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Simultaneous saccharification and ethanol fermentation of waste wheat–rye bread at very high solids loading: Effect of enzymatic liquefaction conditions

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HIGHLIGHTS

- Simultaneous saccharification and fermentation of waste bread at high solids.
- Enzymatic liquefaction was modified on the basis of substrate thermal properties.
- Lower liquefaction temperature decreased hydrolysis efficiency.
- The highest ethanol yield obtained for substrate liquefaction at 59 °C.

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ABSTRACT

The aim of this research was to investigate the ethanol fermentation process course and yield from waste wheat–rye bread in the simultaneous saccharification and fermentation (SSF) conditions at high solids loading (300 g kg^{-1}). Moreover the enzymatic liquefaction conditions were modified on the basis of thermal properties of starch and compared to the temperature optimal for α-amylase activity (85 °C). The liquefaction of raw material at modified conditions decreased the hydrolysis efficiency in comparison to hydrolysis at 85 °C. The course and ethanol yield of the SSF process in the optimized conditions of liquefaction was comparable or even higher in comparison to standard conditions. The best results were obtained when starch was liquefied at the final temperature of gelatinization (59 °C) resulting in final ethanol concentration of 128.01 g L^{-1} yielding 425.04 g kg^{-1} of dry matter and 95.93% practical yield (416.09 g kg^{-1} and 93.91% for liquefaction at 85 °C).

1. Introduction

Bioethanol is currently one of the most promising renewable liquid fuel to replace oil-derived fossil fuels. Nowadays the main substrates for bioethanol production are agricultural crops (corn, wheat, sugarcane, sorghum). These materials are known as very high-yielding and easy to process substrates for the purpose of ethanol production, however its use as the raw materials for fuel production raises concerns about influencing on food prices by representing competing demand for those products [1]. Moreover the price of these substrates is relatively high and it is a major cost in the ethanol production process [2]. Other possible substrates for industrial bioethanol production are agricultural or forestry residues known as the lignocellulosic biomass. These raw materials are cheap, highly available and do not influence on the food prices [3]. However its conversion to ethanol is connected to several processing difficulties and the ethanol yield form them is much lower in comparison to starchy or sugar crops [4].

The great possibility for industrial ethanol production that do not influence food prices and ensures high process efficiency is the use of food industry wastes, by-products and intermediates as the substrates for ethanol fermentation. These substrates usually contain free sugars or easily hydrolysable polysaccharides that are easily converted to monomeric sugars and, subsequently into ethanol. The most notable food wastes that could be converted to ethanol are: potato industry wastes [5], sugar beet processing wastes and intermediates [6], wastewater from soft drinks industry [7] and others. Moreover some food processing wastes could be used as nutrient enrichment sources for improving ethanol fermentation dynamics like soy skim milk [8] or brewer's spent yeast [9].

One of the most promising food industry wastes that could be used as a substrate for ethanol production are the bakery industry

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wastes. Annual global production of bread exceeds 100 million tones [10], and estimated wastage for bakery goods is about 7–10% [11] so the annual production of wastes in this industry can reach even 10 million tones worldwide. The major factor for wastes formation in this field is that part of the bread produced is left unsold and 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 [12]. Bread wastes have limited possibilities for reprocessing in the food industry, they could be used as an animal feed [13] however only when no microbial spoilage occurs. The most promising solution for utilization of bakery wastes is to use it as raw material for ethanol production. Bread has similar composition to its raw materials so it contains significant amounts of starch (500–750 g kg^{-1}), sugars (3–50 g kg^{-1}) and protein (100–150 g kg^{-1}) [14]. Moreover due to the baking process starch is partially gelatinized and depolymerized so its further hydrolysis is facilitated.

Previous studies shown that bread residues are a high yielding substrate for ethanol production. The ethanol yield from waste bread, depending on the processing conditions, could reach approximately 350–370 g kg^{-1} of substrate dry matter [15,16]. Also the possibility of utilizing waste bread which shown significant signs of mold spoilage was studied [17]. The ethanol efficiency was lower in comparison to non contaminated bread but still high yields were obtained ranging, depending on the substrate loading, ca. 240 g kg^{-1}. Industrial plants processing mostly bakery wastes are currently located in Finland, what suggests that industrial scale ethanol production from them is possible [18]. Waste bread could also be used as a substrate for glucoamylase and protease biosynthesis by *Aspergillus awamori* strain [19].

