

Fermentation conditions affecting growth and red pigment production of *Monascus purpureus* FTC 5391

(Keadaan fermentasi yang memberi kesan terhadap pertumbuhan dan penghasilan pigmen merah oleh *Monascus purpureus* FTC 5391)

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Key words: *Monascus purpureus*, pH, inoculum size, monosodium glutamate, red pigment, fermentation

Abstract

Studies on optimization of fermentation conditions for cell growth and red pigment production by *Monascus purpureus* FTC 5391 were carried out in shake flask cultures at 37 °C. The suitable initial culture pH for red pigment production was varied from pH 5.5 to 9, but through this study the optimum initial culture pH was stated at 6.5. The optimal inoculum size for red pigment production was 10% and a decrease in inoculum size resulted in a decrease in mycelial growth and red pigment production. Study on the effect of different nitrogen sources such as $(\text{NH}_4)_2\text{HPO}_4$, $(\text{NH}_4)_2\text{H}_2\text{PO}_4$, NaNO_3 , NH_4NO_3 , $(\text{NH}_4)_2\text{SO}_4$, $(\text{NH}_4)_2\text{S}_2\text{O}_8$, NH_4Cl , peptone, yeast extract, monosodium glutamate (MSG), urea and tryptone, showed cell growth and red pigment production preferred organic nitrogen sources as compared to inorganic nitrogen sources. MSG as nitrogen source gave superior growth and red pigment production compared to other organic nitrogen sources. MSG at 1.2% (12 g/litre) was optimal for cell growth and red pigment production. Using 5% (50 g/litre) glucose and 1.2% (12 g/litre) MSG as the carbon and nitrogen source respectively, we found that K_2HPO_4 (2.5 g/litre), KH_2PO_4 (2.5 g/litre), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1.0 g/litre), KCl , (0.5 g/litre), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.01 g/litre), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.01 g/litre) dan $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (0.03g/litre) gave maximal cell growth and red pigment production.

Introduction

The search for naturally produced food colourants in substitution to chemically synthesized colourants has led to a resurgence of interest in the pigments synthesized by the fungus such as *Monascus purpureus*. This fungus has been used in Asia for many centuries as colour and

flavour ingredients in food and beverages (Lin et al. 1992). The red pigments are of particular interest, because red is the most popular food colour and true natural pigments suitable for applications in food industries are difficult to obtain.

The biosynthesis of the pigments by the fungus is poorly understood. Studies by

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many researchers have revealed that the pigment production in submerged fermentation by *M. purpureus* is affected by numerous environmental factors, particularly the types and concentration of nitrogen source in the medium (Carels and Shepherd 1977, 1978; Chen and Johns 1993). 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 (Chen and Johns 1993). Furthermore, individual pigment concentrations have not been determined.

Wong and Koehler (1981) reported that a *Monascus* sp. isolated from the koji of Kaoliang liquor had a maximum yield of pigment in a medium containing 50 g/litre rice powder and 5 g/litre sodium or potassium nitrate. In addition, they also reported that nitrogen sources such as monosodium glutamate, peptone, and yeast extract or casamino acids are also good nutrients for pigmentation. Carels and Shepherd (1977) reported the effect of different nitrogen sources on pigment production of a number of *Monascus* sp. and concluded that when the source of nitrogen is yeast extract or nitrate, red pigment is formed, whereas with ammonium or ammonium nitrate, orange pigment is formed.

For better control of the pigment production, a well-defined chemical medium, with glutamate as nitrogen source, is used in submerged cultivation of *M. purpureus* (Hajjaj et al. 1999). This medium formulation promotes production and excretion of extracellular pigments (Wong and Koehler 1983) and has also led to the formation of glutamic acid-pigment complex (Blanc et al. 1994).

Recently, *M. purpureus* FTC 5391 capable of producing substantial amount of red pigment has been isolated and characterized (Musaalbakri 2004). The first step in the development of fermentation by this fungus for production of red pigment is to optimize the medium and culture

condition of the process. In this study, the effects of inoculum size, initial pH, type of nitrogen sources, concentration of monosodium glutamate and trace elements on red pigment production in submerged fermentation using shake flasks were investigated. The information gathered from all the experiments was used to propose the optimal medium for *M. purpureus* FTC 5391 fermentation using glucose as substrate.

