Appl Microbiol Biotechnol (1993) 40:132–138

Applied and Microbiology Biotechnology

Effect of pH and nitrogen source on pigment production by *Monascus purpureus*

Ming-Ho Chen, Michael R. Johns

Department of Chemical Engineering, The University of Queensland 4072, Queensland, Australia

Received: 11 November 1992/Accepted: 23 April 1993

Abstract. The effect of pH and nitrogen source on pigment production by *Monascus purpureus* 192F using glucose as the carbon and energy source, was studied in pH-controlled, batch fermentor cultures using HPLC analysis to determine individual pigment concentrations. A maximum of four pigments were detected in fungal extracts. These were the yellow pigments monascin and ankaflavin, the orange rubropunctatin and the red pigment monascorubramine. Monascorubramine was present as the major product in all instances. Fungal growth and ankaflavin synthesis were favoured at low pH (pH 4.0), whereas production of the other pigments was relatively independent of pH. The nature of the nitrogen source affected fungal growth and pigment production, independent of pH. Ammonium and peptone as nitrogen sources gave superior growth and pigment concentrations compared to nitrate. Ankaflavin was not detected in nitrate cultures. The highest red pigment production was obtained using a glucose-peptone medium at pH 6.5, due to the secretion of red pigments into the medium under these conditions.

Introduction

The search for naturally-produced substitutes for chemical food colourants has led to a resurgence of interest in the pigments synthesised by the fungus *Monascus purpureus,* also called *M. anka* or *M. kaoliang.* This microorganisms has been used in Asia for many centuries to colour and flavour food and beverages (Lin 1973; Murakawa 1990). The red pigments are of particular interest, because red is the most popular food colour and true red natural pigments suitable for food use are difficult to obtain.

M. purpureus produces a family of structurally-related, hexaketide pigments, which in aqueous solution range in colour from bright red to bright yellow. Six individual compounds have been isolated from both liquid and solid cultures of the fungus (Fielding et al. 1961; Hadfield et al. 1967; Kurono et al. 1963; Manchand and Whalley 1973). They comprise two red, two orange and two yellow pigments. These pigments have good properties as food colourants, possessing reasonable light and chemical stability, tinctorial strength and water solubility when complexed with appropriate compounds (Wong and Koehler 1983; Francis 1987).

The biosynthesis of the pigments is poorly understood. Studies by many researchers have revealed that pigment production in submerged culture is affected by numerous environmental factors, particularly the nature of nitrogen source and medium pH (Lin 1973; Carels and Shepherd 1977, 1978; Su and Huang 1980; Wong et al. 1981; Broder and Koehler 1980; Lin and Demain 1990). Unfortunately, the results are often difficult to interpret, due to the use of shake-flask cultures, in which the pH changes during cultivation and is dependent on the nitrogen source used. Furthermore, individual pigment concentrations have not been determined. Rather, total pigment concentration has been measured by absorbance at 400, 470 and 500 nm for yellow, orange and red pigments, respectively. Consequently, little is known about how the synthesis of each pigment is affected by changes in pH or nitrogen source. Not surprisingly, there is considerable inconsistency in the results reported to date.

This paper reports the use of pH-controlled fermentor cultures and a high performance liquid chromatograph (HPLC) method for pigment analysis to elucidate the effect of pH and nitrogen source on the production of pigments by *M. purpureus* 192F.

Materials and methods

Organism. M. purpureus UQM 192F (FRR 2190) was obtained from the CSIRO Food Research Laboratories (North Ryde, NSW, Australia) and was maintained on potato dextrose agar (PDA) slopes (Difco, Detroit, Mich., USA) at 4°C.

Correspondence to: M. R. Johns

Inoculum and media. One millilitre of a distilled water (10 ml) suspension from a 6-day-old PDA slope of *M. purpureus,* grown at 30°C, provided a 1% (v/v) inoculum for shake-flask cultures. For the 2-l fermentations, 100 ml of actively growing shake-flask culture grown on identical medium was used. The growth medium was composed of (in g/l): glucose, 50; one of the nitrogen sources, $NaNO_3$, 3; NH_4Cl, 2; or bacto-peptone, 5 (Difco); KH_2PO_4, 1.0; $MgSO_4 \cdot 7\,H_2O$, 0.5, and trace metals (Johns et al. 1982). Sterilisation of the media was performed at 121°C for 15 min. All medium components were analytical grade and used with distilled water.

Cultivation. Shake-flask culture was performed in 250 ml erlenmeyer flasks containing 100 ml medium incubated at 30°C in an orbital water bath (150 rpm) in darkness.

