*Journal of*
*AGROBIOLOGY*

J Agrobiol **28**(2): 85–96, 2011
DOI 10.2478/s10146-011-0010-y
ISSN 1803-4403 (printed)
ISSN 1804-2686 (on-line)
http://joa.zf.jcu.cz; http://versita.com/science/agriculture/joa

## REVIEW

# The required characteristics of ensiled crops used as a feedstock for biogas production: a review

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Received: 29th June 2011
Revised: 31st August 2011

**Abstract**
Maize and grass silages are the main feedstock for anaerobic digestion in agricultural biogas plants. High-quality silage is necessary for high methane yields. Grasses should be cut and ensiled at leafy stages, until full heading, prior to an extensive lignification. Late ripening maize varieties should be harvested towards full ripening due to the increasing starch content in grains, and early to medium ripening varieties at the end of waxy ripeness. The substrate availability for methanogens is improved by fine chopping. Pretreatment processes of a thermal, chemical or biological nature attempting to disrupt lignocellulosic matter are economically demanding, including the application of enzyme hydrolysing structural polysaccharides. Application of lactic acid bacteria inoculants at ensiling seems to have an insignificant effect on methane yields. Some micronutrients necessary for methanogens growth are often deficient in the silages and particularly cobalt, nickel and iron should be supplemented. Maize silage has too low nitrogen content for methanogens growth. The high acidity of silage needs to be partially neutralised prior to anaerobic digestion.

**Key words:** anaerobic digestion; biogas; methane; agricultural biomass; silage

**Abbreviations:**

**AD** – anaerobic digestion; **CFU** – colony-forming unit; **DM** – dry matter; **IFBB** – integrated generation of solid fuel and biogas from biomass; **LAB** – lactic acid bacteria; **ODM** – organic dry matter; **SRI** – silage maize ripening index; **VFA** – volatile fatty acids; **VS** – volatile solids

## INTRODUCTION

European Union policies have set a goal of supplying 20% of the energy demands from renewable energy systems by 2020. At least one quarter of all bioenergy in the future can originate from biogas, produced from organic materials such as fresh or chiefly ensiled whole crops,

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animal manure and slurries, wet food and feed wastes, etc. Moreover, energy production from the anaerobic digestion (AD) of biomass ensures a new opportunity for agricultural production and reduces the emissions of greenhouse gases including those of methane from farm sources.

Methane can be converted into electricity, heat or transport fuel. Carbon dioxide, either as a component of biogas or produced by methane combustion, is considered neutral in greenhouse gas terms. Life cycle assessment indicates that the production of biogas from energy crops partly provides the possibility of a roughly closed cycle of phosphorus, potassium and nitrogen returned in digester effluent to the soil in contrast to cash crop cultivation or combustion technology (Schumacher et al. 2010).

Germany is the largest biogas producer in Europe with dynamic development of agricultural biogas plants since the entry into force of the Renewable Energies Act in 2000 and its amendment in 2004. However, most of these plants have no comprehensive provision for the use of the waste heat from the biogas combustion. Similar trends have been observed in Austria and also in the Czech Republic.

Whereas forages and other crops accumulate seasonally, biogas plants have to be fed continuously. Feedstock has to be thus preserved, with ensiling being the preferred procedure. The technology of forage ensiling for livestock feeding is well developed. However, information on silage-making and storage of ensiled agricultural biomass as a feedstock for AD has so far been limited. Ensiling and AD are two jointed complex biochemical processes. Ensiling changes the properties of plant biomass and directly or indirectly affects AD (Prochnow et al. 2009).

The aim of the review is to collate and assess recent data on the parameters required for ensiled crops enabling maximisation of methane production and energy yields.

## CHARACTERISTICS OF ANAEROBIC DIGESTION OF CROP BIOMASS

Anaerobic digestion (or biomethanation) is the process of organic matter decomposition by a microbial consortium in an oxygen-free environment resulting in biogas. The process has been known from ancient times, was described by Louis Pasteur and developed during the last century in biogas technology used mainly in wastewater and waste treatments. The last decades have brought the dynamic development of biogas production from agricultural biomass.

### General aspects of anaerobic digestion

The biochemical, microbiological and technological principles of AD have been described in detail by Straka et al. (2003).

