Fueling the Blue: Optimizing Bioreactor Fermentations for Maximum Fungal Biomass

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Introduction

Synthetic dyes release harmful chemicals into the environment, motivating researchers to pursue renewable fungal pigments. Before maximizing pigment yield and colour production, it is critical to establish robust fungal growth in bioreactors [1][2][3]. The objective was to identify conditions that produce dense and healthy cultures to enable scalable pigment production. A full-factorial Design of Experiments was employed to evaluate pH (4.0–6.0) and temperature (20–24°C) effects on biomass accumulation, and the productivity [4].

Figure 1: Visual appearance of fungal culture after 14 days of fermentation, illustrating pigment development under controlled conditions

Methodology

A full factorial DoE with two centre points was designed and analysed using MODDE 13.1 to optimize fungal biomass. Pre-cultures were prepared in shake flasks and used to inoculate 5.0L bioreactors (3.0 L working volume). Fermentations ran in batch mode for up to 14 days, with pH (4.0–6.5) and temperature (20–26 °C) as variable factors. DO was maintained at 20% via cascade control, and agitation ranged from 250–450 rpm. Daily samples were taken to measure dry cell weight (DCW) and visually assess pigment formation.

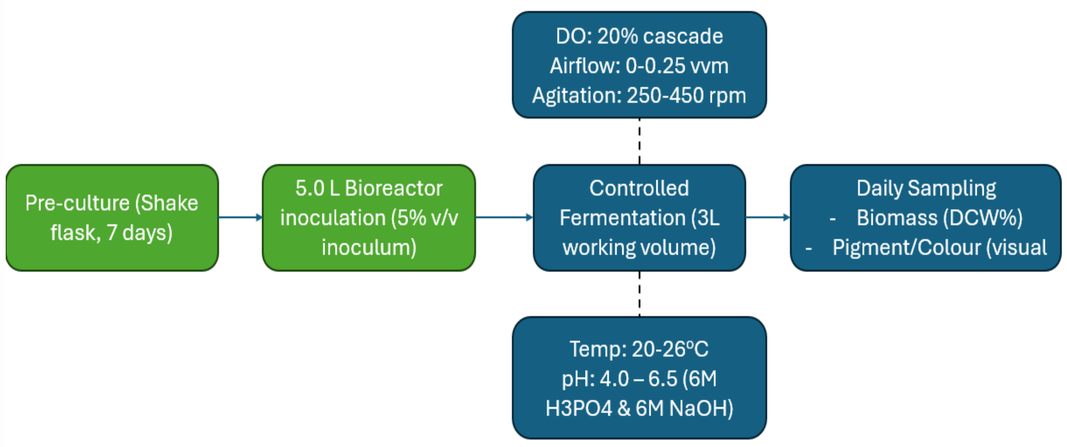

Figure 2: Overview of the experimental workflow used for biomass optimization, including pre-culture preparation, bioreactor fermentation under controlled conditions, and daily sampling for biomass and pigment assessment.

References

[1] Singh, Aarti & Mittal, Anupama & Jangid, Nirmala. (2020). Toxicology of Dyes. 10.4018/978-1-7998-0311-9.ch003.

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[3] Kalra, R., Conlan, X. A., & Goel, M. (2020). Fungi as a potential source of pigments: Harnessing filamentous fungi. Frontiers in Chemistry, 8. https://doi.org/10.3389/fchem.2020.00369

[4] Barad, Miryam. (2014). Design of Experiments (DOE)—A Valuable Multi-Purpose Methodology. Applied Mathematics. 5. 10.4236/am.2014.514206.

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Results

To compare growth efficiency, a variable called productivity was introduced: Productivity (%/hour) = (Maximum DCW %) / (Time of maximum DCW in hours). This was used as the response variable in both the interaction and quadratic models. Biomass yield varied across conditions (Fig. 3), with MT1–MT6 representing different pH and temperature combinations (Table 1). The interaction model (Fig. 4a–b) showed limited predictive power, while the quadratic model (Fig. 5a–b, Fig. 6) revealed promising trends but lacked sufficient data for validation. These results provide a starting point for refining fermentation strategies toward optimal biomass growth.

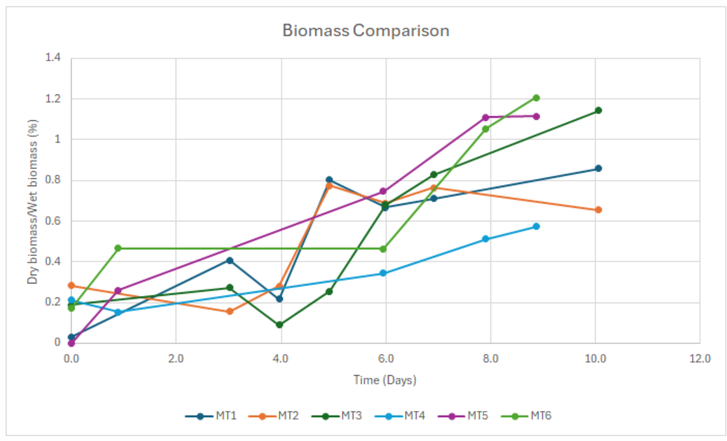

Figure 3: Biomass comparison of Biomass yield across different fermentation runs. MT1–MT6 refer to experimental conditions defined by specific combinations of pH and temperature in the DoE matrix, which is given in Table 1

Table 1: Abbreviations for fermentation conditions used in each experiment.

