AD system is expected to increase the biomethane yields of the system, but only in cases where ammonium levels in the system do not exceed the AD inhibition levels [21].

With respect to methane production, the digestion of DDGS, RM, WS and SE at an I/S ratio of 1.75 (*w/w*) resulted in higher yields (up to 14%) compared to the I/S ratio of 3.5 (*w/w*). AD microorganisms usually utilise the available carbon and nitrogen sources to produce biogas and energy for maintenance. At the same time, part of the available carbon and nitrogen is channelled towards microbial proliferation. It is possible that the increased availability of the feedstock in the cases of the I/S ratio 1.75 (*w/w*) also resulted in an increase in the microbial population, and as a result, the produced gas yields were also increased.

3.4. BMP—Co-Digestion Scenarios

Finally, the effect of different co-digestion scenarios between the two straw samples (WS and SE) and DDGS or RM was evaluated. Specifically, WS and SE samples were co-digested with DDGS and RM in three different ratios based on the VS content of the four AD feedstocks (50:50, 70:0 and 90:0 *w/w*). The C/N ratio was calculated for all co-digestion scenarios based on the composition of each feedstock and is presented in Table 2 along with the ratio of each feedstock used in each co-digestion trial.

Table 2. Co-digestion scenarios and carbon to nitrogen (C/N) ratio calculated for all biochemical methane potential (BMP) trials.

	WS (% *w/w*)	**SE (% *w/w*)**	**DDGS (% *w/w*)**	**RM (% *w/w*)**	**C/N (*w/w*)**
WS-DDGS	50	-	50	-	21
WS-DDGS	70	-	30	-	26
WS-DDGS	90	-	10	-	30
WS-RM	50	-	-	50	19
WS-RM	70	-	-	30	24
WS-RM	90	-	-	10	30
SE-DDGS	-	50	50	-	15
SE-DDGS	-	70	30	-	24
SE-DDGS	-	90	10	-	35
SE-RM	-	50	-	50	15
SE-RM	-	70	-	30	25
SE-RM	-	90	-	10	35

As can be seen in Figure 3A–D, the 50:50 co-digestion scenario offered higher, but not statistically significant, methane yields compared to the rest of the trials (70:30 and 90:10 *w/w*) for both WS and SE co-digested either with DDGS or RM. With regards to the effect of the type of co-substrate on methane production, no statistically significant differences were found in methane production of WS co-digested with either DDGS or RM (Figure 3A,B). Similarly, for SE, a co-digestion with DDGS at 50:50 (*w/w*) resulted in 377 mL CH_4/g VS, while for the same ratio, the co-digestion of SE with RM offered comparable biomethane yields of 373 mL CH_4/g VS. However, in SE trials with less DDGS or RM (ratio 90:10), the produced methane yields were significantly higher in DDGS co-digestions compared to RM co-digestions (Figure 3C,D), while a similar C/N ratio (35 *w/w*) was applied in both cases. According to the results from the mono-digestion BMP experiment, DDGS offered slightly higher methane yields as a sole substrate compared to the RM. This higher biogas potential of DDGS compared to RM can also explain the increased yields offered by SE co-digested with DDGS compared to SE-RM at the 90:10 ratio.

It is also worth mentioning that regardless of the improvement that steam explosion can offer to the digestion of lignocellulosic biomass when WS and SE are co-digested with RM/DDGS, the biomethane potential for the two feedstocks is very similar (Figure 3A–D). As an example, the highest methane production for WS was achieved when co-digested with RM at 50:50 ratio (375 mL CH_4/g VS). Similarly, when SE was co-digested with DDGS at 50:50, the biomethane outcome reached values

close to 377 mL CH_4/g VS. DDGS and RM performed similarly when used as co-substrates in AD, with DDGS showing slightly higher biomethane potential compared to RM.