The production of biogas consists of several successive processes. The main plant biomass components, polysaccharides, proteins and lipids, are initially hydrolysed to more simple chemicals, monosaccharides, amino acids, free long-chain fatty acids and glycerol. The lignocellulosic complex of biomass is hydrolysed only partially; lignin is very recalcitrant and nondegradable. The hydrolytic processes are catalysed by both plant and bacterial enzymes. Initial aerobic conditions are quickly changed to anaerobiosis, which remains during the subsequent fermentation processes.

The fermentable products, called volatile solids (VS; or volatile matter), are fermented by various bacteria, either to volatile fatty acids (VFA; or short-chain fatty acids) with the carbon chain $C_2$–$C_6$, finally resulting in acetic acid (acido- or acetogenesis), or to a mixture of carbon dioxide and hydrogen. Acetotrophic and hydrogenotrophic methanogenic bacteria then convert acetic acid and hydrogen, respectively, into a mixture of methane and carbon dioxide. More recent results suggest that the acetate is completely oxidised into $CO_2$ which in turn is partly reduced to methane (Laukenmann et al. 2010). Information on biogas production from main plant biomass components is given in Table 1. Most of recent digesters work at mesophilic temperatures of 25–39 °C.

Biogas from the AD of agricultural plant biomass is generally composed of 48–65% methane, 36–41% carbon dioxide, up to 17% nitrogen, <1% oxygen, 32–169 ppm hydrogen sulphide, and traces of other gases (cited by Ward et al. 2008).

The technology of AD dealing with various types of digesters, optimum co-digestion of various agricultural substrates, etc. is beyond the scope of this review. Recent knowledge on the topic is available from overviews by Ward et al. (2008), Nizami et al. (2009) and Nizami and Murphy (2010).

A wide variety of inhibitory substances can be the primary cause of disturbance or failure of the anaerobic digesters due to their deleterious effects on functional bacteria. These include free ammonia ($NH_3$), sulphides (preferably $H_2S$ permeating the cell membranes), light metal cations ($Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, $Al^{3+}$), heavy metals (Cr, Fe, Co, Cu, Zn, Cd, or Ni) disrupting enzyme functions, or a range of organics (Chen et al. 2008).

**Table 1.** Biogas production from main plant components (Straka et al. 2003)

| Component | Biogas production ($m^3$ $kg^{-1}$ volatile solids) | Methane proportion (% v/v) |
|---|---|---|
| Saccharides | 0.75–0.90 | 50–60 |
| Proteins | 0.55–0.75 | 70–85 |
| Lipids | 1.10–1.55 | 60–70 |

## The requirements for crop biomass as a feedstock for anaerobic digesters

In this section, the overall biological conditions for both fresh and ensiled crop biomass will be outlined. More detailed data on particularly maize and grass silages will be discussed in section *Main types of silage.*

The aim of supplying crop feedstock for biogas production is to achieve the highest possible methane yield per area unit ($m^3$ $ha^{-1}$). This area-specific methane yield consists of biomass organic dry matter yield ($kg_{ODM}$ $ha^{-1}$) and the feedstock-specific methane yield ($m^3$ $kg_{ODM}^{-1}$) (Prochnow et al. 2009). Feedstock quality can be influenced by numerous factors classable as biomass production management and harvest and preservation management.

The following data on biogas or methane yields should be compared cautiously because of experimental conditions varying from crop production to anaerobic digestion. Comparative results reported from the same laboratory have thus a higher informative value.

### *Crop species and varieties*

Energy crop biomass for biogas production needs to be cultivated in sustainable versatile crop rotations that integrate food, feed, raw materials and energy production. The increasing production of bioenergy on arable land might however lead to competition with food production. Feeding patterns in modern livestock farming have changed from species-rich forage obtained from semi-natural grasslands with low energy content to an increased use of arable forage crops and concentrates, leaving vast area of grassland without management (Richter et al. 2010). Thus, both arable forage crops and grass from semi-natural grassland are available for biogas production.

Information on the AD of various agricultural biomass, preferably of tropical and sub-tropical crops, available until mid-1990’s, has been reviewed by Gunaseelan (1997). At the same time, data were reported for temperate arable crops: wheat, barley, lucerne, red clover, ryegrass, and maize (Pouech et al. 1998). Methane yield from fresh plants harvested at maturity usual for livestock feeding varied between 0.295 and 0.397 $m^3$ $kg^{-1}$ VS. The highest methane yield was observed in maize harvested at the milky grains stage.

