

## Reed canary grass (*Phalaris arundinacea* L.) as a promising energy crop

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### Chrastice rákosovitá (*Phalaris arundinacea* L.) jako slibná energetická plodina

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#### ABSTRACT

Reed canary grass (*Phalaris arundinacea* L.) is a perennial fast-growing C<sub>3</sub> plant belongs to family *Poaceae* with an early season growth, a wide physiological tolerance and with large possibilities of utilization. Recently, the use for bioenergy has become very perspective mainly because its high yield (5–10 t dry matter ha/year) and very good properties for combustion. The mean calorific value is about 16–18 MJ/kg dry matter. It can be usually harvested twice a year at lower cultivation inputs and shows the ability to grow in wide range of soil conditions including on land, which is not appropriate for other agricultural purposes. It has also the potential for different industrial applications, for example for biogas, ethanol, pulp and paper production, or for the production of chemical raw materials, too. The cultivation area rapidly increases, mainly in North Europe, where it is cultivated on thousands of hectares. The cultivation for energy or other industrial purposes has also benefits to the environment because of low intensity on agricultural management, supporting biodiversity and soil preservation against erosion.

**Keywords:** bioenergy crop, cultivation, phytomass utilization, Reed canary grass, yields

#### ABSTRAKT

Chrastice rákosovitá (*Phalaris arundinacea* L.) je vytrvalá rychle rostoucí rostlina patří do čeledi *Poaceae* s časným sezónním růstem, širokou fyziologickou tolerancí a se širokými možnostmi využití. V současné době se využití pro bioenergii stává velice perspektivní hlavně kvůli vysokému výnosu (5–10 t sušiny na hektar za rok) a velice dobrým vlastnostem pro spalování. Průměrná výhřevnost je okolo 16–18 MJ/kg sušiny. Může být obvykle sklizena dvakrát za rok při nižších vstupech pěstování a vykazuje schopnost růstu v širokém rozmezí půdních podmínek, včetně pozemků, které nejsou vhodné pro jiné zemědělské využití. Má potenciál také pro jiné průmyslové využití například v případě bioplynu, etanolu, buničiny, papíru nebo surovin pro chemický průmysl. Oblast pěstování rychle narůstá, hlavně v severní Evropě, kde je pěstována na tisících hektarech. Pěstování pro energii nebo jiné průmyslové účely má také přínosy pro životní prostředí kvůli nízké intenzitě zemědělského managementu podporujícího biodiverzitu a ochranu půdy proti erozi.

**Klíčová slova:** energetická plodina, chrastice rákosovitá, pěstování, výnosy, využití fytohmoty

## INTRODUCTION

At present, one of the most significant environmental problems is the risk of non-renewable energy source depletion. One solution to this problem is the use of bioenergy, which is produced by the release of chemical energy contained on raw materials from biological origin (biomass). The importance of this solution is underscored by the fact that energy from biomass can be considered the most promising source of renewable energy because it is not so dependent on natural conditions as opposed, for example, to wind or solar energy (Zhang, 2016). Biomass energy (i.e. bioenergy) such as energy trees (mainly poplar, willow and eucalyptus) and herbs, wood processing residues (mainly pellets and chips), remains of agricultural production (mainly straw in the European Union, maize straw in North America and India, sugarcane bagasse in Brazil) and production processes, domestic and municipal wastes, and food processing wastes (Gasparatos et al., 2017) can be source raw materials for bioethanol, biogas, bio-diesel, bio-oil and solid biofuel production.

All these products are used in different economic sectors. The most general classification of technologies used in the bioenergy field are the thermochemical and biochemical conversions. The thermochemical conversion includes combustion, gasification and pyrolysis, while the biochemical way covers digestion and fermentation. The products used in thermochemical conversion are the so called solid biofuels, which are a source of heat and electricity, while biochemical conversion transforms raw materials into liquid biofuels (e.g. bioethanol, biodiesel), which can be used for example as car fuels, cooking and lighting. Further, the bioenergy is distinguished by biofuels of first, second and third generation (Gasparatos et al., 2017). The source of first generation bioenergy are agricultural crops that have also food or fodder use (Zhang, 2016). They are mostly oilseeds, starchy and sugar crops (Gasparatos et al., 2017). The above-mentioned products can be industrially produced within the first generation bioenergy. Today, first generation bioenergy is even the second largest global energy source behind fossil fuels (Zhang, 2016). The most important first-

generation bioenergy products are biodiesel elaborated in the European Union and India from oilseed rape (*Brassica napus* subsp. *napus*) and in Brazil from soybeans (*Glycine max* (L.) Merrill), ethanol produced in the United States from maize (*Zea mays* L.) and in Brazil and sub-Saharan Africa from sugarcane (*Saccharum officinarum* L.). However, the first generation bioenergy is also related to a risk in food security and price increases. In addition, the plant production of first generation bioenergy often leads to raising environmental problems. The source of second generation bioenergy are crops exclusively used for energy purposes. Therefore, they are not at risk as in first generation bioenergy. The main problem of second generation is the insufficiently solved technology, which is reflected in the disadvantageous economic evaluation and in the impossibility of quality industrial processing. Nevertheless, there is no doubt that the second generation of bioenergy will play a vital role in the development of this branch (Zhang, 2016). However, nowadays more attention is paid to second generation bioenergy only in Europe and the USA, and their practical use is at an early stage (Gasparatos et al., 2017). One of the authors' objectives of the presented review is to focus on a very suitable crop for second generation bioenergy: reed canary grass (*Phalaris arundinacea* L.) and to contribute to a greater use of crops for second generation bioenergy. In addition to the first and second generations, there is a third generation bioenergy, whose source are algae and microorganisms. However, currently this area is exclusively in an experimental phase (Gasparatos et al., 2017).

This article is a review complemented by authors' own experimental results that describes reed canary grass (*Phalaris arundinacea* L.), further RCG, as a very suitable energy crop. Though besides energy purposes, RCG is widely used including its use as forage crop, a persistent perennial cover for permanent pastures, restoration of degraded soils and waters, phytoextraction of soil contaminants, revegetation and stabilization of shorelines, production of acid slurry impoundments, wastewater treatment for ammonium and nitrate removal, for organic

solute mineralization, bioenergy use, and for pulp, paper, and fiber production (Lavergne and Molofsky, 2004) and others products. For example, Lakaniemi et al. (2011) find out that hydrolysed biomass is suitable substrate for anaerobic reducing bacteria. Reed canary grass is also suitable for research e.g. as phytometer (Robroek et al., 2009). When compared to energy trees, the shortening preparation time to productivity, the annual harvesting and the lower water content are favourable in comparison to wood biomass. As a perennial plant is more productive, it has better net calorific value (Lord, 2015), showing a lower energy consumption associated with soil treatment and cultivation (Pahkala et al., 2008). For example, it presents lower nutrient requirements (Wrobell et al., 2009), which are mainly preferable for part-time, the crop enhances carbon sequestration, reduces carbon dioxide emissions (Pahkala et al., 2008), limits soil erosion and improves water quality, rather than annual crops, as well (Semere and Slater, 2007a). Although, many articles about reed canary grass exist, any scientific reviews including new knowledge gained over the last decade have not been in disposition, especially about its energy utilization.

### ***Taxonomy and genetics***

RCG belongs to family *Poaceae* (e.g. Kinmonth-Schult and Kim, 2011), subfamily *Pooideae*, tribe *Poeae* (Winterfeld et al., 2018) and subtribe *Phalaridinae*. This subtribe still includes genera *Anthoxanthum* L. and *Hierochloa* R. Br. (Voshell et al., 2011). All these genera have a common ancestor, with the *Phalaris* species forming an individual clade sister to the genera *Anthoxanthum* L. and *Hierochloa* R. Br. The species *Phalaris* was classified by different authors based on morphological characters into different groups. The number of reported species reported was not uniform (Voshell et al., 2011). For example, Paunero (1948) recognized only four species that divided into two sections. He sorted RCG into section *Baldingera* and the remaining three genera to the section Paunero (Gaertn.). Also, most other authors rank RCG to genus *Phalaris*. For example, Anderson (1961) lists 15 species of the genus *Phalaris*, and Baldini (1995) compiled a list of 22 species of that and from the newer times Winterfeld et al. (2018)

lists 20 species of this genus. However, Rauschert (1969) and Valdés and Scholz (2006) sorted RCG to the genus *Phalaroides* Wolf. Currently, *Phalaris* is a genus divided to subgenera *Phalaris* and *Phalaroides*, where lies RCG and it is ranked into five sections (*Phalaris*, *Phalaroides*, *Caroliniana*, *Bulbophalaris*, *Heterachne*), wherein RCG belongs to the section *Phalaroides*.

The genetic research of the genus *Phalaris* was provided by e.g. Voshell et al. (2011). These authors found the largest number of DNA pair bases (602) among wild species in the genus *Phalaris* at RCG. Further developed combined ITS and trnT-F phylogeny based on maximum parsimony and Bayesian inference. Here is the RCG put in a common lineage in polytomy with diploid *Phalaris rotgesii*, hexaploid *P. caesia* and *P. peruviana* H. Scholz & Gutte where the number of chromosomes has not been detected. The common morphological feature of these species is the floret type 4. At genetic studies, the research into the organization of the genome is very important (Li et al., 1997). Three genotypes were found within genus *Phalaris*, which Winterfeld et al. (2018) referred to as A, B, C. Genotypes A and B corresponded to diploids, but in genotype A there were six chromosomes in one set, while in genotype B these are 7. The genotype C corresponded to a tetraploid with seven chromosomes per set. Genome B occurs in all subgenera of *Phalaroides*, including also in RCG. Based on the results of this study, the authors assumed that RCG is an autopolyploid containing the quadruple genome B or tetraploid produced by autopolyploid. At the study of plant genome are often used specific tandem repetitive sequences of 5S and 45S rDNA as cytogenetic markers that can be used to compare the 5S and 45S rDNA cytogenetic positions among related species. Nearly all *Phalaris* taxa, except *P. minor*, have been found to have two 45S sites on a set of diploid chromosomes (chromosomes designated A1.2, B1.2, and C1.2). In RCG, two of the four 45 S rDNA sites were found to have intercellular locations, while the other two were near the secondary constriction. In the case of 5S rDNA, the smallest number of sites within the investigated *Phalaris* genera was found in RCG, only two sites per tetraploid chromosome complement,

localized intercalary. The 5S and 45S rDNA variation was probably due to the pericentric inversion of the B1 and B2 chromosomes and the loss of location of one of the 5S rDNA pairs on B2 chromosome.