Materials and methods

Strain and media

The monospore isolate MP 3 (Musaalbakri 2004) of *M. purpureus* FTC 5391, was maintained on potato dextrose agar (PDA) plate for 7 days at 37 °C.

Shake flask culture

All fermentations of *M. purpureus* FTC 5391 were carried out in 500 ml shake flasks containing 250 ml fermentation medium. The inoculum medium (YMP broth) consisted of yeast extract (3 g/litre), malt extract (3 g/litre), peptone (5 g/litre) and glucose (20 g/litre). Four pieces of 4 mm mycelial blocks of *M. purpureus* FTC 5391 were used to inoculate the inoculum cultures in 250 ml flask containing 100 ml YMP broth. The flasks were incubated in orbital shaker at 37 °C, agitated at 250 rpm for 4 days. The 25 ml (10%, v/v) inoculum culture was then inoculated into 500 ml flasks containing 250 ml of production medium. The flasks were incubated in orbital shaker incubator at 37 °C, agitated at 250 rpm for 7 days. All fermentations were performed at least in triplicate.

All fermentations were carried out using a basal medium as suggested by Lin et al. (1992). In this medium formulation, 50 g/litre glucose was used as sole carbon source, with various types and concentration of nitrogen sources according to the needs of each experiment. Initially, the effect of different initial pHs and inoculum sizes on the performance of the fermentation was investigated.

Subsequently, the effects of various organic and inorganic nitrogen sources were studied using optimal pH and inoculum size obtained from the previous experiment. The effect of the concentration of selected nitrogen source (monosodium glutamate) was also studied as well as the requirement of trace elements such as K_2HPO_4 , KH_2PO_4 , $MgSO_4 \cdot 7H_2O$, KCl , $ZnSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$ and $MnSO_4 \cdot 7H_2O$.

Determination of cell concentration

Cell concentration was determined using filtration and oven dry method. A known volume of culture sample (3–5 ml) withdrawn from the shake flask was filtered through a pre-weighed filter paper (Whatman No. 1) by using a vacuum pump. After drying for more than 24 h in an oven at 80 °C i.e., until a constant weight was achieved, the filter paper and cells were re-weighed and the cell dry weight was calculated by difference.

Glucose analysis

Glucose concentration in the culture broth was measured by using Glucose Analyzer (YSI 2700 Select Biochemistry Analyzer). Samples were prepared by filtering the supernatant through sep-pack to remove the particles and pigments that might interfere with the determination.

Determination of red pigment

Samples collected during the fermentation were centrifuged at 3,000 rpm for 10 min using a laboratory centrifuge (Centrifuge 5810R, Germany). The red pigment was present in both fractions; filtrate and cell pellet. In order to measure red pigment in cell pellet, extraction of the pigment was carried out using 95% ethanol. The method of extraction was used as follows: 10 ml of ethanol was added to 1 g wet cell in 20 ml test tube, shaken for a while and then kept at room temperature overnight. The mixture was then filtered through a filter paper (Whatman No. 1) and the filtrate was used for pigment determination.

For measurement of absorbance for filtrate from culture broth, uninoculated medium was used as blank while for filtrate from the extract, ethanol was used as blank. The wavelength at 500 nm represents maximum absorption for the red pigment. Whenever necessary, the samples were diluted with distilled water (filtrate) or ethanol (extract) prior to absorbance measurement. The pigment production was calculated by multiplying the absorbance units by the dilution factor. The spectra of the red pigment were measured using a Cecil CE 2502 2000 series scanning spectrophotometer.

Determination of residual nitrogen

Residual nitrogen in the culture broth was measured by using Kjeldhal method (AOAC 1990).

Results and discussion

Effect of initial culture pH in submerged fermentation

The time courses of red pigment fermentation by *M. purpureus* FTC 5391 at different initial culture pHs are shown in *Figure 1 (A–C)* and the performance of each fermentation is given in *Table 1*. Growth increased with increasing initial culture pH between 5 and 7.5 and no lag phase was observed. However, growth at an initial culture pH of between 5 and 7.5 was not significantly different and growth reached a stationary phase after about 144 h with maximum cell concentration of 11.0 g/litre. At initial pH 6.5 and 7.5, a stationary phase was achieved after about 144 h with a maximum cell concentration of 11.43 g/litre and 10.95 g/litre, respectively. A long lag phase (144 h) and very slow growth was observed at initial pH between 2–4 and pH 10, suggesting that growth of *M. purpureus* FTC 5391 was greatly inhibited at this pH range (*Figure 1A*).

