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Methane production potential of various crop species grown in energy crop rotations

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The methane production potential represents an essential quality parameter of biomass if used as feedstock for biogas production. 769 harvested crop materials from crop rotations were ensiled at standardized conditions and were analyzed regarding their specific methane yields by applying two different experimental setups of batch anaerobic digestion tests. Based on this analysis, reference values for average methane yields per crop species and position within the crop rotation, cutting regime, range of dry matter content, or stage of growth at harvest have been deduced for 93 different crop biomasses. Results provide a comprehensive dataset that can be used in combination with biomass yields for the estimation of methane hectare yields, for economic and ecological evaluation of energy crop rotations, for planning and structural design of biogas plants as well as for decisions regarding the cultivation of alternative co-substrates and the design of sustainable biogas crop rotations.

Keywords
Biogas, methane yield, crop rotation, silage, reference values

In 2050, renewable energies shall account for a major share of domestic energy supply in Germany. Owing to advantageous characteristics of bioenergy carriers such as that they can be stored and flexibly produced or used, energy from biomass will play a significant role in future energy systems (BMEL 2015). At present, forage maize is the most important energy crop for anaerobic digestion in biogas plants. As a C4-crop it possesses optimal utilization characteristics for water and solar radiation which lead to high biomass yields per hectare. Maize can be easily harvested and preserved, and allows for high methane yields at comparatively low production costs due to its good biodegradability. However, divers crop rotations are needed for a sustainable agricultural production. This raises the question: what are efficient cropping systems or crop species that can be cultivated in combination with maize? Since 2005 members of the joint research project ‚EVA' (Development and comparison of optimized cultivation systems for the agricultural production of energy crops under different local conditions within Germany; www.eva-verbund.de) have committed themselves to address this question. The project aims at developing application-oriented regional decision support and contributing to a diversification of production patterns and crop rotations through investigation of varied cropping systems.

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Assessment of the cultivation and adequacy of different crop species or crop rotations comprises the evaluation of energetic, economic and ecological effects (Eckner et al. 2015, Glemnitz et al. 2015). Besides biomass yields, also knowledge on the quality of the crop biomasses in regard to methane production is required. The key issue is the amount of methane that can be produced per mass unit of crop material under appropriate process conditions.

A number of different methodical approaches on the estimation of methane yields already exist. One option is the use of equations that aim at the prediction of specific methane yields of harvested crop materials, usually based on chemical composition analysis (Rath et al. 2015). Such equations have been developed on the basis of comprehensive datasets for individual crop species, mainly for maize (Rath et al. 2015, Stoffel and Köller 2012). However, so far no generally valid equations are available that estimate methane yields for a large variety of crop feedstocks with sufficient precision, especially for new crop species or crop mixtures that are not yet established. Another disadvantage is that substantial chemical analysis are required for calculation of methane yields. In addition, a model validation should be carried out in order to test the validity of these equations. Several studies have shown that differences in specific methane yields are often not sufficiently reflected by existing equations (Czepuck et al. 2006, Rath et al. 2015, Stoffel and Köller 2012).

Another option is the use of values from literature or of reference values for methane production from different feedstocks. Literature or reference values already exist for a large number of crop species that can be grown in energy crop rotations (KTBL 2015, LfL 2016). They are partly compiled from results of laboratory experiments of different institutions (KTBL 2015), but are also partly based on an equation to estimate methane yields according to Baserga (1998) (LfL 2016). Yet, it has been shown that this calculation model does not provide reliable data on methane yields (Czepuck et al. 2006, Rath et al. 2015). The applicability and validity of reference values substantially depends on the underlying data base. Comprehensive data for the estimation of methane yields of a large range of crop species considering the impact of the position within the crop rotation or of cultivation conditions are still lacking as a basis for the design and evaluation of biogas crop rotations. Thus, the aim of the present study is to

- characterize a large range of crop species from energy crop rotations regarding average methane production potentials, based on comprehensive experimental investigations
- with emphasis on cultivation conditions and harvest dates, i.e. position within the crop rotation or maturity at harvest, and
- provide information on the applicability of these reference values. The study includes silages of 59 crop species with reference values derived for 93 different crop biomasses.

Materials and methods

Description of raw materials

Subject of the study are crop biomasses of 59 different crop species or mixtures that were cultivated in the years 2005 to 2015 in field plot trials for investigation of energy crop rotations, and in additional trials for testing of alternative crops at eight different experimental sites in Germany (Figure 1, a). A detailed description of site characteristics and an overview of the energy crop rotations is given by Eckner et al. (2013) and Herrmann et al. (2016). Immediately after harvest, the crop material was cut to a particle size of less than 20 to 30 mm and preserved by ensiling. Ensiling was carried out uniformly in 1.5 liter lab scale silos in triplicates (Figure 1, b). The crop material was manually filled into the lab scale silos and compressed in such way that no headspace remained within the silos. Subsequently, the silos were closed airtight with gases being able to escape from but not infiltrate into the silo. The lab scale silos were stored over a period of 90 days at 25 °C. After taken from the silos, silages were frozen and stored at -18°C until further analysis of chemical composition and methane production.

