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Keywords (separated by '-')	Biomethanation - Constructed wetlands - Lignocellulosic biomass - Pig slurry	
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2 Biomethanation Potential of Wetland Biomass in Codigestion 3 with Pig Slurry

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hydration medium for anaerobic digestion can improve the
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Keywords Biomethanation · Constructed wetlands · 36
Lignocellulosic biomass · Pig slurry 37

Introduction 38

A wetland is a land area that is saturated with water, either 39
permanently or seasonally, so that it takes on the charac- 40
teristics of a distinct ecosystem. A wetland differs from 41
other land environments or water bodies because its veg- 42
etation is adapted to unique soil conditions. Constructed 43
wetlands (CWs) are a technology developed in recent years 44
for treatment and reuse of poor quality waters and for the 45
development of marginal areas. They are systems of 46
purification of municipal, agricultural and industrial 47
wastewater, which reproduce the principle of self-purifi- 48
cation typical of aquatic environments and wetlands. Plant 49
species more frequently utilized are water macrophytes. 50
The most commonly exploited species in Europe are 51
Phragmites australis, and species belonging to the genera 52
Carex, *Scirpus*, *Typha* [36], emergent macrophytes well 53
tolerating high nutrient and pollution levels. 54

The exploitation of herbaceous biomass from wetlands 55
for energy production (heat, electricity and fuels) may add 56
further appeal to the adoption of this practice [15, 19, 22]. 57
In fact, wetland plant species are well adapted to growing 58
in wastewater and are often vigorous, high-productive 59
plants. In recent years wetland biomass utilization for 60
biogas production has received growing attention [1, 2, 25, 61
35]. Earlier studies on conversion of plant biomass into 62
methane [30] revealed particular suitability of water hya- 63
cinth (*Eichornia crassipes* Mart) and napier grass 64

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(*Pennisetum purpureum* L.) for biogas production. Dipu et al. [12] evaluated 6 macrophyte species belonging to genera *Typha*, *Pistia*, *Eichornia*, *Salvinia*, *Azolla*, and *Lemna*, using cow dung as inoculum, and found higher biogas production in codigestion slurries than in cow dung digested alone. Cohen et al. [9] have proposed an integrated treatment system, including CWs for water polishing and anaerobic digestion (AD) of wetland-derived phytomass, for enhancing the economic feasibility of wastewater treatment processes.

Limited amounts of lignocellulosic biomass are commonly used in co-digestion with manure for biogas production in order to enrich manure with volatile solids without excessively enlarging the digester size. However, the frequency of AD using vegetal biomass without manure has recently increased, due to the incentive policies for renewable energies. Government incentives have also raised the interest of the agroindustry (such as olive oil mills, cheese factories, breweries) toward the exploitation of agro-industrial waste for biogas production with no connection with livestock.

Fresh lignocellulosic biomass has usually a high (i.e., >35 %) dry matter content, especially when ensilage or drying is applied to prolong its storage life. Dry fermentation is the most suitable system for biogas production from materials with low moisture content. However, the most spread AD systems nowadays are of the Continuously Stirred Tank Reactor (CSTR) type. Biogas production in CSTR systems requires a dry matter content lower than 10 % [38]. Consequently, lignocellulosic biomass, when used for biogas production in CSTR systems, needs to be diluted. The use of water for biomass dilution is arguable, because water occupies volume without producing biogas. Liquid animal manure (“slurry”) seems the most suitable dilution material because, on the one hand, it hydrates the biomass while supplying it with nutrients; on the other hand, the use of animal manure contributes to the solution of the widespread problem of a proper manure management. The hypothesis at the basis of this research is that the use of animal manure in codigestion with wetland biomass may contribute to biogas yield improvement while fulfilling to the general environmental need of a proper manure management.

The aims of this study were: (1) to evaluate the biomethanation potential (BMP) of wetland biomass, coming either from natural environments or from CWs. Our interest focused on the overall effect of wetland biomass as substrate for AD, regardless of the species; (2) to verify the effect of codigestion of wetland biomass with pig slurry on methane production rate and yield.

