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Production of protein-rich fungal biomass in an airlift bioreactor using vinasse as substrate

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HIGHLIGHTS

- The optimal aeration rate for fungal fermentation was 1.5 vvm.
- The highest fungal biomass yield was 8.04 ($g_{biomass\ increase}/g_{initial\ biomass}$).
- The influent organic matter was reduced by 80%.
- The fungal protein contained approximately 7.8% lysine (on a total protein basis).

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ABSTRACT

The potential for large-scale production of an edible fungus, *Rhizopus oligosporus*, on a liquid residue from sugar-to-ethanol production, vinasse, was investigated. An airlift bioreactor (2.5-L working volume) was used for cultivating the fungus on 75% (v/v) vinasse with nutrient supplementation (nitrogen and phosphorus) at 37 °C and pH 5.0. Aeration rates were varied from 0.5, 1.0, 1.5 to 2.0 $volume_{air}/volume_{liquid}$/min (vvm). The fungal biomass yield depended on the aeration rate, and the highest fungal biomass obtained was 8.04 ± 0.80 ($g_{biomass\ increase}/g_{initial\ biomass}$) at 1.5 vvm. The observed reductions in organic content by 80% (as soluble chemical oxygen demand) suggest the potential of recycling treated effluent as process water for in-plant use or for land applications. The fungal biomass contained ~50% crude protein and the essential amino acids contents were comparable to commercial protein sources for aquatic feeds (fishmeal and soybean meal), with the exception of methionine and phenylalanine.

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1. Introduction

Sugar-based ethanol industries generate up to 13 gallons of a liquid residue, known as vinasse, per gallon of ethanol following the recovery of the alcohol (Pereira, 2008). Vinasse has a characteristic brownish color with a pH of 4.0–6.0 (Nitayavardhana and Khanal, 2012), a very high organic content of 100–130 g/L determined as chemical oxygen demand (COD) (Goldemberg et al., 2008), and may cause serious environmental problems upon direct disposal.

Presently, fertirrigation (fertilization + irrigation) is widely used as a vinasse disposal method as it reduces the need for mineral fertilizers and improves water retention capacities; however, this method can cause soil salification, metal and nutrient leaching into surface and groundwater, alterations in soil quality (such as nutrients imbalance and reduction of alkalinity), phytotoxicity, and odor nuisance (Smeets et al., 2008; Navarro et al., 2000). Other methods of vinasse disposal which have been integrated into modern ethanol plants are vinasse recirculation and vinasse concentration for volume reduction; but these methods are expensive, and the vinasse recirculation in particular has been known to have an adverse effect on ethanol yield (Moura and Medeiros, 2008).

A biorefinery concept, which aims at producing diverse products, including value-added co-products from biofuel residues with concomitant waste remediation, could address the concern over vinasse disposal and enhance the long-term sustainability of sugarcane-to-ethanol plants. Earlier studies showed that fungal fermentation could be applied to produce high-value fungal protein from biofuel residues, such as thin stillage (Rasmussen et al., 2007), vinasse (Nitayavardhana and Khanal, 2010), and crude glycerol (Nitayavardhana and Khanal, 2011). The fungal biomass contained high amounts of crude protein, as much as 45%, with balanced essential amino acids, and could possibly replace the use of fishmeal and soybean meal for aquatic feed applications.

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Fungal protein production on vinasse can increase the overall revenue generation from bioethanol plants as the current market values of fishmeal and soybean meal are approximately $1537 and $515 per metric ton, respectively (USDA, 2012).