### **Morphology of RCG**

RCG (*Phalaris arundinacea* L.) is a fast-growing C<sub>3</sub>-type perennial heterogamous plant (Kinmonth-Schult and Kim, 2011). It is 0.6 to 2 m tall (Christian et al., 2006), exceptionally to 3 m. Culms are straight, solid, smooth and scaly on base (Ust'ak et al., 2012a). The length of panicles is from 7 to 40 cm (Lavergne and Molofsky, 2004). In the middle climate zone of Europe, RCG usually flowers according to the specific area from May to July (Ust'ak et al., 2012a) for some weeks (Christian et al., 2006). RCG contains both fertile and sterile florets (Linding-Cisneros and Zedler, 2001), whose size is 5–6.5 mm. The fruit is a 1.7 mm long grain (Ust'ak et al., 2012a) with a relatively high seed yield (Lavergne and Molofsky, 2004). Weight of thousand grains is about 0.8 g exhibiting seed dormancy. The germination requires light (Linding-Cisneros and Zedler, 2001) and best water saturated soil. High germination is possible in wet soil, too (Lavergne and Molofsky, 2004). Leaves are typical single, flat (Wrobell et al., 2008) and dark green (Ust'ak et al., 2012a) with turgid bulliform cells (Wrobell et al., 2008). Laminas are long pointed. Their length is 10–35 cm and width 0.6–2 cm. Leaf sheaths are narrow and smooth. The bottom ones are sometimes harsh. The ligule is long linear divided with a length of 0.6–1 cm (Ust'ak et al., 2012a). Wrobell et al. (2008) recorded also folded leaves with larger sclerenchyma layer in the extended bundle sheaths. The plants with folded-leaf were shorter, had a smaller panicle length, possessed more panicles, and were more upright in growth habit than those of the flat-leaf plants (Wrobell et al., 2009). The development of leave stops is at flowering stage with a senescence period starting after the seed ripening, however RCG leaves can be green for some months after flowering (Christian et al., 2006). The maximal above-ground live plant biomass is created during summer (Edwards et al., 2006) and during the second vegetative season (Vymazal and Kropfelová,

2005). Contrarily, there is a reduction at the beginning and the end of the growing season (Edwards et al., 2006), and its production is lower in comparison to other grass on the first year (Kätterer et al., 1998). The rhizome root system is broad (Zhang et al., 2013) with strong, long, reptiles, scaly and greenish segmented rhizomes closely below ground that enables RCG spreading (Ust'ak et al., 2012a). A typical trait is to have quite internal airspace in roots (Kercher and Zedler, 2004) with also very high aerenchyma amount, which facilitates both an increase of methane oxidation below ground and an increased passive methane flux to the atmosphere through the plant in wetland.

### **Chemical composition**

RCG is characterized by a natural high concentration and diversity of alkaloids as tryptamine, carboline, gramine and hordenine families, which make this plant poorly tasty (Lavergne and Molofsky, 2004). It contains some anthocyanins: cyanidin 3-glucoside, cyanidin 3-(6"-malonyl-glucoside), cyanidin 3-(3",6"-dimalonylglucoside), peonidin 3-glucoside, peonidin 3-(6"-malonyl-glucoside) and peonidin 3-(dimalonylglucoside). Acylated anthocyanins constitute more than 80% on RCG occurrence (Fossen et al., 2001). The content of lignin, cellulose, holocelullose, ash and soxhlet extractives (40–60 °C processed in petroleum ether) is 23.2%, 37.1%, 68.7%, 2.3% and 0.6% in dry matter, respectively (Aysu, 2012). The non-structural carbohydrates concentration differs during year and it is influenced by cutting, as well. Klimešová (1996) recorded its lowest concentration at rhizomes in June and the highest in November. The average content of boron, iron, manganese, cobalt, cuprum, molybdenum, nickel and zinc in above-ground biomass is 4.6 mg/kg, 34.8 mg/kg, 128 mg/kg, 0.26 mg/kg, 4.12 mg/kg, 0.38 mg/kg, 0.43 mg/kg and 36.5 mg/kg, respectively (Ust'ak et al., 2012a). Other data about macro- and micronutrients contents are showed in Table 1 and 2.

**Table 1.** Macronutrient content in RCG above-ground biomass at different harvesting periods (% DM)

Harvesting time	Country	N	P	K	Mg	Ca	S	Reference
Flowering	Czechia	0.9–1.10	0.18–0.22	1.2–1.8	0.11– 0.13	0.09–0.13	–	Ust'ak et al., 2012a
Flowering	Czechia	1.36	0.23	–	0.70	0.70	–	Strašil, 2014
Autumn	Czechia	0.96	0.17	0.57	0.12	0.40	–	–
Spring	Czechia	0.92	0.14	0.14	0.06	0.25	–	–
Late winter	United Kingdom	0.65–0.94	–	–	–	–	–	Christian et al., 2006
Late winter	United Kingdom	0.59–0.87	–	–	–	–	–	–
Winter	Wales	0.45–0.79	0.07–0.12	0.10–0.24	0.06–0.15	0.11–0.21	0.08–0.14	Smith and Slater, 2011
Summer	Sweden	1.18	0.17	1.12	–	–	–	Landström et al., 1996
Spring	Sweden	0.93	–	0.25	–	–	–	–
Summer	Sweden	1.33	0.17	1.23	–	–	0.17	Burvall, 1997

**Table 2.** Microelement content in RCG above-ground biomass (mg/kg DM)

B	Cu	Fe	Mn	Zn	Country	Reference
3.3–3.9	4.3–10.6	86–1073	207–470	57.2–113	Wales	Smith and Slater, 2010
		0.20–1994	11.1–65.0	16.5–306	Poland	Polechońska and Klink, 2014a
4.6	4.12	34.8	128	36.5	Czechia	Ust'ak et al., 2012a

The nutrients from above-ground biomass are transported to the rhizomes during senescence and they are remobilized during shoot elongation in the next year. For example, Klimešová (1996) recorded the lowest nitrogen content in rhizomes at August and its maximal concentration in June. But, it was observed its earlier decrease or increase content in winter, too (Smith and Slater, 2011).

### **Ecological conditions**

RCG is naturally spread throughout Eurasia and probably North America in mild regions (Laurent et al., 2015). It can grow in wide range of conditions. This is the reason why can be cultivated on land, which is not

suitable or productive for other agricultural uses (Ust'ak et al., 2012b; Perdereau et al., 2017). It includes brownfield sites and capped landfills, where Lord (2015) found out a RCG biomass productivity higher than at conventional crops switch grass, *Miscanthus* and willow, or after-use option on drained, cut-over, peat mining sites, which is often in North Europe (Ge et al., 2012a). Moreover, it was found that the RCG can improve the structure of clay-based soils (Drury et al., 1991). Typical for this plant is its early season growth, rapid vegetative spread, high stem elongation potential, wide physiological tolerance, high architectural plasticity and longevity (Lord, 2015). The optimum temperature for RCG photosynthesis is on average from 20 to 25 °C (Ge et al., 2012a).



However, RCG is also cultivated in North Europe with lower temperatures (Zhang et al., 2013). Water stress changes this optimum to lower temperatures, probably due to deterioration of biochemical process such as the decrease of photosynthetic enzyme activity and regeneration. In addition, the optimum temperature for photosynthesis and the activity of biochemical enzyme is dependent on the yield of photosystem centre (Ge et al., 2012b). Also, Smith and Slater (2011) stated the suitability of RCG cultivation in colder areas, in which do not happen the creation of new shoots out of growing season. Besides, there is necessary to take into account, that even small increase in temperature can have a considerable cumulative impact on early RCG development. For example, Ge et al. (2012b) recorded the decrease of photosynthesis under elevated temperature from mid-growing period. Additionally, the higher temperature causes acceleration of the ontogenetic development, earlier senescence, and a shorter length of the whole growing period, which means less time for carbon fixation and biomass accumulation before seed set and consequently the reduction of biomass (Ge et al., 2012b). A continuous higher temperature can influence distinctly the activity of biochemical enzymes and the integrity of photosystem centre because cumulative effects can increase the negative response to drought (Ge et al., 2012b).

The RCG occurrence is typically in mild climate and under moist conditions such as wet meadows, wetlands, lake shores, stream banks, floodplains (Lavergne and Molofsky, 2004) and wet woodland (Perdereau et al., 2017). However, it can grow in dryer sites, including mountains (Lord, 2015), but chlorophyll fluorescence (Ge et al., 2012b) and stomatal conductance are reduced. Unfortunately, this limits carbon dioxide diffusion to intercellular space (Ge et al., 2012a) and carbon uptake is less effective at low water content (Ge et al., 2012a), which means a reduction of biomass growth. Ge et al. (2012b) found out that soil water availability influenced the variation in photosynthesis and biochemical parameters much more than climatic conditions. RCG can grow in different soils (Maeda et al., 2006), e.g. poorly drained,

heavy, compacted, well-drained, draught (Lord, 2015), flooded (Ust'ak et al., 2012a), salty loam (Christian et al., 2006), artificial (Lord, 2015), contaminated by cattle urine (Maeda et al., 2006) with different pH from 4.0 to 7.5 (Ust'ak et al., 2012a), but heavier (Ust'ak et al., 2012a), clay (Perdereau et al., 2017) and rich-humus (Shurpali et al., 2009), and high concentration of organic nitrogen, total phosphorus and phosphate with a low dissolved oxygen concentrations are the best (Perdereau et al., 2017). According to Ust'ak et al. (2012a) the optimal pH is about 5.0, while according to Perdereau et al. (2017) is a neutral pH.

### **Cultivation area**

Cultivation areas of RCG belong to the biggest in Europe among alternative crops (Ust'ak et al., 2012a). The most often recommended cultivated area for bioenergy purpose is North Europe (Partala et al., 2001). For example, Christian et al. (2006) stated long-term productive RCG potential in Finland. Really, in Finland and Sweden the area of cultivation of this plant achieves thousands of hectares and rapidly increases (Ge et al., 2012a; Zhang et al., 2013). RCG is cultivated on thousands of hectares in Norway and Ireland. However, dormancy is typical for this plant in Scandinavia (Christian et al., 2006). Also, in Latvia biomass of RCG is considered as one of the alternative sources of raw materials for production of pellets in the Baltic and Northern Europe. Unlike Finland and Sweden, the weather conditions are more suitable here, which ensure stable and high yields (Platace et al., 2012). However, Himken et al. (1997) recommended rather *Miscanthus* than RCG in Central Europe as a suitable area for cultivation. In more arid areas, RCG should be cultivated, if folded leaf genotype would be incorporated into a breeding program. In addition, this provision would increase the biofuel quality (Wrobell et al., 2008) and seed retention (Wrobell et al., 2009). Suitable conditions for RCG growing as an energy plant are also in North America in areas with less than 1700 growing degree-days (5 °C basis) (Bélanger et al., 2016).

