Agronomy Research **12**(2), 397–406, 2014

Utilization of urban waste in bioethanol production: potential and technical solutions

M. Raud[1], R. Kesperi[1], T. Oja[2], J. Olt[1] and T. Kikas[1,*]

[1]Institute of Technology, Estonian University of Life Sciences, Kreutzwaldi 56, EE51014 Tartu, Estonia; [*]Correspondence: timo.kikas@emu.ee
[2]Institute of Ecology and Earth Sciences, University of Tartu, Vanemuise 46, EE51014 Tartu, Estonia

Abstract. In urban forestry and greening millions of tons of lignocellulosic waste is produced every year. Although lignocellulosic materials are considered one of the most promising feedstock for the bioethanol production, biomass from urban greening and forestry goes unused. Aim of this research was to investigate the potential of such waste biomass for bioethanol production. Woody and non-woody vegetation from different sources was investigated: old and fresh leaves, and mixed waste from urban greening which contained grass, twigs, and leaves. Cellulose, hemicellulose and lignin contents in the samples were determined using fibre analysis. Dilute acid was used for pre-treatment at temperature of 130°C in order to compare yields from different methods. Thermochemical pre-treatment was followed by enzymatic hydrolysis and fermentation. Produced glucose and ethanol contents were measured using electrochemical analyser. Waste from urban greening had the highest cellulose content of 22.96% and gave the best glucose and ethanol yields, 154.5 g kg^{-1} and 62.5 g kg^{-1}, respectively when the sample was pre-treated with dilute H_2SO_4. In addition, map analysis was used to assess the area manageable by urban forestry and greening in the city of Tartu to estimate the potential of greening and forestry waste as a substrate for bioethanol production that could be used as a fuel in city's public transportation system. In conclusion, it was shown that the waste from urban greening and forestry is a very promising raw material for biofuel production considering its localization and high cellulose content.

Key words: urban green waste, biofuel, lignocellulose, bioethanol.

INTRODUCTION

Today fossil fuels take up 80% of the primary energy consumed in the world, of which 58% alone is consumed by the transport sector (Nigam & Singh, 2011). Continuous depletion of conventional fossil fuel reserves with increasing energy demands and climate change (Agbor et al., 2011, Nigam & Singh, 2011) have led to a move towards alternative, renewable, sustainable, efficient and cost-effective energy sources with smaller emissions (Nigam & Singh, 2011). An attractive option to reduce fossil-fuel dependency is the use of biomass for energy production (Haberl et al., 2011). Conversion of biomass to alternative and renewable fuels is attractive potential to replace fossil-fuel-derived liquid fuels (Abdoulmoumine et al., 2012, de Almeida et al., 2013).

One of the most promising feedstock for the liquid biofuel production is lignocellulosic material (Agbor et al., 2011, Tutt et al., 2012b, Min et al., 2013, Phitsuwan et al., 2013). Lignocellulosic biomass, which is either non-edible residues of food crop production or non-edible whole plant biomass (Nigam & Singh, 2011), make up the majority of the cheap and abundant non-food materials available from plants (Naik et al., 2010, Phitsuwan et al., 2013). One of the underutilized lignocellulosic biomass resources, which is dramatically increasing with rapid urbanization worldwide, is waste from urban forestry and greening (Shi et al., 2013). This kind of biomass contains various ingredients like lawn thatches and cuttings, grass, leaves and twigs from the gardens, green roofs, and recreational parks (Kudakasseril Kurian et al., 2013). Forestry and greening waste in urban areas offers annually substantial and continuous amount of biomass (Shi et al., 2013). Additional benefits are lower feedstock cost and the ability to turn waste product into a useful commodity (Abdoulmoumine et al., 2012) that could contribute significantly to regional bioenergy system (Shi et al., 2013).

