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An investigation into the ability of three fungi and one yeast to grow and capture nutrients in cheese whey

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ABSTRACT

This study addresses the environmental and waste disposal challenges dairy processing poses, namely its most nutrient-rich byproduct – whey. Here, the potential of four fungi/yeast species (*Geotrichum candidum*, *Penicillium corylophilum*, *Penicillium restrictum*, *Pleurotus ostreatus*) was investigated for nutrient reclamation of whey and production of value-added biomass. Conducted in closed and open batch systems, the study evaluates oxygen uptake rates (OUR), biomass yields, and nutrient removal efficiencies at varying mixing speeds. Results show that pellet formation, pH changes, and biomass nutrient content were species-specific. Among the species, *P. corylophilum* shows promise with high biomass yields (13.21 g DW L^{-1}, 2.0 g/g COD removed) and nutrient removal efficiencies of 26 % COD, 35 % N-tot, and 41 % P-tot. While further optimisation is needed, *P. corylophilum* shows potential for bioremediation and improved circularity in the dairy industry. Efficacy could be enhanced through bioreactor optimisation, such as employing a continuously fed bubble reactor.

1. Introduction

Dairy processing generates substantial amounts of wastewater, with up to 10 L produced per litre of raw material, incurring treatment costs of up to 3374 USD/m^3 (Slavov, 2017; Wang and Serventi, 2019). Whey, a predominant dairy byproduct (95 % of milk is turned into whey during cheese making), is produced at a rate of 8–9 L Kg^{-1} of cheese (Slavov, 2017; Estikomah et al., 2023), and is the most polluting dairy waste product due to its high nutrient and fat content (Slavov, 2017; Wang and Serventi, 2019).

Due to whey's high nutrient content, large dairy producers with their vast resources, can leverage whey into profitable whey-based products (Marwaha and Kennedy, 1988; Zotta et al., 2020). However, small dairy producers largely discard whey and globally, half of the whey generated goes untreated (Slavov, 2017). This can lead to the eutrophication of waterways, while land disposal results in indirect eutrophication, malodorous gases, and the release of toxic nitrate ions (Shete and Shinkar, 2013). Whey is rich in lactose, proteins, fats, and salts, posing challenges for treatment due to its high chemical oxygen demand (COD), biological oxygen demand (BOD), suspended solids (SS), and fat, oil, and grease (FOG) levels. For instance, the high levels of lactose present in whey promote filamentous growth and acidification, which disrupts the aerobic processes of activated sludge, while high FOG can cause flotation and wash out of activated sludge from biological wastewater treatment basins or clog screens and filters. A more sustainable solution is urgently needed for the disposal of whey by small dairy producers.

The same characteristics, which make whey challenging to treat, highly biodegradable organic carbon, proteins, and FOG, offer potential as energy sources and building blocks for microbial biomass growth (Slavov, 2017). Fungi and yeast emerge as promising candidates for whey remediation because, 1) they need large amounts of organic carbon for growth (Sankaran et al., 2010), 2) they can reduce inorganic nutrients and FOG (Rosa et al., 2009; Purchase, 2016; Liu et al., 2017) and 3) produce value-added biomass (El-Shora and Metwally, 2008; Vamvakaki et al., 2010; Zotta et al., 2020) for biofuel production, microalgal flocculation, biofertilisation, feed and for production of lipids, proteins, biochemicals, vitamins, and enzymes (Sankaran et al., 2010; Purchase, 2016; Bansfield et al., 2022; Moura et al., 2023).

While bacteria and yeasts have been explored for whey bioconversion, limited studies focus on fungi (Zotta et al., 2020), and only a few investigations have examined fungal growth in unprocessed cheese whey without additional nutrient sources (Bhak et al., 2005; El-Shora

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and Metwally, 2008; Papadaki et al., 2022). In a previous study, growth screens and metabolic activity tests were conducted on ten fungal and yeast species cultivated in five different food waste streams (FWS). Its findings revealed that cheese whey supported the most growth and highest metabolic rates (Bansfield et al., 2023). This follow-up study explores how well whey sustains the growth of selected species and their potential for whey valorisation, in terms of nutrient removal and biomass content, into value-added biomass. The species studied - *Geotrichum candidum*, *Penicillium corylophilum*, *Penicillium restrictum*, and *Pleurotus ostreatus* - were selected based on their high metabolic activity (Bansfield et al., 2023), and their potential to produce high-value biomass (Yadav et al., 2019). The study explored optimization strategies, such as mixing speed and batch cultivation types, on bioremediation in terms of nutrient removal and biomass yield to maximise growth and nutrient recovery from unprocessed cheese whey.

