Renewable and Sustainable Energy Reviews 78 (2017) 1007–1032

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Application of pretreatment, fermentation and molecular techniques for enhancing bioethanol production from grass biomass – A review

Sonali Mohapatra[a], Chinmaya Mishra[a], Sudhansu S. Behera[b], Hrudayanath Thatoi[c,*]

[a] Department of Biotechnology, College of Engineering and Technology, Biju Pattnaik University of Technology, Bhubaneswar 751003, India
[b] Officer at Odisha Public Service Commission, Government of Odisha, India
[c] Department of Biotechnology, North Orissa University, Sriram Chandra Vihar, Takatpur, Baripada 757003, Odisha, India

ARTICLE INFO

Keywords:
Grass biomass
Pre-treatment
Fermentation
Molecular tools

ABSTRACT

Grasses as lignocellulose biomass are promising feed stocks for renewable bioethanol production, since these raw materials have high productivity, require low agricultural inputs, have positive environmental impacts, are easy to process and do not compete with the food crops. However, bioethanol production from grass biomass requires efficient pre-treatment, enzymatic hydrolysis and microbial fermentation processes which varies with types of grass species and the microorganisms used. Pretreatment is an important process for delignification of lignocellulose biomass and is dependent on the type of lignin present in the biomass and the degradation pathway employed for removal of the specific type of lignin. Further, enzymatic hydrolysis converts the cellulose and hemicellulose into monomers, making it feasible for the fermenting microorganisms to convert it into bioethanol where use of improved strain and biomass can yield higher ethanol on industrial scale. This review paper presents an overview of the types of grass species, their composition and cultivation practices, fermentation process used for bioethanol production and genetic tools used for improvement in bioethanol production from grass biomass on a sustainable basis. The current knowledge and future prospect for industrial bioethanol production from grass biomass along with its economic aspects have also been discussed in this review.

1. Introduction

The high dependence on fossil fuels has led to uncertainty of petroleum resources and concern about climatic changes, which mandates for search of an alternative and eco-friendly energy source [1]. It is expected that the global fossil fuel reserves will get depleted by next 40–50 years due to rapid increase in the consumption rate of these non-renewable fuels. Thus, biofuels derived from biomass sources can be an alternate source of energy in future. Among the different biofuels, bioethanol produced from biological sources represents one of the potential renewable resources of energy that can replace fossil fuels and gasoline that are particularly utilised in the transport sector [2]. The fact that bioethanol produces nearly twice as much energy as it consumes, marks its potential as a sustainable biofuel, that can be utilised as an alternative to fossil fuel in commercial scale [2].

Bioethanol can be produced from different biomass such as sugar or starchy materials as well as lignocellulosic biomass which are rich in hexoses and pentosans [2,3]. While, in one hand the use of sugary and starchy biomass as first generation biofuel production leads to high cost of bioethanol production due to the high price of raw materials [4,5], the use of food stocks like sugarcane, corn and cereal grains on the other hand might possibly lead to the food crisis. Biofuels generated from lignocellulosic biomass (second generation biofuel) represent one of the potential renewable sources of energy that are non-polluting and are sustainable [3]. Lignocellulosic biomass are plentily available and can often be locally produced at low cost feedstock for bioethanol