Plant biomass is primarily composed of plant cell walls of which about 75% are polysaccharides (Phitsuwan et al., 2013) that could be used for ethanol production. One possibility for new generation biofuel is production of lignocellulosic ethanol (Chiaramonti et al., 2012, Tutt et al., 2012a) which can be either used directly as fuel or mixed with gasoline in customized engines (Olt & Mikita, 2011, Haghighi Mood et al., 2013). Bioethanol is the most common biofuel in the world and it has been used as a fuel for almost century and a half. Ethanol has a high octane number, good detonation stability and high oxygen content therefore, it allows cleaner combustion process which reduces content of CO, unburned hydrocarbons and NO_x in the exhaust gases (Küüt et al., 2011).

The traditional three step ethanol production process includes pre-treatment of biomass, saccharification to release the fermentable sugars from polysaccharides, fermentation of released sugars to ethanol, and finally distillation to separate ethanol (Nigam & Singh, 2011). However, the complicated structure of lignocellulosic material makes it recalcitrant to chemical and/or biological degradation (Min et al., 2013, Xu et al., 2013). To overcome this, variety of methods for pre-treatment of lignocellulosic material, including biological, physical and chemical processes but also combinations of those, have been studied (Sims et al., 2010, Agbor et al., 2011, Chiaramonti et al., 2012, Xiao et al., 2012, Min et al., 2013). Main effects of these methods are solubilisation of hemicelluloses and alteration or degradation of lignin which makes cellulose more accessible to cellulases (Sun & Cheng, 2002, Alvira et al., 2010, Sims et al., 2010, Xiao et al., 2012, Phitsuwan et al., 2013, Barakat et al., 2014). However, different types of feedstock have different cellulose, hemicelluloses and lignin contents therefore, different pre-treatments are required for particular types of biomass (Alvira et al., 2010, Phitsuwan et al., 2013).

The aim of this study was to assess the potential of the waste from urban forestry and greening as a substrate for bioethanol production using a small size town as an example (Tartu, Estonia – population ca 100,000). For the analysis of bioethanol production efficiency, different pre-treatment methods were applied to woody and non-woody vegetation from different sources in the traditional three step ethanol production process. Glucose and ethanol yields were considered to estimate the most suitable

substrate and pre-treatment method. In addition, map analysis was used to assess the area manageable by urban forestry and greening in the city of Tartu and thereby, estimate the amount of biomass available and the bioethanol production potential.

MATERIALS AND METHODS

Biomass

Three different kinds of biomass samples were collected to investigate the suitability of waste from urban forestry and greening for bioethanol production. Leaves were collected in two different seasons. Mixed leaves from maple, oak and bushes that were left outside over winter were collected in spring. Leaves collected in autumn consisted mainly of maple leaves. In addition waste from urban greening that contained grass, twigs, and leaves was investigated. The samples were dried to a moisture content less than 10% and ground with Cutting Mill SM 100 comfort (Retsch GmbH) and then with Cutting Mill ZM 200 (Retsch GmbH) to a particle-size 3 mm or less.

Pre-treatment

Pre-treatment with 1% of H_2SO_4 solution was used to break the cell structure in the biomass and expose the cellulose to further enzymatic treatment. 1,000 mL of acid solution was added to 100 g of dried and milled biomass. Samples were heated for 60 minutes at temperature of 130 ± 3°C and pressure of 3 bars. After the pre-treatment the samples were cooled to a temperature below 50°C and pH of the samples was neutralized with K_2CO_3 to pH range of 4.5–5.

In case of experiments where the biomass was rinsed after acid pre-treatment, the pre-treated biomass mixture was cooled to 50°C and solid fraction was separated using vacuum filtration with fabric filter (pore size 100 μm). During filtration the solid biomass was rinsed with distilled water. Distilled water was added to the rinsed biomass to regain overall mixture volume of 1,000 ml, and pH was neutralized with K_2CO_3 to pH range of 4.5–5.

Hydrolysis

Enzymatic hydrolysis with enzyme complex Accellerase 1500 was used to convert cellulose in the biomass to glucose. Enzyme mixture was added to samples at a ratio of 0.3 ml per g of biomass. Hydrolysis lasted for 24 hours at a temperature of 50°C under constant stirring in rotating shaker/incubator (Unimax 1010, Heidolph Instruments GmbH & Co.KG). After the hydrolysis, the glucose concentration in all the samples was measured.

