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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.

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

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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 × 10⁶ 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 ((NH₄)₂SO₄) and potassium dihydrogen phosphate (KH₂PO₄) 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- μ m 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
pН	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	55.55 ± 4.15	37.11 ± 0.81
(SCOD) (g/L)		
Total chemical oxygen demand	64.48 ± 2.31	42.99 ± 1.54
(TCOD) (g/L)		
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 \pm standard deviation (sample size (n) = 6).

(ASTM E-11, No. 60, Fisher Scientific, Fair Lawn, NJ, USA), washed with tap water, and dried at 70 °C for 24 h. The dry weights of fungal biomass samples were determined to calculate the fungal biomass yields defined as $g_{biomass increase}/g_{initial biomass}$.

2.4. Bioreactor configuration

A 3.5-L internal loop airlift bioreactor (with 2.5-L working volume) of cylindrical geometry with an inner diameter of 14 cm and a height of 40 cm, was fabricated. The bioreactor and draft tube were made of clear acrylic plastic with a thickness of 0.5 cm. The draft tube had an inner diameter of 10 cm and a height of 16 cm. The top and bottom clearances were 17 cm and 3.5 cm, respectively. The ratio of the downcomer and riser cross-sectional areas (A_d/A_r) was about 1. Air was supplied through porous air diffusers at the bottom of the riser section. Inlet air was passed through a polytetrafluoroethylene (PTFE) membrane filter (0.1 µm pore size) (Whatman, Florham Park, NJ, USA). The pH was controlled at 5.0 using 2% sulfuric acid (H₂SO₄) and/or 2% sodium hydroxide (NaOH) solutions. The temperature was maintained at 37 °C.

2.5. Analytical methods

Total solids (TSs), total suspended solids (TSSs), volatile solids (VSs), and volatile suspended solids (VSSs) in vinasse samples were determined following procedures outlined in the Standard Methods (APHA/AWWA/WEF, 2005). Ethanol, glycerol, lactic acid, and acetic acid concentrations were analyzed using a Waters high performance liquid chromatography (HPLC) system (Waters model 2695) (Waters, Milford, MA, USA). The HPLC was equipped with a Waters model 410 differential refractometer. A Rezex ROA organic acid column (Phenomenex, Inc., Torrance, CA, USA) was used with 5 mM sulfuric acid as the mobile phase at a flow rate of 0.6 mL/min, an injection volume of 40 µl, and a column temperature of 65 °C. During fungal cultivation, liquid samples were withdrawn on a 24-h basis, filtered through a cellulose nitrate membrane filter (0.45 µm pore size) (Whatman, Florham Park, NI. USA) and analyzed for SCOD, nitrogen, phosphorus, and potassium using vial kits from HACH (HACH Company, Loveland, CO, USA).

SCOD was determined according to US Environmental Protection Agency (USEPA) reactor digestion method # 10212 (HACH Company, Loveland, CO, USA). The Nessler method was used to obtain the nitrogen concentration as total Kjeldahl nitrogen (TKN) (# 8075; HACH Company, Loveland, CO, USA). Phosphorus, as PO₄³⁻, was determined using the ascorbic acid method (# 10210; HACH Company, Loveland, CO, USA). Potassium (as K) was measured by the tetraphenylborate method (# 8049 HACH Company, Loveland, CO, USA). The tests were carried out by Agricultural Diagnostic Service Center (ADSC) at the University of Hawai'i at Mānoa (Honolulu, HI, USA).

2.6. Specific oxygen uptake rate (SOUR) determination

In order to quantify undesired microbial contamination during fermentation, a specific oxygen uptake rate (SOUR) was determined as outlined in Standard Methods (APHA/AWWA/WEF, 2005). The experiment was conducted over 3 d in a bioreactor at an aeration rate of 0.5 vvm without fungal inoculation. The SOUR was calculated using the following equation:

 $\begin{aligned} \text{SOUR}(\text{mg O}_2/\text{g VSS} - h) = & [\text{Oxygen consumption rate}(\text{mg O}_2/\text{L} - \text{min}) \\ & \times & (60 \text{ min}/h)] / [\text{biomass concentration}(\text{g VSS}/\text{L})] \end{aligned}$

2.7. Fungal biomass characterization

Fungal biomass from YM broth and vinasse cultures were freeze-dried, and analyzed for crude protein, crude lipid, and amino acid contents at the Department of Aquatic Feeds and Nutrition at the Oceanic Institute (Waimanalo, HI, USA). The trytophan content was analyzed by means of spectrophotometry using the acid ninhydrin method (Pintér-Szakács and Molnán-Perl, 1990).

