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Relation between growth, respirometric analysis and biopigments production from *Monascus* by solid-state fermentation

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Abstract

Solid-state fermentation was carried out to establish relation between growth, respirometric analysis and biopigments production from *Monascus* sp. in columns and in a drum-type bioreactor with forced air. In these reactors, the best aeration rate for biopigment production was 1 ml of air, per gram of wet substrate, per minute. The outlet air composition was determined using gas chromatography (GC), while the pigments produced were measured by spectrophotometry after extraction with ethanol. An ergosterol-dosage method was used to estimate biomass production; in this method, the sterol was extracted and measured by liquid cromatography (HPLC). The results showed that although pigments were a secondary metabolite, its production was proportional to the biomass produced that was estimated by ergosterol analysis, and therefore could be used to estimate biomass formed in the natural support (rice). Specific velocities for pigment and biomass production were estimated by a sigmoid model applied to the data and also with the aid of a computer program nominated FERSOL. Under ideal conditions in column fermentation, a maximum specific growth velocity of 0.039 h^{-1} and a specific pigment production velocity of 27.5 AU/g biomass h was obtained, at 140 h, with 500 AU/g dry fermentate after 12 days. The specific product formation velocity in the bioreactor was 4.7 AU/g h, at 240 h fermentation, and the total pigment production was 108.7 AU/g dry fermentate after 15 days.

Keywords: *Monascus*; Aeration; Solid-state fermentation; Bioreactor; Kinetic parameters; Natural pigments

1. Introduction

Microbial pigments are a promising alternative to other color additives extracted from animals or vegetables, because they are also considered natural, pose no seasonal production problems and show high productivity. Among pigments produced by fermentation, one of the most important is *Monascus* pigments, which have been used for centuries as food colors in eastern countries, and which present potential use in meats, beverages, sauces and soups. This fungus produces a mixture of polyketidic pigments among which the red ones are regarded as the most important [1]. *Monascus* pigments are moderately stable in slightly acidic to basic pHs and at temperatures up to 60 °C [2]. Other important *Monascus* metabolites are citrinin (a mycotoxin, undesirable in the product), and a series of anti-hyperlipidemic substances such as monacolins K and L [3]. During *Monascus* cultivation, CO_2, ethanol and acetate are also produced [4]. The conditions for *Monascus* fermentation are species dependent, but the optimal temperature is in the range of 28–32 °C for growth and pigment production for most species. As for the initial pH, growth occurs in a wide range, from 2.5 to 8.0, and the ideal range for pigment production is 4.0–7.0 [5].

Fungi from the genus *Monascus* are unable to grow anaerobically using glucose as a substrate, but may grow with oxygen limitation. In this condition, there is a higher production of ethanol and CO_2, but there is a lower pigment production. With a higher aeration, ethanol production decreases while pigment increases [6]. In high glucose concentrations (above 20 g/L, in liquid cultures), a Crabtree-like effect occurs, i.e., a shift to a mainly anaerobic metabolism, with ethanol production, even in good aeration conditions [7]. With lower glucose concentrations, or with other sugars, in liquid fermentation, it is possible to divide the production of pigments in two phases: at first, glu-

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cose is converted to ethanol and biomass; later on, ethanol is converted to biomass and pigments [8].

Traditionally *Monascus* has been cultivated on rice (forming ang-kak or red rice), although several other media, either liquid or solid, have been tested for pigment production. This is the natural substrate which gives the best production, compared to other typical cereals, tubers and leguminous plants.

Solid-state fermentation (SSF) is a traditional method of fermentation, used since centuries in the production of traditional foods in Orient, such as ang-kak itself [9–11]. Although liquid fermentation is used in most industrial fermentative processes, there are advantages for the production of *Monascus* pigments by SSF [11,12], with high productivity of pigments at a relatively cheap cost. Furthermore, the extraction of pigments from SSF matter seems to be more efficient, since the production in solid media is in concentrated form.

The analysis of pigment production for *Monascus* is usually done by measuring the absorbance of pigment in the ranges near 400, 470 and 500 nm for yellow, orange and red pigments, respectively [1,13]. Since the samples must be, in several cases, diluted for the reading in the spectrophotometer, the absorbance values may not be directly comparable and should be converted to a "specific absorbance", accounting the dilution factor of the sample [14]. The best procedure for pigment analysis is liquid chromatography which, although is a extended analysis, allow separation and quantification of individual pigments; using this method, Hajjaj et al. [15] determined that 1 unit of absorbance at 480 nm (AU_{480}) corresponds to 15 mg/L of red pigment with $M = 498$ g/mol, and used this equivalence for the conversion of absorbance readings of extracts into pigment masses.