### **Agronomy of stands**

RCG can be seeding nearly all after forecrops. However, very suitable forecrops are root crops fertilized by cattle manure, legume-cereal mixture and cereals, which followed after a forage crop or a rape. After the forecrop should be carried out stubble cultivation, phosphorus and potassium fertilization, followed by medium ploughing. Before sowing, the pre-sowing preparation of the soil including the rolling, must be perfectly carried out (Ust'ak et al., 2012a). The poor contact between the soil and the plant threatens the emergence and survival of the low-resistance RCG seedlings (Casler and Andersander, 2006). Before seeding, weeding should be provided, especially at occurrence of couch grass (*Elymus repens* (L) Gould) and other perennial weeds (Pahkala et al., 2008). But, here is also an intense competition of annual weeds (Casler and Andersander, 2006). Seeds are sown into narrow rows (Pahkala et al., 2008) and firmed fine seedbed prepared by ploughing, cultivating and rolling in the spring (Semere and Slater, 2007a), which is better option than later sowing, when seedlings suffer because of drought, without a cover crop (Pahkala et al., 2008). RCG seeds are usually sowed by pneumatic precision drill (Semere and Slater, 2007a). The recommended seed quantity is 20–25 kg/ha and the distance between lines 12.5 to 30 cm. Herbicide application is recommended in time, when RCG has two to five leaves. It can use the same herbicides, which are applied to spring cereals (Ust'ak et al., 2012a). However, Massé et al. (2011) achieved favorable RCG yields (6–10.7 t/ha DM, in average 8.2 t/ha DM) even without herbicide post-emergence application. Nutrients requirements are usually low even in common soils (Zhang et al., 2013), especially for combustion. For example, nitrogen needs at this purpose are stated only 40–60 kg/ha in the sowing year and 60–90 kg/ha during the next years. Fertilizer dosage depends on supply nutrient in soils, cultivation area and nutrient losses at harvesting, which are quite high, especially by potassium. This is why nutrient demands for biogas production are higher and nutrient dosage should cover losses of nutrients at harvest. Such as optimal nitrogen dosage is 80–120 kg/ha/yr (Ust'ak et al., 2012a). In general, RCG has lower nitrogen and

energy use efficiency than e.g. *Miscanthus*, which means to apply higher dosage of nitrogen, especially on sandy soil. However, despite of lower nitrogen content in water logged-organic soil, RCG naturally grows and gives high yields of both above-ground biomass (Wile et al., 2014) up to 1.2 kg DM/m<sup>2</sup>/yr (Lewandowski et al., 2003; Askaer et al., 2011) and below-biomass. The reason of large biomass production could aid in greater oxygen transport to anoxic sediments, which can stimulate the consumption of methane by rhizospheric bacteria (Zhang et al., 2013).

The nutrients can be supplied by mineral fertilizers or digestate. Recommended digestate dosage having 8% dry matter is 25 t/ha/yr. Specific digestate dosage is necessarily stated according to the chemical and dry matter content in digestate and yields. Digestate should be applied in spring and after the first cut. The mineral fertilization by potassium, phosphorus or sulphur can be provided in spring or after harvest (Ust'ak et al., 2012a). Though, Kätterer et al. (1998) did not find any nitrogen influence of fertilization on RCG growth. Also, Ust'ak et al. (2012a) stated that high yield of RCG is possible to gain without nitrogen fertilizer. Prochnov et al. (2009) reported that nitrogen fertilization has no effect on fertile soils. On the contrary, Massé et al. (2011) recorded an increase in average RCG yield from 7.1 t/ha DM at nitrogen dosage of 40 t/ha to 9.2 t/ha at nitrogen dosage of 160 t/ha. Smith and Slater (2010) researched the influence of organic fertilizer application, specifically cattle manure, chicken litter, pig manure, limed sewage cake and no limed sewage cake, but they do not record any growth response. However, in case of chicken litter, they found out high nitrogen, phosphorus and potassium content at harvest. It can mean that the more effective uptake and translocation of nutrient from all examined organic manure was in the chicken litter. Patterson et al. (2009) explored the possible utilization of waste water (specifically municipal effluent, Kraft pulp mill effluent and activated sewage sludge) for irrigation and as a source of nutrients. They recommended the use of Kraft pulp mill effluent as water and nutrient source. Besides, they found out that water, municipal effluent, Kraft pulp mill effluent

and activated sewage sludge significantly increased the biomass yield and the effluent applications did not cause toxic accumulation in tissues. However, waste activated sludge significantly increased phosphorus, potassium, sulphur, boron, magnesium, and zinc soil available concentrations. The irrigation can be a disadvantageous for reasons of lodging. Also, the decomposition rate of crops residues is probably higher in irrigated areas (Kätterer et al., 1998). Semere and Slater (2007a) stated no need of chemical fertilizers, insecticide and fungicide because they did not record, e.g. fungal disease on RCG. The pest green peach aphids (*Myzus persicae*) was found, but it was recorded no influence on the biomass yield result in infestation after maturity and the time when leaves were starting to dry out and senescence. However, there is necessary take into consideration that RCG is the host plant of *Apamea ophiogramma* and therefore, its occurrence within RCG is inevitable (Christian et al., 2006). No virus disease was recorded by Semere and Slater (2007a) including barley yellow dwarf, although Lamprey et al. (2003) infected RCG by this virus. RCG can be sometimes attacked by leaf disease *Stagonospora* and *Helminthosporium* (Ust'ak et al., 2012a).

The threat to weeds in RCG stands is one of the greatest risks at plant cultivation in the first year (Pahkala et al., 2008). The most dangerous is the weed activity at a time when RCG does not nourish autotrophically and does not create an establishment sward. Therefore, RCG should be included on non-weed land. According to Stražil et al. (2011) the most dangerous weeds are grass such couch grass as and rough bluegrass (*Poa trivialis* L.). However, the intensive competition of annual weeds, especially after the stand establishment is also dangerous (Casler and Andersander, 2006). Semere and Slater (2007b) dealt not only with RCG stand weeding and recorded their lower diversity in RCG growth than at *Miscanthus* growth, although they applied less herbicide to RCG. The lowest weed biodiversity was recorded in wheat growth, where was applied the greatest herbicide amount. Higher diversity of weeds in *Miscanthus* growth was caused by its slow initial growth, early development in the season, planting in wider rows and thus showing a

lower plant density that enables weeds gain soil nutrient and light enough. However, the weed cover was similar in second and third year of both *Miscanthus* (1–5%) and RCG (1–7%). Thus, RCG suppresses the weed growth and the effect of applied herbicide is not so important, unlike such as wheat. On the other hand, the ability of RCG to control weeds can reduce biodiversity in stands of this plant. For example, Semere and Slater (2007a) recorded that a significant weed decrease in the second year at RCG stand means a decrease of butterfly numbers.

Nevertheless, the cultivation of RCG has rather favourable environmental effect due to low intensity of agricultural management and its growth also provide habitat and food for many native organisms, e.g. plants, insects such as beetles, butterflies, e.g. meadow brown (*Maniola jurtina* L.) or large skipper (*Ochlodes venatus* Esper), Dipterans, Hymenopterans, Hemiptera, Psocoptera, Neuropteran, Collembola, Thysanoptera, Orthoptera, Dermaptera and other invertebrates (Semere and Slater, 2007a), small mammals and birds (Semere and Slater, 2007b) mainly in native area. In relation to insects, there is important that RCG growth enables insect overwintering (Semere and Slater, 2007a).

The comparison of RCG cost assessment at cultivation for energy purposes according to different data from the Czech Republic was provided by Ust'ak et al. (2012b). The mean cost of previous growth extermination, ploughing, harrowing, skidding, purchase of seeds, seeding, rolling and herbicide application against dicotyledonous weeds in the first year of cultivation are 32, 40, 11, 18, 15, 94 and 20 Euro/ha/yr, respectively. It shows a cultivation cost on average of 228 Euro/ha/yr. Fertilization, single cut, turning, raking and pressing determine the costs of 53, 28, 47, 20 and 45 Euro/ha/yr, respectively. Overall maintaining costs are 195 Euro/ha/yr with fertilization (and 165 Euro/ha/yr without fertilization). Hallam et al. (2001) stated the production cost for RCG cultivation at combustion purposes of about 560.3–676.3 EUR.



### **Biomass production and harvest season**

Reed canary grass is an important plant for bioenergy because of relatively high biomass yields (see Table 3) in range of 5–10 t DM/ha/yr (Ust'ak et al., 2012a, 2012b), which can be further increased, e.g. by applications of carbon dioxide (Kinmonth-Schult and Kim, 2011; Ge et al., 2012a). For example, Kinmonth-Schult and Kim (2011) recorded doubling RCG biomass at carbon dioxide increasing. The rise of carbon dioxide probably results in long-term fructan storage in rhizomes, which may be an advantageous in terms of overwintering and vegetative spread. On the other hand, the increase of carbon dioxide can cause a decrease of nitrogen content in leaves and provide the creation of root biomass to ensure a sufficient nutrient supply (Zhang et al., 2013). Increasing carbon dioxide is the most effective at ambient temperature. Contrarily, higher temperature reduces the stimulatory effect of elevated carbon dioxide. The natural yield is probably influenced by the genetic diversity. Thus, many genotypes can produce more biomass than commercial varieties, and the yield can be improved by genotype selection and breeding (Christian et al., 2006), climatic and soil conditions (Ust'ak et al., 2012a). In other words, Kätterer et al. (1998) recorded retarded growth and decreased the RCG efficiency at converting radiation into biomass caused by cold temperature in May, although they used high nitrogen dosage. Otherwise, the relationship between RCG yield and geographic region of genotype was not found. The highest yield is acquired in years with higher rainfall and in soil, where height of bottom water is 30–40 cm. Generally, the average harvest yield is 7–8 t/ha/yr on clay soils and 10 t/ha/yr on mull soils after the second harvest, while the average harvest yield is 3–6 t/ha/yr after the first spring harvesting. The yield of dry matter mainly depends on the growth during the previous year (Pahkala et al., 2008), harvest date (Massé et al., 2011), harvesting method (Tahir et al., 2011) harvest losses, the cultivar (Pahkala et al., 2008), fertilization level (Massé et al., 2011). Tahir et al. (2011) at RCG yield assessment by mean of variance analysis mostly recorded two- and three-way interaction between the habitat, age, harvesting method and cultivar. Although RCG yield

is usually high, the energy crops *Miscanthus x giganteus* and switchgrass have often higher yields (Christian et al., 2006; Laurent et al., 2015). However, a higher RCG biomass productivity than switchgrass was recorded in northern Ohio and occasionally in North Iowa (Tahir et al., 2011). The RCG yields verified in various studies are shown in Table 3.

