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Utilization of Permanent Grassland for Biogas Production

Pavel Fuksa¹, Josef Hakl¹, Zuzana Hrevušová¹,
Jaromír Šantrůček¹, Ilona Gerndtová² and Jan Habart³

¹*Department of Forage Crops and Grassland Management,
Czech University of Life Sciences, Prague*

²*Research Institute of Agricultural Engineering*

³*Department of Agro-Environmental Chemistry and Plant Nutrition,
Czech University of Life Sciences, Prague
Czech Republic*

1. Introduction

Permanent grasslands represent undoubtedly an inseparable part of landscape, which has historically both agriculture and environmental importance. Considering restricting agriculture-food-processing production, especially in Central and Eastern European countries, the main aim of European policy is to support mainly environmental function of permanent grasslands. To fulfil non-productive function of permanent grasslands, there is the base of their utilization and harvest of biomass. Therefore, agriculture is also focused on non-food processing production where the first place is taken by energy production.

This chapter is dealing with utilization of permanent grasslands for energy production and their energetic balance. The main attention deals with a particular way of production of biogas, which is the most applied method as far as energy production of this vegetation in Europe is concerned. The production of biomass for energy, as well as traditional forage produce, cannot omit functions of permanent grasslands. Therefore, a part of the chapter is also focused on biological and environmental aspects of permanent grasslands. It is not possible to incorporate all related topics in their entirety because of limited scope of the chapter. The aim of this chapter is to give general knowledge with emphasis on reciprocal coherence of mentioned issues. Detailed information can be found in cited literature.

2. Importance of permanent grassland

Permanent grasslands are important parts of natural landscape, as well as, element of management of agricultural land not only in the Europe territory. Grassland covers approximately $3.4 \cdot 10^9$ ha, i.e. 69 % of the world's agricultural area or 26 % of total land area. In Europe, grasslands also cover a considerable amount of landscape. Currently they represent almost 38 % of agricultural land area. Area in the Czech Republic has been expanding over last few years. At present grasslands cover over 23 % of agricultural land area (Food and Agriculture Organization Statistic [FAOSTAT], 2011).

Permanent grassland is defined as a land used permanently (for five years or more) for herbaceous forage crops, either cultivated or growing wild (FAOSTAT, 2011). Under favourable conditions, the grass species prevail in grasslands. However varied ecological conditions allow expansion of large amount of different plant species, where legumes are, for many reasons, very important. The plant composition is the result of interaction of ecological factors of placement and the methods of cultivation. The mediation of succession, such composition of natural species is created on sites that in given ecological conditions and specific way of utilization thrives the best. Throughout the years this balance is also created on newly founded stands seeded with mix of grasses and legumes.

Depending on place of origin we can discern between two basic types of permanent grasslands:

- **Natural grasslands** - were created without human intervention. They are situated on steppe, marshland and peat bog locality, in areas above high altitude tree-lines. Production of biomass is negatively affected by less favourable conditions of locality. These grasslands are used mostly extensively.
- **Semi-natural grasslands** - are situated in areas originally planted with trees. Their existence is dependent on continuous human intervention and cultivation such as grazing or/and cutting. They often have a potential for large yields and they can be used intensively. Among this group we can also find grasslands created by seeding of mix cultural grasses and species of legumes.

Permanent grasslands belong to agricultural systems with very high environmental value. They are among the most biologically active and most productive vegetation types with fast cycle of growth and high capability of transferring chemical elements in biosphere. Their importance comes from two key aspects: productive and non-productive functions.

3. Functions of permanent grassland

Agriculture traditionally puts emphasis mainly on productive function of permanent grasslands. They are source of both livestock fodder and plant biomass used for alternative purposes. For productive purposes, however, only a part of permanent grassland can be used. In some regions, the requirements of grass fodder are lower than the amount that permanent grasslands are able to produce. Therefore, great deal of area can remain unused. Nevertheless the grasslands that are not used for production are also very valuable parts of landscape, as they hold broad scale of so-called non-productive functions.

3.1 Productive functions

Production of permanent grasslands is in a close relationship with amount and quality of produced biomass. Productive function of permanent grasslands is historically connected particularly with providing fodder for livestock. In this way, permanent grasslands significantly contribute to human diet by providing fodder for livestock and thus allowing production of human foodstuff (milk and meat). It is for this reason that the research of production potential of grasslands was for the major part focused on optimizing relationship between production of fodder, its quality and productivity of animals.

The usage of biomass for animal nutrition is still dominant way of its utilization, but in the recent period the importance of this traditional relation has been reduced. The main reasons for this can be considered reducing the quantity ruminants, especially in the countries of Central and Eastern Europe. As a result of increasing milk and meat productivity of animals, in suitable regions there is an increase of amount of fodder being produced from arable land (legume-grass mixture, maize). Because of this and other reasons (for example political and financial support for renewable energy) there has been in last 10 – 15 years significant increase in usage of biomass produced from permanent grasslands for alternative purposes (Hohenstein & Wright, 1994; Prochnow et al., 2009a, 2009b; Rösch et al., 2009).

According to Prochnow et al. (2009b), the grassland biomass is suitable in many ways for producing energy. Currently it is used in practice as a feedstock for biogas production and as solid biofuel for combustion. Future pathways can include the production of lignocellulosic bioethanol, synthetic biofuels or synthetic natural gas. Feedstock from grassland will also be used as raw material for the bio-industry within Green Biorefineries (Kromus et al., 2006, as cited in Prochnow et al., 2009b).

If suitable management of permanent grasslands regarding productive and non-productive functions stays the same, the change in how the final product is being used will not have a negative impact on farming of permanent grasslands. Permanent grasslands also have an important function in relation with arable land. Ruminants transfer the biomass through digestion and partially use it for their need. The remaining 35 – 50 % of organic matter is excreted in form of excrements. Organic matter in form of farm fertilizer is then used primarily on arable land and there it is important factor contributing to its fertility. When we utilize grass biomass for energy, however, loss of organic matter is higher. Biogas fermentation can degrade cellulose to an extent of about 80 % (Ress et al., 1998). Usage of biomass for direct combustion leads to 100 % loss of organic matter. Energy utilization of biomass can therefore lead to reduction in return of organic matter into soil, in comparison with traditional system, where fodder is utilized by ruminants.

With reduction of return of the organic matter into the soil, there can be disruption in organic balance of the agricultural system, which can lead to number of negative consequences (reduction in fertility of the soil, increase in leaching nutrients into the underground water, increased hazard of erosion etc.). It is necessary to reduce hazards to arable lands such as these by applying effective countermeasures, for example by increasing the share of legumes in crop rotation or by growing catch crops (Brant et al., 2011). The risk is not significant in permanent grasslands. The root system of the plants creates sufficient amount of organic matter inside the soil, so it is not necessary to fertilize them organically at a regular base.

