PRODUCTION OF BIO-ETHANOL FROM BARLEY STRAW AND REED CANARY GRASS: A RAW MATERIAL STUDY

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ABSTRACT: The aim of the study was to estimate the optimal harvest time and overall potential of barley straw and reed canary grass (RCG) as feedstocks for bio-ethanol production. The estimate was conducted based on samples from field trials in Finland collected during the seasons 2005/06 and 2006/07. The total dry matter (DM) yield for barley was 8-10 metric tons ha^{-1}, whereof proportion of straw comprised 30%. For RCG the highest DM yield, more 11 tons ha^{-1}, was obtained one month after seed maturing. The content of soluble sugars decreased drastically when plant got older being the lowest in mature barley straw and in dead plants in spring for RCG. Cellulose and lignin content of barley straw increased until dough stage of grain and changed only slightly thereafter. Changes in hemicellulose content were equal but lesser. Cellulose and lignin content for RCG was higher the later the plant material was harvested. Only minor changes in hemicellulose content were discovered. Barley straw taken at harvesting time, and RCG taken at seed maturing time and in spring were treated by steam explosion, where after the success of enzymatic hydrolysis and yeast fermentation were evaluated in laboratory-scale. The enzymatic hydrolysis of steam exploded washed raw materials followed by yeast fermentation was evaluated in laboratory-scale. Both RCG and barley straw were easily pre-treated with steam explosion, hydrolysed to monosaccharides and fermented to ethanol, and thus suitable raw materials for ethanol production.

Keywords: straw, reed canary grass, bio-ethanol

1 INTRODUCTION

The EU has established a goal to replace up to 10 energy-% of all fuel used in road transport with renewable fuels by the year 2020. The bulk of EU's car fleet is running on either diesel or petrol. Ethanol can be mixed into petrol, but the current petrol specification, however, limits this mixing ratio to about 5 volume-%. Ethanol is predominantly produced from renewable raw materials and thus, the use of it as fuel should potentially decrease the consumption of fossil energy and the emission of greenhouse gases (GHG). The production of ethanol from sugar cane juice, corn starch and cereal grains using fermentation process is the conventional way to make ethanol. This process is, especially when corn and cereal grains are used as the raw material, rather cost- and energy-intensive as well as not optimal from a GHG emission perspective [1,2]. Using lignocellulosic waste materials or high biomass-yielding grasses instead as the raw material for ethanol production could be a way to reduce the production costs, reduce the GHG emissions and avoid competition with the food sector.

MTT and VTT studied the availability and quality requirements of lignocellulose-based raw materials such as cereal straw and reed canary grass (RCG) (*Phalaris arundinacea* L.) to be used in bio-ethanol production. The selected raw materials are especially interesting from a Finnish perspective (and other countries with similar growth conditions). RCG is currently grown for large-scaled energy production [3], and spring barley is the best adapted cereal crop in the northern conditions. Optimal plant development stage and chemical composition of the biomass, as well as the technological preconditions to modify the raw material into a fermentative form were the key questions to be answered in the current cooperation project.

State-of-the-art technologies (pre-treatment, enzymatic hydrolysis and fermentation) were used to assess the bio-ethanol production efficiency with the aim to also evaluate the economic feasibility of a hypothetical factory utilizing the studied raw materials. In this paper, some preliminary results, especially in regard to affects of the raw materials are presented and discussed.

2 MATERIAL AND METHODS

2.1 Field trials

Barley straw was harvested at six different phases of growth during the seasons 2005 and 2006 in Jokioinen, Finland. The first harvesting was performed at the early ear emerging stage, and the last at grain threshing (Table I). RCG was harvested six times in 2005 and nine times in 2006 in the field sown 2003. The first harvesting was at the panicle emergence and the last at yellow stage in the following spring (Table I). The samples for both raw materials were analysed for biomass yield and dry matter (DM) content, fibre components (cellulose, hemicellulose and lignin) and soluble sugars.

2.2 Chemical analysis of the raw material

For chemical analysis the plant samples were dried at temperature less than 40^{o}C, and pulverized to less than 1 mm diameter. The content of cellulose and hemicellulose was measured using the results for acid detergent fibre (ADF), acid detergent lignin (ADL), and neutral detergent fibre (NDF). ADF, ADL, and NDF were measured by the Fibertec™ 1020 (M6) System which follows methods with official approval as ISO 6865, 92/89 EEC and AOAC 2002.04 (feed) [4]. Soluble sugars such as fructose, glucose and saccharose were measured by liquid chromatographic determination [5].

