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Demo-scale production of protein-rich fungal biomass from potato protein liquor for use as innovative food and feed products

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ABSTRACT

Innovative food and feed products have recently attracted the attention of both producers and consumers. Filamentous fungi are important biomass producers with their high protein contents. In this study, fungal biomass production from edible potato protein liquor (PPL), generated during starch production processes, was investigated through different fungal strains (*Rhizopus oryzae*, *R. oligosporus*, *R. delemar*, *Aspergillus oryzae* and *Neurospora intermedia*). The effects of PPL concentration, incubation time, initial pH, and cultivation conditions (in shake flaks and different scale reactors) were examined to determine the amount of biomass and its crude protein level. It was determined that the fungal biomass produced by *R. delemar* in industrial scale contained 53% crude protein. For this strain, the amino acid and fatty acid profiles as well as metals (iron, manganese, copper, and zinc) of the produced biomass were also investigated to assess possible use as a food or feed source. The *R. delemar* fungal biomass can be a promising raw material for feed and food production, for example, considering its protein and fatty acid profiles with 41% essential amino acids and 33% polyunsaturated fatty acids.

1. Introduction

Innovative food and feed products are one of the rapidly rising trends of the food industry. At the same time, providing high-quality food to the rapidly growing world population is another challenge. Protein-rich food products, produced from algae, fungi (filamentous fungi and yeast), and bacteria, have attracted the attention of consumers as more healthy and sustainable alternatives to red meat and are also promising ingredients in animal feed (Amorim et al., 2021; Gharibzahedi et al., 2018; Ritala et al., 2017). Mycoprotein is known as generally recognized as safe (GRAS) and its products (tofu, tempeh, miso, oncom, red rice, hamanatto, and shoyu) have traditionally been produced by using various kind of filamentous fungi e.g. *Rhizopus oryzae*, *Aspergillus oryzae*, *Neurospora intermedia* and *Monascus purpureus* in Far East countries (Denny et al., 2008; Filho, Andersson, et al., 2019; Moore & Chiu, 2001; Sar, Ferreira, & Taherzadeh, 2020).

Filamentous fungi have been used for biomass production on a variety of food industry side-streams (fish industry wastewaters, vinasse, olive oil mill wastewater, thin stillage, etc. (Ferreira et al., 2015; Karimi et al., 2019; Sar, Ferreira, & Taherzadeh, 2020; Sar, Ozturk, et al., 2020; Sar et al., 2021)) as they are potential producers of amylase, invertase, cellulase, xylanase, lipase or protease (Ferreira et al., 2016). Filamentous fungi have also been used to improve the protein content of various food by-products such as potato-based starch water and bread waste (Filho et al., 2017b, 2019b; Svensson et al., 2021). In America and Europe, potatoes are important substrate for fungal cultivation, as they form a large amount of by-products generated following processes to produce starch, potato chips and French fries (Arapoglou et al., 2010; Bradshaw & Ramsay, 2009). After the starch production process from potatoes, the remaining liquid is heated at 110 °C using steam and then converted into concentrated potato protein liquor (PPL), which consists of about 40% solids (Filho et al., 2017a, 2017b). Although PPL, which has high chemical oxygen demand (COD) and biological oxygen demand (BOD) levels, is of food origin, it should not be discharged without waste treatment. On the other hand, although it is recommended to be used as a cattle feed additive or as a fertilizer in agricultural activities, it cannot be considered good for any of these purposes due to its low protein concentration and the formation of bad odour during long-term storage (Filho, Zamani, & Taherzadeh, 2017). Instead of these applications, potato peel can be converted into value-added products such as lactic acid (Cunha et al., 2018), paramylon (β-1,3-glucan) (Šantek et al., 2010), and ethanol (Arapoglou et al., 2010) through microbial

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Table 1
Compositional contents of potato protein liquor (PPL).

Components	
pH	5.10 ± 0.04
Total COD (g/L)	288 ± 0.00
Total Solid (g/kg)	371.97 ± 2.34
Dissolved Solid (g/kg)	276.59 ± 3.52
Volatile Solid (g/kg)	254.64 ± 0.05
Ash (g/kg)	116.71 ± 0.49
Nitrogen (g/kg)	15.03 ± 2.84
Protein (g/kg)	93.94 ± 17.72
Glucose (g/L)	8.48 ± 0.58
Other Sugars (g/L)	6.10 ± 0.44
Glycerol (g/L)	0.36 ± 0.01
Lactic acid (g/L)	48.89 ± 1.90
Acetic acid (g/L)	4.69 ± 0.14
Ethanol (g/L)	0.35 ± 0.07

fermentation (Pathak et al., 2018). Additionally, Filho, Zamani, and Taherzadeh (2017) investigated fungal biomass production from PPL through *R. oryzae* and determined the optimum aeration rates to obtain the high protein concentration.

This study was aimed to compare fungal biomass production of *R. oryzae* to other fungal species, namely *R. microsporus* var. *oligosporus*, *R. oryzae* var. *delemar*, *Aspergillus oryzae* var. *oryzae* and *Neurospora intermedia*. Then, the effects of different parameters such as initial pH, incubation time and fed batch were studied in both shake flasks and bioreactors (4.5 L capacity) to determine the crude protein levels of biomass of fungal strain, which gave the highest amount of biomass and protein. The second part of the study aimed at producing biomass from this fungal strain in a demo-scale bioreactor (1200 L capacity) and to determine its potential for use as food or animal feed based on e.g., amino acid and fatty acid compositions.

2. Materials and methods

2.1. Substrate

The potato protein liquor (PPL) was obtained in fresh form from Lyckeby Starch AB (Kristianstad, Sweden) and was stored at 4 °C for three months throughout the study, while it can be stored for up to one year since it is concentrated. Prior to use, the substrate diluted with water was sterilized by autoclaving at 121 °C for 20 min. The composition of PPL was given in Table 1.