3.2 Non-productive functions

Permanent and semi-natural grassland are very important not only as a source of fodder, but they also play a significant role in environment (Stypiński et al., 2009). These non-productive functions of permanent grasslands interfere in different fields such as:

- Protection and stabilization of biodiversity – plant and animal genetic resources.
- Protection against erosion of soil – against both wind and water erosion.
- Water management – high infiltration of rainfalls and flood waters, maintenance of water reserves in the soil.
- Function of biological filtration – they filter considerable amount of agents that are dangerous to health (nitrates, phosphates, biocides) and they prevent them from penetrating into deeper layers of the soil and subsequently into the underground water.
- Increasing soil fertility – they create large amount of dead organic matter and they enrich the soil with humus, improving the soil structure.
- Great supply of both above-ground and underground active living matter.
- Fixation of air nitrogen – both symbiotic and non-symbiotic.
- Balancing changes in temperature and humidity of surrounding air.
- Aesthetic and landscape functions.
- Health-hygienic function – production of oxygen, capturing of gas emission, reduction in dustiness and level of noise etc.
- Social economic function – particularly in marginal regions in connection with livestock breeding, they are used as source of living for people.

Permanent grasslands are able to fulfil these and other functions, provided that correct management is applied. Underutilized and neglected permanent grasslands are able to maintain these functions only in limited amount, or they can even contribute negatively in those areas, according many literal sources (Hopkins & Holz, 2006; Rychnovská, 1993; Rychnovská & Parente, 1997). Absence of regular utilization and grassland management cause degradation to fallow, and consequently, establishment of high number pioneer shrubs and trees. Planning of grassland management is necessary to conserve total diversity and retain its important functions in landscape (Moog et al., 2002).

Maintains of present status of grasslands and introduction of agro-environmental programs and agreements is one of the solution for sustainable development, it means the optimal and environmentally friendly utilization of nature resources like soil, water, plants and animals communities (Stypiński et al., 2009).

4. Primary productions and energy balance of permanent grassland

Productivity of permanent grassland is a determinative component influencing affectivity of use of biomass, whether it involves fodder for ruminants or biomass for energy use. From the point of view of possibility of affecting productivity of permanent grasslands, it is necessary to understand that we are talking about open systems with many structures and functions, which are affected by many known and even larger number of unknown feedbacks. The site conditions (such as soil composition, supply of water) and the system of management that is being used have a huge impact on botanical composition and with it connected yield of biomass (Rychnovská & Parente, 1997).

Primary production of permanent grasslands is traditionally expressed in yield of dry matter ($t_{DM} \text{ ha}^{-1}$). Variability in yield of permanent grassland is, considering different ecological conditions and different management, very broad and can vary in range of 1 – 15 (in rare cases even more) $t \text{ ha}^{-1}$.

As far as energy flows in ecosystem are concerned, it is more apposite to monitor the amount of produced energy from specific area of land. Expressing primary production of stands in energy units allows considering the suitability of applied management from the point of view of expressed energy inputs and outputs in the system. To calculate these balances the energy requirements of individual applied arrangements must be known and it is also necessary to determine the amount of energy contained in biomass.

4.1 Calorific value

The amount of energy in biomass is possible to determine on the basis of calorimetric measurement. The principle of calorimetric determination of the volume of gross weight is based on burning down a sample in oxygen atmosphere and recording resulting increase of temperature in calorimetric system. The gross calorific value of the substance that is being burned down is counted using the following formula (1):

$$Q = \frac{C \cdot \Delta T - Q_1}{m} \quad (1)$$

Q – Gross calorific value of the sample (J g⁻¹)

C – Heat capacity of the calorimeter system (J K⁻¹)

Δ T – Increase in temperature of the calorimeter system during a combustion experiment (K)

Q₁ – Extraneous energy from combustion of the cotton thread (J)

m – Mass of the sample (g)

It is possible to use acquired gross calorific value to determine other parameters, such as:

- Net calorific value (calorific value of combustible substance; the weight of sample reduced by weight of ashes after burning down).
- Heating value of biomass (the usage of biomass as a fuel for direct combustion).
- The number of energetic balances when growing plants etc.

The usage of calorimetric method for studying plants has been already presented by Long (1934). The content of energy in plant material is given by the chemical composition in the plants and it can differ for individual plant species (Yajing et al., 2007). In mixed association, such as permanent grasslands, the content of energy is dependent on composition of species, but it can be changed during the vegetation (Neitzke, 2002). It depends on proportion of individual parts of plants (Sims & Singh, 1978), on ecological or climatic conditions (Long, 1934) and on other parameters.

As far as anthropogenic aspects are concerned, frequency of mowing and dosage of nutrients are considered to be the most important factors influencing the production of permanent grasslands. When appraising the significance of those as well as other ways of management on any of the indicators (the quality of the fodder, the content of energy in the fodder etc.), it is necessary to consider mainly experiments, where the chosen type of management is being applied on long-term basis. Permanent grasslands are dynamic associations, where stabilization occurs after long-term application of applied treatments. These experiments have higher testifying value than the short term ones.

The data presented below are results of long-term meadow experiments, where the dominant species was meadow foxtail (*Alopecurus pratensis*), alliance *Deschampsion cespitosae*. The experimental locality is situated near village Černíkovice, Czech Republic (49°46'26"N, 14°34'52"E), on alluvial meadow in 363 m a.s.l. Average annual rainfall is 664 mm, average annual temperature of locality is 7.2 °C. The soil type is Gleyic Fluvisol with level of underground water in range of 0.1 – 0.5 m under the surface.

The experiment with application of various doses of nutrients was started in 1966 and it is sorted by method of randomized blocks in four replications. The area of individual plots is 15 m² (3 x 5 m). The stand is harvested in three subsequent cutting. There are six different treatments:

- N₀P₀K₀ – no fertilization
- N₀P₄₀K₁₀₀ – application of 40 kg P ha⁻¹ + 100 kg K ha⁻¹ year⁻¹
- N₅₀P₄₀K₁₀₀ – application of 50 kg N ha⁻¹ + PK
- N₁₀₀P₄₀K₁₀₀ – application of 100 kg N ha⁻¹ + PK
- N₁₅₀P₄₀K₁₀₀ – application of 150 kg N ha⁻¹ + PK
- N₂₀₀P₄₀K₁₀₀ – application of 200 kg N ha⁻¹ + PK

There was another experiment with different frequency of mowing found in 2001 at the same locality. The harvests are realized in May and in October for the two cuts per year treatment and in October for the one cut per year treatment. The monitored plots are not fertilized.

The samples of biomass for determination the content of energy were taken during vegetation period in years 2007 - 2009. The calorific value in the dry biomass was measured by the automatic adiabatic calorimeter system IKA C 5000 control. The calorific value was calculated according to the Czech State Standard ČSN ISO 1928 (1999), without the dissolving temperature of sulphuric acid and nitric acid correction.