The first yield is recommended to obtain in the third year after sowing. The best harvest time for combustion purposes should be at late winter or early spring, when the moisture content of dead plants is the lowest (10% to 15%) according to the cultivation area (Pahkala et al., 2008). For example, Smith and Slater (2011), who conducted their research in Wales, recorded the highest dry matter content in January and February. The greatest moisture losses are from November to February (52–76%). However, the feasibility of harvesting in early spring depends on local climatic conditions (Heinsoo et al., 2011). In this case, snow cover may cause lodging damage, which may make impossible harvesting. Harvesting after melting of snow is also problematic, as it is necessary to use machines that allow the material to be lifted from the ground, with significant harvest losses and soil contamination, and the difficulty of this operation due to soil water saturation. Therefore, this method of harvesting is disadvantageous in areas where snow cover is common over winter (Tahir et al., 2011). In any case, RCG is very suitable for combustion in terms of dry matter. The risk of corrosion at combustion can be significantly decreased, if RCG is harvest in time, when has low mineral content, especially potassium, thus some weeks after RCG senescence. In general, RCG biomass overwintering leads to a decrease in potassium, phosphorus, calcium, sulphur and chlorine content (Tahir et al., 2011). Christian et al. (2006) recorded the reduction of potassium concentration by an average of 54%, but no nitrogen reduction in case of delayed harvest. This harvest is also advantageous because the low water content. Otherwise, Kätterer et al. (1998) recorded an increase of nitrogen, phosphorus, potassium and sulphur concentration at harvest after nitrogen fertilization. In case of biogas production, two cuts per year are usually provided. But, it is also possible

**Table 3.** Reed canary grass yields (t DM/ha)

Yield	Time harvest	Country	Reference
8.41	Summer	Czechia	Strašil et al., 2005
8.00	Autumn	Czechia	-"
6.04	Early spring	Czechia	-"
6.09	Autumn	Czechia	Strašil and Moudrý, 2011
4.73	Spring	Czechia	-"
8.80	Summer	Czechia	Ust'ak et al., 2012a
7.95	Summer	Czechia	-"
7.15	Summer	Czechia	-"
5.50	Summer	Estonia	Heinsoo et al., 2011
6.80	Early spring	Estonia	-"
8.60	Late autumn	Estonia	-"
9.49	Late winter	Wales	Christian et al., 2006
7.25	Late winter	Wales	-"
9.14	Late winter	Wales	Christian et al., 2006
7.01	Late winter	Wales	-"
3.2–4.5	Flowering	Lithuania	Tilvikiene et al., 2016
3.2–5.1	Autumn	Lithuania	-"
3.0	Early spring	Belgium	Muyllé et al., 2015
7.85	Summer	Canada	Bélangier et al., 2016
7.56	Summer	Canada	-"
7.08	Autumn	Canada	-"
8.17	Summer	Canada	Massé et al., 2016
10.70	Summer	Canada	-"
6.43	Summer	Canada	-"
9.86	Summer	Canada	-"
8.22	Late summer	Canada	-"
9.15	Late summer	Canada	-"
6.09	Late summer	Canada	-"
9.31	Late summer	Canada	-"
7.50	Early autumn	Canada	-"
8.76	Early autumn	Canada	-"
6.03	Early autumn	Canada	-"
7.66	Early autumn	Canada	-"

to provide only one cut. Three cuts are possible at optimal conditions. The water content at harvest should be 65–75% (Ust'ak et al., 2012a), which corresponds to the time, when the greatest increase in phytomass occurs (Kára et al., 2005). For example, Bélanger et al. (2006) found the highest biomass yield in RCG stand based in Canada (Research and Development Centre of Agriculture and Agri-Food Canada at Lévis) in late July, while yields decreased with later harvest dates (late August and mid-October, see Table 2). According to Pahkala et al. (2007) is the best harvest time for RCG production to ethanol in spring as dead material. Specifically, the RCG spring harvest was carried out at the beginning of April or May. However, in order to obtain a high yield and high fiber and sugar content, it would be the best to harvest RCG in late autumn in a green state (in this study the harvest was made at the end of October). But, during this period a high water content was detected in the biomass, which could complicate the possible storage.

### **Harvesting and machinery**

The common existing farming machines can be used for harvesting, which is very favorable at any method of energy use (Kätterer et al., 1998). For example, Digman et al. (2010) used for the RCG harvest, which was later tested for ethanol production, a direct-cut-forage harvester with a theoretical cut length of 5 mm. The same machines as in the autumn harvest of straw cereals can be used at the RCG spring harvest for combustion (Nilsson and Hansson, 2001). RCG intended for combustion is harvested mostly in the spring by a mowing machine, which is cut into rows where drying takes place (Kára et al., 2005). According to Pahkala et al. (2008) is used a disc mower without conditioner followed by a rotary rake and large baler the best. A wind-rower is also suitable. Otherwise, it is not recommended the usage of conditioner because of great biomass losses (50% even more) since plants are very fragile in spring. After drying on the line, the phytomass is pressed into either cylindrical or cubic bales. In the case of cylindrical bales, a higher density of the compressed material and a higher press performance are achieved. However, large cubical bales are the most economical

for transport. The bales are mostly crushed by terminal chaff cutter or a mobile crusher to triturate together with the wood matter from forest before using in power plants since often these ones do not have a possibility to manipulate the bales or to mix the new material with other fuels. If RCG is harvested in earlier dates (summer to autumn) with a forage harvester, the cutting is taken to a place where it usually needs to be dried (Kára et al., 2005). The transport of chopped grass biomass, which was cut by regular forage harvesters is economical, if the distance to the power plant is less than 10 km. If the transport distance is longer, the use of a baling system is better (Pahkala et al., 2008). At drum harvesters, it is advisable to reduce the drum speed thereby increasing the throughput of the harvester and reducing the losses of leaves (Kára et al., 2005). When harvesting phytomass for biogas production, the plant biomass after a previous cutting can be transferred by a forage harvester to a conveyor, it may be optionally collected by a self-loading forage wagon or molded by presses. From the point of view of biogas production, it is important at these machines to reduce the size of the phytomass. The minimum theoretical cut length can be 2.5 mm, 20 mm and 39 mm for a cutter, a self-loading forage wagon and for presses, respectively. After harvesting, the phytomass can be transported to a biogas plant. However, there is a greater risk of wear on the biogas plant because the phytomass thus produced causes its abrasion and the risk of soil clogging from the field. But, the biggest problem is that biogas production is usually low in this phytomass. Another option is firstly to let the phytomass wilting until the water content of the phytomass is 30–40% and then store it in the silo (Prochnow et al., 2009).

### **Storage**

For storage, it is advantageous that the same systems can be used as in other areas of agriculture, for example at storing cereal straw (Nilsson and Hansson, 2001). The storage of RCG compressed into packages depends on the shape of the package. Cubic bales should be stored in covered areas against rain, while round bales with foil can be stored in the field. The cubic packages are

advantageous in terms of handling and limited storage space (Kára et al., 2005). At RCG storage for combustion purposes, the moisture has to be below 23% to prevent self-ignition (Smith and Slater, 2011) and to enable the material manipulation (Pahkala et al., 2008). If RCG is used to produce biogas, the harvested phytomass is transported directly to the biogas plant or treated by means of its ensilaging or haylaging (Ust'ak et al., 2012a). The problem of storage for ethanol production is insufficiently solved not only in RCG, but also in other grasses, although a huge amount of biomass is needed for ethanol production. For example, at ethanol production of 75 ML/year in a suitable plant will be required by about 408–870 tonnes per day, depending on the conversion efficiency and the type of raw material. In theory, the long-term storage of both dried and ensiled phytomass or various modifications of these methods could be considered. However, harvesting the dried phytomass and baling it is disadvantageous in terms of biomass conversion, since additional liquid material will have to be added for biomass conversion. Furthermore, it is disadvantageous that is necessary to ensure, for example the transport to the biorefinery, a particle size reduction and a rehydration. Thus, wet storage seems to be better and less expensive to use this biomass for ethanol production. Other advantages of this storage method are the reduction of dry matter losses, greater product uniformity, increased susceptibility to enzymatic hydrolysis, reduced fire risk and the possibility to add valuable chemicals and biological preparations. The classic wet forage storage is designed silage. However, with conventional silage there is a risk that the organic acids formed will adversely affect microbes performing ethanol fermentation. One solution to that could be the use of chemical stabilizers, which slow down ensiling and can also at the same time promote the enzymatic conversion of plant carbohydrates into fermentable monosaccharides. For this reason, sulfuric acid and calcium hydroxide have been tested at RCG preparing. The RCG was harvested with a direct-cut-forage harvester with a theoretical cut length of 5 mm in June and about 45% humidity. After harvesting, the homogenization was carried out in a reel