Fermentation

In order to start the fermentation process, 2.5 g of dry yeast *Saccharomyces cerevisiae* was added to all samples. Fermentation process was carried out at room temperature under low oxygen conditions in 1,000 mL glass bottles, sealed with a fermentation tube and lasted for 5 days after which, the ethanol concentration was measured.

Chemical analysis

The percentage of lignin, Acid Detergent Fiber (ADF), and Neutral Detergent Fiber (NDF) in the dry mass (DM) of the biomass samples was determined at the Plant Biochemistry Laboratory of Estonian University of Life Sciences (Tecator ASN 3430; AOAC, 1990; Van Soest et al., 1991). The glucose and ethanol concentrations in the mixture were determined using Analox GL6 analyser (Analox instruments Ltd.).

Bioethanol production potential analysis

The area of different land cover types was measured from the Estonian Base Map distinguishing the differences between public lawn areas, parks with trees (mapped as forests in the base map), private gardens having a very similar to each other with a couple of trees, some bushes and lawn on each. Separate type of park is graveyard which in Estonia's case is mostly forest type with large old-grown trees. As the classes on the base map do not match specifics of urban green areas in detail the map was visually compared to orthophoto image of the town (as Published by the Estonian Land Board http://xgis.maaamet.ee/xGIS/XGis) and necessary corrections between different land-use classes were made considering the litter production capacity.

Also, data from municipality about the actual litter removal contracts and public green areas was used. The latter could not be used directly as it does not follow the same classification considering the litter productivity and does not cover all areas (like private gardens).

The average greening waste productivity in a square measure was estimated using data from municipality about the regularly maintained parks and public greenery areas and collected greening waste amounts from these areas.

The bioethanol production potential from Tartu greening waste was estimated from calculated yearly amounts of greening waste from different land cover types and ethanol yield. The information about city's public transportation and current biogas and diesel fuel usage was gained from local municipality and it was used to estimate the bioethanol potential as a substitute for diesel fuel in Tartu's public transportation system.

RESULTS AND DISCUSSION

Biomass analysis

A biomass can be characterised on the basis of its relative proportion of cellulose, hemicellulose, and lignin (Table 1). Among the biomasses used the waste from urban greening had the highest cellulose content. Cellulose content in spring leaves is comparable to that while in autumn leaves, it is considerably lower. Lignin concentration is the highest in spring leaves. Lignin concentration in the waste from urban greening is similar to that in spring leaves while in autumn leaves it is much lower. On the other hand, the highest hemicellulose concentration was measured in autumn leaves sample. The most important property of biomass for bioethanol production is high cellulose content (Tutt & Olt, 2011) and therefore, waste from urban greening containing grass, twigs, and leaves is expected to be the most suitable substrate.

Table 1. The results of biomass analysis for cellulose, lignin and hemicellulose

Sample	Hemicellulose (%)	Cellulose (%)	Lignin (%)
Spring leaves	6.00	21.06	27.74
Waste from urban greening	6.86	22.96	22.73
Autumn leaves	8.45	14.54	11.16

Ethanol production

To evaluate the suitability of waste from urban greening and forestry for bioethanol production, samples of various types of biomass were collected and investigated. Enzymatic hydrolysis was used for the conversion of cellulose into glucose and fermentation for its later conversion to ethanol. However, the cellulose fibres are packed into the hemicellulose / lignin matrix which makes them inaccessible to enzymes. To overcome this limitation, enhance hydrolysis, and increase ethanol yield, acid pre-treatment was used to break the hemicellulose / lignin cover. Glucose yield and hydrolysis efficiency (Fig. 1) and ethanol yield and fermentation efficiency (Fig. 2) were used to characterize the effect of pre-treatment on different types of biomass.

