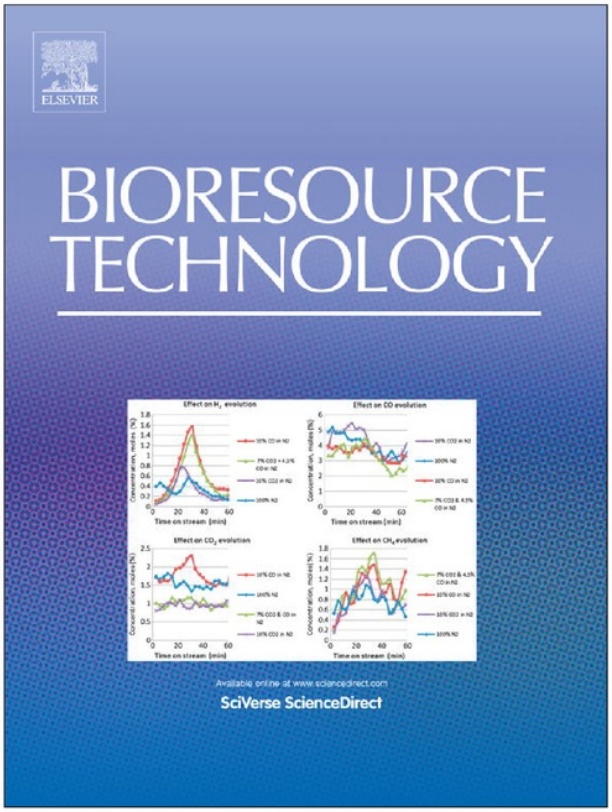
Chemical composition and methane yield of reed canary grass as influenced by harvesting time and harvest frequency

by Sutaryo Sutaryo



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Chemical composition and methane yield of reed canary grass as influenced by harvesting time and harvest frequency

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HIGHLIGHTS

- Harvest time and frequency had significant influences in methane yield.
- 45% more methane was produced in two-cut management compared to one-cut management.
- Chemical composition of biomass influenced concentration of methane in the biogas.
- Biogas produced from young biomass had lower fraction of CH_4 at the start of assay.

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ABSTRACT

This study examined the influence of harvest time on biomass yield, dry matter partitioning, biochemical composition and biological methane potential of reed canary grass harvested twice a month in one-cut (OC) management. The regrowth of biomass harvested in summer was also harvested in autumn as a two-cut management with (TC-F) or without (TC-U) fertilization after summer harvest. The specific methane yields decreased significantly with crop maturity that ranged from 384 to 315 and from 412 to 283 NL (normal litre) (kg VS)$^{-1}$ for leaf and stem, respectively. Approximately 45% more methane was produced by the TC-F management (5430 Nm^3 ha^{-1}) as by the OC management (3735 Nm^3 ha^{-1}). Specific methane yield was moderately correlated with the concentrations of fibre components in the biomass. Larger quantity of biogas produced at the beginning of the biogas assay from early harvested biomass was to some extent off-set by lower concentration of methane.

1. Introduction

Biogas production by anaerobic digestion of various feedstocks has increased in recent years in European countries. Traditionally, biogas is produced from manure, industrial waste, and sludge but there is growing interest in using biomass from plants as energy rich substrate for biogas production (Amon et al., 2007; Gunaseelan, 2007; Chandra et al., 2012). In addition to biogas production, anaerobic digestion of biomass also produces digestate that can be used as a valuable fertilizer in crop production (Herrmann et al., 2012).

Although a wide range of agricultural crops and their residues can be used for biogas production, perennial grasses are considered as a better option because of their high biomass yield potential and little environmental impacts in crop cultivation (Lewandowski et al., 2003). Reed canary grass (RCG, *Phalaris arundinacea* L.) is one of the promising perennial grasses to be used as energy crop under Nordic climatic conditions because of its higher biomass yield potential in colder climate (Lewandowski et al., 2003; Wrobel et al., 2008). RCG can be cultivated in water logged peat soils in river valleys as it has aerenchymatous tissues that allow oxygen supply to the root system. Furthermore, RCG is a relatively short crop suitable to cultivate in river valleys where open landscapes are desired (Venendaal et al., 1997). In Denmark, commercial production of RCG for bioenergy purpose has not started yet but there is a growing interest to cultivate this crop for biogas production (Raju et al., 2011; Triolo et al., 2011).