Batch fermentor cultures were performed in a 2-l glass vessel (B. Braun, Sydney, Australia) sealed with a stainless steel headplate. The working volume was 1.3 l. Agitation was provided at 500 rpm by a flat-bladed impeller and sterile air was supplied at 1.3 l/min. Automatic temperature (30°C) and pH control was performed, the latter using a pH control module (LH Fermentation, Stoke Poges, UK) equipped with a steam-sterilisable pH electrode (Ingold, Urdorf, Switzerland) and sterile solutions of 1 M NaOH or 1 M HCl. A foam controller (LH Fermentation) added silicon antifoam (Dow Corning, Sydney, Australia) as required.

The glass vessel was autoclaved at 121°C for 15 min and sterile medium was added aseptically upon cooling. After inoculation (7.5%, v/v), the fermentor was maintained in darkness.

Analysis. Fermentation samples (20 ml) were collected aseptically and filtered through no. 1 Whatman filter paper, washed twice with 20 ml distilled water and the resulting mycelial mat dried in vacuo at 65°C to a constant weight. Glucose and nitrate concentrations were determined by HPLC and suppressed ion chromatography, respectively, according to the method of Johns and Stuart (1991). Prior to analysis, protein was removed from samples using Centriflo ultrafilters (Amicon, Danvers, Mass., USA). Sample peak area was compared with that of a known standard, which was injected at the beginning of analysis and after every five samples.

Organic nitrogen and ammonium concentrations were determined by Kjeldahl semi-micro analysis (Morrison and Boyd 1987), with omission of sample digestion for ammonium determination.

Pigment concentration was determined by both spectrophotometric and HPLC analysis. Solution absorbance was measured on a spectrophotometer (Hitachi, Tokyo, Japan) at 400, 470 and 500 nm, corresponding to yellow, orange and red pigment concentrations, respectively (Carels and Shepherd 1977). The concentration of water-soluble, extracellular pigments in the sample filtrate was estimated using uninoculated medium as a blank. Intracellular pigments in a known weight of dried biomass were estimated by the method of Johns and Stuart (1991) using 20 ml of 95% ethanol.

Individual intracellular pigment concentrations were measured by a HPLC method modified from Sweeny et al. (1981). Five millilitres of the above ethanol extract was dried in vacuo at 65°C. The residue was dissolved in 5 ml mobile phase and filtered through a 0.2-μm hydrophobic membrane (Sartorius, Gottingen, Germany). Ten microlitres of filtrate was injected into a Waters HPLC using a C_{18}-μBondapak (300 mm × 3.9 mm) column (Bio-Rad, Richmond, Calif., USA) operated at 25°C with a tunable UV absorbance detector (Waters, Milford, Mass., USA) set at 392 nm. The mobile phase consisted of acetonitrile-water (70:30 v/v) at a flow rate of 1.0 ml/min.

Ankaflavin, monascorubrin and monascorubramine concentrations were determined by comparing sample peak area with that of authentic standards (gift of J. Sweeny, Coca Cola, Atlanta, Ga., USA). In the absence of sufficient quantities of authentic monascin and rubropunctatin, their concentration was estimated by assuming an identical peak area: concentration response as their respective analogues, ankaflavin and monascorubrin. Orange pigments (i.e. rubropunctatin) were differentiated from yellow (i.e. monascin) by the shift in retention time of the former, but not the latter, when treated with NH_4OH (Kurono et al. 1963). Retention times for pigments detected in this work are listed in Table 1 and correspond in order of elution to those of Sweeny (personal communication).

Table 1. HPLC identification of pigments produced by *Monascus purpureus* 192F

Monascus pigments	Retention time (min)	Standard error
Monascorubramine	1.6	0.02
Monascin	2.5[a]	0.05
Rubropunctatin	3.6[a]	0.01
Ankaflavin	7.3	0.08
Monascorubrin	7.8	0.14

[a] Retention time is the mean of thirty determinations

Results

Effect of pH

The results from batch fermentations using glucose-ammonium medium and performed at pH 4.0 and pH 6.5 are presented in Figs. 1 and 2, respectively. The pH values were chosen to permit comparison with previous studies by Carels and Shepherd (1978) and Yongsmith et al. (1989).

At pH 4.0, the mycelia formed dense pellets (1–2 mm diameter) and the broth became orange-red in colour within 2 days. Fungal growth began almost immediately and ended after 5 days due to nitrogen exhaustion, at which point glucose uptake also ceased (Fig. 1A). The observed growth yield was low (Table 2).

Pigment production, as measured by absorbance, lagged growth and peaked at 6 days for both intracellular and extracellular pigments (Fig. 1B). A decrease in absorbance was observed after this time. Maximum specific rates of both biomass growth and glucose consumption occurred after 2.5 days, whereas that for pigment synthesis occurred at day 4.