Differences in calorific value in above-ground biomass of the permanent grassland according to supply of nutrients and sequence of cutting are presented in Fig. 1 and Fig. 2. Calorific value in above-ground biomass was, in average of three cuts, significantly influenced by treatment of fertilizing ($P = 0.0004$), where the lowest value (16891 J g⁻¹) was recorded with the variant which was not fertilized and the highest value (18143 J g⁻¹) with the variants fertilized by nitrogen. There was no proof of any significant influence of increasing the dosage of nitrogen. The content of energy in biomass differed in individual cuts ($P = 0.0053$). The highest one was in the first cut (18131 J g⁻¹) and the lowest was in the third cut (17237 J g⁻¹).

Although the presented results document significant effect of fertilization and cutting sequence on energy content in biomass, it is necessary to emphasize that the differences between minimal and maximal values range up to 10 %. This fact can be also noticed from results of another experiment in which permanent grassland that is cut once a year is compared with a different treatment cut twice a year. Significantly highest content of energy ($P = 0.0003$) in years 2007 – 2008 was recorded in biomass during spring harvest (18620 J g⁻¹), and lowest (18006 J g⁻¹) during autumn harvest of the twice cut treatment. Content of energy in biomass from treatment, which was cut once a year in autumn (18203 J g⁻¹), did not differ from autumn harvest from treatment which was cut twice a year.

Fig. 1. The effect of fertilization on calorific value (kJ g⁻¹) of above-ground biomass of permanent grassland, 2009, Černíkovice locality, Czech Republic

Fig. 2. The effect of cutting sequence on calorific value (kJ g⁻¹) of above-ground biomass of permanent grassland, 2009, Černíkovice locality, Czech Republic

A difference in energy value of plants with different supply of nutrients was already recorded by Long (1934). Neitzke (2002) detected an influence of an increase in the nutrient supply on the calorific values only in some types of grasslands. On the contrary, Úlehlová (1980) while studying content of energy of permanent grasslands with low production of biomass, did not record any differences when using fertilization. The differences among weed species (*Elytrigia repens*, *Cirsium arvense*, *Chenopodium album*, *Amaranthus retroflexus*, *Echinochloa crus-galli*) recorded Fuksa et al. (2006). The calorific value of dry matter ranged from 16800 J g⁻¹ (*A. retroflexus*) to 18210 J g⁻¹ (*E. crus-galli*).

It is possible to conclude, that the change in content of energy in harvested biomass can be caused by change in chemical composition of plants within the species as a reaction to fertilization, and also by change of species composition of the vegetation. If the fertilization in a specific experiment has a low impact on composition of species or on increase of biomass in context of plants' chemical composition, its impact will be also low as far as content of energy in plants is concerned. According to Fuksa et al. (2006) and Brant et al. (2011) it is necessary to replenish that calculation of energy produced from certain area of land is dependent primarily on yield of biomass, as content of energy in biomass has smaller variability than the yield. For precise calculation of energy balances, however, determining the content of energy in biomass is important.

4.2 Factors affecting primary production of permanent grasslands

Ability of yield of permanent grasslands is dependent on botanical composition, which is an outcome of interaction of stands' conditions, competitive relationships among plants and a way of stand management. Composition of stand is usually affected the most by water and nutritional regime of the locality. Other edaphic, climatic and orographic factors, similarly to biotic factors (interaction with plants, animals and microorganisms) have lower impact in relation to botanical composition.

The most important agrotechnical intervention that can be used to affect primary production of permanent grasslands is regular cutting or pasture. The absence of management usually leads to degradation of grassland. Another important and very effective factor is fertilization. Other interventions (for example changes in water regime) are applied only on small areas, or they are not very effective in affecting yield (harrowing, dragging, rolling, additional seeding etc.).

4.2.1 The impact of cutting on primary production

While cutting, large part of assimilation area of plants is removed. Number of cuttings, date of mowing and the height of growth that is being mowed affects not only yield and quality of harvested matter, but also the ability of plants to regenerate for further growth.

High **frequency of cutting** has rather negative impact on yield of biomass, especially during the first half of vegetation period. The plants that are cut regenerate from nutrients stored in their root system. In residue of leaves and stalks there is still photosynthesis going on, however, because of small assimilation area, the production of carbonaceous agents is quite low. As a result the plants grow initially very slowly. However, with larger assimilation area also increases speed of growth. The more often plants reach this period, the less biomass is

produced during the year. The process of regeneration of growth can be significantly sped up by applying high dose of N-fertilizer (Frame, 2000). On the other hand, short intervals between individual cuttings lead to increased quality of harvested biomass. Plants in early phase of growth have lower content of fiber and higher content of proteins and water-soluble carbohydrates. This is favourable from the point of using biomass as a fodder or while the process of biogas formation.

Table 1 presents results of experiment with varying frequency of mowing from experimental location Nicov, Czech Republic. This stand of permanent grassland is located in 880 m a.s.l. (49°7'35.027"N, 13°37'0.435"E), on Loamy-sand type of soil. Long-term average of temperature is 6.0 °C, and long term average of rainfalls is 819 mm per year. The experiment was arrangement in the block design in three replications. The area of one plot is 18 m² (1.5 x 12 m). Factors being monitored are various doses of nitrogen (40 kg N ha⁻¹, 80 kg N ha⁻¹ and variant without fertilization) and double frequency of mowing (two-cut and four-cut variant). The dose of 40 kg N ha⁻¹ was applied on a one-time basis at the beginning of the vegetation period, dose of 80 kg N ha was divided into 40 kg N ha⁻¹ at the beginning of the vegetation period and 40 kg N ha⁻¹ after first mowing.

Number of cuts	Fertilization (kg ha ⁻¹)	1 st cut	2 nd cut	3 rd cut (t ha ⁻¹)	4 th cut	Total yield
2 cuts	N ₀ P ₀ K ₀	3.92	1.97	-	-	5.89
	N ₄₀ P ₀ K ₀	4.77	2.14	-	-	6.91
	N ₈₀ P ₀ K ₀	5.41	2.76	-	-	8.17
4 cuts	N ₀ P ₀ K ₀	2.68	1.44	1.23	0.40	5.76
	N ₄₀ P ₀ K ₀	2.84	1.48	1.26	0.34	5.92
	N ₈₀ P ₀ K ₀	3.16	1.90	1.44	0.39	6.89

Table 1. Effect of cutting and fertilization on biomass yields of permanent grassland (t_{DM} ha⁻¹), average of years 2007 – 2009, Nicov locality, Czech Republic

There was no significant yield difference between two-cut and four-cut utilization in the locality of Nicov. Increasing size of doses of nitrogen led to an increase in yield in both variants of mowing, and to higher yield when using same dose of nitrogen were recorded when using the two-cut variant. These results show that the most suitable regime for mowing should come out of conditions of locality, as well as of the anticipated level of fertilization (Table 1).

From the result's listed in the Table 2, we can clearly see that one-cut variant used in the locality of Černíkovice is not compatible with the length of growth at the stand, and this represents significant loss in yield, or more precisely in energy, when compared to the two-cut variant.