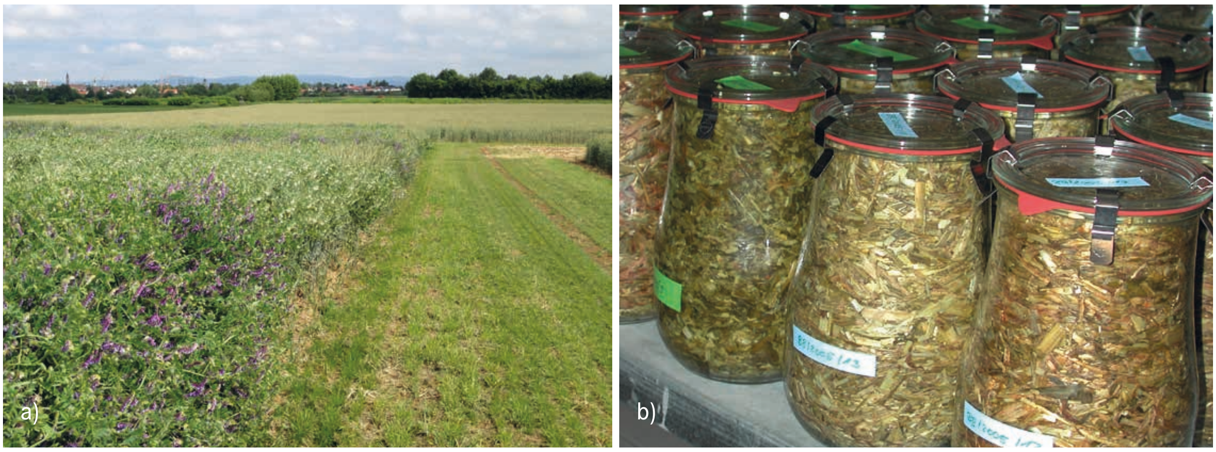

Figure 1: a) Field plot cultivation trials for investigation of energy crop rotations (Photo: M. Fritz, TFZ) and b) lab scale silos for preservation of harvested crop material (Photo: C. Idler)

Analysis of methane yields in batch anaerobic digestion tests

Methane production from ensiled samples was determined by applying two different experimental setups for batch anaerobic digestion tests (Figure 2). In total 769 silages were analyzed with 2 to 6 repetitions (predominantly with 3 repetitions).

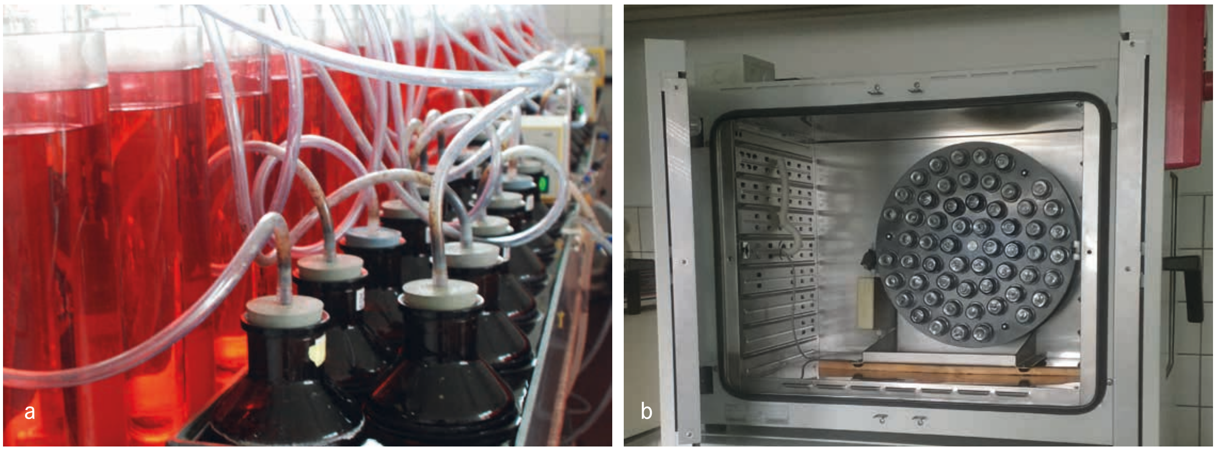

Figure 2: Experimental setup of the batch anaerobic digestion tests: a) 2 liter reactors with wet gas meters at the ATB (Photo: C. Herrmann) and b) "Hohenheim biogas yield test" system at the TLL (Photo: F. Hengelhaupt)

About 80% of the silages were measured and evaluated regarding their methane production without further pretreatment in 2 liter reactors at the Leibniz Institute for Agricultural Engineering and Bioeconomy e.V. (ATB). Each reactor was filled with 1.5 liter inoculum consisting of digestate of previous laboratory anaerobic digestion tests with crop biomasses, and 50 g of the silage to be analyzed (organic dry matter (ODM) ratio $ODM_{substrate} : ODM_{inoculum} = 0.5 \pm 0.2$). The reactors were incubated at 35 °C over a period of 30 days in a water bath. The biogas produced was collected in wet gas meters and the gas volume was measured by applying the liquid displacement method using a NaCl saturated solution as barrier solution. The gas composition (methane, carbon dioxide) was detected by means of a portable gas analyzer equipped with infrared sensors (GA94, Ansyco). Gas sensors were calibrated each time before starting a batch assay run. The procedure of the batch anaerobic digestion tests has been previously described in detail (Herrmann et al. 2011).