Materials and Methods

Materials

Samples of wetland biomass were collected in autumn, at the end of the growing season, in their natural environment (Italy, Po Valley, Veneto region, 45°38'N, 11°40'E, 10 m a.s.l.) or in CWs experimental plants, located in the same area and managed by the DAFNAE Department of the Padua University. Samples belonging to 23 plant species were obtained (Table 1). The environment of these species is characterized by high levels of soil moisture. For this reason they have been assessed in experimental tests for their potential use in constructed wetlands, for removing high levels of N and organic load from animal slurry or digestate [26]. Some of them are typical macrophytes, others live in riparian environments or uncultivated lands, some others grow in humid areas close to the sea, in saline environments. Representative subsamples were dried at 65 °C at constant weight, and then milled at 1 mm (Cutting Mill SM 100 Comfort, Retsch, Germany). Each sample was a composite of aboveground biomass from 5 plant individuals collected in the same site. As each plant species was represented by only one sample collected at a single site, no statistical inference was drawn on the species effect on AD, which is beyond the scope of this work.

Fresh pig slurry to be used in co-digestion with wetland biomasses was drawn after biomass mixing with a pumping system from the CREA farm storage tank collecting faeces, urine, tap water used for cleaning pens from a fattening piggery, and rainwater. Its average composition was: total solids (TS), 1.39 % fresh weight (FW; SD, 0.045 %); volatile solids (VS), 0.98 % FW (SD, 0.040 %); ashes, 0.41 % FW (SD, 0.006 %); organic C, 396 g kg⁻¹ TS (SD, 2.40 g kg⁻¹); total N, 56.1 g kg⁻¹ TS (SD, 1.83 g kg⁻¹); ammonium N, 26.3 g kg⁻¹ TS (SD, 0.75 g kg⁻¹); pH in water, 7.14 (SD, 0.08); total P, 22.4 g kg⁻¹ TS (SD, 0.72 g kg⁻¹); lignin, 5.7 % TS (SD, 0.09 %); hemicellulose, 10.6 % TS (SD, 0.05 %); cellulose, 6.1 % TS (SD, 0.08 %). These composition values are consistent with historical data from our laboratory regarding pig slurry produced in our experimental farm.

Experimental Set-Up

A preliminary experiment was carried out to test the average biomethanation potential (BMP) that can be expected when using wetland biomass as AD substrate, using 23 wetland biomass samples. This experiment was also used to examine the relationship between plant composition and AD performances.

Table 1 Wetland plant species used as a source of biomass for AD

Species	Common name	Natural environment of growth
<i>Arctium lappa</i> L.	Greater burdock	DA, NRS
<i>Artemisia caerulea</i> L.	Sea mugwort	Salty soils
<i>Arundo donax</i> L.	Giant reed	Riparian
<i>Aster tripolium</i> L.	Sea aster	Moist salty soils
<i>Calamagrostis epigejos</i> (L.) Roth	Wood small reed	Moist salty soils
<i>Carex acutiformis</i> Ehrh.	Lesser pond sedge	Wetland
<i>Carex riparia</i> L.	Great pond sedge	Wetland
<i>Cynodon dactylon</i> (L.) Pers.	Bermudagrass	DA, NRS, moist sites along rivers
<i>Elytrigia atherica</i> (Link) Kerguelen	Wheatgrass	Moist salty soils
<i>Glyceria maxima</i> (Hartm.) Holmb	Reed mannagrass	Wetland
<i>Halimione portulacoides</i> (L.) Aellen	Sea purslane	Moist salty soils
<i>Helianthus tuberosus</i> L.	Jerusalem artichoke	Riparian
<i>Inula crithmoides</i> L.	Golden samphire	Moist salty soils
<i>Iris pseudacorus</i> L.	Yellow flag	Wetland
<i>Juncus maritimus</i> Lam.	Sea rush	Wetland
<i>Limonium narbonense</i> Mill.	Sea lavender	Moist salty soils
<i>Miscanthus x giganteus</i> Greef et Deu.	Giant miscanthus	Moist meadows
<i>Phalaris arundinacea</i> L.	Reed canarygrass	Wetland
<i>Pucciniella palustris</i> (Seen.) Hayek	Alkaligrass	Moist salty soils
<i>Sarcocornia fruticosa</i> (L.) A.J.Scott	Glasswort	Moist salty soils
<i>Scirpus sylvaticus</i> L.	Woodland bulrush	Wetland
<i>Symphytum x uplandicum</i> Nyman	Comfrey	DA, UL, NRS
<i>Typha latifolia</i> L.	Broadleaf cattail	Wetland