2.2. Fungal cultivation

Five edible filamentous fungal strains (*Rhizopus oryzae* CCUG 28958, *R. microsporus* var. *oligosporus* CBS 112586, *R. oryzae* var. *delemar* CBS 145940, *Aspergillus oryzae* var. *oryzae* CBS 819.72, *Neurospora intermedia* CBS 131.92) were used. The fungal strains were maintained on Potato Dextrose Agar (PDA; pH 5.6, containing 4 g/L potato extract (Sigma-Aldrich, Buchs, Switzerland), 20 g/L glucose (Fisher Scientific, Loughborough, UK) and 15 g/L agar (Sigma, Buchs, Switzerland)). For fungal inoculation, pre-grown plates were treated with 20 mL of distilled water, and then 100 μL of spore suspension were inoculated into fresh PDA using a L-shape sterile disposable plastic spreader. The fungal strains were cultured at 30 °C for three days and then stored at 4 °C until use for a maximum of one month.

2.2.1. Cultivation in shake flasks

First, the fungal strains were cultivated in 250 mL baffled Erlenmeyer flasks containing 100 mL of diluted PPL (1:10). Each flask was inoculated with 2 mL of spore suspension (1.14×10^5 spores/mL for *R. oryzae*, 8.70×10^5 spores/mL for *R. oligosporus*, 1.24×10^6 spores/mL for *R. delemar*, 1.69×10^7 spores/mL for *A. oryzae*, 6.30×10^5 spores/mL for N. *intermedia*), and the cultivation was carried out in a water bath shaking incubator at 35 °C and 125 rpm for 48 h. Then, the factors of initial pH (varied within 3.0–6.0 adjusted with 2 M H_2SO_4 (Sigma-Aldrich, Darmstadt, Germany)) and different dilutions of PPL (1:5, 1:10, and fed batch) were studied in both shake flasks and bubble column reactors. All cultivations were carried out in duplicate.

2.2.2. Cultivation in bench scale bubble column reactors (4.5 L capacity)

The fungal cultivation in PPL at different dilutions was performed in 4.5 L glass bubble column bioreactors (Belach Bioteknik, Stockholm, Sweden). The bioreactors filled with 3 L of PPL (diluted to 1:10 and 1:5 with distilled water and initial pH adjusted to 4.50 with 2 M H_2SO_4 (Sigma-Aldrich, Darmstadt, Germany)) were sterilized at 121 °C for 20 min. Then, 20 mL/L of spore suspension of *R. delemar* (1.24×10^6 spores/mL) were inoculated, and the cultivations were set at 35 °C and using an aeration rate of 0.5 vvm (volume of air per volume of medium per minute) (Filho, Zamani, & Taherzadeh, 2017). All cultivations were carried out in duplicate.

2.2.3. Cultivation in pilot scale bioreactor (26 L capacity)

The fungal cultivation in PPL was carried out in 26 L total volume of bioreactor (2 m high × 15 cm diameter, Bioengineering AG, Wald, Switzerland). First, the pilot-scale reactor was in-situ sterilized at 130 °C for 20 min, using a steam injector. Then, 20 L of sterilized PPL (diluted to 1:10 with tap water and initial pH adjusted to 4.50 with 2 M H_2SO_4 (Sigma-Aldrich, Darmstadt, Germany)) and 1 L overnight grown fungi (*R. delemar*) in Erlenmeyer flasks were pumped into the reactor. Cultivation was set at 35 °C and using an aeration rate of 0.5 vvm for 48 h (Ferreira et al., 2015). In addition, fungal cultivation produced in 26 L reactor for 24 h was used as a pre-culture for demo-scale reactor.

2.2.4. Cultivation in demo scale reactor (1200 L capacity)

The PPL was also used for cultivation in demo-scale reactor (1200 L capacity, 4 m high × 0.65 m diameter, Process- & Industriteknik AB, Kristianstad, Sweden). First, the reactor was in-situ sterilized at 122 °C for 20 min, using a steam injector. Then, the PPL (diluted to 1:16 with tap water) was transferred to reactor and then heat-sterilized at 120 °C for 20 min using a steam injector. After cooling the reactor, the pre-grown fungi from 26 L reactor were transferred to demo-scale reactor. Cultivation was set at 35 °C and using an aeration rate of 0.5 vvm for 48 h. Every 12 h, 250 mL of sample was collected to determine the levels of biomass, pH and COD. The pH during the cultivation was manually adjusted to be between 4.5 and 5.0 using concentrated sulfuric acid (98%).

2.3. Analytical methods

2.3.1. Substrate characterization

Total solids (TS) and dissolved solids (DS) of substrate (PPL) were determined according to Sluiter et al. (2008). The ash content was determined using a muffle furnace (Gallenkamp, London, UK) at 550 °C for 4 h. The total COD levels were analysed using a COD kit (Nanocolor® COD 15000, Düren, Germany). Glucose (6.25–50 g/L; Fisher Scientific, Loughborough, UK), lactic acid (1.25–10 g/L; Sigma-Aldrich, Buchs, Switzerland), acetic acid (1.25–10 g/L; Sigma-Aldrich, Buchs, Switzerland) and ethanol levels (1.25–10 g/L; Sigma-Aldrich, Buchs, Switzerland) of substrate were analysed using high-performance liquid chromatography (HPLC) (Waters 2695, Waters Corporation, Milford, USA) equipped with an analytical ion exchange column based on hydrogen ions (Aminex HPX-87H, BioRad Inc., California, USA) (Sar, Ferreira, & Taherzadeh, 2020).