Interesting Austrian results for important crops are collated in Table 2 (Amon et al. 2007a). Maize, as the most productive crops, has been studied in detail in thirteen varieties cultivated at several sites (Amon et al. 2007b). The chemical composition and specific methane yields for late ripening varieties are given in Table 3. Late ripening varieties produced more biomass than medium or early ripening varieties. Methane yield declines as the crop approaches full ripeness. Within late ripening maize varieties (FAO ca. 600), the methane yields were 0.312–0.365 and 0.268–0.286 $m^3$ $kg^{-1}$ VS at milk and full ripeness, respectively. These varieties may be harvested later, towards full ripeness. The optimum harvesting time for early to medium ripening varieties (FAO 240-390) is at the end of waxy ripeness. The highest methane yield per hectare was achieved from the digestion of whole crop maize with 5,300–8,500 and 7,100–9,000 $m^3$ $ha^{-1}$ from early/medium and late ripening varieties, respectively.

Results from a comparison of AD in a 100–200d assay of 12 crops suitable for Finnish conditions, Jerusalem artichoke (*Helianthus tuberosus*), timothy-red clover mixture and reed canary grass (*Phalaris arundinacea*) gave the highest potential methane yields of 2,900–5,400 $m^3$ $h^{-1}$ (Lehtomäki et al. 2008).

**Table 2.** A comparison of methane yields from some temperate crops under the conditions of experimental anaerobic digestion (adapted from Amon et al. 2007a)

| Crop | Methane yield ($m^3$ $ha^{-1}$) | Optimum varieties and vegetation stage for harvest. |
|---|---|---|
| Maize | 7,500–10,200 | Locally suitable varieties with a high biomass yield, FAO number 300-600. Harvest during milk to wax ripeness. |
| Cereals (winter wheat, triticale, winter rye) | 3,200–4,500 | Fast growing varieties with a high biomass yield. Harvest during milk to dough grain stages. Rye and triticale are suitable as intercrops. |
| Sunflower | 2,600–4,550 | At dry matter of about 15%. Optimum varieties were not yet determined. |
| Grasses from permanent grasslands | 3,200–3,500 | First cut should not be before the stage of ear emergence. |

**Table 3.** Chemical composition and specific methane yield of late ripening whole-crop maize. Values of four varieties harvested at three developmental stages (adapted from Amon et al. 2007b)

| Days of vegetation | Milk ripeness | Wax ripeness | Full ripeness |
|---|---|---|---|
| | 97 | 122 | 151 |
| Dry matter (% fresh matter) | 18.7±0.8 | 29.3±0.9 | 46.8±4.7 |
| Volatile solids (% fresh matter) | 17.9±0.6 | 27.8±0.8 | 45.2±4.4 |
| Starch (% DM) | 2.35±1.4 | 23.9±3.2 | 33.3±8.1 |
| Crude protein (% DM) | 8.75±1.1 | 7.3±0.6 | 6.7±0.6 |
| Crude fat (% DM) | 1.28±0.1 | 2.3±0.2 | 2.2±0.5 |
| Cellulose (% DM) | 36.1±1.6 | 26.8±1.6 | 23.6±4.0 |
| Hemicellulose (% DM) | 25.8±0.5 | 35.0±2.3 | 34.2±2.7 |
| Lignin (% DM) | 6.95±1.4 | 5.35±0.7 | 5.1±1.1 |
| C:N ratio | 32.1±8.9 | 39.8±4.6 | 46.4±4.2 |
| Methane yield ($m^3$ $kg^{-1}$ VS) | 0.338±0.027 | 0.308±0.020 | 0.278±0.010 |

DM = dry matter; VS = volatile solids

In a report by Petersson et al. (2007), 0.36, 0.42 and 0.44 $m^3$ $kg^{-1}$ VS was produced from straws of winter rye, oilseed rape and faba bean, respectively. The respective methane yields were 72, 78 and 77% of theoretical yield.

Overall, feedstock-specific methane yields rise by to the earlier cutting of more digestible grasses, while area-specific yields mainly increase due to higher biomass yields (Prochnow et al. 2009).

### *Crop biomass pretreatments to improve the digestibility*

Each crop biomass contains a proportion of lignocellulosic material consisting mainly of polymers of cellulose, hemicellulose and lignin. The components form a rigid network in which cellulose and lignin fibres are connected via hemicellulose.

High-molecular weight cellulose, consisting of D-glucosopyranose units linked with ß-1,4-glycosidic bonds, partially forms a crystalline and partially an amorphous structure. Cellulose fibrils are a characteristic arrangement.