DA disturbed areas, UL uncultivated land, NRS nitrogen rich soils

In the second experiment, the rate and yield of methane production were compared for wetland biomass samples (*Plant material*), in monodigestion (PS−) or in codigestion (PS+) with pig slurry (*Treatment*), in a completely randomized block design with 3 replications. The plant materials which had given the best or the worst results in the first experiment were selected for this comparison: *Arundo donax*, *Carex riparia*, *Cynodon dactylon*, *Elytrigia atherica*, *Halimione portulacoides*, *Inula crithmoides*, *Scirpus sylvaticus*, *Phragmites australis*, which is one of the dominant wetland species in Europe [36], was also included.

Anaerobic Digestion and BMP Determination

Digestate from pig slurry was used as inoculum source. It was prepared as follows: 200 mL of a definite synthetic medium for methanogens (phosphate buffered basal medium, PBBM; [14]) without energy sources was mixed in 500-mL serum bottles with 200 mL fresh liquid fraction of pig slurry collected from the farm storage tank after separation of the solid phase, in a N₂–CO₂ (80:20) atmosphere. This mixture was left to incubate in strictly anaerobic conditions and the head space composition was analyzed

for CH₄ accumulation. The inoculum was considered as ready for use when CH₄ production had stopped, indicating exhaustion of endogenous energy sources.

Anaerobic digestion was carried out using dried and milled wetland biomass samples as substrates. In the first experiment, the reaction mixture included 1.25-g dried sample (2.5 %; “substrate”), 50 mL of PBBM (“hydration medium”) without energy sources, and 5 mL inoculum, in 100-mL reactors (118.5 mL effective volume), in triplicate (69 reactors, in total). The pH of the reaction mixtures varied between 6.0 and 7.7. In the second experiment, each reactor contained 1 g VS. Precisely, in each PS− reactor, 1 g VS of plant biomass was added to 50 mL PBBM; in each PS+ reactor, 0.51 g VS of plant biomass were added to 50 mL pig slurry, containing 0.49 g VS, for a total of 1 g VS. The total VS concentration in all the reactors was 2 %. Pig slurry alone was inoculated as control. Fifty-one reactors were prepared in total (8 plant materials × 2 substrate levels + pig slurry alone, ×3 replicates). Five-mL inoculum was added to all 100-mL reactors (118.5 mL effective volume). The average pH of wetland biomass after mixing with PBBM was 6.72 (SD, 0.52), while in the presence of pig slurry it was 6.80 (SD, 0.50). The head-space of the reactors was gassed with N₂–CO₂ (80:20)

throughout the preparation steps before the start of the experiment. Reactors were plugged with butyl rubber stoppers and aluminum seals and incubated at 35 °C for 90 days.

The biogas production (volume and composition) was measured according to Owen et al. [24] 2 days after the start of the incubation and then weekly for 3 months. Biogas was collected by means of 100-mL glass syringes. The incubation period was completed when there was no more biogas production in any of the reactors. No methane production was detected in the control reactors (inoculum in PBBM without energy source).