Harvest time significantly influences biomass yield of RCG (Seppälä et al., 2009; Tahir et al., 2011). Moreover, chemical composition of the biomass changes significantly with crop development which subsequently may affect biodegradability and specific methane yield (Amon et al., 2007; Massé et al., 2010; Hübner et al., 2011). Less lignified biomass with high concentration of easily degradable components such as non-structural carbohydrates, soluble carbohydrates and soluble cell components is considered suitable for high specific methane yield (Seppälä et al., 2009; Massé et al. 2010; Triolo et al., 2011). Harvest time also affects the proportion of leaf and stem in harvested biomass

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(Hübner et al., 2011). Usually, the fraction of leaves in grasses decreases with increasing maturity. Biomass with higher proportion of leaves is considered better for biogas production as the leaves of the grasses are less lignified and they contain more protein than the stems (Bruinenberg et al., 2002). Thus, harvesting at early stage of crop development may provide a better quality of the biomass for biogas production, but the total methane yields per hectare may not be improved if the biomass yield at early harvest is too low (Schittenhelm, 2008; Massé et al. 2010; Hübner et al., 2011).

Therefore, it is important to determine the optimum stage of harvesting to balance the trade-off between quantity and quality of biomass for biogas production (Schittenhelm, 2008; Bruni et al., 2010). In case of perennial grasses like RCG and switchgrass, this trade-off could be avoided by harvesting biomass multiple times in a year where an increase in both biomass quality and yield may be achieved (Seppälä et al., 2009; Massé et al., 2010). However, the extra biogas produced by multiple harvesting should also pay for extra harvesting costs and probably also for increased fertilizer requirement (Reynolds et al., 2000). Moreover, the growing season should be long enough for sufficient regrowth of the plants when multiple harvesting is practiced. Under Danish climatic conditions, RCG normally starts to grow at the end of March and it starts to flower at the beginning of June. The growing season lasts until September which permits to harvest RCG at least two times in a year.

Although RCG is recognized as a candidate crop for biogas production in Denmark, there is no information available about the effect of harvesting time and harvest frequency management on methane yields. Knowledge on effects of harvest time and harvest frequency on dry matter partitioning, biochemical composition of the harvested biomass, and their subsequent effects on specific methane yield are still lacking. Therefore, this study aims to determine the influence of crop maturity and harvest frequency on aboveground biomass yield, dry matter partitioning, biochemical composition and biological methane potential of RCG biomass.

2. Methods

2.1. Field experiment and plant material

The experiments were conducted on a cultivated peatland located in Nørreå river valley of Denmark (56°44'N, 9°68'E) near to Viborg. Details of soil properties at the experimental site can be found in Kandel et al. (2012). In brief, average peat depth was more than 1 m, and bulk density at the surface of the peat (0–20 cm depth) was 0.29 g cm^{-3}. Total organic carbon (TOC) and total nitrogen (TN) at 0–30 cm depth were 38.5% and 3.2%, respectively.

The field was ploughed in 2009 and three large (18 × 24 m) plots were sown with RCG (cv. Bamse). RCG biomass was not harvested in the first year but it was harvested regularly from second year as green biomass in autumn. This experiment was performed in 2011, so the biomass in this experiment represents the biomass from the third year after establishment of the RCG stand. When regrowth of the RCG was observed after the winter, the three plots were fertilized with standard mineral fertilizer at a rate of 60–13–77 kg N–P–K ha^{-1} on 4 April 2011. Each large plot was divided into two subplots (18 × 12 m). An area of 5 × 4 m in each subplot was used for two-cut (TC) management and the rest of the subplot area was used for one-cut (OC) management (i.e., harvested once in a growing season). Aboveground biomass from an area of 1 × 1 m was harvested manually from each OC subplot area twice a month until the end of September 2011 to monitor the development in biomass yield and quality during growing season. A new 1 × 1 m area was selected on every sampling date. The reason for taking biomass samples from two subplots in a plot was to increase the representativeness of the large plot. Biomass harvest started very early in this experiment to obtain biomass with a broad range of chemical composition which would allow to better understand the effect of biochemical composition on biogas production. Ten tillers from each harvested sample were chosen randomly and leaves and stems were separated manually to determine leaf/stem ratio and dry matter partitioning. The panicle represented a very small portion of the plant (less than 15% in its maximum); therefore the total culm including panicle is referred to as stem. The biomass was oven dried at 70 °C to constant weight for dry matter (DM) determination. Then the two subsamples taken from the two subplots of each plot were mixed well and ground in a mill with 1 mm sieve size for further analysis of chemical composition and biological methane production (BMP) as suggested by Hübner et al. (2011).

To study the effect of harvest frequency on dry matter yield and biogas production, biomass from the TC subplots was harvested on 15 June 2011 as a first cut of the TC management. After the summer harvest, one of the harvested TC subplots in each large plot was fertilized with an additional amount of 60–13–77 kg N–P–K ha^{-1} standard mineral fertilizer whereas the other TC subplot was left unfertilized. The objective of this fertilizer treatment was to understand the fertilization requirement of RCG for effective regrowth after summer harvest. Regrowth of RCG biomass in the TC subplots were harvested again on 22 September 2011 and the samples from both cuts were handled in a similar way as described previously. Biomasses harvested as regrowth in fertilized and unfertilized subplots are referred to as TC-F and TC-U, respectively. Biomass yield from the summer harvest and regrowth from the same plot were pooled to get the total biomass yield for comparison with the maximum biomass yield in OC management.