The food grade fungal species, *Rhizopus microsporus* (var. *oligosporus*), was cultivated on vinasse (Nitayavardhana and Khanal, 2010) at laboratory-scale using 250-mL Erlenmeyer flasks containing 100 mL of medium. The highest fungal biomass yield obtained under optimal growth conditions (100% (v/v) vinasse, pH 5.0, 37 °C, and nutrient supplementation) was 1.12 ± 0.07 ($g_{biomass\ increase}/g_{initial\ biomass}$) with an organic removal efficiency of up to 42% measured as soluble chemical oxygen demand (SCOD). Since fungal fermentation in a bioreactor provides better control of operating conditions such as pH, mixing, and air supply, a study on the production of fungal protein in a bioreactor system is critically important for obtaining reliable data on fungal biomass yields and organic removal for process scale-up.

Growth and morphology of filamentous fungi in submerged fermentation depend on a wide range of parameters, including cultivation medium, agitation intensity, and shear stress (Cui et al., 1998; Wang et al., 2005). Fungal morphology has an effect on the rheological properties of the fermentation broth. Generally, three morphological types can be distinguished, namely free filamentous mycelia (suspended mycelia), clumps, and pellets (Wang et al., 2005). The formation of free filamentous mycelia or clumps in a bioreactor may increase medium viscosity, fungal growth around the impellers, and a reduction in mass transfer, thereby reducing fungal growth, productivity, and reactor performance (Wang et al., 2005; Liu et al., 2008). In contrast, fungal growth in the form of pellets provides good mass and oxygen transfer, minimizes adverse effects on reactor performance, and does not increase fermentation broth viscosity (Wang et al., 2005; Liu et al., 2008). Moreover, pellet formation often facilitates efficient settling and recovery of fungal biomass. Formation and stability of the pellets depend on reactor configurations that avoid extensive mechanical forces (shear stress).

An airlift bioreactor is a simple design and provides good mixing that facilitates high mass transfer with low energy consumption (Luo and Al-Dahhan, 2008; Sivasubramanian and Prasad, 2009). Mixing in the airlift bioreactor is facilitated by air bubbles supplied through diffusers at the bottom of the reactor. Therefore, shear forces are avoided and fungal pellet formation is promoted. The objectives of the current study were (i) to examine fungal biomass yields and organic removal at different aeration rates of 0.5, 1.0, 1.5, and 2.0 $volume_{air}/volume_{liquid}$/min (vvm) in an airlift bioreactor; (ii) to evaluate the feasibility of fungal protein production on commercially derived sugarcane-ethanol vinasse; and (iii) to characterize the crude protein and amino acid content of the fungal biomass with an eye on its suitability as an aquatic feed ingredient.

2. Methods

2.1. Vinasse samples

The first set of vinasse samples was prepared in the laboratory using sugarcane syrup obtained from Hawaiian Commercial & Sugar Company (HC&S) (Puunene, HI, USA). The sugarcane syrup was diluted to obtain a desired concentration for ethanol fermentation. Ethanol fermentation was carried out by *Saccharomyces cerevisiae* for 72 h. The reaction was terminated by autoclaving the fermentation broth at 121 °C for 20 min. Ethanol was recovered using a rotary evaporator operating at 50 °C and 120 rpm (Rotavapor R-215, Buchi Labortechnik AG, Flawil, Switzerland), and a vacuum pump was employed to maintain a pressure of 93 kPa (Self-Cleaning Dry Vacuum System-2025, Welch, IL, USA). The ethanol recovery process was conducted for 30 min. The second set of samples, referred to as Brazilian vinasse, was obtained from a commercial sugarcane-to-ethanol facility in Brazil. Both vinasse samples were kept at 4 °C until use. The characteristics of both vinasse samples are presented in Table 1.

