Fuel 134 (2014) 250–256

Contents lists available at ScienceDirect

Fuel

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

Ethanol fermentation of waste bread using granular starch hydrolyzing enzyme: Effect of raw material pretreatment

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HIGHLIGHTS

- Waste wheat rye-bread ethanol fermentation using granular starch hydrolyzing enzyme.
- Three raw material pretreatment methods studied for ethanol yield improvement.
- Separate hydrolysis and fermentation process studied for comparison.
- Fermentation of unpretreated waste bread yielded 354.36 g ethanol kg^{-1} raw material.
- Application of raw material pretreatment further improved ethanol yield.

ARTICLE INFO

Article history:
Received 7 March 2014
Received in revised form 20 May 2014
Accepted 27 May 2014
Available online 12 June 2014

Keywords:
Ethanol fuel
Waste bread
Ethanol fermentation
Granular starch hydrolyzing enzyme (GSHE)
Saccharomyces cerevisiae

ABSTRACT

The subject of this research project was assessment of direct starch to ethanol conversion process course of waste wheat-rye bread using granular starch hydrolyzing enzyme (GSHE). Several pretreatment methods (enzymatic prehydrolysis, microwave irradiation, sonification) were used to improve the course of fermentation and were compared with separate hydrolysis and fermentation (SHF). Due to high water binding capacity of raw material fermentations were conducted at a substrate loading of 150 g kg^{-1}. Only during enzymatic pretreatment and the SHF process the raw material was preliminary liquefied so its higher concentrations could be applied. The dynamics of fermentation was similar in all studied variants. The fermentation of unpretreated waste bread ended with 80.00% ethanol yield (354.36 g kg^{-1} of raw material). Pretreatment of raw material improved ethanol yield by ca. 3–8%.

1. Introduction

Ethanol is considered as one of the most promising renewable fuel that can replace fossil fuels-based transportation fuels. It is most commonly produced by microbial (most often yeast) catalyzed fermentations using plant biomass as a raw material. Starchy raw materials (i.e. corn, wheat, sorghum) are still the most common feedstocks for fuel ethanol production in temperate climate regions of the world (Europe, North America, Central Asia). However its use as fuel production resources may affect on the prices of food products manufactured from them [1]. The use of non-edible parts of the plant (straws, stalks), known as the lignocellulose biomass, as the raw material in distillery is nowadays considered as the most promising opportunity for ethanol production that does not affect the prices of foodstuffs [2]. However, the conversion of lignocellusosic biomass into fermentable sugars and, subsequently into ethanol requires high temperature pretreatment which is often catalyzed using corrosive, non-ecological or costly agents like acids, alkali, ionic liquids and others [3]. Moreover the efficiency of saccharification and fermentation of lignocellulose is still much less efficient in comparison to starches [4], but starchy raw materials are very costly and the cost of the feedstock can exceed 65% of the price of final product [5]. The solution to the problems of affecting food prices by using agricultural crops for fuel production and the technological difficulties with conversion of lignocellulosic biomass is utilization of food industry wastes for production of biofuels. One of the most promising food waste that can be processed into ethanol is waste bread. It contains significant amount of starch that is easily hydrolyzed to monomeric sugars using amylases, the amount of starch and simple sugars in bread ranges 500–750 and 3–50 g kg^{-1} respectively [6]. Moreover bread contains 100–150 g kg^{-1} of protein which, after hydrolysis to peptides and amino acids, is essential for yeast growth and accelerated fermentation [7]. Waste bread is also highly accessible raw material for ethanol processing. The estimated wastage for bakery products ranges 7–10% of its total

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http://dx.doi.org/10.1016/j.fuel.2014.05.081
0016-2361/

production [8], taking into consideration estimated world annual production of bread which is about 100 million tones [9] the amount of generated waste can reach even 10 million tones per year worldwide. The major factor for bread waste formation is that part of the produced product is left unsold and is returned to the bakery due to significant level of staling and large amount of available assortment of bakery products which are produced in excess to fulfill the consumers demands [10]. There are limited possibilities for reprocessing bread waste in the bakeries. Some wastes can be processed into bread crumbs, as a replacement of part of flour in sourdough preparation or as animal feed. However due to often microbial spoilage its use for human and animal nutrition could be risky for health of the consumers. These problems are the reason why waste bread is most often left on landfills or used as a fuel for combustion. The most promising solution for waste bread utilization is use it as a raw material for ethanol fuel production. Earlier studies shown that waste bread is a high-yielding material for ethanol fermentation [11,12]. The amount of ethanol produced from bread waste that shown no signs of mould contamination, depending on processing technology, ranged as mentioned by the authors ca. 350–370 g kg^{-1} of feedstock dry matter. Kawa-Rygielska and Pietrzak [13] studied the possibility of using waste bread showing high level of surface mould contamination for ethanol production, this resulted in a decrease of ethanol yield in comparison to non contaminated material (ca. 230–250 g kg^{-1} depending on the raw material loading in the fermentation feed). The other possibilities for waste bread utilization via biotechnological processes are in example: solid state fermentation by *Aspergillus awamori* for production of amylases and proteases [14], biohydrogen production using rhizosphere microflora [15], succinic acid production by *Actinobacillus succinogenes* [16] or aromatic compounds production by *Geotrichum candidum* [17].