Hemicellulose is a complex of polymeric carbohydrates varying among crops. Both pentoses, particularly xylose and arabinose, and hexoses, particularly mannose, glucose and galactose, form the building units. The molecular weight is lower than that of cellulose. The structure is branched and short lateral chains are more easily hydrolysable than the backbone. Solubility in water depends on the proportion of various units, and increases with increasing temperature. Mannans and xylans are better soluble than hemicelluloses with other building units. Moreover, hemicelluloses are more sensitive to thermal chemical treatment than cellulose and lignin.

Lignin, present in plant cellular walls, is an intricate variable heteropolymer constructed of phenylpropane ($C_6 + C_3$) alcohols. It solidifies plant tissue structure and participates in impermeability, resistance against microbial attack and oxidative stress. Its chemical structure makes it very resistant to degradation.

Thus, effective AD demands a degree of disruption of the poorly digestible lignocellulosic complex. The rate of hydrolysis is limited by the crystallinity of cellulose, the degree of polymerisation, the available surface area and the contents of lignin and moisture.

The processes of various pretreatments to enhance digestibility were recently evaluated in a book (Himmel 2008) and a review (Hendriks and Zeeman 2009). The main goal is increasing the surface area accessible for hydrolytic enzymes. The pretreatments are classified as *i)* mechanical (milling of dry materials), *ii)* thermal (with liquid hot water; with steam at about 150–180 °C or as a steam explosion based on biomass heating with high-pressure saturated steam in a pressure vessel for up to 20 minutes at 140–260 °C. The vessel is then rapidly decompressed into an atmospheric pressure which causes significant disruption and defibration of the biomass, *iii)* acidic (with strong inorganic acids), *iv)* alkaline (e.g. with lime or ammonia), *v)* oxidative (e.g. with hydrogen peroxide or peracetic acid) and *vi)* various combinations of the above.

The effects of hydrothermal pretreatment at 185 or 190 °C were tested on maize, rye and red clover silages for ethanol production (Xu et al. 2010, Oleskowicz-Popiel et al. 2011). Cellulose hydrolysis and the following ethanol recovery were considerably improved. Nevertheless, such a process is economically demanding. If anything, hot water treatment seems to be applicable for silages as a pre-step of AD.

Ultrasonic treatment is another emerging physical procedure. The induced cavitation of the substrate causes an increase in the specific area of particles, cell lysis and the destruction of biological macromolecules. More efficient and faster AD is then carried out. In an experiment with maize silage, sonication increased biogas and methane production by 13–29.5% and 16.9–29.5%, respectively, as compared with AD without ultrasonic pretreatment (Zavacký et al. 2010).

### *Trace elements requirement*

Trace elements are the micronutrients necessary for the growth of anaerobic bacteria because cobalt, nickel, molybdenum or tungsten are cofactors of the enzymes participating in methane formation. A deficiency of trace elements in AD of agricultural biomass (energy crops, crop residues and animal excreta) is probably the first reason for poor process efficiency without any other obvious reason, despite proper management and control of other operational and environmental parameters. An suboptimal digester performance can occur particularly in agricultural biogas plants operating with energy crops as monosubstrates (Demirel and Scherer 2011).

Jarvis et al. (1997) reported a significant increase in the organic loading rate of a grass-red clover silage-fed mesophillic AD following cobalt addition to reach its concentration of 2.0 g $m^{-3}$ of active reactor volume. The positive effects of iron, nickel, molybdenum, or a mixture of the four trace elements were not recorded.

Cobalt and moreover molybdenum and selenium were determined as limiting elements during long-term monodigestion of maize silage, while the effects of further supplemented elements (Fe, Mn, Cu, Zn, Ni, and B) were not significant (Lebuhn et al. 2008). In a further report from that laboratory, the process was not sustained below an availability of $10^{-8}$ μg Co per methanogenic cell (Munk et al. 2010). Nevertheless, Hinken et al. (2008) observed biogas production from CCM silage (corncob mix) to be increased by 35% in the variant supplemented with cobalt, iron and nickel as compared to the reference silage.

Using a model substrate (xylan, starch, urea and potassium phosphate) simulating maize silage, Pobeheim et al. (2010, 2011) proved the positive effects of supplementation with nickel or cobalt at levels of 10.6 and 0.4–2.0 μM, respectively, on methane production. The addition of molybdenum did not significantly affect methane yields.

Thus, at least cobalt, nickel and iron are trace elements, which may limit methane production from energy crops and threaten the stability of AD. The requirements of the digester can be provided by a co-substrate with elevated trace element contents such as animal excreta or wastewater sludge. Another way is supplementation with chemical additives.

