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Assessing the impact of manipulation of fermentation broth on the texture, flavour, and nutritional quality of fungal biomass at bench-top and lab scale.

Hannah Dallas, PhD[1] (Fermentation Scientist), Paul Brett, PhD[1] (Lead Scientist), Shichen Deng, MSc[1] (Senior Food Technologist)

Affiliations: [1] Adamo Foods, [2] University of Nottingham

Abstract

This work exemplifies the positive effect on yield, texture, and nutritional composition on fungal mycelium by the inclusion of exogenous Iron and Cobalamin (Vitamin B12) when grown with the INFORS HT Techfors-S bioreactor. Elemental concentration as detected by ICP-MS, yield, texture, and flavour are compared to control biomass at both the bench and lab scale. The results highlight the importance of media optimising and the utility of stirred tank bioreactors for filamentous fungi growth as a method of providing improved growth conditions for the overall yield of a fermentation as well as a vehicle for easily examining the effects of broth adaptation on both the nutritional composition and the sensory profile of the biomass produced.

Keywords
Fungal mycelium, Biomass, Iron, Cobalamin (Vitamin B12), Wet cell weight (WCW), Bioaccumulation, Shake Flask (SF), Sterilise-in-place (SIP)

Introduction

Filamentous fungi applications in biotechnology and food technology are diverse, as are the ways in which they are grown. Harnessing the mycelium produced through both liquid and solid state fermentation, the cultural impacts of mycelium can be felt worldwide, from beer, to bread, to the more exotic tempeh. Adamo foods is using a unique process to produce a nutritious and tasty whole cut meat replacement. To do this, Adamo is producing large quantities of biomass in a reproducible, sterile manner using the INFORS HT Techfors-S bioreactor.

This work reviews the scale-effects of using the INFORS HT Techfors-S as a stable vehicle to investigate and understand how changes to media composition affect the nutritional composition of the biomass produced as well as the yield when compared to small-scale productions using 500 mL Erlenmyer shake flasks (SFs).

Using an in-house system to categorise the quality of biomass for the purposes of steak formation, there can be a trade-off between nutritional and sensory qualities.

Objective

- Assess the impact of exogenous Fe(III) and Cobalamin (Vitamin B12) on growth characteristics and nutritional profile of a fungal mycelium
- Validate performance of INFORS HT Techfors-S for the consistent and high yield production of fungal mycelium
- Understand the textural and flavour impacts of nutritional manipulation of fermentation broth on the steaks produced

Methods and materials

- **Equipment:** 3 lots of 15 L (10 L working volume) INFORS HT Techfors-S bioreactors (BR), operating with 4-way adapter for sterile addition, and 3 lots of Rushton-type impellers per vessel.
- **Media:** Fermentation media supplemented with an easily accessible sugar source. Inclusion of Fe(III) as Iron Citrate at 5 mg/L and/or B12 at 3 mg/L.

- **Analytical tests:** Iron analysis via elemental ICP-MS (performed by Campden BRI), and B12 as total (also performed by Campden BRI). Biomass weights were recorded before and after a deactivation step.
- **Validation:** Initial tests of concentrations to achieve a minimum detection limit were performed in SFs and tested using the previous analytical methods. Iron and B12 concentrations between 5-5000 µg/L and 3-3000 µg/L were tested for bioaccumulation. Samples of the broth as well as both the active and inactive biomass were tested for Fe and B12 concentrations to check for consistent "binding" within the mycelium.
- **Fermentation conditions:** Agitation, pH, and aeration of the vessels was controlled using the proprietary Infors data system, Iris. A sterile sugar solution was added post SIP. To one vessel, Fe (III) in the form of Ferric citrate was added to a concentration of 5 mg/L, to another was added B12 at a concentration of 3 mg/L, and to a final vessel 5 mg/L Fe (III) and 3 mg/L B12.

Results

The results from the scale-up test within the INFORS HT Techfors-S bioreactor demonstrated that the vessels were capable of producing fungal mycelium of matching, or improved, nutrient composition when compared to shake flasks as detected by ICP-MS analysis of the post fermentation biomass. The INFORS HT Techfors-S vessels also allowed for confirmation of yield, texture, and flavour changes as a result of nutritional changes of the broth when compared to a control fermentation run.

Nutrient concentration change

Where Fe (III) was included (Fe+B12 = BR_1, Fe = BR_3), the detected bioaccumulation of Iron more than doubled compared to SF control, and increased by 23-35 % compared to the Iron SF test. Similarly, biomass produced in the INFORS HT Techfors-S showed a 34-67x increase in B12 concentration (Fe+B12 = BR_1, B12 = BR_2) when grown in the INFORS HT Techfors-S depending on media composition, when compared to the SF control. When compared to the B12 SF test, this was still 1.3-2.5x more than the detected concentration in the SFs.

	Test	Conc (mg/100g)	% of control	Change (from SF control) (mg/100g)	% of SF Test	Change (from SF test) (mg/100g)
SF	Fe Test	2.6	186	1.2	-	-
SF	Fe Control	1.4	-	-	-	-
SF	B12 Test	3.33×10^{-2}	2620	3.20×10^{-2}	-	-
SF	B12 Control	1.3×10^{-3}	-	-	-	-
BR_1	Fe	3.2	229	1.9	123	0.6
BR_3	Fe	3.5	250	2.1	135	0.9
BR_1	B12	8.5×10^{-2}	6693	8.37×10^{-2}	3269	5.17×10^{-2}
BR_2	B12	4.3×10^{-2}	3386	4.17×10^{-2}	1654	9.7×10^{-3}

Table 1. Results of ICP-MS testing of biomass after fermentation in shake flasks (SF), and INFORS HT Techfors-S Bioreactors (BR). Fe (III) Citrate included at 5 mg/L, and Cobalamin included at 3 mg/L in the supplied broth for both SFs and BRs.

Sensory changes

In a blind test of steaks made from biomass produced by fermentations using the INFORS HT Techfors-S there was a detectable difference in both the texture and the flavour profiles when changing the nutrient composition of the fermentation broth.

Sample	Blind codes	Texture	Flavour
Control	440	Slightly firmer and, more fibrous texture than the other samples.	Representative of Adamo's stadard product, with beef notes and no discernible off-notes. Not overwhelmingly strong in any specific flavour direction.
Fe (III)	624	Tender, similar to sample 710 but less firm than sample 440.	Similar to 440 but described as having hints of "cooked oil" and a bit of tanginess.
B12	710	The most tender of the three samples.	Different from the other samples with strong off-notes.

Table 2. Sensory report from biomass collected from BR fermentations as reported after biomass had been processed into steaks for analysis of effect on flavour and texture.

Conclusion

The addition of exogenous nutrients resulted in changes in the nutritional profile as measured by ICP-MS. With increases in concentration detected for both Fe (III) (Ferric citrate) and Vitamin B12 (Cobalamin), when compared to control conditions. The scale effect of a INFORS HT Techfors-S bioreactor showed further increases in the concentration of both nutritional elements when compared with the same conditions in shake flasks.

It appears that the addition of nutrients to the fermentation broth in the INFORS HT Techfors-S may have affected the texture of the biomass produced. The effect on flavour seems limited in the case of added Fe (III) with the flavour profile being mostly comparable to the control and more pronounced in the case of adding B12 which produced off-notes and had a greater effect on taste overall.

Figure 1. Example control steak produced by Adamo Foods using biomass produced using the INFORS HT Techfors-S.