2.3 Pre-treatment for enzymatic hydrolysis

The samples for straw taken at harvesting time and for RCG at seed maturing time (4th sampling) and in spring (9th sampling) were treated by steam explosion (2% SO_2, 190°C-200°C, 10 min), where after the success of enzymatic hydrolysis and yeast fermentation were evaluated in laboratory-scale experiments.

Table I: Sampling dates for barley straw and reed canary grass biomass.

Sampling phase	Sampling dates	
Barley	2005	2006
Sowing time	2-May	6-May
1 20% ear emergence (EE)	27-Jun	21-Jun
2 Full ear emergence	4-Jul	29-Jun
3 Two weeks after full EE	18-Jul	12-Jul
4 Early dough stage of grains	26-Jul	20-Jul
5 Yellow ripeness of grains	1-Aug	1-Aug
6 Harvest ripeness	8-Aug	7-Aug
Reed canary grass		
Sowing time 4-Jun 2003		
1 20% panicle emergence	22-Jun	22-Jun
2 Beginning of flowering	5-Jul	5-Jul
3 End of flowering	14-Jul	19-Jul
4 Seed matured	3-Aug	31-Jul
5 A month from sampling 4		30-Aug
6 Two months from sampling 4		27-Sep
7 Three months from sampling 4		26-Oct
8 Plants senescent	16-Nov	11-Dec
9 Delayed harvesting in spring	2-May 06	4-Apr 07

2.4 Enzymatic hydrolysis and yeast fermentation

The enzymatic hydrolysis of steam exploded washed raw materials was performed with commercial cellulase mixture (Celluclast 1.5L FG, Novozymes) supplemented with β-glucosidase (Novozym 188, Novozymes). The enzyme dosage used was 10 FPU g^{-1} dry matter and β-glucosidase 100 nkat g^{-1}). Hydrolysis of washed fibre was carried out in test tubes at 1% concentration, pH 5 at 45°C with magnetic stirring. Hydrolysis of raw materials at higher DM concentrations were carried out in fermentors at 10% concentration with cellulase dosage of 20 FPU g^{-1} dry matter supplemented with β-glucosidase 200 nkat g^{-1}.

Simultaneous saccharification and fermentation studies were carried out in a bioreactor (Braun CT2) in 1 liters volume at 10% DM concentration using both RCG and barley straw. The enzyme dosage of cellulase mixture was 20 FPU g^{-1} dry matter and β-glucosidase 200 nkat g^{-1}. Pre-hydrolysis was carried out for 5 hours at pH 5 and at 45°C. For the fermentation phase the temperature was reduced to 30°C and inoculated with 3.5 g l^{-1} yeast. Fermentation at 15% DM concentration was carried out with barley straw in the same bioreactor by using enzyme dosage of cellulase mixture 10 FPU g^{-1} dry matter and β-glucosidase 100 nkat g^{-1}. Over night prehydrolysis was carried out at 45°C. For the fermentation phase the temperature was reduced to 30°C and inoculated with 3.5 g l^{-1} yeast.

3 RESULTS

3.1 Biomass production

The total biomass yield of barley doubled between the ear emergence stage (1st sampling) and the yellow ripening stage (5th sampling) (see Fig. 1). At highest, the total DM yield was 8 and 10 tons ha^{-1} in 2005 and 2006, respectively. The straw yield was about 30% of the total biomass.

The biomass yield of RCG doubled between the panicle emergence stage (1st sampling) and the seed ripening stage (4th sampling), the latter being more than 11 tons DM ha^{-1} (see Fig. 2) in 2005. In 2006, the highest yield was obtained one month after seed maturing (5th sampling). The DM content of the green biomass was between 20 and 50%, and more than 90% at spring harvesting.

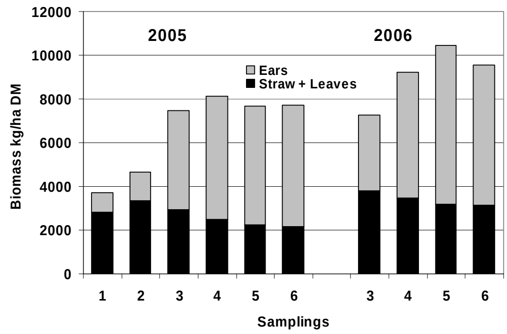

Figure 1: Dry matter (DM) yield for barley in 2005 and 2006. Sampling dates from Table I.