Nickel contents of 1.40±0.94, 2.47±0.75, 2.73±0.77 and 2.47±0.91 mg $kg^{-1}$ DM were determined in maize, grass, red clover-grass and oat silages, respectively (Kalač 1986). However, data on the total contents of the metals in crop feedstock have only limited value because of the unknown bioavailability for bacteria participating in AD.

## REQUIRED CHARACTERISTICS OF SILAGES

Within the used energy crops, maize is harvested and ensiled directly, while most of the other crops, e.g. grasses, clovers, lucerne or cereals, are ensiled, either directly in a fresh state, or more frequently after wilting. The crop matter can be ensiled either in various types of silos or in bales.

Quality criteria can be divided into two groups – the rate of preservation of digestible matter and the effectiveness of the suppression of microbiota deteriorating crop matter. The manufacturing process must quickly establish the anaerobic conditions enabling fermentation of water-soluble carbohydrates by various lactic acid bacteria. The lactic acid produced and to a lesser extent acetic acid increase acidity, which must reach the so-called critical pH value, depending mainly on the dry matter content (Table 4).

**Table 4.** Active acidity necessary for efficient silage preservation (so-called critical pH value) in relation to dry matter of ensiled forage

| Dry matter (%) | pH |
|---|---|
| 15 | 4.10 |
| 20 | 4.20 |
| 25 | 4.35 |
| 30 | 4.45 |
| 35 | 4.60 |
| 40 | 4.75 |
| 45 | 4.85 |
| 50 | 5.00 |

Sufficient acidity under anaerobic conditions preserves silage until its use (see section *Silage parameters and anaerobic digestion*). A further deterioration arises after a silo or a bale opening due to air access. Both unfermented carbohydrates and lactic and acetic acids are metabolised by activated yeasts to carbon dioxide and water. Aerobic stability is usually lower in well-preserved silages (particularly of maize) than in poorly preserved silages characteristic in elevated contents of butyric acid and ammonia. Overall information on biochemistry and microbiology of silage is available in the books of McDonald et al. (1991) and Woolford (1984), respectively.

### Particle size

As reviewed by Prochnow et al. (2009), the data from the literature on the effects of the particle size of ensiled crops on methane yields are ambiguous. Chopping of various harvested crops to the size usual for silage-making for livestock feeding seems to be acceptable, but reduced particle size is preferred. Crop matter can be chopped both during harvest and during silage removing from a silo or a bale. Further comminution is only reasonable if an achievable methane yield exceeds the additional energy demand.

### Silage additives

The application of additives helps to affect the preservation process in several ways:

i) chemical preservatives (mainly formic acid or a mixture of carboxylic acids or their salts) suppress undesirable microbiota, particularly butyric acid bacteria and putrefactive bacteria;
ii) inoculants of lactic acid bacteria (LAB) help to accelerate lactic acid fermentation in the initial stage of preservation (some 15 days), during which the necessary acidification must pass. While homofermentative strains are preferred for silages for ruminants feeding, heterofermentative strains could be more beneficial for AD, since they facilitate the production of intermediates (particularly acetic acid) for methanogens;
iii) via increasing fermentable carbohydrate contents, either directly (e.g. with molasses addition) or indirectly via polysaccharide hydrolases (particularly cellulase and/or hemicellulases).

The additives are not necessary for the ensiling of crops with a high content of fermentable carbohydrates such as maize or wilted tetraploid ryegrasses. On the contrary, they are widely used for poorly ensilable crops such as unwilted lucerne, clovers or some grasses.

In a report by Pakarinen et al. (2008), biological additives such as LAB applied at a very high dose of $1.5 \times 10^{11}$ CFU $g^{-1}$ of ensiled crop together with enzymes (cellulase, pectinase and xylanase), did not affect methane yield from ensiled mixtures of grasses, both from unwilted (15.6% DM) and wilted (30.4% DM) biomass.

The possibility was also tested of increasing methane yield from maize silage using biological additives as a pretreatment. Ellenrieder et al. (2010) observed effective starch hydrolysis following the application of an amylase-based preparation on finely ground maize silage. Nevertheless, no positive effect of the pre-hydrolysis on methane yield was established. The results from a report by Vervaeren et al. (2010), indicate that divergent biological additives involving yeasts or enzymes during maize ensilage seem to be more efficient than the spontaneous fermentation or the addition of LAB inoculants for the effective decomposition of polysaccharide structures.