Biomethanation potential (mL CH₄ g⁻¹ VS) was expressed as the maximum amount of CH₄ cumulated over time that can be produced by a given substrate per g of volatile solids, including the amounts of CH₄ released in the syringe at each measurement date as well as the CH₄ volume remaining within the reactor.

In the second experiment, the parameters of the cumulative CH₄ production curves were evaluated by means of a modified 3-parameter Gompertz equation [17]:

$$M(t) = M_{max} \exp \left\{ -\exp \left[\left(\frac{e R_{max}}{M_{max}} \right) (\lambda - t) + 1 \right] \right\}$$

where M(t) (mL) is the total amount of CH₄ produced at the culture time t (d); *e* is exp(1); *M*_{max} (mL) is the maximum cumulative CH₄ production; *R* (mL d⁻¹) is the daily rate of CH₄ accumulation in the linear phase of CH₄ accumulation; and *λ* is the lag time duration (d), that is the time of microbial adaptation before exponential CH₄ production. This function is often utilized for interpolating growth curves, in general, and microbial growth curves, in particular [42].

Since in this experiment each reactor contained 1 g of VS, the *M*_{max} value (mL CH₄) coincided with the BMP value (mL CH₄ g⁻¹ VS).

Analytical Methods

The following parameters describing plant composition were determined: pH of the reaction mixture, TS, VS, total N, total P, organic C, C to N ratio (C/N), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (ADL), hemicellulose, cellulose, total polyphenols (TP), soluble carbohydrates (SC), starch (Sta), total carbohydrates (TC = SC + Sta).

Total solids were determined gravimetrically by thermal treatment at 105 °C at constant weight. Analyses of the plant materials were conducted on samples dried at 65 °C at constant weight and milled at 1 mm. Organic C was determined by dichromate oxidation with external heating and reflux condenser. Total N was determined with the Kjeldahl apparatus. Total P was determined on ashes by colorimetry with ammonium molybdate, after solubilization by means of HCl

1 N. The pH was determined after suspension, 2-h stirring and sedimentation of 1.5 g dry matter in 50 mL distilled water. Fiber fractions (NDF, ADF, ADL) were determined according to Van Soest et al. [33]. The hemicellulose content was estimated as the difference between NDF and ADF; the cellulose content as the difference between ADF and ADL. For SC and Sta determination, plant tissues (20 mg) were washed with pure acetone to remove the interfering pigments and then centrifuged [21]. Soluble carbohydrates were extracted twice with 2.5 mL ethanol 80 % and determined on the centrifuged supernatant by the anthrone method [23]. Five mL HCl 1.1 % were added to the centrifuged residual pellet, and diluted to 10 mL with distilled water after heating in a water bath at 100 °C for 10 min. Soluble carbohydrates after hydrolysis were determined with the anthrone method. Soluble carbohydrates, starch and TC are expressed as mg glucose g⁻¹ of dry matter. Total polyphenols were determined according to the Folin–Ciocalteu colorimetric assay [31] and expressed as mg tannic acid per g of dry matter.

Pig slurry and digestates were analyzed according to APHA [3]. In the first experiment, digestate analysis was performed on a composite sample obtained by mixing the digestate of the 3 treatment replicates. In the second experiment, the single replicates were used for analysis.

Methane concentration in the biogas was determined by means of a MicroGC Agilent 3000 gas chromatograph, equipped with 2 columns, Molsieve and Plot U; detector: TCD. Carrier gas: argon.

Statistical Analysis

The correlation matrix between AD and BMP was obtained by means of the PROC CORR of the SAS package [28]. ANOVA was applied to compare the effect of wetland digestion with or without pig slurry in the second experiment. Comparisons of the means were based on the Tukey test at $\alpha = 0.01$.

Model fitting for the description of CH₄ accumulation curves was performed using the PROC NLIN of the SAS package. The parameter values were estimated according to the Gauss–Newton method. The time (d) necessary to reach *M*_{max} was estimated by calculating the ratio *M*_{max}/*R*. Data from 3 replicates was merged for the parameter value estimation.

