Biotechnol Lett (2011) 33:273–276
DOI 10.1007/s10529-010-0429-z

ORIGINAL RESEARCH PAPER

Utilization of dry distiller's grain and solubles as nutrient supplement in the simultaneous saccharification and ethanol fermentation at high solids loading of corn stover

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Received: 14 June 2010 / Accepted: 1 October 2010 / Published online: 15 October 2010

Abstract Dry distiller's grain and solubles (DDGS) is a major by-product of corn-based ethanol production and is usually used as animal feed. Here, it was added to the simultaneous saccharification and ethanol fermentation (SSF) carried out at high solids loading of steam explosion pretreated corn stover using a mutant strain *Saccharomyces cerevisiae* DQ1. The performance of SSF process with DDGS was comparable to those using the expensive yeast extract supplementation. With 30% (w/w) solids plus the addition of cellulase and 1 g DDGS l^{-1}, the final ethanol reached 55 g l^{-1} (7% v/v). The results indicated that the expensive supplement of yeast extract could be replaced by DDGS.

Keywords Corn stover · Dry distiller's grain and solubles (DDGS) · High solids loading · Simultaneous saccharification and ethanol fermentation · Yeast extract

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Introduction

The major barrier for the commercial production of ethanol using lignocellulosic feedstocks is the cost of processing lignocellulose (Lynd et al. 2008). The replacement of the high costly nutrient supplements by cheap additives in the fermentation could be an important way for the overall cost reduction. Dry distiller's grain and solubles (DDGS) is a major by-product of ethanol production using corn as the feedstock and is generally used as an animal feed due to its rich nutrient contents such as protein, oil, fiber, yeast cells, and other unfermented components of the grain (Ham et al. 1994; Spiehs et al. 2002; Kim et al. 2008a). In previous studies, DDGS was only used as a feedstock of ethanol fermentation by hydrolyzing its cellulose component into fermentable sugars (Dien et al. 2008; Kim et al. 2008a, b; Ezeji and Blaschek 2008). The potential of DDGS as a nutrient supplement replacing the expensive nutrient additives such as yeast extract has not been well demonstrated. Lau et al. (2008) showed that the DDGS addition improved the ethanol fermentation performance of the hydrolysate of the ammonia fiber expansion (AFEX) pretreated corn stover using recombinant *E. coli* strain. In this study, we demonstrated that DDGS was able to replace yeast extract as the nutrient supplement for the simultaneous saccharification and ethanol fermentation at high solids loading of steam explosion pretreated corn stover. The result indicated that DDGS could

assist the well accomplishment of the process and reduce the cost of yeast extract addition in the cellulose ethanol fermentation.

Materials and methods

Raw materials and pretreatment

Corn stover was collected in Jilin Province, China in 2007. The corn stover was milled, steam explosion pretreated at the condition of 200°C, 2.0 MPa for 4 min. The pretreated corn stover contained approx. 50% (w/w) dry solids matter (DM), and was ground in a juice blender for a few seconds to disperse the aggregates formed during the storage at 4°C before feeding into the bioreactor.

DDGS was a commercial animal feed product of Jilin Fuel Alcohol Co., Jilin, China. It is produced from the dry mill corn fermentation for ethanol production. The dry matter of DDGS was 87% (w/w). Yeast extract was purchased from Oxoid Ltd. (Basingstoke, Hampshire, England). All other chemicals were from local suppliers.

Enzymes and strain

The cellulase used was Accellerase 1000 (Genencor International, Rochester, NY, USA) with the filter paper activity of 65.8 FPU ml^{-1} and cellobiase activity of 152 IU ml^{-1} (Zhang et al. 2010).

A mutant *Saccharomyces cerevisiae* DQ1 strain with the high tolerance of inhibitor substances derived from lignocellulose pretreatment was used in all the fermentation experiments (Zhang et al. 2010). The synthetic medium contained 20 g glucose l^{-1}, 2 g KH_2PO_4 l^{-1}, 1 g $(NH_4)_2SO_4$ l^{-1}, 1 g $MgSO_4 \cdot 7H_2O$ l^{-1}, and 1 g yeast extract l^{-1}. The medium was autoclaved at 115°C for 20 min before use.