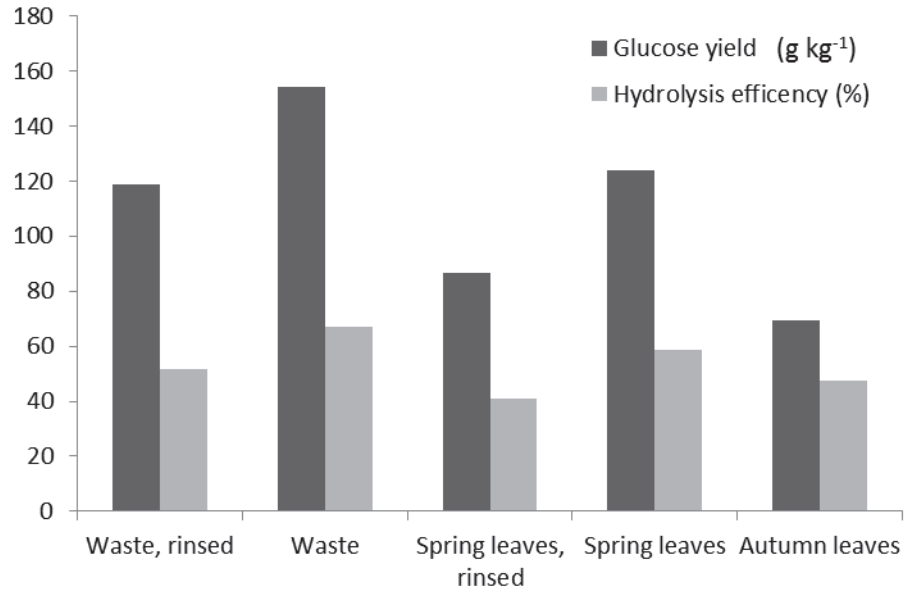


Figure 1. Glucose yields and hydrolysis efficiencies in case of different biomass types and pre-treatments.

Results indicate that from three types of biomasses investigated, the waste from urban greening is the best type for ethanol production. It has the highest cellulose content and resulted in the highest glucose and ethanol yields – 154.5 g kg^{-1} and 62.5 g kg^{-1} of biomass, respectively. Least glucose was gained from autumn leaves – 69.3 g kg^{-1} that had the lowest cellulose content. Glucose and ethanol yields from

different samples depended directly on the cellulose content of the sample. The higher the cellulose content the higher the respective glucose and ethanol yields.

Hydrolysis efficiency was also the best in case of the waste from urban greening – 67.3% while the lowest result was gained with spring leaves that were rinsed with water after acid pre-treatment – 41.2%. Relatively low hydrolysis efficiency indicates inefficiency of the pre-treatment and shows that large amount of cellulose was not converted to glucose. Acid pre-treatment at these conditions did not fully degrade the hemicellulose and lignin covering from cellulose fibres and therefore, enzymes did not have good access to cellulose to degrade it to glucose.

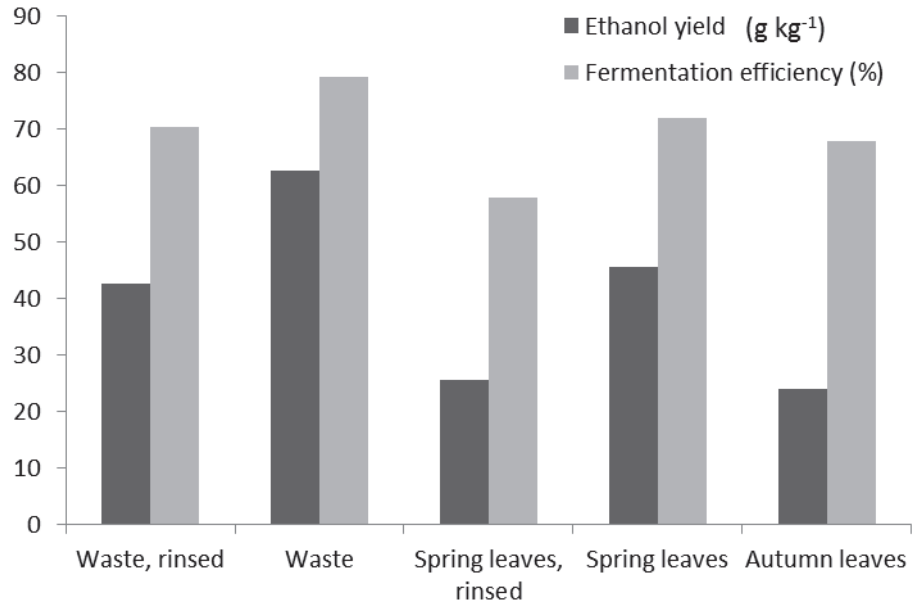


Figure 2. Ethanol yields and fermentation efficiencies in case of different types of biomass and pre-treatments.