Results and Discussion

Biomethanation Potential of Wetland Biomasses

The BMP of the plant materials was on average 213 mL CH₄ g⁻¹ VS (*n* = 23, CV = 18.6 %). Nearly 75 % of the plant materials (17 out of 23 plant species; Fig. 1) showed

a BMP > 200 mL CH₄ g⁻¹ VS, with 5 among them producing more than 250 mL CH₄ g⁻¹ VS. These amounts are lower than those reported for energy crops and other agricultural by products, which may produce even more than 400 mL CH₄ g⁻¹ VS [5, 6]. However, they were of the same level or even higher than those that can be obtained from agro-industrial waste [11] or wheat straw [34].

Residual VS in wetland biomass digestates were on average 51.7 % of the initial VS content (Table 2). The mean VS decrease was then 48.3 %, lower than that reported by Bouallagui et al. [6] for AD of fruit and vegetable waste (58–65 %). Klimiuk et al. [16], for silages of four crop species, found large differences in residual VS content at the end of AD, depending on the species. The decrease in organic C content and C to N ratio caused by CH₄ and CO₂ release during AD was accompanied by an increase (nearly doubling) of N and P concentrations in the digestate, compared to those measured at the start of the process (Tab. 3) in agreement with the results of Tambone et al. [32].

Relationship Between Anaerobic Digestion and Plant Composition

Among plant composition parameters, most varying (CV > 60 %) among species were: C to N ratio, soluble carbohydrates and starch (and, consequently, total carbohydrates; Table 3). Possible reasons for differences among plant materials in suitability to AD were evaluated by means of correlations between plant composition parameters and BMP. Biomethanation potential was positively correlated with plant N content ($r = 0.59$, $P < 0.01$) and negatively correlated with C to N ratio ($R = -0.63$, $P < 0.01$), ADF (i.e., lignin + cellulose) content ($R = -0.71$, $P < 0.001$), and cellulose content ($R = -0.53$, $P < 0.01$).

Table 2 Simple statistics of selected composition parameters, for digestate coming from AD of wetland biomass (n = 23)

Parameter	Mean	Minimum	Maximum	CV (%)
TS (%)	1.36	1.10	1.78	14.8
VS (% initial VS)	51.7	36.0	73.3	19.2
N (g kg ⁻¹ TS)	47.4	4.8	96.6	59.4
P (g kg ⁻¹ TS)	5.4	0.9	11.3	59.1
Organic C (g kg ⁻¹ TS)	310	223	367	10
C/N	13.1	3.2	75.8	128

The N content and the C to N ratio are important factors for the improvement of biogas production, even though contrasting effects on BMP were reported, probably depending on the range of explored values [29, 37, 41]. These results suggest the opportunity to increase biogas yields from wetland biomass by appropriate modulation of the C to N ratio.

It is well known that lignin, among VS components, is especially recalcitrant to AD [8]. Alvinge [2] tested for biogas production two macrophyte species, *Typha latifolia* and *Phalaris arundinacea*, with or without treatment of demolition of the lignocellulosic tissues (mechanical milling, alkaline treatment with lime and fungal degradation), and he was able to obtain increased CH₄ production by 16–27 %, depending on the kind of applied pretreatment.

Polyphenols, common tissue components of several plant species, had been included in the analysis because they could exert an inhibiting effect on microbial activities [10]. In this experiment the total polyphenols concentration was not significantly correlated to BMP. The presence of a VS fraction containing lignocellulosic molecules recalcitrant to digestion may explain the only partial removal of VS during the AD process.