HPLC analysis revealed the presence of four pigments: monascorubramine (red), rubropunctatin (orange) and the yellow pigments, monascin and ankaflavin (Fig. 1C). The HPLC data differ substantially from the absorbance data (Fig. 1B) in two respects, First, whereas by HPLC analysis the monascorubramine concentration is much greater than yellow pigment concentrations, the absorbance data indicate the opposite [i.e. A_{400nm} (yellow) largely exceed A_{500nm} (red) readings]. Second, the pigment concentrations (by HPLC) were highest at day 4, compared to day 6 by absorbance.

Ankaflavin synthesis at pH 4.0 corresponded to a decrease in monascin concentration, but the maximum ankaflavin concentration was much lower. Concentra-

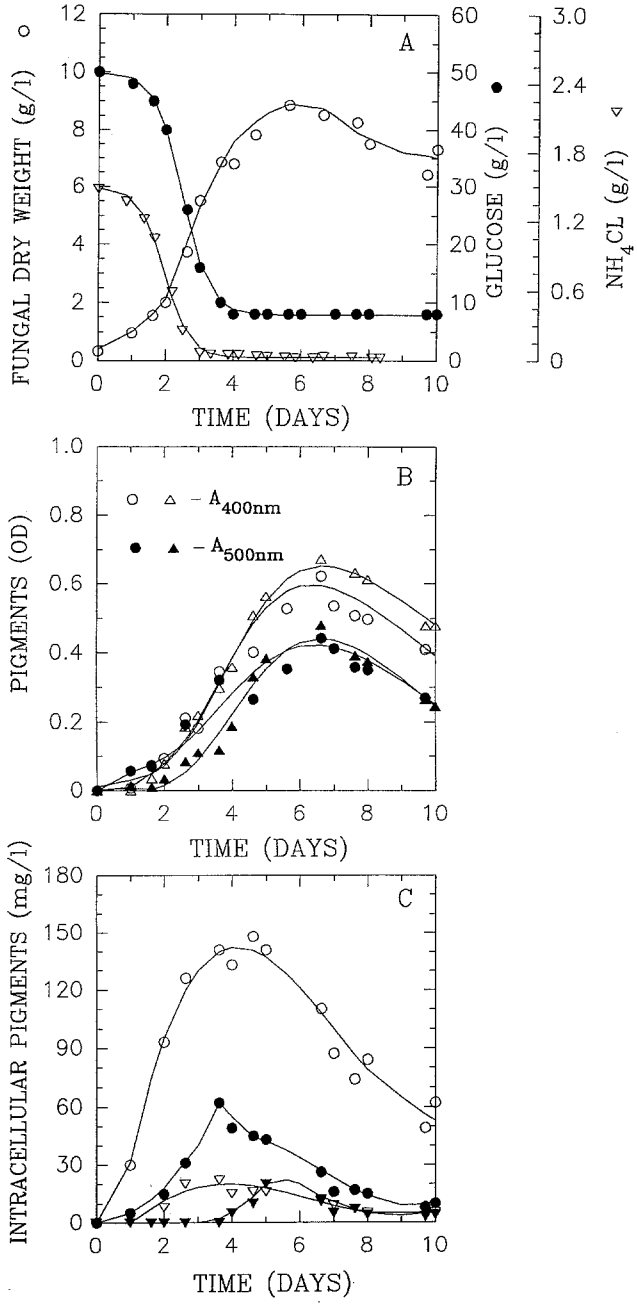

Fig. 1A–C. Growth and nutrient consumption (**A**), absorbance of intracellular (○, ●) and extracellular (△, ▲) pigments (**B**) and intracellular pigment (○, monascorubramine; ●, monascin; ▽, rubropunctatin; ▼, ankaflavin) concentration (**C**) in batch fermentor culture of *Monascus purpureus* 192F on glucose-ammonium medium at pH 4.0