Two recent developments in the field of bioethanol from starchy raw materials deserves special attention, namely, the simultaneous saccharification and fermentation (SSF) and the very high gravity (VHG) fermentation. The SSF process is distinguished from the traditional separate hydrolysis and fermentation (SHF) by the fact that the liquefied (by the action α-amylase) slurry is cooled to the fermentation temperature, pitched with yeast and the glucoamylase is added to saccharify the dextrins. It has several advantages in comparison to the SHF process. The saccharification and fermentation acts in one reactor so the investment cost for processing plant is decreased, for the same reason less energy and cooling water is needed in comparison to SHF [20]. Moreover the concentration of monomeric sugars in the mashes which were subjected only to enzymatic liquefaction is much less than in those which were fully hydrolyzed. This is important due because high concentration of glucose in the medium causes inhibitory effect upon the yeast so the cell viability decreases and the fermentation process could be prolonged [21]. The VHG technology is defined as the process of fermentation of media containing high concentration (above 270 grams per liter) of dissolved solids. Its major advantage over 'normal' gravity fermentation is higher ethanol productivity and possibility of obtainment of very high ethanol concentration (above 15% by volume) which is the cause for energy reduction for distillation [22]. However the VHG process causes few technological problems, including reduction in fermentation dynamics by high osmotic pressure upon the yeast. The solution to this problem is the proper yeast nutrition with assimilable nitrogen [23]. Also the hydrolysis of protein present in the raw material increases formation of free amino nitrogen and consequently the fermentation efficiency of VHG media [24]. The application of the SSF and VHG technologies in the process of waste bread to bioethanol utilization was not previously studied. The benefits of these processes combined with the use of cheap raw material could result in a highly efficient fermentation processes. Moreover the selection of enzymatic liquefaction conditions (mostly temperature) could further increase the fermentation yield and reduction of process costs.

The aim of the study was to investigate the ethanol fermentation course and final efficiency using the SSF process under VHG conditions with the use of waste wheat–rye bread as the sole substrate. Moreover the effect of the enzymatic liquefaction temperature, based on the thermal characterization of starch present in the substrate, on the fermentation dynamics and efficiency was studied and compared to the process where applied liquefaction temperature was set on the basis of optimal conditions for the used α-amylase activity.

2. Materials and methods

2.1. Raw material

Waste wheat–rye bread, the most common bread type in Poland, was obtained from a large local producer. The material constituted returns of unsold bread from shops, it did not shown signs of surface mold spoilage. The whole loafs were manually cut into ca. 2–4 cm dices and dried in a forced air oven (WTC Binder, Germany) at 40 °C for 12 h. Afterward the material was ground in a knife mill (Rotary Mill, Brabender, Germany) with 1.5 mm internal mesh sieve and stored at room temperature in airtight jar until used. The moisture content in raw material was measured using WPS 50P weighing dryer (Radwag, Poland) and it ranged 41.43 ± 1.38 g kg^{-1} (mean value ± standard deviation at $n = 3$). Starch content in the substrate was measured using Ewers polarimetric method [25] and it ranged 689.13 ± 4.52 g kg^{-1} of dry matter. The content of total sugars in waste bread sample 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 acid solution ratio). Total sugars content in the raw material ranged 866.87 ± 2.85 g kg^{-1} dry matter.

2.2. Enzymes and yeast

Thermostable α-amylase (EC 3.2.1.1) preparation Termamyl SC DS was used in the enzymatic liquefaction step in the amount of 1.25 mL kg^{-1} of raw material dry matter. Glucoamylase (EC 3.2.1.3) preparation SAN Extra L and protease (EC 3.4.23.28) Neutrase 0.8 L were used in the simultaneous saccharification and fermentation. The enzyme doses were 1.25 and 0.9 mL kg^{-1} of dry matter for SAN Extra L and Neutrase 0.8 L respectively. All enzymes were kindly provided by Novozymes (Denmark). Active dry yeast *Saccharomyces cerevisiae* Ethanol Red were purchased from Fermentis (France). The yeast were rehydrated in sterile distilled water (1:10 m/v yeast to water ratio) for 30 min at ca. 30 °C prior to inoculation.

2.3. Determination of thermal properties of gelatinization of starch present in the raw material

The temperatures of phase transitions of starch present in waste bread were determined using differential scanning calorimetry (DSC) as described by Zięba et al. [27]. Briefly 10 mg (dry matter basis) amount of waste bread was weighed into semi-pressure aluminum crucible (ME-2990, Mettler-Toledo) and bidistilled water was added to obtain raw material to water ratio of 1:3 m/v. The crucibles were sealed and conditioned for 1 h at room temperature. The analysis were performed using DSC 822^e differential calorimeter (Mettler-Toledo, Switzerland) in a temperature range of 25–100 °C at 4 °C min^{-1} heating rate. Empty crucible was used as the reference sample. The analysis was performed in triplicate and obtained thermograms were integrated using Star^e software (Mettler-Toledo, Switzerland). The initial temperature of gelatinization (T0), endotherm peak temperature (Tp) and gelatinization final temperature