2.2. Fungal culture and mycelia inoculum preparation

The food-grade fungal species, *Rhizopus microsporus* (var. *oligosporus*) was obtained from the American Type Culture Collection (ATCC # 22959, Rockville, MD, USA). The freeze-dried culture was reactivated in sterile deionized water and grown on Potato Dextrose Agar (PDA) (Difco Laboratories, Sparks, MD, USA) plates at 24 °C for 5 d. To prepare fungal spore suspension, the fungal spores were harvested and kept in a solution containing 0.1% (w/v) peptone and 0.2% (v/v) Tween 80 (Fisher Scientific, Fair Lawn, NJ, USA). Glycerol (20% v/v) was added to the harvested spore solution prior to storage at −30 °C. A spore suspension of 1 mL (4.37×10^6 spores/mL) was inoculated into a 1-L flask containing 500 mL Yeast Mold (YM) Broth (Difco Laboratories, Sparks, MD, USA) to prepare a mycelia fungal inoculum. The culture was kept at 37 °C and 150 rpm for 3 d in an incubator shaker, and was then used as a starter for fungal cultivation on vinasse.

2.3. Fungal cultivation and fungal biomass yield determination

Fungal cultivations were conducted in two 2.5-L working volume airlift bioreactors. A fermentation broth containing 2 L of sterile vinasse (75% v/v) was inoculated with 500 mL fungal mycelia inoculum. Fungal cultivation was conducted at pH 5.0 and 37 °C with nutrient supplementation (Nitayavardhana and Khanal, 2010). Nutrient supplementation was maintained at a SCOD:N:P ratio of 100:5:1. Ammonium sulfate ($(NH_4)_2SO_4$) and potassium dihydrogen phosphate (KH_2PO_4) were supplemented to the vinasse as sources of nitrogen (N) and phosphorus (P), respectively. Fungal biomass was harvested after 3 d of fermentation using the USA Standard Test Sieve with 250-μm nominal opening size

Table 1
Characteristics of vinasse obtained by fermentation of sugarcane syrup with *Saccharomyces cerevisiae* in the laboratory (vinasse) and vinasse obtained from a commercial sugarcane-to-ethanol plant located in Brazil (Brazilian vinasse).

Parameters	Vinasse	Brazilian vinasse
pH	4.25 ± 0.32	4.15 ± 0.04
Total solid (TS) (%)	2.23 ± 0.28	3.68 ± 0.00
Volatile solid (VS) (%)	1.46 ± 0.06	2.38 ± 0.00
Total suspended solid (TSS) (%)	0.76 ± 0.05	0.67 ± 0.03
Volatile suspended solid (VSS) (%)	0.46 ± 0.40	0.52 ± 0.02
Soluble chemical oxygen demand (SCOD) (g/L)	55.55 ± 4.15	37.11 ± 0.81
Total chemical oxygen demand (TCOD) (g/L)	64.48 ± 2.31	42.99 ± 1.54
Total Kjeldahl nitrogen (TKN) (mg/L)	365.86 ± 80.90	748.04 ± 32.92
Ethanol (g/L)	20.98 ± 0.00	2.33 ± 0.00
Glycerol (g/L)	6.80 ± 0.00	2.33 ± 0.00
Lactic acid (g/L)	7.43 ± 0.00	3.91 ± 0.01
Acetic acid (g/L)	0.78 ± 0.00	0.89 ± 0.00
Potassium (K) (mg/L)	1734.83 ± 194.60	4451.15 ± 139.80
Phosphorus (P) (mg/L)	28.58 ± 2.61	31.20 ± 2.83
Calcium (Ca) (mg/L)	559.83 ± 147.60	1166.50 ± 14.85
Magnesium (Mg) (mg/L)	376.70 ± 37.09	426.90 ± 17.11
Sodium (Na) (mg/L)	954.40 ± 252.91	91.11 ± 68.72
Iron (Fe) (mg/L)	1.84 ± 0.22	11.63 ± 0.02
Manganese (Mn) (mg/L)	3.12 ± 0.42	3.56 ± 0.01
Zinc (Zn) (mg/L)	0.80 ± 0.14	0.55 ± 0.29
Copper (Cu) (mg/L)	0.15 ± 0.09	0.20 ± 0.08
Boron (B) (mg/L)	0.48 ± 0.14	0.26 ± 0.16

Mean value ± standard deviation (sample size (n) = 6).

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