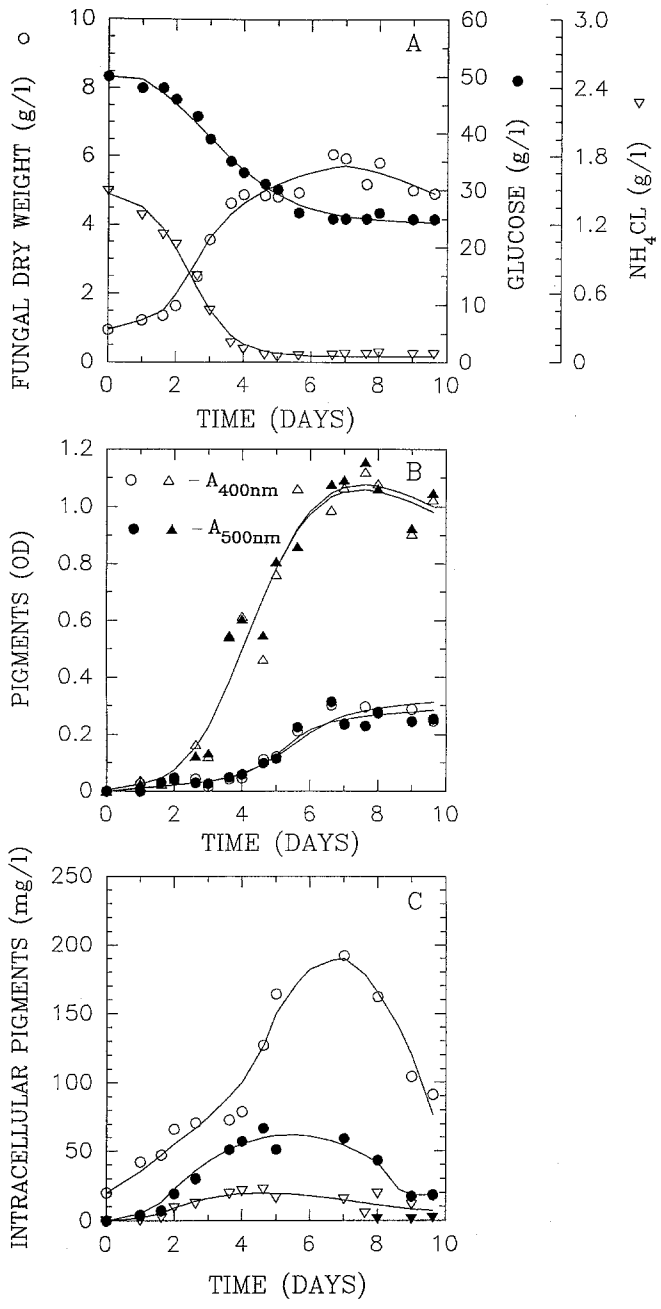

Fig. 2A–C. Growth, nutrient consumption (**A**) and pigment production (**B, C**) in batch fermentor culture of *M. purpureus* 192F on glucose-ammonium medium at pH 6.5. Symbols as for Fig. 1

Table 2. Effect of pH and nitrogen source on growth of *M. purpureus* 192F in batch fermentor cultures

	pH 4.0			pH 6.5		
Medium	GA	GN	GP	GA	GN	GP
$Y_{x/s}$ (g/g)	0.15	0.09	0.14	0.28	0.14	0.26
q_s (g/g/day)	7.8	10.5	11.4	2.9	5.2	6.1
μ_{max} (1/h)	0.036	0.073	0.035	0.028	0.011	0.025

GA, glucose-ammonium medium; GN, glucose-nitrate medium; GP, glucose-peptone medium; $Y_{x/s}$, observed growth yield; q_s, maximum specific rate of glucose consumption; μ_{max}, maximum specific growth rate

tions of all pigments decreased with time within 24 h of the maximum concentrations being attained.

At pH 6.5, the fungal morphology was similar to that at pH 4.0, but the culture turned red within 3 days of cultivation. Fungal growth and glucose consumption were relatively poor, with 25 g residual glucose/l remaining after fungal growth ceased (Fig. 2A). The observed growth yield, however, was higher than that at pH 4.0 (Table 2).

Pigment production, as measured by absorbance, lagged growth and peaked at day 7 (Fig. 2B) with a small decrease thereafter for the extracellular pigments. Yellow (A_{400nm}) and red (A_{500nm})absorbance was equivalent during the fermentation, but the majority of the absorbance was extracellular.

HPLC analysis revealed the presence of monascorubramine, monascin and rubropunctatin (Fig. 2C). In contrast to the pH 4.0 culture, only trace levels of ankaflavin were detected. The monascorubramine concentration peaked at day 7 at 180 mg/l. Whereas this concentration was higher than at pH 4.0, the concentrations of monascin and rubropunctatin were similar to those at pH 4.0. Monascorubramine and monascin concentrations decreased substantially after 6 days of cultivation, which corresponded to the end of fungal growth.

Effect of nitrogen source

The effect of the nitrogen source on pigment production was studied at both pH 4.0 and pH 6.5, using batch fermentor cultures with glucose-nitrate, or glucose-peptone medium. Selected data for nitrate and peptone cultures are presented in Figs. 3 and 4, respectively. Important fermentation variables are summarised in Table 2 for all the combinations of nitrogen source and pH tested.