2.3.2. Protein analysis

The harvested biomass was dried at 70 °C to constant weight. The crude protein contents of substrate and oven dried biomass were analysed according to the Kjeldahl method. After the Kjeldahl analysis, a factor of 6.25 was used to determine the crude protein content (Mæhre

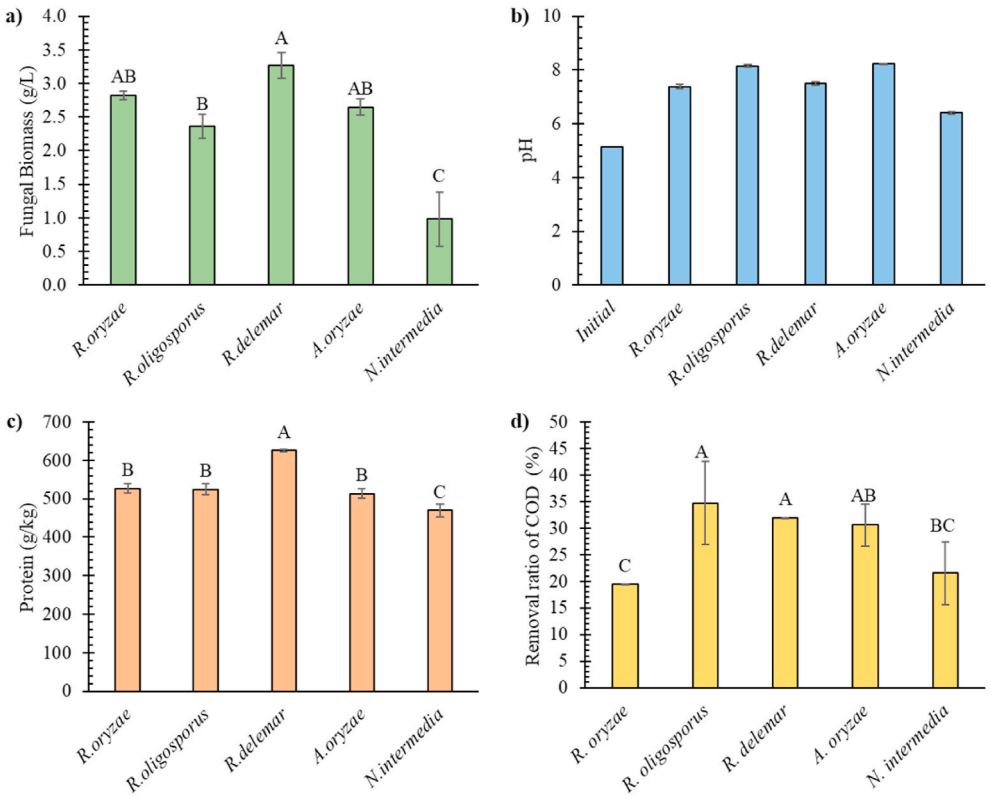

Fig. 1. Fungal biomass production from PPL through different filamentous fungal strains; a) fungal biomass levels (g/L), b) pH values of medium and cultivated broths, c) crude protein levels of produced biomass (g/kg oven dried biomass), d) COD reduction levels (%) after fungal cultivation. All experiments were performed in duplicate, and error bars indicate sample standard deviations. Different letters indicate significant ($p < 0.05$) differences.

et al., 2018).

2.3.3. Amino acid composition

Acid hydrolysis of ~30 mg freeze-dried samples was done by adding 4 mL of 6 M HCl and incubation at 110 °C for 20 h. The solution was filtered (0.45 μm PES, Fisherbrand) and diluted 20 times with 0.2 M HAc before the amino acid composition was analysed with LC-MS as described by Harrysson et al. (2018). Quantification was done using Amino Acid Standard H (Thermo Scientific, Rockford, Illinois, USA) as external standard. Results are expressed as the anhydro-amino acid values.

2.3.4. Total lipids and fatty acid composition

Total lipids were determined gravimetrically after chloroform/methanol extraction starting with ~0.2 g freeze-dried sample. Extracted lipids were dissolved in toluene and heptadecanoic acid (C17:0, Larodan AB, Solna, Sweden) was added as internal standard for quantification. The composition of fatty acids was then determined according to the method described by Cavonius et al. (2014). Fatty acids were *trans*--esterified by addition of 10% acetylchloride in methanol and incubation at 70 °C for 2 h. Reaction was stopped my addition of Milli-Q water and fatty acid methylesters (FAME) were extracted using petroleum ether: diethyl ether (4:1). After evaporation, FAME were dissolved in iso-octane prior injection onto a GC (Agilent Technologies Inc., California, USA) equipped with a VF-VAX column (30 cm × 0.25 mm x 0.25 μm) and interfaced with an MS detector (Agilent Technologies Inc., California, USA). Identification of fatty acids were done using GLC Reference Standard 463 (Nu-Check Prep Inc., Minnesota, USA).

2.3.5. Minerals

Minerals were analysed in ~0.35 g freeze-dried sample using atomic absorption spectroscopy (Agilent Technologies Inc., California, USA) after microwave digestion (Milestone SRL, Sorisole, Italy) as described in Gmoser et al. (2020). Quantification was made using standard curves of iron (Sigma-Aldrich, Buchs, Switzerland), manganese (Reagecon Diagnostics Ltd, Clare, Ireland), zinc (Fisher Scientific, Loughborough, UK) and copper (Fisher Scientific, Loughborough, UK).

2.3.6. Tocopherols

Tocopherols was measured according to Larsson et al. (2007) and Gmoser et al. (2020). For chromatographic separations, a Phenomenex C18, Luna 100 A, 150 × 3 mm micron column was used. The mobile phase consisted of 98% methanol (HPLC, Laboratory-Scan LtD, Dublin, Ireland) in water, and the flow rate was 0.5 mL/min. Standards used was γ-tocopherol (Sigma Aldrich, Saint Louis, USA), β-tocopherol (Sigma Aldrich, Saint Louis, USA).

2.4. Statistical analysis

The software MINITAB® (Minitab Inc., State College, PA, USA) was used to statistically analysis of the obtained results with ANOVA (analysis of variance) tables using general linear models. Significant differences were considered at p-value < 0.05 within a 95% confidence interval.

