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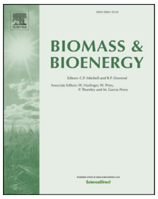

Research paper

How well does *Miscanthus* ensile for use in an anaerobic digestion plant?

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ABSTRACT

This study examined the ability for early-harvested *Miscanthus* (*Miscanthus x giganteus* and *Miscanthus sacchariflorus*) to be stored in silage for later use in anaerobic digestion. Two silage additives favouring a homo and hetero-fermentation pathway were examined. The results show that silage additives are necessary to effectively ensile *Miscanthus*, otherwise untreated *Miscanthus* grasses incurred dry matter losses of 4% during three months' storage. The silage additives improved the lactic and acetic acid production in the *Miscanthus* silages however did not have any effect on the biogas yield. On a 'per tonne volatile solids'-basis, *Miscanthus* produces half the biogas yield of maize. The outlook for the use of *Miscanthus* AD therefore depends on the yield when harvested in autumn. A minimum yield of 19–26.5 t DM/ha is needed for *Miscanthus* to match the biogas production from a similar area of maize yielding 10–14 t DM/ha.

1. Introduction

There is a steadily growing role of anaerobic digestion (AD) in the biomass energy sector, playing an important role in dealing with organic wastes and slurries while providing a renewable form of natural gas [1]. Anaerobic digestion technologies generally are well-proven, having already been used in the UK for over 100 years to treat sewage sludge [2]. The process of AD involves the microbial breakdown of biodegradable matter in an anaerobic environment, generating a biogas typically containing 60% methane and 40% carbon dioxide [1]. The remaining digestate is rich in plant nutrients and is a valuable bio-fertiliser [3]. The process occurs extensively in landfills and in the rumens of cows, but AD plants provide opportunities to control and optimise the operation while generating a renewable fuel [2].

There are currently 259 AD plants in the UK, and 163 are listed as being 'farm-fed', as opposed to being integrated with waste water management [4]. Main economic incentives originate from Feed-in Tariffs for renewable electricity generation, which currently provide rates of 10.13, 9.36, and 8.68 p/kWh_e for generation in small ($<250\ kW_e$), medium (250–500 kW_e) and large scale installations ($>500\ kW_e$), respectively [5]. Larger-scale ($>5\ MW_e$) facilities can currently claim two Renewable Obligation Certificates (ROCS) per MWh_e generated. Also installations completed after 15th July 2009 can claim a fixed tariff from the Renewable Heat Incentive (RHI) of 7.5, 5.9, 2.2 p/kWh_{th} for small ($<200\ kW_{th}$), medium (200–600 kW_{th}), large ($>600\ kW_{th}$) producers, or 7.5 p/kWh for biomethane injection into the grid [4].

Wet AD plants run on an inoculum derived from livestock manure or slurry that essentially provide the bacterial culture for methanogenesis. Biomethane production is then stimulated by feeding the substrate with a source of organic matter. Slurry-only systems can generate enough methane to achieve GHG emission savings of about 14% compared to marginal electricity production, however the co-digestion of crops is sometimes necessary to increase biomethane yields from slurry to improve the economic balance of the plant [6,7]. Typical substrates for co-digestion include purposely grown crops such as forage maize, fodder and sugar beets, grass silages and grain crops [8], or wastes from water treatment, sewage sludge and waste food [9]. In 2014 29,373 ha of forage maize (*Zea mays*), or 0.5% of England's arable area, was used for AD [10]. Maize is one of the most rapidly growing crops in the UK and the National Farmers Union aims for an additional 125,000 ha of maize grown for AD in England by 2020 [11].

There are concerns that widespread forage maize cultivation will lead to direct damage to soil quality, soil erosion and pollution

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of surface and groundwater [12]. Although biogas production from maize is highly efficient, there are concerns that its wide scale use in AD could result in competition between energy and food uses [13]. A study by Styles et al. [7] strongly advised against the use of purpose-grown crops for AD due to adverse impacts of this competition on land use change. The authors identified that using food waste is a more environmentally sound option, assuming a reference case where 60% of the waste would have otherwise been landfilled. They also compared the GHG savings of utilising maize silage in the AD plant with those from growing the energy crop *Miscanthus* on the equivalent amount of land to generate heat. They showed that the latter generated far better GHG savings, mainly because the indirect land use impacts of using land for *Miscanthus* were somewhat offset by sequestration of carbon under the crop. The use of the grass itself as a biogas substrate was explored by Mayer et al. [12], who examined the biomethane potential (BMP) of *Miscanthus* along with 13 other possible AD substrates such as hemp, immature rye, sorghum, spelt, sunflower, switchgrass and tall fescue. They identified *Miscanthus* to be the most promising alternative to maize for AD in terms of biogas yield, and it was also suggested that the crop would help reduce erosion from agricultural soils.

A challenge with using *Miscanthus* in AD is that the crop undergoes considerable changes in yield, moisture content and composition during the growing season [14]. Having an optimal harvesting window is common with most silage crops, for example King et al. [15], examined the impact of harvest period on the ensilability of five common grasses (*Lolium* spp., *Dactylis* spp., *Festuca* spp., *Phleum* spp.) and discovered a negative relationship between silage-quality and harvest date over a 2 month period (May to July). In grass silage, earlier cuts tend to be more digestible than later ones [9,16]. Maize is shown to have improved BMPs when ensiled at the optimum time [17]. In *Miscanthus* grown for combustion purposes, the harvesting window traditionally occurs after the winter period as this provides a drier material with lower contents of minerals such as chlorine, sulphur, nitrogen, potassium and ash that are known to cause corrosion and slagging in biomass boilers (Lewandowski & Kicherer 1997) (Lewandowski and Heinz, 2003). In addition to this, harvesting in the winter allows the translocation of nutrients from the aerial biomass to the rhizome and leaching of non-structural components, which is believed to be the reason that *Miscanthus* is a low-input crop (Cadoux et al., 2012).

In AD systems the optimal harvest window for *Miscanthus* differs to that of combustion. Firstly, during the over-wintering phase there is a considerable loss of one third of the maximum yield achieved in autumn [18,19]. Secondly, by harvesting the crop 'early' or 'green', in the autumn means that the sugar content is maximised, as little remains in material harvested in a late winter harvest [12,20]. Storing the biomass in silage will preserve the quality of the material and allow a constant supply of biomass to be fed into an AD plant [21]. Other studies find that the process of ensilage can improve the BMP [22]. For example there was a 15% higher CH_4 potential from ensiled elephantgrass (*Pennisetum purpureum* Schum.) and energycane (*Saccharum* sp.) compared to fresh crops [23]. The biogas yield from *Miscanthus* harvested after the winter is generally low (84 m3/t VS [24]).