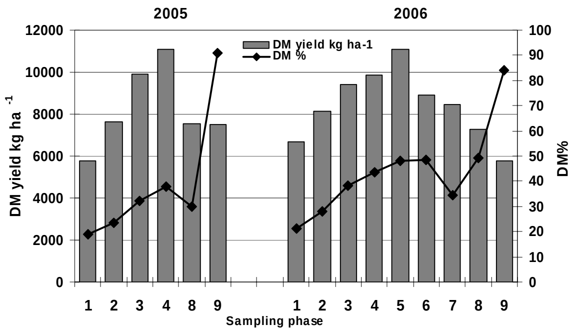

Figure 2: Dry matter (DM) yield and DM content for reed canary grass at different sampling phases in 2005/06 and 2006/07. Sampling dates from Table I.

3.2 Chemical composition of the raw material

The content of soluble sugars for barley straw was high at the ear emergence stage but decreased to a low level as grains started to grow. For RCG, the content of soluble sugars was higher in green plants than in late autumn or in dead plants in spring, when the sugar content was as low as in barley straw (Table II).

Cellulose and lignin content of barley straw (incl. leaf blades and sheaths) increased until dough stage of grain (4th sampling) and changed only little until harvest. Changes in hemicellulose content were lesser (Fig. 3). Cellulose and lignin content for RCG was higher the later the plant materials were harvested. There were only minor changes in hemicellulose content (Fig. 4).

Table II: Total soluble sugar content for barley straw and reed canary grass at different development stages in 2005/06 and 2006/07.

Barley	Sugars, g kg^{-1} DM			
2005	Fructose	Glucose	Sucrose	Total
27-Jun	37.3	31.1	58.5	127.2
4-Jul	43.7	30.1	71.3	145.0
18-Jul	37.2	16.8	22.6	76.7
26-Jul	11.2	8.9	0	20.1
1-Aug	0	4.3	0	4.3
8-Aug	0	4.7	0	4.7
2006				
12-Jul	65.2	40.6	69.8	175.6
20-Jul	50.4	26.4	42.7	119.3
1-Aug	11.4	7.0	0	18.4
7-Aug	7.8	4.7	0	12.5
Reed canary grass				
2005	Fructose	Glucose	Sucrose	Total
22-Jun	23.3	22.2	7.5	53.0
5-Jul	19.3	17.5	8.9	45.7
14-Jul	25.3	22.1	10.2	57.5
3-Aug	25.2	19.4	23.1	67.7
16-Nov	9.2	8.0	0.0	17.2
2-May	1.4	3.4	0.0	4.8

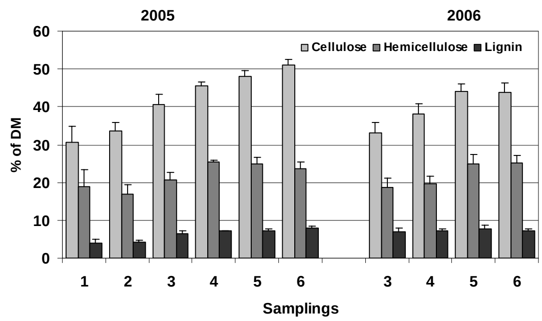

Figure 3: Cellulose, hemicellulose and lignin content for barley straw at different development stages in 2005 and 2006. Sampling dates from Table I.

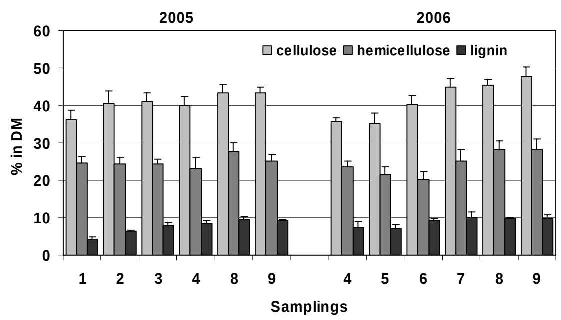

Figure 4: Cellulose, hemicellulose and lignin content for reed canary grass at different development stages in 2005/06 and 2006/07. Sampling dates from Table I.

3.3 Processibility of barley straw and reed canary grass into ethanol

Raw materials obtained by steam explosion could be hydrolyzed efficiently with relative low enzyme dosage in laboratory conditions with sugar yields of about 80-100% in 72 hours (Fig. 5). The hydrolysis level of RCG harvested in the autumn was lower than in material harvested in spring. Studies at higher (10%) DM concentrations resulted hydrolysis levels up to 95% of the theoretical amount of sugars in 72 hours.

In simultaneous saccharification and fermentation at 10% DM concentration both RCG and barley straw were hydrolyzed and fermented similarly. Neither of the materials was toxic to the enzymes or yeast even at high DM concentration, and they could be efficiently hydrolyzed and fermented to ethanol. In addition, barley straw was fermented at as high as 15% DM content. Steam exploded material was pre-hydrolyzed over night before yeast inoculation. Yeast started readily to produce ethanol from glucose (Fig. 6). Ethanol concentration of around 20 g l^{-1} was obtained already in 24 hours.