2. Materials and methods

2.1. Species maintenance and whey collection

The yeast *Geotrichum candidum* (NRRL Y-552) and three filamentous fungi —*Penicillium corylophilum* (NRRL 802), *Penicillium restrictum* (NRRL 3381), and *Pleurotus ostreatus* (NRRL 3526) — were sourced from the USDA-ARS Culture Collection (NRRL). All species were maintained according to the procedures set out in Bansfield et al. (2023).

Cheese whey was obtained directly from the cheese manufacturing process immediately following separation, stored at −20 °C, and autoclaved (120 °C, 30 min) before use. Autoclaved cheese whey was characterised by concentrations of COD (as per SFS 5504:1988) at 82.51 g O_2 L^{-1}, total organic carbon (TOC) (measured as per SFS-EN 1484:1997 on a Shimadzu TOC-L/TNH-L autosampler, Shimadzu corp., Japan) at 25.51 g L^{-1}, total nitrogen (N-tot) (Shimadzu TOC-L/TNH-L autosampler, Shimadzu corp., Japan) at 1.37 g L^{-1}, total phosphorus (P-tot) (samples were autoclaved with $K_2S_2O_8$ and H_2SO to release all phosphates which were then measured as per SFS ISO 15923-1:2018 on a Skalar, BluVision Analyzer) at 0.49 g L^{-1}, total suspended solids (TSS) (SFS-EN 872:2005) at 15.42 g L^{-1}, heavy metal (As, Hg, Cd, Cr, Cu, Pb, Ni) concentrations <0.6 mg Kg^{-1} (at Metropolilab, Finland as per SFS-EN ISO 17294-2) and a pH of 6.5 (Fisherbrand pH Fix 2.0–9.0 strips).

2.2. Oxygen uptake experiments (closed batch)

Cheese whey was inoculated using cultures of each species in the exponential phase. These were prepared by adding 2 mL of maintenance culture to 18 mL of Yeast Malt Broth (YMB) (3.0 g of Yeast extract (Thermo Fisher), 3.0 g Malt extract (Thermo Fisher), 10.0 g Glucose (Sigma-Aldrich) and 5.0 g Peptone (Fluka analytical) in sterile MQ for a total volume of 1 L). Each culture was cultivated until its species-specific exponential phase and used to inoculate 80 mL of cheese whey for a final concentration of 0.003 g DW L^{-1} in a manometric respirometer apparatus (closed batch system), following the procedure described in Bansfield et al. (2023). Cultures were incubated at 23 °C with agitation at three different mixing speeds (100, 150, and 200 rpm) with continuous oxygen uptake measurements (56 min intervals) until the Oxitop system's measuring limit (~2100 mg O_2 L^{-1}) was reached. Culture pH was measured with Fisherbrand pH Fix 2.0–9.0 pH strips, 1) before inoculation with fungi and, 2) at the end of all tests. For each mixing speed, the experiments were conducted in quadruplicate for each species.

2.3. Total biomass measurements and nutrient analysis

After all closed-batch experimental runs, replicates were pooled, and the biomass was separated from the spent whey by centrifugation at 3000 rpm for 3 min. The freeze-dried biomass was subsequently weighed, and samples at the mixing speed with the highest total biomass yields (g DW L^{-1}, g/g COD removed) were analysed for carbon (C), nitrogen (N), phosphorus (P), and heavy metal (arsenic, mercury, cadmium, chromium, copper, lead, and nickel) content either in-house (Unicube trace, Elementar) or at Metropolilab, Finland (SFS-EN ISO 17294-2). The spent whey from the pooled replicates was analysed for COD, TOC, N-tot and P-tot and nutrient removal efficiencies for each species were calculated.

2.4. Open batch cultivation

Oxygen availability is likely a limiting factor in the metabolism and growth of fungi in the closed system (Bansfield et al., 2023). To investigate the effect of oxygen availability, the species/mixing speed combination with the highest biomass yield and nutrient removal rates in the closed batch system was tested in an open batch system. Aseptic techniques were employed to inoculate 1 L bottles with the same inoculum concentration and whey volume as in the oxygen uptake measurements. This open batch set-up pumped air into the top of the bottle above the culture while venting CO_2. The air was pumped through a sterile 0.2 μm filter and sterile MQ in gas-washing bottles to maintain sterility and prevent sample desiccation (Fig. 1). The replicates from this test were pooled, and total biomass measurements and nutrient analysis were carried out as described in Section 2.3 above.