3. Results and discussion

In this work, the fungal biomass production from PPL was compared through different fungal strains and different initial pH levels of the substrate. Then, the effects of PPL concentration and incubation time on the amount of fungal biomass and its crude protein content were evaluated in both shake flasks and bubble column reactors (4.5 L). In addition, for the most promising strain, fungal biomass production capacities were investigated using two large-scale reactors (26 L and 1200

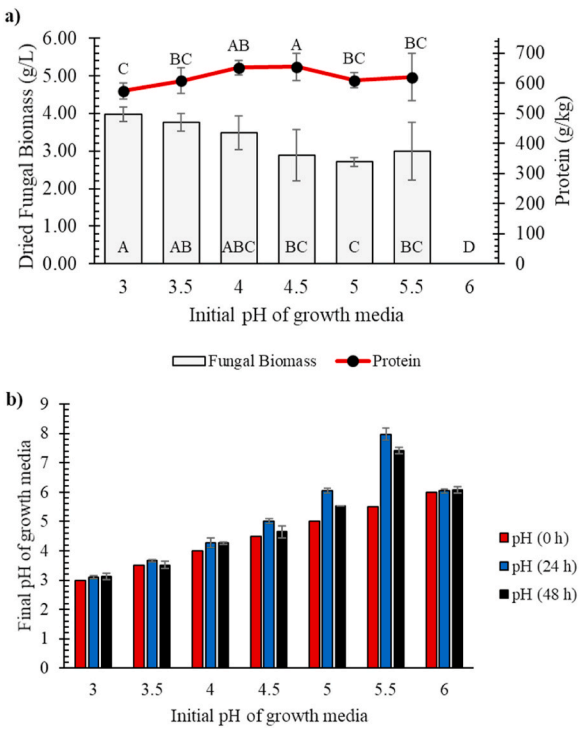

Fig. 2. Fungal biomass production from PPL through *R. delemar* at varying initial pH levels (ranging from 3.0 to 6.0); a) fungal biomass levels (g/L) and their crude protein levels (g/kg oven dried biomass), b) pH values of growth media and cultivated broths after 24 and 48 h. All experiments were performed in duplicate, and error bars indicate sample standard deviations. Different letters indicate significant ($p < 0.05$) differences.

L). The amino acid and fatty acid profiles as well as selected metals of the biomass produced in the demo-scale reactor were also investigated to determine its potential for use as food or animal feed.

3.1. Characterization of PPL

The chemical composition of PPL is presented in Table 1. Although the PPL content varied according to the thermal process, it contained high COD levels. The COD level of the PPL used in this work was found to be 288 g/L and the glucose content was determined as 8.48 g/L by the HPLC analysis in this work. These numbers can be compared with a previous publication that reported PPL obtained from the same company contained 126 g/L total carbohydrates, 72 g/L of which was glucose, by total carbohydrate analysis (Filho, Zamani, & Taherzadeh, 2017).

3.2. Fungal cultivation

3.2.1. Effects of fungal strains

In the first approach, five different fungal strains were cultivated in diluted PPL (1:10) for 48 h in Erlenmeyer flasks to compare their biomass production and protein levels (Fig. 1). A PPL:water ratio of 1:10 was chosen because Filho, Zamani, and Taherzadeh (2017) examined the effects of different dilutions of PPL (1:1.5 to 1:39 (PPL:water) dilutions to give different COD levels) and found high fungal biomass yield at 1:10 PPL dilution. Dilution of PPL was required for its use as a substrate for microbial cultivation due to its high COD levels and concentrated form, as in the use of concentrated vinasse for fungal cultivation (Karimi et al., 2019). Since the concentrated medium will affect the water activity, it will also affect the fungal growth (Han & Nout, 2000). On the other hand, lactic acid is potential inhibitor of cell growth, enzymatic hydrolysis and microbial activity (Huang et al., 2003). Concentrated PPL contains high levels of lactic acid (Table 1). Although *Rhizopus* species can produce lactic acid (Millati et al., 2005), the addition of a neutralizing agent or dilution are necessary to avoid potential inhibition of lactic acid.

The highest fungal biomass (based on dry weight) after incubations was obtained with *Rhizopus delemar* (3.27 g/L; $p < 0.05$), followed by *R. oryzae* (2.82 g/L), *Aspergillus oryzae* (2.65 g/L), *R. oligosporus* (2.36 g/L), and *Neurospora intermedia* (0.98 g/L) (Fig. 1a). It was seen that the final pH values of culture broths of *R. oligosporus* and *A. oryzae* exceeded pH 8.0 (Fig. 1b). These high pH-values may occur due to accumulation of ammonia in the medium, which can affect the growth of fungi (Ali et al., 2017; Filho, Zamani, & Taherzadeh, 2017). Similar to the amount of biomass, the highest crude protein content, 62.5% (w/w), was observed for the *R. delemar* ($p < 0.05$) (Fig. 1c). The levels of protein may differ depending on the substrate and fungal strains. Parchami et al. (2021) determined that the highest fungal biomass and protein content were obtained with cultivations of *A. oryzae* (6.1 g/L biomass containing 40.5% protein) and *N. intermedia* (3.2 g/L biomass containing 59.6% protein), respectively. These differences in levels of protein and fungal biomass were also seen with different kinds of substrates such as vinasse and olive oil mill wastewater (Karimi et al., 2018, 2019; Sar, Ozturk, et al., 2020).

Glucose, ethanol and acetic acid were consumed by all fungal species, whereas lactic acid was not consumed during the cultivation (Fig. S1). The COD reduction ratios in PPL reached up to 35% by cultivation of *R. oligosporus*, however there was no significant difference

Table 2
Fungal biomass production (g/L and g/L undiluted PPL) from PPL in Erlenmeyer flasks and Bubble column reactors (4.5 L capacity) and their crude protein levels (g/kg oven dried biomass).