Frequency of mowing also affects yield of biomass in indirect way, through changes in botanical composition of the growth. In general, frequent mowing reduces presence of high-growing species and supports increase in share of low-growing and shade-intolerant species, including leguminous species. Positive impact of leguminous species comes from their ability to assimilate aerial nitrogen with the help of rhizobia (Soussana & Tallec, 2010).

Changes in botanical composition have been described by number of authors. For example Kramerger & Gselman (1997) found that when using higher doses of N (180 kg ha⁻¹) + PK, we can find in grasslands that are mowed often (6 times a year) more species, then in grasslands that are mowed only 2-3 times a year. Authors contribute this to competition over light between the plants. On the opposite, Zechmeister et al. (2003) describes negative correlation between richness of species variation and intensity of mowing. The highest amount of cuts that were included in the study, however, was 4 times a year. Level of fertilization was also lower.

Number of cuts	Fertilization (kg ha ⁻¹)	Yield (t ha ⁻¹)	Energy outputs (GJ ha ⁻¹)	Energy inputs (GJ ha ⁻¹)	Energy gain (GJ ha ⁻¹)	Energy effectiveness (GJ GJ ⁻¹)
1 cut	N ₀ P ₀ K ₀	4.80	87.38	1.58	85.77	55.19
2 cuts	N ₀ P ₀ K ₀	7.91	145.39	2.81	142.58	51.66
3 cuts	N ₀ P ₀ K ₀	6.08	102.97	3.11	99.86	33.13
	N ₀ P ₄₀ K ₁₀₀	6.53	115.40	6.55	108.84	17.61
	N ₅₀ P ₄₀ K ₁₀₀	8.56	154.51	11.34	143.17	13.63
	N ₁₅₀ P ₄₀ K ₁₀₀	10.83	199.24	20.21	179.03	9.86

Table 2. Effect of cutting (average of years 2007 – 2008, data in the upper part of the table) and effect of fertilization (2009, data in the lower part of the table) on biomass yield (t_{DM} ha⁻¹) and energy balance of permanent grassland, Černíkovice locality, Czech Republic

The optimal date for **first mowing** is from the beginning to full earing of the predominant grasses in the sward. Earlier mowing means increase of the quality and lower yield of fodder, later mowing results in the opposite (Frame, 2000). When we utilize early mowing, we support growth mainly of lower-growth species that are little affected by defoliation. When we utilize late mowing, there is decrease in quality of overgrown sod, which is felt the most in dry areas.

Height of mowing determines how much of assimilation area and reserve material is kept. Optimal height for mowing permanent grassland's is 30 – 40 mm. Lower height of mowing is more tolerable to creeping species (for example *Poa pratensis*, *Festuca rubra*, *Agrostis gigantea*, *Alopecurus pratensis*) than bunch type of grasses (*Dactylis glomerata*, *Phleum pratense*, *Festuca pratensis*, *Lolium perenne*, *Arrhenatherum elatius* etc.).

4.2.2 Effect of fertilization on primary production

Nutrients removed by harvest of permanent grasslands can be compensated for one part from soil's resources, for another part from the atmosphere (most importantly N) and also from application of fertilizers. The question of fertilizing permanent grasslands represents complex problem, which is composed of diversity and colorfulness of composition of swards in relationship to water and nutritional regime, the way sward is utilized, weather conditions, type of fertilizers, date and method of applying fertilizers etc.

Fertilizing supports development of species which have higher ability to use nutrients for creating a large amount of biomass and gain competitive advantage in this way. As a consequence plants of lower growth that reside in shade are eliminated from growth

(Lepš, 1999). Those are usually less valuable components of grasslands. Fertilization also supports creating of new tillers and larger foliage and in general it creates more robust habitus of plants.

The effect of fertilization is usually bigger on swards that are less productive, but are composed of species which react well to fertilization. If favourable humidity conditions are present, it is possible with help of fertilization by mineral fertilizers to increase the yield by 100 to 200 % as show for example Honsová et al. (2007).

Hopkins (2000) points out that at some localities, even if grasslands are based on very productive species (for example *Lolium perenne* and *Trifolium repens*), it is necessary to apply high doses of nutrients for maintaining of high yield, while original swards usually have much lower demands in this area.

Source of production stability is high diversity of species in swards, whose auto-regulative mechanisms allow alternating dominance of group of species which are best adapted to climatic conditions of the particular year.

Nitrogen is considered to be the most important nutrient for increasing yield, but its overall effect has to be considered in more broad perspective. The response of sward to nitrogen fertilization was researched in many experiments all over the Europe. Most of grasslands were mowed at the same time, doses of N were ranging from zero to the extreme of 600 kg ha⁻¹. The specific reaction of the sward is also dependent on other factors – the availability of water, weather of season, type of sward (content of leguminous species, density of grass shoots, the size of root system etc.), soil's characteristics, and frequency of defoliation (Hopkins, 2000).

The increase of biomass when applying N is usually linear (15 – 25 kg of dry matter for 1 kg of N) up to doses of 250 – 350 kg ha⁻¹. When applying higher doses of N (350 - 450 kg ha⁻¹) the increase of yield drops down to 5 – 15 kg for 1 kg of applied N. Increase in yield stops at doses of 450 – 600 kg ha⁻¹. When using mixture of grass and white clover, the yield increases in linear fashion up to 250 – 300 kg ha⁻¹. The increases of biomass yield are lower, as clover gradually declines, until it disappears altogether and the grassland is then composed only of grass component (Frame, 2000; Whitehead, 1995).

Excessive input of nitrogen leads to undesired changes in vertical structure of growth, to mutual casting of shadow on leaves, to turning yellow of lower levels of sward and to reduction in photosynthesis. Natural fixation of nitrogen is stopped, unused nitrogen is leached into underground water and increased content of free nitrates in the plants appears.

Annual individual nitrogen fertilization usually leads to initial increase in mass, but after several years the production decreases again as a result of exhaustion of other nutrients (Van Der Woude et al., 1994). At some localities the application of nitrogen does not have to produce any effect, because the growth of grassland is limited by different source (Malhi et al., 2010) – usually by phosphorus or potassium. Niinemets & Kull (2005) recorded significant increase in yield of grasslands on calcite soil even when phosphorus was applied solely. When phosphorus and calcium were applied in combination, the increase in yield was higher than when it was applied individually.

Impact of **phosphorus** on yield is, due to its low mobility, visible only after several years have passed. Its usage is increased when there is sufficient humidity and low reserve of available potassium in soil and it is dependent on botanical composition of the growth. Production effectiveness of P-fertilization is in average about 5.3 kg of dry matter to 1 kg of added P. With simultaneous application of K the production effectiveness increases to 22.5 kg of dry matter to 1 kg of added P (Klapp, 1956). The yield variability is usually higher than in sward that are not being fertilized, as a result of variations in cover by leguminous species. On soil with low content of P its influence can be supported by simultaneous N-fertilization (Frame, 2000).