The typical pretreatment method for enzymatic hydrolysis of starches to fermentable sugars is based on a two-step method. In the first step starch is liquefied by heat-stable α-amylase (EC 3.2.1.1) in order to decrease the viscosity of gelatinized starch solution and to produce short-chained dextrins, by breaking down the α-1,4-glycosidic bonds in the middle of amylose and amylopectin chains. During the second step of starch hydrolysis (saccharification) the dextrins are saccharified by glucoamylase (amyloglucosidase, EC 3.2.1.3) to obtain monomeric sugars (glucose). Often supportive enzymes, like proteases, cellulases, pullulnases and others, are used to increase the amount of fermentable sugars, decrease the viscosity of the mash and produce free amino nitrogen that is used as a nutrient for yeast [18,19]. After the hydrolysis the mash is inoculated with yeast and subjected to ethanol fermentation. This kind of process is named separate hydrolysis and fermentation (SHF). The enzymatic pretreatment is a costly process because the liquefaction step is conducted in high temperature (80–100 °C) what demands large amount of energy, also the saccharification step is costly because glucoamylase acts slowly and optimal temperature for its activity is ca. 50–60 °C. The optimization for energy demand for starch hydrolysis for ethanol production led to development of simultaneous saccharification and fermentation (SSF) process, in which liquefied starch slurry is cooled to temperature where yeast are able to ferment and glucoamylase is added so the sacchrification of dextrins and utilization of resulting monomeric sugars occurs at the same time [20]. The most recent development in the field of processing starchy raw materials to ethanol is the direct starch to ethanol conversion using granular starch hydrolyzing enzyme (GSHE). GSHE is obtained from genetically modified *Trichoderma reesei* and it shows the activity of α-amylase and glucoamylase displaying on the surface of starch granules. Earlier studies shown that the efficiency of direct starch to ethanol conversion process is comparable to 'traditional' technologies [21] and its advantage is lower energy demand because of lack of the starch gelatinization and liquefaction steps. Also some improvements for direct conversion of starch to ethanol technology were done. Balcerek and Pielech-Przybylska [22] studied, among other, the effect of thermal prehydrolysis of triticale meal using α-amylase and application of protease on the process of raw starch hydrolysis and fermentation. They discovered that better efficiency of fermentation was obtained without thermal activation but with added proteolytic enzyme. Montalbo-Lomboy et al. [23] studied the effect of sonification of corn meal slurry prior to direct conversion to ethanol. The results of this research proved that sonification of raw material improved the ethanol yield by ca. 20% in comparison to the control samples, moreover the ethanol yield in sonificated samples was similar to jet-cooked corn meal. The authors also conducted the economical evaluation and energy usage for jet-cooking and ultrasonics installations in industrial environments. They stated that the overall cost for installation and maintenance for ultrasonic apparatus is lower in comparison to hydrocooking, also the energy analysis proved that much less energy is needed for sonification of raw material than for jet cooking. Also microwave treatment improves enzymatic hydrolysis of starch [24]. The usage of cheap, waste raw material like bread leftovers in the cost-effective process of direct starch hydrolysis and fermentation could be very attractive for ethanol fuel production. Until now the usage of GSHE for the waste bread processing into ethanol was not studied. This could be very effective solution including high ethanol yield and vast reduction of processing costs in comparison to traditional processes.

The object of this study was to asses the ethanol fermentation course and efficiency of waste bread using granular starch hydrolyzing enzyme in comparison to separate hydrolysis and fermentation process. Also different methods of raw material pretreatment (enzymatic, ultrasonic and microwave) was conducted to improve raw starch hydrolysis and fermentation.

2. Materials and methods

2.1. Raw material

Waste wheat-rye bread (after shelf-life, returns from shops) was obtained from local bakery. Whole loafs were manually cut into dices of ca. 2–4 cm size. Obtained dices were dried in a forced air oven at 40 °C for 12 h and ground in a knife mill (Rotary Mill, Brabender, Germany) with 1.5 mm internal mesh sieve. Raw material was stored in an air tight jar at room temperature until used. Starch content in waste bread was determined using Evers polarimetric method [25] and it ranged 689.13 ± 4.52 g kg^{-1} of raw material dry matter. The amount of total sugars (as glucose) was measured using the DNS method [26] after mild acid hydrolysis (70 °C, 10 min) with 80 g L^{-1} HCl solution (1:7.5 m/v raw material to HCl solution ratio) and it ranged 866.87 ± 2.85 g kg^{-1} of raw material dry matter. Raw material moisture content was measured using WPS 50P weighing dryer (Radwag, Poland) and it ranged 41.43 ± 1.38 g kg^{-1}.

2.2. Enzymes and yeast

GSHE preparation STARGEN 002 was obtained from Genencor International (USA). STARGEN 002 contains *Aspergillus kawachii* α-amylase expressed in *T. reesei* and glucoamylase from *T. reesei*. As declared by the manufacturer, the preparation activity is 610 glucoamylase units per gram and has an optimal temperature range of 35–45 °C. Neutrase 0.8 L (Novozymes, Denmark) is a bacterial protease form *Bacillus amyloliquefaciensis*, its declared activity was 0.8 proteolytic units per gram. Ceremix 6X MG (Novozymes) is a preparation displaying multidirectional substrate