Abbreviation	pH	Temperature
MT1	6.5	26
MT2	6.5	20
MT3	4	26
MT4	4	20
MT5	5.25	23
MT6	5.25	23

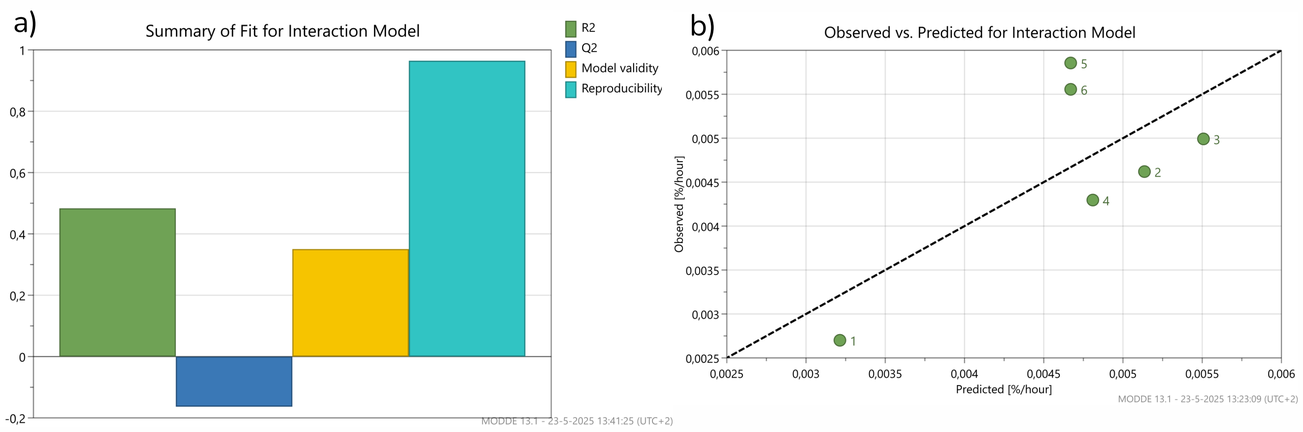

Figure 4: (a) Summary of fit for the interaction model; (b) Observed vs. predicted biomass values based on the interaction model. The model shows limited predictive accuracy.

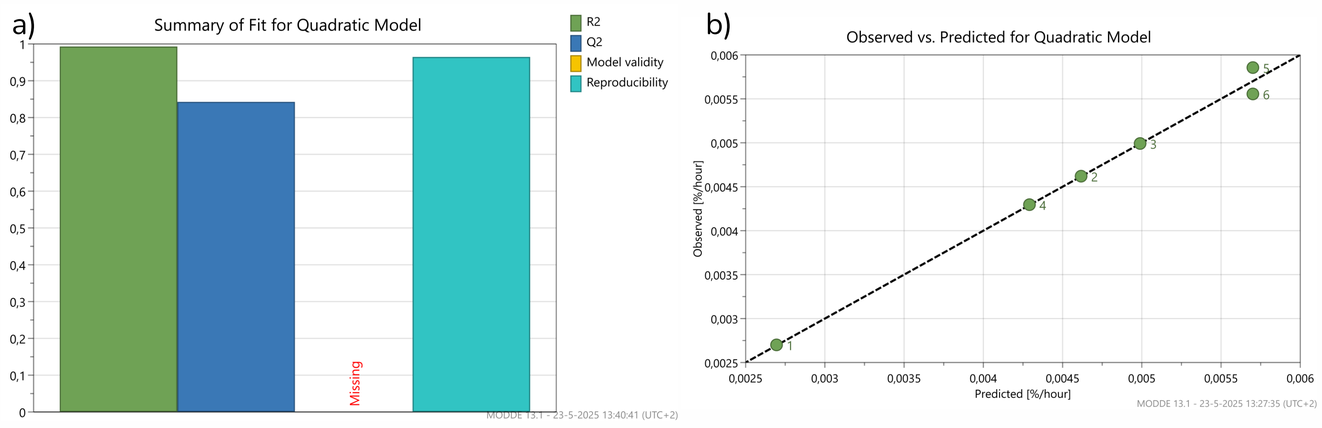

Figure 5: (a) Summary of fit for the quadratic model; (b) Observed vs. predicted values using the quadratic model. Model performance improves, but reliability is limited due to insufficient data.

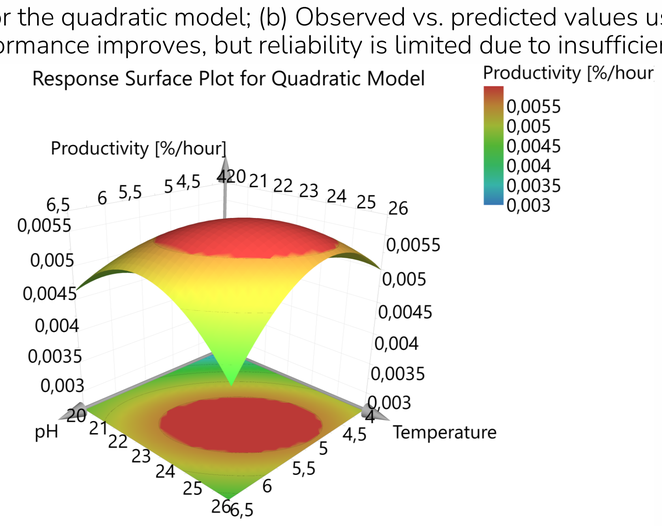

Figure 6: Response surface plot of the quadratic model illustrating potential trends in biomass optimization under varying pH and temperature conditions.

Conclusion

The research proceeded with the two centre point conditions, which showed the highest biomass productivity, to focus on pigment production optimization. While initial trends appear promising, the current dataset is too limited to confirm the true optimal growth conditions. Additional experiments are needed to improve model reliability and prediction.