Cell growth assay

Growth of *S. cerevisiae* DQ1 was conducted in the 250 ml shake-flasks with 50 ml synthetic medium at 30°C, 150 rpm for 60 h. Growth was determined from OD_{600} values; an OD_{600} of 1 = 0.5 g dry cell mass l^{-1}.

Simultaneous saccharification and fermentation

The simultaneous saccharification and fermentation (SSF) was carried out in a 2 l bioreactor with a helical stirrer. The procedure for a three-step inocula adaptation and SSF was described by Zhang et al. (2010). Each experiment was conducted for three times and the error bar was added to the experimental data.

HPLC analysis

Analysis of liquid samples was performed by HPLC with a refractive index detector and an Aminex HPX-87H column at 65°C with 5 mM H_2SO_4 as eluant at 0.6 ml/min. Samples were centrifuged to remove cells and other water-insoluble substances, diluted and filtered through a 0.2 μm filter before injection.

Results and discussion

Cell growth

Growth of *S. cerevisiae* in the synthetic medium containing different nutrient supplement (yeast extract or DDGS) is shown in Fig. 1. Growth with yeast extract as the nutrient supplement was better than that with DDGS at both 1 and 5 g l^{-1}. Growth only showed limited improvement when DDGS was digested with 50 FPU cellulase per dry matter, indicating that the partial solubility of DDGS in the

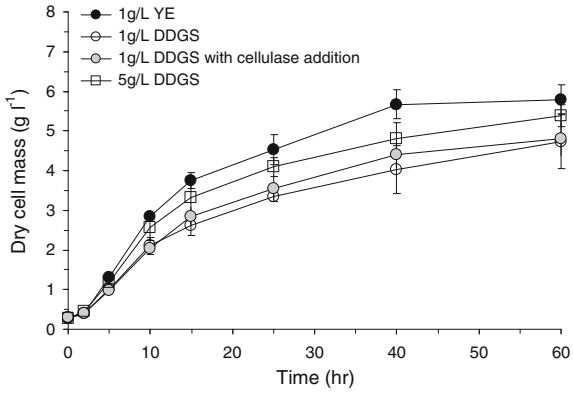

Fig. 1 Cell growth under different nutrient supplementations. 30°C, 150 rpm for 60 h. 50 FPU DM g^{-1} of Accellerase 1000 and 1 g DDGS l^{-1} were added to the synthetic medium followed by inoculation and cultivation

medium was not the reason for the growth performance. DDGS addition as a nutrient supplement is therefore not as good as yeast extract in the synthetic medium.

Simultaneous saccharification and fermentation

DDGS as a nutrient supplement in the cellulosic ethanol fermentation was compared with yeast extract using steam-exploded, pretreated corn stover and cellulase at 14.7 FPU DM g^{-1} (Fig. 2). Glucose consumption and the final ethanol concentration with 1 g DDGS l^{-1} were better than that with 1 g yeast extract l^{-1}, while glucose consumption and the final ethanol concentration were similar with either 5 g yeast extract or DDGS l^{-1}. Ethanol with 1 or 5 g DDGS l^{-1} reached 55 and 57 g l^{-1}, corresponding to the volumetric concentration of 7 and 7.2% (v/v), respectively.

As a negative control, SSF experiments without yeast extract or DDGS were conducted in a 2 l bioreactor; no growth or ethanol accumulation was found (data were not shown). A similar result occurred using shake-flasks with the solids loading decreased to 10% (w/w) to lessen the effect of inhibitors. Again, no growth or ethanol formation was found. Thus supplementation with either yeast extract or DDGS is important for SSF using steam-exploded, pretreated corn stover.

