ENHANCED BIOETHANOL PRODUCTION FROM EXTRACTED SUGAR BEET CHIPS

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Abstract

In this study generarlly/preliminary bioethanol production were analyzed from sugar beet *(Beta vulgaris)* chips to bioethanol. The sugar beet chip is a by-product of the sugar industry; it is created after pressing of the extracted beet slices. The chips are poor in sugar but rich in cellulosic components. Therefore the main aim of our project was to examine and intensify the enzymatic hydrolysis of cellulose to monosaccharides in order to obtain higher ethanol yield. Cellulase enzyme, from *Trichoderma reesei,* and cellobiase enzyme, from *Aspergillus niger* was applied for hydrolysis. During hydrolysis cellulose is degraded by the cellulases to sugars, which can be fermented by yeasts to ethanol. Sugar beet is the most promising biomass-derived energy feedstock crop and it has received considerable attention for the production of value-added products.

Keywords

bioethanol, sugar beet chips, by-product, enzyme hydrolysis

Introduction

The use of ethanol as an engine fuel has as long a history as the car itself. It began with the use of ethanol in the internal combustion engine invented by Nikolas Otto in 1897. Alcohols have been used as fuels since the inception of the automobile. The term “alcohol” often has been used to denote either ethanol or methanol as a fuel. With the oil crises of the 1970s, ethanol became established as an alternative fuel. Many countries started programs to study and develop fuels in an economic way from available raw materials. The interest then waned as the price of oil dropped, until 1979 when we had another oil crisis. Since the 1980s, ethanol has been considered as one possible alternative fuel in many countries [1].

The worldwide transport sector depends almost totally on fossil fuels. The vegetable oil and the vegetable alcohol are/even could be the environmentally friendly motor fuels for replacing crude oil. Ethanol (C_2H_5OH) (as ethyl alcohol or fuel ethanol or bioethanol) is fermented from sugars, starches or from cellulose biomass, so cellulosic materials can be used to produce bioethanol as well. Bioethanol represents an important, renewable liquid fuel for motor vehicles. The bioethanol can be used as petrol additive up to 20%, the optimal ratio of petrol: ethanol is 85:15 [2]. The bioethanol is appropriate for the mixed fuel in the gasoline engine also because of its high octane number furthermore, due to its low cetane number and high heat of vaporization it can impede the self-ignition in the diesel engine [3].

Production of bioethanol from biomass is a possible way to reduce consumption of crude oil with lower environmental load, and it is an environmentally friendly motor fuel for replacing crude oil. Bioethanol can be produced from several biomass feedstock using different conversion technologies, and it can be produced from raw materials containing fermentable sugars, especially sucrose containing feedstock, such as sugarcane or sugar beet. Beside these, ethanol can be produced from cellulose-containing materials, e.g. maize stalk, forest-products wastes, grasses or sorghum. In European moderate climate area the most convenient renewable raw materials for bioethanol production are grains and sugar beet [4].

Sugar beet is a biennial plant belonging to the family *Quenopodiaceae*. Its scientific name is *Beta vulgaris*. The roots are pivoting, almost totally buried, with a yellow-greenish rough peel. The root is the organ where most sugar is accumulated in the plant [5].

The beets arrive at the production plant without crown and loaded in silos by mechanical means through channels with circulating water. The beets are washed and passed through systems retaining diverse solid materials, such as stones, leaves and small roots. Once washed, the beets are transported to the choppers where they are shredded into very thin slices that called chips or cossettes, and passed to the diffuser to extract the sugar content into a water solution. The diffusers are large rotary drums where the chips are put in contact with a hot water stream flowing in the opposite direction. The sucrose is extracted from the vacuoles of the beet cells into the flowing water generating the raw juice or diffusion juice. The spent chips are called pulp and leave the diffuser with about a 95 % moisture content, but with low sucrose content. To recover part of the sucrose contained in the pulp, it is pressed in screw presses reducing the moisture to 75 % [6].

