Fuel 236 (2019) 544–553

Contents lists available at ScienceDirect

Fuel

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

Full Length Article

Bioethanol production from various lignocellulosic feedstocks by a novel "fractional hydrolysis" technique with different inorganic acids and co-culture fermentation

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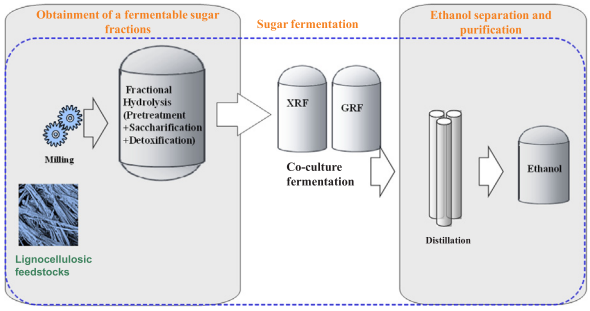
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Bioethanol
Inorganic acids
Saccharification
Fractional hydrolysis
Co-culture fermentation

ABSTRACT

Second generation (2G) ethanol production is facing major research challenges primarily because of an absence of suitable technology for the extraction of maximum fermentable sugars from complex lignocellulosic structure as well as unavailability of suitable fermentation technique and single microorganism to convert these sugars (pentose and hexose) efficiently into ethanol. Present study is focused on the exploration of various lignocellulosic feedstocks using different inorganic acids for the recovery of maximum amount of fermentable sugars as separate fractions, direct from the biomass by a novel process called "fractional hydrolysis" with minimum toxics generation. Different physical and chemical parameters were optimised for the process previously. Four different inorganic acids (HCl, H_3PO_4, HNO_3, and H_2SO_4) up to 30% concentration (v/v) were used in 7- and 8-stage fractional hydrolysis processes to treat dry biomass in a fractional hydrolysis column. Using kans grass biomass, H_2SO_4 resulted in maximum extraction of pentose and hexose sugars separately with negligible toxics. Furthermore, the technique was explored using three different wide and easily available lignocellulosic feedstocks, resulting in saccharification (%): Kans grass 84.88; Sugarcane bagasse 82.55; Wheat straw: 81.66. Hydrolysate fractions without any detoxification were taken into a co-culture system containing *Zymomonas mobilis* (for glucose fermentation) and *Candida shehatae* (for xylose fermentation) at bioreactor level. 93.28% of the sugar present in xylose-rich fraction (initial total reducing sugar: 59.74 g/L) and 95.44% of glucose-rich fraction (initial total reducing sugar: 100.25 g/L) were utilised to produce 67.28 g/L ethanol from the kans grass biomass hydrolysate; thereby achieving 82.45% of the maximum theoretical ethanol production.

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https://doi.org/10.1016/j.fuel.2018.09.024
Received 31 March 2018; Received in revised form 16 August 2018; Accepted 6 September 2018

1. Introduction

It is widely known that transportation sector is almost entirely dependent on fossil fuels; primarily on petroleum-based fuels (liquefied petroleum gas, gasoline, compressed natural gas and diesel fuel gas). Amount of petroleum availability is depleting day by day; therefore alternatives are required to produce liquid fuels for reducing the future effects of the shortage in supply of transportation fuels. Biofuels are considered as a relevant research field because of a. Energy security reasons b. Environment concerns c. Foreign exchange savings and d. Various socioeconomic issues, by both developing and industrialised countries [1–7]. All these advantages of biofuels over conventional fossil fuels will be resulting in an increase in the share of biofuels in the automotive fuel market over the next decade [8–11].

Term biofuel refers to solid (biochar), liquid (biodiesel, bioethanol, and vegetable oil) or gaseous (biohydrogen, biosyngas, and biogas) fuels that are mainly produced from biomass [12–16]. They are renewable; most common is bioethanol (petrol additive or gasoline substitute). Bioethanol has the potential to reduce both crude oil consumption, and environmental pollution [5,17–26]. Bioethanol can be produced from plentiful and domestic cellulosic biomass resources (agricultural and forestry residues, herbaceous and woody plants as well as municipal and industrial solid waste streams). Currently, world ethanol production is about 60% from feedstocks of food and sugar crops, requiring high-quality agricultural land for their growth; thereby giving rise to food vs. fuel conflict. Bioethanol production overall cost varies widely by feedstock type, conversion process, production scale, and region. Feedstock cost (crops) is a significant component in the ethanol production cost [18–19,27].[1]

As ethanol demand is expected to increase more than double in the near future, new technologies must be moved from the laboratories to commercial reality to meet this requirement [28]. The focus is shifting towards lignocellulosic biomass for ethanol production; also known as second generation (2G) ethanol. Lignocellulosic biomasses are mainly harvested from agricultural wastes materials and forest residues crops. They are easily available in almost every region and different climatic condition [29]. Lignocellulosics consist of cellulose (40–60%), hemicellulose (20–40%), and lignin (10–25%) on an average. Typically cellulose and hemicelluloses part comprise 2/3rd of the total dry biomass. Carbohydrate part (cellulose and hemicellulose) of lignocellulosic biomasses can be saccharified to obtain soluble sugars and it is further converted into ethanol by fermentation [30]. Lignocellulosic biomasses are the most promising alternative for sugar crops because of (a) low cost (b) high yield (c) wide availability throughout the year and (d) ability to grow in marginal lands with almost nill water supply requirement.