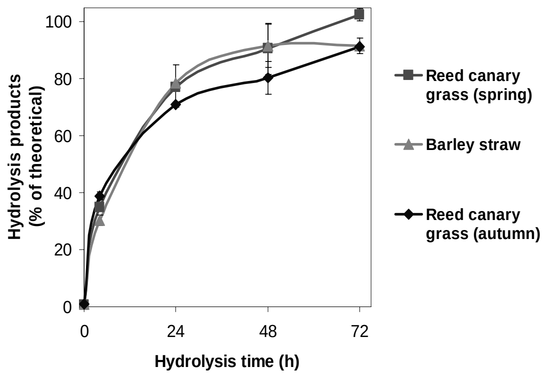

Figure 5: Hydrolysability of steam exploded reed canary grass and barley straw.

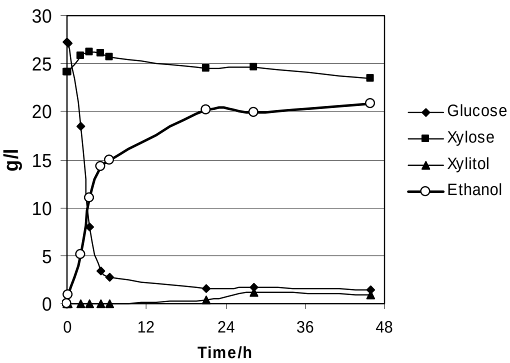

Figure 6: Production of ethanol in simultaneous saccarification and fermentation of steam exploded barley straw.

4 DISCUSSION

The total biomass yield of barley doubled between the ear emergence stage and the yellow ripening stage (Fig. 1). At maturity about half of the above ground biomass is located in the grains [6,7]. The storage of photosynthate in the leaf and in stem tissues along the path of transport between the source and sink provides an important control in relation to the partitioning of carbon in plants [8]. Carbohydrate reserves (fructans, fructose, glucose, sucrose) build up in the stem at the time of ear emergence and anthesis and subsequently these reserves are utilized during grain growth (Table II). Pre-anthesis assimilation was estimated to have contributed 44% of grain dry matter in a very dry, hot year, but only 11 per cent in a wetter, cooler year [7].

Celluloses and lignin content of barley straw (incl. leaf blades and sheaths) increased until dough stage of barley grain and changed only little until harvest. In maize more than half of the cell wall material accumulated after internode elongation had ended [9]. Galactose and arabinose began to accumulate early in cell wall development, which was presumed to be associated with primary wall growth during internode elongation. The major secondary wall constituents did not begin to accumulate rapidly until shortly before internode elongation ended [9].

The DM yield of straw comprised 30% the total biomass of barley at grain maturing stage. However, the height of the cutting affects the straw yield. For example the stubble height of 15 cm will mean 25% less in straw yield [10].

In this study, for RCG the highest DM yields, more than 11 metric tons, were obtained in the 5th sampling in end of August in 2006. In early 2006 harvested spring yield was 7.6 t DM ha^{-1} that was same as yields in average [11]. In earlier studies, RCG has remained productive longer if it is harvested as dead material in spring [11]. The DM content of the green material varied from 20 to 50% being too moist for storing as bales. The material harvested in spring was very dry and could be easily stored long time without drying.

The growth phase depending changes in chemical composition of RCG were parallel but less drastic than those with barley straw. The cellulose contents in 2005 remained lower than those for barley and the numbers for RCG in 2006. The plant stand was harvested for the first time in 2005, and the younger plantations often produce less fibre [11]. RCG produces new shoots in spring and again in autumn resulting increase of green material in biomass. The total soluble sugar content in RCG remained constant to the seed maturing stage differing from barley straw. Sugar content in spring harvested RCG was equivalent to that of barley straw at grain maturing stage. If we think about biomass, fibre or sugar yield alone, RCG should be harvested in late autumn at green stage. However, the water content of biomass is high and thus, the storage without energy intensified drying is not possible. RCG and mature barley straw were hydrolysed and fermented similarly in laboratory conditions.

5 CONCLUSIONS

Both RCG and barley straw were found to be suitable raw materials for ethanol production. Materials could be easily pre-treated with steam explosion, hydrolysed to monosaccharides and fermented to ethanol.

The optimum harvesting times for bio-ethanol raw material are at grain maturity for barley straw, and in spring as dead material for RCG. The other potential raw materials, barley harvested at full ear emergence and RCG harvested after seed maturing stage, need further research.

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