Direct biomass estimation in solid-state fermentations is usually impossible, because of the difficulty in efficiently separate biomass from the substrate matrix; this problem excludes gravimetric methods. The biomass may be indirectly measured by the determination of cell components such as glucosamine (present in chitin, a fungal cell wall component), ergosterol (present in the cell membrane), or proteins or nucleic acids [11]. It is also possible to estimate the biomass produced in the process through a CO_2 balance, taking into account the composition of the biomass and the stoichiometry of the process. Ergosterol is a cell component which may be advantageously used for indirect determination of fungal biomass, because of its easiness of extraction and analysis (the technique is described in the next section). This compound is usually quantified for the determination of fungal biomass in soils, because it is the most important sterol in the cell membrane of fungi and some microalgae [16], and besides is a compound practically absent from plants and animals [17]. Ergosterol analysis was initially proposed for contamination estimation of cereals by fungi, but it was soon adopted as a method for estimation of living fungal biomass in soils [18], presupposing that ergosterol is a labile marker, which is quickly decomposed after cell death. In recent works, it was determined that fungal ergosterol is not labile – persisting in important amounts, depending on the conditions to which it is submitted. Therefore, care should be taken when ergosterol is used as a *marker* for *living* cells [19,20]. This fact, however, reinforces the utility of this method for analysis of SSF, since ergosterol dosage would represent the quantification of *total* biomass, because of the permanence of ergosterol during the whole process.

The aim of this work was to study the production of pigments by a strain of *Monascus* sp. using rice as substrate in SSF in column and drum type bioreactors.

2. Material and methods

2.1. Strain and preservation

A strain of *Monascus* sp. LPB 31, an isolate from a *Monascus* contaminated rice, obtained at the Biotechnology and Bioprocess Engineering Division (DEBB) of the Federal University of Paraná (UFPR) was used. It was routinely grown in potato-dextrose-agar medium at 32 °C for 10 days and preserved at 4 °C for 3 months, after which it was re-inoculated. A previous comparative study with *Monascus* strains showed that LPB 31, chose for further fermentations, produces less citrinin (18 mg/g dry substrate) and has a higher productivity than eight other strains referenced in [21].

2.2. Inoculum preparation

A loopful of spores was transferred from the stock culture to PDA medium in Petri dishes and incubated at 32 °C for 10 days. After incubation, spore suspension was prepared by the addition of 5 mL 0.1% Tween-80, followed by gentle rubbing with a Drigalski spatula. Spore suspension was standardized by addition of sterile distilled water to 0.5–2.5 × 10^6 spores/mL. Spores were counted by microscopy, using a Neubauer chamber in a microscope (Leica DMLS). The inoculum was transferred to the flasks, columns or reactor in which fermentations were performed using pipettes, followed by medium homogenization. The inoculation ratio used was equivalent to 5% of the material to be fermented.

2.3. Fermentation

Whole rice (2–4 mm) was used as substrate. It was moistened with water (53% initial moisture), autoclaved, cooled, inoculated with 5 × 10^4 spores/g wet substrate and incubated at 32 °C in glass columns with 20 cm length, 4 cm diameter containing 60 g of substrate. Force aeration was carried out in the columns with a flux of 1 N mL/g substrate min (milliliter of air in standard conditions, per gram of wet substrate, per minute) of air for 1–8 days. Samples, as whole column, were withdrawn at intervals of 24 h. SSF was also carried out under similar conditions for 16 days in a drum reactor. The fermentation samples were vacuum-dried (200 mmHg) at 40 °C for 12 h and pulverized in an electric mill.

2.4. Biomass analysis

Samples of 0.5 g of dry fermented substrate from each column were used. To prepare biomass standards, 100 mL liquid medium [13] were inoculated with a loopful of mycelium

(around 0.5 cm^2) from a Petri dish, and incubated at 32 °C and 120 rpm for 7 days. The biomass obtained (a sample of 0.4 g) was filtered through paper, washed with distilled water and dried at 40 °C in a dessicator with silica gel as adsorbent for 12 h.

2.5. Ergosterol extraction

The fermented samples (column and pure biomass) were taken in flasks to which 2 mL analytical grade ethanol and 1 mL 2 M NaOH were added. The flasks were agitated, closed and incubated at 70 °C for 30 min, with periodic agitation. After incubation, 2 mL of 1 M hydrochloric acid were added, mixed by agitating the flasks and then 1 mL of 1 M $KHCO_3$ and 2 mL *n*-hexane were added. The mixture was agitated, transferred to a test tube and centrifuged at 3000 g for 10 min, at 20 °C to aid separation of light and heavy phases. The light phase (*n*-hexane) was separated and a new extraction was made again with 2 mL *n*-hexane, followed by a third extraction with 1 mL *n*-hexane. After pooling the extracts, the organic phase was evaporated under vacuum (200 mmHg) at 35 °C, resuspended in 200 mL *n*-hexane and filtered through a PVDF membrane.