For about 20% of the silages, determination of specific methane yields was conducted at the Thuringian State Institute of Agriculture (TLL) using the 'Hohenheim biogas yield test (HBT)'. Silages were dried for 24 hours at 60 °C and were subsequently milled to a particle size of < 1 mm. For each digestion test, about 35 g of inoculum and 0.4 g of sample material (ratio $ODM_{Substrate} : ODM_{Inoculum} = 0.5$) was filled into 100 ml glass syringes which were used as fermenters and for gas collection. The glass syringes were inserted into a slowly rotating rotor which results in a continuous mixing of the reactor content, and are tempered in an incubator at 37 °C over a period of 30 days. The produced gas pushes the plunger out of the syringes, and the gas volume can be read from the graduated scales on the syringes. Analysis of the methane content was conducted using an external measuring device with infrared sensor (Sensors Europe GmbH, AGM 10). The function and experimental method of the HBT has been described in detail by Helffrich and Oechsner (2003).

The specific methane yield was calculated as the sum of the gas volume that was produced during the test period, corrected by the gas production of the inoculum according to the VDI guideline 4630

(VDI 2006), and referring to the added ODM of the substrate sample. All gas volumes wer normalized to standard conditions (dry gas, 273,15 K, 1013 hPa). In the experimental setup with 2 liter reactors, the gas which fills the headspace at the beginning of each experiment results in a dilution of the produced biogas components. Thus, methane contents that were measured during the test procedure were subjected to a headspace correction as suggested by the VDI guideline 4630 (VDI 2006). Microcrystalline cellulose as well as an internal laboratory standard consisting of dried and to less than 1 mm milled crop material were analyzed as reference substrates in each experimental run in order to ensure the validity of results and a sufficient activity of the inoculum. Anaerobic digestion tests were generally stopped after a duration of 30 days. At this point of time, the criterion for termination of the anaerobic digestion tests according to the VDI guideline (VDI 2006) was reached for all samples investigated, and gas production was largely completed.

Chemical analysis

The dry matter (DM) and organic dry matter (ODM) content of the silages which provide the reference basis for the calculation of methane yields were analyzed by oven drying at 105 °C and by subsequent ashing of the dried samples at 550 °C according to standard procedures (VDLUFA 2006). For evaluation of silage quality, pH-values of the silages were determined using a pH measuring electrode, and lactic acid, volatile fatty acids (acetic, propionic, i-butyric, n-butyric, i-valeric, n-valeric, caproic acid) and alcohols (ethanol, propanol, 1,2-propanediol, 2,3-butanediol, partly methanol) of the silages were analyzed. Analysis of volatile fatty acids and alcohols was conducted by means of gas chromatography while lactic acid is measured using high pressure liquid chromatography (HPLC) (Herrmann et al. 2016). Volatile organic compounds of the silages are lost during DM analysis of the silages and lead to an underestimation of the actual dry matter content. Thus, the measured dry matter content was corrected by losses of organic acids and alcohols (in the order of 0.2 to 2.6% of the fresh matter) as suggested by Weissbach and Kuhla (1995), Weissbach and Strubelt (2008a), and Weissbach and Strubelt (2008b). Methane yields that were measured from fresh silages using 2 liter reactors refer to the corrected organic dry matter (ODM_C). Methane yields that were measured from dry input materials using the HBT were not subjected to an additional correction since volatile organic compounds are already lost before adding the feedstock to the fermenter (Mukengele and Oechsner 2007).

Calculation of reference values

Reference values for methane production that can be obtained by anaerobic digestion under favorable process conditions were calculated based on the measured specific methane yields of different silages. Microcrystalline cellulose used as reference substrate in both laboratories achieved on average 96.4% (ATB experimental setup) and 98.6% (HBT) of the maximum value attainable for cellulose. Results suggest that a highly active inoculum was applied for experimental investigations (VDI 2006). The relative standard deviation of the methane yields lay at 4.5% (ATB experimental setup) and 4.0% (HBT) and was comparatively low (Heuwinkel et al. 2009). Methane yields that are determined by different laboratories often reveal deviating values. Thus, reproducibility of methane yields in different laboratories is limited (Raposo et al. 2011). Main reasons are the use of different experimental setups accompanied by varying pretreatment of samples and varying experimental procedures, and the impact of different inocula which are used in each laboratory (Raposo et al. 2011). In the present study, preliminary comparative analysis of identical samples were conducted in both experimental setups that were applied. Minor deviations of on average 2.6% between methane yields measured in both laboratories were determined in an interlaboratory comparison (Döhler and Wulf 2010). In addition, comparative analysis were conducted for 36 identical substrate samples (silages). Deviations of on average 4.7% and a close correlation of biogas yields (R^2 = 0,83) were found. In order to further minimize variations caused by the experimental setup, reference values were calculated as mean values of the measured methane yields relative to the methane yield of maize silage cultivated as main crop which was measured in the respective laboratory. Maize silage was chosen as reference basis as it is the most frequently utilized feedstock in agricultural biogas plants. The reference basis 'maize main crop (MC)' was thereby deduced from a large number of measured values (n = 71) from all experimental sites, obtained in 8 different years of cultivation. Maize varieties were selected for cultivation based on site-specific conditions. Thus, calculation of the reference basis 'maize (MC)' comprised data from in total 14 different, early to very late ripening maize varieties (maturity class S220 to S440). All maize samples used for calculation of the reference basis were exclusively harvested at the early dough to hard dough (BBCH 83-87) stage of maturity.