2.2. Biochemical analyses of the biomass

Part of the ground sample was used for biochemical composition analysis and another part was used for the BMP assay. The ash concentration was determined as the residue after incineration at 525 °C in a muffle furnace. Total nitrogen (N) and carbon (C) concentrations were determined by the LECO dry combustion system (LECO Corporation, St. Joseph, MI, USA). The C concentration was determined only in the biomass from last harvest in the OC management and from regrowth after first cut in the TC managements. Neutral detergent fibres (NDF), acid detergent fibres (ADF) and acid detergent lignin (ADL) of the plant samples were determined by the van Soest and Wine (1967) method with the Fibertec™ 2010 Systems (Foss Electric, Hillerød, Denmark). Cellulose was calculated as the difference between ADF and ADL, and hemicelluloses as the difference between NDF and ADF. The ADL was considered as lignin assuming that the fraction of lignin-bound nitrogen is trivial.

2.3. Inoculum preparation and properties

Inoculum for anaerobic digestion of the biomass was obtained from a post digestion tank of a mesophillic biogas plant at research centre Foulum, Denmark. The inoculum was degassed for 3 weeks before it was used in the BMP assays to ensure that the biogas production from the inoculum itself was minimal. The inoculum was further strained with a manual sieve with a mesh size of 500 µm (Retsch, Inc., Haan, Germany) to remove the solid fractions. Biophysical and biochemical analysis of the inoculum were performed after removing the solid fractions. The average pH of the inoculum was 7.74. The average total solid (TS) and volatile solid (VS) of the inoculum were 2.63% and 1.44%, respectively. The average total ammoniacal nitrogen (TAN) in the inoculum was 1.78 g L^{-1} and total volatile fatty acid (VFA) was 111 mg L^{-1}.

2.4. BMP assay

The BMP assay was carried out as described by Møller et al. (2004). In brief, inoculum (200 g) was added to a 0.5 litre infusion glass bottle which served as the batch reactor. Four grams of dry and ground plant sample was added to the batch reactor making a $VS_{substrate}/VS_{inoculum}$ ratio close to 1.25. Three batch reactors with inoculum only were used as controls. After adding inoculum and the plant material, the batch reactor was closed with a butyl rubber stopper and sealed with an aluminium crimp. Subsequently, the batch reactor was flushed with N_2-gas for 2 min to create anaerobic condition by removing residual oxygen. The batch reactor was then placed in an incubator at a constant temperature of 35 °C. Volume of the biogas produced was measured every week at the beginning of the batch assay and then gradually at longer time intervals using an acidified (pH < 2) water displacement method. The batch assay was continued for 69 days. Each batch reactor was mixed thoroughly by shaking to prevent dry layer formation and to encourage degassing just before the gas volume measurement. A 22 mL sample bottle was flushed with biogas from the batch reactor to determine CH_4 concentration in the biogas. The gas samples were then analyzed by a gas chromatograph (HP 6890 series; Agilent Technologies) equipped with a thermal conductivity detector. The temperatures of injection port, oven, filament and detector were 120, 35, 140, and 120 °C, respectively. Helium was used was the carrier gas with a flow rate of 30 ml min^{-1}.

The methane produced from the samples was adjusted by the methane produced by the inoculums itself. The specific methane yields were calculated as NL CH4 $(kg\ VS)^{-1}$ (NL = normal litre, i.e. gas volume corrected to 0 °C and 1.013 bar). Methane yield per hectare were calculated as product of dry matter yield, percentage of VS in biomass and specific methane yield.

2.5. Statistical analysis

Total annual biomass and methane yield of the TC managements were compared with maximum biomass and methane yields of the OC system using one-way ANOVA. Simple and multiple linear regression analyses were performed using specific biogas or methane yield as the dependent variable and biomass fibre components as the potential predictor variables. At first, simple linear regressions were performed, and later, multiple linear regressions