Potassium is more mobile in soil than phosphorus and plants accept it easily. Its production effectiveness is lower than effectiveness of phosphorus. Klapp (1956) lists relative increase of yield by 12.5 %, and it increases throughout the years, as K-fertilization is continuously applied. The biggest effect is achieved on impoverished peat and peaty soils. To achieve good effect, it is important that sufficient humidity is present. Lack of K can be felt during dry years and particularly on stands with very low reserve of K in soil. In general, with insufficient potassium nutrition there is a reduction of photosynthesis, growth is slowed down, and there is a decrease in yield and quality of fodder, regardless of any potential N-fertilizing. There is a constraint in root system and together with decrease in effectiveness of regulating transpiration of leaves, the plant are more susceptible to dry weather (Frame, 2000).

The inaccessibility of phosphorus in soil for plants is often caused by too low level of pH. This can be improved by appropriate **liming**. Applying of calcium independently has only small effect on yield of biomass. It can cause only transitory mobilization of nutrients in soil, which leads to short-term increase in biomass yield. After exhausting all available nutrients, yield is reduced yet again. The increase in yield after liming varies within broad limits and is strongly dependent on placement and specific conditions (Klapp, 1956).

The effect of fertilization on above-ground biomass in permanent grassland is well visible in Fig. 3, which records long-term yields of four variants of fertilization used in experiment in Černíkovice. Yields were significantly affected by climatic conditions of the particular year. Lowest yields were always recorded in $N_0P_0K_0$ variant, which was not fertilized. Yields from areas fertilized by PK started to show differences, from unfertilized areas, after approximately 25 years of periodical fertilization. Higher yields were recorded particularly in years that were favourable to growth of leguminous species. NPK-fertilizing, in most cases, significantly increased the yield. When 200 kg N ha⁻¹ was applied, higher effect was recorded, in contrast with dose of 100 kg N ha⁻¹.

In table 3 yields of biomass and production of energy of permanent grassland are shown which were evaluated from the same experiment in time period of 2007 – 2009. Total yield was significantly increasing as a result of applying nutrients: from 6.08 t ha⁻¹ for non-fertilized variant to 9.67 t ha⁻¹ for the variant $N_{150}P_{40}K_{100}$. Significant effect of fertilization was recorded during monitored time period particularly during first mowing. It was not recorded in the course of following mows. This aspect can contribute to application of dose of nitrogen which happened before first mowing, which is also visible in share of first mowing on the total yield (44 – 57 %) rising proportionately to the dosage of fertilizer being applied.

Fig. 3. Development of biomass yields ($t_{DM} \text{ ha}^{-1}$) of permanent grassland in 1967 – 2006, Černíkovice locality, Czech Republic (Honsová et al., 2007)

Fertilization (kg ha^{-1})	1 st cut	2 nd cut	3 rd cut (t ha^{-1})	Total yield
$\text{N}_0\text{P}_0\text{K}_0$	2.66	2.54	0.88	6.08
$\text{N}_0\text{P}_{40}\text{K}_{100}$	3.52	3.00	0.95	7.46
$\text{N}_{50}\text{P}_{40}\text{K}_{100}$	4.64	3.00	0.97	8.62
$\text{N}_{150}\text{P}_{40}\text{K}_{100}$	5.46	3.13	0.95	9.54

Table 3. Effect of fertilization on biomass yields of permanent grassland ($t_{DM} \text{ ha}^{-1}$), average of years 2007 – 2009, Černíkovice locality, Czech Republic

It is particularly important how high the yield was in relationship to the total production of energy that was determined for year 2009, which means that the calculated values were increasing proportionately to the increase in level of fertilization (Table 2). Highest values for the average of all variants of fertilization were recorded during first mow (67 GJ ha^{-1}) and the lowest during third one (16 GJ ha^{-1}). Total production of energy reached on these grasslands in Černíkovice ($102.97 - 199.24 \text{ GJ ha}^{-1}$) is comparable to total production of plants grown on arable soil. For example Fuksa et al. (2006) presented data on total energy production of silage maize in range from 107.27 to $231.04 \text{ GJ ha}^{-1}$, depending on particular

year and the way growth was treated to improve its protection against weed. Rösch et al. (2009) set the production of energy from 66 GJ ha⁻¹ (low-input grassland) to 119 GJ ha⁻¹ (high-input grassland).

From the perspective of total energy balances (chapter 4.3) it is not only the total production of energy that is significant, but it is also important to consider necessary energy inputs, which are particularly high for nitrogenous fertilizers.

4.2.3 The effect of fertilization on composition of species

One of main factors affecting composition of species in grassland is the availability of nutrients, which is possible to significantly alter by usage of fertilizers (Hejcman et al., 2007). The most significant effect of fertilization is a direct influence on individual species, which will result in change of reciprocal abilities to compete. At the same time, indirect effect of nutrients will start, for example there will be increase in density of the sward, which leads to less light being able to penetrate into lower levels, which can provoke even more pressure to compete.

The number of species of plants has very close relationship to production of above-ground biomass (for example Hejcman et al., 2007; Oomes, 1992). On more impoverished localities, a number of low-growing species coexists. When the placement is gradually enriched by nutrients, the production of the above-ground biomass increases. At the same time, plants that are more demanding and higher are also able to spread and competition over light in the sward increases. Lower-growth species of plants disappear from grassland and only few species most capable in competition remain (Guo & Berry, 1998).

N-fertilization has the most profound effect in the starting period (3 – 6 years). It increases the cover of grasses (directly in proportion to the doses of N), mainly higher bunch and rhizomatic species. On oligothropic soils, the low grasses are replaced by medium-grown ones. At the same time, there is a significant decrease of cover of legumes – critical level of N doses, which can still maintain 10 – 15 % of legumes in meadows, is according to ecological conditions 50 – 60 kg ha⁻¹. There is also a significant reduction in cover of other dicotyledonous species, particularly the lower ones (Mrkvička et al., 2006; Whitehead, 1995).

The following period is prominent for increase in cover of rhizomatic grasses, which gradually become the main part of the sward. The speed of their spread and size of the share of the sward they have is in direct proportion to dosages of N being applied (Honsová et al., 2007). Rhizomatic grasses have higher capacity of reserve organs, bigger leaf area left after mowing and higher ability of vegetative reproduction. Even though they are of lower growth, they are better adapted to applications of high doses of available N than bunch grasses are.

N fertilization not only significantly affects botanical composition, but its effect is also long-term. Systematic fertilization using higher doses of N (above 100 kg ha⁻¹) gradually creates more simple, largely grass stands, and their grow shows higher dependence on meteorological conditions. This effect does not have to affect all localities, however, in some cases it is conditioned by adding PK-fertilization at the same time (Schellberg et al., 1999).

Negative effect of N-fertilization is a reduction of number of species in the sward. When the doses of nitrogen are increased, the number of species is reduced by 50 – 60 % (Willems et al., 1993; Zechmeister et al., 2003). N does not have to be the single factor affecting number of species, however. Permanent grasslands that are rich in species and are limited by N have about 30 – 40 species on area of 1 m², swards limited by N and P can be even more rich in species. For example Niinemets & Kull (2005) recorded 70 – 90 species in area of 1 m². Hejcman et al. (2007) found that many undemanding species that can spread in sward are fertilized solely by N, but when the PK-fertilization is added, they quickly start to decline.

