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Effect of Yeast Extract on Growth Kinetics of *Monascus purpureus*

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Abstract

Growth kinetics and red pigment production of *Monascus purpureus* CCT 3802 was studied. A reproducible inoculum with extremely dispersed hyphae for bioreactor runs was obtained through a two-step cultivation in a shaker. First, the spores were cultivated in a complex medium rendering a suspension of vegetative cells. In the second step these cells were grown in a semisynthetic medium. Two types of media were employed in the bioreactor runs: a semisynthetic (glucose, salts, and yeast extract), and a synthetic, without yeast extract. The inclusion of yeast extract, caused an increase in cell yield on glucose ($Y_{x/s}$) as high as 40%. Also, yeast extract probably yielded a higher proportion of red pigment associated with the cell, relative to the synthetic medium. On the other hand, cells grown on the synthetic medium were slightly higher producers of red soluble pigments.

Index Entries: *Monascus purpureus*; yeast extract; red pigment; kinetics.

Introduction

Interest in natural food coloring has increased in recent years. Patents on natural pigments are outnumbering the synthetic ones by five to one *(1)*. The number of permitted synthetic colorings has decreased because some, such as azorubin and tartrazin, have been shown to cause allergies *(2)*. Therefore, the development of processes for the production of microbial organic pigments has become important.

Monascus spp. are millinery used in the East Asian countries. Cultivation is done in semisolid medium (rice) *(3)*, and the whole medium has been used for centuries as a medicinal agent and as a food coloring *(4)*.

Interest in the red pigments of *Monascus* spp. relies on the substitution of the nitrite and nitrate in cured meats, for they are significantly less toxic. Other additives also can be substituted such as cochinila carmim *(5)*. In addition, the fungi synthesize mevinolin, which is a cholesterol-reducing medicine *(6)*.

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Growth kinetics and pigment production in the submerged cultivation of *Monascus* spp. have been discussed in several articles *(7–10)*. However, the influence of yeast extract on growth and production activities of *Monascus* spp. has not been determined.

In the present work, two parts of the *Monascus purpureus* culture process were studied: (1) the standardization of the inoculum cultivation procedure in order to get vegetative cells with extremely dispersed hyphae, and (2) the growth kinetics and red pigment production through batch runs on a semisynthetic medium (composed of glucose, salts, and yeast extract) and on a synthetic medium (without yeast extract).

Materials and Methods

Microorganism and Storage

M. purpureus CCT 3802 was obtained from the Centro de Culturas Tropicais of Fundação Tropical de Pesquisas André Tosello, Campinas, SP, Brazil. This strain corresponds to ATCC 36928. A standardized suspension of spores in glycerol (15% [v/v]) was stored at –20°C.

Inoculum for Bioreactor Runs

The inoculum was precultivated in two phases. First, the spores were activated through the cultivation in 500-mL shake flasks (10^6 spores/mL) containing 100 mL of a complex medium (described subsequently) at pH 5.5. The flasks were incubated for 48 h at 30°C and 300 rpm.

Second, 20 mL of the first preculture was used to inoculate 80 mL of a semisynthetic medium (described subsequently). This second preculture was incubated in 500-mL shake flasks for 30 h at 30°C and 200 rpm.

Cultivation Medium

Three types of media were utilized: complex, semisynthetic, and synthetic. The complex medium contained (in distilled water) 10 g/L of glucose, 3.0 g/L of meat extract, and 5.0 g/L of peptone. The semisynthetic medium contained (in distilled water) 20 g/L of glucose, 4.8 g/L of $MgSO_4 \cdot 7H_2O$, 1.5 g/L of KH_2PO_4, 1.5 g/L of K_2HPO_4, 0.01 g/L of $ZnSO_4 \cdot 7H_2O$, 7.6 g/L of monosodium glutamate, 0.4 g/L of NaCl, 0.1 g/L $FeSO_4$, and yeast extract. The semisynthetic medium without yeast extract was called synthetic medium. pH values were adjusted to 5.5. The media were sterilized in an autoclave at 120°C for 20 min.

A solution of yeast extract and the salts (except $MgSO_4$) was sterilized inside the bioreactor (or in an Erlenmeyer flask for the shaker runs). Another solution, composed of glucose and $MgSO_4$, was sterilized separately at pH 4.0, to avoid undesirable reactions.

Bioreactor Cultivation

The two experiments were done in a 5-L bioreactor Bio Flow III (New Brunswick Scientific, Edison, NY).

Temperature was kept at 30°C and agitation was kept at 500 rpm. pH was controlled at 5.0 ± 0.3 through automatic addition of an NaOH solution (2 *N*) or an HCl solution (2 *N*). Airflow rate was kept at 1.0 L/(L·min). Four liters of the media described previously was inoculated with 400 mL of the inoculum.