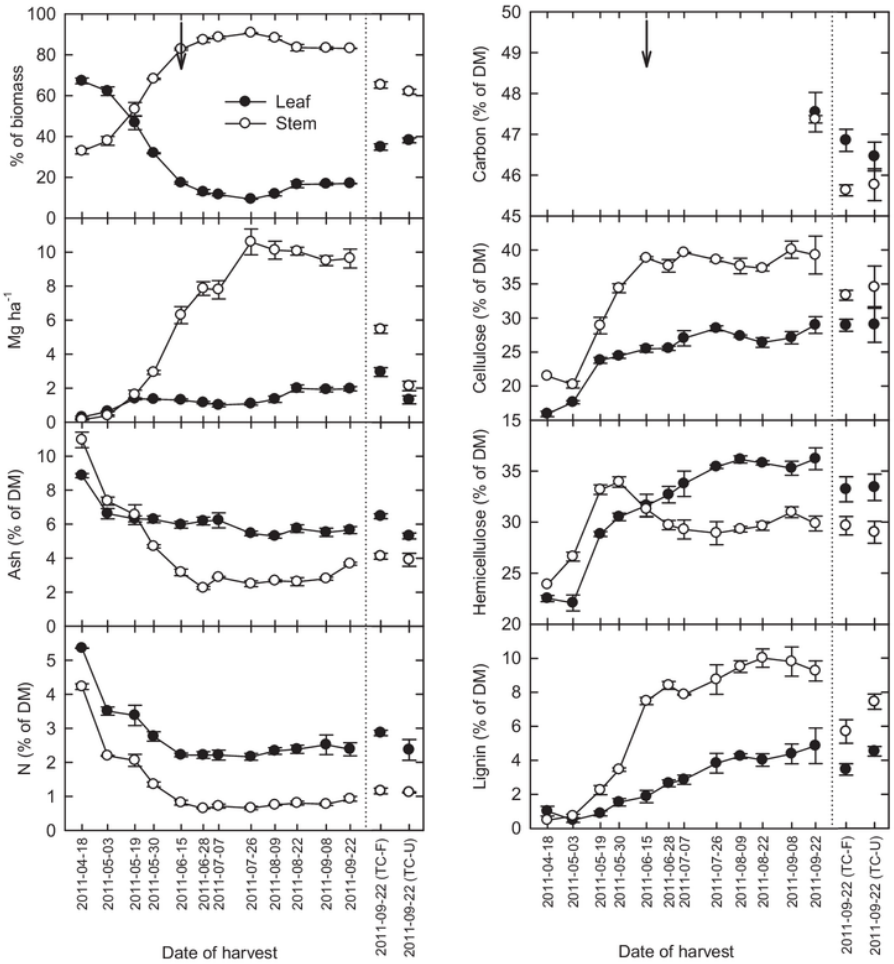

Fig. 1. Seasonal development in leaf/stem partitioning, yield, and chemical composition of reed canary grass biomass. Dotted vertical lines separate measurements in two-cut managements from one-cut management. The arrows indicate first harvest date in two-cut managements. Standard errors of the means ($n = 3$) are shown as vertical bars.

were performed using statistically significant variables. Pearson's correlation coefficient (r) was calculated for the relation between NDF concentration of biomass and CH_4 concentration in the biogas produced at the beginning (within 9 days, i.e. first two pumping) of the BMP assay. All statistical analyses were performed using the statistical package in SigmaPlot (11.0) (Systat Software, Chicago, IL, USA).

3. Result and discussion

3.1. Yield, dry matter partitioning and chemical composition of RCG

The RCG plants started to sprout by the end of March. The biomass harvested at the start of the growing season had higher proportion of leaves which declined with crop growth until mid summer (Fig. 1). Such a trend in decreasing fraction of leaf biomass with increased maturity is common for grasses (Ge et al., 2012). A slight increase in leaf biomass was observed after flowering (mid-June) as the plants produced new branches with higher proportion leaves after flowering. Both TC-F and TC-U biomass had higher proportion of leaves compared to the biomass in the OC management harvested in autumn. Biomass yield of the OC management reached a maximum level of 12.04 ± 0.3 Mg ha^{-1} (mean ± standard error, n = 3) that was almost stable from August to September. The biomass yields of the TC managements, TC-F and TC-U, were 8.45 ± 0.4 and 3.45 ± 0.5 Mg ha^{-1} in the second cut producing a total annual biomass yield of 16.0 ± 1.0 and 11.0 ± 0.8 Mg ha^{-1}, respectively. Total annual biomass yield of TC-F was significantly higher ($p < 0.05$) than the OC and TC-U managements. The results are in line with findings from other study which have also reported higher biomass yield from TC management of RCG cultivated for bioenergy production compared to OC management (Tahir et al., 2011).

Concentrations of ash and N in both leaf and stem of young plants were very high but the concentration decreased rapidly during peak growth as an effect of nutrient dilution with CO_2 assimilates (Fig. 1). After peak growth of plant biomass, the concentrations of ash and N did not change significantly. Concentration of N in the leaves was higher than in the stems throughout the growing season. Concentration of ash was higher in the stems at the start of the growing season but it became higher in the leaves before flowering and remained the same thereafter. The concentration of cellulose in both stems and leaves increased rapidly before flowering and with a slower rate during the rest of the growing season. Cellulose concentration in the stems was significantly higher than in the leaves throughout the growing season.