Nitrate gave the lowest yields of *M. purpureus* of all the nitrogen sources tested (Table 2). At pH 4.0, the maximum biomass concentration was only 3.6 g/l dry weight, despite rapid growth and almost complete glucose consumption (Fig. 3A). At pH 6.5, the fungus grew only slowly. Glucose-nitrate cultures were notable for their selective synthesis of monascorubramine (Figs. 3B, 3C). Monascin and rubropunctatin concentrations were low compared to other nitrogen sources, especially at pH 4.0, and ankaflavin was not detected. The monascorubramine concentration was higher at pH 6.5 than pH 4.0, but in both fermentations the monascorubramine concentration increased only during the growth phase.

In contrast to nitrate, *M. purpureus* grew as well on peptone as on ammonium at both pH 4.0 and 6.5 (Table 2). The specific rates of glucose consumption were higher in peptone cultures. At pH 4.0, the glucose was almost completely consumed after 6 days (Fig. 4A) whereas at pH 6.5, the lower growth rate was accompanied by a high residual concentration of glucose (20 g/l). In both peptone fermentations, total nitrogen concentration profiles were similar and the total nitrogen concentration was not completely depleted.

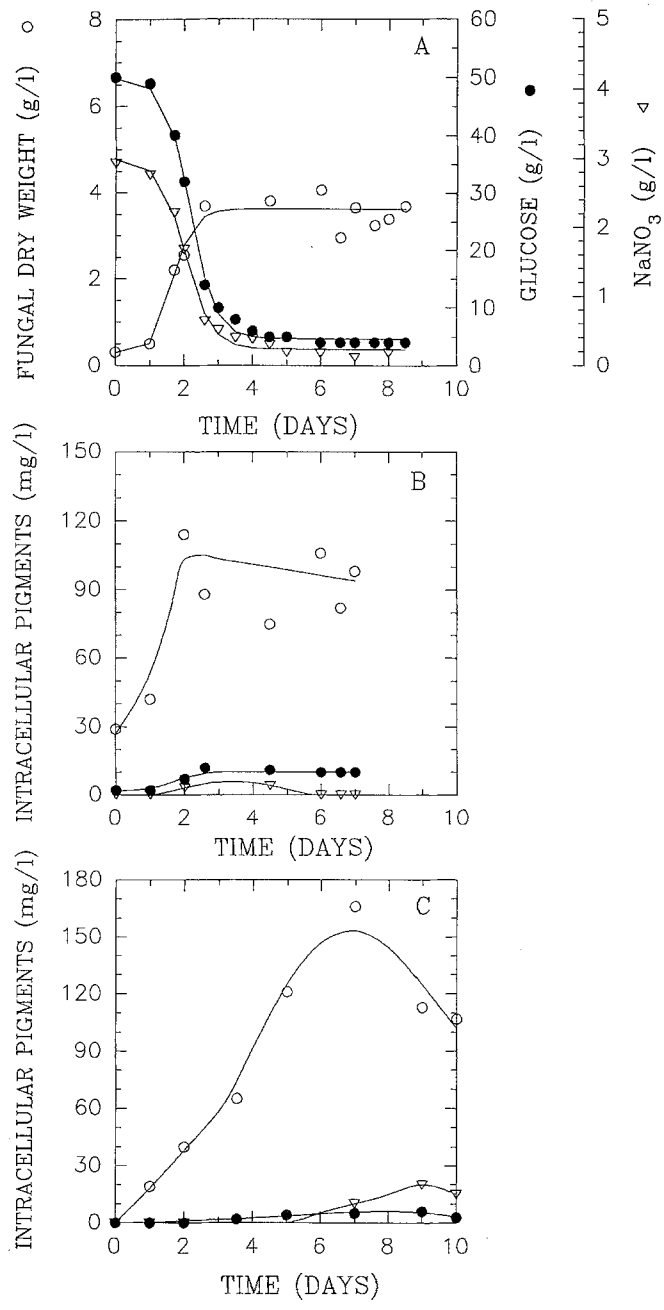

Fig. 3A–C. Growth and nutrient consumption at pH 4.0 (**A**), intracellular pigment concentrations at pH 4.0 (**B**) and pH 6.5 (**C**), in batch fermentor culture of *M. purpureus* 192F on glucose-nitrate medium. Symbols as for Fig. 1

Glucose-peptone cultures at pH 4.0 produced a mixture of pigments similar to the glucose-ammonium culture at pH 4.0 (Fig. 4B). Monascorubramine was the predominant pigment and its maximum concentration of 200 mg/l was the highest intracellular concentration observed in any of the media. Both monascin and ankaflavin were produced in relatively large concentrations, the concentration of the latter again increasing late in the fermentation as the monascin concentration fell.