It seems from the limited data available that the application of polysaccharide-hydrolysing enzymes is of limited usefulness because of high economic cost.

### Silage parameters and anaerobic digestion

While the critical pH value for the steady preservation of silage varies between 4.1 and 5.0 at dry matter of 15 and 50%, respectively (Table 4), the ideal pH range for AD is greatly higher and very narrow: 6.8–7.2. The growth rate of methanogens is considerably reduced below pH 6.6. Thus, silage can hardly be used as a monosubstrate without treatment. Partial neutralisation is therefore necessary, e.g. with $NaHCO_3$. The optimum acidity for the hydrolysis of substrate components and acidogenesis has been reported as being between pH 5.5 and 6.5 (Ward et al. 2008).

Short-chain fatty acids are a key intermediate in the process of anaerobic digestion but in high concentrations they are capable of inhibiting methanogens. Acetic acid is usually present in higher concentrations than other VFA during anaerobic digestion, but propionic and butyric acids are more inhibitory to the methanogens (Ward et al. 2008). An increased level of butyric acid is typical for silages of poor quality. Moreover, ammonia, being another inhibitor of methanogens, is elevated in such silages. However, it is toxic in its non-ionised form above pH 7. The content of free ammonia is a function of the total ammonia content, pH value and the temperature of the matter.

In contrast to the rumen of cattle and other ruminants, anaerobic and microaerophilic fungi and ciliates do not play a prominent role in plant biomass fed biogas reactors. A combined culture of thermophilic *Clostridium thermocellum* and *C. stercorarium* with optimum pH above 6.5 was shown to efficiently degrade cellulose in maize silage (Zverlov et al. 2010).

## MAIN TYPES OF SILAGE

Two types of silage have been widely used for AD within Europe, maize silage and grass silage. Silages of other crops have been used and studied to a limited extent only.

### Maize silage

In addition to the data on the composition and suitability of fresh maize for the AD in section *Crop species and varieties*, further information on silage maize and maize silage is available.

Compared to other lignocellulosic substrates, maize has a low degree of lignification. Kruse et al. (2008) have reported a moderate effect of the environment on the variability in the fibre components of silage maize. Coefficients of variation ranged between 2.6% for hemicelluloses and 8.9% for cellulose. The variation in fibre contents was more strongly affected by environmental than by genotypic factors.

In Germany, a special energy maize breeding program aims to increase dry matter yield to 30 t $ha^{-1}$. Future energy maize hybrids will be larger sized and later maturing than recent silage maize hybrids. Moreover, such hybrids should have a high specific methane yield and dry matter content above 28%, to prevent effluent seepage during ensiling. In a two-year experiment, late energy maize prototypes had a lower content of fat and protein, but higher contents of ash, detergent fibre and lignin as compared with the climatically adapted medium-early hybrids. However, despite a substantially different content of nutrients among the hybrids, no clear-cut association between chemical composition and specific methane yield was observed (Schittenhelm 2008).

Amler (2010) recommends for the selection of maize variety and the determination of harvest date the Silage maize Ripeness Index (SRI), which is the ratio of the dry matter content of grains to the dry matter of residual plants. Under the conditions of central Germany, the optima for maize ensiling and yield maximum correspond with physiological ripeness and are close to grain DM of 63% and SRI between 2.6 and 2.9. Under these conditions it is possible to reach the optimum ripeness of 30–35% DM of the whole-plant silage maize and stover DM of 22–24%.

Under Hungarian conditions also, silage maize varieties with later maturity than those generally used for silage production for feeding of ruminants were recommended for biogas production (Hadi 2009).

Maize and naturally also maize silage have a low nitrogen content. For instance, Klimiuk et al. (2010) calculated the chemical formula $C_{56\cdot5}H_{112}O_{48.1}N$ for maize silage. The most suitable C:N ratios for AD are quoted as 15–30:1. Using maize silage as a monosubstrate thus causes the instability of AD. Moreover, the high acidity with pH values usually below 4.0 produces a low degradation rate during the initial period of AD. Co-digestion is therefore preferred, particularly with manure or excess sludge from wastewater treatment plants as alkalising materials, containing moreover essential micronutrients for methanogens (Bruni et al. 2010, Hutňan et al. 2010). The highest methane production was achieved at the ratio of maize silage to liquid pig manure (w/w) between 1:1 and 2:1 (Dębowski et al. 2009).