Some crops are more suited to ensiling than others, though what constitutes a 'good' or 'poor' silage may differ between a livestock farmer or AD operator [21]. In general, the aim for producing good quality silage is to maximize the conversion of water soluble carbohydrates (WSC) in the biomass into lactic acid that will preserve the biomass against spoilage losses (McDonald et al., 1991). Whole crop maize is a popular silage crop as it has a high yield, low buffering capacity (BC), high dry matter (DM) and WSC concentrations [25]. *Miscanthus* may be a difficult to ensile crop, as warm-season grasses generally have low soluble carbohydrates, and a high BC [26]. Pilat et al. [27], compared the forage quality of silage produced from *Miscanthus saccharifloru*s at three different time-points, and with a range of additive treatments. Their experiments produced good quality silage at all growth stages, and it was further improved when combined with addition of lactic acid and enzymatic silage additives. In contrast, Klimiuk et al. [13], ensiled *Miscanthus x giganteus* and *M. sacchariflorus* at the flowering phase and produced poor silage, despite adding formic acid additive.

1.1. Aim of study

This study examined the potential for *Miscanthus* to be utilised within an AD plant and to offset the demand for conventional crops such as forage maize. The aim of the study was to test whether two species of *Miscanthus* early-harvested (*Miscanthus x giganteus* and *M. sacchariflorus*) can successfully be ensiled. The grass varieties were compared with forage maize. In addition, the impacts of two additive treatments encouraging homo and hetero-fermentation were tested on *Miscanthus x giganteus*.

2. Methods

The experiment was carried out at Rothamsted Research in autumn 2014. During this time the mean annual temperature was 11.1 °C and the total rainfall was 714.4 mm.

2.1. Substrate: Miscanthus

The *Miscanthus x giganteus* used in this study is a naturally occurring triploid hybrid of diploid *Miscanthus sinensis* and a tetraploid *M. sacchariflorus* [20]. In autumn 2014 the crop was 10 years old. The site has a silty clay loam soil with flints [28]. It was grassland for the majority of the previous 100 years and an adjacent *Miscanthus* crop had not responded (in terms of yield) to nitrogen fertiliser [29]. Therefore no nitrogen or other fertilisers were applied. During the building phase, 2005–2007 some herbicides for broad leafed weed control were applied. For this experiment the crop was harvested on the 11th September 2014, which corresponds with an 'early' or 'green' harvest' (Lewandowski and Heinz, 2003). The site had previously been harvested in March 2014, This was the first time the crop had been cut in autumn. The crop was harvested from a single 3.75 × 6.67 m plot and yielded 16.4 t DM/ha. It is recognised that strong diurnal changes in carbohydrates occur in *Miscanthus*, therefore to maximise WSC content the crop was harvested between 11 am and 12 pm [20]. Whole stems were harvested by hand, cutting between 10 and 15 cm from the ground. The material was harvested and ensiled within a 2 h period to avoid changes in carbohydrate concentrations [30]. In the field, random samples of stems were flash frozen in liquid nitrogen and freeze-dried for WSC analysis. The biomass was then chopped as described in the following section.

The *M. sacchariflorus* used in this study is a tetraploid that was part of a seed population collected from central Japan, by TINPLANT, in 1992 [20]. It was also grown on a silty clay soil with slightly fewer flints than the other site [28]. In autumn 2014 the crop was 6 years old. In contrast to the *M.* x. *giganteus* site the *M. sacchariflorus* was growing on a former arable field where yield responses to nitrogen fertiliser may be expected [31]. The plots were given 100 kg ha^{-1} N as ammonium nitrate each spring following harvest. The crop had previously been harvested in February 2014. For the experiment, harvests were made on the 12th September 2014, again between 11am and 12pm. Instead of a single plot, the crop was taken from four replicate plots within a random block design of a larger trial including *Miscanthus* species (*Miscanthus x giganteus*, *sinensis*,

sinensis Goliath and *sacchariflorus*). Again samples were taken in the field for soluble sugar analysis, and then the biomass was chopped.

2.2. Substrate: maize

Maize (*Z. mays*), variety Hudson, was used as a comparison in the experiment. The site gives average yields of *Z. mays* between 10 and 14 t DM/ha. This is based on a mean of 17 data points between 1997 and 2014 during a five course rotation where maize is grown only once every 5 years. The crop was grown on the same soil type as the *Miscanthus x giganteus*. The crop is given adequate P, K, S & Mg and the pH is controlled. The value presented (11.66 t ha^{-1} DM) is from plots given 144 kg ha^{-1} N, the upper end of the recommended application in the Fertiliser Manual, Defra's good practice guide [32]. This was harvested on the 1st October by the time the crop in the field had dried to approximately 35% D.M. Random whole stems were harvested from a field that has grown continuous maize for 18 years and had received 96 kg N ha^{-1} in the seedbed.

2.3. Biomass ensilage

Three treatments of *Miscanthus* and one *Z. mays* control were examined. There were four replicates of each crop and/or treatment. The following treatments were examined:

a) *Miscanthus x giganteus*, without silage additive
b) *Miscanthus x giganteus*, treated with a homo-fermentative silage additive solution ‘A’ containing *Lactobacillus plantarum*, *Pediococcus acidilactici and L. paracasei*.
c) *Miscanthus x giganteus* treated with a hetero-fermentative silage additive solution ‘B’ containing *L. brevis* and *L. fermentum*.
d) *M. sacchariflorus*, without silage additive
e) *Z. mays* – silaged.

The biomass was chopped using a traditional chaff cutter prior to ensilage. Inspection of the cut material showed the average chop size was 15 mm (standard deviation 9 mm, from 260 random samples), with 83% of cut material falling under a chop length of under 16 mm. Previous in-house trials with a forage harvester (JF willow harvester, Prados Itapira, Brazil, model JF192 Z10) and a PTO disc chipper (Jenson Service GmbH, Bahnhofstrasse, Germany) failed to cut consistently under 30 mm, which the literature suggests is necessary for successful ensilage [13,26,33].

The small-scale silage silos were 30 L fermentation tanks with tightly fitting lids designed for brewing. The silo dimensions were 30 cm in diameter, 40 cm tall and a total volume of 0.03 m^3. Approximately 7 kg of chopped biomass was added to each silo, leaving a 10 cm headspace for a 10 kg sandbag to compress the biomass [34]. The density of the ensiled biomass was therefore at least 320 kg/m^3, which is identified as a minimum required density to reduce DM losses [35]. The lid of the silo was sealed to ensure air tightness.

Two types of silage additive: homo (A) and hetero (B)-fermentative treatments that are specialised for the ensilage of high DM crops, were provided from an industrial contact. The additives were applied to *Miscanthus* x *giganteus* to achieve a colony-forming unit (cfu) application rate of 2.5×10^8 cfu/kg. The biological activity of the powdered additive is 1×10^{11} cfu per gram. The additive solution was made my mixing 0.25 g of additive powder with 500 ml distilled water. The solution was mixed for 30 min on a mixer tray before applying to the biomass, which followed the methodology described in Ref. [25]. A pre-weighed sample of biomass was spread evenly over a tarpaulin sheet onto which the silage additive solution was applied. Additive was then applied evenly at a rate of 5 ml per kg biomass using a handheld sprayer. The material was then placed within the silo in the same way.

After four months of storage the silos were re-weighed and samples were collected for analysis.