2.6. Ergosterol analysis

The extracts were analyzed in an HPLC (Varian ProStar) with a C_{18} column and a PDA (photodiode array) detector set to 282 nm. The solvents used were HPLC-grade from SIGMA. The elution conditions were based on previous reports [16–20]. An injection volume of 10 μL of the sample was used. The mobile phase used was methanol (from 0 to 3 min), acetonitrile from 3 to 10 min, pure methanol from 10 to 15 min with a flow of 2 mL/min. The retention time obtained for the standard was 3.35 min. As standard, a solution of ergosterol PA with 10000 μg/mL with dilutions to 5000 and 1000 μg/mL was used. The baseline was determined with 10 mL of pure hexane. The ergosterol content in the biomass was considered to be 5.06 mg/g [21], and this value was used to convert chromatographic results into a biomass estimate.

2.7. Pigment and citrinin analysis

The pigments produced in the fermentation were extracted with ethanol and determined by spectrophotometry at 500 nm, corresponding to red pigments. Samples were also assayed for citrinin content, determined by HPLC (both analyses according to methods described in Ref. [21]).

2.8. Respirometry studies in columns

With the aim of determining the kinetic parameters for growth and pigment production by *Monascus* LPB 31, a column fermentation system was used with controlled temperature in a water bath at 32 °C. Ten glass columns with 20 cm length, 4 cm diameter were used with 60 g of rice (preinoculated before packing) (Fig. 1). The columns were closed at both ends with cotton plugs, connected to humidifiers and aerated with 1 N mL/min g medium for each column (value set with the aid of a rotameter). The production of CO_2 and O_2 consumption by the cultures were measured through a GC (Shimadzu GC-8A, Shimadzu Co., Japan), which was linked to a program for chromatograph control and integration (Chroma Biosystèmes, Ltd., France). The column used in the GC was a Porapak 80/100 at 60 °C, with 2 m length, with helium as carrier gas and a thermal conductivity detector. Samples, as whole columns were withdrawn at intervals of 24 h and the fermented media from the columns were vacuum-dried (10 mmHg, 45 °C) for 24 h, followed by pigment and biomass analysis as described above.

2.9. Respirometry studies in drum bioreactor

The respirometric studies in horizontal drum bioreactor was carried out by taking 2400 g of rice and fermented under the

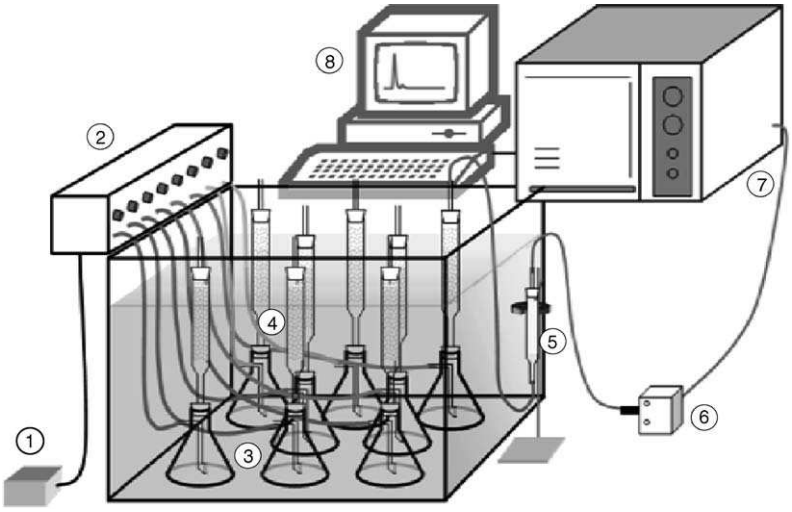

Fig. 1. Column set-up for respirometry studies. (1) Air pump; (2) air distribution system; (3) humidifiers; (4) fermentation columns—these are immersed in a water bath with controlled temperature; (5) drying column attached to a column exit; (6) sampling valve; (7) gas chromatograph; (8) computer with data acquisition and control software.

similar conditions as above. The samples were withdrawn at regular intervals directly from the bioreactor, dried and analyzed for pigments as above.

2.10. Regression, curve fitting and kinetic parameters

Three computer programs were used for the determination of the kinetic parameters: for the sigmoid biomass fitting it was used the software Origin Pro 6.1 (Origin Lab). For linear regression and other graphics, the software used was Excel 2002 (Microsoft). For kinetic parameters determination, details were followed as described by Pandey et al. [11] and a program FERSOL (developed by Rodriguez-León [22]).

3. Results and discussion

Two fermentation systems (columns with 60 g of wet substrate and a drum reactor with 2400 g of wet substrate) were used to cultivate Monascus over rice, with the same inoculum, air flow, substrate composition and temperature. The first experiment consisted in determining the adequate airflow in columns; then, respirometric analysis was performed in columns and the reactor.