Abbreviations: **ABTS**, 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); **AFEX**, Ammonia fibre explosion; **ARP**, Ammonia recycles percolation; **CBP**, Consolidated bioprocess schemes; **CF**, Co-fermentation; **CMC**, Carboxymethylcellulose; **CMCase**, Carboxymethylcellulase; **Crl**, Crystallinity index; **DBU**, 1, 8-diazabicyclo [5.4.0] undec-7-ene; **DMC**, Direct microbial conversion; **DP**, Degree of polymerization; **FPase**, Filter paper activity assay for cellulases; **GAXs**, Glucuararonoxylans; **GCE**, Guaiacylglycerol-β-guaiacyl ether; **GlcA**, Glucuronic acid; **HBT**, Hydroxybenzotriazole; **ILs**, Ionic liquids; **LHW**, Liquid hot water pretreatment; **LMS**, Laccase mediator system; **Mg**, Megagallons; **MEA**, Monoethanolamine; **MLG**, Mixed-linkage glucan; **NREL**, National renewable Energy Laboratories; **PDC**, 2-pyrone-4,6-dicarboxylate; **PEF**, Pulsed-electric- field; **QTL**, Quantitative trait loci; **SHF**, Separate hydrolysis and fermentation; **SILs**, Switchable ILs like; **SmF**, Submerged fermentation; **SOC**, Soil organic carbon; **SPORL**, Sulfite pretreatment to overcome recalcitrance of lignocelluloses; **SSCF**, Simultaneous saccharification and co-fermentation; **SSF**, Simultaneous saccharification and fermentation; **SsF**, Solid state fermentation; **TCA**, Tricarboxylic acid; **Xyl**, Xylose

* Corresponding author.
E-mail address: hnthatoi123@gmail.com (H. Thatoi).

http://dx.doi.org/10.1016/j.rser.2017.05.026
Received 14 November 2016; Received in revised form 9 March 2017; Accepted 5 May 2017
1364-0321/

production. Biofuels from lignocellulosic biomass have been shown to offer most environmentally attractive and technologically feasible near-term alternative bioethanol with 94% lower greenhouse gas emissions than that of gasoline [6,7,8]. In a study by Ryan et al., [9] it has been stated that for every 1000 L of bioethanol from lignocellulosic biomass, approximately 2.6Mg carbon dioxide emission is saved.

There are different types of lignocellulosic biomasses such as wood based, non-wood biomasses that include agricultural residues, sugar cane bagasse, switch grass, cotton fibre etc. Among the different lignocellulosic biomasses short rotation crops like grasses have high yields of upto 40 Mg/ha/year as compared to corn feedstocks which have yields of 7 Mg/ha/year [10]. In future, these fast growing plants can be targeted as potential energy crops not only because of their high productivity per hectare but also due to their abundancy, availability and utilisation of the whole plants, high percentage of total cellulose and hemicellulose content and comparatively less lignin content [11]. Further, grasses can grow globally in a wide range of geographies, climate and soil types [12]. However, the main bottle neck in large scale ethanol production from grass as lignocellulosic biomass is the technological impediments of breaking down plant biomass (lignin in the cell walls) and releasing carbohydrate polymers (cellulose and hemicellulose) that can be fermented into fermentable sugars and further refined fuels [11].

In order to obtain bioethanol, lignocellulose biomasses have to be pre-treated followed by enzymatic hydrolysis and fermentation. Pre-treatment of grass biomass prior to enzymatic hydrolysis is necessary to break the recalcitrant lignin. But grasses have the advantage of low lignin content, which eventually leads to milder pre-treatment conditions [13]. Further, enzymatic hydrolysis converts the cellulose and hemicellulose into monomers, making it feasible for the fermenting microorganisms to convert it into bioethanol. However, improvement in grass biomass and microorganisms (specifically pentose fermenting) by application of molecular techniques in genetic modification of plant biomass and microbes would play significant role for commercialization of bioethanol production from grasses. Further, biofuel production depends upon the availability of the feedstock, production and supply pathways, availability of the technologies and the cost-effectiveness, which varies from region to region.

2. Grass biomass around the world for bioethanol production

2.1. Types of grass biomass

There are around 11,369 accepted grass species that have been known worldwide till now [14]. The habitat of these grasses ranges from infertile land mass to well drained fertile soil in varied climatic conditions. Grasses are composed primarily of carbohydrate polymers (cellulose and hemicellulose) and phenolic polymers (lignin) along with other compounds, such as proteins, acids, salts, and minerals. The accumulation of carbohydrate can be attributed to the photosynthetic cycle in plants. The carbohydrate content is not similar for all the types of grasses, and significantly varies due to a lot of factors such as 1) variety of the grass 2) developmental stage of the grasses and 3) the environmental conditions in which it is grown.