2.4. Analyses

Biomass samples were taken between harvesting and chopping to record instant losses in WSC between the field and the point of ensiling, and samples were taken of the chopped material before and after ensiling. The following subsections describe the analyses performed on the samples. On opening the silage silos the state of fungal growth was determined visually. The samples for WSC, pH, volatile fatty acids (VFA) and volatile solids (VS) were sampled from further than 9 cm below the top and above bottom of the silo [26].

2.4.1. pH and buffering capacity (BC)

The BC is calculated from the measured amount of acid that will reduce the pH of a prepared sample to a given pH. This is done using HCl as lactic acid has a tendency to act as a buffer between pH 6 and 4 [36]. The sample was prepared by mixing 10 g of dried and milled material with 100 ml distilled water and shaking for 30 min. The initial pH was then assessed using a pH detection probe. An amount of 3 ml of 0.01 M HCl was added in 1 ml stages and the resulting pH change was recorded each time. The BC (β) was calculated according to [37], where:

$$\beta = \varDelta B / \varDelta pH$$

Where ΔB is the molar equivalents required to cause a given unit change in pH of a solution.

The pH of the final silage material was tested promptly after the silage silos were opened. In this instance the biomass was prepared by mixing 10 g of fresh material with water, and shaking for 30 min. The liquid was drained off and pH tested.

2.4.2. Material composition

For both crops four samples were taken of the homogenised material, after cutting and of the resulting silage. The moisture content was determined by measuring mass changes after heating at 80 °C for 48 h. The VS and ash contents were then determined by from mass loss after heating for a further 4 h in a muffle furnace at 550 °C [38]. Samples of silage were sent to Sciantec (Stockbridge Technology Centre, Cawood, North Yorkshire, YO8 3SD) for determination of lactic acid, acetic acid, propionic acid, and n-butyric acid content. According to their standard operating procedure, this test involves grinding a portion of sample in the presence of solid CO_2, which is then shaken with a known volume of water to extract the Fatty Acids. The extract is spiked with an internal standard and then passed to the GC to determine the individual component composition by comparison with a series of standard solutions. The same company were used to determine the fibre composition by analyses of the acid-detergent lignin, acid-detergent fibre and neutral-detergent fibre content of the ensiled material. This was performed according to the Van Soest method for determination of the digestible/fractions [39], and NDL specifically was assessed according the method detailed in Ref. [40].

For WSC determination, three samples were taken of whole stems in the field, after chopping and of the final silage. For *Z. mays* the cobs were separated from the leaves and stems in order to monitor loss of sugars and starches between harvesting and ensilage in these components alone. Homogenised samples of the ensiled maize were also taken. The samples were flash frozen in liquid nitrogen, freeze dried and then cryo-milled. Concentrations of starch, sucrose and glucose in the biomass were determined by the method of Purdy et al. [20].

2.5. Biomethane potential (BMP)

The BMP was measured using an automated laboratory incubation system (AMPTSII, Bioprocess Control, Lund, Sweden) consisting of three main units: a thermostatic water bath (Lauda, Germany) holding 500 ml reactor bottles (Simax, Czech Republic), each stirred by a motorised mixing rod; a CO_2 fixing unit containing 80 ml of 3 M sodium hydroxide (NaOH) and thymolphthalein pH-indicator solution, this removes non-methane gases; and a gas volume measuring device which relies on liquid displacement and buoyancy opening ca. 10 ml tipping cells. This process is explained in more detail in Refs. [38,41]. The biogas produced is saturated by water, therefore to allow for comparisons with other studies the results produced by AMPTSII are normalised to standard conditions, i.e. converted to 0 °C, 1 atm and no humidity (i.e. removing the water content and considering the gas dry). This is done by the system recording the temperature and pressure at each measuring point and using a series of equations to normalise the results [38,41]. The standard error of laboratory (SEL) of the process is below 1% (i.e. 0.1 mL/10 mL) for the range observed in this experiment (i.e. flow rates up to 60 L/day).

Samples were prepared for incubation in the 500 ml reactor bottles; each bottle contained 400 g of inoculum and substrate at a 2:1 ratio by mass of inoculum VS to substrate VS. The inoculum was obtained as digestate from an 80 kWe farm-fed mesophilic anaerobic digester which co-digests maize silage, cattle slurry and poultry manure. In order to reduce the direct production of biogas from the inoculum, it was incubated for 5 days at a temperature of 37 °C; this depletes the residual biodegradable organic material present. To create anaerobic conditions, the head space of each reactor bottle was purged using nitrogen gas for 2 min. The reactor bottles were incubated at 37 °C and stirred automatically at 110 rpm for a period of 60 s every 2 min. The system was controlled via a PC and gas volume produced from each sample was recorded continuously for 45 days.

2.6. Statistical analysis

GenStat (16th edition, © VSN International Ltd, Hemel Hempstead, UK) was used to analyse the results. In the analysis the results were nested to examine the impact of *Z. mays*, *M. sacchariflorus* and finally *Miscanthus x giganteus* and its three treatments (control, additive A and B). Analysis of variances was performed on each measured parameter for the ensiled material, the resulting silage and the final BMP. A two-sided test of correlations was performed to identify key positive and negative correlations in the parameters affecting the BMP. Finally, linear regression was used to model the BMP based on the key parameters.

3. Results and discussion

3.1. Biomass suitability for ensilage: biomass controls

The composition and qualities of the ensiled material are shown in Table 1. The DM content of the crops was between 33.5% and 39.7%, with *M. sacchariflorus* being slightly drier than the other two crops. Untreated *Miscanthus x giganteus* and *M. sacchariflorus* silage was of poor quality compared to *Z. mays*, as indicated by higher pH and lower lactic and acetic acid content (Fig. 1). An indication of successful establishment of lactic acid bacteria (LAB) is a pH of 4 which inhibits further degradation by other bacteria [25]. The untreated grasses failed to drop in pH after ensilage, both having a silage pH higher than 5. The average pH of *Z. mays* was 4.7, and an average lactic and acetic acid formation of 1.7 and 0.6%, respectively, suggesting that the cut size of the material and the conditions within the silage silos were sufficient for the material to ensile.

Table 1
Characteristics of the crops entering the silage silo (Different superscripts within rows denote significant differences ($P < 0.05$)).

Details	Units	Crop		
		M. giganteus	*M. sacchariflorus*	*Z. mays*
Dry matter content	% W.M	35.7	39.7	33.5
Ash	% D.M	1.3^b	1.3^b	1.0^a
Buffering capacity	–	0.026^a	0.032^b	0.044^c
pH	–	5.5^a	5.7^b	5.6^a
Sugar	% D.M	4.85^a	4.16^a	8.42^b
Starch	% D.M	3.53^a	4.07^b	17.7^c
Cellulose*	% D.M	37.6^b	38.5^b	13.3^a
Hemicellulose*	% D.M	23.1^b	21.2^b	16.1^a
Lignin*	% D.M	9.4^b	9.1^b	2.3^a

*Have been corrected by the ash content.