Two different pre-treatment approaches were used. After the acid pre-treatment solid part of some of the samples was separated and rinsed with water before adding enzymes while other samples were immediately processed with enzymes. Rinsing of the biomass should remove by-products from the hemicellulose degradation that inhibit enzymatic activity of the yeast and thus, increase the fermentation efficiency (Tutt et al., 2012a). However, the results show that rinsing of the biomass with distilled water after acid pre-treatment decreases the glucose and ethanol yield from 154.5 g kg^{-1} to 118.7 g kg^{-1} and 62.5 g kg^{-1} to 42.7 g kg^{-1}, respectively in case of waste from urban greening. Similar decrease can be seen in case of spring leaves. The lack of effect on fermentation efficiency can be attributed to low concentration of hemicellulose in all three samples. Decrease in glucose and ethanol yields was caused by the removal of some of the sugars already hydrolysed during the acid pre-treatment with rinsing water.

Biofuel potential in the city of Tartu

Tartu is the 2-nd largest city in Estonia with approximately 100,000 inhabitants. The city is located in south-eastern part of Estonia and its territory is 38.8 km^2 From this area 3.9 km^2 is determined as park area and public greenery and 5.1 km^2 consists of natural meadows and bushy areas. From the park and public greenery areas 3.15 km^2 is regularly maintained by the city (Tartu municipal administration, 2010). However, various kinds of verdant areas, like private yards, forest stands, graveyards, etc. which also produce greening waste are not considered in given area.

Map analysis was used in order to gain more comprehensive information about types and acreages of the green areas within Tartu city including private yards, forest stands, graveyards, etc. Various types of greeneries and park areas, estimated amounts of the greening waste they produce in a year, corresponding bioethanol potentials, and percentage of the fuel consumed by public transportation of the city that it would cover are outlined in table 2. Water-meadows, forests and railway protection zones were not includes in the estimation of these areas. Majority of the maintained greenery area in Tartu – 7.09 km^2 make up private gardens and courtyards. Another major part of the green area includes public greenery – 3.72 km^2. Park and graveyard areas however have relatively small area – 0.42 and 0.41 km^2, respectively. In addition, some avenues and boulevards are lined with trees which total greening area is 1 km^2. Also, it can be seen that not all public greeneries and parks are regularly maintained. A relatively large unmaintained green area is mostly formed by the meadows of River Emajõgi (in total exceeding the private gardens). These areas as not maintained were not included in the analysis.

Table 2. Comparison of various types of green areas, estimated amounts of the greening waste they produce in a year, corresponding bioethanol potentials, and percentage of the fuel consumed by public transportation of the city that it would cover based on official city records and map analysis

Type of green area	Area (km^2)	Greening waste (t $year^{-1}$)	Bioethanol potential (t $year^{-1}$)	Coverage of fuel (%)
Public greenery	3.72[2]	4,285	268	21.8
Private yards	7.09[2]	8,170	511	41.5
Parks	0.42[2]	484	30.3	2.46
Graveyards	0.41[2]	472	29.5	2.40
Avenues	1.00[2]	1,152	72.1	5.85
TOTAL	**12.64**	**1,456**	**911**	**74**
Regularly maintained parks and public greenery[1]	3.15	3,628.7	227	18.4

[1] Data according to official city records (Tartu municipal administration, 2010)
[2] Data based on map analysis

During 2007–2009, the average yearly biodegradable greening waste collected from regularly maintained parks and public greeneries was 3,628.7 tons (Tartu municipal administration, 2010). Therefore, it can be estimated that approximately 1,151.9 tons of urban greening waste is produced yearly from km^2 of this type of area. The biomass (greening waste) potential for the city was calculated based on this estimation. For calculation of bioethanol production potential, the highest ethanol

yields gained from urban greening waste were considered – 65.2 g kg^{-1} of biomass. As seen from table 2, approximately 227 tons of bioethanol could be produced in a year if all collected greening waste from regularly maintained park and public greenery area would be used for bioethanol production. Private gardens and courtyard areas have the highest biomass and bioethanol production potentials, followed by public greenery. Bioethanol production potential could be more than three times higher if greening waste collection would be extended to private gardens and courtyards. Smaller part of bioethanol potential comes also from avenues, graveyards and park areas, from which the latter are already mostly maintained and greening waste collected.