Fig. 1 Biomethanation potential (BMP) of biomass samples from selected wetland species. Error bars are standard deviations

Table 3 Average composition of wetland biomass samples, and minimum, maximum and coefficient of variability (CV) (n = 23)

Parameter	Mean	Minimum value	Maximum value	CV (%)
pH of the reaction mixture	6.7	6.0	7.7	7.7
Total solids, %	94.7	91.5	96.1	1.1
Volatile solids, %	89.0	78.8	96.5	5.5
Ashes, %	11.0	3.5	21.2	44.6
Total N, g kg ⁻¹	23.4	5.0	40	50.7
Total P, g kg ⁻¹	2.9	0.7	6.3	54.9
Organic C, g kg ⁻¹	429	368	465	5.6
C/N	26	11	85	73.4
Hemicellulose, % ^a	19.2	5.9	38.7	47.3
Cellulose, %	23.6	4.1	45.1	54.0
Lignin, %	13.4	5.3	33.4	54.1
Total polyphenols (mg tannic acid g ⁻¹)	15.4	6.1	48.1	56.4
Soluble carbohydrates (SC, mg glucose g ⁻¹)	61	25	279	85.5
Starch (Sta, mg glucose g ⁻¹)	63	26	207	67.4
Total carbohydrates (TC = SC + Sta, mg glucose g ⁻¹)	123	57	354	62.9

All the concentration values are referred to the total solids content

Biogas Production by Wetland Biomasses in Codigestion with Pig Slurry

The codigestion with pig slurry reduced the AD lag phase (Fig. 2a) while increasing the R (Fig. 2b) and Mmax values (Fig. 2c). The lag phase duration varied from 0, in the majority of cases, to 0.6 days in the PS− reactors (higher than 0 in 4 out 23 cases). In the PS+ reactors it varied between 0 and 0.31 days (higher than 0 in only 2 cases). Lag phase duration depends on several factors including the level of recalcitrance of the substrate. As in this experiment the lag phase duration was 0 or very short, no negative effects of the substrate on microbial activities could be deduced.

The R values averaged 9.2 mL CH₄ day⁻¹, in the PS−, and 16.0 mL CH₄ day⁻¹, in the PS+ reactors (Tukey value for the difference between the PS− and the PS+ treatments, at $P < 0.01$: 1.53 mL CH₄ day⁻¹), with a 25 % reduction on average of the time needed to reach Mmax (from 27.7 to 20.7 days) in PS+. The average Mmax was 255 mL CH₄ g⁻¹ VS, in the PS− reactors, and 332 mL g⁻¹ VS, in the PS+ reactors (Tukey value for the difference between the PS− and the PS+ treatments, at $P < 0.01$: 16.7 mL of CH₄), with a 30 % increase in methane production, for the same amount of initial VS content. In this experiment the Mmax values (i.e., cumulated mL CH₄) coincided with BMP values (cumulated mL CH₄ g⁻¹ VS), because the substrate of all the reactors contained 1 g of VS.

Besides the general improvement of methane production rate and yield, codigestion reduced the differences in AD performances between plant materials. The CV of R

and Mmax values in the PS+ treatment (9.7 and 5.0 %, respectively) was lower than in the PS− treatments (25.3 and 10.0 %, respectively). The increase of Mmax for the plant materials in codigestion with pig slurry, in comparison with monodigestion, was particularly high for those which had given the worst results in monodigestion such as *S. silvaticus*, *I. crithmoides* and *P. australis*. Codigestion has been reported to be advantageous because it results in a substrate better balanced and assorted in terms of nutrients [13, 18]. Positive effects of codigestion with pig slurry could be attributed in particular to an enrichment in mineral salts and to an increase in N availability for microorganisms. In fact, the initial ash concentration was 67 % higher in codigestion than in monodigestion (Table 4), for the same initial amount of volatile solids, whereas the total N content was 48 % higher and the concentration of ammonium N was nearly 6 times higher than that in monodigestion. As pig slurry is particularly rich in methanogenic microorganisms [27] a possible contribution of the pig slurry microbial populations to the methanogenic activity could also be hypothesised. However, it is also known that the type and relative richness of the various microbial groups in the anaerobic digesters is driven by the substrate characteristics [40]. Therefore, the quantitative and qualitative relationship between the initial and the consolidated microbial populations in batch reactors is not obvious.