Ethanol concentrations were significantly higher for the two *Miscanthus* controls than for *Z. mays* and the treated silages ($p < 0.001$). Ethanol is a product of hetero-fermentative lactic acid bacteria (LAB), which follows the 6-phosphogluconate pathway or phosphoketolase pathway, producing a combination of lactic acid, acetic acid, ethanol, carbon dioxide and some heat [42]. Homo-fermentative LAB is generally preferred in animal feed as it converts C_6-sugars solely into lactic acid, without the loss of carbon or heat, therefore a higher energy recovery compared to hetero-fermentation, and lower DM losses between the ensiled material and final product [43]. Both hetero and homo LAB are naturally present on plant material, although many factors can affect the type of fermentation that takes place in the silage and thus, there is usually a mixture of end products [44]. The *Z. mays* silage had an average ethanol content of 2.7%, and a typical ethanol production of 1–3% is considered reasonable: the *Miscanthus* silages had concentrations of 6–7%; indicating excessive metabolism by yeasts [45]. This is reported to be an issue with feedstocks with a limited availability of WSC with prolonged storage [42]. An analysis of the Pearson product–moment correlation (Fig. 2) found a significant ($p < 0.001$) positive correlation ($r = 0.83$) between the ethanol content of silage and the corresponding silage pH, suggesting that ethanol does not contribute towards preservation of the material [44]. Significant negative correlations were observed between lactic ($r = -0.79$ $p < 0.001$) and acetic acid ($r = -0.58$, $p < 0.0075$) contents and the silage pH, the correlation was lower with acetic acid as this is a weaker acid.

The two grasses studied just about satisfy the minimum sugar content required (50 g/kg) for successful silaging [44]. Klimiuk et al. [13], also found that *Miscanthus x giganteus* and *M. sacchariflorus* ensiled poorly and attributed this to the low contents of WSC. In this study, *Z. mays* had a significantly higher sugar content than the two *Miscanthus* types ($p < 0.001$); which were not significantly different to each other. *Z. mays* also contained the highest starch content, and *Miscanthus x giganteus* had the lowest ($p < 0.001$). There were no significant differences in the WSC between the times of cutting and ensiling for any of the crops, or the separated cobs and leaves, suggesting the timeliness of the experiment was sufficient to preserve the qualities of the biomasses.

The reduction in WSC concentrations during ensiling was significant for all crops (sugar: $p < 0.001$, paired $t = 1.7$, starch: $p = 0.03$, paired $t = 1.8$, both with $df = 11$), indicating its substantial use as a substrate for fermentation [15]. The Pearson

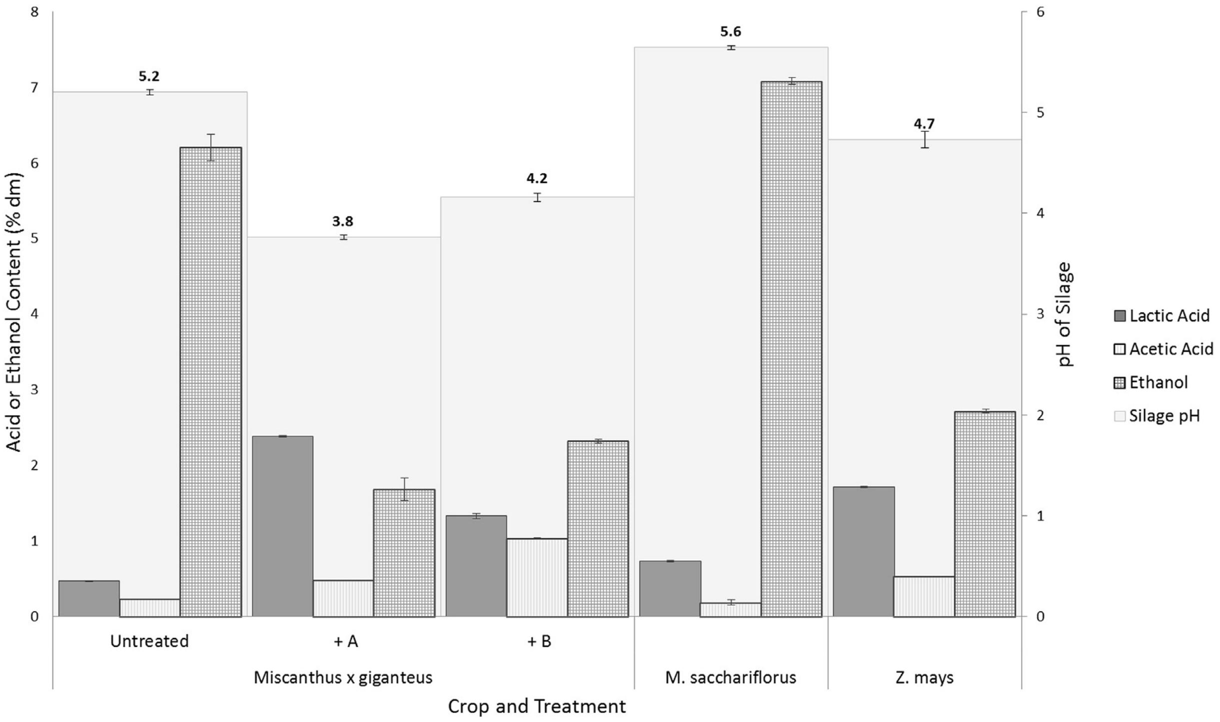

Fig. 1. Lactic acid, acetic acid and ethanol production in the silages and the resulting silage pH.

product–moment correlation found a correlation between the starch ($r = 0.97$, $p < 0.001$) content of the original biomass and that of the resulting silage, of which a slightly weaker correlation was observed in sugar ($r = 0.66$, $p = 0.0014$), suggesting the depletion of sugar was greater than starch; an indication of its use as a substrate for anaerobic fermentation during the ensiling process (Fig. 3). Therefore, *Z. mays* silage contained a higher WSC content than all other silages ($p < 0.001$). Klimiuk et al. [13], also observed a drop in cellulose and hemicellulose contents during *Miscanthus* ensiling, showing these can also provide a substrate for LAB fermentation. Lignin contents, on the other hand, were unchanged as lignin is generally not degradable under anaerobic conditions. In this study both of the grasses had higher ($p < 0.001$) cellulose, hemicellulose and lignin content compared to *Z. mays*. Changes in structural content were not observed in this study; therefore it is not possible to detect the utilisation of cellulose or hemicellulose by the LAB.

The analysis of the BC (Table 1) of the ensiled biomass showed that all the biomass samples had the capacity to resist pH change compared to distilled water (BC 0.01). All three crops had different BCs with *Z. mays* having the highest (most resistant to change) and *Miscanthus x giganteus* the lowest. This should attribute the grasses with a greater suitability for ensilage; however no correlation was found between BC and silage pH. The BC is determined by the content of organic acid salts, orthophosphates, sulphates, nitrates and chlorides, rather than the protein content (Playne & McDonald, 1966), which is believed to attribute legumes with poor ensilability [37]. In this study, the BC was strongly ($p < 0.001$) correlated with the measured starch ($r = 0.93$) and sugar ($r = 0.92$) contents of the biomass, and negatively correlated with the cellulose ($r = -0.93$), hemicellulose ($r = -0.85$) and lignin ($r = -0.94$) contents. Pilat et al. [27], also found the BC reduced as the crop matured, which was associated with an increase in crude fibre content. Therefore, having a suitable substrate for fermentation is more important in determining ensilability than the BC.