Extensive research has been completed on the conversion of lignocellulosic materials to ethanol production in the last two decades. This conversion includes two processes: hydrolysis of cellulose in the lignocellulosic materials to fermentable reducing sugars and fermentation of the sugars to ethanol. The hydrolysis is usually catalyzed by cellulase enzymes and the fermentation is carried out by yeast or bacteria. These enzymes are produced by several microorganisms, commonly by bacteria and fungi. These microorganisms can be aerobic or anaerobic, mesophilic or thermophilic. There are different factors that affect the enzymatic hydrolysis of cellulose, namely: substrate concentration, cellulase activity, reaction conditions (temperature, pH as well as other parameters), and a strong product inhibition [7]. Substrate concentration is one of the main factors that affect the yield and initial rate of enzymatic hydrolysis of cellulose. At low substrate levels, an increase of substrate concentration normally results in an increase of the yield and reaction rate of the hydrolysis. To improve the yield and rate of enzymatic hydrolysis, research has been focused on optimizing the hydrolysis process and enhancing the cellulase activity [8]. During hydrolysis cellulose is degraded by the cellulases to reducing sugars, which can be fermented by yeasts or bacteria to ethanol.

The factors that have been identified to affect the hydrolysis of cellulose include porosity, i.e., accessible surface area of the waste materials, cellulose fiber crystallinity and lignin and hemicellulose content. The presence of lignin and hemicellulose makes the access of cellulose enzymes to cellulose difficult, thus reducing the efficiency of the hydrolysis. Pretreatment must meet the following requirements: improve the formation of sugars or the ability to subsequently form sugars by enzyme hydrolysis; avoid the degradation or loss of carbohydrate; avoid the formation of byproducts inhibitory to subsequent hydrolysis and fermentation processes; and be cost effective [8].

One major problem with bioethanol production is the availability of raw materials for the production. The availability of feedstock for bioethanol can vary considerably from season to season and depends on geographic locations. Locally available agricultural biomass will be used for the bioethanol production. In Europe the main raw material of bioethanol is the beetroot, wheat, maize, in North-America it is the maize and wheat, and while in South-America it is the sugar cane. The different beet

varieties are used for human food, animal feed and sugar production. Sugar beet is an important crop in Europe, North America, and Asia. France is the major producer of sugar beet followed by Germany and the United States [9].

Sugar beet contains from 12 to 15 % sucrose. Fuel ethanol production includes the generation of a large quantity of residues. Effluent treatment will always be an important topic of research [9].

In this work the utilization of organic waste from sugar beet processing was examined in ethanol production. Sugar beet chips after extraction and pressing are poor in sugar but rich in cellulosic components. Therefore the main aim of our project was to examine and intensify the enzymatic hydrolysis of cellulose to monosaccharides in order to obtain higher ethanol yield. The optimal conditions of fermentation the ethanol yield were investigated also.

Methods and materials

Sugar beet chips were used as the polysaccharide source for our experiences. They have gained after extaction and pressing. The particle size of sample was 0.5-2.0 cm after chopping. There was made different composition suspensions from sugar beet chips (min. 7.5 g/cm^3, max. 30 g/cm^3) for investigation.

For enzymatic hydrolysis cellulase (Cellulast 1.5L, Novozymes A/S, Denmark; 700 U/g) from Trichoderma reesei (Sigma) and cellobiase (Novozym 188, Novozymes A/S, Denmark; 250 U/g) from Aspergillus niger (Sigma) was applied dosed in a different concentration of 50; 100; 300 and 600 μLg_{TS}^{-1}.

The temperature and pH of enzymatic hydrolysis were controlled at 40±0.2°C and pH 4.0; 4.5; 5.0±0.1. The samples was incubated and mixed in a thermostat with magnetic stirrer for 7 days.

Reduction of the sugar concentration was estimated by 3.5-dinitrosalicylic acid (DNS) photometric method, with glucose as standard. This spectrophotometrically (S2000 UV/VIS) method means: using 3.5 dinitro-salicylic acid method after calibration with Invertas enzyme [10-12]. Mixture of cellulase and cellobiase enzymes were used to decompose the cellulose fraction of beet chips. In our experiments the ratio of enzymes to substrate, the pH of suspension, and the temperature were varied to find the optimal condition for hydrolysis.

