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Water reclamation and value-added animal feed from corn-ethanol stillage by fungal processing

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HIGHLIGHTS

- Innovative water reclamation, energy savings and additional coproducts.
- Removes recycling inhibitors, i.e. lactic/acetic acid, glycerol, suspended solids.
- Fungal biomass has high protein, valuable amino acids suitable for *non-ruminants*.
- Fungal biomass could also potentially be raw material for chitin and chitosan.
- Fungal cultivation on thin stillage could make ethanol production more sustainable.

GRAPHICAL ABSTRACT

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ABSTRACT

Rhizopus oligosporus was cultivated on thin stillage from a dry-grind corn ethanol plant. The aim of the research was to develop a process to replace the current energy-intensive flash evaporation and make use of this nutrient-rich stream to create a new co-product in the form of protein-rich biomass. Batch experiments in 5- and 50-L stirred bioreactors showed prolific fungal growth under non-sterile conditions. COD, suspended solids, glycerol, and organic acids removals, critical for in-plant water reuse, reached ca. 80%, 98%, 100% and 100%, respectively, within 5 d of fungal inoculation, enabling effluent recycle as process water. *R. oligosporus* contains 2% lysine, good levels of other essential amino acids, and 43% crude protein – a highly nutritious livestock feed. Avoiding water evaporation from thin stillage would furthermore save substantial energy inputs on corn ethanol plants.

1. Introduction

Fuel ethanol production in the US has been growing with 13 billion gallons produced in 2012. Biofuels, such as corn ethanol, help alleviate oil dependence and improve energy security, while boosting the rural economy and reducing greenhouse gas emissions. The corn ethanol industry has advanced in efficiency, but still has considerable room for improvement. Innovations to date include no cook-raw starch hydrolysis (POET BPX™ process), back-end crude corn oil recovery, and corn fractionation (e.g., POET BFRAC™ process). Fuel ethanol companies are also pursuing renewable energy sources in order to reduce natural gas

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consumption, for instance, by integrating biomass gasification and methane digesters.

Reducing energy inputs, creating value-added coproducts from stillage, and recycling water are important to sustainable corn ethanol production. Innovations for improved sustainability help to contest recent ethanol issues, such as indirect land use changes and food versus fuel, with negative impact on public perception of corn ethanol. Most conventional dry-grind corn ethanol plants generate 5–6 gal stillage per gal ethanol after distillation, of which up to half is recycled directly as backset (Ethanol Producer Magazine, 2006), requiring ca. 3.5 gal of fresh water per gal ethanol (RFA, 2013).

Dry-grind processing produces ethanol from corn by milling, hydrolyzing with enzymes (with or without cooking), fermentation, and distillation. Approximately one-third of the corn is converted to ethanol, one-third to carbon dioxide, and one-third remains as dissolved and suspended organics in the stillage. Most suspended solids – wet distillers' grains – are removed by centrifugation. The liquid centrate – thin stillage – contains mainly dissolved organic materials (solubles) of 75–100 g/L as COD (Schaefer and Sung, 2008; Singh et al., 2007).

The ethanol-to-stillage ratio in the beer after fermentation needs to be high to lower distillation cost, but is limited by ethanol toxicity to the yeast. The profitability of dry-grind ethanol production depends on the sale of both the ethanol and the coproducts from stillage. Recycling water is important as there are limits to supply, and the ethanol plants are not permitted to discharge processing water.

Up to half of the thin stillage is directly recycled as process water, and the remainder is evaporated to produce condensed solubles, *syrup* of 30% solids (RFA, 2013), requiring a substantial part of plant energy inputs. The syrup is blended with wet grains and dried to produce DDGS. Dry-grind corn ethanol plants produced 39 million metric tons of distillers' grains in 2011 (RFA, 2013), the year of peak production, before the 2012 drought. Distillers' grains are sold primarily as livestock feed for cattle and dairy. The low content of digestible amino acids limits its use for nonruminants. Only 12% and 8% of distillers' grains were consumed by swine and poultry, respectively, in 2012, in spite of higher proportions of hog and chicken production in the US Corn Belt (RFA, 2013). The syrup has suffered a decline in demand as it is not nutritionally expedient to include syrup; it is sold for very low prices and is even given away (Johnson, 2013).

It would be much more advantageous to use the nutrients in thin stillage for the cultivation of beneficial microbes or microbial products. One possibility is bacterial cultivation and harvesting a metabolite, e.g., lactic acid such as done by Djukic-Vukovic et al. (2013).