2.8. Statistical analysis

Experiments and analyses were conducted in triplicates. The results of fungal biomass yield and SCOD removal were statistically analyzed by Predictive Analytics Software (PASW) Statistics version 18 (SPSS Inc., Chicago, IL, USA). The statistical differences were determined using a one-way analysis of variance (ANOVA) followed by a Duncan's comparison at a 95% confidence level. All values are mean values with standard deviation.

3. Results and discussion

3.1. Effect of aeration rate on fungal biomass yield, SCOD removal, and fungal biomass characteristics

3.1.1. Fungal biomass yield at different aeration rates

Among the aeration rates examined, 0.5, 1.0, and 1.5 vvm resulted in the formation of spherical pellets (~0.5-0.8 cm in diameter) within 1 d of inoculation. During cultivation, the pellets grew larger in size with a more pronounced extension zone (hairy surface structure). Physical networking between some fungal pellets was observed. By day 2, a mixture of spherical pellets and chunks of fungal biomass settled at the bottom of the reactor. Clumps of fungal biomass attached at the annulus section between the draft tube and the external column were observed during fungal fermentation at aeration rates of 1.0 and 1.5 vvm. The clumps of fungal biomass attached at the downcomer section when operating the reactor at the aeration rate of 1.5 vvm were bigger than those at 1.0 vvm. The presence of big fungal biomass chunks at the bottom of the reactor at a low aeration rate (e.g., 0.5 vvm) suggested that the aeration rate was insufficient to maintain circulatory movement of the biomass within the bioreactor. At aeration rates of 1.0 and 1.5 vvm, the fungal biomass chunks at the downcomer section were occasionally circulated in the reactor in spite of their larger size. The fungal biomass yields at different aeration rates are presented with the superscript letters representing the correlation based on statistical analyses. A maximum fungal biomass yield of 8.04 ± 0.80^c g_{biomass} increase/g_{initial} biomass $(3.79 \pm 0.31 \text{ g/L})$ was obtained at an aeration rate of 1.5 vvm. Yields of $3.73\pm0.47^{a}~g_{biomass~increase}/g_{initial~biomass}~(1.82\pm0.12~g/L)$ and $5.86 \pm 0.40^{b} g_{biomass}$ increase/ $g_{initial}$ biomass $(3.03 \pm 0.20 \text{ g/L})$ were obtained at aeration rates of 0.5 and 1.0 vvm, respectively.

Increasing superficial gas velocities results in improving the volumetric mass transfer coefficient (*kLa*), gas holdup, and liquid circulation velocity (Sivasubramanian and Prasad, 2009). Fig. 1 shows the dissolved oxygen (DO) concentrations during fungal cultivation at various aeration rates. Only at an aeration rate of 1.5 vvm, it was possible to maintain DO concentrations of 2.0 mg/L or higher, which is an essential concentration required in the suspended growth biological wastewater treatment process (Tchobanoglous et al., 2002). The fungal biomass yield decreased to $5.14 \pm 0.40^{\text{b}}$ g_{biomass} increase/g_{initial} biomass (2.38 ± 0.07 g/L) at an aeration rate of 2.0 vvm presumably because the intensive turbulent flow in the liquid phase in the riser area caused a shear force that impacted the form of fungal growth. Instead of spherical fungal pellets, thread-like mycelia were

observed that could have increased the apparent viscosity of fermentation broth.

Because the opening between the annulus section of the draft tube and the reactor wall in the reactor was 2.0 cm, the agglomerated fungal biomass was not able to easily flow downward to generate a recirculation pattern. To address this issue, future bioreactor designs should consider optimizing the A_d/A_r ratio such that it is greater than 1. In a series of experiments without the draft tube in the airlift reactor (known as a bubble column reactor) to determine fungal growth, it was found that the bubble column reactor resulted in elimination of the formation of fungal biomass chunks and a fungal biomass yield increase by as much as 28% $(11.18 \pm 0.13 \text{ g}_{\text{biomass increase}}/g_{\text{initial biomass}} \text{ or } 5.16 \pm 0.06 \text{ g/L}) \text{ in com-}$ parison to the yields in an airlift reactor at an aeration rate of 1.5 vvm. Other than superficial gas velocity and reactor geometry, sparger structure is also a critical factor because it affects the gas holdup, liquid circulation velocity, and the mass transfer coefficient in an airlift reactor system. Thus, future research is needed to examine how these parameters affect the hydrodynamics and mass transfer in the bioreactor.