3.1. Effect of aeration rates in columns

SSF was carried out in glass columns for 8 days with different flow rates of aeration. The results obtained are presented in Fig. 2, with the specific absorbance as a function of airflow: apparently most adequate air flow was 1 N mL/g min, i.e., 1 mL of air in standard conditions, per gram of wet substrate, per minute. Lower pigment production for airflows below 1 N mL/g min could be due to the deficiency in oxygen transfer to the culture. However, this could not be case with higher flow rates, but these also resulted in lower pigment production. Although temperature and air humidity were supposedly controlled, it is conceivable that there was a slight alteration of medium humidity, or other factors capable of altering the growth of the microorganism. It could be possible too that the excessive

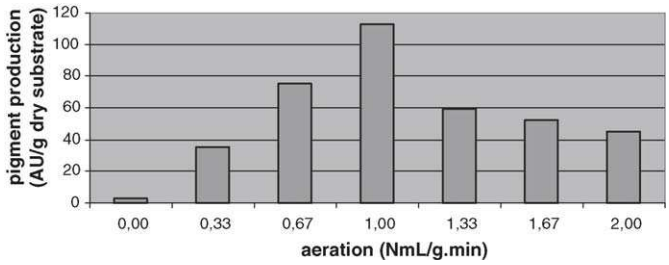

Fig. 2. Effect of aeration on pigment production (as specific absorbance at 500 nm) in rice SSF.

aeration decreased the pigment production because of a shift on the fungal metabolism. However, it would need further investigation. In the subsequent experiments in columns, 1 N mL/g min airflow was used.

3.2. Respirometric analysis in columns

Fig. 3 shows results of the respirometric analysis in columns, using rice as substrate: O_2 consumption, production of CO_2, pigments (as SPABS), biomass and respiratory quotient Q in the course of time. Sigmoid curves (Boltzmann equation) were chosen to fit the produced biomass and pigment data, and the following equations were obtained:

$$\text{Biomass (g)} = \left[\frac{0.1778 - 4.7352}{1 + e^{(\text{time}-152.09)/16.83}}\right] + 4.7352 \quad (R^2 = 0.950) \qquad (1)$$

$$\text{SPABS (AU/g)} = \left[\frac{0 - 5459}{1 + e^{(\text{time}-166.30)/18.13}}\right] + 5459 \quad (R^2 = 0.980) \qquad (2)$$

These equations were used to generate the fitting curves also present in Fig. 3, using the program MS-Excel. The estimate of the specific velocities was made point to point, using the expressions: $\mu \sim X^{-1}\Delta X/\Delta t$, and $q_p \sim X^{-1}\Delta P/\Delta t$; the corresponding data is presented in Fig. 4.

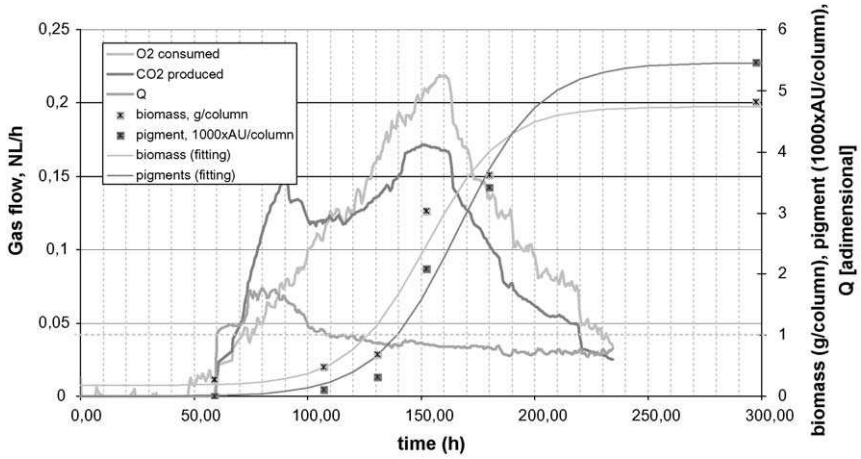

Fig. 3. Results of the respirometric analysis in columns, using rice as substrate: O_2 consumption, production of CO_2, pigments (as SPABS), biomass and respiratory quotient Q in the course of time.

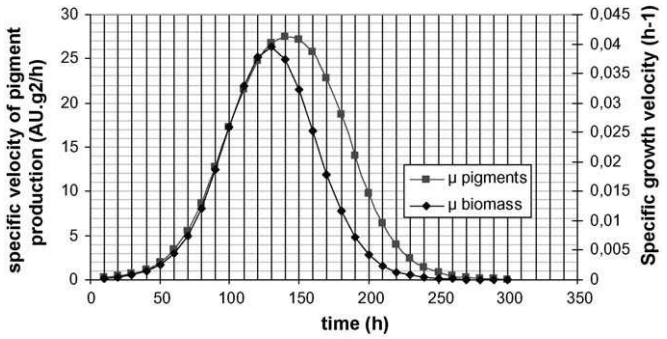

Fig. 4. Specific velocity of pigment and biomass formation for column fermentation, based on sigmoid models.

To discuss the data on Figs. 3 and 5 the fermentation was divided in phases, based on the analysis of the curve for Q (respiratory quotient), biomass and pigments, as reported in Fig. 3:

Lag phase, 0–60 h: This phase represented the adaptation to the culture medium and the spore germination, hence the amount of CO_2 produced (and diluted by the air flow) was almost nil and the respiratory quotient Q was zero (for periods less than 60 h). The biomass and pigment production also showed low values. In should be noted that 60 h was a rather long lag phase regarding industrial fermentations, and this fact denoted the need to increase the inoculum concentration or the inoculation ratio, working with a pre-fementer.