At pH 6.5, the glucose-peptone culture had a similar pigment profile to the pH 4.0 culture, except that intra-

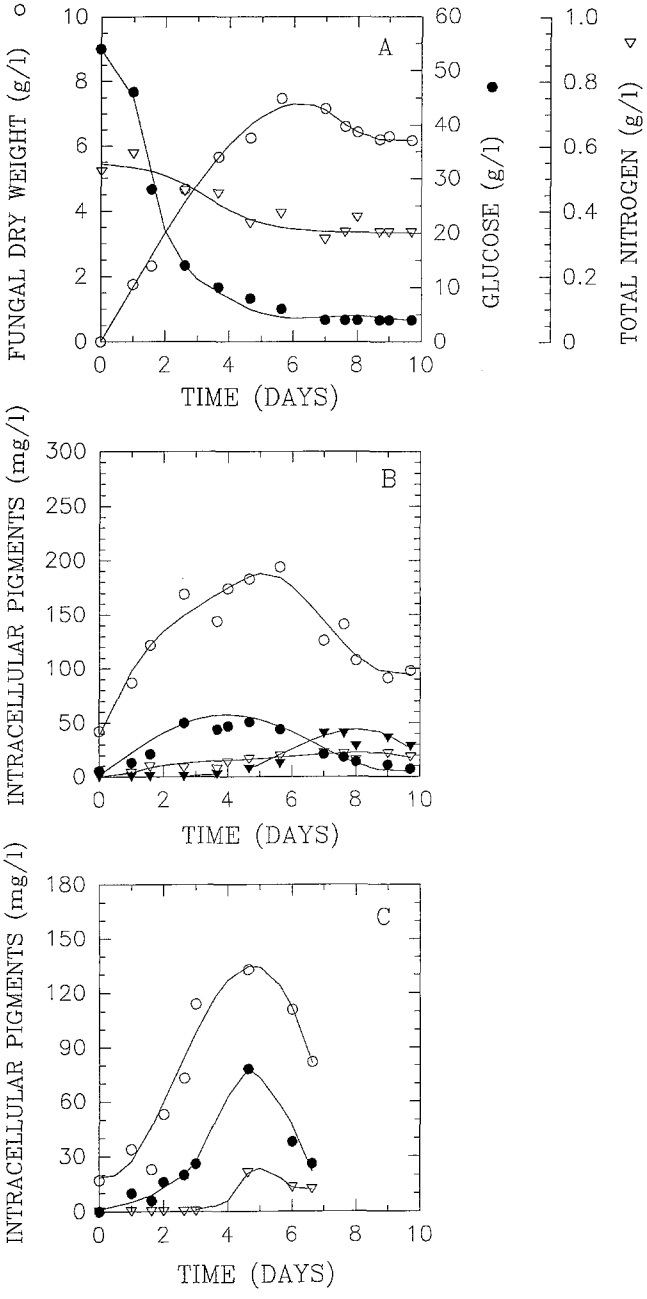

Fig. 4A–C. Growth and nutrient consumption at pH 4.0 (**A**), intracellular pigment concentrations at pH 4.0 (**B**) and pH 6.5 (**C**), in batch fermentor culture of *M. purpureus* 192F on glucose-peptone medium. Symbols as for Fig. 1

cellular concentrations were lower and ankaflavin was not detected (Fig. 4C). A significant decrease in intracellular pigment concentrations occurred once growth stopped.

The effect of pH and nitrogen source on the partitioning of pigments between the fungal mycelium (intracellular) and the medium is given in Table 3. Pigment secretion into the medium was not only clearly enhanced at pH 6.5 compared to pH 4.0, irrespective of the nitrogen source, but also demonstrated considerable dependency on the nature of the nitrogen source at pH 6.5. Although red pigments are reportedly more

Table 3. Effect of pH and nitrogen source on pigment secretion

pH	Media[a]	$A_{400\,mm}/A_{400\,mm}$[b]	$A_{500\,mm}/A_{500\,mm}$[b]
4.0	GN	1.2	1.0
4.0	GA	1.2	1.1
4.0	GP	0.9	1.7
6.5	GN	8.2	8.7
6.5	GA	3.6	12
6.5	GP	17	21

[a] Media symbols as for Table 2
[b] Ratio of extracellular to intracellular pigment absorbance

water-soluble than yellow pigment, the ratio of extracellular to intracellular absorbance was generally similar for A_{400nm} and A_{500nm}. The only exception was in the pH 6.5, glucose-ammonium culture (Table 3).

Discussion

All pigments, except ankaflavin, were produced in varying concentration under all the conditions tested, with the red pigment monascorubramine being the predominant pigment produced. Rubropunctamine, the C_5H_{11}-side-chain red analogue of monascorubramine was not detected.