3.1.2. Organics removal at different aeration rates

The SCOD concentrations in the fungal bioreactor at various aeration rates are presented in Fig. 2. During the first day of fermentation, SCOD was reduced more rapidly at a higher aeration rate; however, after the second day of fermentation, SCOD concentrations were nearly the same at all aeration rates. Although fungal cultivations at different aeration rates provided different fungal biomass yields, SCOD reductions at the end of fungal fermentation were not significantly different. Fungal cultivation at 1.5 vvm, the aeration rate that yielded the highest fungal biomass production, resulted in an SCOD removal of $77.60 \pm 3.73\%$ and the effluent SCOD was 7.43 ± 0.52 g/L. Lower aeration rates resulted in lower oxygen solubility in the fermentation broth. Consequently, fungal growth was negatively impacted, leading to lower fungal biomass vields at 0.5 and 1.0 vvm than at 1.5 vvm. However, at a high aeration rate (2.0 vvm), a lower fungal biomass yield with a significantly lower SCOD removal was observed. Part of the SCOD removed was not directly associated with fungal growth and was likely consumed by other microbes in the bioreactor. Therefore, experiments were conducted in an airlift reactor at an aeration rate of 0.5 vvm without fungal inoculation to investigate SCOD removal by other microbes present during fermentation. The influent SCOD was reduced by nearly 49% when conducting fermentation without fungal inoculation and the effluent SCOD was 19.24 ± 0.26 g/L. Since the SCOD was reduced regardless of fungal inoculation, specific oxygen uptake rate (SOUR) and the SCOD reduction of the fermentation medium without fungal inoculation were investigated to determine the microbial (non-fungal) growth in the bioreactor (Fig. 3). The SCOD reduction along with SOUR suggested that the contamination had occurred during the fermentation process. The rapid SCOD reduction observed on the first day of cultivation



Fig. 1. Dissolved oxygen (DO) concentration in fungal cultivation on vinasse at various aeration rates (0.5, 1.0, and 1.5 vvm) (sample size (n) = 3).

was in close agreement with the highest SOUR at the same period. Low SOUR of about 3.8 mg O_2/g VSS-h was observed at the end of cultivation, suggesting the exhaustion of readily degradable organic matter in the growth medium. SCOD removal with fungal inoculum on Day 2 to the end of cultivation was higher with respect to SCOD removals of the sample without fungal inoculum while lower SOUR was obtained from fermentation without fungal inoculum. This finding suggests that the SCOD reduction during that period was predominantly from the fungal growth.

3.1.3. Fungal biomass characteristics

The crude protein contents in fungal biomass obtained at different aeration rates did not differ significantly at 95% confidence level. Under optimal aeration (1.5 vvm), the fungus contained approximately $49.70 \pm 0.52\%$ of crude protein (dry weight basis). In contrast, the crude lipid content in the fungal biomass was dependent on the aeration rate. The highest crude lipid content was $3.44 \pm 0.10\%$ (dry weight basis), and was obtained at an aeration rate of 1.5 vvm. Crude lipid contents of the fungal biomass cultivated at 0.5 and 1.0 vvm were 1.81 ± 0.04 and $2.10 \pm 0.04\%$ (based on dry basis), respectively. The improvement in lipid content at higher aeration rates is likely attributable to oil accumulation due to nitrogen limiting conditions. Fungal growth was more rapid at higher aeration rates and resulted in faster consumption of nutrients. Lipid accumulation in microbial cells occurs when an element, especially nitrogen in the medium becomes limiting while the carbon source is present in excess (Beopoulos et al., 2009). However, the low crude lipid content of fungal biomass cultivated at $2.0 \text{ vvm} (1.34 \pm 0.07\% (based on dry basis)) \text{ was in close agreement}$ with the low fungal biomass yield indicating that the fungal growth did not result in nitrogen limitations in the fermentation medium.