Reference values are solely based on analysis of ensiled crop materials. Silage quality can significantly influence the methane yield as has been shown in other studies (Herrmann et al. 2011). Thus, silages with poor to very poor silage quality according to DLG (2006) were not considered for estimation of the reference values. Furthermore, losses of ODM occur during ensiling. Losses are not included in the reference values of methane yields, i.e. values refer to the ODM added to the biogas process. In order to achieve an increased accuracy, reference values of crop species were further specified regarding their stage of maturity at harvest, cut or position within the crop rotation. The stages of maturity of the crops at harvest were determined as phenological growth stages with BBCH identification keys as described by Meier (2001). Classification of different positions within the crop rotations include main crops, secondary crops, winter and summer cover crops. When cultivated as main crop, the vegetation period of the crop is not limited. When cultivated as secondary crop, the crop is established after a winter cover crop. This is usually realized by a slightly later harvest of the winter cover crop in order to increase its biomass yield, and a subsequently later sowing of the secondary crop. The biomass yield of the secondary crop is often reduced due to the later sowing date. Winter cover crops are sown after the summer crop in late summer or autumn, and the main biomass formation and utilization usually occurs in the following year until the end of heading or begin of flowering. In contrast, the main biomass of summer cover crops is formed until the end of the vegetation period, and harvest and utilization is conducted from late summer to early winter.

Results and discussion

Reference values for methane yields of different crop silages

Results on methane yields relative to maize cultivated as main crop, and on average methane contents of the biogas produced from different crop biomasses are given in Table 1. Mean methane yields of 354.6 L_N/kg ODM_c (n = 47, ATB) and 363.9 L_N/kg ODM (n = 24, TLL) are determined for maize cultivated as main crop which represents the reference basis. Results are in good agreement with methane yields of maize typically found in literature (Rath et al. 2013, Stoffel and Köller 2012). Reference values for methane yields of investigated crop species and mixtures range from 35 to 114% of the methane yield of maize (MC) (Table 1). For a number of crop species data on methane yields already exist in literature which are in the same range as found in the present study (Haag et al 2015, Mahmood and Honermeier 2012, Mast et al. 2014, Molinuevo-Salces et al. 2013, Schumacher 2008). Results indicate that several crop species achieve similar or higher methane yields compared with maize silage (Table 1). This applies to early cut grasses or forage grass and legume mixtures (BBCH < 55, middle of heading) such as annual ryegrass, clover grass, alfalfa grass, and ryegrass mixtures; whole crop cereals harvested in their vegetative stage of growth such as winter barley, winter rye and winter triticale; and further silages of mustard harvested before flowering, a mixture of spring barley and ryegrass, Jerusalem artichoke (tubers), and sugar and fodder beet. Methane yields of whole crop cereals harvested during the reproductive stage of growth at early milk to soft dough stage of maturity, and of sorghum are in the range of 85 to 100% of the methane yields of maize (MC). With regard to cereal silages, methane yields decrease in the order from winter barley, winter triticale, winter rye and winter wheat. This trend is also reflected by crop mixtures that include different cereal species. In general, methane yields tend to decrease with increasing contents of the fiber fraction, especially of lignin and cellulose (results not shown). Thus, crop biomasses with high concentrations of lignin such as giant knotweed, the overwintered wild flower mix, miscanthus, buckwheat (after flowering), sunflowers or Jerusalem artichoke halm exhibit low methane yields in the range of 35 to 75% of maize (MC).

Table 1: Reference values for methane yields relative to maize (MC) ± standard deviation from silages of different crop species and mixtures, average methane contents, stages of maturity at harvest, and dry matter and crude ash contents of the silages (range of measured values are given in parenthesis)