	PPL Concentration	Incubation Time	Final pH	Biomass (g/L)	Biomass (g/L)/Undiluted PPL	Crude protein[a] (g/kg oven dried biomass)
Flask	1:10	24	4.94 ± 0.04	3.24 ± 0.33	32.40	614.39 ± 20.76
		48	5.03 ± 0.01	1.74 ± 0.55	17.42	621.34 ± 38.75
Reactor	1:10	24	4.99 ± 0.27	4.18 ± 0.30	41.76	511.18 ± 27.19
		48	5.83 ± 0.07	6.14 ± 0.12	61.38	463.70 ± 0.13
Flask	1:5	48	4.91 ± 0.08	9.58 ± 4.06	47.90	467.38 ± 41.49
		72	5.17 ± 0.31	9.56 ± 2.04	47.82	454.63 ± 8.06
Reactor	1:5	48	4.96 ± 0.28	13.21 ± 4.91	66.07	439.23 ± 57.05
		72	4.96 ± 0.08	8.94 ± 2.20	44.70	458.47 ± 53.18
Flask	FedBatch (1:3.77)	48	4.50 ± 0.07	6.39 ± 0.49	24.08	484.75 ± 51.64
	FedBatch (1:2.71)	72	4.59 ± 0.00	9.57 ± 1.10	25.93	425.73 ± 5.62
Reactor	FedBatch (1:3.77)	48	4.92 ± 0.04	10.02 ± 2.24	37.79	388.20 ± 75.79
	FedBatch (1:2.71)	72	4.80 ± 0.04	12.37 ± 3.97	33.53	354.87 ± 44.51

[a] A nitrogen-to-protein-conversion factor of 6.25 was used.

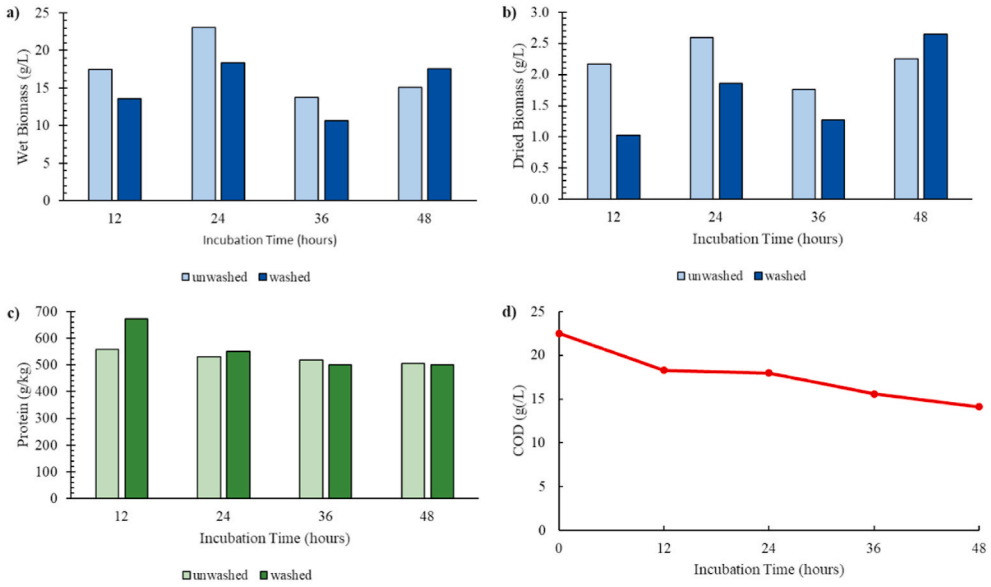

Fig. 3. Fungal biomass production from PPL through *R. delemar* in the demo-scale reactor; a) wet fungal biomass levels (g/L), b) oven dried fungal biomass levels (g/L), c) crude protein levels of oven dried biomass (g/kg), d) COD levels (%) of growth medium during the fungal cultivation. Unwashed biomass refers to just pressed, washed biomass refers to washed with tap water and then pressed.

compared to *R. delemar* for COD reduction (32%, $p = 0.25$, Fig. 1d). *R. delemar* was chosen for further investigations as both the amount of fungal biomass and the crude protein level of this strain was found to be comparatively higher than the other fungal strains.

3.2.2. Effects of pH

R. delemar was cultivated in diluted PPL (1:10) at varying initial pH levels (between 3.0 and 6.0, Fig. 2a) to determine the biomass and crude protein levels. The amount of biomass production tended to increase under acidic conditions, while the productivity was decreased in the pH-range 4.5–5.5, and even more, no biomass production was seen at pH 6.0. On the other hand, the highest crude protein content with 65% (w/v) was observed at pH 4.0–4.5 ($p < 0.05$). The final pH levels also increased when the initial pH was higher than 4.5 (Fig. 2b). Therefore, fungal cultivation in reactors was carried out in diluted PPL at pH 4.5 since higher amount of biomass and protein levels were obtained at these conditions.

3.2.3. Fungal cultivation in flask vs. bubble-column reactors

Fungal biomass production and their crude protein levels were investigated when using PPLs at both 1:10 and 1:5 concentrations in both Erlenmeyer flasks (1-Liter capacity) and bubble-column reactors (4.5-Liter capacities). In addition, the effect of fed-batch on fungal cultivation was also examined (Table 2). Although the amount of biomass increased as the PPL concentration increased in the Erlenmeyer flasks, there were significant decreases in the crude protein levels, however, it was determined that the incubation time did not have a significant effect on neither biomass nor crude protein levels (at the same concentrations). In the bubble-column reactors, the highest fungal biomass was determined to be 61–66 g/L undiluted PPL after 48 h. Extending the incubation time to 72 h caused a decrease in biomass production (44.7 g/L undiluted PPL). In such aerated conditions, the fungus may grow faster, resulting in faster decomposition of the biomass obtained. As a result of rapid fungal growth, reductions in crude protein levels of biomass produced under aerated conditions can be observed due to late biomass harvest (Sar, Ferreira, & Taherzadeh, 2020). Additionally, the aeration rate also affects the protein levels of harvested biomass (Ferreira et al., 2012).