2.5. Statistical analysis

Linear regression was employed to ascertain the mean maximum oxygen uptake rate (OUR) for each species across various rotational speeds (rpm). Differences between OURs at different mixing speeds were assessed using one-way ANOVA with Tukey's post hoc tests (homogeneous variances) or Welch's one-way ANOVA with Games-Howell post hoc tests (heterogeneous variances). Normality of distribution was tested with Shapiro–Wilk's test, and homogeneity of variances was assessed using Levene's test. All statistical analyses were performed using SPSS version 29.

3. Results

3.1. Pellet formation and pH changes

Filamentous fungi exhibited pellet formation across all agitation speeds, with noticeable variations in pellet size and abundance. For *P. corylophilum* and *P. ostreatus*, an inverse relationship was observed between pellet size and mixing speed, with pellet size diminishing as

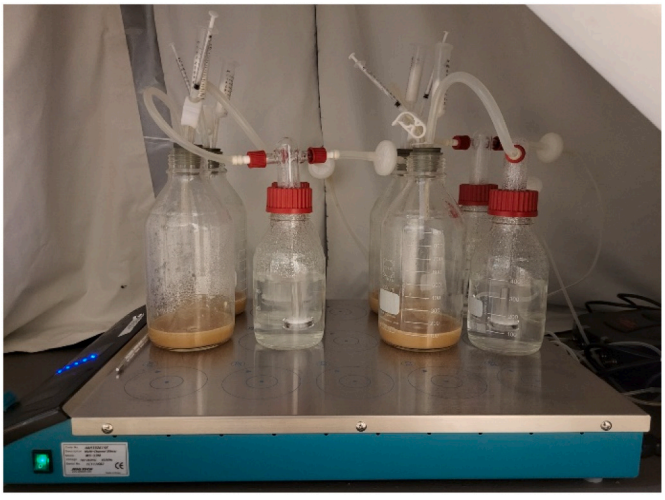

Fig. 1. Open batch cultivation experimental set-up.

mixing speed increased. Conversely, *P. restrictum* displayed an increase in pellet size with escalating mixing speeds (Table 1).

P. corylophilum and *P. restrictum* produced several pellets at each rpm (>10 pellets), while *P. ostreatus* produced no or few pellets (<10 pellets). In the open batch system, *P. corylophilum* produced several smaller pellets compared to the closed system.

The initial pH of whey (pH 6.5) remained largely unchanged for both *G. candidum* and *P. restrictum* across all mixing speeds (Table 2). However, *P. corylophilum* showed a marked increase in pH, while *P. ostreatus* displayed a notable reduction at all mixing speeds. The pH in the open-batch cultures of *P. corylophilum* was the same as that of the closed-batch cultures.

3.2. Oxygen uptake rates

OURs increased with rising mixing speed, peaking at 200 rpm for all species (Fig. 2a).

Significant increases in OUR for *G. candidum* were observed when the mixing speed was adjusted from 100 to 150 rpm and from 100 to 200 rpm ($F(2,9) = 41.32$, $p < 0.001$; Tukey post hoc, $p > 0.001$ in both cases). For *P. corylophilum* and *P. ostreatus,* the increase was significant only between the lowest and highest mixing speeds ($F(2,9) = 5.547$, $p = 0.027$; Tukey post hoc, $p = 0.017$, ($F(2,8) = 6.57$, $p = 0.020$; Tukey post hoc, $p = 0.017$, respectively). On the contrary, mixing speed did not affect the OUR of *P. restrictum*. Naturally, OURs increase most significantly between 100 rpm and 200 rpm, followed by 100 rpm and 150 rpm (Fig. 2a). The increases in metabolic activity were generally <20 % except for *G. candidum* and *P. ostreatus* which had OUR percentage increases of 20–37 % for some or all increases in rpm (Table 3). Thus, OUR showed a weak positive trend with increasing mixing speed.

3.3. Biomass yields

In the closed batch system, biomass yields ranged from 4.19 g DW L^{-1} and 0.20 g/g COD removed (*G. candidum*) to 10.84 g DW L^{-1} and 0.55 g/g COD removed (*P. restrictum* and *P. corylophilum* respectively) (Fig. 2b & Table 4). The highest biomass yields generally occurred at 200 rpm, except for *P. restrictum*, which peaked at 150 rpm. Overall, increases in biomass yields were most pronounced between 100 rpm and 200 rpm (Fig. 2b). A weak trend of increasing OUR with increasing mixing speed was observed (Fig. 2a).

