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Simultaneous hydrolysis and co-fermentation of whey lactose with wheat for ethanol production

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HIGHLIGHTS

- Whey permeate can contribute to ethanol production as a co-substrate to wheat.
- *A. oryzae* β-galactosidase can effectively hydrolyse lactose during fermentation.
- Whey permeate can be incorporated into both STARGEN and jet cooking systems.

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ABSTRACT

Whey permeate was used as a co-substrate to replace part of the wheat for ethanol production by *Saccharomyces cerevisiae*. The simultaneous saccharification and fermentation was achieved with β-galactosidase added at the onset of the fermentation to promote whey lactose hydrolysis. *Aspergillus oryzae* and *Kluyveromyces lactis* β-galactosidases were two enzymes selected and used in the co-fermentation of wheat and whey permeate for the comparison of their effectiveness on lactose hydrolysis. The possibility of co-fermentations in both STARGEN and jet cooking systems was investigated in 5 L bioreactors. Ethanol yields from the co-fermentations of wheat and whey permeate were evaluated. It was found that *A. oryzae* β-galactosidase was more efficient for lactose hydrolysis during the co-fermentation and that whey permeate supplementation can contribute to ethanol yield in co-fermentations with wheat.

1. Introduction

Whey is the residue produced by the precipitation of casein from milk during the production of cheese and casein. The major components in whey are lactose and whey protein, which account for approximately 75% and 10% of the total solids, respectively (Mawson, 1994). Whey protein can be separated from whey by ultrafiltration, resulting in a lactose-rich stream so-called whey permeate. Lactose is the most abundant component in both whey and whey permeate besides water. Current whey and whey permeate utilization consumes only a small portion of lactose generated from cheese production (OECD-FAO, 2015). The large surplus of whey lactose can be used as a potential low-cost substrate for industrial chemical and fuel production.

Numerous studies have focused on utilizing whey lactose for ethanol production. The first studies to ferment whey lactose directly for ethanol were carried out in the 1940s (Browne, 1941; Rogosa et al., 1947), while commercial ethanol production from whey lactose dates back to at least the 1970s (Lyons and Cunningham, 1980; Guimaraes et al., 2010). Utilization of whey lactose for ethanol production has been considered as a promising strategy with both academic and industrial attempts. *Kluyveromyces* strains, particularly *K. marxianus*, have been used that are able to ferment lactose directly. However, the low ethanol titre of 2.5–4.2% (v/v) with a low starting lactose content of 5.0%, and the prolonged fermentation time with high lactose concentrations were the major drawbacks of direct lactose-to-ethanol production by *Kluyveromyces* yeasts. Based on the literature, the reason for these drawbacks is likely the low osmotic tolerance of *Kluyveromyces* yeasts, which leads to slow growth rates (Guimaraes et al., 2010). Currently, the global ethanol supply mainly depends on fermentations utilizing natural sugars and starch as feedstocks (Bai et al., 2008; Renewable Fuels Association, 2016). In North America, the bio-ethanol industry has been dominated by grain-to-ethanol production, which accounts for more than 99% of the annual production capacity (Renewable Fuels Association, 2016; Canadian Renewable Fuels

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Association, 2015). Therefore, integration of whey lactose into conventional grain-to-ethanol production as a co-substrate is an interesting and attractive strategy for whey lactose utilization and reduced grain consumption. Whey lactose can be supplemented to replace part of the grain, leading to a reduction of feedstock cost, less dependence of production cost on grain price fluctuations, and lower costs associated with enzymes used for starch hydrolysis. In addition, incorporation of whey lactose into grain-to-ethanol fermentations would be highly compatible with the current process of ethanol production. Therefore, setup of new major facilities is not required, resulting in low construction costs for ethanol plants to adopt this approach.

In this study, concentrated whey permeate was directly blended with wheat for mash preparation without pre-hydrolysis. *S. cerevisiae* was used for ethanol production because of its high tolerance to substrate and product inhibition (Cot et al., 2007). *A. oryzae* or *K. lactis* β-galactosidase was added at the onset of the fermentations for simultaneous lactose hydrolysis. The goal of this study was the partial replacement of the wheat and process water used for ethanol production with whey permeate, and to evaluate the effectiveness of simultaneous lactose hydrolysis during the fermentations as well as the contribution of whey permeate to ethanol production. This is the first study to apply simultaneous lactose hydrolysis in co-fermentations of whey lactose and grains by *S. cerevisiae*.

2. Materials and methods

2.1. Grain, enzymes, and others

Spring wheat (AC Andrew) was provided by Seed Solutions (Viking, AB, Canada). Wheat was ground by a laboratory hammer mill (Model 3100, Perten, Sweden) equipped with a mill feeder (Model 3170, Perten, Sweden). Sieve sizes of 0.5 mm and 1.98 mm were used to mill wheat for STARGEN- and jet cooking-based fermentations, respectively. The moisture and starch contents of wheat flour were determined using AOAC official method 934.01 and a Total Starch Assay Kit (Megazyme, Country Wicklow, Ireland), respectively. Whey permeate, a byproduct from dairy processing obtained from reverse osmosis and ultrafiltration of whey, was provided by a large dairy company in Canada and was shipped on ice and stored at 4 °C upon receipt. The compositional profile of whey permeate has been discussed in our previous study (Parashar et al., 2016).