The average residual VS content of the digestates was 47.7 % of the initial VS content in the PS− treatment and 44.8 % in the PS+ treatment, without significant differences between PS− and PS+ treatments and no significant

Fig. 2 Values of the Gompertz parameters for the curves of methane accumulation from wetland biomasses digested with (Pig slurry +) or without pig slurry (Pig slurry -). **a** lag phase duration (λ); **b** maximum CH_4 accumulation rate (R); **c** maximum potential methane production (M_{max}). Error bars are standard deviations

correlation between residual VS content of the digestates and BMP.

The better biomethanation performances observed when plant materials were in codigestion with pig slurry, for the same starting amount of VS, can be related to differences in the quality of these VS. The most productive plant materials were those having higher N concentrations in their tissues (*E. atherica* and *H. portulacoides*, Table 4). Pig slurry further increased N availability while lowering the C to N ratio. Codigestion with lignocellulosic plant material has been suggested for animal effluents, in order to increase the carbon amount available for AD [4] and to adjust the C to N ratio at levels suitable for AD [39]. According to our results, the opposite is also true: as lignocellulosic materials supply high amounts of carbon we can improve AD performances by adding animal manure rich in nitrogen that compensates for these high amounts of C, lowering and improving the C to N ratio. The amount of ammonia-N supplied in the reactors by pig slurry (0.033 % fresh weight; 1.18 % TS, on average, as the difference between PS+ and PS- mean values; Table 4) was not so high as to inhibit the methanogenic activity [7]. It has been reported that ammonia supply to lignocellulosic manure can even enhance biogas yield [20].

Conclusions

Interesting BMP levels were associated to anaerobic digestion of wetland biomass. The variability of BMP among wetland samples was linked to their nutrient content. An important role was played by the C to N ratio. The time for the joining of the maximum methane production was on average 25 % shorter and the amount of methane was 30 % higher for wetland biomass in codigestion with pig slurry than in monodigestion. The advantage of

Table 4 Changes in substrate composition during anaerobic digestion of wetland plant samples digested with or without pig slurry

Parameter	Digestion without pig slurry		Digestion with pig slurry	
	Mean	CV	Mean	CV
Total solids (TS), %				
Input material	2.54	2.77	2.77	1.30
Digestate	1.8	10.4	1.6	8.7
Volatile solids (VS), % initial ^a				
Digestate	47.7	14.8	44.8	10.8
Ashes, % TS				
Input material	0.45	15.7	0.68	5.3
Digestate	0.75	9.3	0.64	7.3
Total N, % TS				
Input material	3.0	35.0	4.4	10.9

Table 4 continued

Parameter	Digestion without pig slurry		Digestion with pig slurry	
	Mean	CV	Mean	CV
Digestate	5.1	33.4	9.7	16.2
NH ₄ -N, % TS				
Input material	0.24	2.72	1.42	1.29
Digestate	2.7	55.5	6.6	20.2
Organic C, % TS				
Input material	40.8	2.4	40.1	1.2
Digestate	33.1	10.0	31.9	3.8
C/N				
Input material	15.7	40.9	9.2	11.6
Digestate	7.2	31.3	3.4	14.7

^a Initial volatile solids content in the reactors (substrate + inoculum): 2.09 g L⁻¹

codigestion with pig slurry was particularly evident (35–43 % more CH₄ production) when using plant materials that had not given the best results in monodigestion, such as *A. donax* and *P. australis*. Pig slurry in codigestion with wetland biomass modified the N content and the C to N ratio of the methanogenic substrate, with an overall improvement of the methane production rate and yield. Liquid animal manure is therefore a better hydration medium for AD of wetland biomass, in comparison with water, because its supply permits to adjust the C to N ratio in favour of higher methane production rates and yields.

The joint evaluation of attitude to biomethanation and agronomic performance will allow the selection of the wetland species most advantageous as substrates for biomethanation. These materials could represent a more valid and environmentally sustainable alternative for AD than energy crops.

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