Production of natural red dye by submerged fermentation in a 3 L Minifors 2 bench-top bioreactor

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Abstract

The production of natural red dye through submerged fermentation was evaluated using controlled bioreactor cultivation in the INFORS HT Minifors 2. Critical process parameters were monitored and controlled, while INFORS HT eve® bioprocess software enabled digital traceability and supported process optimization. The approach facilitated the generation of reproducible process data for natural ingredient biomanufacturing.

Keywords
Submerged fermentation, fungal fermentation, precision fermentation, natural red dye, bioprocesses, organic red pigment, scalability, bioreactor, Minifors 2, eve®

Objective

To develop a fungal fermentation process for natural red dye production and scale it to 3 L using bench-top bioreactors, with control and automation of key process variables to support high dye yields, accuracy, reproducibility, and regulatory compatibility.

Specific objectives

- Develop suitable conditions for natural red dye production through batch fermentation of *Talaromyces spp.*
- Produce a natural red dye through a verifiable biological process.
- Scale production to 3 L per batch.
- Standardize control of main cultivation variables: pH, temperature, dissolved oxygen (DO), and agitation.
- Optimize fermentation laboratory workspace.
- Optimize processing time.



Installation of eight Minifors 2 bench-top bioreactors.

Introduction

Global demand for natural colorants that remain stable across a range of pH values and temperatures has driven the development of fungal platforms for scalable pigment production. Michroma has developed a patented precision fermentation process using *Talaromyces spp.* for natural red dye production.

The process was optimized for Minifors 2 bench-top bioreactors, enabling programmable process control and traceable fermentation data. Developed at bench scale, the process is intended for subsequent scale-up to industrial production systems.

The process was operated in batch mode, using buffer solutions for pH adjustment and air for dissolved oxygen control. The dye is extracellular, which facilitates recovery because it is released into the culture medium. Dye production was monitored using absorbance measurements (Abs).

Fungal conidia were inoculated into a vegetative growth medium containing phosphate and simple carbon and nitrogen sources to obtain actively growing mycelium in the exponential growth phase. The resulting vegetative culture was then aseptically transferred to the production medium at 1% (v/v), where the fungus produces the natural red pigment.

The final product can be formulated as a liquid solution or a dry powder, depending on the intended industrial application.

Applications and market segments

- Food industry (natural ingredients)
- Cosmetics
- Nutraceuticals
- Aquaculture (substitution of synthetic dyes)
- Sustainable biomanufacturing

Materials and methods

Microorganisms

Cultures of *Talaromyces spp.* were grown by submerged fermentation to support fungal growth and production of an azaphilone-based colorant released into the culture medium.

Culture medium

A basic culture medium for fungal growth was prepared locally using glucose, starch, yeast extract, amino acids, and mineral salts. The medium supported fungal growth and natural red dye production without the addition of synthetic colorants.

Bioreactor cultivation and equipment configuration

Submerged fermentation was performed in a stirred-tank bioreactor. Based on the required performance, flexibility, and operating volume, a Minifors 2 bench-top bioreactor with a 3 L total volume and 2 L working volume was selected.
The following process variables were controlled:

- pH operating range: 3.5–5.0 with ± 0.5 tolerance (controlled by automated peristaltic acid/base pumps)
- Temperature: 30 ± 2 °C
- DO: >30% (sparger aeration with compressed air)
- Agitation: 600–800 rpm (with Rushton impellers)

The process variables were controlled automatically. eve® bioprocess software was used to monitor, trace, and analyze process data generated during development and production.

Product recovery

Downstream processing involved unit operations that did not require the addition of inorganic chemicals or substances reported to be harmful to the intended use of the natural red dye.

- **Centrifugation:** The fermentation broth was centrifuged for 30 minutes to separate the biomass from the dye-rich supernatant.
- **Filtration:** Commercial 0.22 µm filters were used to remove residual fungal cells and cellular debris, which can reduce dye purity and affect its physical properties.
- **Purification:** Chromatographic techniques were used; details were not disclosed for patent and intellectual property reasons.

Results

Bioreactor cultivation produced high concentrations of the desired natural red dye. The dye showed the following characteristics relevant to its intended food applications:

- Thermal stability: >100 °C without degradation
- pH stability: pH 3 and 8
- Solubility: high in aqueous media and emulsions

Bioreactor operation maintained product properties with high batch-to-batch reproducibility under controlled cultivation conditions..