The low initial pH of 4 and high organic content make thin stillage an ideal feedstock for fungal cultivation. Fungal treatment of thin stillage has the potential to recover water and enzymes for in-plant reuse and to produce a high-quality animal feed (distillers dried grains with fungal protein or fungal protein only). The fungal coproduct could command an increased market value, while improving profits and minimizing environmental impacts. Based on 6 gal stillage per gal ethanol and recycling 50% as backset, there is 3 gal or more thin stillage available for fungal cultivation. The use of fungi is advantageous as they produce a wide array of biochemicals and enzymes, which tend to be more effective in degrading complex carbohydrates than bacteria (Sankaran et al., 2010; van Leeuwen et al., 2013). The food-grade fungus *Rhizopus microsporus* var. *oligosporus* (shortened *Rhizopus oligosporus*) produces numerous enzymes, including β-glucosidase, glucoamylase, lipase, phytases, and chitinases (Gautam et al., 2002; Jin et al., 1999a–c, 2001a,b).

Rhizopus species are also known to produce α-amylase (Jin et al., 1998). Under aseptic conditions in a nutrient medium, *R. oligosporus* was reported to produce about 12% chitin (Blumenthal and Roseman, 1957) as well as 4% and 40% yields of lysine and protein, respectively (Rhodes et al., 1961). *R. oligosporus* was successfully cultivated on wheat-milling and corn wet-milling streams, achieving significant reductions in COD of up to 80–90% (Jin et al., 1999a, 2002; Gautam et al., 2002; Jasti et al., 2008; Sankaran et al., 2008). The filamentous nature of fungal mycelia and potential for pellet formation aids in fungal biomass recovery (Gautam et al., 2002; Van Leeuwen et al., 2013).

Based on the utility of fungi and the readily available low-cost substrate, bench- (5 L) and pilot-scale (50 L) experiments were conducted to evaluate fungal treatment of thin stillage for fungal biomass production and organics removal to obtain recyclable effluent for in-plant use. Aeration rates in stirred bioreactors were varied from 0.2 to 1.0 L air/L working volume/min (vvm). Feed stillage and bioreactor samples were analyzed to determine the removal of total and soluble COD (TCOD, SCOD), total and volatile suspended solids (TSS, VSS), glycerol, and lactic and acetic acids, critical for recycling the effluent as process water. Fungal biomass production was quantified, and samples were analyzed for protein and amino acid contents.

2. Methods

2.1. Thin stillage

Thin stillage samples were obtained from Lincolnway Energy (Nevada, IA, USA), dry-grind corn ethanol plant. Samples were collected in sterile 10- and 20-L carboys and stored at 4 °C prior to use. The pH of fresh thin stillage was acidic (pH 3.8–4.7), and the COD averaged 90 g/L, of which 55 g COD/L was dissolved solids. The total and reducing sugar contents averaged 17 and 6 g/L, respectively. Suspended solids were 20–30 g/L, and the total nitrogen content was 6 g/L. Thin stillage suspended solids settled during storage at 4 °C. Supernatant from settled thin stillage (0.2 g/L suspended solids) was used as substrate for preparation of fungal mycelia inoculum and to determine the effect of thin stillage particles on fungal growth and morphology.

2.2. Fungal culture

Freeze-dried culture of the fungus *R. oligosporus* was obtained from the American Type Culture Collection (ATCC 22959, Rockville, MD, USA). The culture was revived in yeast mold (YM) broth (Difco Laboratories, Sparks, MD, USA) at 25 °C. Plates of potato dextrose agar (Difco Lab) were inoculated with the revived culture and incubated at 25 °C for 5–7 d. Fungal spores were harvested from the plates using sterile deionized water containing 0.1% (w/v) peptone (Difco Lab) and 0.2% (v/v) Tween 80 (Fisher Scientific, Fair Lawn, NJ, USA). Glycerin (20% [v/v]) was added to the spore suspension prior to ultra-low freezer (−75 °C) storage in sterile 2-ml cryovials. The harvested spore count of 5×10^6 spores/ml was determined by haemocytometer. Mycelia inoculum was prepared by heat sterilization (121 °C for 20 min) of YM broth or settled thin-stillage supernatant (1 L), inoculation with 1 vial (2 ml) of spore suspension, and incubation at 150 rpm shaking and 37 °C for 3 d.

2.3. Bioreactor set-up and operation

Batch cultivations of fungi on thin stillage were performed in bench-top stirred bioreactors with 5-L working volumes. Bench-top bioreactors were sterilized with water by autoclaving for 45 min at 121 °C, drained, and filled aseptically with thin stillage using a peristaltic pump. Culture conditions were chosen based on previous fungal research treating corn ethanol wet-milling