3.2. Fungal cultivation on sugarcane-ethanol-derived vinasse

The characteristics of the vinasse from the commercial facility are summarized in Table 1. Brazilian vinasse was high in solids. TKN, potassium, calcium and iron, but low in organic content (expressed as COD) and sodium in comparison to the laboratoryprepared vinasse. The high amounts of sodium in laboratoryprepared vinasse were due to the use of NaOH solution (2%) for controlling the pH of the fermentation medium. Since the Brazilian vinasse has significantly lower organic content compared to laboratory-prepared vinasse, the Brazilian vinasse was used without dilution for fungal fermentation. The experiment was conducted under an optimal aeration rate of 1.5 vvm. The result showed that fungal biomass yields on Brazilian vinasse and laboratory-prepared vinasse were not significantly different at a 95% confidence level and the fungal cultivation in Brazilian vinasse resulted in a fungal biomass yield of 8.52 ± 2.44 gbiomass increase/ginitial biomass $(4.91 \pm 1.46 \text{ g/L})$. This finding showed that the results obtained from laboratory prepared vinasse can be applied to vinasse obtained from a commercial sugarcane-ethanol plant. The elimination of vinasse dilution could possibly save costs for fungal protein production by reducing clean water use and wastewater remediation.

The fungal biomass derived from laboratory-prepared vinasse had crude protein ($49.70 \pm 0.52\%$) and lipid ($3.44 \pm 0.10\%$) contents similar to those obtained from Brazilian vinasse ($47.62 \pm 0.32\%$ crude protein and $3.70 \pm 0.06\%$ crude lipid). As shown in Fig. 4, the overall essential amino acids of fungal biomass from both laboratory-prepared vinasse and Brazilian vinasse were comparable to fishmeal and soybean meal, the commercial protein sources for aquatic feed (Lim and Akiyama, 1992; Miles and Chapman, 2009) except for methionine and phenylalanine. Importantly, fungal lysine content (based on protein basis) was slightly higher than the commercial protein sources. Lysine is the most critical amino acid required in aquatic feed as it is often the most limiting amino



Fig. 2. Soluble chemical oxygen demand (SCOD) concentrations for fungal fermentation on vinasse under various aeration rates (0.5, 1.0, 1.5, and 2.0 vvm) (sample size (*n*) = 3).



Fig. 3. Soluble chemical oxygen demand (SCOD) concentrations and specific oxygen uptake rate (SOUR) for fermentation of vinasse without fungal inoculation under aeration rate of 0.5 vvm (sample size (n) = 3).

acid in feed ingredients (Cheng et al., 2003; Craig and Helfrich, 2009). Wilson (1989) also indicated that lysine is critical for optimal growth of fish. As previously observed, fungi inherently contain low sulfur-containing amino acids (Gregory et al., 1976), a low methionine content for both vinasse samples were observed. The high tryptophan contents in fungal protein is beneficial to aquatic feed applications since it reduces the aggressive behavior and controls juvenile cannibalism in fish (Winberg et al., 2001; Hseu et al., 2003).

Improvements in protein and amino acid contents in fungal biomass would be possible by elimination of the washing step during fungal biomass harvest. The yeast cells in vinasse could possibly improve protein and amino acid profiles of the fungal biomass. Co-feeding of the fungal protein with commercial protein might be necessary for maintaining adequate amino acid requirements for feed applications.



Fig. 4. Comparison of essential amino acid content (% protein basis) of fishmeal, soybean meal, and fungal biomass cultivated on laboratory-prepared vinasse and Brazilian vinasse under an optimal aeration rate (1.5 vvm). ¹Modified from NRC (1993). ²Experimental analysis (sample size (*n*) = 1).

4. Conclusions

Fungal protein production from sugar-ethanol residue, vinasse, in an airlift bioreactor was determined to be feasible with a high fungal biomass yield $(8.04 \pm 0.80 \text{ g}_{\text{biomass}} \text{ increase}/\text{g}_{\text{initial}} \text{ biomass}$ $(3.79 \pm 0.31 \text{ g/L})$ under optimal aeration rates (1.5 vvm). Fungal fermentation technology is promising for sugarcane-to-ethanol industries as illustrated by fungal biomass yields on commercial sugarcane-ethanol derived vinasse. The fungal protein contained high percentages of lysine, which is a limiting amino acid in aquatic feeds. Co-feeding the fungal protein with commercial protein would address the low methionine and phenylalanine content in the fungal biomass and reduce the cost of protein ingredient for feeds.

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