Among the different varieties of wild and cultivated grasses, bluestems, Indian grass, and switchgrass are some of the most common examples of wild grasses. The cultivated grasses such as smooth broom grass, timothy, meadow foxtail are some of the species that are derived from wild species of grasses. They are developed through different breeding methods viz., pure line selections, mutants, polyploids and inter-generic/interspecific hybrids. Both wild and cultivated grasses are considered as suitable plant biomass because of their high carbohydrate content, their longevity, redevelopment after the cut off, and effective capability to tolerate the drought [15].

The maturity of the grass is the key factor that determines the quantity of cell wall (cellulose, hemicellulose and lignin) and other cell components (protein, lipid and sugars) in the grass. It has been reported that while the structural carbohydrates in cell wall increase with the maturity of the grasses, the reverse phenomena is observed for the cell components [16]. Whenever the rate of photosynthesis supersedes the rate of plant growth or the plant gets into a stress, carbohydrate accumulation starts in the plants. In these conditions high concentrations of carbohydrates like starch, sugar and fructans can be seen in dry hay of cool season grasses. The carbohydrates are mostly accumulated in the lower regions of the grass stem like stem bases, stolons, corms, and rhizomes [17,18]. More particularly, substantial amounts of soluble carbohydrates are stored in the parenchyma cells that surround the vascular bundles located within internode tissues [19,20]. Hence, these cells can be good targets for enhanced carbohydrate storage in grass biomass, which would result in increased carbohydrate yields successively, not interfering in the plant growth pattern.

Further, depending on the temperature or climatic conditions, grasses are divided into tropical and temperate region grasses. Tropical grasses or C4 grasses can withstand optimum temperatures and are high yielding varieties as compared to temperate or C3 grasses due to their highly efficient mode of photosynthesis. Sucrose and fructose are the predominant reserve constituents of temperate-origin grasses while sucrose and starch, are the major constituents of tropical-origin grasses [21,22].

The type of grass variety and the environmental conditions in which it is cultivated play an important role in determining the chemical composition of the grasses and thereby it's potential to be considered as a bioethanol crop. Therefore, attentive considerations of the ecological aspect serve as an integral part for bioethanol production from grasses. The consideration of the above mentioned factors will be crucial in utilising many underutilised grass varieties in future for sustainable bioethanol production.

2.2. Composition and potential of grass biomass for bioethanol production

The most favourable biomass resource for biofuel production should be readily available, should have high yielding biomass per dry weight, unwavering desirable chemical concentrations and should be economical [23]. Other features like elevated carbon and hydrogen concentrations and minimum concentrations of oxygen, nitrogen and other organic components are also crucial for a biomass to be considered for bioethanol [24]. In addition to the above mentioned features the importance of a biomass also lies on the fact that the industries using the biomass should produce less effluent and offer low CO_2 emission ability.

Grasses have the advantage of possessing possibly all these features. The general composition of grasses along with the process of conversion of grasses to bioethanol is represented in Fig. 1. Grasses grow naturally and do not require any special requirements for cultivation, which makes the biomass growth cost effective, as application of fertilizers and pesticides is not a necessity [25]. It also has good above-ground foliage and much denser growth which maximizes the amount of biomass that an acre of land can produce. Additionally, grasses are composed primarily of carbohydrate polymers (cellulose and hemicellulose) and phenolic polymers (lignin) and lower concentrations of various other compounds, such as proteins, acids, salts, and minerals. These carbohydrate polymers, which typically make up two-thirds of cell wall dry matter, are polysaccharides that can be hydrolysed to sugars and then fermented to ethanol. Further the carbohydrate concentration in grasses is directly related to the bioethanol yield from biomass and the maturity of the grass is the key factor that determines its quantity in the grass. Another feature that makes grass an attractive energy crop is its potential to increase carbon storage by increasing above and below ground biomass, specifically in C4 grasses.