Fermentation, 60–90 h: From 60 h until around 85 h the value of Q increased, reaching a maximum value of 2, which denoted a high respiratory activity, with the produced CO_2 far higher than to consumed O_2. This showed that there must be another metabolite formed (other than CO_2) related with anaerobiosis, because the production of CO_2 (with carbon oxidated in comparison with carbon on the substrate) implies the reduction of part of the substrate. In effect, the smell of the exhaust gas in the beginning of the fermentation resembled that of ethanol (data not shown), which was also observed by other authors [6,4]. The aeration, however, should be enough to guarantee the aerobic development of the small amount of biomass present in this phase. The production of ethanol could be explained by a Crabtree-like effect, also observed by other authors [7,8] in submerged fermentation.

Shift to respiration, 90–140 h: In this phase Q decreased to a value near 0.8 and the production of biomass and pigments started to increase; the uptake of oxygen and production of CO_2 reached a maximum ($t = 150$–160 h). In this phase, the mycelium development was quite high, reaching a maximum estimated value of specific growth velocity $\mu = 0.039\ h^{-1}$, at $t = 130$ h. The specific pigment production also reached a maximum, with 27.50 AU/g^2 h at $t = 140$ h (values obtained by the derivation of the model curve $X = f(t)$ (Eq. (1)) (Fig. 4).

Respiration and pigment synthesis, 140 h to the end of the fermentation: In this phase, Q remained constant around 0.8. The uptake of oxygen and production of CO_2 were also constant. There was formation of large amounts of pigments, which was consistent with the secondary metabolite formation in SSF [11]. The specific velocity of biomass and pigments synthesis decreased (Fig. 4). This indicated the deceleration of the growth, which ceased its exponential phase. From 280 h on, the pigment productivity (0.76 AU/g h) was roughly 10 times inferior to the maximum productivity of approximately 74 AU/g h at 170 h of fermentation.

3.2.1. Determination of fermentation parameters using the program FERSOL

The program FERSOL used the consumed oxygen and produced biomass data to estimate the fermentation parameters for the model:

$$\frac{dO_2}{dt} = \frac{1}{Y_{X/O}}\frac{dX}{dt} + mX \tag{3}$$

where dO_2/dt is the oxygen uptake rate, in g/h; $Y_{X/O}$ is the relation between biomass formed and consumed oxygen [biomass

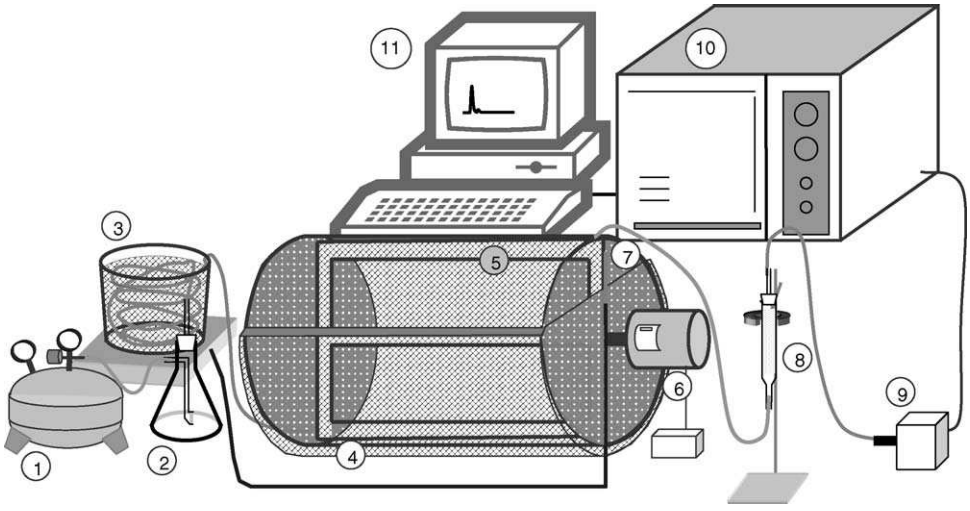

Fig. 5. Horizontal drum bioreactor and respirometry system. (1) Air compressor; (2) humidifier and filter system; (3) air heater: the tubing is immersed in water, warmed by a heating plate; (4) bioreactor with an air diffusion jacket; (5) mixing pad; (6) timer-controlled motor for mixing; (7) thermocouple near the air outlet; (8) drying column; (9) sampling valve; (10) gas chromatograph; (11) computer with data acquisition and control software.