Fertilization by phosphorus and potassium has usually smaller effect on botanical composition of the sward, but it still has mostly positive effect. Its significance is higher on soils with lower content of available P and K. When PK-fertilizers are systematically applied, there is an increase in a share of legumes from average of 15 % for unfertilized swards up to 20 – 25 % (Klapp, 1956). At the same there is a decline in other dicotyledonous species (Mengel & Steffens, 1985). PK-fertilization also slightly increases a share of lower-growth and medium-growth grasses.

At some localities, P subsidiaries can negatively affect the number of species. Wassen et al. (2005) describes a case where limitation of N was more important than limitation of P. Enrichment of soil by phosphorus can lead to decline in species adapted to its low accessibility. Some of the more rare species can be suppressed that way and are often replaced by the more common species.

Over fertilization by potassium leads significantly to negative effects. When extreme doses are applied and inappropriate ratio of N : P : K is used, it leads not only to decline of legumes, but later even grasses are forced out and ruderal weeds spread, such as *Rumex obtusifolius* etc. Shortage of K, on the other hand, reduces the ability of plants to survive winter. *Trifolium repens* is particularly sensitive to its shortage and it can result in a complete disappearance from the growth (Frame, 2000).

Positive effect of P and K on the spread of legumes can be supported by suitable **application of Ca**. Ca itself has generally small effect on botanical composition of the growth. However when the pH is modified on acid soils, there can be suppression of some of the more sensitive species.

Conclusive **effect of variants** of fertilizing on botanical composition is shown on long-term fertilized grassland in Černíkovice. In the total evaluation of years 2007 – 2009 the variants of fertilization explained 21.6 % of data variability. Ordination diagram (Fig. 4) shows the spread of intensity of fertilization along 1. axis (15.8 % of variation). The difference between unfertilized variant and PK variants and the variants that used N-fertilization is noticeable. Along the second axis there is a gradual increase in doses of applied N, which caused differences between botanical composition of N₅₀P₄₀K₁₀₀ and N₁₀₀P₄₀K₁₀₀ variants and the variants N₁₅₀P₄₀K₁₀₀ and N₂₀₀P₄₀K₁₀₀. Species that prospered on unfertilized and PK treatments were from monocotyledonous species, for example *Luzula campestris*, *Agrostis capillaris* and *Anthoxanthum odoratum*, furthermore all legumes (particularly *Lathyrus pratensis*), and number of other dicotyledonous species, for example *Alchemilla* sp. and genus *Ranunculus*. For variants N₅₀P₄₀K₁₀₀ and N₁₀₀P₄₀K₁₀₀, *Holcus lanatus* and *Deschampsia cespitosa* were dominant. For variants N₁₅₀P₄₀K₁₀₀ and N₂₀₀P₄₀K₁₀₀, high grass *Alopecurus pratensis* was dominant in the first place, and furthermore also *Urtica dioica* and *Elytrigia repens*. Analysis

of redundancy (RDA) has also shown conclusive **effect of year** on botanical composition, which has explained 3.2 % of data variability.

Note: *Agrostis capillaris* – AgroCap, *Agrostis stolonifera* – AgroSto, *Achillea millefolium* – AchiMil, *Ajuga reptans* – AjugRep, *Alchemilla sp.* – AlchSp, *Alopecurus pratensis* – AlopPra, *Anthoxanthum odoratum* – AnthOdo, *Anthriscus sylvestris* – AnthSyl, *Cerastium holsteoides* – CeraHol, *Cirsium arvense* – CirsArv, *Cirsium palustre* – CirsPal, *Cirsium vulgare* – CirsVul, *Cynosurus cristatus* – CynoCri, *Dactylis glomerata* – DactGlo, *Deschampsia caespitosa* – DescCes, *Elytrigia repens* – ElytRep, *Equisetum palustre* – EquiPal, *Festuca pratensis* – FestPra, *Glechoma hederacea* – GlecHed, *Holcus lanatus* – HolcLan, *Lathyrus pratensis* – LathPra, *Luzula campestris* – LuzuCam, *Lysimachia nummularia* – LysiNum, *Plantago lanceolata* – PlanLan, *Poa pratensis* – PoaPra, *Poa trivialis* – PoaTri, *Ranunculus acris* – RanuAcr, *Ranunculus auricomus* – RanuAuri, *Ranunculus repen* – RanuRep, *Rumex acetosa* – RumeAce, *Stellaria graminea* – StelGra, *Taraxacum sect. Ruderalia* – TaraSp, *Trifolium dubium* – TrifDub, *Trifolium hybridum* – TrifHyb, *Trifolium repens* – TrifRep, *Trisetum flavescens* – TrisFla, *Urtica dioica* – UrtiDio, *Veronica arvensis* – VeroArv, *Veronica chamaedrys* – VeroCha, *Veronica serpyllifolia* – VeroSer

Fig. 4. RDA analysis of relationship between species composition of permanent grassland and variants of long term fertilization, average of years 2007 – 2009, Černíkovice locality, Czech Republic

Suitable management of permanent grasslands can lead to increase in diversity, although this usually has negative impact on production. Biodiversity has value not only from the point of ethical and esthetical view, but it also is important for preserbing species and their genotypes, as the diversity of ecosystem leads to its stability (Nösberger & Kessler, 1997).

4.3 Energy balance of permanent grassland

Energy balance of permanent grassland comes from comparison of inputs and outputs of energy. Energy outputs are divided into energy of plant production, residue of plants and irreversible energetic losses. For permanent grassland, the largest share is composed of above-ground biomass (industrially or alternatively usable biomass), as mentioned in chapter 4.2.

Input energy consists of energy of outer environment (sunlight, energy in soil, atmosphere and infrastructures of surround environment) and energy of technological inputs, which consist of direct part (energy of human work, fossil energy, other energy sources – draught animal etc.), and indirect part (energy of mechanisms, products of chemical industry, organic fertilizers, seeds etc.) (Hülsbergen et al., 2001). Additional energy can increase volume of sunlight energy that is captured in biomass (Jones, 1989).

Table 2 shows calculated energy inputs of permanent grassland with different levels of fertilization and frequency of mowing being applied, at the experimental location of Černikovice. Energy of human work, technological interventions (application of fertilizers, mowing and hay-making) and applied fertilizers were included in energy inputs. The largest share of inputs for the fertilized permanent grasslands is formed from energy in form of nitrogenous fertilizers. For grassland that is mowed three times a year, there was more than six times larger difference in the total value of energy inputs between unfertilized variant and the highest level of fertilization variant ($N_{150}P_{40}K_{100}$). On the contrary, very low values were found for unfertilized grasslands that were harvested once or twice a year.