- Reproducibility: coefficient of variation (CV) below 5% for all measured variables, including pH, dissolved oxygen, red pigment concentration, and biomass.
- Fermentation time: 70 h in batch mode

The benchtop studies supported development of the dye production process at laboratory scale. The process is intended for operation at laboratory volumes of 0.5–10 L and subsequent scale-up to industrial volumes above 100,000 L. The development approach was reported to be compatible with:

- Supervisory control and data acquisition (SCADA) systems
- Single-use or stainless-steel bioreactors
- Clean-in-place (CIP) and sterilization-in-place (SIP) protocols

Fungal growth kinetics

Periodic samples were collected during bioreactor cultivation to monitor biomass and viability and characterize fungal growth kinetics. Biomass was measured offline as dry cell weight (DCW; Figure 1).

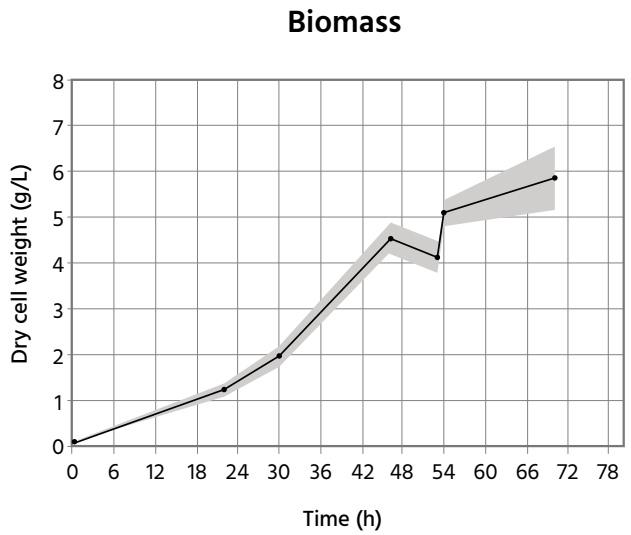


Figure 1. Biomass growth expressed as dry cell weight (DCW) over time. Shaded area represents the 95% confidence interval (n = 13).

Bioreactor operation enabled online process monitoring and periodic offline sampling. Red pigment concentration was measured offline (Figure 2), while dissolved oxygen concentration was monitored online using a Hamilton sensor (Figure 3).

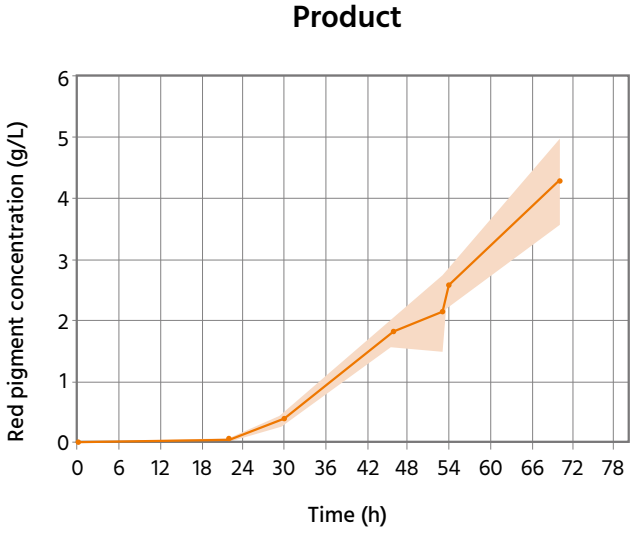


Figure 2. Red pigment concentration over time. Shaded area represents the 95% confidence interval (n = 13).

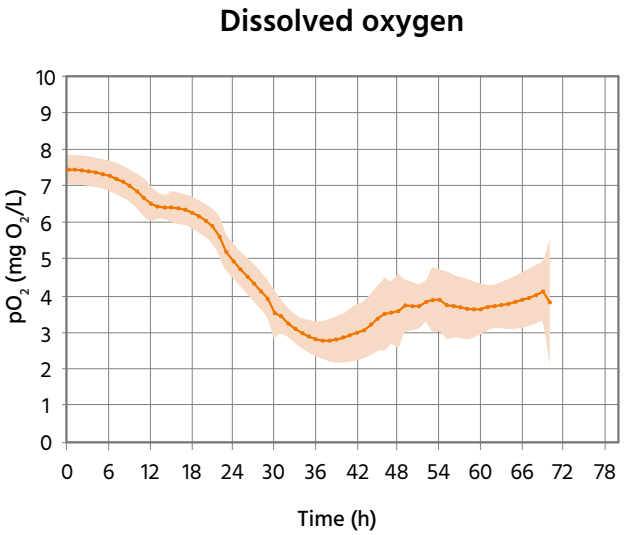


Figure 3. Online dissolved oxygen concentration over time. Shaded area represents the 95% confidence interval (n = 13).