Similar to Erlenmeyer flask cultivations, glucose, ethanol and acetic acid were consumed when the fungus was cultivated in bubble column reactors. Additionally, lactic acid partially consumed by 24%–31% (Figs. S2a and b). In addition, the consumption of metabolites was found to be relatively slow at higher PPL concentrations. It was determined that approximately 30%–40% of the COD content was removed after fungal biomass production under both cultivation conditions. A similar range of total COD reduction of 35%–44% was reported by Sar, Ozturk, et al. (2020) after 72 h of cultivation of *Aspergillus oryzae* in olive oil mill wastewater. Similarly, total COD reduction by up to 58% was reported during fungal cultivation in vinasse (Karimi et al., 2019; Nair & Taherzadeh, 2016; Nitayavardhana & Khanal, 2010) and in fish industry wastewaters (Sar et al., 2020a, 2021). In a previous study, higher COD reduction (50%) was reached by the addition of invertase enzyme to improve the sucrose consumption although the same amount of biomass production (65 g/L undiluted PPL) and same protein (50%) content were obtained in PPL medium with *R. oryzae* (Filho, Zamani, & Taherzadeh, 2017). However, high amounts of enzymes will be required to scale up the fungal cultivation to large-scale reactors, increasing the cost of bioproducts (Liu et al., 2016). Therefore, enzyme addition was not applied in this study, as large-scale reactors (26–1200 L capacities) were performed for fungal cultivation.

To improve the COD consumption concomitantly with biomass production from PPL, a fed-batch approach was conducted. Fed-batch cultivation was initiated in diluted PPL (1:10) medium, and undiluted PPL was added every 12 h for 48–72 h. Although the amount of final biomass (10.0–12.3 g/L) gave similar results with submerged cultivations (8.9–13.2 g/L) in bubble column reactors, biomass/undiluted PPL yields (33–37 g/L) and crude protein levels (35%–38%) were also relatively lower. Lactic acid accumulation was observed during the fed-batch cultivation, and it reached 30 g/L after 72 h of incubation (Fig. S2c).

3.2.4. Fungal cultivation in pilot and demo scale reactors

Initially, fungal cultivation was performed in bubble column reactor with a capacity of 26 L (pilot-scale), and this fungal biomass was used as a pre-culture for the demo (industrial)-scale reactor (1200 L capacity). After 48 h of cultivation in the pilot-scale reactor, 32.8 g dry biomass/L undiluted PPL containing 41% crude protein was produced in diluted PPL (1:10). Then, the cultivation in demo-scale reactor gave 37.6 g dry biomass/L undiluted PPL containing 53% crude protein after 48 h incubation (Fig. 3). The crude protein content of harvested material

Table 3
The amino acid and fatty acid profiles of produced fungal biomass from PPL through *R. delemar* in demo-scale reactor. Results are expressed as mean ± standard deviation of three analytical replicates.

Crude protein content and amino acid profile				Lipid content and fatty acid profile		
Components	mg/g dried fungal biomass	%	Soybean meal [a]	Components	mg/g dried biomass	%
Crude Protein	529.8 ± 6.6	–	49.6	Total Lipid	66.9 ± 3.3	–
Glycine	13.12 ± 0.58	4.85	4.77	C10:0	ND	0.04
Alanine	16.50 ± 2.91	6.10	4.81	C12:0	ND	0.13
Serine	13.87 ± 2.87	5.13	4.81	C14:0	0.27 ± 0.00	0.78
Proline	10.16 ± 1.20	3.76	5.31	C15:0	0.37 ± 0.01	1.09
Valine [a]	15.44 ± 1.51	5.71	5.55	C16:0	7.22 ± 0.23	21.10
Threonine [a]	14.83 ± 2.43	5.49	4.24	C16:1n7	0.31 ± 0.01	0.90
Isoleucine [a]	13.39 ± 1.33	4.95	5.33	C18:0	2.20 ± 0.06	6.44
Leucine [a]	21.35 ± 1.41	7.90	8.85	C18:1n9	9.81 ± 0.30	28.68
Aspartic acid	34.23 ± 5.45	12.66	12.70	C18:1n7	1.08 ± 0.04	3.16
Lysine [a]	23.11 ± 2.90	8.55	7.19	C18:2n6	8.35 ± 0.27	24.43
Glutamic acid	47.49 ± 7.53	17.57	16.65	C18:3n6	1.31 ± 0.08	3.84
Methionine [a]	3.75 ± 1.31	1.39	1.59	C18:3n3	1.44 ± 0.07	4.21
Histidine [a]	7.32 ± 0.76	2.71	3.10	C20:0	0.33 ± 0.01	0.96
Phenylalanine [a]	12.43 ± 1.11	4.60	5.58	C20:1n9	ND	0.74
Arginine	12.55 ± 2.58	4.64	7.89	C20:2n6	0.15 ± 0.02	0.44
Tyrosine	10.75 ± 2.33	3.98	N/A	C22:0	0.24 ± 0.01	0.71
				C22:1n9	0.02 ± 0.01	0.07
				C24:0	0.80 ± 0.01	2.35
Total AA [b]	270.28	100.00		Total FA [c]	34.22 ± 0.79	100.00
Total essential AA [b]	111.62	41.30		SFA [c]	11.48 ± 0.28	33.56
TEAA/TAA [b]	0.41	–		MUFA [c]	11.48 ± 0.36	33.54
				PUFA [c]	11.26 ± 0.40	32.90

N/A: not assessed, ND: not detected.
Data were modified from Mjoun et al. (2010)
[a] Essential amino acids.
[b] AA: amino acids, TEAA: total essential amino acids, and TAA: total amino acids.
[c] FA: Fatty acids, SFA: Saturated fatty acids, MUFA: monounsaturated fatty acid, and PUFA: polyunsaturated fatty acids.