Crop species or crop mixture	**Specification/ characteristics**	**n**	**BBCH at harvest**	**ODM methane yield (relative to maize MC)[1)]**		**Methane content**	**DM_c content**	**Crude ash**
				in %		**in %**	**in %**	**in % DM_c**
Maize	MC	71[A,H]	83-87	100	±5.5	55.3	31 (25-38)	4 (3-6)
Amaranth		2[A]	75-79	84	±3.9	58.8	21 (19-23)	14 (13-14)
Wild flower mix	biogas, 1st year	2[A]	61-69	69	±0.6	59.2	20 (20-21)	11
Wild flower mix	biogas, overwintered	1[A]	85	56		64.2	44	9
Bokhara clover (Honey clover)	MC	9[A]	63-69	78	±7.8	58.7	29 (20-35)	7 (5-9)
Buckwheat	BBCH ≤ 59	1[A]	51	89		63.0	12	9
Buckwheat	BBCH 61 - 89	8[A]	75-87	72	±2.7	56.4	30 (25-32)	9 (7-10)
Buckwheat	BBCH ≥ 91	6[A]	91-95	67	±8.5	58.1	24 (18-30)	11 (8-15)
Buckwheat/ phacelia	SCC	1[A]	71	71		57.6	26	9
Cup plant		5[A]	77-85	67	±5.2	59.6	28 (24-31)	12 (11-13)
Annual ryegrass	SCC, early cut, BBCH < 55	6[A]	33-51	103	±14.9	57.9	34 (15-58)[3)]	12 (9-17)
Annual ryegrass	SCC, late cut, BBCH ≥ 55	13[A,H]	59-71	92	±9.9	58.5	26 (16-41) [3)]	11 (8-14)
Fodder beet	roots, heads and leaves removed	2[A]	49	112	±2.4	57.3	19 (19-20)	6 (5-8)
Spring barley/ Italian ryegrass/ hybrid ryegrass	MC	2[A]	77-83	100	±1.5	54.8	27 (25-30)	9 (7-11)

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Crop species or crop mixture	Specification/ characteristics	n	BBCH at harvest	ODM methane yield (relative to maize MC)[1]		Methane content	DM_c content	Crude ash
				in %		in %	in %	in % DM_c
Oat	Milk to soft dough	$15^{A,H}$	73-85	92	±7.2	57.0	37 (26-54)	7 (6-9)
Oat/fodder vetch	MC	1^{A}	71/65	109		63.7	21	12
Giant knotweed	1st cut	1^{A}	k.A.	35		66.7	25	7
Giant knotweed	2nd cut	1^{A}	k.A.	41		62.9	19	9
Clover grass mixture	1st cut, early [4]	7^{A}	47-51	108	±12.1	57.7	26 (15-35) [3]	10 (10-13)
Clover grass mixture	1st cut, late [5]	7^{A}	57-59	97	±9.3	57.4	27 (21-37) [3]	9 (8-11)
Clover grass mixture	Following cuts, early [4]	5^{A}	59	94	±12.5	57.6	38 (26-53) [3]	11 (9-12)
Clover grass mixture	Following cuts, late [5]	14^{A}	59-67	89	±10.7	58.8	35 (22-53) [3]	10 (8-12)
Landsberger mixture: Fodder vetch/ crimson clover/ Italian ryegrass	1st cut	$3^{A,H}$	59-61	99	±6.1	59.7	22 (17-28)	11 (10-12)
False flax	MC	5^{A}	80-83	83	±4.6	63.1	30 (29-31)	13 (10-20)
Alfalfa	1st cut, early [4]	2^{A}	49	98	±1.4	59.9	22 (21-24) [3]	11
Alfalfa	1st cut, late [5]	2^{A}	57-59	87	±2.1	59.2	34 (30-37) [3]	10 (9-11)
Alfalfa	Following cuts, early [4]	3^{A}	49-51	80	±7.8	57.7	49 (42-56) [3]	11
Alfalfa	Following cuts, late [5]	5^{A}	59-67	77	±5.8	58.8	43 (34-52) [3]	10 (8-11)
Alfalfa grass mixture	1st cut, early [4]	$5^{A,H}$	49-55	101	±9.0	58.0	29 (19-42) [3]	11 (11-12)
Alfalfa grass mixture	1st cut, late [5]	$14^{A,H}$	57-61	91	±6.2	60.0	23 (13-44) [3]	11 (9-16)
Alfalfa grass mixture	Following cuts, early [4]	4^{A}	51	82	±8.8	56.9	47 (36-59) [3]	11 (10-12)
Alfalfa grass mixture	Following cuts, late [5]	$24^{A,H}$	55-67	83	±6.5	58.6	33 (16-50) [3]	11 (9-15)
Maize	SC	$27^{A,H}$	83-87	102	±5.4	55.4	31 (22-42)	4 (3-7)
Maize	Insufficiently ripened	$11^{A,H}$	69-79	98	±5.5	55.4	21 (15-28)	5 (4-6)
Marrow stem kale	SC	2^{A}	41-50	98	±0.0	54.8	15 (15-16)	13 (11-14)
Miscanthus		4^{A}	k.A.	64	±8.7	61.1	34 (30-40)	6 (5-7)
Fodder radish	SCC	5^{A}	59-61	79	±7.0	58.8	13 (9-17)	15 (12-18)
Phacelia	Till end of flowering	$6^{A,H}$	51-65	96	±14.6	61.5	13 (9-19)	16 (13-23)
Phacelia	From fruit development	4^{A}	69-71	87	±8.4	69.8	18 (12-26)	17 (14-18)
Quinoa	Soft dough to fully ripe	7^{A}	85-95	82	±4.3	58.5	21 (19-22)	16 (11-18)
Rapeseed	MC, flowering to fruit development	2^{A}	65-73	88	±0.2	57.6	24 (21-26)	8
Rapeseed	MC, seed ripening	3^{A}	85-86	77	±2.7	60.4	29 (27-32)	9 (8-10)
Tall wheatgrass	Flowering	8^{A}	61-69	83	±6.8	57.7	30 (19-42)	8 (5-13)
Forage pea	Flower buds visible	1^{A}	55	84		62.1	13	9
Forage pea	MC	1^{A}	76	72		60.5	25	10
Forage pea/ oat/ false flax	Milk to soft dough	5^{A}	71-83	92	±8.4	56.9	39 (35-43)	6 (5-7)
Spring barley	Milk to soft dough	$8^{A,H}$	77-85	96	±4.4	55.8	38 (31-59)	6 (3-7)
Spring rye	Milk to soft dough	7^{A}	71-83	86	±6.0	55.6	40 (20-57)	5 (4-7)
Spring triticale/ oat	Milk to soft dough	1^{A}	75	91		56.6	39	5