3.2. *Effect of the silage additives*

3.2.1. *Volatile fatty acid (VFA) formation*

There was a significant effect of treatment of the *Miscanthus x giganteus* with both silage additives on the silage pH ($p < 0.001$). The average pH treatments with additive homo-fermentative silage additive (A) and the hetero-fermentative (B) additive were 3.7 and 4.2, respectively. Both of the treated *Miscanthus* silages were more acidic than *Z. mays* ($p < 0.001$). Analysis of the VFA composition of the silages indicated that these differences in pH were due to their higher lactic and acetic acid concentrations compared to all other silages tested ($p < 0.001$). Propionic acid and n-butyric acid were produced in very small quantities in all silages. As expected, a higher concentration of acetic acid was produced in the silage treated with the hetero-fermentative additive, and the resulting pH is higher.

There are few other studies examining the effect of silage additives in *Miscanthus*. One study found no improvement in silage quality from *M. sacchariflorus* treated with an unspecified *Lactobacillus*, however a combined bacteria and cellulase additive produced a much improved silage [27]. In other ‘difficult to ensile’ crops it was found that additions of *L. plantarum* increased the lactic acid contents and decreased the pH of silages produced from high DM tropical legumes [46] and triticale [47], whereas a contrasting study found that only *Lactobacillus buchneri* improved the quality of triticale silage [48].

3.2.2. *Fresh and dry matter losses*

Low-loss preservation of whole crop plant material is essential for economical and sustainable use of biogas crops for AD [42]. In this study, the net loss of fresh material was measured after the silaging process and after re-opening the silos. Overall, the fresh material losses were small (2% or lower, Fig. 4). The two treated sets of *Miscanthus x giganteus* silage had a lower loss ($p = 0.028$), and *M. sacchariflorus* had the highest ($p < 0.001$). This crop was the

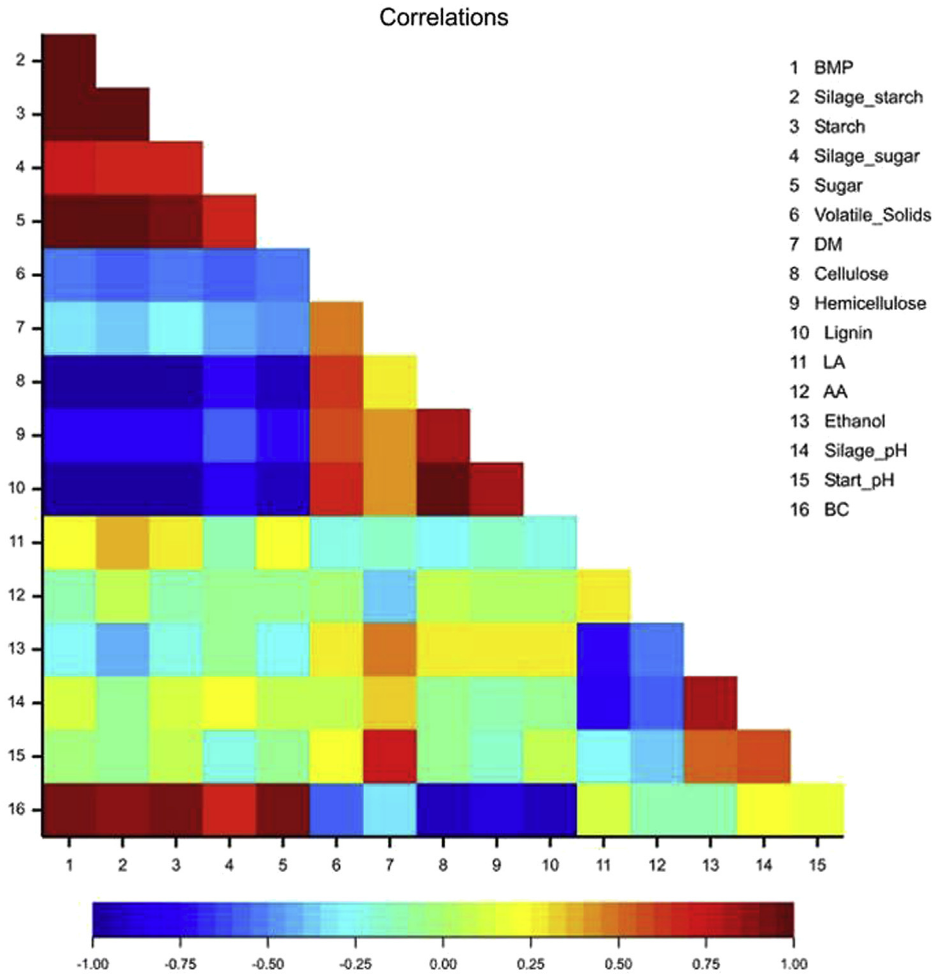

Fig. 2. Pearson product–moment correlation of factors affecting BMP.

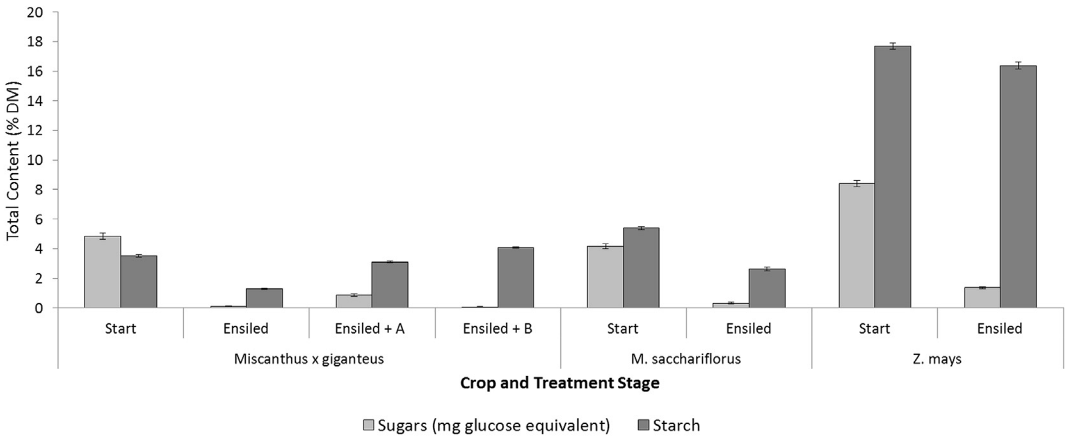

Fig. 3. Sugar and starch contents of crops and resulting silages.

driest out of all crops, which may explain the observed result. Paired t-tests showed the relative D.M of the two control *Miscanthus*' dropped during ensilage (paired $t = 8.14$, $p > 0.001$, and $t = 2.57$, $p = 0.042$, $df = 6$, respectively). In both crops an average DM loss of 4% was measured. There was no significant change in the VS content after ensiling. The *Z. mays* silages had an average fresh material loss of 1% and no changes in average DM content or VS content was observed.