reached by only pressing the biomass was 67%, while the crude protein content was stable after 24 h and there was no significant effect from washing. Contrary to previous cultivations, it was determined that the lactic acid concentration decreased by 74% in the demo scale reactor (Fig. S3). Consumption of metabolites, including lactic acid, may be thought to promote fungal growth. Overall, up to 12 kg of wet biomass (up to 85% water content) was harvested at the end of cultivation. This significant amount of biomass with a high crude protein content could subsequently be evaluated as food or animal feed raw material. The crude protein content of the biomass found in the current study was similar to commercially available fungi-based products such as Quorn™ containing up to 50.7% protein (Gmoser et al., 2020; Wiebe, 2002) and biomass from thin stillage containing 48%–53% protein (Ferreira et al., 2015). In addition, biomass from PPL can be an alternative to biomass obtained in large-scale reactors using bread waste and oat flour as substrates. The crude protein levels of these substrates were increased from 11% to 37% when *R. delemar* and *A. oryzae* were cultivated, respectively (Rousta et al., 2021; Svensson et al., 2021). On the other hand, typical animal feeds used for fish, shrimp, chicken, and pig contain on average 32%–45%, 25%–42%, 25%, and 13% total protein, respectively (Bjørkli, 2002, p. 41; Miles & Chapman, 2006). The fungal biomass produced in this study might thus be a replacement for soybean meal (40%–50% crude protein) which has been frequently used as protein source in animal feed (Banaszkiewicz, 2011; Liu, 1997).

3.3. Nutritional properties of fungal biomass

The percentage of amino acids based on dry fungal biomass (*R. delemar*) was compared with soybean meal as a commercial product (Table 3). Essential amino acids for human represented 41% of the analysed amino acids, although the highest amount of individual amino acids in the fungal biomass consisted of aspartic acid and glutamic acid, which are non-essential. These both amino acids are defined as excitatory neurotransmitters and glutamic acid plays an important role in the 'malate-aspartate shuttle' required for glucose metabolism (Randall et al., 2002; Tang et al., 2020). Although glutamate is not accepted as a nutrient needed in diets for humans and animals, it has been determined that glutamate-supplemented diet improved the growth performance of pigs and protected their intestinal health (Hou & Wu, 2018).

Among the essential amino acids, the fungal biomass contained high lysine and leucine while less methionine and histidine. Previous studies determined that the percentages of lysine and leucine in fungal biomass produced from vinasse were found to be lower than fishmeal (Karimi et al., 2019; Nitayavardhana & Khanal, 2010), whereas, in this study both amino acids were determined to be higher than in fishmeal, which agreed with the results of Karimi et al. (2018). The lower content of the sulphur-containing amino acid methionine could be related to the lack of sulphur in PPL.

The high concentration of nitrogen-containing compounds other than protein contributes to an overestimation of the crude protein content of the biomass when 6.25 is used as the correction factor in a Kjeldahl analysis (Karimi et al., 2019; Mæhre et al., 2018). The total amino acid level (27%) was different from the crude protein level (53%) could be due to in the presences of glucosamine derivatives in cell wall and nucleic acids (Ferreira et al., 2012; Zamani et al., 2007). Other nitrogenous components (amines, urea, ammonia, nitrates, nitrites, phospholipids, nitrogenous glycosides) also affect the crude protein content (Mariotti et al., 2008).

The percentage of fatty acids based on freeze dried fungal biomass is also presented in Table 3. Total fat content of the freeze-dried biomass was 6.69%. The fatty acids present at highest relative levels in fungal biomass were palmitic acid (C16:0, 21.1%), oleic acid (C18:1n9, 28.7%) and linoleic acid (C18:2n6, 24.4%). The proportion of saturated fatty acids (SFAs), monounsaturated fatty acid (MUFAs) and polyunsaturated fatty acids (PUFAs) were found to be similar. Linoleic acid, and the percentage of PUFA, were found in higher levels when using substrates such as thin stillage, bread waste, and vinasse to cultivate *N. intermedia*, *R. delemar*, and *R. oryzae*, respectively (Ferreira et al., 2015; Karimi et al., 2019; Svensson et al., 2021). However, in our study, a significant amount of α-linolenic acid (C18:3n3) was found at 1.44 mg/g dry

Table 4
Vitamin E and metal (iron, manganese, copper, and zinc) levels (mg/kg dried biomass) of produced fungal biomass from PPL through *R. delemar* in demo-scale reactor. Results are expressed as mean ± standard deviation of three analytical replicates.

Components	mg/kg
Vitamin E (Beta/gamma)	1.9 ± 0.7
Iron (Fe)	547 ± 10
Manganese (Mn)	32 ± 1
Copper (Cu)	47 ± 1
Zinc (Zn)	606 ± 13

biomass. α-Linolenic acid is also a precursor to the longer chained omega-3 fatty acids such as EPA and DHA, and is an essential fatty acid for energy supply, regulation of enzymatic activity and regulation of gene expression (Guillou et al., 2010). However, palmitic acid, an SFA, when used in feeds could improve milk yield, milk fat concentration and the efficiency of milk production (Rico et al., 2016; Sears et al., 2020). Overall, the fatty acid and amino acid composition suggested that the fungal biomass can be used both as an animal feed or food source.

Vitamin E (tocopherol) is known as an antioxidant essential for human health that can be naturally found in vegetables (Grilo et al., 2014). The two major forms of vitamin E, α-tocopherol and γ-tocopherol exhibit anti-inflammatory activity *in vitro* and *in vivo* (Reiter et al., 2007). Potatoes can also contain vitamin E (0.7 μg/g fresh weight), which consists of 90% α-tocopherol and 10% β- and γ-forms (DellaPenna & Mène-Saffrané, 2011). However, the biomass obtained in this study was found to contain only γ- and β-tocopherol (Table 4) which implies that no α-tocopherol remains after PPL and subsequent fungal biomass production.