Major research challenges of 2G ethanol production at commercial level are 1. Maximum extraction of fermentable sugars (cellulose and hemicelluloses) from lignocellulosic biomass during saccharification. 2. Selection of suitable microorganism (more tolerant toward fermentation inhibitors) and fermentation technique to convert maximum amount of sugars present in the lignocellulosic biomass hydrolysate into ethanol for higher productivity. 3. Process integration to minimise the total number of steps involved in overall production.

Various techniques have been developed during recent years to overcome these challenges for efficient bioethanol production at the commercial level. However, most of the current technologies used for fuel ethanol production are cost ineffective and unable to eliminate process steps significantly. In one of the major findings during recent years, a new popping pretreatment technique has been developed and performed on rice straw for enhancing cellulose conversion efficiency; resulting into sugar production of 0.567 g/g straw after 48 h under the optimum conditions and ethanol yield was 0.172 g/g of straw (80.9% of the maximum theoretical) after 24 h fermentation [31]. In another study, extrusion pretreatment method was developed for pine; optimum conditions included 150 rpm of screw speed, barrel temperature 180 °C and moisture content 25%. Maximum cellulose, hemicellulose, and total sugar recoveries were 65.8, 65.6% and 66.1% respectively during the process [32]. Corn cob acid hydrolysate was used as a substrate for microbial lipid production, and the remaining solid residue was enzymatically hydrolysed where 71.6% conversion efficiency of fermentable sugars into valuable products was achieved [33]. Furthermore, a novel method (treatment with NaOH in a twin-screw extruder for continuous pretreatment) has been developed for barley straw; maximum ethanol concentration of 46 g/L was achieved with 77.4% yield [34]. Unique enzymatic hydrolysis approach using phosphoric acid impregnated and steam exploded sugarcane bagasse was tried under high solid (18–22%) and low enzyme loading that resulted in maximum sugar concentration of 76.8 g/L under the optimum conditions, while total glucan conversion was 69.2% [35]. On a pilot scale, bioconversion of wheat straw was done by dilute acid pretreatment followed by bioabatement of fermentation inhibitors, and simultaneous saccharification and fermentation (SSF) was performed using *Escherichia coli* FBR5 with fermentation time 83 h. Maximum ethanol productivity of 0.43 g/L/h was achieved with maximum ethanol yield 0.29 g/g (86% of maximum theoretical ethanol yield) [36]. Technological approach improvements and optimisation of various factors were prioritised in these studies. Nevertheless, 2G ethanol production still has some challenges that need to be properly addressed in the development of a sustainable bioethanol industry.

Therefore, in the present work, a unique approach with just two process steps (fractional hydrolysis and fermentation) was adapted for the conversion of lignocellulosic biomass into fuel ethanol with high conversion efficiency and thus hoping to bring down overall 2G ethanol production cost effectively. A novel “fractional hydrolysis” technique was developed which gives soluble pentose and hexose sugars as separate fractions directly from lignocellulosic biomass. As known, there is no naturally occurring microorganism fermenting pentose and hexose sugars simultaneously with the same efficiency; obtaining separate xylose-rich fraction (XRF) and glucose-rich fraction (GRF) of hydrolysate hold a tremendous advantage. Additionally, fractional hydrolysis process merges two conventional 2G ethanol production steps (pretreatment and hydrolysis). Furthermore, toxic compounds in the hydrolysate were found negligible; therefore, hydrolysate can be taken directly for fermentation without any detoxification, thereby reducing the overall production cost. Development and optimisation of various parameters for the fractional hydrolysis technique have been discussed in another communicated manuscript. In this study, four different strong inorganic acids (HCl, H_3PO_4, HNO_3, and H_2SO_4) were tested during 7- and 8-stage fractional hydrolysis processes for the maximum sugar recovery with minimum toxics. Also, three different lignocellulosic biomass were selected (kans grass, sugarcane bagasse, and wheat straw) for the study because of their more even geographical distribution and higher polysaccharide content compared to other feedstocks.

For glucose fermentation, *Saccharomyces cerevisiae* and *Zymomonas mobilis* are the most commonly used microbes. *Z. mobilis* gives higher ethanol yield (5–10%) and is about 2.5 times faster productivity compared to *S. cerevisiae* [37]. Under anaerobic conditions, *Z. mobilis* can produce almost a theoretical amount of ethanol from glucose via Entner-Doudoroff pathway. Among xylose fermenting microorganisms, *Candida shehatae* has shown fermenting ethanol faster compared to other microbes [38]; also specific ethanol production rate has been found highest [39]. *C. shehatae* NCIM 3501 (for XRF) and *Z. mobilis*

[1] **2G:** Second generation **MTCC:** Microbial Type Culture Collection and Gene Bank **IMTECH:** Institute of Microbial Technology **NCIM:** National Collection of Industrial Microorganisms **NCL:** National Chemical Laboratory**NREL:** National Renewable Energy Laboratory **LAP**: Laboratory Analytical procedure **XRF**: Xylose-rich fraction **GRF:** Glucose-rich fraction **TRS:** Total reducing sugar **DNS:** Dinitrosalicylic acid, **SSF:** Simultaneous saccharification and fermentation