STARGEN™ 002 (enzyme blend of α-amylase and glucoamylase, 570 glucoamylase unit/g), Optimash™ TBG (thermostable β-glucanase, 5625 U/g), GC 626 (acid α-amylase, 10,000 soluble starch unit/g), and Fermgen™ (protease, 1000 spectrophotometric acid protease unit/g) were provided by Genencor International (Palo Alto, CA, USA). Viscozyme Wheat FG (xylanase and pentosanase), Liquozyme SC DS (α-amylase, 240 Kilo Novozymes Unit/g), and Spirizyme Ultra (glucoamylase, 900 amyloglucosidase unit/g) were supplied by Novozymes (Franklinton, NC, USA). *Aspergillus oryzae* (8 U/mg) and *Kluyveromyces lactis* (2600 lactase activity unit/g) β-galactosidases were purchased from Sigma-Aldrich (St. Louis, MO, USA). D-glucose and Lactose/D-Galactose Assay Kits were obtained from Megazyme (Country Wicklow, Ireland). SuperStart distiller's yeast was supplied from Lallemand Ethanol Technology (Milwaukee, WI, USA).

2.2. Mash preparation and STARGEN-based fermentation in shake-flask scale

Fermentations in 500 mL shake-flask scale were carried out with a starting broth mass of 250 g. For the standard STARGEN-based fermentation of 30% wheat (w/w, wet basis), wheat flour (0.5 mm) was weighed into a 500 mL Erlenmeyer flask and mixed thoroughly with distilled water. The pH of wheat mash was adjusted to 4.0 using 4 N HCl. The flask was covered with foil and heated in an incubator shaker (Innova 44/44R, New Brunswick Scientific, Edison, NJ, USA) at 55 °C and 200 rpm. When the temperature of mash reached 53–55 °C, Fermgen™ (940 μL/kg of grain), Optimash™ TBG (80 μL/kg of grain), and GC 626 (440 μL/kg of grain) were added. The flask was kept at this temperature for 1 h at 200 rpm. After that, diethyl pyrocarbonate (DEPC, Sigma-Aldrich, St. Louis, MO, USA) was added to the flask as a chemical disinfectant at a dosage of 105 μL/kg mash. The flask containing mash was stored at 4 °C for 72 h prior to fermentation.

For the whey permeate-blended STARGEN-based fermentation, 25% wheat (w/w, wet basis) was prepared. An amount of whey permeate was added to contribute fermentable carbon from lactose in order to provide for the same amount of total fermentable carbon (carbon from wheat starch and whey lactose) as that of 30% wheat. Theoretically, 1 g of starch can be converted to 1.111 g of glucose containing 0.444 g of fermentable carbon; 1 g of lactose can generate 0.526 g of galactose and 0.526 g of glucose with a total of 0.421 g of fermentable carbon. Therefore, 1 g starch can be replaced by 1.05 g of lactose to maintain the same amount of fermentable carbon. The following treatments were identical to the standard fermentation stated above.

After 72 h storage at 4 °C, the mash was heated up to 53–55 °C in the incubator shaker at 200 rpm. STARGEN™ 002 (2.8 mL/kg of grain) was added to the mash, followed by 1 h incubation at 53–55 °C, 200 rpm. Urea (1 M) was added to the mash to obtain a final concentration of 16 mmol/kg. When the temperature of the mash decreased to 30 °C, hydrated yeast was added at an approximate initial viable cell concentration of 2×10^7 cfu/mL to the mash. β-Galactosidase was added together with the hydrated yeast to conduct simultaneous lactose hydrolysis and fermentation for the whey permeate-blended group. The flask was then sealed with a gas trap, which has an S-lock filled with water to allow CO_2 escape during fermentation. All fermentations were carried out at 30 °C, 200 rpm for 72 h.

2.3. Analytical assays

2.3.1. Glucose, galactose, lactose, lactic acid, and acetic acid

Fermentation samples were centrifuged at 15,000×*g* (Model 5424, Eppendorf, Hamburg, Germany) for 10 min. Clear supernatant was transferred to a sealed tube and boiled for 5 min to inactivate enzymes, followed by filtration through a 0.22 μm membrane filter (Mandel Scientific, Guelph, ON, Canada). The filtrate obtained was used for the analysis of sugars and organic acids. Glucose, lactic acid, and acetic acid concentrations were analyzed by high performance liquid chromatography (HPLC, 1200 series, Agilent Technologies, Mississauga, ON, Canada) equipped with a refractive index detector (1100 series, Agilent Technologies, Mississauga, ON, Canada) and an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA). The analyses were carried out using a mobile phase of 5 mM H_2SO_4 at 60 °C with a flow rate of 0.5 mL/min for 30 min. Different concentrations of glucose, lactic acid, and acetic acid (HPLC grade, Sigma-Aldrich, St. Louis, MO, USA) standard solutions were prepared and analyzed each time for preparation of standard curves. Samples with a glucose concentration lower than 1 g/L were analyzed using a D-Glucose Assay Kit. Lactose and galactose concentrations were measured using a Lactose/D-Galactose Assay Kit. The lactose concentrations in the fermentation samples collected at 0 h (initial) and 72 h (residual) were used for the calculation of lactose hydrolysis% as per the following Eq. (1):