The produced bioethanol can be used as a fuel in public transportation system of the city. The buses used for local public transportation in Tartu drive yearly ca 3.5 million km and consume diesel fuel or natural gas. Currently, approximately 10% of used fuel is natural gas. The fuel consumption of buses is 40 l of diesel fuel or 41 kg of natural gas per 100 km. Therefore, it can be estimated that if only diesel fuel would be used 1.44 million litres of diesel fuel or 1.29 million litres of diesel fuel and 0.14 tons of natural gas would be necessary in a year. Since the calorific value of bioethanol is smaller than that of a regular diesel fuel – 29 MJ kg^{-1} vs. 35 MJ kg^{-1}, respectively (Ritslaid et al., 2010), the fuel consumption of bioethanol is around 20% higher than that of a diesel fuel.

The current biogas and diesel fuel usage was taken into account when bioethanol potential as a substitute of diesel fuel in Tartu's public transportation system was calculated (Table 2). 18.4% of diesel fuel could be replaced with bioethanol if currently collected greening waste would be used to produce bioethanol. In addition 41.5% of diesel fuel could be additionally replaced with bioethanol if greening waste from courtyards and private gardens would also be processed to bioethanol. Latter would require widening the collection system to greening waste from private residences.

CONCLUSIONS

The potential of the waste from urban forestry and greening as a substrate for bioethanol production using a small size town as an example (Tartu, Estonia – population ca 100,000) was investigated. Different pre-treatment methods were applied to woody and non-woody vegetation from different sources in the traditional three step ethanol production process. It was found that the best glucose and ethanol yields were gained with urban green waste, containing grass, twigs and leaves, pre-treated with H_2SO_4. The city of Tartu was analysed using remote sensing to assess the area manageable by urban forestry and greening and thereby, to estimate amount of biomass available for bioethanol production. According to public records, in years 2007–2009 Tartu had 3.15 km^2 of regularly maintained park and public greenery areas from where greening and forestry waste was collected. If all private garden and courtyard areas would be included in the greening waste collection system in addition to city public greenery and park areas and used for bioethanol production, more than 60% of diesel fuel used in city's public transportation could be replaced.

REFERENCES

Abdoulmoumine, N., Kulkarni, A., Adhikari, S., Taylor, S. & Loewenstein, E. 2012. Economic analysis of municipal power generation from gasification of urban green wastes: case study of Fultondale, Alabama, USA. *Biofuels, Bioproducts and Biorefining* **6**(5), 521–533.

Agbor, V.B., Cicek, N., Sparling, R., Berlin, A. & Levin, D.B. 2011. Biomass pretreatment: Fundamentals toward application. *Biotechnology Advances* **29**(6), 675–685.

Alvira, P., Tomás-Pejó, E., Ballesteros, M. & Negro, M.J. 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresource Technology* **101**(13), 4851–4861.

Barakat, A., Chuetor, S., Monlau, F., Solhy, A. & Rouau, X. 2014. Eco-friendly dry chemo-mechanical pretreatments of lignocellulosic biomass: Impact on energy and yield of the enzymatic hydrolysis. *Applied Energy* **113**(0), 97–105.

Chiaramonti, D., Prussi, M., Ferrero, S., Oriani, L., Ottonello, P., Torre, P. & Cherchi, F. 2012. Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method. *Biomass and Bioenergy* **46**(0), 25–35.

de Almeida, M.N., Guimarães, V.M., Falkoski, D.L., Visser, E.M., Siqueira, G.A., Milagres, A.M.F. & de Rezende, S.T. 2013. Direct ethanol production from glucose, xylose and sugarcane bagasse by the corn endophytic fungi Fusarium verticillioides and Acremonium zeae. *Journal of Biotechnology* **168**(1), 71–77.