## Grass silage

As mentioned above, high-quality silage is regarded as a precondition for high methane yields. As compared with maize silage, the production of the desired silage quality from grasses is more demanding. Grasses have a generally lower content of fermentable water-soluble carbohydrates and a higher proportion of nitrogenous substances, mainly proteins. The fermentation is slower and the elevated buffering capacity retards the decrease of pH value necessary for efficient preservation. Lactic acid bacteria have thus rather difficult conditions in their competition with clostridia and putrefactive bacteria.

The decomposition of proteins is under way even in ensiled grasses of very good quality. The proportion of ammonia nitrogen ($NH_3$-N) in such silages can be up to 8% of total nitrogen.

Moreover, grasses have a tendency to lignificate extensively after full heading. As a rule, feedstock-specific methane yields thus rise due to earlier cutting dates, while area-specific methane yields mainly increase due to higher biomass yields. A leafy and nonlignified stage of grass growth with a high leaf : stem ratio enables the production of crop matter with a relatively high content of fermentable substrate and a low fibre content. The content of water-soluble carbohydrates increases during the day because photosynthetic processes are highest in early evening.

As reviewed by Nizami et al. (2009), the first cut of grasses offers more methane than later cuts. Three cuts are usually recommended under favourable growing conditions (Prochnow et al. 2009). Nevertheless, in Ireland, a country with extraordinarily productive grass growing (about 12 t of volatile dry solids per ha yearly), the three-cut system for silage production for livestock feeding is generally deemed uneconomic due to the high costs of harvesting and lower yields from third (or subsequent) cuts (Smyth et al. 2009).

According to data cited by Smyth et al. (2009) on Irish grass silages, the mean content of volatile dry solids was found to be 92% of dry solids and a hypothetical formula $C_{28.4}H_{44.5}O_{17.7}N$ was generated for dry grass. Thus, the ratio 28:1 was determined for C:N. Biogas production is related to mass of VS rather than mass of silage; typically one kg of VS produces 0.30 $m^3$ of methane (Nizami et al. 2009).

Pakarinen et al. (2008) reported high methane yields from well preserved silages of both unwilted and wilted grass mixtures, but considerably lower yields from poorly preserved silages with secondary butyric fermentation and proteolysis, resulting in elevated contents of VFA with $C_4$–$C_6$ carbon chain.

Comparing methane production from grass silage in a one-stage process with the combined thermophilic hydrogen and mesophilic methane production in a two-stage process, the highest methane yield (0.495 $m^3$ $kg^{-1}$ VS) was obtained from grass silage pretreated with NaOH. The silage was then separated to the solid fraction, which was digested in the two-stage process, while the liquid fraction was treated directly in the one-stage AD (Pakarinen et al. 2009).

An extended model suitable for the modelling of AD of grass silage has been developed (Koch et al. 2010), taking into account both data on silage chemical composition and parameters describing biochemical processes during AD.

Various aspects affecting the decision-making on digester configurations for the production of methane from grass silage have been reviewed by Nizami and Murphy (2010).

### *Integrated generation of solid fuel and biogas from grass silage*

Most of biogas plants have no comprehensive provision for the use of the waste heat from the biogas combustion. The highest energy efficiency and the lowest costs could be realised by combined heat and power production from biogas and the provision of heat from the solid biofuels. A new technology has been recently developed in the University of Kassel, Germany, called the Integrated Generation of Solid Fuel and Biogas from Biomass (IFBB). Grass biomass is subjected to hydrothermal conditioning and is subsequently processed using a screw press, which results in a press fluid for biogas production and a press cake for direct combustion as solid fuel. Drying of the cake with the waste heat from the biogas combustion is a key aspect of the procedure (Richter et al. 2009).

In an experiment with grass silage (Richter et al. 2011a, b), herbage from a lowland hay meadow (*Arrhenaterion*) was cut and ensiled eight times during the first cut between the very early and the very delayed stages of vegetation. All silages were well preserved. Hydrothermal conditioning was carried out with a mash of silage with water (1:4, w/w) stirred for 10 min at five temperatures between 10 °C and 90 °C. Subsequent mechanical dehydration was conducted with a screw press. Maximum net energy yields were 10.2 MWh $ha^{-1}$ for the IFBB treatment without hydrothermal conditioning and 9.0 MWh $ha^{-1}$ for the treatment with hydrothermal conditioning at 50 °C. Comparative whole-crop digestion achieved a maximum net energy yield of 3.7 MWh $ha^{-1}$ (Richter et al. 2011b). Increasing sward maturity had a positive effect on methane yields of press fluids and on the energy conversion efficiency of the IFBB process. A medium temperature of 50 °C of the hydrothermal conditioning seemed to be optimal. Increasing contents of silage neutral detergent fibre and of dry matter, i.e. delayed cuts, were strong predictors for energy output parameters and energy conversion efficiency.