type mixer and then transferred to sealed bags of 158 L. Then half of the biomass was stored at -20 °C and the remainder was dried to a humidity of about 30%. During the withering process, the material was irregularly mixed manually and monitored to obtain the desired humidity and then stored at -20 °C. At the desired humidity, the stored substrate was transferred to a refrigerator (5 °C) one day before use to thaw. Sulfuric acid was applied as an 18 N solution and calcium hydroxide as a powder. Prior to pretreatment, the substrate was rehydrated to about 40% and 60% humidity. The samples with both lower and higher humidity were supplemented with the same amount of water. Both acid and hydroxide were applied to 250 g of DM rehydrated substrate and mixed by hand. Then the samples were compressed to mini-silos where the anaerobic conditions took place. The ambient temperature was 22 °C. The application of sulfuric acid resulted in a pH decrease from 4.3 to 1.5 for samples with lower humidity and from 3.5 to 1.4 for samples with higher humidity, and the decrease in lactic acid level from 5.0 to 0.82 g/kg organic matter (OM) for samples with lower humidity and 4.8 to 0.41 for samples with higher humidity. At the same time, it was also an increase of acetate from 9.5 to 29.5 g/kg OM for samples with lower humidity and from 5.1 to 14 g/kg OM for samples with higher humidity. The application of calcium hydroxide resulted in a pH increase from 4.0 to 10.0 for samples with lower humidity and from 3.6 to 10.9 for samples with higher humidity, lowering the lactic acid level from 4.5 to 1.3 g/kg OM for samples with lower humidity and from 3.8 to 0.58 g/kg OM. In this case, the increase of acetate from 10.0 to 38.0 was for samples with lower humidity and from 6.0 to 20.0 g/kg OM for the ones with higher humidity. Thus, both the application of sulfuric acid and calcium hydroxide resulted in a decrease in lactic acid probably due to the inhibition of lactic acid bacteria, and conversely an increase in acetates probably due to de-acetization of arabinoxylan. Also, despite the increase in the acetate content, no inhibition of fermentation was observed. The above storage method has led to the preservation of glucose and xylose in the cell wall. In contrast, arabinose was released from the cell wall

as a result of sulfuric acid treatment. The greater the acidification, the greater the effect. Acid treatment also promoted the conversion of glucose to ethanol (maximum conversion efficiency was 83%) and storage efficiency. However, for the higher humidity samples treated with sulfuric acid, the conversion was lower. A possible cause could be that higher humidity lead to a decrease in acid efficiency. However, overall sulfuric acid treatment was more effective than calcium hydroxide (Digman et al., 2010).

### Energy utilization

#### Combustion

RCG can be used for heating and electricity generation in form of pellets (Kätterer et al., 1998; Wile et al., 2014), powders, wafers, cobs (Kätterer et al., 1998) at good conversion efficiencies (Wile et al., 2014) in modern power generation plants. Though, chlorine in RCG can cause corrosion (Pahkala et al., 2008) and higher minerals content and sulphur, especially potassium, additionally produces slagging and fouling in the combustion chamber (Pahkala et al., 2008). In contrast, according to Tahir et al. (2011), the RCG zinc content does not cause problems. The high content of minerals as chlorine, sulphur (Pahkala et al., 2008) and nitrogen (Tahir et al., 2011) means environmentally harmful emissions. But, the negative influence of chlorine does not threaten the total energy of fuel, if plant biomass is mixed with peat or wood chips to 10% (Pahkala et al., 2008). Calcium and magnesium content can lead to increase the problems related to

the ash melting point, while high potassium content contributes to solve these problems and limit thus the corrosion and slagging of combustion chamber. Therefore, a low content of calcium and magnesium, and a high content of potassium are also suitable for combustion. Contrarily, the high content of nitrogen is not good for combustion because of nitrogen oxide emissions. Specifically, the content of nitrogen, potassium, sulphur and calcium should be for combustion 0.1–0.6%, 0.2%, 0.1–0.3%, 2.5% and 15–35% of total weight dry matter, respectively. A higher content of nitrogen was recorded in Wales during October and December. In a later period, RCG cultivated in Llwynprenteg area showed significant gradual increase, which probably means that those plants in this area were still taking up nitrogen for further plant growth, while RCG cultivated in Llysdinam showed a highly significant decrease of nitrogen in later winter. Higher nitrogen content and probably higher temperature in winter were likely the cause of new shoots creation (Smith and Slater, 2011), which results in the decrease of harvest quality because new shoots had higher water and mineral content (Christian et al., 2006). Low potassium values suitable for combustion in January and February probably results in leaching from the senescent plants (Smith and Slater, 2011), while their higher values were recorded in October (Smith and Slater, 2011). Kätterer et al. (1998) recorded 2.5 to 5-fold lower potassium content in early spring than autumn. In the Table 4 are presented different levels of energy values at RCG biomass.

**Table 4.** Energy values of RCG

Country	Heating value [MJ/kg DM]	Energy yield [GJ/ha]	Reference
Germany	18.0	-	Kasper, 1997
Sweden	17.9	-	Burvall, 1997
United Kingdom	-	97	Lord, 2015
Wales	17.49	-	Smith and Slater, 2011
Estonia	16.6–17.2	-	Heinsoo et al., 2011
Czechia	17.8	142	Stražil, 2014
European Union	18.7	-	Muyllé et al., 2015
USA	16–18	-	Smith et al., 2015



### **Biogas production**

Other possible RCG bioenergy utilization is for biogas production. Biogas is produced by degradation of digestible organic matter. Thus, high digestible organic matter in biomass is desirable. In case of RCG, the digestible organic matter decreases with age, but its content can be increased by many times harvesting at a year. However, RCG belongs to less grass tolerant to often cutting. This is probably the reason why Geber (2002) recorded a better total amount of volatile solids (further VS), as well dry matter (further DM), in the two-cut regime than in three or four ones. The digestible organic matter can be influenced by the cut height and whether conditions, too. Really, Geber (2002) found out positive impact on digestible organic content at the first cut, when increased stubble weight and stated that hot and dry weather can cause a low content digestible organic matter. However, the height of stubble did not have any impact on the digestibility of the different cutting regime. In conclusion, two cuts of RCG for biogas production are the best. But a suitable option would be to extend the growth period in three-cut regimes with earlier first cut and delaying the last cut. This procedure should increase the total dry matter production, too. Also, Kandel et al. (2013) recorded 45% more methane in two-cut management compared to one-cut. The wild plants have lower biogas content than cultivated ones. More specifically, Oleszek et al. (2014) found that wild plants had a biogas yield of 120 NL/kg VS, while the cultivated variety 406 NL/kg VS among lignocellulosic cultivars belong to Palaton (Ust'ak et al., 2012a; Muylle et al., 2015) and Bamse (Muylle et al., 2015), respectively. Furthermore, nitrogen fertilization has a significant effect on methane yield. But in general, it cannot be said that a higher nitrogen dose would increase the methane yield. Conversely, the increased nitrogen fertilization may have a negative effect on digestibility (Bélanger and McQueen, 1998) and a positive effect on lignin and nitrogen concentrations (Kätterer et al., 1998). For example, Massé et al. (2011) reported a specific methane yield of 0.195 NL/g VS at a nitrogen dose of 40 kg/ha while with a dosage of 160 kg/ha produced a decreased specific methane yield of 0.178

NL/g. Rather, such methane yields are lower compared to both annual crops and perennial grass species usable for biogas production. However, in the case of methane yield per hectare, which is the product of specific methane yield and biomass yield, nitrogen fertilization had a positive effect on this parameter as nitrogen fertilization led to an increase in biomass yield of 31%. Some biogas and methane yields are showed in Table 5.

### **Ethanol production**

Some second generation bioenergy grasses (hereinafter referred to as grasses in this chapter), including RCG, are suitable raw materials for ethanol production because of their high yields, a relative low-cost and low inputs (sometimes no pesticides or fertilizers) and therefore, cheapness in cultivation (or wild grasses can be used and there are no growing costs), positive environmental benefits (e.g. erosion and nutrient leaching control), higher carbon and hydrogen concentrations and minimum content of oxygen and nitrogen in the biomass (Mohapatra et al., 2017). Grasses are lignocellulosic crops, with cellulose being the primary substrate for ethanol production in this case (Dien et al., 2006). Last but not least in the ethanol production from grasses, it is also the advantageous that it does not compete with food crops. Currently, *Miscanthus* sp. followed by switchgrass (*Panicum virgatum* L.), Napier grass (*Pennisetum purpureum* Schumacher) and *Vilfa stellata* (*Cynodon dactylon* (L.) Pers) are considered to be the most attractive grasses for ethanol production. Although RCG is also suitable for ethanol production, mainly due to the favorable carbohydrate and salt content, the persistence (e.g. Dien et al., 2006), drought resistance (Mohapatra et al., 2017) and flooding (Dien et al., 2006), and to other stress conditions, it does not reach a comparable annual production of dry matter per hectare as mentioned grass (Mohapatra et al., 2017). In addition, for example Dien et al. (2006) found a lower glucose content (2–4 g/kg DM) in RCG than switchgrass (6–14 g/kg DM) and also Lucerne (*Medicago sativa* L.) (15–18 g/kg DM). In particular, a higher amount of glucose is advantageous since the conversion of glucose results in a higher ethanol

**Table 5.** Biogas production from reed canary grass

Country	Biogas yield (l/kg VS)	Biogas yield (l/kg DM)	Specific methane yield (m <sup>3</sup> /t DM)	Methane yield (m <sup>3</sup> /ha)	Reference
Germany	540 <sup>a</sup>	-	-	-	Baserga and Egger, 1997
Lithuania	-	238–361 <sup>a</sup>	-	-	Tilvikiene et al., 2016
Denmark	283–412 <sup>b</sup>	-	-	3735–5430 <sup>b</sup>	Kandel et al., 2013
Canada	187 <sup>b</sup>	-	-	1370 <sup>b</sup>	Masse et al., 2011
Czechia	-	-	240–260 <sup>b</sup>	2160–2600 <sup>b</sup>	Usták et al., 2012a

yield compared to other monosaccharides, in particular pentose (Mohapatra et al., 2017). On the other hand, Dien et al. (2006) found that RCG is significantly more accessible to glucose conversion than switchgrass. Most of the glucose is in the cellulose. However, mature plants contain a small amount of free glucose, and in addition to cellulose, lignocellulosic biomass can also be a sucrose and starch source (Dien et al., 2006). An effective pretreatment with the purpose to de-lignify this biomass is necessary at ethanol production from grasses, due to their lignocellulosic nature (Mohapatra et al., 2017). Indeed, because otherwise lignocellulosic biomass would not be subject to enzymatic hydrolysis (Bradshaw et al., 2007), converting cellulose and hemicellulose into monomers that are capable to be converted to ethanol by microorganisms (Mohapatra et al., 2017). The older the grass, the more it contains lignin and the pretreatment intensity increases (Bradshaw et al., 2007). The lignin content is different for different grass species. In the case of RCG, Digman et al. (2010) recorded less lignin (130 g/kg DM Klason lignin) than switchgrass (150 g/kg DM Klason lignin). The pretreatment may include physical (mechanical disruption), chemical (most often dissolution in alkali or acids) and physico-chemical (e.g. steam explosion, ammonia fiber expansion) and biological methods.