Haberl, H., Erb, K.-H., Krausmann, F., Bondeau, A., Lauk, C., Müller, C., Plutzar, C. & Steinberger, J.K. 2011. Global bioenergy potentials from agricultural land in 2050: Sensitivity to climate change, diets and yields. *Biomass and Bioenergy* **35**(12), 4753–4769.

Haghighi Mood, S., Hossein Golfeshan, A., Tabatabaei, M., Salehi Jouzani, G., Najafi, G.H., Gholami, M. & Ardjmand, M. 2013. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. *Renewable and Sustainable Energy Reviews* **27**(0), 77–93.

Kudakasseril Kurian, J., Raveendran Nair, G., Hussain, A. & Vijaya Raghavan, G.S. 2013. Feedstocks, logistics and pre-treatment processes for sustainable lignocellulosic biorefineries: A comprehensive review. *Renewable and Sustainable Energy Reviews* **25**(0), 205–219.

Küüt, A., Ritslaid, K. & Olt, J. 2011. Study of potential uses for farmstead ethanol as motor fuel. *Agronomy Research* **9**(1), 125–134.

Min, D., Yang, C., Shi, R., Jameel, H., Chiang, V. & Chang, H. 2013. The elucidation of the lignin structure effect on the cellulase-mediated saccharification by genetic engineering poplars (Populus nigra L. × Populus maximowiczii A.). *Biomass and Bioenergy* **58**, 52–57.

Naik, S.N., Goud, V.V., Rout, P.K. & Dalai, A.K. 2010. Production of first and second generation biofuels: A comprehensive review. *Renewable and Sustainable Energy Reviews* **14**(2), 578–597.

Nigam, P.S. & Singh, A. 2011. Production of liquid biofuels from renewable resources. *Progress in Energy and Combustion Science* **37**(1), 52–68.

Olt, J. & Mikita, V. 2011. Diesel engine and fuel-supply system characteristics for testing ethanol as additive fuel. *Agronomy Research* **9**(1), 175–187.

Phitsuwan, P., Sakka, K. & Ratanakhanokchai, K. 2013. Improvement of lignocellulosic biomass in planta: A review of feedstocks, biomass recalcitrance, and strategic manipulation of ideal plants designed for ethanol production and processability. *Biomass and Bioenergy* **58**, 390–405.

Ritslaid, K., Küüt, A. & Olt, J. 2010. State of the Art in Bioethanol Production. *Agronomy Research* **8**(1), 236–254.

Shi, Y., Ge, Y., Chang, J., Shao, H. & Tang, Y. (2013) Garden waste biomass for renewable and sustainable energy production in China: Potential, challenges and development. *Renewable and Sustainable Energy Reviews* **22**(0), 432–437.

Sims, R.E.H., Mabee, W., Saddler, J.N. & Taylor, M. 2010. An overview of second generation biofuel technologies. *Bioresource Technology* **101**(6), 1570–1580.

Sun, Y. & Cheng, J. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology* **83**(1), 1–11.

Tartu municipal administration. 2010. Statistiline ülevaade Tartu 2009, p. 100.

Tutt, M., Kikas, T. & Olt, J. 2012a. Comparison of different pretreatment methods on degradation of rye straw. *11th International Scientific Conference Engineering for rural development.* Jelgava, Latvia, pp. 412–416.

Tutt, M., Kikas, T. & Olt, J. 2012b. Influence of different pretreatment methods on bioethanol production from wheat straw. *Agronomy Research* **10**(1), 269–276.

Tutt, M. & Olt, J. (2011) Suitability of various plant species for bioethanol production. *Agronomy Research* **9**(1), 261–267.

Xiao, W., Yin, W., Xia, S. & Ma, P. 2012. The study of factors affecting the enzymatic hydrolysis of cellulose after ionic liquid pretreatment. *Carbohydrate Polymers* **87**(3), 2019–2023.

Xu, F., Shi, Y.-C. & Wang, D. 2013. X-ray scattering studies of lignocellulosic biomass: A review. *Carbohydrate Polymers* **94**(2), 904–917.