P. corylophilum at 200 rpm displayed the highest biomass yield and the highest nutrient reduction efficiencies (Fig. 2b & 5), leading to its selection for oxygen optimisation studies in the open batch cultivation system. In the open batch system at 200 rpm, *P. corylophilum* exhibited increased biomass yields from 10.81 to 13.21 g DW L^{-1} and 0.55 to 0.83 g/g COD removed compared to the closed system (Fig. 3 & Table 4).

3.4. Biomass composition

In the closed batch system, all species had similar C content (~46 %), while the N and P varied among the species (Fig. 3 a-c).

G. candidum had the highest N content, *P. corylophilum* exhibited the highest C and P content, while *P. ostreatus* had the lowest N and P concentrations. For all species, As, Cd, Ni, Hg, and Pb concentrations were low (<0.5 mg Kg^{-1}), with slightly higher Cu concentrations (2.6 mg Kg^{-1} for *G. candidum*, 1.0 mg Kg^{-1} for *P. corylophilum*, 0.9 mg Kg^{-1} for *P. ostreatus*, and 1.6 mg Kg^{-1} for *P. restrictum*). The biomass composition of *P. corylophilum* in the open batch system closely mirrored that observed in the closed system, with a marginal reduction in overall nutrient content (<5.5 % decrease in open batch cultures), characterized by high C and low N and P levels (Fig. 3 a-c). As, Cd, Ni, Hg, and Pb concentrations remained low (<0.5 mg Kg^{-1}), with slightly higher Cu concentration at 0.6 mg Kg^{-1}.

Table 2
Modal pH of all species in cheese whey at different mixing speeds at the end of experimental runs.

Cultivation system	Species	100 rpm	150 rpm	200 rpm
Closed batch	*G. candidum*	6.5	6.5	6.0
	P. corylophilum	8.5	8.0	8.0
	P. ostreatus	3.5	3.5	3.5
	P. restrictum	7.5	7.5	7.5
Open batch	*P. corylophilum*	NA	NA	8.5

*NA (not applicable).

3.5. Nutrient removal efficiency

The nutrient removal efficiencies achieved through the cultivation of fungi and yeast typically did not exceed 30 % (COD: 16–26 %, TOC: 6–11 %, N-tot: 11–21 % and P-tot: 3–22 %) (Fig. 4a-d). Except in the case of *P. corylophilum,* which had ~41 % P removal and ~35 % N removal and *P. restrictum,* which had ~31 % P removal. *P. corylophilum* effected the highest nutrient removal efficiencies overall, which were similar in both the open and closed batch cultures (efficiencies were <7 % lower in open than closed batch cultures).

4. Discussion

4.1. Pellet formation

Both Penicillium species demonstrated enhanced pellet formation, exceeding previous observations with a proliferation of >10 pellets and sizes ranging from 0.2 to 2 cm, in contrast to the previously observed <10 pellets of <0.3 cm in diameter or absence of pellet formation (Bansfield et al., 2023). This may partly be attributed to the reduced concentration of total suspended solids (TSS) in the current batch of cheese whey (~15 g L^{-1}), compared to the earlier study (~17 g L^{-1}), leading to a lower viscosity. Viscosity inversely correlates with pellet formation (Galaction et al., 2004), leading to increased pellet formation and larger pellet sizes in the present study. However, consistent with our prior research, *P. ostreatus* exhibited inhibited pellet formation, indicating a preference for filamentous growth even under more turbulent conditions.

The impact of mixing speed on pellet size in filamentous fungi was species-specific. *P. corylophilum* and *P. ostreatus* displayed an inverse relationship between mixing speed and pellet size, consistent with most species (Gibbs et al., 2000; Veiter et al., 2018). In contrast, *P. restrictum* exhibited a concomitant relationship, a phenomenon observed in some cases (Kelly et al., 2004). This divergence may be attributed to species-specific sensitivities to shear forces in pellet form, where the mixing speed required for effective agitation and pellet breakup differs for each

Table 1
Amounts and sizes of filamentous fungal pellets observed at three mixing speeds at the end of experimental runs.

Cultivation system	Species	100 rpm	150 rpm	200 rpm
Closed batch	*P. corylophilum*	ø = 0.5–1 cm	ø = 0.5 cm	ø = ~0.3 cm
	P. ostreatus	ø = 0.2–1 cm	ø = 0.2–0.5 cm	ø = ~0.3 cm
	P. restrictum	ø ≤ 0.2 cm		
Open batch	*P. corylophilum*	NA	NA	ø = 0.2–1 cm

*NA (not applicable).