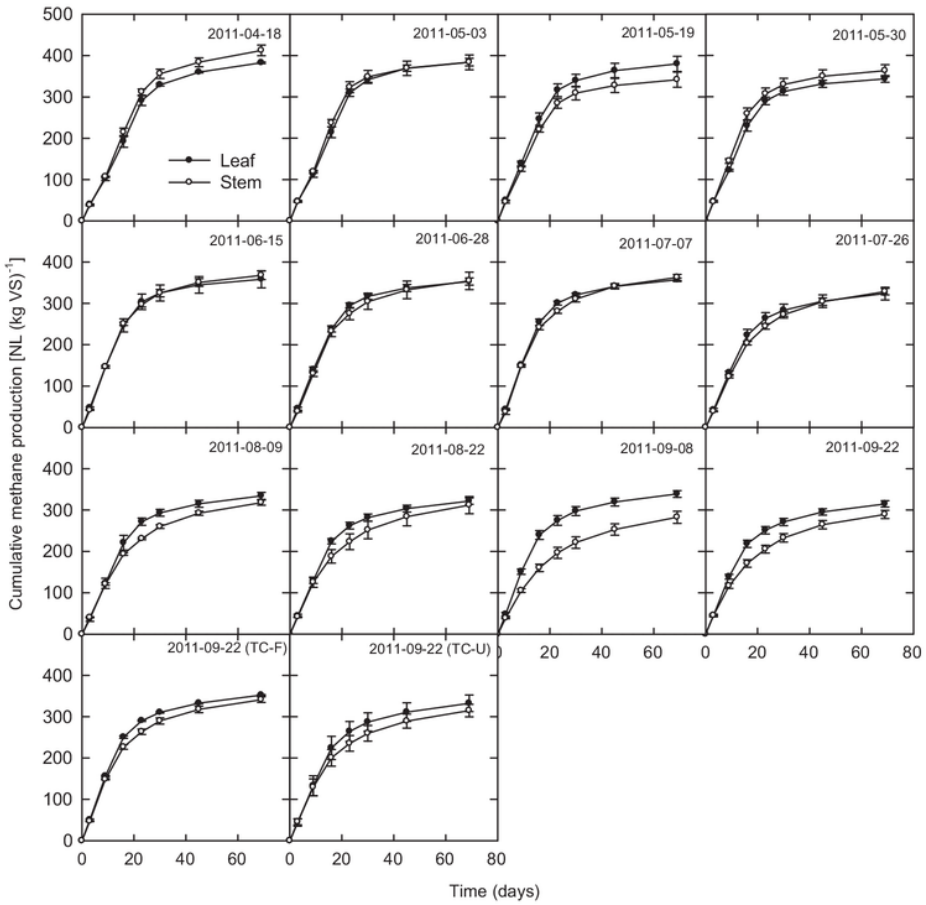

Fig. 2. Accumulated methane production over time from anaerobically digested leaf and stem fractions of reed canary grass. Biomass harvest dates are shown in upper right corners. Standard errors of the means (n = 3) are shown as vertical bars.

Concentration of hemicelluloses in the stems increased at the start of the growing season before flowering and then dropped and remained constant for the rest of the standing period. A rapid increase in cellulose and lignin concentration in the stems during flowering may be the reason for decreased proportion of hemicellulose. Unlike the stems, a decline in concentration of hemicellulose was not observed in the leaves during flowering stage. Similar to the cellulose, the concentration of lignin in the stems increased rapidly just before flowering and with a lower rate thereafter. Concentration of lignin in the leaves increased with a relatively slow rate throughout the growth period. As expected, the leaves had significantly lower lignin concentration in mature biomass as compared to the stems. These results are in line with the findings from other studies where typical indicators of maturity stage is increasing concentrations of fibre components (cellulose, hemicellulose, and lignin) along with declining ash and nitrogen concentrations (Collins and Casler, 1990; Schittenhelm, 2008; Čop et al., 2009).

Both ash and N concentrations were slightly higher in TC-F biomass. Total C concentration ranged from 45.8% to 47.5% in the biomass from the final harvest of the three different types of managements. Biomass in OC management had slightly higher concentration of C which may be due to lower concentrations of ash and N in the biomass. Lignin concentration in the stems of TC-F and TC-U were lower than in OC biomass harvested in autumn suggesting that quality of the biomass can be improved by TC management (Triolo et al., 2011).

3.2. Methane yield

Cumulative methane production from leaf and stem RCG biomass harvested at different dates throughout the growth season are presented in Fig. 2. A sharp and linear increase of methane production was observed at the beginning of the batch assay. A major part of methane was produced from both leaves (86–91%) and stems (78–91%) within 30 days of the batch assay. The rates and the asymptotic maximum of the curves illustrate more easily biodegradable biomass with higher specific methane yield when it was harvested at the early stages of growth. The difference between specific methane yield between leaf and stem biomass harvested at the early stages of growth were either very small or non-existent, but later on the differences were more pronounced. A small but continuous production of methane was observed from stems of mature biomass towards the end of the batch assay when production from the leaves had reached the maximum suggesting slower biodegradation of fibre components of the stems towards senescence.