The objective of physical methods is primarily to achieve a reduction in grass size, which also reduces the polymerization and crystallinity of cellulose. They are usually carried out in combination with other pretreatment methods. The importance of physical pretreatments is that they improve the efficiency of other pretreatments. Physical modifications include chipping, milling, grinding,

extrusion and pyrolysis. While milling and grinding are frequent pretreatments in grasses, chipping is mainly used in woody plants. A significant advantage of physical treatments is that they do not produce any toxic or inhibitory intermediates, and significant disadvantages are the high costs due to high energy consumption and the need for maintenance and replacement of worn parts (Mohapatra et al., 2017). As the RCG physical was preceded by further adjustments, the procedure used is listed along with these methods.

The purpose of chemical and physicochemical pretreatment methods is to increase the elimination of lignin and/or hemicellulose, and to decrease the crystallinity index and the degree of cellulose polymerization. Then, the chemical treatment leads to the internal degradation of lignin and hemicellulose. The RCG chemical pretreatment in acid dissolution was performed by Dien et al. (2006) at 120 °C in an autoclave and at 150 °C in a pipe reactor. At the first variant, the procedure was as follows: 2 g of phytomass were mixed with 18 ml of dilute sulfuric acid solution in a glass sealed tube for 1 hour in an autoclave set at 121 °C. In the second variant, the phytomass pretreatment was carried out using a steel pipe reactor and in a fluidized heating sand bath. In this case, 2 g of phytomass were mixed with 18 ml of dilute acid solution in a pipe reactor. Heating was then carried out at 150 °C (the heating time was on average ten minutes), the incubation was continued for 20 minutes and a rapid cooling was performed by immersion in a cold water bath. The first pretreatment resulted in RCG when no stem elongation, resulting in the release of 58 g/kg DM glucose, and at the seed maturity stage, the release of 49 g/kg DM glucose, whereas the

second case of pretreatment resulted in RCG where no stem elongation recording a higher glucose release (60 g/kg DM when no stem elongation and 53 g/kg glucose at full seed stage). The second treatment was therefore preferable. Dien et al. (2011) affirmed that in the case of RCG pretreatment with acid, the high fructose content is disadvantageous with this grass. For example, Dien et al. (2006) found in RCG when no stem elongation a gain of 5 g/kg DM of fructose and at full seed stage 12 g/kg DM, and states that if acid pretreatment is performed at 150 °C fructose is completely degraded in ten minutes into hydroxymethylfurfural, thus causing a loss in sugar yield. Moreover, hydroxymethylfurfural is a potential inhibitor of microbial fermentation and is difficult to remove. Furthermore, chemical and hydrothermal treatment of RCG was performed by Dien et al. (2011). The procedure was as follows: 1.5 kg DM of RCG (ground) was mixed with either water or ammonia in 316 stainless steel mini-batch reactors. These reactors were heated to 150–180 °C in a fluidized heating aluminum oxide bath. The separated reactor was provided with an internal temperature monitoring cell. The reactions were terminated by the transfer to a water bath. About eight minutes needed to reach the reaction temperature of the reactors, while cooling to ambient temperature took less than two minutes. In a hydrothermal treatment at 180 and 190 °C for 30 minutes, 15% w/w (i.e. the mass percent) of water was applied. A pretreatment with ammonia was carried out at 130–150 °C in 2–4% w/v (i.e. the volume percent) of ammonium hydroxide solution for 20 minutes and digested in combination with celluloses and pectinases. This pretreatment resulted in a glucose yield of 190 (2% of sodium hydroxide solution, reaction temperature) to 220 mg/g (4% of ammonium hydroxide solution, reaction temperature 130 °C). In general, grasses are preferable to use alkali rather than acid chemical treatments, as the solubility of lignin also increases due to the dissolution of free phenolic compounds. In addition, the use of alkali causes less degradation of monosaccharides (Mohapatra et al., 2017). Also, another problem found specifically in RCG is the high consumption of acids (in RCG e.g. 100 g/kg biomass). Such high acid

consumption would be problematic in commercial applications. The high consumption is also associated with the use of alkali, but in the case of ammonia, it is possible that evaporation would allow its recycling (Dien et al., 2011). An alternative to chemical solvents is the use of ionic liquids, which are liquid salts formed by organic cations and inorganic or organic anions (Mohapatra et al., 2017). Unlike other methods, this pretreatment makes possible to decrystallize portions of the cellulose from the lignocellulosic biomass while disrupting lignin and hemicellulose, which has a significant positive effect on later enzyme saccharification. Another advantage of this process is the removal of lignin and its recovery in a separate, i.e. more valuable form. In addition, ionic liquids are less volatile compared to aqueous solutions, allowing biomass to be treated at atmospheric pressure and at boiling temperatures (Brandt et al., 2013). The major drawbacks of this process are the high cost of chemicals and the long retention time what accounts with several days (Mohapatra et al., 2017). In the case of RCG, DBU – MEA–SO<sub>2</sub> ionic fluid solvents (DBU: 1,8-diazabicyclo [5.4.0] undec-7-ene; MEA: monoethanolamine) and DBU–MEA–CO<sub>2</sub> have been tested. At enzymatic hydrolysis was achieved an excellent conversion of glucan to glucose in the range of 75–97% (Soudham et al., 2015). From RCG physicochemical pretreatment methods was investigated for example the ammonium fiber expansion (AFEX), a process in which concentrated ammonia is used to treat biomass at a given time (a treatment of biomass 25 g usually takes 30–40 minutes), moisture and ammonia content. Also, Bradshaw et al. (2007) investigated the effect of AFEX at 80–120 °C and ammonia levels of 0.8 kg/kg DM, 1.0 kg/kg DM, and 1.2 kg/kg DM, with the best results at 100 °C, 60% humidity and 1.2 kg of ammonia per kg of DM phytomass. The rapid release of pressure causes the biomass to expand. The treated biomass is then placed in a fume hood overnight to remove ammonia. Ammonia evaporation also occurs at the explosion. The higher the explosion temperature, the more ammonia will evaporate and the phytomass fiber structures will be destroyed. After the ammonia has been evaporated, the biomass can

undergo hydrolysis. Furthermore, the temperature affects the amount of ammonia used. For example, at 80 °C, more than 1.2 kg of ammonia per 1 kg of DM biomass had to be used to achieve higher conversion to glucose, while at 100 °C 1 kg of ammonia per 1 kg of DM biomass was sufficient. However, at temperatures above 100 °C, a reduction in both glucose and xylose conversion was observed. Among other physicochemical methods, steam explosion has been applied to RCG, which is a process based on a combination of steam release and pressure, leading to the disruption of lignin barriers and increasing the susceptibility of cellulose to enzymes, as biomass treated in that way has larger pores and surface area, which facilitates enzyme catalyzed hydrolysis (Alfami et al., 2000). The specific conditions of RCG steam explosion were carried out at 190–200 °C for 10 minutes using 2% of sulfur dioxide. In this way, a maximum conversion to glucose of 80–100% was achieved (Pahkala et al., 2007). Significant advantages of this process include the low chemical and energy consumption (Alfami et al., 2000). There are significant disadvantages associated with chemical and physicochemical methods such as high energy requirements, although for example RCG has been found that acid treatment can be performed under much milder conditions than switchgrass (Dien et al., 2011), further the corrosion, the formation of intermediates and the negative impact on the environment. The biological pretreatments that are not associated with these disadvantages could be a suitable alternative to these methods. At biological delignification, bacteria and fungi can be theoretically used, however, an effective depolymerization of lignin can only be generally achieved by white-rot-fungi and basidiomycetes. An alternative to biological methods is the enzymatic delignification, which is faster and more efficient than biological methods (instead of weeks, only hours, while no carbohydrate consumption). The ultimate goal of all pretreatment methods is to achieve depolymerization of the cellulose chain and to obtain glucose. At the biochemical level, this process is based on hydrolysis catalyzed by the cellulase enzyme complex (Alfani et al., 2000). These enzymes are also commercially available.

For example, Celluclast 1.5 L (Mohapatra et al., 2017) was used by Dien et al. (2006) together with 188 beta-glucosidase in a 1 : 1 ratio.

The common goal of the pretreatment and saccharification is to optimize the fermentation process. The existing methods of fermentation in grasses can be varied, where are including the separate hydrolysis and fermentation (SHF) and the simultaneous saccharification and fermentation (SSF). In the case of SHF, the enzymatic hydrolysis of pretreated grass into monosaccharides by cellulases and xylanases (first phase) is performed separately from fermentation to ethanol (second phase) in separate units, while in the other cases cellulose hydrolysis is carried out in the presence of fermentative microorganisms such as yeast, bacteria and fungi that can ferment ethanol in the lignocellulosic hydrolyzate. The main advantage of SHF is that both hydrolysis and fermentation can be carried out under optimal conditions, which is significant because the action of cellulases is effective at 45–50 °C, while commonly used fermentative organisms work the best at temperatures of 30–37 °C (Mohapatra et al., 2017). However, SHF is disadvantageous over SSF in higher enzyme loading, higher osmotic stress and the risk of microbial contamination. The SSF method has been tested in RCG by Dien et al. (2011). Here the process of enzyme hydrolysis was performed at 50 °C and 125 rpm using an incubator/shaker for 72 hours. For ethanol fermentation was used *Saccharomyces cerevisiae* strain D5A, Optiflow RC2 (15 FPU/g glucan), Novo188 (40 CBU/g glucan) and Multifect pectinase (50 U xylanase activity/g biomass), and the yeast strain YRH 400, the Multifect Xylanase (50 U/g biomass), ferulic esterase (5 U/g biomass) and beta-xylosidase (100 ml/g biomass) were added. In this study, xylose was fermented instead of glucose. The application of pectinases resulted in an increase in ethanol production using the yeast strain *Saccharomyces cerevisiae* D5A, and even with the application of all four enzymes, there was no greater yield of ethanol than using only pectinases. The highest conversion efficiency into ethanol in this process was 84%. Using the YRH 400 strain, all of the above enzymes were mixed. The final ethanol concentration here was



from the RCG harvested in the vegetative state of 15.0 g/l, which corresponded to a maximum conversion to ethanol of 68.2% and from the seed maturity phase of 14.2 g/l, which corresponded to a maximum conversion to ethanol of 53.9%. The application of *Saccharomyces cerevisiae* capable of fermenting xylose resulted in a higher theoretical yield of ethanol than if glucose had been fermented (Dien et al., 2011). Although several successful methods of lignin removal have been tried in RCG as well as several other grass species and bioconversion of the remaining cellulose to fermentable sugar has been tried, it is currently not possible to obtain ethanol from RCG at industrial way. The technology of ethanol recovery from lignocellulosic biomass on an industrial scale would require the consolidation of pretreatment, enzymatic degradation and fermentation processes to achieve full substrate utilization. While such technologies do not exist yet, they are being developed. Another way to ensure the industrial production of ethanol from lignocellulosic biomass would be to use advanced molecular genetics techniques, which would eliminate pretreatment or enzymatic hydrolysis and thus significantly reduce the cost of the end product. This pathway is also subject of research (Mohapatra et al., 2017).