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Crop species or crop mixture	Specification/ characteristics	n	BBCH at harvest	ODM methane yield (relative to maize MC)[1)]		Methane content	DM_c content	Crude ash
				in %		in %	in %	in % DM_c
Bristle oat	Development of fruit	2[A]	72-75	98	±1.5	61.4	27	15 (13-17)
Bristle oat	Soft dough to full ripe	6[A]	83-91	89	±3.1	59.3	23 (22-24)	9 (8-10)
Mustard	Till end of inflorescence emergence	2[A]	39-51	104	±3.2	58.8	14 (13-15)	14 (13-15)
Mustard	From flowering	1[A]	65	83		59.8	22	10
Sunflowers		11[A]	71-87	75	±7.8	56.9	24 (17-36)	12 (10-19)
Sorghum *bicolor x bicolor*	DM < 26% [6)]	16[A,H]	34-82	94	±6.5	57.2	22 (15-28)	7 (5-10)
Sorghum *bicolor x bicolor*	DM ≥ 26% [6)] + BBCH < 69	5[A]	59-65	88	±4.4	58.2	27 (24-29)	5 (4-6)
Sorghum *bicolor x bicolor*	DM ≥ 26%% [6)] + BBCH ≥ 69	9[A]	75	86	±5.8	56.0	27 (23-29)	5 (4-6)
Sorghum *bicolor x sudanense*	DM < 26% [6)]	35[A,H]	33-83	94	±7.6	58.2	21 (15-25)	7 (5-13)
Sorghum *bicolor x sudanense*	DM ≥ 26% [6)] + BBCH ≥ 69	37[A,H]	69-85	88	±5.8	56.5	29 (24-36)	5 (4-8)
Jerusalem artichoke	Tuber	1[H]	47	107		55.1	19	8
Jerusalem artichoke	Haulm	10[A,H]	39-63	69	±6.2	55.0	28 (14-42)	10 (8-14)
Winter barley	WCC, till end of heading	7[A,H]	29-59	106	±4.6	57.2	23 (17-39)[3)]	9 (5-13)
Winter barley	Flowering	3[A,H]	63-69	98	±2.8	56.7	26 (24-28)	7 (5-8)
Winter barley	MC, milk to soft dough	37[A,H]	71-85	99	±5.7	56.4	32 (24-52)	6 (4-9)
Winter barley/ turnip rape	Till end of heading	2[A]	43/65-51/61	87	±13.5	58.9	33 (18-47)	11 (10-12)
Winter barley/ winter triticale/ winter wheat	MC, milk to soft dough	3[H]	79-83	99	±1.7	56.2	43 (35-47)	6 (5-7)
Winter barley/ forage pea	Barley: milk to soft dough	2[A]	83/67-83/69	94	±8.3	59.1	27 (25-29)	7
Winter rye	WCC, till end of heading	30[A,H]	41-59	105	±5.4	57.9	22 (14-39)[3)]	8 (6-11)
Winter rye	Flowering	4[A,H]	61-65	95	±4.9	57.2	29 (27-32)	6 (5-7)
Winter rye	MC, milk to soft dough	19[A,H]	70-87	95	±7.9	56.0	35 (26-53)	5 (4-8)
Winter rye / Hungarian vetch		2[A]	85/79-87/78	87	±0.9	58.7	34 (31-36)	6 (6-7)
Winter rye / forage pea/ meadow fescue	Rye: milk to soft dough	1[A]	65/75/69	86		57.2	28	7
Winter rye / winter triticale/ meadow fescue[2)]	Milk to soft dough	6[A]	73-83	91	±6.5	56.1	33 (29-36)	6 (5-8)
Winter rye / fodder vetch[2)]	MC, milk to soft dough	13[A]	71-85	87	±5.4	56.3	31 (19-39)	6 (5-8)
Winter rye / hairy vetch	Rye: milk to soft dough	2[A]	85/75-87/73	85	±0.9	58.4	33 (31-35)	6 (5-7)
Turnip rape		2[A]	65-73	99	±14.2	57.1	16 (14-18)	8 (8-9)