The specific methane yields decreased significantly with crop maturity ranging from 384–315 and 412–283 NL CH_4 $(kg\,VS)^{-1}$ for leaves and stems, respectively (Fig. 3). In the OC management specific methane yield of the stems decreased rapidly after July when the plant reached towards senescence. However, specific methane yield from leaves did not decline sharply which may be due to increased proportion of young leaf on new stems produced after flowering. Specific methane yield of biomass from TC-F was comparable with the specific methane yield of biomass harvested on 15 June as the first cut of the TC management and significantly higher ($p < 0.05$) compared to the TC-U and biomass harvested in autumn in OC managements. The higher specific methane yield might be due to the lower lignin concentration in TC-F biomass. This result is contrary to findings from Seppälä et al. (2009) who reported significantly lower specific methane yield from biomass at second harvest in a TC management, but the finding in the present study is in line with Massé et al. (2010) who also found similar specific methane yield of biomass from both harvests. The results from the TC-F and TC-U treatments suggest that availability of nutrients in the soil affect the chemical composition of biomass, and thus the specific methane yield from autumn harvest in a TC management.

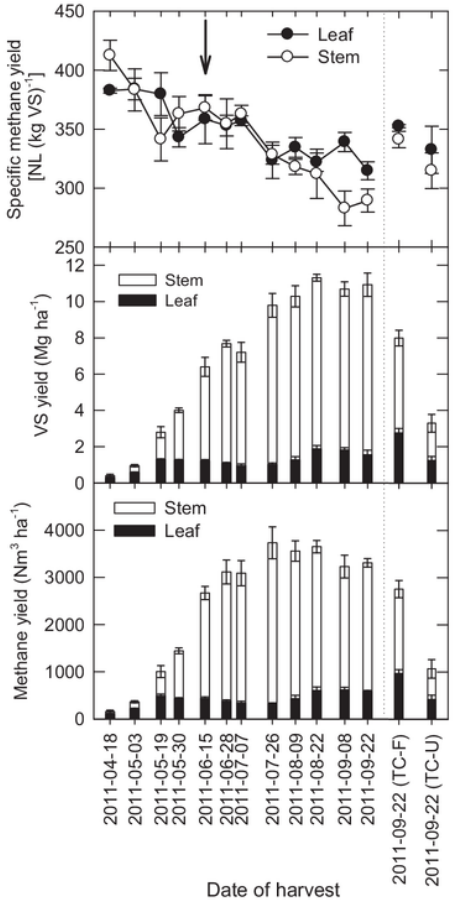

Fig. 3. Specific methane yield, total volatile solid (VS) and total methane yield per hectare of biomass from different harvest dates. The dotted vertical lines separate measurements in two-cut managements from one-cut management. The arrow indicates first harvest date in two-cut managements. The errors bars represent standard errors of the means ($n = 3$).

Although specific methane yield decreased with crop maturity, methane yield per hectare increased until the end of July as a result of increased VS production. Beyond July no further increase in the biomass yield was seen while the specific methane yield continued to decrease which resulted in a decline in total methane yield per hectare. As the farmer aims to produce maximum methane yield per hectare, the results suggest harvesting the biomass by the end of July for OC management. The plants start to yellow and to drop the panicles at this stage. Approximately 45% more ($p < 0.001$) methane was produced by the TC-F management (5430 Nm^3 CH_4 ha^{-1}) compared to the maximum methane production from the OC management (3735 Nm^3 CH_4 ha^{-1}). Therefore, the finding suggests harvesting the biomass two times in a growing season – first in summer and second in autumn. However, optimization of nutrients in the soil seems to be important to support the regrowth of biomass after first harvest. The current results support the finding of Massé et al. (2010) who also suggested harvesting switchgrass two times in a growing season cultivated in

north-eastern Canada. In contrast, Seppälä et al. (2009) did not find any benefit in harvesting RCG two times as total methane yield per hectare was not improved by a TC management in Finland. This opposite conclusion might be due to shorter growing season in Finland and different nutritional status of the soil cultivated for RCG production.

3.3. Concentration of CH_4 in the biogas

The methane concentration in the biogas ranged from 25% to 75% throughout the course of the batch assay (Fig. 4). At the start of the assay, methane concentration of the biogas produced from young biomass was very low compared to methane concentration of the biogas produced from mature biomass. For example, after the first week of incubation methane concentration in the biogas produced by biomass from the first harvest was about 25–30% whereas the concentration was above 50% in biogas produced by biomass from the last harvest. The lower methane concentration in the biogas produced from young biomass at the beginning of the batch assay might be due to rapid fermentation of non-structural components of the biomass resulting in a small build-up in acid products and partial inhibition of the hydrogenotrophic methanogens (Rincón et al., 2010; Chandra et al., 2012). Although the methane concentration in biogas produced from young biomass was significantly lower at the beginning of the batch assay, it increased sharply reaching about 75% by the third week. After a peak, methane concentration in all batch reactors decreased slightly and remained constant during the rest of the assay run. This variation in methane concentration during assay progress may be associated with a change in source carbon being converted to biogas, from readily available such as non-structural and soluble carbohydrates to structural fibre fractions like NDF, long chain proteins and some oils which stoichiometrically produce a higher proportion of methane (Rincón et al., 2010).