There have also been studies the fate of the mineral compounds of dried press cakes unsuitable for combustion (Richter et al. 2011a). During combustion, nitrogen is almost completely oxidised into air polluting nitrogen oxides ($NO_x$). Potassium and chlorine are involved in a corrosion process in the furnace. Potassium and magnesium promote melting of the ash resulting in slagging and fouling processes inside the combustion chamber. The contents of these elements were significantly reduced in the press cake compared to the silage. Press cakes of late cutting dates were considered best suitable for combustion.

The IFBB process has been shown to be suitable for a mixture of maize silage, grass silage and cattle slurry conditioned at 60 °C for 15 min. Chlorine was concentrated in the press fluid. Nevertheless, liquid wastes of AD (fugate) are too diluted and the content of mineral nutrients is very low. Thus, their agrochemical value for the fertilisation is very limited (Kolář et al. 2010).

### Other crops silage

In a comparative testing of AD of maize, sorghum (*Sorghum saccharatum*), *Miscanthus* × *giganteus* and *Miscanthus sacchariflorus* silages (Klimiuk et al. 2010), mean methane yields were 0.33, 0.33, 0.10 and 0.19 $m^3\ kg^{-1}$ VS. The efficiency of cellulose conversion varied between 83.6% for sorghum and 52.1% for *Miscanthus* × *giganteus*, and that of hemicellulose between 88.9% for maize and 59.7% for *Miscanthus* × *giganteus*. Silages of both *Miscanthus* species had a higher lignin content than maize or sorghum.

Another study (Herrmann et al. 2011) compared the methane production from silages of maize, a sorghum hybrid (*Sorghum bicolor* × *sudanense*), forage rye and triticale. All forages were ensiled as control variant without any additive, and preserved either with chemical preservatives or with LAB inoculants and stored for up 1 year. Prolonged storage resulted in ODM losses. Methane yields from maize and triticale silages were comparable, but higher than those from sorghum and rye. The yields from silages with chemical preservatives were lower by 2–7% than those from the control variants, while the impact of applying LAB inoculants was negligible.

Sugar beet silage without leaves has an extremely low pH value of 3.3–3.4. Moreover, it is a poor substrate for AD due to low availability of nutrients and low buffering capacity. Supplementation with nitrogen and buffering agents (particularly $KHCO_3$ or $NaHCO_3$) has therefore to be performed regularly (Demirel and Scherer 2008, Demirel et al. 2009).

Phosphate seems to be the limiting macronutrient for AD of fodder beet silage (Scherer et al. 2009).

## CONCLUSION

Maize and grass silages are the main feedstock for anaerobic digestion in European countries with a dynamic development of agricultural biogas plants. Generally, high-quality silage is necessary for high methane yields. However, some differences exist in demands for parameters between silage for livestock feeding and for anaerobic digestion.

Rumen microbiota is much more effective in hydrolysis of lignocellulosic matter than are methanogens. Thus, grasses should be cut and ensiled at leafy stages, generally prior to or until anthesis, but the first cut should not be before the stage of ear emergence. Fine chopping of crops improves the availability of the substrate for methanogens. Pretreatment processes of mechanical, thermal, chemical or biological nature, including the application of polysaccharide-hydrolysing enzymes, in an effort to disrupt lignocellulosic matter are economically demanding. Some micronutrients have often been deficient in methanogens and particularly cobalt, nickel and iron should be supplemented at silage digestion. Application of various lactic acid bacteria at ensiling seems to have a negligible effect on methane yields. High acidity of silage needs to be partially neutralised prior to anaerobic digestion. Moreover, maize silage has too high a C:N ratio.

Late ripening maize varieties should be harvested towards full ripening due to increasing starch content; early to medium ripening varieties at the end of waxy ripeness. A breeding program for special energy maize is in progress.

## ACKNOWLEDGEMENT

The author acknowledges financial support of the Ministry of Education, Youth and Sports of the Czech Republic (Project No. MSM 6007665806). The helpful comments of Professor Ladislav Kolář are acknowledged.

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