These results give strong evidence for low dosage supplementation by cheap DDGS for excellent SSF performance, even better than that using the same amount of expensive yeast extract. DDGS is a cheap additive used in animal feed and the price of a typical DDGS product is only $0.25 per kg (www.grains.org/images/M_images/market_perspectives/2010/7-2-10/MP07022010.pdf, June 24, 2010, US Grain Council). The price of a typical yeast extract product (Oxoid Ltd, Basingstoke, Hampshire, UK) is $55 per kg (http://www.biodee.net/ProductDetail.aspx?id=822&lang=en). The ethanol fermentation using the lignocellulose feedstock produced 55 kg ethanol with 1 kg yeast extract supplement, equivalent to 18.2 kg yeast extract with the cost of approx. $1,000 for producing 1 ton ethanol, if the current result was maintained to the commercial scale. On the other hand, the cost of

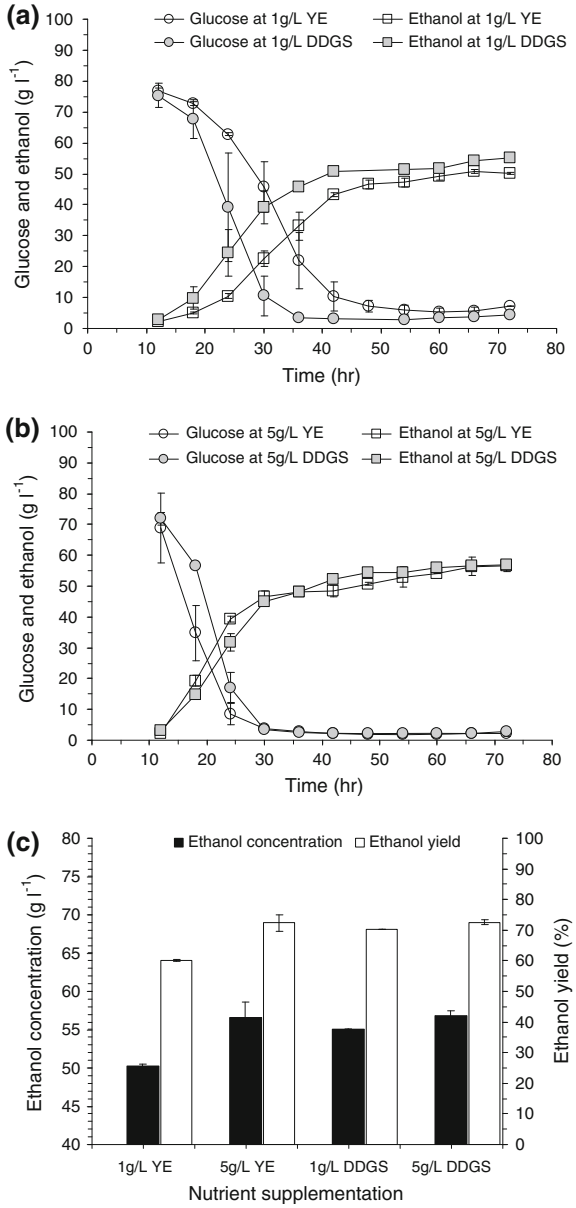

Fig. 2 Effect of nutrient supplementation on SSF at high solids loading. 50°C, 200 rpm for 12 h. 14.7 FPU DM g^{-1} of Accellerase 1000, batches of corn stover and 1 g yeast extract l^{-1}, 5 g yeast extract l^{-1}, 1 g DDGS l^{-1}, or 5 g DDGS l^{-1} were added to the synthetic medium followed by inoculation and SSF. 37°C. 200 rpm for another 60 h. **a** Glucose consumption and ethanol accumulation during the SSF process with 1 g yeast extract and DDGS l^{-1}. **b** Glucose consumption and ethanol accumulation during the SSF process with 5 g yeast extract and DDGS l^{-1}. **c** Final ethanol concentration and ethanol yield between the use of yeast extract and DDGS

DDGS supplement was only $4.3 for producing 1 ton ethanol using lignocellulose. This cost is negligible for the total ethanol cost, and a significant cost reduction comparing to using yeast extract supplement. The result provided a way to reduce the cost for the cellulose ethanol production by replacing the expensive supplement with the cheap one, DDGS derived from the corn ethanol production.

Acknowledgments This research was supported by Innovation Programs for Undergraduates by Shanghai Municipal Education Commission and East China University of Science and Technology (S0822), National Basic Research Program of China (2011CB707406), Natural Science Foundation of China (20976051), Ministry of Education of China (20090074110013), Shanghai Leading Academic Discipline Project (B505), Fundamental Research Funds for the Central Universities of China, and National Special Fund for the State Key Laboratory of Bioreactor Engineering of China (2060204).

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