Previous studies have usually reported methane concentration in the range of 50–70% in biogas produced from grasses (Seppälä et al., 2009; Massé et al. 2010; Hübner et al., 2011). However, all these studies represent analysis of biogas produced from mature biomass with high concentration of fibre components. The lower concentration of methane in this study represents the biogas produced from very young biomass which was not included in the previous studies. A significant positive correlation ($r = 0.81$ for leaf and 0.84 for stem, $p < 0.001$ for both) was observed between concentrations of NDF (NDF represents the structural part in the

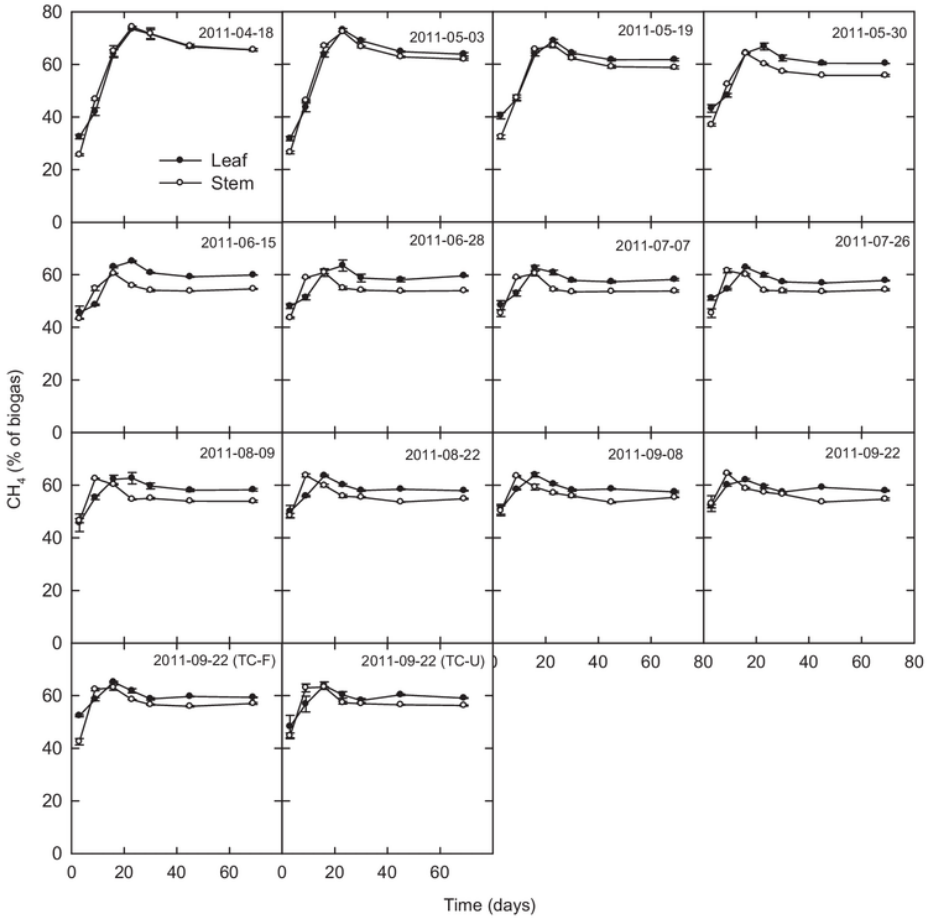

Fig. 4. Methane concentration of the biogas produced throughout the time course of the batch assay. Biomass harvest dates are shown in upper right corners. Standard errors of the means ($n = 3$) are shown as vertical bars.

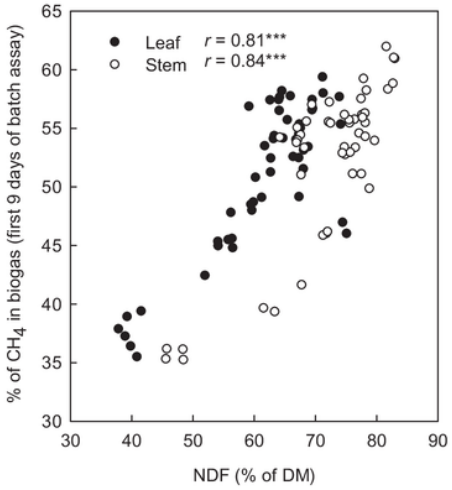

Fig. 5. Correlation between neutral detergent fibre (NDF) concentration in biomass (% of DM) and methane concentration of the biogas (%) at the beginning of the batch assay (first 9 days). The Pearson's correlation coefficient (r) is presented. ***, Significant at the 0.001 probability level.