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Crop species or crop mixture	Specification/ characteristics	n	BBCH at harvest	ODM methane yield (relative to maize MC)[1)]		Methane content	DM_c content	Crude ash
				in %		in %	in %	in % DM_c
Winter triticale	WCC, till end of heading	5[A,H]	29-59	104	±8.8	56.3	26 (16-42)[3)]	9 (6-10)
Winter triticale	Flowering	7[A,H]	65-69	101	±4.8	57.4	33 (27-37)	6 (5-8)
Winter triticale	MC, milk to soft dough	38[A,H]	71-85	97	±5.3	55.6	35 (25-55)	5 (3-10)
Winter triticale / Hungarian vetch		2[A]	85/78-85/79	91	±2.3	56.8	32 (31-33)	7
Winter triticale/ faba bean	Triticale: milk to soft dough	2[A]	83/78-85/80	91	±3.9	58.2	31 (30-32)	7 (6-7)
Winter triticale/ forage pea[2)]	Triticale: milk to soft dough	4[A]	73/88-85/79	89	±4.1	58.1	29 (20-33)	8 (7-10)
Winter triticale/ winter wheat	MC	1[H]	75	101		57.4	26	7
Winter triticale/ fodder vetch[2)]	milk to soft dough	3[A]	75/55-83/65	86	±10.0	59.9	29 (19-40)	8 (6-13)
Winter triticale/ hairy vetch	Triticale: milk to soft dough	2[A]	k.A.	92	±0.9	57.0	31 (29-33)	7 (7-8)
Winter wheat	MC, milk to soft dough	3[A]	83-85	91	±8.9	54.3	49 (40-58)	4 (4-5)
Ryegrass mixture	1st cut, early [4)]	19[A]	25-51	114	±9.6	58.3	24 (11-37)	10 (9-16)
Ryegrass mixture	1st cut, late [5)]	18[A]	57-69	105	±9.2	57.2	27 (14-44)[3)]	9 (7-14)
Ryegrass mixture	Following cuts, early [4)]	10[A]	51-60	103	±2.5	57.2	28 (22-33)[3)]	10 (7-12)
Ryegrass mixture	Following cuts, late [5)]	28[A]	57-81	96	±9.7	57.3	33 (19-48)[3)]	10 (7-15)
Meadow fescue	1st cut, late	2[A]	65-69	86	±9.0	57.6	36 (27-44)[3)]	8 (7-8)
Meadow fescue	Following cuts, late	1[A]	75	87		54.7	21	11
Sugar beet	roots, heads and leaves removed	6[A]	39-49	107	±5.6	53.0	21 (17-25)	6 (3-12)

n: number of silages investigated
BBCH: growth stage according to Meier (2001)
ODM: organic dry matter; DM_c: corrected dry matter
MC: main crop; SC: secondary crop; WCC: winter cover crop; SCC: summer cover crop;
[A] measured values from 2 liter reactors with wet gas meters (experimental setup at ATB);
[H] measured values from the Hohenheim biogas test (experimental setup at TLL);
[1)] Reference basis ODM-methane yield of maize MC: 354.6 L_N/kg ODM (ATB-test stand), 363.9 L_N/kg ODM (HBT).
[2)] partly with undersown ryegrass or meadow fescue.
[3)] partly wilted after harvest
[4)] early cut: high cutting frequency or BBCH < 55; [5)] late cut: low cutting frequency or BBCH ≥ 55; [6)] classification according to the DM content at harvest

Effects of cultivation and harvest conditions on methane formation

Besides crop species, the maturity at harvest, the cutting regime for crops that are cut several times per year, and the position within the crop rotation also substantially influence specific methane yields. Thus, reference values for methane yields of several crop species in Table 1 are further specified, mainly depending on the stage of maturity of the crops at harvest. The reason for changes in methane production potentials of crops during the growing season is an increasing lignification of the crop biomass with an advancing stage of maturity. Since lignin or complex lignocellulosic compounds are hardly or not digestible within the biogas process, an advancing maturity usually decreases the specific methane yield related to the ODM of the biomass.

When cultivated as secondary crop or cover crop, harvest is commonly conducted at a slightly earlier stage of growth compared with the cultivation as main crop. This is reflected in values of specific methane yields (Table 1). Methane yields of winter cereals harvested until the end of heading are on average 7 to 12% (relative to maize MC) lower than when harvest occurs at milk to soft dough stage of maturity. Early (BBCH < 55, till middle of heading) or with high frequency cut grasses, clover or alfalfa grass mixtures, and alfalfa show on average 4 to 19% higher methane yields (relative to maize MC) as compared with the late cut (BBCH > 55) or with low frequency cut biomasses. In addition, about 7 to 19% higher methane yields (relative to maize MC) are obtained from biomasses of the first cut as compared with the following cuts. Decreasing methane yields with increasing stages of maturity were further found for buckwheat, phacelia, rapeseed, bristle oat, forage pea and mustard (Table 1). An exception to this is sorghum. Remarkable differences in development of DM, lignification and stage of maturity at harvest were observed for sorghum. Hence, a further specification of sorghum depending on a combination of DM content and stage of maturity at harvest is suggested (Table 1).

