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Short communication

Dewatering investigations on fungal biomass grown in thin stillage from a dry-mill corn ethanol plant

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ABSTRACT

An innovative bioprocess utilizing thin stillage from a dry-grind corn ethanol plant was used to produce a useful filamentous fungus (*Rhizopus microsporus* var. *oligosporus)* in a pilot-scale bioreactor. The fungal process can improve the economics of corn ethanol production by producing an excellent food supplement for livestock or serving as a feedstock material for producing chitin, chitosan, and glucosamine. However, in order to be economically viable, effective and low-cost mechanical dewatering of the fungal biomass grown in thin stillage is required. In this study, dewatering tests were performed on fungal biomass using gravity and centrifugal sedimentation, gravity screening, a belt filter, a filter press, and centrifuge filtration in order to determine the most effective dewatering methods for this application. Utilizing a gravity-fed concave screen followed by a centrifuge filter proved to be the most effective dewatering approach and increased the screenable solids (i.e., larger than 20 mesh) content of the fungal biomass from the bioreactor from 1% to 30%. Achieving a solids content greater than 30% with mechanical dewatering is unlikely because of theoretical limits due to intracellular water. Nonetheless, this degree of dewatering greatly reduces thermal drying costs necessary to obtain a final product with a moisture content of 10%.

1. Introduction

Between 2004 and 2014, the U.S. production of ethanol increased from 3.4 to 14.3 million gallons annually [1], with dry-mill plants accounting for over 80% of the ethanol produced [2]. In the dry-mill process, a low-value byproduct stream known as thin stillage is produced. Some of the thin stillage, which contains solubles and some residual suspended solids, is recycled back to the fermentation tanks. However, the amount of thin stillage that can be recycled is limited by its lactic acid, acetic acid, and glycerol concentrations, which build up in the fermentation tanks and inhibit ethanol production. The remaining thin stillage is evaporated to obtain syrup that can be used to produce distiller's dried grains with solubles (DDGS).

Producing additional co-products would improve the economics of the ethanol industry, and cultivating the filamentous fungus (*Rhizopus microsporus var. oligosporus,* or simply *R. oligosporus*) on thin stillage is one possible option [3–5]. Other fungal species have also been successfully grown in thin stillage [6,7]. The fungi consume many organic compounds in the thin stillage, including lactic acid, acetic acid, and glycerol [3,4]. In addition, a nutritional co-product having high concentrations of crude protein and essential amino acids for use as an animal feed is produced [8]. The fungi can also serve as a feedstock material for producing chitin, chitosan, and glucosamine. However, in order to be economically viable, extensive and cost-effective mechanical dewatering of the fungi grown in thin stillage in the bioreactor is required. Various mechanical dewatering approaches were tested for that purpose. The impact of the dewatering technologies on the nutrient content of the fungi was beyond the scope of this study.

This work is not intended to present a thorough or comprehensive evaluation of the various dewatering technologies investigated. Rather, it is intended only to serve as exploratory tests to determine which of the specific methods and specific pieces of equipment tested yielded encouraging results, and to use that information to gain insights into suitable dewatering approaches for our continuing research on utilizing fungal biomass grown in thin stillage.

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2. Experimental methods

2.1. Fungal cultivation

Fungal biomass was grown in thin stillage in a 1600-L pilot-scale bioreactor containing 1500 L of thin stillage. Thin stillage collected from the Lincolnway Energy (Nevada, IA) plant was used as the growth medium. Spore suspensions of *R. oligosporus* were initially prepared and stored according to Ozsoy et al. [5]. Fungal inoculant for the reactor was then prepared by adding one 2-mL vial of spore suspension (containing 10^5 to 10^6 spores/mL) to each of eight 2-L flasks containing one liter of sterilized YM broth. Those seed cultures were incubated at 37°C for 24 h while shaking at 180 rpm. After the thin stillage in the bioreactor reached 37°C, it was inoculated with the seed cultures and the reactor was operated for 48 h in batch mode. Air was introduced through fine-bubble ceramic diffusers at a rate of 200–300 L/min in order to provide dissolved oxygen and promote mixing. The effluent from the bioreactor consisted of cultivated fungal biomass suspended in residual thin stillage. Unless otherwise noted, this reactor effluent was used for each of the solid-liquid separation techniques investigated.

Prior to fungal cultivation, the thin stillage contained 3% total suspended solids. However, the concentration of the suspended solids that could be removed with a 20-mesh screen (hereafter referred to as "screenable solids") was only 0.1% (w/w). After fungal cultivation, the concentration of total suspended solids in the reactor effluent remained at 3%, but the concentration of screenable solids was 1% (w/w) and consisted of fungal mycelia which grew in the form of pellets.

2.2. Biomass dewatering

A variety of techniques for dewatering the effluent from the bioreactor were explored. Samples of dewatered fungal biomass were analyzed for moisture content according to Mitra et al. [6], while total solids, total suspended solids, and total dissolved solids were determined using standard procedures for wastewater [9]. Screenable solids were calculated after passing the fungal biomass over a 20-mesh screen.