In difficult to ensile crops, such as the *Miscanthus* controls in our study, it is generally found that the slow development of lactic acid bacteria provides opportunities for spoilage organisms to proliferate [25]. The results from this experiment provide evidence that both bacterial silage additives helped to establish communities of fermentation organisms to produce satisfactory levels of VFA to

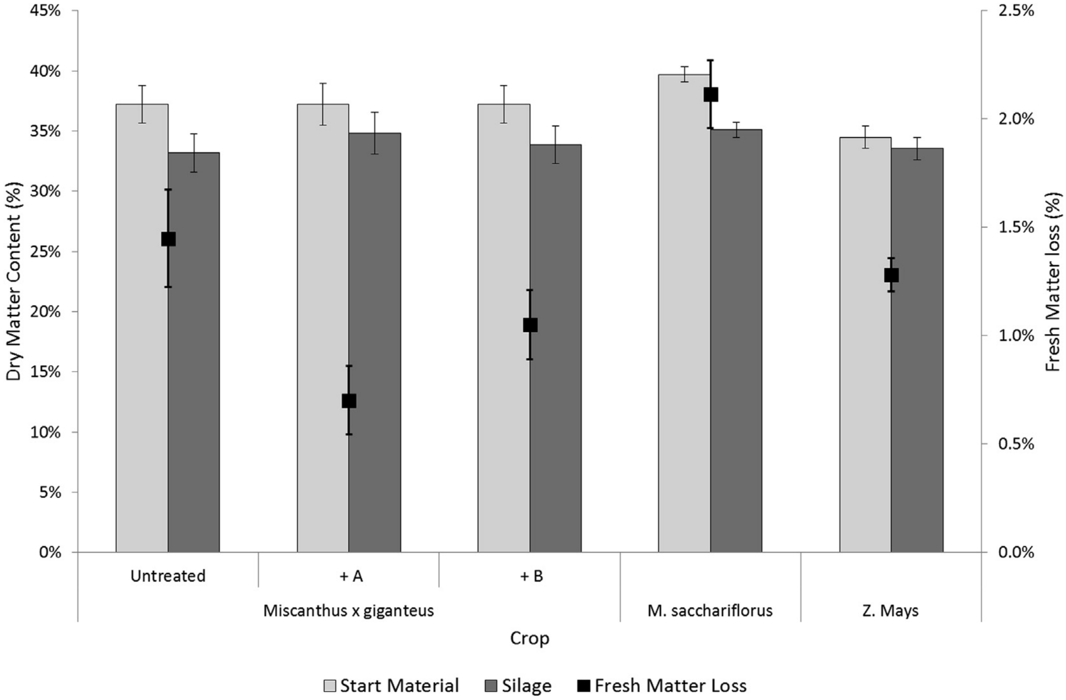

Fig. 4. Dry matter content before and after silaging and fresh matter losses during ensilage.

reduce the DM losses incurred during the silage process in *Miscanthus x giganteus*. The impact of the additives was significant, indicating that they were applied effectively, which is suggested to be difficult in high DM crops [48]. In the literature, DM losses during ensilage range between 3 and 27%, with a median of 11%, and are expected to occur in the first few weeks of storage [49].

Due to the small-scale nature of the experiment, the full potential of the reduction of DM losses were not explored. Further research is needed to test whether additives convey increased stability of the silage during feed out at a larger scale. It is known that higher concentrations of acetic acid, associated with hetero-lactic fermentation, increase aerobic stability once the silage is opened [44]. The rate of loss of fresh material was reduced in the two treated silages, particularly those treated with the hetero-fermentative additive (Fig. 4). The difference was not significant, however, as all the *Miscanthus* samples had significantly lower losses than *Z. mays* after 1 and 2 weeks of opening ($p = 0.04$). Changes in DM content or temperature were not monitored; therefore this should be explored further.

3.3. Biomethane potential (BMP)

The measured BMP yields from the crops (m^3 CH_4/t VS) and treatments are shown in Table 2. The VS content of the *Miscanthus* grasses were higher than the *Z. mays* ($p = 0.01$). Fig. 5 shows the average rate of methane production in the four crops over the 45 day incubation period.

Table 2
Biomethane potential (BMP) for each crop silage (values in parentheses indicate standard deviations).

Crop	Treatment	DM (%)	VS (%)	BMP (m^3 CH_4/t VS)
M. giganteus	Control	33.2% (1.5%)	32.7% (1.4%)	186 (18)
	+ A	34.8% (2.1%)	34.9% (1.7%)	173 (15)
	+ B	33.8% (1.4%)	35.3% (1%)	172 (6)
M. sacchariflorus	Control	35.1% (0.7%)	34.2% (1.1%)	189 (5)
Maize	Control	34.5% (0.7%)	28.5% (1%)	381 (16)

3.3.1. Comparing Miscanthus controls

The *Z. mays* silages had significantly higher ($p < 0.001$) biomethane yields, averaging 381 m^3 CH_4/t VS. There was no difference in accumulated BMP between the two *Miscanthus* controls, although *M. sacchariflorus* showed a slightly more rapid production rate, which may be due to the higher starch content in the crop. Otherwise, at the point of harvesting and ensiling, the two grasses had similar structural fibres and sugar contents. One study [13], found the BMP of *M. sacchariflorus* was almost twice that of *Miscanthus x giganteus*, which was attributed to a higher cellulose content (41.9% vs. 31.9%) while having similar lignin contents. In other studies BMP values of between 179 and 218 m^3 CH_4/t VS [50] or have been reported for *Miscanthus*. A good quality maize crop is expected to yield between 205 and 450 m^3 CH_4/t VS [51].

3.3.2. Effect of silage additives

There was no difference between the BMP of treated and untreated *Miscanthus* silages, with an average BMP of 180 m^3 CH_4/t VS. Despite the significant differences in VFA content, there was no effect of either silage additive on the BMP of the *Miscanthus x giganteus* samples. Few studies have examined the effect of silage additives on the BMP of *Miscanthus*. A study examining the treatment of *M. sinensis* silage with a 1% solution of ‘Bacta-sile’: a combination of *Pediococcus*, *Enterococcus* and *Lactobacillus* bacteria as well as cellulase, hemicellulase and amylase, found it gave a small increase in methane production, however the rate of production was accelerated [52]. A study examining the effect of a homolactive bacterial additive on ryegrass observed no benefits in BMP, even though the pH was reduced [22]. In other crops, LAB additives have been shown to increase methane production from sorghum silages by 0.5–3%, due to increased organic acid production [42], whereas Vervaeren et al. [25], study found LAB only improved the BMP in maize while incorporated with enzymes (α-