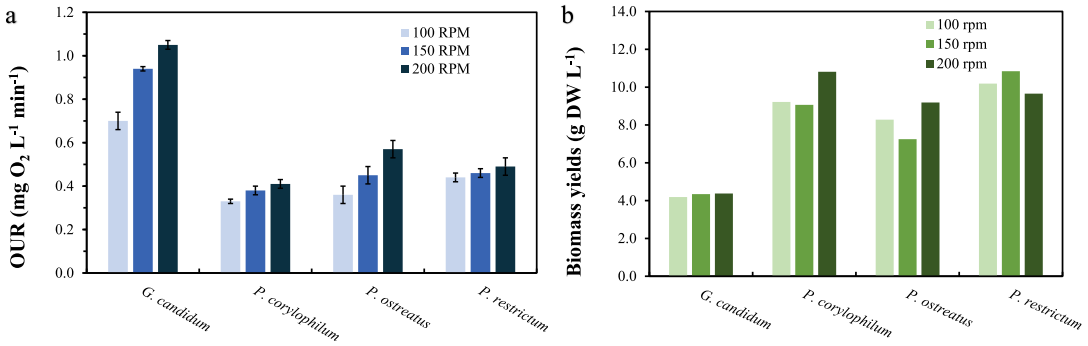

Fig. 2. a) Mean maximum oxygen uptake rates (OURs) (mean ± SE, $n = 4$), and b) Biomass yields (g DW L^{-1}) of all species in cheese whey at three different mixing speeds (100, 150 and 200 rpm) in a closed batch system.

Table 3
Increases (%) in mean maximum oxygen uptake rates (OURs) with increasing mixing speeds for each species.

Species	100 to 150 rpm	150 to 200 rpm	100 to 200 rpm
G. candidum	20 %	10 %	33 %
P. corylophi	13 %	7 %	20 %
P. ostreatus	20 %	21 %	37 %
P. restrictum	4 %	6 %	10 %

Table 4
Biomass yields (g/g COD removed) of all species at optimal mixing speeds in a closed batch reactor and *P. corylophilum* in an open batch reactor.

Cultivation system	Species	g/g COD removed	rpm
Closed batch	*G. candidum*	0.20	200
	P. ostreatus	0.42	200
	P. restrictum	0.49	150
	P. corylophilum	0.55	200
Open batch	*P. corylophilum*	0.83	200

species (Papagianni, 2004). It is plausible that *P. restrictum* pellets possess higher tensile strength, resisting breakup, while *P. corylophilum* may break up, resulting in smaller and more numerous pellets with increasing mixing speed. Notably, when cultivated in an open batch system, *P. corylophilum* produced larger pellets, highlighting that increased aeration contributes to larger pellet size, as previously documented (Veiter et al., 2018).

4.2. Metabolic activity

Generally, mixing speed exerted a positive influence on metabolic activity, although the extent of this effect varied by species. For instance, *G. candidum* and *P. ostreatus* had larger OUR percentage increases with rising mixing speeds, contrasting with the smaller percentage increases observed for both *Penicillium* sp. While faster agitation generally improves nutrient and oxygen distribution, fostering metabolism and growth (Galaction et al., 2004; Espinosa-Ortiz et al., 2016), the connection between metabolic activity and mixing speed was weak. The study indicates that the interplay between several factors, including agitation intensity, aeration, pH, and the dynamics of gas-liquid mass transfer, broth rheology, liquid-particle mass transfer and shear sensitivity, renders the relationship between mixing speed and metabolic activity complex. However, the interplay between these factors is intricate and difficult to parse, as underscored by *P. ostreatus*' high metabolic activity despite its extremely low culture pH, which should have induced entry into the stationary phase (Walker and White, 2017).

4.3. Biomass yield

Species-specific responses were also observed in the influence of mixing speed on biomass yields. For instance, *G. candidum* had an