As part of process improvement, four culture media were tested. Their formulations are confidential. Only medium composition was varied; cultivation conditions and automated bioreactor control were kept constant. The comparison identified a formulation with higher red pigment concentration during upstream processing, while retaining the required product properties (Figure 4).

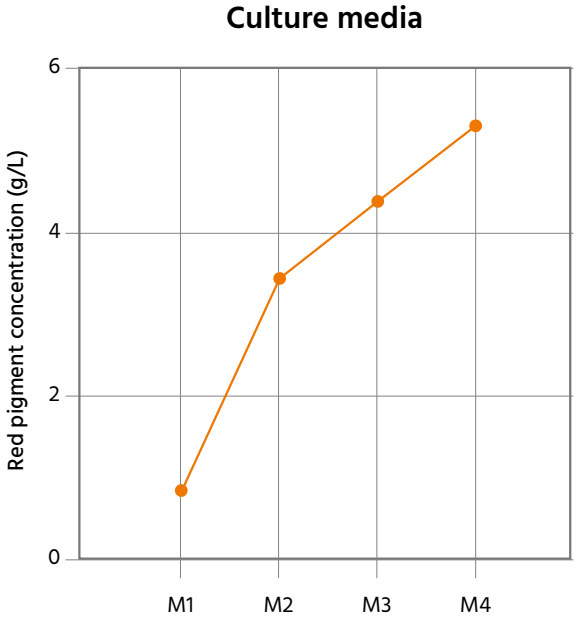


Figure 4. Red pigment concentration obtained using four culture medium formulations (M1–M4) during upstream processing.

Process overview

The dye production process combines upstream fermentation with downstream separation and purification, including centrifugation, filtration, and chromatography, followed by drying and quality control to prepare the product for its intended market application (Figure 5).

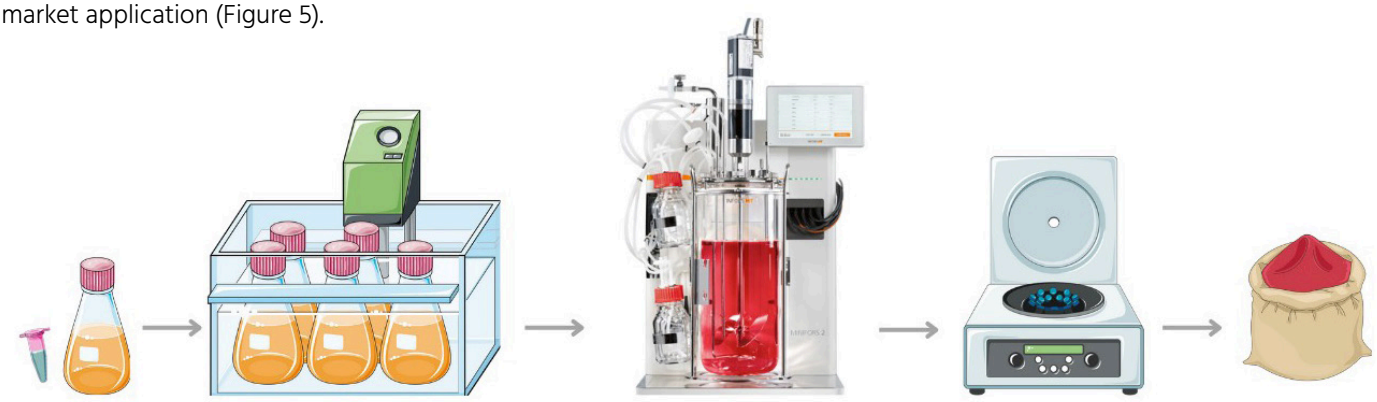


Figure 5. Overview of the dye production process.

Conclusion

The Minifors 2 bench-top bioreactor and eve® bioprocess software supported controlled natural red dye production, digital traceability, and process optimization. The reported results showed reproducible batch operation and supported further development of the process for natural ingredient production.

The compact and ergonomic design accommodated multiple bioreactors on the same laboratory bench. The installation was reported to reduce demand for auxiliary utilities such as chilled water and compressed air.

The bioreactor can also be used for research and production processes involving fungi and yeasts.



Minifors 2 bench-top bioreactor used for natural red dye production.



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