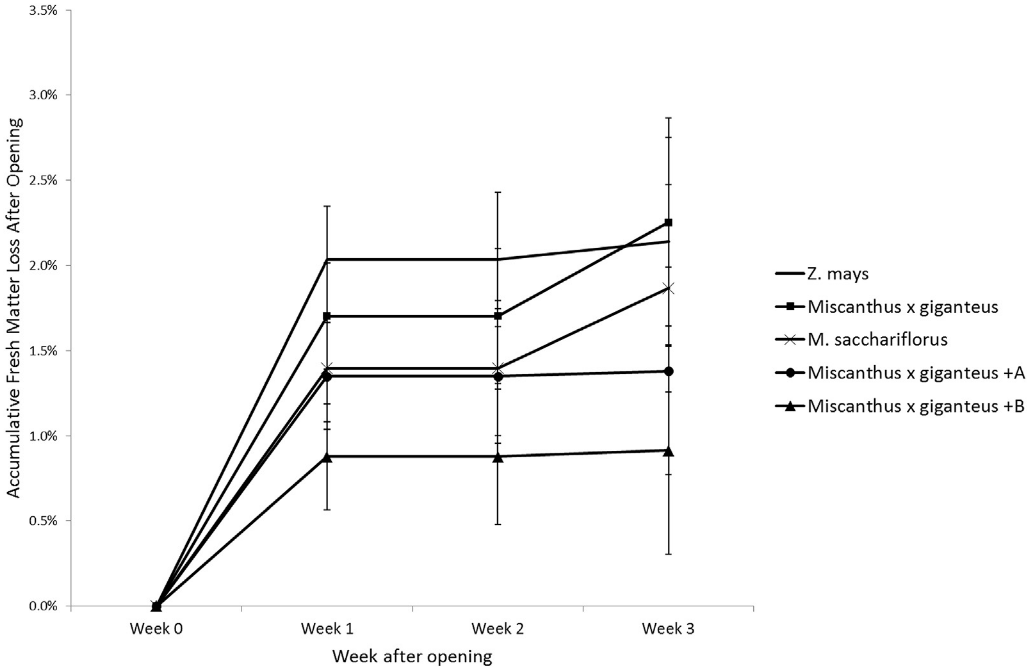

Fig. 5. Accumulative fresh matter losses after opening silage silos.

amylase, cellulase, hemicellulose) or yeasts.

Linear regression of the results showed that the cellulose content of the biomass explained 93.5% of the variation in BMP, with a negative slope (Table 3). The silage starch content explained 92.9% of the variation. The lactic, acetic acid and ethanol content did not show a significant r^2 against the BMP. An analysis of the Pearson product–moment correlation of the factors affecting BMP indicated positive correlations between the starch ($r = 0.97$) and sugar contents ($r = 0.74$). A negative correlation was observed between BMP with the cellulose ($r = -0.97$), lignin ($r = -0.96$) and hemicellulose ($r = -0.77$) content of the biomass. Although there is evidence that cellulose and hemicellulose can be used as a substrate for BMP, in the literature the lignin content is generally negatively correlated with BMP [16,53,54]. Mayer et al. [12], found that the higher levels of structural compounds present in *Miscanthus* showed a negative relationship with BMP.

After testing each variable with a stepwise forward selection the best fit was achieved when modelling cellulose and starch. No further additional explanatory variables were significant and there was no significant interaction between cellulose and starch. Checking the effect of crop type showed significantly higher first-order rate constants ($p < 0.001$) between maize and the *Miscanthus*, but the two grasses were not different. Li et al. (2013) reported a lower first-order rate constant for feedstocks with lignin content higher than 15% [53]. Final modelled relationships between starch, sugar and BMP were therefore determined for the crops as:

$$(Z.mays)\;\; BMP = 1000{*}(0.4225 + 0.00242{*}Cellulose - 0.00418{*}Starch)$$

$$(Miscanthus)\;\; BMP = 1000{*}(0.1016 + 0.00242{*}Cellulose - 0.00418{*}Starch)$$

Where BMP = accumulated m^3 CH_4/t VS, starch and cellulose are reported in %. Other studies showed that acetic acid, butyric acid and ethanol accounted for 75–96% of the variation in methane yield [42].

It has been shown that poor quality silages do not necessarily mean they are poor substrates for AD, on a ‘per t VS’ basis [21]. Silages for use as cattle feed require adequate crude protein and digestible energy contents in order to ensure body weight gain and milk production [30], and should have an acetic acid content of no more than 6%, because it provides little energy for microbial growth in rumen [48]. In contrast, silages which contain high levels of acetic acid are good for anaerobic digestion, as acetic acid is a precursor of methane [25,55]. The results from the experiment show that the benefit of applying silage additives was limited to reducing the DM losses during ensilage. The improved lactic and acetic acid content of the treated silages had no benefit on the BMP compared to the ‘poor’ control silages with higher ethanol contents. Ultimately, the higher fibre components and low WSC of the

Table 3
Crop constituents with a significant influence on BMP.

Parameter	r^2	Constant	Slope	p	SE
Silage sugar	51.7	0.16	0.010	$p < 0.001$	0.058
Silage starch	92.9	0.14	0.001	$p < 0.001$	0.022
Cellulose	93.5	0.48	−0.002	$p < 0.001$	0.021
Hemicellulose	56.6	0.63	−0.019	$p < 0.001$	0.055
Lignin	92.3	0.46	−0.029	$p < 0.001$	0.023
Cellulose + Silage starch	94.6	0.33	(cellulose) −0.004 (starch) 0.001	(cellulose) $p < 0.001$ (starch) 0.045	0.019

grasses attributed the grass with a lower BMP compared to *Z. mays*.

3.3.3. Biomethane production kinetics

The BMP is not the only important parameter when selecting a potential AD substrate: the kinetics of biomethane generation must also be considered as this affects the rate of production that will be expected in real-scale, continuous plants [56]. In this study, similar production kinetics to that found by Mayer et al. [12], were observed between the *Z. mays* and *Miscanthus* samples. Maize showed more rapid methane production compared to the *Miscanthus* samples (Fig. 6), with rapid production over the first two weeks and levelling out after day 25. There was no difference in accumulated BMP between the two *Miscanthus* controls, although *M. sacchariflorus* showed a slightly more rapid production rate, which may have been attributed to the higher starch content. Mayer et al. [12], observed a tilted profile in the BMP production curve of *Miscanthus*, indicating it had not yet reached an apparent asymptote plateau of production. They suggest that further conversion to biomethane was possible if the digestion time was left for longer than 42 days. In this study, however, which ran for the same duration, the curvature of the methane production profile appears to have plateaued in a similar manner to *Z. mays*; with the rate of increase declining after 25 days, suggesting that longer digestion would only marginally increase the BMP. Treatment with silage additives had a small effect of increasing the rate of production compared to the control grass, however after day 20 there was no difference.

3.4. Outlook: the biomethane potential of Miscanthus

An important factor affecting the biomethane potential of any crop is the dry matter yield per hectare [57]. As mentioned in Section 2.2, at Rothamsted *Z. mays* tends to yield between 10 and 14 t DM/ha. A range of yields are reported for early harvests of *Miscanthus*. A trial in the UK making 47 observations of *Miscanthus* yields between May and February showed a peak in yield around 24.3 t DM/ha around early October [58]. Across four sites in the UK, Germany and Portugal (3 sites) *Miscanthus x giganteus* yields averaged 16.0, 24.5 and 37.4 t DM/ha, while *M. sacchariflorus* averaged 11.1, 12.6 and 35.2 t DM/ha, respectively [59]. More recent studies based in France and Denmark reported yields of up to 20 t VS/ha and 27 t DM/ha, respectively [12,60]. Overall *Miscanthus* silages produced 49% of the BMP from maize on a per t VS basis. Factoring in the effect of yield requires that at least 19–26.5 is needed for *Miscanthus* to reach the biomethane production of a hectare of *Z. mays* yielding 10–14 t DM/ha. Therefore, the outlook of the BMP of *Miscanthus* is highly dependent on the yield of dry matter per hectare.