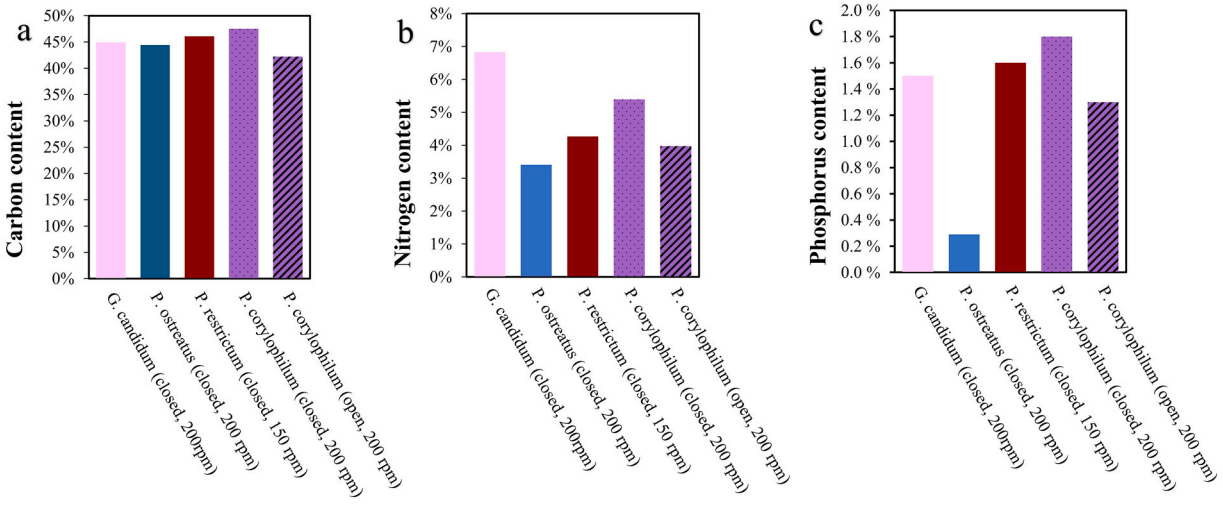

Fig. 3. Nutrient content of all species as a percentage of dry biomass: a) Carbon, b) Nitrogen, and c) Phosphorus at species-specific optimum mixing speeds in closed batch systems except for *P. corylophilum* which was also cultivated in an open batch system.

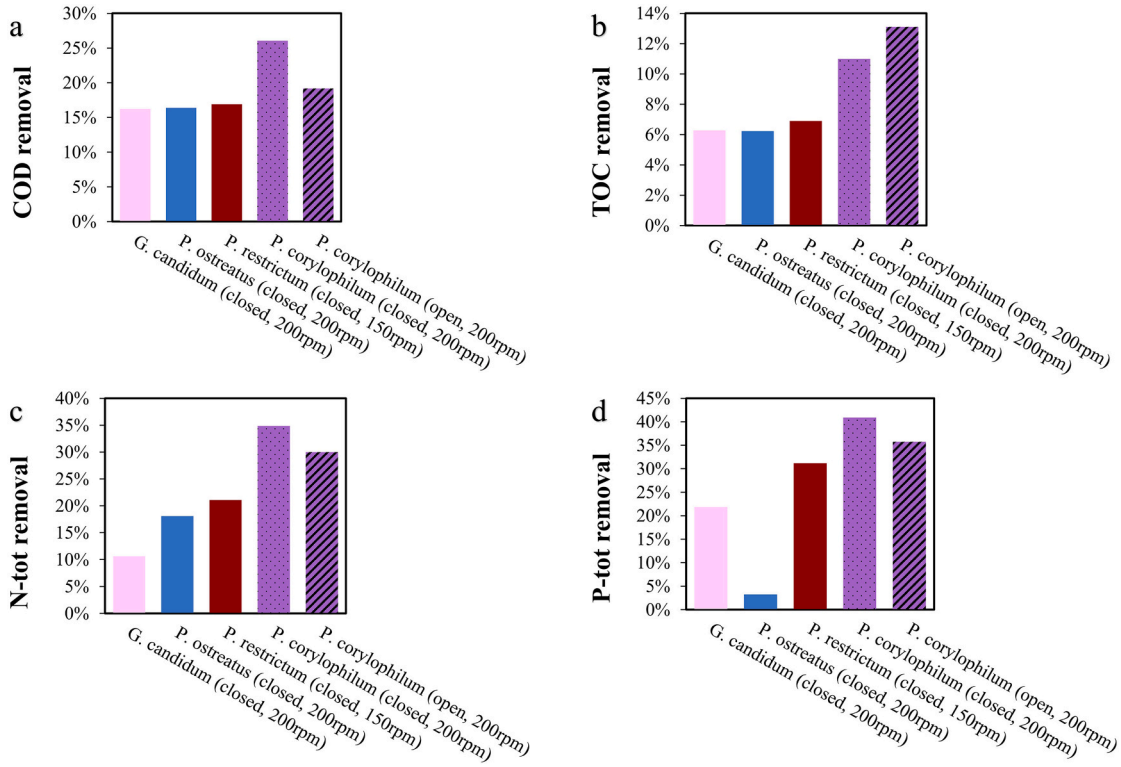

Fig. 4. Nutrient removal efficiencies (%) of all species in cheese whey after fungal cultivation: a) COD, b) TOC, c) N-tot, d) P-tot at species-specific optimal mixing speeds in closed batch systems except for *P. corylophilum* which was also cultivated in an open batch system.