As is above-mentioned, total production of energy is dependent mainly on levels of yield reached, because the content of energy in biomass of permanent grassland has low variability. Assessment of energy balance further allows to evaluate total effectiveness of production of energy, when considering the same energy inputs into system. We calculated values of **energy gain** (difference between energy production and inputs of energy) and **energy effectiveness** (how much energy is produced from one unit of energy input) from the results of evaluation of experiments with different ways of managing grasslands in locality of Černikovice.

From the evaluated energy balances follows that with the increase in level of fertilization, there is a significant increase of energetic gain (Table 2). For the $N_{150}P_{40}K_{100}$ variant the energy gain was higher by 79.17 GJ ha^{-1} when compared to unfertilized variant. This difference was gained by increasing inputs from 3.11 to 20.21 GJ ha^{-1} , which means by 17.10 GJ ha^{-1} .

In comparison to energy gain, highest values of energy effectiveness were reached in grassland with minimal inputs (no fertilization, low frequency of mowing). With the intensification of management (particularly fertilization), this value decreases. Energy effectiveness of crops grown on arable soil is according to findings of Hülsbergen et al. (2001) for example for potatoes 4.3, winter wheat 14.4, winter barley 9.4, spring barley 9.9 and sugar beets 11.1 GJ GJ^{-1} . With the increase of additional energy inputs the energy effectiveness in permanent grasslands decreases, however it still reaches higher values in comparison with crops grown on arable soil.

On the basis of the present results it can be concluded, that for swards with suitable botanical composition while the energy in form of fertilizers is added, it leads to adequate yield reaction and the value of energy gain reached is comparable with intensive crops on arable soil. In contrast to regular crops on arable soil, permanent grasslands reach noticeably higher levels of energy effectiveness as a result of very low energy inputs into system.

5. Biogas production from permanent grassland

The aim of supplying crop feedstock for biogas production is to achieve the highest possible biogas yields per area unit ($\text{m}^3 \text{ha}^{-1}$). The area biogas yield consists of the substrate biogas yield ($\text{l kg}^{-1}_{\text{ODM}}$; ODM - organic dry matter) and the biomass yield ($\text{kg}_{\text{ODM}} \text{ha}^{-1}$). The substrate biogas yield depends on biomass quality on one hand and on biogas technology and process of engineering on the other hand (Prochnow et al., 2009b). It is possible to affect effectiveness of production of biogas by using suitable combination of biomass yields and its quality from the standpoint of biogas production. The most significant controllable factors, which determine the potential of yield of permanent grasslands, have been already described in the previous parts of the chapter. Quantity of biomass is, however, closely connected with its quality. The factors, which affect grasslands production, therefore have an impact on its quality as well.

5.1 Quality of biomass used for biogas production

In general, all types of biomass (liquid manure, energy plants, bio waste, sewage sludge) can be used as substrates, as long as they contain carbohydrates, proteins, fats, cellulose, and hemicellulose as main components (Deublein & Steinhauser, 2008). According to Amon et al. (2007), the methane production from organic substrates mainly depends on the content of nutrients (crude protein, crude fat, crude fiber, N-free extracts), which can be degraded to CH_4 and CO_2 . The biogas represents mainly the mixture of CH_4 and CO_2 with minority ratio of other gases (N_2 , O_2 , etc.). Ratio of CH_4 in biogas usually varied from 60 to 65 % (Straka et al. 2006).

The organic matter is, the only source of utilizable energy, which was stored in it by the energy of sunlight while the plants were growing. This organic matter allows storing the energy up to the time when it is released again, in the process of its degradation while influencing of microorganisms in rumen or biogas plant.

Requirements on the biomass quality are different when crops are anaerobically digested in biogas plants compared to being fed to cattle. The digester at the biogas plant offers more time to degrade the organic substance than the rumen does. In addition, it is likely to assume that the microorganism population in the digester is different from that in the rumen (Amon et al., 2007). As with fodder for animals, chemical analysis of biomass can be considered as basic evaluation of quality, which assesses the content of individual nutrients. There is a difference in the specific methane yield of crude fat ($850 \text{ l kg}^{-1}_{\text{ODM}}$), crude protein ($490 \text{ l kg}^{-1}_{\text{ODM}}$), and carbohydrates (crude fiber and N-free extracts: $395 \text{ l kg}^{-1}_{\text{ODM}}$) (Karpenstein-Machan, 2005, as cited in Amon et al., 2007). The assessed content of nutrients gives no picture of how well they are degradable, which is dependent also on technological aspects of biogas transformation itself. For this reason, the chemical analyses of total content and mutual ratios of individual nutrients represent in the

first place starting potential for following degradation. Real yield of biogas from plant biomass is then affected mainly by technological parameters of the process of degradation, from the size of input particles, parallel fermentation of various substrates, to compliance with suitable conditions for all levels of micro-bacterial degradation.

In the following sub-chapters 5.2 there are results of experiments with various types of management of permanent grasslands and the impact on yields of substrate and total production of biogas described.

5.2 Substrate biogas yield

Assessment of yield of biogas from biomass represents basic qualitative characteristic in this process of energy production. As mentioned before, the basic thing is content of individual nutrients. This fact leads to logical effort to theoretically calculate production of biogas from its content. Amon et al. (2007) described the methane energy value model, which estimates methane yield from the nutrient composition of energy crops in mono fermentation via regression models. This model investigates and considers the impact of the content of crude protein, crude fat, crude fiber and N-free extracts on the methane formation. It is necessary to put a reminder here, however, that the calculations based on content of nutrients or laboratory tests of amount of yield described below, show potential degradation of biomass with ideal conditions present. As above-mentioned, real values reached depend on technological aspects of fermentation in a specific biogas plant.

Table 4 presents results of substrate biogas yield in litter per kg of dry matter (1 kg⁻¹_{DM}) from two experimental locations (Nicov and Černíkovice) in 2009. Characteristics of locality conditions and design of these experiments are mentioned in chapters 4.1 and 4.2.

Locality	Fertilization (kg ha ⁻¹)	1 st cut	2 nd cut	3 rd cut	4 th cut
		(l kg ⁻¹ _{DM})			
Nicov	N ₀ P ₀ K ₀	318	380	-	-
	N ₄₀ P ₀ K ₀	364	367	-	-
	N ₈₀ P ₀ K ₀	338	317	-	-
	N ₀ P ₀ K ₀	445	331	407	503
	N ₄₀ P ₀ K ₀	358	425	453	445
	N ₈₀ P ₀ K ₀	375	418	405	414
Černíkovice	N ₀ P ₀ K ₀	520	410	541	-
	N ₅₀ P ₄₀ K ₁₀₀	545	484	483	-
	N ₁₅₀ P ₄₀ K ₁₀₀	446	467	588	-

Table 4. Substrate biogas yield (l kg⁻¹_{DM}) from permanent grassland, 2009, Nicov and Černíkovice localities, Czech Republic

The substrate biogas yield was assessed while using laboratory batch test. The plant material was processed in fresh state, immediately after harvest of monitored grasslands. Basic homogenization and grinding of matter followed. Tested biomass was put together with inoculum in doses into fermentors that were gas-sealed. Biomass from experimental locality of Nicov was tested in 2 litres bottles in three replications for each variant. Dosage of mixed substrate was 1000 g (100 – 200 g of biomass and 800 – 900 g of inoculum). Cultivation took place in thermo box at 37 °C. Time of delay for mixed substrate in fermentors was 35 days. Biomass from experimental location of Černíkovice was tested in 120 ml bottles in five replications for each variant. Two grams of tested biomass and 80 ml of inoculum were dosed into fermentors. Cultivation took place in thermo box at 40 °C for a period of 49 - 50 days. Production of biogas in laboratory tests of biomass was evaluated once a day from both locations, using gas-metric burette. Besides tests of production of biogas with substrates, cultivation of inoculum itself in the same conditions was done and it was subsequently discounted from production from test bottles with substrates. In this way, net substrate production of biogas was obtained. Active mesophile anaerobic sediment from biogas plant was used as the inoculum.