### **Phytoremediation potential**

RCG could be used for phytoremediation in terrestrial and aquatic environments (e.g. Lojko et al., 2015) because it can accumulate some pollutants such as metals, including these heavy, even in large quantities. For example, in the area of Mostoon Brook England, where industrial waste was previously deposited, RCG of the 27 large-scale plants in the area belonged to the species that accumulated the largest amount of selected heavy metals in the shoots (arsenic 0.8 mg/kg DM, chromium 0.2 mg/kg DM, copper 1.6 mg/kg DM, manganese 201 mg/kg DM, nickel 5.4 mg/kg, lead 3.4 mg/kg and zinc 84.6 mg/kg, totaling 307 mg/kg). Larger concentrations of these elements were found only in the shoots of the root of the snake root (*Bistorta major* S.F. Gray 618.1 mg/g), distemper (*Agrostis canina* L., 371.6 mg/g DM), common foxtail (*Phleum pratense* L., 334.8 mg/g DM)

and black wormwood (*Artemisia vulgaris* L., 310.6 mg/g DM). At RCG, the hyperaccumulation in this study was predicted for nickel and manganese (here even high). For these elements, the translocation factor, i.e. the ratio between the element concentration in shoots and roots, was not only greater than one (nickel 1.76, manganese 5.42), but also the largest of all plants studied (Nworie et al., 2019). From the study of Vymazal et al. (2007) is also suggested that RCG may be a good phytoextractor because it accumulates a significant amount of chromium, nickel, copper, zinc, cadmium and lead, and Antonkiewicz et al. (2016) reported that RCG could be used as a good phytoextractor, because it accumulates more chromium, copper, zinc, lead and nickel than *Miscanthus giganteus* J.M. Greef and Deuter ex Hodk and Renvoize. Neuschütz and Greger (2010) in turn recommended RCG to phytostabilize sulfidic mine tailing because the presence of this plant in combination with sludge and ash layer can prevent metals and nutrient leakage, especially reducing the amount of leachate. Fialkowski et al. (2019) reported a high total concentration of heavy metals in RCG and *Miscanthus x giganteus* comparable to hyperaccumulators on soils where sludge was applied. However, the cause of this phenomenon was a large amount of biomass, while the bioconcentration factors (the proportion of the concentration of the substance in the organism and the medium) of heavy metals were low. The phytoremediation of RCG on soils where sludge has been applied has also been studied by Antonkiewicz et al. (2016). Here, the uptake of heavy metals was investigated in RCG Bamse as well as in *Miscanthus x giganteus* on soils where sludge was applied at 10, 20, 40 and 60 t/ha DM prior to sowing or, in the case of *Miscanthus x giganteus*, planting. The land where the sludge was not applied served as a control. The sludge contained 25.4 mg/kg DM of chromium, 14.8 mg/kg DM of nickel, 111 mg/kg DM of copper, 1005 mg/kg DM of zinc, 2.35 mg/kg DM of cadmium and 42.9 mg/kg DM of lead. As the sludge dose increased, RCG increased the intake of zinc (846.8 g/ha control, 2777.5 g/ha at the highest sludge dose) and cadmium (2.3 g/ha control, 15.3 g/ha at the highest sludge dose). For the other heavy metals evaluated, this trend was observed only up to a



slurry dose of 40 t/ha DM. Thus, the lowest intake of these heavy metals was at the control (chromium: 29.3 g/ha, nickel: 32.8 g/ha, copper: 81.1 g/ha, lead: 14.6 g/ha) and the highest at 40 t/ha DM (chromium: 117.5 g/ha, nickel: 152.9 g/ha, copper: 429.5 g/ha, lead: 56.6 g/ha). At this dose, the intake of chromium, nickel, copper, zinc and lead was also significantly higher than that of *Miscanthus x giganteus*. On the other hand, at a 20 t/ha of sludge dose, chromium, copper, zinc, cadmium and lead intake were significantly greater in *Miscanthus x giganteus* than in RCG.

Overall, the highest heavy metal intake was found in *Miscanthus x giganteus*, therefore the authors of this study consider *Miscanthus x giganteus* to be more suitable for sludge phytoremediation than RCG. However, RCG is expected to accumulate more of these elements than *Miscanthus x giganteus* with the exception of cadmium. The following average heavy metal content was found in RCG at all doses of sludge including control: 1.30 mg/kg DM chromium, 1.58 mg/kg DM nickel, 4.32 mg/kg DM copper, 37.79 mg/kg DM zinc, 0.61 mg/kg DM lead and 0.15 mg/kg cadmium DM, while for *Miscanthus x giganteus* this average heavy metal content was as follows: 1.21 mg/kg DM chromium, 0.86 mg/kg DM nickel, 3.82 mg/kg DM copper, 28.67 mg/kg DM zinc, 0.18 mg/kg DM of lead and 0.61 mg/kg DM of cadmium. Increasing the amount of sludge lead to an increase in the heavy metal content of the plants. In case of RCG, the heavy metal content of the control was as follows: chromium 0.93 mg/kg DM, nickel 1.04 mg/kg DM, copper 2.56 mg/kg DM, zinc 26.76 mg/kg, cadmium 0.07 mg/kg and lead 0.85 mg/kg DM. At the lowest sludge dose there was already a significant increase in the content of heavy metals in plants. For RCG it was chromium at 1.07 mg/kg DM, nickel at 1.09 mg/kg DM, copper at 3.12 mg/kg DM, zinc at 32.82 mg/kg DM, cadmium at 0.11 mg/kg DM and lead at 0.48 mg/kg DM. At the highest sludge dose, RCG contained chromium 1.70 mg/kg DM, nickel 2.24 mg/kg DM, copper 6.34 mg/kg DM, zinc 42.86 mg/kg DM, cadmium 0.27 mg/kg DM and lead 0.85 mg/kg DM. The highest increases in heavy metals compared to controls were in the case of cadmium by 285%, lead by 84% and chromium by 82%.

From heavy metals for example, nickel phytoremediation was separately investigated in RCG, where Korzeniowska and Stanislawska-Glubiak (2019) found that RCG had a higher ability to bioaccumulate nickel in above-ground biomass than in wicker baskets (*Salix viminalis* L.) and maize. The bioaccumulation was evaluated as the ratio of metal concentration in above-ground biomass and in the soil expressed in milligrams per kilogram, the so-called bioaccumulation ratio. The soils, in which these plants were cultivated, there were intentionally contaminated with nickel at doses of 60 mg/kg, 100 mg/kg and 240 mg/kg, and soils containing no nickel served as controls. The authors of this study do not consider any of these plants to be suitable for phytoextraction because the bioaccumulation ratio was significantly lower than 1 for all plants. Moreover, the bioaccumulation of nickel in RCG roots was too low to be considered at least a typical phytostabilizing plant, immobilizing nickel in the soil. Also, the transport of nickel from roots to above-ground biomass was low in all plants, with RCG averaging 20% in the first season and 28% in the second one.

A common problem in phytoremediation of heavy metal contaminated soils is that these soils tend to lack organic matter as well as low abundance of microorganisms. It would be desirable to apply suitable microorganisms to such soils. The presence of soil organisms in plant rhizosphere may intensify the process of phytoremediation by degrading contaminants in the rhizosphere, increasing heavy metal accumulation in plant tissues and indirectly promoting plant growth. For example, fungi of the genus *Trichoderma*, which are able among other things to reduce certain toxins and promote plant growth and development, may be used. Two *Trichoderma* strains designated as MS01 and MS02 were used to evaluate improvements in the phytoremediation process for cadmium, chromium, copper, lead, zinc and nickel not only in RCG, but also in *Miscanthus x giganteus*, switchgrass and several willow species (*Salix* sp.). The strain MS01 was isolated from forest soil and MS02 from degraded soil. The experiment was carried out in 20 plastic containers of 10 L containing soil, additives and the above mentioned plants (1 species was placed

in five pots). In eight pots was tested the strain MS01, in eight MS02 and in four there was no strain (control). The experiment lasted 21 weeks. The uptake of heavy metals by plants was assessed using the bioaccumulation coefficient, i.e. the proportion of metal concentration in the plant dry matter and the initial content of the element in the soil (in milligrams per kilogram). The application of *Trichoderma* fungi strains caused an increase in bioaccumulation factor of heaviest metals compared to control. The decrease in bioaccumulation factor compared to control occurred for lead in all plants, cadmium, chromium and zinc in *Salix* ssp., and chromium and nickel in RCG. At RCG, the bioaccumulation factor for most metals was greater, except for chromium and lead, when MS02 was applied. In other plants also, the bioaccumulation factor of MS02 was higher in most of the heavy metals examined. The ability of plants to allocate heavy metals from roots to above-ground biomass was evaluated using the translocation index, i.e. the ratio of the metal concentration in the above-ground part of the plant and the metal concentration in roots (in milligrams per kilogram) expressed as a percentage. In the case of lead, in both *Trichoderma* fungi strains decreased the translocation index in all plants compared to the control (except *Miscanthus x giganteus* with MS01), with RCG showing the lowest translocation index of all plants ( $2 \pm 1\%$ ). However, for other metals in RCG increased the translocation index over control, when both *Trichoderma* fungi strains were applied. Overall, the application of both *Trichoderma* fungi strains lead to an increase in bioaccumulation and translocation factor for all metals evaluated here except lead in all investigated plants and the application was effective in mobilizing and extracting cadmium, chromium, copper, zinc and nickel irrespective of the plant species used (Kacprzak et al., 2014).