increasing trend in biomass yields with rising mixing speeds, while *P. restrictum* exhibited a decrease only at specific speed transitions. Each species had distinct optimal mixing speeds for biomass yield. The biomass yield of *P. restrictum* peaked at 150 rpm, while *P. corylophilum* and *P. ostreatus* yields peaked at 200 rpm. This phenomenon of a species-optimal mixing speed has long been documented (Dronawat et al., 1995). Although metabolism and growth are interlinked (Walker and White, 2017), the magnitude of increase in biomass yield with mixing speed did not align with similar increases in the metabolic activities of some species. Additionally, the connection between biomass yields and mixing speed was not as apparent as between metabolic activity and mixing speed. This suggests a complex relationship between metabolism and growth, possibly because not all the energy from the carbon substrate is converted to ATP (Stryer, 1995), and of this, not all is used for growth (Walker and White, 2017).

Pellet formation and size affect culture broth rheology and oxygen availability, which in turn affects metabolism and growth, i.e., increasing pellet size has a negative effect. In contrast, more numerous pellets of smaller size have a positive impact on metabolism and growth (Veiter et al., 2018). The same relationships were observed in this study, with *P. corylophilum* forming numerous small pellets (improving rheology and oxygen availability) exhibiting higher biomass yields than *P. ostreatus,* which predominantly grew as free filaments (increases viscosity decreasing oxygen availability) (Garcia-Ochoa et al., 2010). In addition to oxygen availability, other culture conditions, such as pH, carbon source, C:N ratio, further influenced biomass yields (Gao et al., 2007; Liu et al., 2017; Walker and White, 2017). For instance, the C:N ratio of whey, though high (18.6:1 on a mass-to-mass ratio), may not have been optimal for the cultivated species (Castillo et al., 2020).

The OxiTop system is a closed batch cultivation system where oxygen levels decrease as carbon dioxide levels increase during the experiment lowering metabolism and growth (Papagianni, 2004; Taniwaki et al., 2010), likely decreasing nutrient removal. This premise was tested by cultivating the species *P. corylophilum,* which had the highest biomass yields and nutrient removal rates in an open batch system allowing for enhanced oxygen supply and CO_2 escape. Given that oxygen is usually the limiting factor for growth in submerged cultures (Garcia-Ochoa et al., 2010), it was hypothesised that increased oxygen flow would increase oxygen availability and growth substantially. However, the biomass increased only slightly, possibly due to the high viscosity of whey, limiting oxygen transfer despite increased airflow (Gibbs et al., 2000; Garcia-Ochoa et al., 2010). Nevertheless, biomass yields achieved here were close to or higher than those of other studies where similar fungi/yeast species were cultivated in whey or whey permeate (Kim and Lebeault, 1981; Wu and Hansen, 2009; Aouidi et al., 2010).

4.4. Biomass content

Fungi are known for their capacity to generate biomass enriched with organic carbon, notably in the form of carbohydrates and nitrogenous substances such as proteins and amino acids, and to accumulate phosphate (Johri et al., 2015). The biomass of the four species, high in C and low in N and P, aligns with the literature findings (Adour et al., 2004; Hoa et al., 2015; Agarwal et al., 2017; Zhang and Elser, 2017; Bakratsas et al., 2021). While all four species had similar C, N and P content, some species-specific variances existed. These variances were most pronounced for N and P content. For instance, *P. ostreatus* had lower N and P content (N: ~3 %, P: 0.3 %) than other species (N: 4–7 % and P: 1.3–1.8 %).

Though substrate type can influence the nutrient and mineral content of mushrooms and yeasts to some degree, the fact that the values of our biomass align with the values found in the literature suggests that whey did not impact the composition of the test species. Interestingly, the fungal and yeast biomass had slightly higher concentrations of Cu and Pb than whey, suggesting they bioaccumulate these elements in the biomass. However, these levels (Cu: 0.9–2.6 mg Kg^{-1}, Pb: 0.05–1.0 mg Kg^{-1}) are well below toxic levels (EFSA, 2004, 2016, 2023), making the biomass suitable for a wide array of applications. On the other hand, the

low N (<7 %) and P (<2 %) content of the biomass means it would not be appropriate as fertiliser or feed. However, the C content of ≥42 % positions it as an excellent supplement to fertiliser and feed.