Table 1
Values of time, biomass and pigment produced, consumed O_2 and conversions of substrate in biomass ($Y_{X/S}$), substrate in product ($Y_{P/S}$), oxygen in biomass ($Y_{X/O}$) and oxygen in product ($Y_{P/O}$) used for the estimations of kinetic parameters through the program FERSOL

Fermentation time (h)	Produced biomass (mg/column)	Pigment produced (AU/column)	O_2 consumed (mmol/h)	O_2 acum. (mmol)	$Y_{P/S}$ (mg/g)	$Y_{X/S}$ (g/g	$Y_{P/O}$ (g/mol)	$Y_{X/O}$ (g/mol)
0	30	0	0	–	–	–	–	–
59	278	0	0.224	0.01	0	0.0219	0	–
107	480	109.7	4.804	136	0.109	0.0298	0.0121	3.3100
131	681	313.5	7.227	275	0.268	0.0372	0.0171	2.3687
152	3040	2082.3	9.283	454	1.503	0.1449	0.0688	6.6290
180	3625	3423.3	6.261	683	2.088	0.1463	0.0751	5.2635
297	4816	5458.9	0	915	3.335	0.1950	0.0894	5.2307

units/O_2 units]; dX/dt is the biomass production rate, in g/h; m is a maintenance coefficient, reflecting the amount of O_2 used in other functions besides biomass production, in h^{-1}; X is the amount of biomass in the system, in g.

The data applied to the program are presented in Table 1, which represents a material balance on a column. From the values of time, biomass and consumed O_2, using the program FERSOL, the following values were obtained: $Y_{X/O} = 3.703$ g biomass/mol O_2, $m = 0.000\ h^{-1}$ and $\mu_{max} = 0.038\ h^{-1}$, in the range of 59–180 h, with a regression coefficient $R^2 = 0.989$—a value that indicated good correlation between the data and the model. The value of $m = 0$ indicated, in fact, that the value was too small to be estimated by the program, which showed that biomass production was probably far superior to that of pigments (or other metabolites, which needed oxygen uptake). This hypothesis was supported by the small values of $Y_{P/S} = 0.00335$ and $Y_{P/O} = 0.0894$ at the end of the fermentation, showing that substrate and oxygen conversions in pigments were far inferior to the conversions in biomass. The value of $Y_{X/O}$ obtained by the program was similar to that obtained by direct calculation of kinetic parameters in the range near 107 h of fermentation. The value found for μ_{max} was similar to the one obtained using the sigmoid model for biomass, with $\mu_{max} = 0.039\ h^{-1}$. This value indicates that *Monascus* fermentation could have its time reduced, provided that the conditions for inoculation and incubation were adequate to provide a higher specific growth velocity *during the whole fermentation*. The absence of a lag phase is improbable, but better adaptation of the inoculum should provide a reduction of fermentation time.

At the end of the column fermentation, 17 mg of pigment/g biomass were obtained, a value near to that obtained in submerged fermentation by Hajjaj et al. [15], estimated through a graph as 16.8 mg pigment/g biomass. The fact that in SSF these pigments are less dispersed, in the proportion of approximately 5 g pigment/kg wet fermented matter (with around 60% water), against around 0.1 g pigment/L in submerged fermentation, show that the advantage of cultivation of *Monascus* in SSF lies in the concentration, rather than absolute production.

3.2.2. Correlation biomass-pigment

A correlation analysis (not shown) of the data from whole columns (biomass and pigments produced) presented in Table 1, showed the correlation coefficient $R = 0.977$, which suggested a fair proportionality between both factors. Since a correlation for biomass concentration × specific absorbance could be used to estimate biomass in further fermentations, concentration data for columns (presented in Table 2), allowed the following expression to be found:

$$\text{Biomass} = -2.10^{-06}\text{SPABS}^2 + 0.0028\text{SPABS} + 0.0302, \quad \text{with } R^2 = 0.9955 \tag{4}$$

where biomass: biomass in the dry fermentate (g/g); SPABS: specific absorbance, in AU/g dry substrate.

Whereas this expression should be used with care, since it probably depends on the strain used and on the cultivation conditions, it is a simple way to estimate the biomass production during the course of the fermentation, since the extraction of pigments and the subsequent absorbance reading is a analysis far more simple than ergosterol extraction and chromatographic analysis.

3.2.3. Fermentation kinetics in a horizontal drum bioreactor

Fig. 5 shows the set-up of horizontal drum bioreactor. SSF was carried out under similar conditions of column with aeration of 1 N mL/g min (i.e., 2.4 N L/min). Consequently, the scaling-up criterion used was a constant ratio of airflow/fermentation medium. A thermal insulation was provided for the reactor, and a temperature sensor linked to a thermostat and a heater was placed near the gas exhaust from the reactor, in order to control the system temperature. There were variations in temperature, especially on the fifth day of fermentation. However,

Table 2
Pigment and biomass production related to dry fermented substrate, at different phases fermentation—data used for correlation

SPABS (AU/g dry product)	Biomass (g/g dry product)
0	0.01591
1.29	0.04040
3.68	0.03499
12.57	0.06044
112.8	0.35717
315.8	0.71838
269.5	0.58909
232.8	0.59636
291.7	0.68121
499.9	0.93737