For example, Marchard et al. (2014) studied the phytoremediation of heavy metals at RCG in the aquatic environment, investigating the copper removal from water in a so-called bio-rack system not only in RCG, but also in reed (*Phragmites australis* (Cav.) Trin. Ex Steud) and rush (*Juncus articulatus* L.). The bio-rack system was designed by Valipour et al. (2009), which is a new design

of a constructed wetland (CW). The unique feature of this system was the presence of many vertical pipes where there was no sediment, but were planted with reed. The system was designed as a rack that allowed the plant growth and supported a matrix for the presence of bacteria. The bio-rack system allows several cycles, so blockage due to accumulation, biofilm formation and saturation of sorption sites can be prevented. In this study, 15 perforated tubes filled with a mixture of gravel (diorite 80%) and perlite (20%) were used in the bio-rack system. In addition, the bio-rack system consisted of 12 constructed wetlands, which were either planted with the above plants or were plant free and served as a control. The system was designed in three repetitions. Plants were transferred to CW from copper contaminated sites. Each constructed wetland was filled with a mixture of fresh water from the Jalle d'Eysines River (30%) in Bordeaux (France) and from the water supply (70%). The water was contaminated with copper (158.5  $\mu\text{g/L}$ ) and three trials were made. The first trial was provided in early spring, the second at the beginning of the vegetation season (both at pH 8) and the third at the peak of the growth season at pH 6. In all experiments, the water was recirculated in the CW for 14 days. The amount of copper removed was calculated by subtracting from the hundred the proportion of copper measured on the fourteenth and starting (zero) days multiplied by one hundred. Copper removal efficiency was evaluated using the Relative Treatment Efficiency Index (RTEI), which is the proportion of difference at copper removal in planted and unplanted constructed wetland and the sum of copper removed in planted and unplanted wetland as a percentage. In the first trial, the total copper was removed in 52% (RTEI 0.1), 68% (RTEI 0.2), and 87% in the RCG, which was the highest in all cases of plant CW. In addition to total copper removal,  $\text{Cu}^{2+}$  removal was also evaluated. In the first experiment, CW with RCG had 2%  $\text{Cu}^{2+}$  removal, which was more than CW with *Juncus articulatus* L., but less than CW with reed where 10% of the copper was removed.

Conversely, in the second experiment at CW with RCG was achieved the greatest removal of  $\text{Cu}^{2+}$  from all plants

(77%) for RTEI 0.1. In the third experiment,  $\text{Cu}^{2+}$  was in all cases 99% removed (RTEI 0). Thus, the best copper removal occurred in all plants in the third experiment, which could be due to the higher density and activity of plants and microorganisms at the peak of the vegetation season. The reason for the success of CW with RCG as well as CW with reed in copper removal may be that these plants produce large amounts of underground biomass and thus roots can take a significant amount of copper. Moreover, it is generally accepted that parts of RCG accumulate metals in decreased order: roots (Polechońska and Klink, 2014a), rhizomes (Vymazal et al., 2007), leaves and stems (Polechońska and Klink, 2014a), while most of macronutrients are accumulated in above-ground biomass, which make RCG suitable for nutrient phytoextraction from water and bottom sediments of eutrophic lakes and rivers (Polechońska and Klink, 2014b). However, the maximum standing stock values occur at different times for each heavy metal, which complicate the optimum time for above-ground biomass harvest in order to remove the maximum of all heavy metals at once. But it seems that the harvest during the early growing season is the best (Březinová and Vymazal, 2015). Furthermore, it has been found that a similar amount of underground biomass, such as RCG is also produced by rush, which showed the lowest copper removal (Marchard et al., 2014). A possible cause of this phenomenon could be that copper was complexed with low molecular weight organic acids such as oxalate, which was observed in *Juncus maritimus* Lam., with increased copper exposure. This would lead to a reduction in copper removal. Furthermore, it should be taken into account that if the above-ground biomass is higher than the underground biomass, the accumulation of heavy metals may be greater in the above-ground part. For example, just in RCG, Vymazal (2016) noted that higher amounts of above-ground biomass ( $1196 \text{ g/m}^2$ ) versus underground ( $244 \text{ g/m}^2$ ) resulted in greater accumulation of copper and mercury in above-ground biomass. In addition to heavy metal phytoextraction, RCG could be used to remove radioisotope cesium 137 from other inorganic substances because it was found to be able

to accumulate it. However, an even greater ability to accumulate radiocesium was found by Lasat et al. (1997) at increasing order in brown mustard (*Brassica juncea* (L.) Czern.), Bean (*Phaseolus acutifolius* Gray) and especially in cabbage (*Brassica oleracea* convar. *capitata* L.).

The phytoremediation of organic compounds has also been studied in RCG. For example, together with the fescue (*Festuca arundinacea* Schreb.), soybean, alfalfa, ryegrass (*Lolium multiflorum* Lam.), the greater straw sedge (*Carex normalis* Mackenzie) and three varieties of gourd (*Cucurbita pepo* L. ssp. *pepo*) were tested for phytoextraction of polychlorinated biphenyls (PCBs) by Zeeb et al. (2006). The study was conducted in a greenhouse. Soil contamination of PCBs was performed by Aroclor 1260. The concentration of PCBs in soils was from 90 to 4200  $\mu\text{g/g}$ , while the organic content ranged from 0.06 to 2.02%. In this study, the most effective varieties in the extraction of PCBs were from *Cucurbita pepo* L. In this short-term trial (8 weeks) no detectable decrease in PCB was observed at monitored soils, but the results obtained here suggest that this goal could be achieved when apply many times. Furthermore, it was found that all plants showed signs of stress in the most contaminated soils (4200  $\mu\text{g/g}$ ). In contrast, no symptoms of stress were observed in the plants used in the least contaminated soils (90 and 250  $\mu\text{g/g}$ ). Also, Dzantor and Woolston (2001) used for PCB phytoextraction RCG together with meadow pea (*Lathyrus sylvestris* L.) and medick (*Medicago polymorpha* L.). In this case, the experiment was carried out under laboratory conditions and the plants were in soil containing 50 mg/kg PCB. The soil contaminated by PCB was performed with Aroclor 1248 and byphenyl at 1000 mg/kg, and plant residues that induced PCB degradation (ground pine needles or orange peels) were also added. After nearly 100 days, the PCB content in the soil, where RCG was cultivated, showed 59%, while in the soil without plants, the PCB content was 69%. However, a lower PCB content in the soil was observed in so many varieties (55%) and also in soils without plants, but with biphenyl (48%) and orange peels (45%). The combination of the used plants and the additives in this study increased the effect of plant

PCBs phytoremediation. Dzantor et al. (2000) has also investigated PCB phytoremediation in RCG and several other grasses (*Festuca arundinacea* Schreb, *Dichanthelium clandestinum* L. (Gloud), switchgrass.) and leguminoses (*Medicago sativa* L., *Securigera varia* L., *Lespedeza cuneata* (Dum. -Cours) G. Don., *Lathyrus sylvestris* L.). The best results were obtained with RCG, *Panicum variegatum* L. and *Lathyrus sylvestris* L. In addition, RCG along with rapeseed brassica (*Brassica napus* L.) were used for phytoremediation of polycyclic aromatic hydrocarbons using compost supported by the addition of silicon dioxide in nano-forms. In this study, better results were obtained in RCG than in rapeseed (Wloka et al., 2019). Chekol et al. (2002) evaluated seven fodder plants, including RCG in terms of phytoremediation of soils with high (6.3%) and low organic matter content (2.6%) contaminated with pyrene and trinitrotoluene at 100 mg/kg, with trinitrotoluene as the best results achieved in RCG and switchgrass on soils with low organic matter content. However, none of the fodder plants studied had a significant effect on the pyrene content. Dzantor et al. (2000), based on their experiments comparing the degradation of pyrene as well as trinitrotoluene in natural soil and soil contaminated with microbial contaminant, found that the soil microbial transformation had the greatest influence on the degradation of these substances.

The standardization of agronomic practices at contaminated sites should be developed for RCG and other species used as energy and phytoremediation plants to achieve high yields. Furthermore, it is also important to ensure that farmed animals would not graze on contaminated soils (or to cultivate species avoided by livestock), so that plants can be used for a given energy purpose and that potentially invasive species do not spread to the surrounding area (Pandey et al., 2016). For example, just in the case of RCG its invasiveness can be a problem in many countries, mainly in North America (Semere and Slater, 2007a). RCG is naturally spread throughout Eurasia and probably North America in mild regions (Laurent et al., 2015). However, its cultivars introduced from Europe shortly after 1850 are invasive (Lavergne and Molofsky, 2004). The invasive cultivars mainly occurred in Illinois

and Midwestern United States (Spyreas et al., 2010). They reduced native vegetation and canopy vegetation complexity, changed the wetland soil environment due to decreasing soil organic matter, increased soil moisture (Weilhoefer et al., 2017), reduced nutrient retention time and carbon storage, and accelerated the turnover cycles reducing carbon sequestration (Askaer et al., 2011). The quantitatively and qualitatively decrease of biodiversity can be consequently caused by the reduction of herbivores and their predators. For example, Spyreas et al. (2010) found a negative correlation between the occurrence of RCG and the Homopterous. RCG abundance is probably supported by high soil nitrogen and calcium content (Martina and von Ende, 2008). The extermination of RCG is difficult because the rhizomes vitality, which manifests its recovery, whenever chemical treatment is suspended. At this way, tillage should be provided before herbicide application (Annen, 2008). According to Semere and Slater (2007a) the best solution is to use RCG natural genetic variation in breeding programs. It would be also appropriate to ensure that the aesthetics of the landscape after harvest do not deteriorate, for example by introducing rotary and successive harvests. In addition to these areas, the interaction of microorganisms and energy plants on contaminated soils should be further investigated. It is important that not only scientists would be involved in the solution of phytoremediation of contaminants by energy plants, but the widest possible range of other subjects. These are mainly farmers, phytoremediation companies and entities that determine the rules related to this issue (Pandey et al., 2016).

## CONCLUSION

A recent global problem is the risk of non-renewable energy source depletion. The utilization of a renewable energy source will be necessary in the early future. This article emphasizes the importance of canary grass (*Phalaris arundinacea* L.) for this purpose. The very important advantages of this plant are its high yield, low inputs for cultivation and the ability to grow in different conditions. Recently, RCG has been used specially for combustion. But, it has potential for other bioenergy utilization as for



biogas, ethanol, pulp, paper and fibre fuels or chemical raw materials production. Besides bioenergy utilization, it can be used for phytoremediation and nitrogen compounds removal. Older ways of utilization are for forage crop, persistent perennial cover in permanent pastures and restoration of degraded soils and waters.

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