2.2.1. Sedimentation

Laboratory tests were conducted to evaluate dewatering by sedimentation. Settling characteristics of the suspended solids in the bioreactor effluent were assessed by determining the sludge volume index (SVI) [10]. The SVI value, reported in mL/g, is the volume (mL) occupied by 1 g of suspension after settling for 30 min. It is defined according to Eq (1), where "SV_{30}" is the volume of settled sludge after 30 min in a 1-L graduated cylinder (mL/L), and "x" is the suspended solids concentration of the bioreactor effluent (g/L).

$$\mathrm{SVI} = SV_{30}/x \tag{1}$$

Subsequent tests were performed using a laboratory centrifuge to determine the dewatering potential of the bioreactor effluent at various g-forces. Centrifuge bottles were filled with 250 ml of the reactor effluent and run at 2000–5000 g's for 10 min. Sludge volumes were calculated as the percent of settled fungal biomass after centrifuging. In addition to using a laboratory centrifuge, 200-L samples of reactor effluent were run through a pilot-scale horizontal decanter centrifuge (Centrisys, Kenosha, WI, USA). The speed of the decanter centrifuge was increased from 1000 g's to its upper limit of 3500 g's while adjusting the screw conveyer speed.

2.2.2. Filtration

The specific resistance to filtration (SRF) was tested according to Coackley and Jones [11]. This test provides insight into the dewaterability of solid-liquid slurries and has been extensively utilized for sludge dewaterability in the wastewater treatment industry. For this test, a Buchner funnel was used with Whatman 11-cm, Grade-4 disk filters having a pore size of 20–25 μm. The Buchner funnel was inserted into the top of a graduated cylinder that was modified by adding a side-arm, and a vacuum pump was attached to the side-arm to provide suction for the filtration. For each test, 200-mL samples of reactor effluent were vacuum-filtered at a negative pressure of 30 kPa. The cumulative volume of filtrate was plotted vs. time and the slope of the line was used to calculate the SRF value. After filtration, the mass of filter cake per unit volume of filtrate was determined by drying and weighing. The specific resistance to filtration ("r", in m/kg) was then calculated using Eq (2), where "ΔP" is the transmembrane pressure (Pa, or kg m^{-1} s^{-2}), "A" is the filter area (m^2), "b" is the slope of the line from the time vs. cumulative volume plot (s/m^6), "μ" is the dynamic viscosity of the filtrate (kg $m^{-1}s^{-1}$), and "w" is mass of cake per unit volume filtrate (kg/m^3).

$$r = 2\Delta P A^2 b \Big/ \mu w \tag{2}$$

Gravity screening was investigated using a 20-mesh, inclined, variable-slope screen that was 1.2 m in length (see Fig. 1). As the bioreactor effluent was discharged over the screen, most of the dewatering occurred at the top of the screen, which was inclined at 65°. The biomass accumulated at the bottom of the screen and was scraped into a hopper, while the filtrate was collected underneath the screen and discharged through a flow meter. The volume of filtrate and the mass of screened biomass were measured.

Tests with a pilot-scale belt filter were conducted by Siemens Water Technologies. An initial test involved using reactor effluent that had been passed over a 20-mesh screen to increase the screenable solids content from 1% to 15%. However, the solids content was too high and could not be fed into the belt filter. Two tests were subsequently performed using the bioreactor effluent

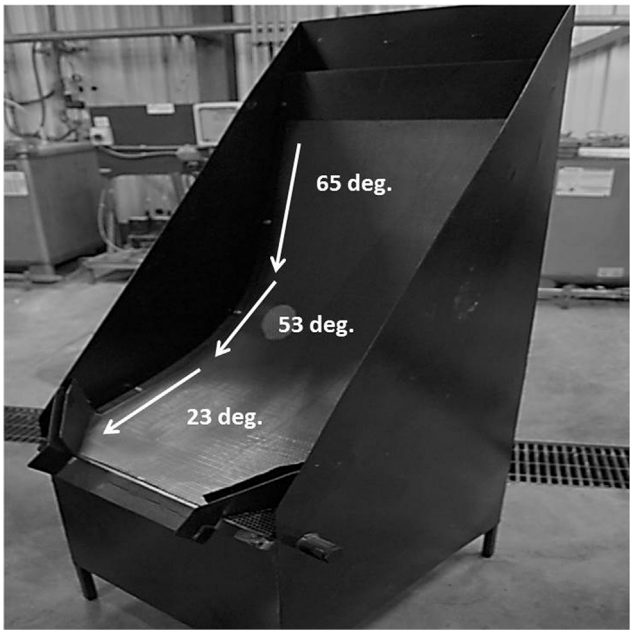

Fig. 1. Simple gravity screen used for filtration tests.