Analytical Methods

Samples were periodically taken from the bioreactor cultivations in order to analyze cell concentration (*X*) as dry cell weight, glucose concentration, and pigment production (*Abs*). The assessment of *X* was done after samples were vacuum filtrated through a 1.2-μm membrane followed by drying the pellet in a microwave (180 W, 15 min). Glucose concentration was determined by the glucose-oxidase method (Merck, Darmstadt, Germany). Red soluble pigment production was evaluated through the absorbancy measurement at $\lambda = 500$ nm, using the filtrate samples.

Ethanol and acetate concentrations were determined by a Waters 600E high-performance liquid chromatograph (Waters, Milford, MA) equipped with a Waters 410 refractometer (35°C) at its outlet. The Shodex Ionpak KC-811 column (Shodex, Japan) at a working temperature of 40°C was employed with a flow of 1 mL/min of H_3PO_4 (0.1%) as the mobile phase. Samples injected to the high-performance liquid chromatograph were filtered through a Waters NH_2 filter, to remove the pigments, which could damage the column.

Results and Discussion

The procedure for inoculum culture was established through shaker runs, whose properties are described on Table 1. According to Table 1, complex media were more appropriate to induce spore germination, because the lag phase became shorter while the media became complex (runs I–III).

The initial spore concentration was standardized on 10^6 spores/mL, in order to not render pellets of germinated cells. To obtain a reproducible inoculum for the bioreactor runs, the two-step procedure of run V was established, which is the same as described in Materials and Methods. Through this procedure, the shorter lag phase was verified in the spore germination and a suspension of extremely dispersed hyphae was obtained, which rendered no lag phase in the subsequent bioreactor cultivation. This is an important feature for industrial purposes because it shortens the growth phase, also reducing the possibility of contaminant growth.

From the shaker runs, it was visually verified that the addition of yeast extract to the medium yielded a higher pigment production associated with the cells relative to the synthetic medium, in addition to higher values of cell concentration. Taking into account the significant influence of the yeast extract on process performance, batch runs R1 (semisynthetic

Table 1
Properties of Runs

Run	Medium	Inoculum	Lag phase (h)
I	Synthetic	Spores	40
II	Semisynthetic	Spores	24
III	Complex	Spores	20
IV	Synthetic	Vegetative[a]	0
V	Semisynthetic	Vegetative[a]	0

[a]Inoculum deriving from spores cultivation in complex medium (III).

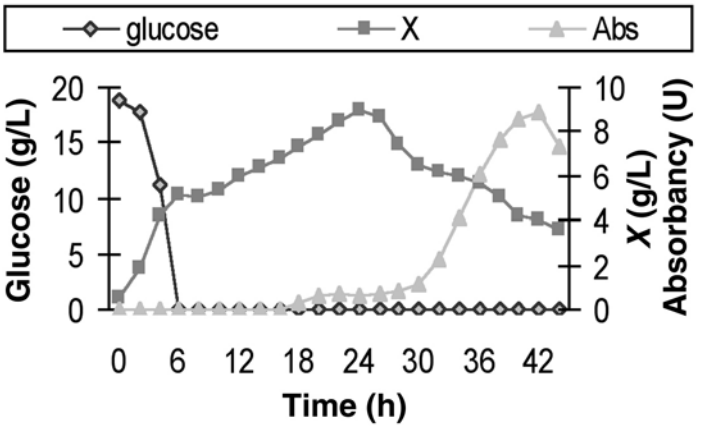

Fig. 1. Cell concentration (*X*); red pigment (*Abs*) and glucose concentration in R1, semisynthetic medium.

medium) and R2 (synthetic medium) were done in the bioreactor, in order to study the effect of yeast extract in well-controlled conditions (Figs. 1 and 2).

In both experiments, R1 and R2, a clear separation between growth and production phases was verified, a typical behavior for secondary metabolites. Red soluble pigment production began when cell growth almost finished. Table 2 summarizes growth features for both runs.

Growth activity was significantly more effective in the medium with yeast extract relative to the synthetic one, regarding the increase of 40% on cell yield on glucose, reported in Table 2. Also, the medium with yeast extract allowed the maintenance of cell viability when glucose was exhausted, opposing the behavior verified with the synthetic medium, in which a sharp decrease in cell concentration was verified before growth on carbon sources other than glucose was established. However, the closer specific growth rate values in both runs indicate that the rates of the metabolic pathways of the growth were not limited in the synthetic medium. Therefore, yeast extract acts only as an additional nutrient supplier.