Application of reference values – opportunities and limits

Reference values (Table 1) can be employed as approximate values in combination with biomass yields for calculation of methane hectare yields and for the evaluation of energy crop rotations e.g. regarding energetic, ecological and economic effects. Furthermore, reference values are of use for planning and design of biogas plants, or for decisions concerning the cultivation and utilization of alternative crops as feedstock or co-substrate in existing biogas plants. The application of relative values enables a relation to laboratory-specific or practically relevant methane yields through the choice of the reference basis according to the underlying question, or to generally used standard values as for example given by KTBL (2014) (Table 2). By applying own measured values or mean values of methane content and DM or crude ash contents as listed in Table 1, biogas yields as well as gas production with reference to DM or fresh matter can be calculated. In general, additional losses that occur during ensiling need to be considered for balancing or estimations of methane hectare yields. Losses that occur during harvest and ensiling include field losses, effluent losses, fermentation losses during ensiling, respiration losses during storage and losses during feed-out of silages. They can be estimated e.g. according to Jeroch et al. (1993).

Reference values detailed in Table 1 are applied within the framework of the joint research project 'EVA' for a comprehensive economic and ecological evaluation of biogas crop rotations. The use of reference values for calculation of methane hectare yields and energy yields are explained in Table 2 for winter triticale as an example:

Table 2: Application of reference values of methane yields for the calculation of methane hectare yields and energy yields exemplified by winter triticale, cultivated in Dornburg in 2007

Calculation step	Unit	Value	Categorization
Crop species		Winter triticale, whole crop	Field value
BBCH		75 (medium milk)	Field value, rated
Fresh matter yield	t/ha	38.79	Field value, measured
DM content	% FM	31.9	Field value, measured
DM yield	t/ha	12.4	Field value, calculated
Field loss	%	2	Field value, according to (Jeroch 1993)

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Calculation step	Unit	Value	Categorization
DM yield reduced by field loss	t/ha	12.1	Field value, calculated
Effluent losses	% DM	0	Silage, according to (Jeroch 1993)
Fermentation losses	% DM	5	Silage, according to (Jeroch 1993)
Respiration losses during storage	% DM	2	Silage, according to (Jeroch 1993)
DM yield reduced by field and silage losses	dt/ha	11.3	Silage, calculated
Losses during feed-out	% DM	1	Silage, according to (Jeroch 1993)
DM yield of silage	**t/ha**	**11.2**	Silage, calculated
Total silage losses	% DM	9.8	Silage, calculated
Crude ash content	% DM	5.0	Methane yield, according to Table 1
ODM yield of silage	t/ha	10.6	Methane yield, calculated
Maize (MC) - reference basis; methane yield (KTBL)	m^3_N/t ODM	338	Methane yield, according to KTBL (2014) (a different reference basis can be chosen)
Winter triticale, relative methane yield (KTBL)	% of maize	97	Reference value for methane yield, according to Table 1
Reference value methane yield related to ODM	m^3_N/t ODM	327.9	Methane yield, calculated
Methane hectare yield	**m^3_N/ha**	**3475**	Methane yield, calculated
Net heating value methane	MJ/m^3_N	35.9	Energy yield, calculated
Energy yield	**GJ/ha**	**124.8**	Energy yield, calculated

Information on approximately achievable methane yields related to the ODM of silages from different crop species and classifications can be obtained from reference values listed in Table 1. However, the exact methane production potential of a crop biomass depends on its actual chemical composition, especially on the fiber content or content of lignin and cellulose, and on the crude nutrient content (Herrmann et al. 2016). The chemical composition is subject to natural fluctuations and is influenced by many other factors such as the chosen variety, site and weather conditions during cultivation, or the course of ensiling. In case of crop mixtures, the share of the individual mixing partners within the mixture is of importance. Thus, in order to address scientific questions which require detailed knowledge on the specific methane yields of crop biomasses, laboratory analysis for determination of methane yields and chemical characteristics of the feedstocks are still advisable.

When transferring reference values into practice it needs to be considered that, besides feedstock characteristics, also process conditions during continuous operation of the biogas plant, especially the retention time within heated reactor stages, the organic loading, process temperature and the feedstock mix, i.e. the availability of macro and micro nutrients, will influence gas production. Process inhibition or disturbance can lead to a remarkable reduction of gas yields and of methane content within the produced biogas. The application of a practice-relevant reference basis for 'maize MC' can partly compensate for these influences. The impact of individual factors, for example of the hydraulic retention time, is also dependent on the kinetics of degradation of the feedstock which is not taken into account by the use of relative values. The relation between methane yields of different silages can change, especially at short hydraulic retention times. In general it should be considered that reference values are based on data obtained under favorable process conditions.

Conclusions

The methane production potential is an essential quality parameter of crop biomasses used as feedstock for biogas production. Results of the present study confirm that the choice of crop species, its integration into crop rotations or cultivation systems accompanied by the maturity of the crops at harvest are main influencing factors concerning methane yields that can be obtained under favorable process conditions in biogas plants. Reference values for methane yields relative to maize (MC) of 93 ensiled crop biomasses grown in energy crop rotations that are based on comprehensive experimental investigations, enable the estimation of specific methane yields depending on crop species, position within the crop rotation, cutting regime, DM content or stage of maturity at harvest for a large variety of crop feedstocks. This provides a comprehensive database that can be utilized in combination with biomass yields for the design and evaluation of sustainable biogas crop rotations. However, despite extensive measurements the database from anaerobic digestion tests is still limited. Future addition of measured values and increase of the database will further enhance the validity of the reference values, especially for silages that are so far investigated with low sample size. Furthermore, the addition of so far not considered but promising crop species seems beneficial and is recommended.

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