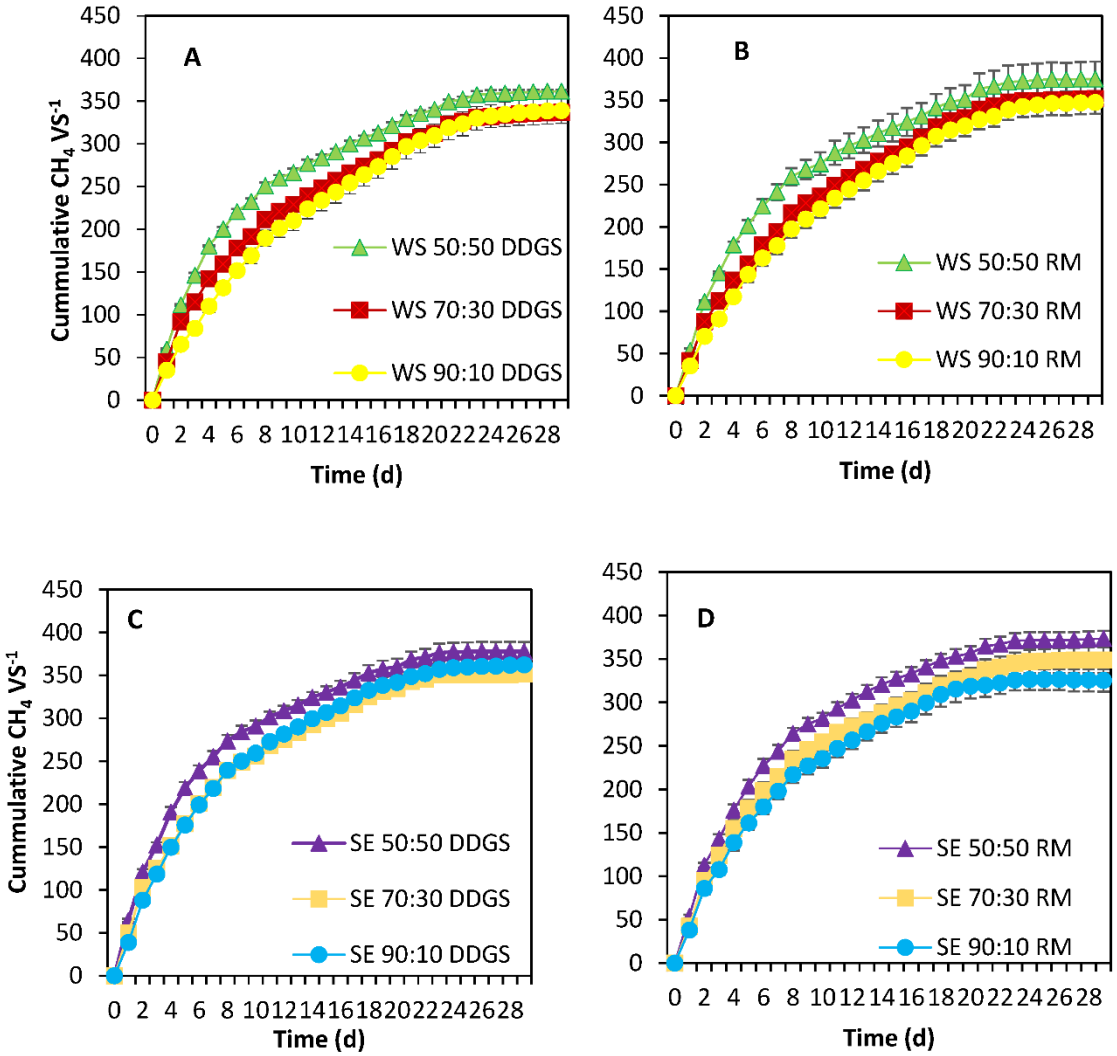

Figure 3. Cumulative methane production examining the co-digestion of (**A**) untreated wheat straw with DDGS; (**B**) untreated wheat straw with RM; (**C**) steam-exploded wheat straw with DDGS and (**D**) steam-exploded straw with RM.

Furthermore, to evaluate the effect of co-digestion on the gas production solely from WS and SE straw, methane concentrations recorded in mono-digestion trials of DDGS and RM were subtracted from the cumulative final production of the six co-digestion scenarios (Figure 4). Despite the notable increase in gas production in DDGS and RM co-digestion at 50:50, the produced yields from SE seemed to be significantly increased when lower amounts of either DDGS or RM were added to the system (Figure 4). This observation could be explained by the differences in the C/N ratio between the different BMP trials (Table 2). The C/N ratio was calculated within the range of 12 to 15 (*w/w*) in all cases where a 50:50 digestion was applied (Table 2). At the same time, the C/N ratio reached values higher than 35 (*w/w*) when SE was digested either with RM or DDGS at a 90:10 ratio (Table 2). Generally, the produced gas yields for SE significantly increased as the concentrations of the two food processing by-products decreased. The same increasing trend was observed for WS; however, the difference in the produced yields from the different co-digestion trials was not always significant. As an example, the methane production from WS, after subtracting the methane production from RM (70:30 ratio), was 229 mL CH_4/g VS, reaching 230 mL CH_4/g VS when a 90:10 ratio with RM

was applied. Based on the results from the first BMP experiment, the addition of a nitrogen source affected, to a higher extent, the digestion of SE compared to WS. As reported in previous studies [38], the steam explosion pre-treatment can partially disrupt the structure of straw, and as a consequence, the carbohydrates present in the lignocellulosic biomass become more accessible to the microorganisms of AD. It is possible that this increased availability of degradable components of straw along with the increased availability of nitrogen resulted in an increase in the microbial population of the system and, as a consequence, the biotransformation of the feedstock to biogas was also enhanced.