Since potatoes are reported to contain high levels of micronutrients such as iron, zinc, manganese and copper (Hajslova et al., 2005), these essential micronutrients (Lal et al., 2020) were also analysed in the obtained biomass (Table 4). It was found that the biomass is rich in zinc (606 mg/kg) and iron (547 mg/kg), and also contains significant amounts of copper (47 mg/kg) and manganese (32 mg/kg).

3.4. Biorefinery of fungal biomass from PPL

A schematic diagram of submerged cultivation using PPL as the carbon source for fungal biomass production is shown in Fig. 4. After the fungal cultivation, 3.1 kg of dry biomass containing 53% crude protein and 6.7% lipid can be obtained when 100 kg of PPL is used (Table 5). It is thought that fungal biomass could be a potential food/feed source and an alternative to soybean meal with its protein amount, amino acid profile, and fatty acid profile. The wastewater released after the cultivation contained a low amount of total solids (13.5 ± 0.2 g/kg) and COD (14.1 ± 0.2 g/L) levels. Despite the consumption of monosaccharides, lactic acid (0.86 ± 0.04 g/L) that remains unconsumed plays a major role in determining the COD value. In addition to some components of PPL like lactic acid, microbial metabolites, ammonium, and unspecified starch/oligosaccharides are also responsible for the COD value (Anastasi et al., 2012; Filho, Brancoli, et al., 2017). This wastewater can be evaluated to produce a different microbial metabolite such as through an activated sludge process.

4. Conclusion and future perspectives

Filamentous fungi are potential producers of protein-rich biomass. In this work, fungal biomass containing 53% crude protein was obtained from potato protein liquor through *Rhizopus delemar* in a demo-scale reactor. The fungal biomass obtained from this edible substrate can be an alternative source of food and feed given the amino acid and fatty acid compositions. There were also relatively high levels of zinc and

Table 5
Mass balance during the fungal cultivation in demo-scale airlift reactor.

Initial substrate	Final Product
• 100 kg PPL • ~1230 kg water	⁂ 20 kg wet fungal biomass (~3.1 kg dried fungal biomass) ※ 53% ± 1% crude protein (dried biomass) ※ 6.7% ± 0.3% lipid (dried biomass) ⁂ ~1300 kg wastewater ※ pH - 7.50 ※ COD (g/L) - 14.1 ± 0.2 ※ Total solid (g/kg) - 13.5 ± 0.2 ※ Dissolved solid (g/kg) - 10.5 ± 0.6 ※ Volatile solid (g/kg) - 7.2 ± 0.7 ※ Ash (g/kg) - 6.3 ± 0.1 ※ NH_4 (g/L) - 0.41 ± 0.03 ※ Lactic acid (g/L) - 0.86 ± 0.04 ※ Sugars (g/L) - 0.10 ± 0.02

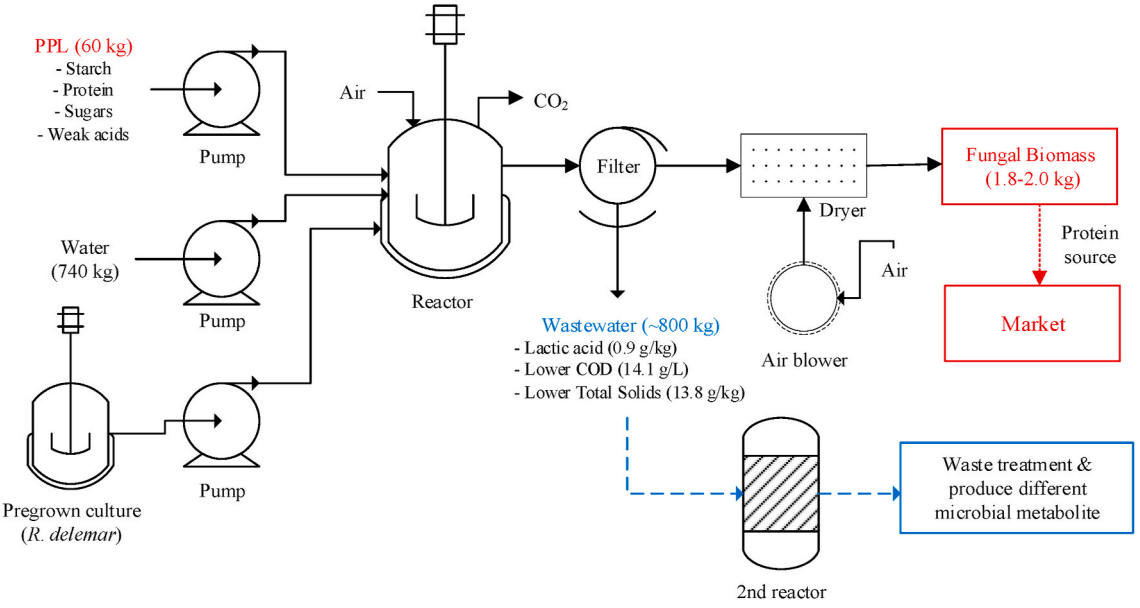

Fig. 4. Schematic illustration of fungal biomass production using PPL through *R. delemar* in demo-scale airlift reactor.

iron. However, additional studies are needed to determine whether this food product is safe for humans and animals and how it comply with established regulations (e.g., the European Commission's Food Safety policy or Food and Agriculture Organization of the United Nations). In addition, it will be useful to carry out industrial integration and techno-economic analysis studies to produce bio-protein from food industry by-products.

Author contribution

Conceptualisation: Taner Sar and Mohammad J. Taherzadeh; Methodology: Taner Sar, Karin Larsson and Rikard Fristedt; Formal analysis and investigation: Taner Sar, Karin Larsson and Rikard Fristedt; Writing - original draft preparation: Taner Sar; Writing - review and editing: Taner Sar, Karin Larsson, Rikard Fristedt, Ingrid Undeland and Mohammad J. Taherzadeh; Supervision: Ingrid Undeland and Mohammad J. Taherzadeh, Projection administration: Mohammad J. Taherzadeh.

Declaration of competing interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2022.101637.

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