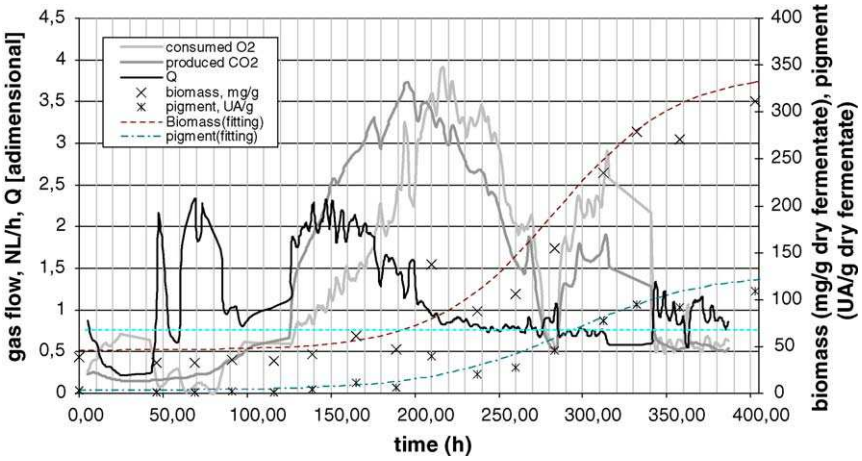

Fig. 6. Results of the respirometric analysis in drum-type horizontal bioreactor using rice as substrate–oxygen uptake, CO_2 and pigment production (as SPABS), biomass production and respiratory quotient in the course of time.

same general tendency was observed as with column fermentations (Fig. 6).

The analysis of Fig. 6 showed the same fermentation pattern in the drum bioreactor as in column fermentation, but with a longer lag phase and a overall worse development of the culture. Again, the respiratory quotient Q was used as reference to divide the fermentation time in the following phases: before 80 h there was a phase of very slight growth; from 80 to 150 h mainly anaerobic metabolism, with $Q > 1$ and a slight increase in biomass production; from 150 to 220 h there was a decrease in Q, which stabilized around 0.8, but still with an increase in pigment and biomass production; and from 220 to 350 h the production of pigments and biomass was high, with Q in a approximately constant value. The important decline in oxygen uptake and CO_2 produced, at $t = 280$ h, suggested analysis problems, since it contrasted with the high biomass and pigments production.

Adapting again a sigmoid model to the values of estimated biomass (using equation (4)) and pigment produced in the course of the fermentation, the following expressions were obtained:

$$\text{Biomass (g)} = \left[\frac{46.114 - 341.55}{1 + e^{(\text{time}-283.8)/35.666}}\right] + 341.55 \qquad (R^2 = 0.953) \qquad (5)$$

$$\text{SPABS (AU)} = \left[\frac{3038 - 129000}{1 + e^{(\text{time}-292.9)/40.894}}\right] + 129000 \qquad (R^2 = 0.957) \qquad (6)$$

These equations were used to generate graphics (figure not presented), in the same way done with Eqs. (1) and (2), and Fig. 4, using MS-EXCEL program. The determination of the specific velocity of growth was done point-to-point, using the expressions: $\mu \sim X^{-1}\Delta X/\Delta t$, and $q_p \sim X^{-1}\Delta P/\Delta t$.

The regression coefficients for Eqs. (5) and (6) show that the sigmoid model satisfyingly represented the estimated biomass production. The maximum specific growth velocity was $\mu_{max} = 0.013\ h^{-1}$, a value three times lower than that obtained in columns. The specific product formation velocity was 70 μg of pigment/g biomass h, or 4.7 AU/g h, and the total pigment production was 108.7 AU/g dry fermentate—a value 4.6 times lower than that obtained in columns. Since the medium composition and inoculum rate were the same as in column fermentations, the reasons for the poorer performance of the drum reactor remain to be determined. Technical reasons might be the deficient temperature control and eventual formation of preferential flows in the fermentation bed, decreasing actual aeration rates (although in each daily sampling the medium was homogenized to avoid clogging). However, it is important to remember that the two reactors have different geometries, and the criterion for scaling-up (maintenance of the airflow as related to substrate amount) may not be adequate.

4. Conclusions

For the production of *Monascus* pigments in SSF, best conditions were forced aeration (with water-saturated air) of 1 N mL/min g of wet substrate in an 8 cm height bed at 32 °C for 8 days. Under these conditions, a maximum specific growth velocity of 0.039 h^{-1} and a specific pigment production velocity of 27.5 AU/g biomass h were obtained. The same airflow, applied to a horizontal drum-type bioreactor, gave a slower growth, possibly due to deficiencies in the aeration. This indicated that aeration played a critical influence in pigment formation, due to the alteration in humidity and oxygen transfer and, as a consequence, on the metabolism. On the other side, although *Monascus* pigments are a secondary metabolite (i.e., not associated to growth), the amount of pigments produced was directly proportional to the biomass produced for a given substrate and cultivation conditions. Therefore, it could be likely the manipulation of aeration in different fermentation phases works as a tool to be explored regarding the increase in productivity of *Monascus* pigments.