Monosodium glutamate probably was consumed as carbon and energy source, in addition to nitrogen source, after glucose exhaustion,

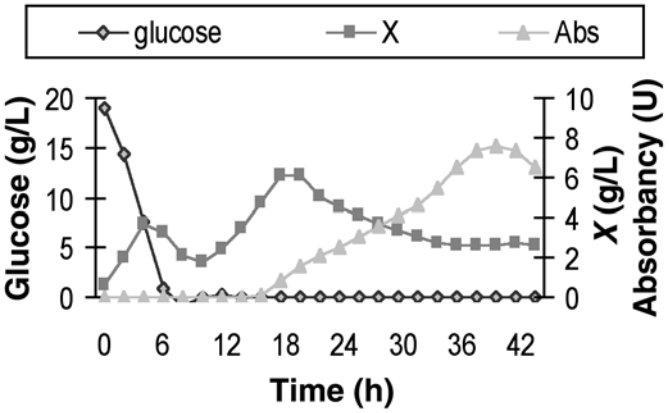

Fig. 2. Cell concentration (*X*); red pigment (*Abs*) and glucose concentration in R2, synthetic medium.

Table 2
Growth Features of Runs R1 and R2[a]

Run	X_{max} (g/L)	$Y_{x/s}$	μ_6 (h^{-1})	μ_{40} (h^{-1})
R1	9.0	0.42	0.44	0.23
R2	6.1	0.30	0.40	0.28

[a]X_{max}, cell concentration at the end of the growth phase; $Y_{x/s}$, cell yield on glucose, at the end of the growth phase; μ_t, specific growth rate at time *t*.

Table 3
Production Features of Runs R1 and R2[a]

Run	Abs_{max} (500 nm)	*Pec* (*Abs*/[g·L])	*Pep* (*Abs*/[g·L])	*Pp* (*Abs*/h)
R1	8.8	0.08	2.2	0.21
R2	7.8	0.13	2.7	0.19

[a]Abs_{max}, maximum absorbancy at λ = 500 nm; *Pec* and *Pep*, specific production (relative to cell concentration) at the end of the growth and production phase, respectively; *Pp*, productivity.

in both runs. Although *Monascus* spp. are reported as ethanol and acetate producers *(11,12)*, which also could be consumed, concentrations of these molecules in the range of 10–40 mg/L by the end of the growth on glucose does not explain the increase in cell concentration of about 4 g/L. Table 3 summarizes features of the red pigment production of the runs.

Cells cultivated in the synthetic medium showed a higher level of red soluble pigment production relative to that of the medium enriched with yeast extract (see *Pec* and *Pep* values in Table 3). Regarding that productivity (*Pp*) was almost the same for both runs, the synthetic medium could be the better one. However, the production of red insoluble pigments (not

measured) was probably higher in the semisynthetic medium, which can lead to a higher level of total production (soluble plus insoluble red pigments). Further studies of the process including measurements of total red pigment production on both media must be conducted.

Conclusion

A reproducible inoculum with extremely dispersed hyphae for the bioreactor runs was obtained through a two-step cultivation in a shaker. First, the spores were cultivated in a complex medium rendering a suspension of vegetative cells. Second, the culture was subsequently grown in a semisynthetic medium, also utilized in the bioreactor. Moreover, no lag phase was observed in the bioreactor through this procedure.

Cells grown on the synthetic medium were higher producers (23%) of red soluble pigments, relative to cells in semisynthetic medium.

On the other hand, cells grown in the semisynthetic medium (with 1 g/L of yeast extract added), showed a more effective growth (increase of 40% on cell yield on glucose, $Y_{x/s}$) and probably a higher proportion of red pigment associated with the cells.

Further studies on the influence of medium composition on red metabolite solubility must be conducted.

References

1. Francis, F. J. (1989), *Crit. Rev. Food Sci. Nutr.* **28(4),** 273–314.
2. Multon, J. L. (1992), *Additifs et auxiliares de Fabrication dans les Industries Agro-alimentares*, Tec. et Doc. Lavoisier, ed., Apria.
3. Rosenblitt, A., Agosin, E., Delgado, J., and Pérez-Correa, R. (2000), *Biotechnol. Prog.* **16,** 152–162.
4. Went, F. A. F. C. (1985), *Ann. Sci. Nat. Bot. Ser.* **8,** 111–148.
5. Fabre, C. E., Santerre, A. L., Baberian, R., Pareilleux, A., Goma, G., and Blanc, P. J. (1993), *J. Food Sci.* **58(5),** 1099–1110.
6. Wong, H. C. and Bau, Y. S. (1977), *Plant Physiol.* **60,** 578–581.
7. Lin, T. F. and Demain, A. L. (1991), *Appl. Microbiol. Biotechnol.* **36,** 70–75.
8. Juzlová, P., Martinková, L., and Kren, V. (1996), *J. Ind. Microbiol.* **16,** 163–170.
9. Pastrana, L., Blanc, P. J., Santerre, A. L., Loret, M., and Goma, G. (1995), *Process Biochem.* **30(4),** 333–341.
10. Hamdi, M., Blanc, P. J., and Goma, G. (1997), *Bioprocess Eng.* **17(2),** 75–79.
11. Hamdi, M., Blanc, P. J., and Goma, G. (1996), *Process Biochem.* **31(6),** 543–547.
12. Pastrana, L. and Goma, G. (1995), *Process Biochem.* **30(7),** 607–613.