Results and discussion

The time-depending cellulose hydrolysis to monomer sugar was investigated without pre-treatment from sugar beet chips in the first series of experiments. We focused on the determination of the maximum value of cellulose hydrolysis. The sugar content was measured at the received ferment-juice and it was given per unit dry material weight basis (g/g $_{TS}$).

The Figure 1 demonstrates the effect of the pH on the enzyme hydrolysis. The results have showed that the optimum pH of enzymatic hydrolysis of cellulose is pH 4.5. This value is the same as it is publicated in the international Journals as the pH optimum of cellulose enzymes.

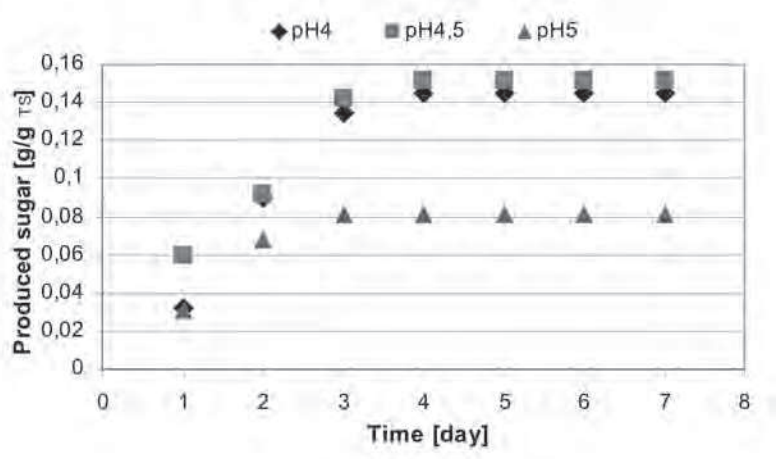

Figure 1. Produced sugar from sugar beet chips during enzymatic hydrolysis (7.5 g/cm^3 substrate concentration; 300 $\mu LgTS^{-1}$ enzymes concentration)

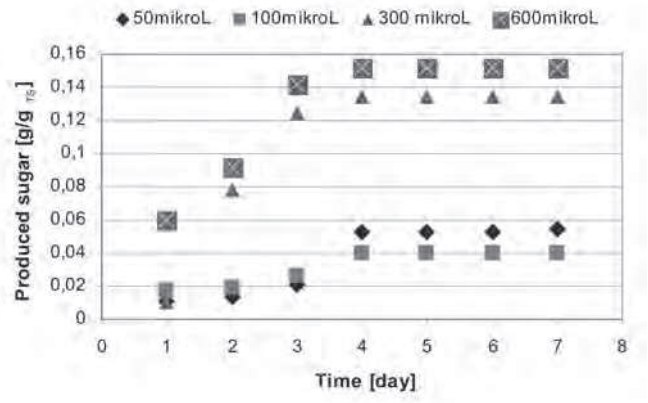

Figure 2. Produced sugar from sugar beet chips during enzymatic hydrolysis (7.5 g/cm^3 substrate concentration, pH 4.5)

In the Figure 2, we can follow the effect of the enzyme amount on the sugar yield. The results have showed that the highest sugar production have been measured at the suspension 300 and 600 $\mu LgTS^{-1}$ and there isn't significant difference between these two values. There isn't relevant sugar production at the smaller enzyme concentrations

The Figure 3, shows the optimal substrate concentration is 7.5 g/cm^3 at a given enzyme quantity (300 $\mu LgTS^{-1}$); at the more concentrated suspensions the sugar yield was smaller. The sugar content of the suspensions was increased until the 4th day of fermentation and after that the sugar content of the samples was constant. It shows us, that the real useful period of the enzymatic degradation is only 4 days, after this period is no longer appropriate to continue it due to there will not be changes in the amount of converted sugar yield.