Culture pH clearly affected fungal growth and ankaflavin synthesis. Growth and activity of the fungus was favoured at pH 4.0 over pH 6.5, independent of the nitrogen source. This is evidenced by markedly higher maximum specific rates of glucose consumption and faster specific growth rates at pH 4.0, and high residual glucose concentrations in pH 6.5 cultures. However, the final mycelial dry weight was often similar and the observed growth yield was always higher at pH 6.5.

Ankaflavin synthesis occurred only at pH 4.0 in significant amounts. In these fermentations, ankaflavin concentration increased at the expense of monascin concentration during the stationary phase of the culture. This suggests that ankaflavin is synthesised from monascin by the addition of a C-2 unit to the saturated C_5H_{11} side-chain of monascin. Ankaflavin production at low pH presumably accounts for the increased yellow colour of these broths in both our studies and those of other workers (Carels and Shepherd 1977, 1978) since the monascin (yellow) and monascorubramine (red) concentrations were relatively independent of pH.

Both ammonium and peptone gave superior cell yields compared to nitrate, despite nearly equivalent, or better, specific rates of glucose consumption in nitrate cultures. There exists some controversy in the literature as to the best nitrogen source for red pigment production with organic nitrogen (Yoshimura et al. 1975; Broder and Koehler 1980; Su and Huang 1980), and nitrate (Lin 1973; Carels and Shepherd 1977) being favoured. The effect of organic nitrogen is complicated, because it may serve as a carbon source and can promote the protein-bound dissolution of red pigments into the culture broth (Broder and Koehler 1980).

Unfortunately, extracellular pigment concentrations could not be analysed by HPLC, probably because they were complexed with nitrogenous medium components (Broder and Koehler 1980). Nevertheless, absorbance data revealed that pigment secretion into the medium was favoured at pH 6.5, particularly for red pigments, and by the use of peptone. The higher polarity of the red pigments is known to enhance their binding to water-soluble nitrogen-containing organic compounds (i.e. peptone), which has been proposed as the solubilisation mechanism (Lin and Iizuka 1982; Su and Huang 1980). The high ratio of extracellular to intracellular absorbance at 500 nm in the pH 6.5, glucose-peptone culture suggests that total (intra- and extracellular) monascorubramine production at pH 6.5 greatly exceeded that at pH 4.0, but that the bulk of the pigment was secreted into the medium. Consequently, the intracellular monascorubramine concentration in the pH 6.5, glucose-peptone culture remained low. Peptone, therefore, was the best nitrogen source for monascorubramine production at both pH values. These results support suggestions that solubilisation of pigments into the medium, enhances red pigment synthesis (Evans and Wang 1984).

The data reveal that both ammonium- and peptone-containing cultures produced higher total monascorubramine concentrations than those with nitrate as the nitrogen source. However, the latter were remarkable for their selectivity for monascorubramine production, even at low pH. Virtually no yellow pigments (monascin and ankaflavin) were present, in contrast to ammonium and peptone-containing cultures. Therefore, nitrate is the preferred nitrogen source, if monascorubramine is required, with a minimum of yellow pigments.

Clear evidence of sizeable pigment loss late in batch cultures was observed from the absorbance and HPLC measurement of pigment concentrations. This has not been remarked on by previous authors. The loss was observed in both intracellular and extracellular pigment concentrations and cannot be explained as a change in pigment location. It is possible that the pigments are degraded by an enzymic pathway, which may be induced by nutrient exhaustion. Enzyme degradation of secondary metabolites is a common phenomena in fungi (Johns et al. 1982).

The results from HPLC pigment analysis do not correlate well with absorbance measurement of intracellular pigments. The use of absorbance tends to overpredict yellow pigment concentation, probably because both red and yellow pigments contribute to absorbance at 400 nm (Hiroi and Shima 1975). Furthermore, HPLC measurement revealed that the synthesis of monascorubramine, monascin and rubropunctatin was more closely associated with the active growth of the fungus than absorbance data suggest. This has considerable importance for the interpretation of previous work in which absorbance has been used to estimate pigment concentrations. HPLC measurements are more useful, since individual pigments are identified, although there was sizeable scatter in HPLC data. In contrast, absorbance measurements are unable to separate the contributions of different pigments to absorbance at a given wavelength, but were less variable.

It has been suggested that red pigments are derived by chemical reaction from orange precursors (Kurono et al. 1963). The time profile of individual pigment concentration during batch fermentations in this work provide only equivocal evidence for this hypothesis. Monascorubramine concentrations considerably exceeded those of the orange pigment, rubropunctatin. Furthermore, monascorubramine has a C_7H_{13} side-chain, whereas both rubropunctatin and monascin possess a C_5H_{11} side-chain. Therefore, at least two reaction steps would be required in the conversion of rubropunctatin to monascorubramine. The addition of a C-2 unit to the side chain is unlikely to occur by spontaneous chemical reaction. Finally rubropunctamine was not detected, despite the presence of rubropunctatin, the orange C_5H_{11} analogue.