biomass) in the biomass and CH_4 concentration in the biogas at the beginning of the assay (Fig. 5). As discussed earlier, the large amount of non-structural and soluble sugars present in young biomass might have fermented rapidly at the beginning of batch assay to give rise to high amount of biogas with lower percentage of methane. On the other hand, mature biomass with higher percentage of NDF must have degraded slowly, thus avoiding rapid fermentation at the beginning of the batch assay. The findings from the current study suggest that there is a possibility of wasting carbon resources as carbon dioxide if biomass with very low concentration of structural components is used for biogas production.

3.4. Correlations between biochemical composition of biomass and biogas and methane yield

Similar coefficients of determination (R^2) were observed when different fibre components of leaf biomass were correlated with specific biogas and methane yield (Table 1). However, both specific biogas yield and specific methane yield from stem biomass correlated better with lignin than cellulose which supports the results from previous studies (Gunaseelan, 2007; Triolo et al., 2011). The reason for the better correlation with lignin in case of stem (which had larger range of lignin concentration) may be due to higher resistance of lignin itself and its role in providing resistance to cellulose for enzymatic digestion by forming a matrix with cellulose (Mussatto et al., 2008). Concentration of cellulose, NDF and ADF in the biomass also had a significant but moderate to low correlation with specific biogas or methane yield. The correlations of fibre groups with specific biogas yield were generally higher compared to their correlations with specific methane yield indicating that fibre components could define overall biodegradability of biomass better than final composition of the product (biogas). The coefficients of determination (R^2) was increased slightly when both lignin and cellulose were used as predictor variables in a multiple linear regression equation for leaf biomass but this was not the case for stem biomass. The results suggest that the methane yield can be predicted by the concentration of fibre components in the biomass with a fairly good precision (see also, Gunaseelan, 2007; Amon et al., 2007; Triolo et al., 2011).

4. Conclusions

Harvest time had a significant impact on biomass yield, specific methane yield and methane production per hectare. TC-F management increased methane yield per hectare by 45% as compared to OC management. Specific methane yield was predicted by the concentration of fibre components in the biomass with a fairly good precision. Biochemical composition of biomass not only influenced specific methane yield but it is also affected quality of the biogas produced, i.e. concentration of methane in the biogas. Methane concentration in the biogas produced from young biomass was lower at the beginning of batch assay.

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Table 1
Coefficients of determination (R^2) and level of significance (p) for linear regression of specific biogas and methane yields [NL (kg VS)$^{-1}$] with various fibre fractions (% of DM).

Part	Product	Variable	R^2	p	Equation
Leaf	Biogas	Lignin	0.66	<0.001	Biogas = −28.7 * Lignin + 658.8
		Cellulose	0.61	<0.001	Biogas = −10.7 * Cellulose + 846.1
		NDF	0.69	<0.001	Biogas = −4.9 * NDF + 871.3
		ADF	0.69	<0.001	Biogas = −8.7 * ADF + 821.142
		Lignin, cellulose	0.73	<0.001	Biogas = −18.3 * Lignin − 5.4 * Cellulose + 765.8
	Methane	Lignin	0.48	<0.001	Methane = −11.9 * Lignin + 383.1
		Cellulose	0.51	<0.001	Methane = −4.8 * Cellulose + 469.4
		NDF	0.55	<0.001	Methane = −2.1 * NDF + 477.7
		ADF	0.56	<0.001	Methane = −3.8 * ADF + 456.0
		Lignin, cellulose	0.57	<0.001	Methane = −6.1 * Lignin − 3.0 * Cellulose + 442.6
Stem	Biogas	Lignin	0.46	<0.001	Biogas = −17.8 * Lignin + 690.5
		Cellulose	0.33	<0.001	Biogas = −7.7 * Cellulose + 839.7
		NDF	0.38	<0.001	Biogas = −5.4 * NDF + 952.6
		ADF	0.41	<0.001	Biogas = −6.0 * ADF + 819.7
		Lignin, cellulose	0.46	<0.001	Biogas = −15.4 * Lignin − 1.5 * Cellulose + 728.3
	Methane	Lignin	0.37	<0.001	Methane = −7.6 * Lignin + 385.7
		Cellulose	0.28	<0.001	Methane = −3.4 * Cellulose + 452.2
		NDF	0.34	<0.001	Methane = −2.4 * NDF + 505.3
		ADF	0.35	<0.001	Methane = −2.6 * ADF + 442.5
		Lignin, cellulose	0.38	<0.001	Methane = −6.3 * Lignin − 0.9 * Cellulose + 406.8

the chemical analyses, and Britt Malthesen and Claudia Nagy for the biogas tests.

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