Values of substrate biogas yield were in range of 317 to 588 l of biogas for kg_{DM} (Table 4). It is clear from the presented results, that the main influence on yield of biomass had term and sequence of mowing, and the highest values were reached when earlier term and higher frequency of mowing were applied. That is in concordance with results summarized by Prochnow et al. (2009b), who found that the yield of methane in general declines as the vegetation phase proceeds. Amon et al. (2007) came to similar conclusions that substrate methane yield declines from value around 300 l kg⁻¹_{ODM} during stem elongation and before inflorescence, to 171 l kg⁻¹_{ODM} during flower stage.

Results in Table 4 also show that in the framework of individual experiments, higher values in yield were reached during the third and the fourth mowing. The reason for that can be the generally applicable negative relationship between quality and quantity, where increasing yield decreases degradability of substrate, because of changes in chemical composition and higher share of lower quality tissues. The yield is usually lower during the third and the fourth mowing, with higher content of leaves in harvested material. Leaves are in general considered to have higher quality and are more easily degradable than stems (Pearson & Ison, 1997). Fertilization had no consistent effect on yield but it is possible to conclude that with strong increase in yield while fertilizing, there was also a slight decrease in yield of substrate. This difference was more apparent during earlier terms of mowing. It is therefore possible to affect quality of harvested biomass mainly by numbers and terms of mowing, and it is necessary to consider earliness and height of plants in the grassland as well.

Another influencing factors, however, has to be considered as well. The specific methane yields of grassland showed significant differences (Fig. 5) between the mountainous and the valley regions (Amon et al., 2007). A low specific methane yield (128 – 221 l kg⁻¹_{ODM}) only was measured from the biomass coming from the hill site, independent of the number of cuts. The grass grown at the valley site produced 190 – 392 l kg⁻¹_{ODM}. The highest specific methane yield was reached in the biomass from the second cut of the four-cut variant. The yield of biogas gained thus depends significantly on specifically varying compositions of species in permanent grasslands different locations. Grasslands always represent mixed associations of different botanical composition, from grasslands that are intense and poor in

species, to grasslands that are quite rich in species. This can lead to different management focused on different goals, which takes into consideration environmental functions.

Barring the content of nutrients in biomass, yield of biogas can also be influenced by modification of substrate. According to Hendriks & Zeeman (2009), pretreatment (mechanical, thermal, chemical) can be done to improve the hydrolysis yield and total methane yield. Mshandete et al. (2006) studied the effect of particle size on biogas yield from sisal fiber waste. Methane yield increased by 23 %, when the fibers were cut to 2 mm size, compared to untreated fibers.

Fig. 5. Methane yield (l kg⁻¹ODM; m³ ha⁻¹) from permanent grassland at two sites (hill and valley) and under different management intensity (Amon et al., 2007)

5.3 Area biogas yield

The yield of biogas from one unit of area represents basic indicator for calculating economical effectiveness of the grown plants. Acceptable supply costs can be achieved at high grass yields, moderate distances of transport and favourable field conditions for machinery operation (Blokchina et al., 2011). As was noted by Deublein & Steinhauser (2008), it is only profitable from an economic point of view, when the materials are sourced from a location within a distance of 15 – 20 km.

Results shown in Fig. 6 and Fig. 7 clearly demonstrate that although the effect of fertilization on substrate biogas yield was inconsistent, suitable doses of nutrients significantly increase production of biogas per hectare. Fig 6 shows furthermore that although higher frequency of mowing increased quality of biomass (Table 4), total production of biogas per hectare was comparable to two-cut and four-cut systems. Four-cut system has higher need for energy inputs (see chapter 4.3), which means the two-cut system can be evaluated as the more suitable system in this case.

Amon et al. (2007) found that area methane yield tends to increase with increasing number of cut and fertilization levels. The biomass yields seem to be more important factor to achieve high area methane yield. According to Gerin et al. (2008), extensity of management of permanent grasslands leads to decrease in yield of dry matter and substrate biogas yield. However, too high intensity does not necessarily have to produce satisfactory results either.

Fig. 6. Area biogas yield (m³ ha⁻¹) from permanent grassland under different management intensity, 2009, Nicov locality, Czech Republic

Fig. 7. Area biogas yield (m³ ha⁻¹) from permanent grassland under different management intensity, 2009, Černíkovice locality, Czech Republic

Therefore, it is possible to conclude that the basic element for determination of suitable management methods is still permanent grassland with specific composition of species, on specific locality, with respect to its environmental functions. According to variability of permanent grasslands, there is no universal optimal management for production of biomass for animals or energy use. The first and the basic step for optimizing production of biogas is a well-chosen number of mows, which will suitably utilize present vegetation period and natural fertility of locality. Terms of mows consequently must be based on planned number of mows considering earliness of sward and actual biomass yield. It is necessary to understand that frequent mows in early vegetation periods do increase substrate biogas yield ($1 \text{ kg}^{-1}_{\text{ODM}}$), but because of lower yield from higher number of mows, reduction of area biogas yield ($\text{m}^3 \text{ ha}^{-1}$) can occur. This aspect of reduction in yield can be partially eliminated on grasslands with suitable composition of species by using adequate fertilization. This significantly increases yield in various regimes of harvest. At the same time, it does not significantly reduce yields of substrate.

6. Conclusion

Utilization of permanent grasslands for production of biogas represents a system with different final adjustment in comparison with utilization of forage for feeding purposes. The basic management of permanent grasslands abides preserved, however optimization of this process can differ from traditional use of biomass. The chapter shows that the term grassland is very wide and includes varied groups of stands. Therefore, it cannot be provided any all-purpose instructions for biogas produce from permanent grasslands. It is also necessary to point out that optimal management, which would cover both productive and non-productive functions of this vegetation, does not exist.

According cited literature and our own results, it is evident that for determination the optimal management of permanent grasslands for production of biogas it is necessary to take into consideration an influence of locality, species composition and other reciprocal biological relations. The system of biogas production from the permanent grasslands can fulfil productive as well as non-productive functions of grasslands. Considering the type of stand it is possible to modify the management towards maximization of production or strengthen their environmental value.

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Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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