These higher yields are only achievable if the crop is harvested 'green', as a daily loss of 31.1 kg DM/ha/day after peak yield is reported [58], or a total loss of one third of the top yield during the winter phase [61]. There is, however a trade-off between the yield and moisture content of the biomass, and harvesting later means that the crop is more suited to heat and power uses [62]. This is not the case with silage or biomethane production, and as the crop matures over the winter period a number of chemical changes occurs in the biomass to makes it less suitable for AD, in terms of both BMP and biomethane production kinetics [8,56]. This is attributed to an increase in the cell wall components, and a drop in above-ground biomass WSC contents after October [20].

The long-term sustainability of harvesting *Miscanthus* in autumn has yet to be assessed [12]. One study showing three consecutive years of autumn harvesting found no negative affect on *Miscanthus* yield [12], however observed variation across the literature regarding the crop's response to fertilisation [63], suggest that more research is required to explore variation with location, soil type and agronomy. Yates et al. [64], describe a crop grown in northern France which produced yields in excess of 20 t ha^{-1} DM from autumn harvests for 4 years. However, where no additional nitrogen was added yield suddenly fell to a little over 15 t ha^{-1} DM in year 5, whereas when 120 kg ha^{-1} N was added in each spring yields remained greater than 20 t ha^{-1} DM. Similar unpublished work by the authors suggest that only two consecutive autumn harvests may be made on less fertile sandy soils in the UK, but that

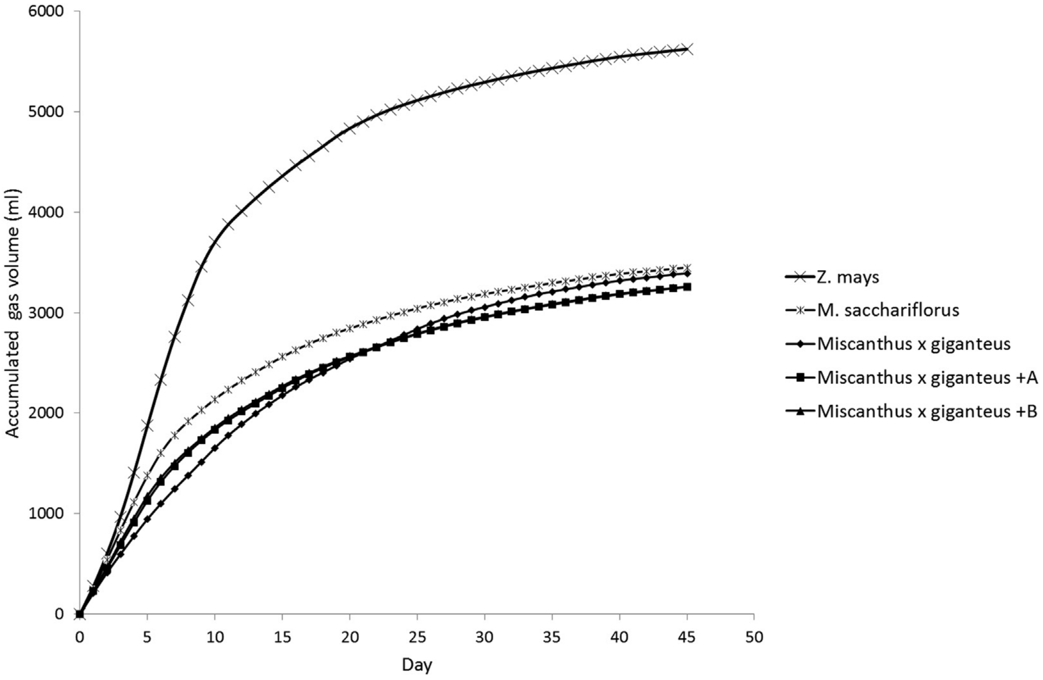

Fig. 6. Accumulative methane production from the *Miscanthus* and *Z. mays* silages.

subsequent return to spring harvesting allows a full recovery of the crop. Autumn harvest yields in the UK have not exceeded 20 t DM ha^{-1} and the case for *Miscanthus* replacing maize as a feedstock for AD relies upon the environmental benefits that may be achieved rather than simply the BMP, and which land is available for it to be grown.

Concerns over early harvesting are around potential disruption of nutrient and carbohydrate translocation that is important for over-wintering and regrowth in the following spring. Understanding the timing of nutrient remobilization from the stems to the rhizome is important when shifting the timing of harvest [20]; harvesting before this point may prematurely exhaust the crop (Clifton-Brown et al., 2007). The translocation of nitrogen into the rhizome has been demonstrated using ^{15}N-labelled fertilisers (Christian et al., 2006). Recycling of K_2O is reported to be less efficient than N and P_2O_5, and a greater proportion of K_2O is removed in the harvested biomass [63]. This may explain the higher K_2O-based fertiliser demands. Though it may be possible to adjust the agronomy of the crop to compensate for higher levels of nutrient offtake in early-harvested material, it is not possible to supplement the crop's carbohydrate stores. It is also possible that increased fertiliser, particularly nitrogen additions, affects the suitability of the feedstock for ensilage and AD, due to increased BC and decreased WSC [15]. Monitoring carbohydrate stores in the rhizome show that after October, starch is mobilised from above ground biomass and by November the rhizome reserve is equal to that measured in the previous February, suggesting that harvesting after this time would not detrimental to the carbohydrate store [20].

Further exploration into pre-treatment of *Miscanthus* for AD applications could be performed in order to boost the BMP. These could be applied in the form of silage additives, for example exploring the addition of enzymes to aid degradation of cellulosic structures that can act as a pre-treatment for AD crops [52,65,66]. Pre-treating the biomass with steam-explosion could also make the biomass more amenable to digestion [24].

4. Conclusion

The results of this study suggest that it is possible to produce biogas from *Miscanthus*, however the BMP on a 'per t VS' basis is limited compared to *Z. mays*. This was attributed to the *Z. mays*' significantly higher starch content and a lower cellulose content; characteristics which were ultimately identified as the main determinants of BMP. A minimum yield of 25.5 t DM/ha is needed for *Miscanthus* to match the biogas production from a typical yielding area of *Z. mays*.

The results suggest that silage additives are necessary in order for *Miscanthus* to ensile effectively. In this study bacterial inocula favouring a homo and a hetero-lactic fermentation both significantly improved the lactic and acetic acid production in the silage, reduced DM losses during ensiling, but did not improve the BMP. Further research is required to test whether DM losses are reduced when applied at a larger scale and whether the additives improve the stability of the silage.

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