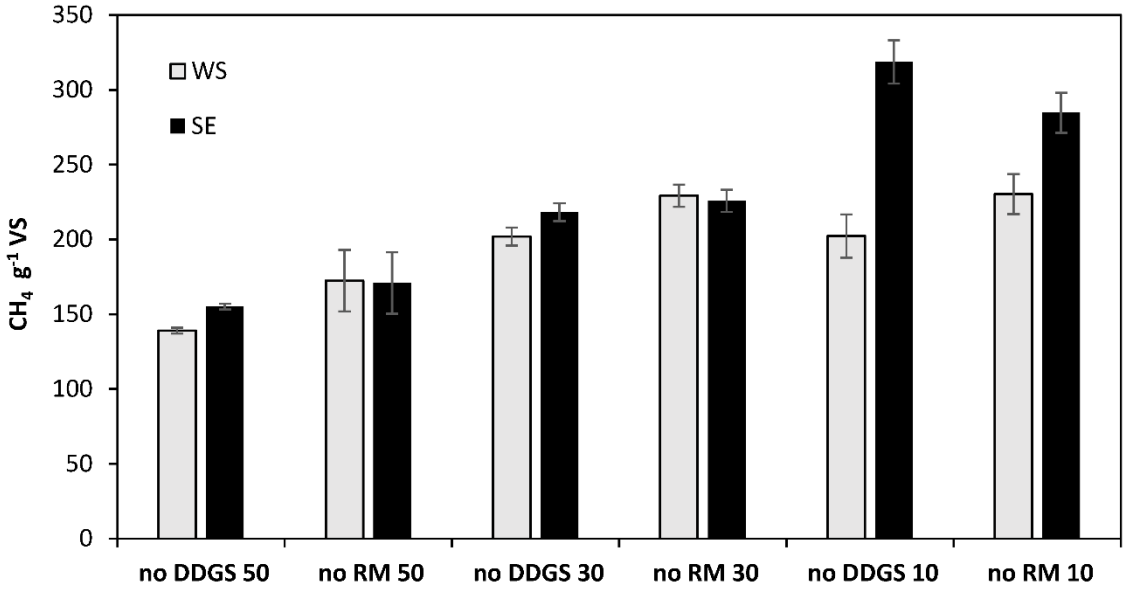

Figure 4. Methane production after 30 days of co-digestion of untreated and steam-exploded wheat straw after subtracting the expected methane production of the RM and DDGS, respectively.

Another possible explanation for the results presented in Figure 4 is the selective consumption of the feedstock by AD microorganisms. It is already proven that when different feedstocks or feedstock components co-exist inside a bioreactor, the microorganisms will first consume the more easily degradable material [39]. As shown earlier, the digestion of DDGS or RM offered higher biomethane yields compared to WS and SE, without any signs of inhibition or lag phase occurring. It is likely that DDGS and RM (especially in cases of 50:50 ratio or higher) were preferable substrates for AD microorganisms and their depletion coincided with micronutrient depletion. As such, WS and SE digestion was limited in these cases. On the other hand, when significantly lower amounts of DDGS and RM were present in the AD system, they offered a balanced C/N ratio, enhanced the metabolic activity and were quickly depleted, allowing for a further breakdown of WS and SE. According to these results, small amounts of either DDGS or RM can be used as an AD supplement to stimulate gas production and improve straw biodegradability.

4. Conclusions

The effect of steam explosion pre-treatment of WS on AD was evaluated in parallel with the provision of nitrogen to balance the high C/N ratio of WS. The steam explosion pre-treatment offered a 12–21% enhancement in methane production in all examined scenarios, while the adjustment of the C/N ratio was clearer when combined with a steam explosion pre-treatment step. Furthermore, all four different examined feedstocks (WS, SE, DDGS and RM) performed well as sole substrates in a batch AD system with a preferable I/S of 1.75 over that of 3.5 (*w/w*). It was also found that the addition of nitrogen, either inorganic (NH_4Cl) or organic, as a co-substrate was more efficient towards gas production for steam-exploded straw than towards untreated straw. This study has shown, for the first time, that DDGS or RM can be used as an AD supplement to stimulate gas production and

improve wheat straw biodegradability. Additionally, it was shown that the addition of 10% of either DDGS or RM on an AD system operating with WS can enhance gas yields at levels similar to those achieved by steam-exploded straw. Further techno-economic evaluation and life cycle assessment (LCA) could determine the financial sustainability of commercial biomethane production based on these co-digestion strategies.

Author Contributions: A.C., K.-A.G.K. and F.K. conceived the original idea and designed the experiments for this study. The experiments were conducted by F.K. and J.D. while F.K. and J.D. also collected the experimental data and ran the data analysis. K.-A.G.K., A.C. and D.C. verified the analytical methods and provided supervision during the experiments and data analysis. The original manuscript was written by F.K. and was reviewed/edited by A.C., K.-A.G.K. and D.C. All authors have read and agreed to the published version of the manuscript.

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