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References

[1] M.R. Johns, D.M. Stuart, Production of pigments by *Monascus purpureus* in solid culture, J. Ind. Microbiol. 8 (1991) 23–38.

[2] J.C. Carvalho, B.O. Oishi, A. Pandey, C.R. Soccol, Biopigments from Monascus: strains selection, citrinin production and color stability, Braz. Arch. Biol. Technol. 48 (6) (2005) 885–894.

[3] J. Ma, Y. Li, Q. Ye, J. Li, Y. Hua, D. Ju, D. Zhang, Constituents of red yeast rice, a traditional chinese food and medicine, J. Agric. Food Chem. 48 (2000) 5220–5225.

[4] A. Rosenblitt, E. Agosin, J. Delgado, R.P. Correa, Solid substrate fermentation of *Monascus purpureus*: growth, carbon balance, and consistency analysis, Biotechnol. Prog. 16 (2000) 152–162.

[5] B. Yongsmith, W. Tabloka, W. Yongmanitchai, R. Bavavoda, Culture conditions for yellow pigment formation by *Monascus* sp. KB 10 grown on cassava medium, World J. Microbiol. Biotechnol. 9 (1993) 85–90.

[6] L. Pastrana, P.J. Blanc, A.L. Santerre, M.O. Loret, G. Goma, Production of red pigments by *Monascus ruber* in synthetic media with a strictly controlled nitrogen source, Proc. Biochem. 30 (4) (1995) 333–341.

[7] M.H. Chen, M.R. Johns, Effect of carbon source on ethanol and pigment production by *Monascus purpureus*, Enzyme Microb. Technol. 16 (1994) 584–590.

[8] M. Hamdi, P.J. Blanc, G. Goma, Effect of aeration conditions on the production of red pigments by *Monascus purpureus* growth on prickly pear juice, Proc. Biochem. 31 (6) (1996) 543–547.

[9] A. Pandey, Recent developments in solid-state fermentation, Proc. Biochem. 27 (1992) 109–117.

[10] A. Pandey, C.R. Soccol, D.A. Mitchell, New developments in solid-state fermentation. Part 1. Bioprocesses and products, Proc. Biochem. 25 (2000) 1153–1169.

[11] A. Pandey, C.R. Soccol, J.A. Rodriguez-Leon, P. Nigam, Solid State Fermentation in Biotechnology—Fundamentals and Applications, first ed., Asiatech Publishers Inc., New Delhi, 2001.

[12] C.R. Soccol, L.P.S. Vandenberghe, Overview of applied solid-state fermentation in Brazil, Biochem. Eng. J. 13 (2003) 205–218.

[13] T.F. Lin, A.L. Demain, Formation of water-soluble *Monascus* red pigments by biological and semi-synthetic processes, J. Ind. Microbiol. 9 (1992) 173–179.

[14] S.W. Chiu, Y.K. Poon, Submerged production of *Monascus* pigments, Mycologia 85 (2) (1993) 214–218.

[15] H. Hajjaj, P. Blanc, E. Groussac, J.L. Uribelarrea, G. Goma, P. Loubiere, Kinetic analysis of red pigment and citrinin production by *Monascus ruber* as a function of organic acid accumulation, Enzyme Microb. Technol. 27 (2000) 619–625.

[16] P. Gong, X. Guan, E. Witter, A rapid method to extract ergosterol from soil by physical disruption, Appl. Soil Ecol. 17 (2001) 285–289.

[17] P. Matilla, A.M. Lampi, R. Ronkainen, J. Toivo, V. Piironen, Sterol and vitamin D2 contents in some wild and cultivated mushrooms, Food Chem. 76 (2002) 293–298.

[18] H.J. Montgomery, C.M. Monreal, J.C. Young, K.A. Seifertd, Determination of soil fungal biomass from soil ergosterol analyses, Soil Biol. Biochem. 32 (2000) 1207–1217.

[19] C.M. Lindblom, E. Wachenfeldt, L.J. Tranvik, Ergosterol as a measure of living fungal biomass: persistence in environmental samples after fungal death, J. Microbiol. Methods 59 (2004) 253–262.

[20] X.R. Zhao, Q. Lin, P.C. Brookes, Does soil ergosterol concentration provide a reliable estimate of soil fungal biomass? Soil Biol. Biochem. 37 (2) (2005) 311–317.

[21] J.C. Carvalho, Desenvolvimento de bioprocesso para a produção de pigmentos a partir de Monascus por fermentação em substrato sólido. Curitiba, 2004. Tese (Doutorado em Processos Biotecnológicos). Setor de Tecnologia, Universidade Federal do Paraná.

[22] J.A. Rodriguez-León, L. Sastre, J. Echevarria, G. Delgado, W. Bechstedt, A mathematical approach for the estimation of biomass production rate in solid state fermentation, Acta. Biotechnol. 8 (1988) 307–310.