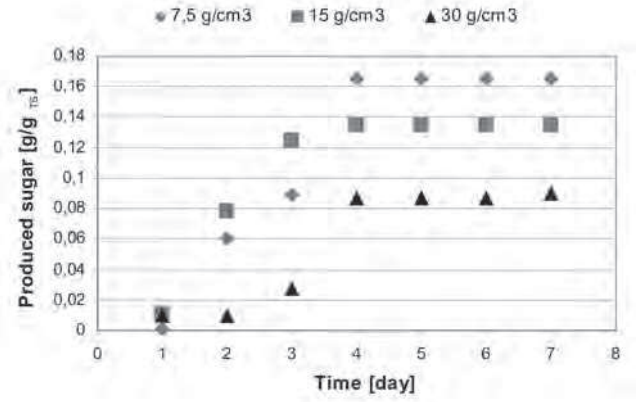

Figure 3.: Produced sugar from sugar beet chips during enzymatic hydrolysis (300 $\mu LgTS^{-1}$ enzymes concentration, pH 4.5)

Conclusions

In this experiments the ratio of enzymes to substrate, the pH of suspension, and the temperature were varied to find the optimal condition for hydrolysis. The amount of fermented sugar was determined from sugar beet chips. The chips were not pre-treated. Our preliminary results showed that the sugar beet chips have a great potential to biofuel production. The bioethanol yield can be large-scale increased by the optimized condition of cellulose hydrolysis prior to the fermentation.

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References

1. Ayhan Demirbas, Bioethanol from cellulosic materials: A renewable motor fuel from biomass, Energy Sources, 27:327-337, 2005, ISSN: 0090-8312 print/1521-0510 online

2. Zsuzsanna László, Sándor Beszédes, Szabolcs Kertész, Cecilia Hodúr, Gábor Szabó, Imre Kiricsi, Bioethanol from sweet sorghum, Hungarian Agricultural Engineering 20. p. 15-17.

3. G. Najafi, B. Ghobadian, T. Tavakoli, T. Yusaf*, Potential of bioethanol production from agricultural wastes in Iran, Renewable and Sustainable Energy Reviews 13 (2009) 1418–1427

4. Vasso Oreopoulou, Winfried Russ, Utilization of by-products and treatment of waste in the food industry, Integrating Safety and Environment Knowledge Into Food Studies towards European Sustainable Development, 2007 Springer Science+Business Media, LLCISBN-10: 0-387-33511-0, ISBN-13: 978-0387-33511-7

5. Sin-Yie Liu, Chien-Yih Lin, Development and perspective of promising energy plants for bioethanol production in Taiwan, Renewable Energy 34 (2009) 1902–1907

6. C. A. Cardona, Ó. J. Sánchez, L. F. Gutiérrez, Process synthesis for fuel ethanol production, CRC Press 2010, ISBN 978-1-4398-1597-7

7. Mustafa Balat, Production of bioethanol from lignocellulosic materials via the biochemical
pathway: A review, Energy Conversion and Management xxx (2010) xxx–xxx

8. S. Prasad, Anoop Singh, H.C. Joshi, Ethanol as an alternative fuel from agricultural, industrial and urban residues, Resources, Conservation and Recycling 50 (2007) 1–39

9. Peter Ho, Margarida Vieira: Case studies in food safety and environmental health, Integrating Safety and Environment Knowledge Into Food Studies towards European Sustainable Development, 2007 Springer Science+Business Media, LLC ISBN-10: 0-387-33514-5, ISBN-13:978-0-387-33514-8

10. Prihardi Kahar, Kazuo Taku, Shuzo Tanaka, Enzymatic digestion of corncobs pretreated with low strength of sulfuric acid for bioethanol production, Journal of Bioscience and Bioengineering VOL. 110 No. 4, 453–458 (2010)

11. Bélafi-Bakó, K., Koutinas, A., Nemestóthy, N., Gubicza, L., Webb, C., Continuous enzymatic cellulose hydrolysis in a tubular membrane bioreactor, Enzyme and Microbial Technology 38 (2006) 155-161

12. Melinda Gáspár *, Gergely Kálmán, Kati Réczey, Corn fiber as a raw material for hemicellulose and ethanol production, Process Biochemistry 42 (2007) 1135–1139