4.5. Nutrient removal efficiency

Nutrient removal was species-specific, with notable species variations (e.g. the *Penicillium* sp.) in removal capacities for different nutrients. N removal is very species-specific, with most fungal denitrifiers belonging to the Ascomycota phylum, to which both *Penicillium* and *Geotrichum* belong. However, of the two, only *Penicillium* is a denitrifier (Mothapo et al., 2015). This may account for the *Penicillium* sp. higher N removal efficiencies compared to *P. ostreatus* and *G. candidum*. *Penicillium* species, notably *P. corylophilum*, excelled in P removal (30–40 %), followed by *G. candidum* (22 %), further emphasising the species-specific nutrient uptake pattern. Given that phosphorus uptake in fungi/yeasts is intricately linked to carbon uptake and metabolism (Fellbaum et al., 2012), it is logical that *Penicillium* sp. and *G. candidum*, adapted for lactose metabolism, would exhibit enhanced phosphorus uptake in whey. Furthermore, the oxygen dependence of carbon metabolism (Walker and White, 2017), means low oxygen availability in batch cultures hinders phosphorus uptake. Therefore, the low lactose utilisation capacity of *P. ostreatus* (Adebayo-Tayo et al., 2011), combined with limited oxygen availability in the closed batch culture, likely suppressed P uptake and removal by *P. ostreatus*.

COD and TOC removal efficiencies were relatively low. *G. candidum*, *P. ostreatus*, and *Penicillium* sp. are known for effective COD reduction in submerged cultures (Olivieri et al., 2006; Asses et al., 2009; Leitão, 2009; Purchase, 2016), but actual removal rates in this study were suboptimal. Despite initial assumptions that open batch cultures might offer better oxygen availability and thus superior COD removal than closed batch systems, both likely faced similar oxygen limitations due to oxygen's low solubility in liquids combined with increasing oxygen uptake as biomass growth increased. This, together with the high COD concentrations, could have reduced COD removal efficiency as higher nutrient removal efficiencies often occur with lower COD/TOC concentrations in bubble reactors, which facilitate better oxygen transfer within the broth, which in turn supports more effective C metabolism and growth (Asses et al., 2009; Doran, 2013).

Fungi and yeasts have better degradation rates at acidic and neutral pH (Purchase, 2016). Surprisingly, *P. corylophilum*, cultured at a high pH (8–8.5), demonstrated higher nutrient removal and degradation rates than those with acidic or neutral pH. Therefore, adjustment of the pH of *P. corylophilum* cultures, in addition to the use of a bubble reactor, may significantly improve its nutrient removal efficiency. Nevertheless, *P. corylophilum* emerged as the most promising species for nutrient removal, particularly for P removal.

5. Conclusion

This study showed: 1) distinct species-specific responses in metabolic activity, biomass yields, nutrient removal and pellet morphology to variations in mixing speed; and 2) a complex interaction between oxygen availability, nutrient concentrations, carbon source, C:N ratio and pH, influencing the metabolism, growth, and nutrient processing capabilities of fungi and yeasts in whey. Although overall biomass yields and nutrient removal efficiencies were modest, *P. corylophilum* demonstrated potential for efficient biomass production and nutrient removal. Further improvements in the performance of *P. corylophilum* could be made with pH adjustments and better reactor design such as a continuously fed bubble reactor which would optimize oxygen availability and lengthen the exponential growth phase. Nevertheless, cultivation of *P. corylophilum* in whey presents a promising valorization strategy, with the potential for generating high-value biomass and by-products, which would not only bring extra revenue but would also make the system economically self-sustainable. This system would enhance the sustainability and resource efficiency of small dairy producers which often dispose of whey, as opposed to large dairy firms which already leverage side streams into additional revenue.

CRediT authorship contribution statement

D. Bansfield: Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **K. Spilling:** Writing – review & editing, Supervision, Resources, Conceptualization. **A. Mikola:** Writing – review & editing, Supervision, Resources, Conceptualization. **J. Piiparinen:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets supporting the conclusions of this article are included within the article and its additional online files.

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Declaration of generative AI and AI-assisted technology usage

During the preparation of this work, the authors used Grammarly to improve readability, ensure inclusivity, and check grammar and punctuation. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for its content.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2024.101854.

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