In conclusion, the results of this work clearly demonstrate the separate effect of pH and nitrogen source on the production of individual pigments by *M. purpureus* and reveal new information useful for designing an efficient fermentation process for red pigment production.

References

Broder CU, Koehler PE (1980) Pigments produced by *Monascus purpureus* with regard to quality and quantity. J Food Sci 45:567–569

Carels M, Shepherd D (1977) The effect of different nitrogen sources on pigment production and sporulation of *Monascus* species in submerged shaken culture. Can J Microbiol 23:1360–1372

Carels M, Shepherd D (1978) The effect of pH and amino acids on conidiation and pigment production of *Monascus major* ATCC 16362 and *Monascus rubiginosus* ATCC 16367 in submerged shaken culture. Can J Microbiol 24:1346–1357

Carels M, Shepherd D (1979) The effect of changes in pH on phosphate and potassium uptake by *Monascus rubiginosus* ATCC 16367 in submerged shaken culture. Can J Microbiol 25:1484–1488

Evans PJ, Wang JY (1984) Pigment production from immobilised *Monascus* sp. utilizing polymeric resin adsorption. Appl Environ Microbiol 6:1323–1326

Fielding BC, Holker JSE, Jones DF, Powell ADG, Richmond KW, Robertson A, Whalley WB (1961) The chemistry of fungi. Part XXXIX. The structure of monascin. J Chem Soc 4579–4589

Francis FJ (1987) Lesser-known food colorants. Food Technol 41:62–68

Hadfield JR, Holker JSE, Stanway DN (1967) The biosynthesis of fungal metabolites. Part II. The β-oxo-lactone equivalents in rubropunctatin and monascorubrin. J Chem Soc 751–755

Hiroi T, Shima T (1975) Studies on the structure of two pigments obtained from *Monascus* species. J Jp Soc Food Nutr 28:497–501

Johns MR, Stuart DM (1991) Production of pigments by *Monascus purpureus* in solid culture. J Ind Microbiol 8:23–28

Johns MR, Chong R, Maddox IS (1982) Hydrolysis of some natural and synthetic bile acid conjugates by *Cerospora melonis*. Can J Microbiol 28:457–461

Kurono M, Nakanishi K, Shindo K, Tada M (1963) Biosynthesis of monascorubrin and monascoflavin. Chem Pharm Bull 11:359–362

Lin CF (1973) Isolation and cultural condition of *Monascus* sp. for the production of pigment in a submerged culture. J Ferment Technol 51:407–414

Lin TF, Demain AL (1990) Effect of nutrition of *Monascus* sp. on formation of red pigments. Appl Microbiol Biotechnol 36:70–75

Lin CF, Iizuka H (1982) Production of extracellular pigment by a mutant of *Monascus kaoliang* sp. nov. Appl Environ Microbiol 43:671–676

Manchand PS, Whalley WB (1973) Isolation and structure of ankaflavin: a new pigment from *Monascus* anka. Phytochemistry 12:2531–2532

Morrison RT, Boyd RN (1987) Organic Chemistry, 5th edn. Allyn and Bacon, Boston, pp 494–495

Murakawa S (1990) Functions and applications of *Monascus* (in Japanese). Gekkan Fudo Kemikaru 6:42–46

Su YC, Huang JH (1980) Fermentative production of anka-pigments (*Monascus*-pigments). Proc Nat Sci Council Repub China 4:201–215

Sweeny JG, Estrada-Valdes MC, Iacobucci GA, Sato H, Sakamura S (1981) Photoprotection of the red pigment of *Monascus anka* in aqueous media by 1,4,6-trihydronaphthalene. J Agric Food Chem 29:1189–1193

Wong HC, Koehler PE (1983) Production of red, water-soluble *Monascus* pigments. J Food Sci 48:1200–1203

Wong HC, Lin YC, Koehler PE (1981) Regulation of growth and pigmentation of *Monascus purpureus* by carbon and nitrogen concentrations. Mycologia 73:649–653

Yongsmith B, Tabloka W, Bavavoda R, Vaisirifoj V, Yoshida T, Tanaka A (1989) Characterization of cassava-utilization *Monascus* and their yellow pigments. Microb Util Renew Res 6:340–348

Yoshimura M, Yamanaka S, Mitsugi K, Hirose Y (1975) Production of *Monascus*-pigment in a submerged culture. Agric Biol Chem 39:1789–1795