Different initial cultures pHs greatly influenced the red pigment production by *M. purpureus* FTC 5391 (*Figure 1B*). At initial culture pH of between 6 and 8, high

Fermentation affecting growth and red pigment production

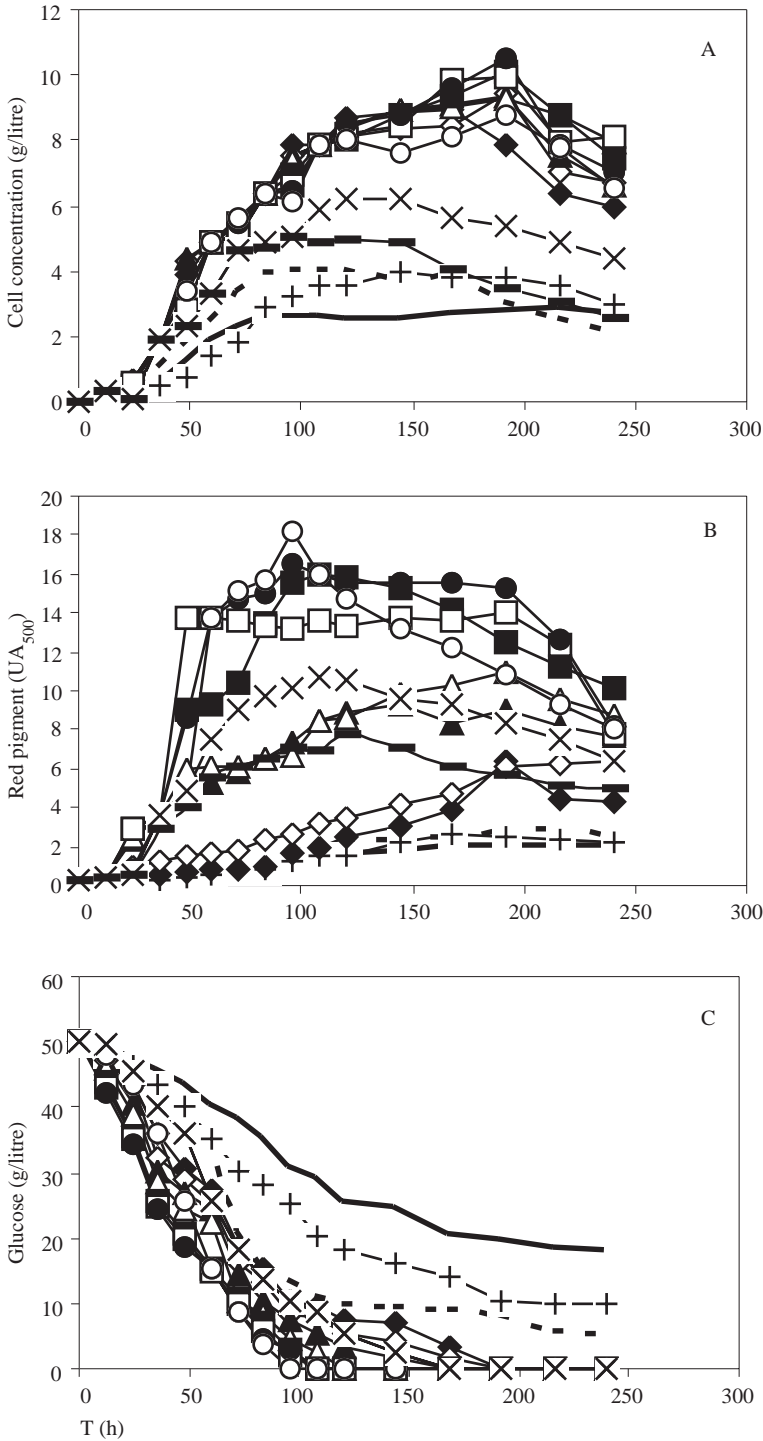


Figure 1. Effect of different initial culture pHs on growth of *Monascus purpureus* FTC 5391 and red pigment production: — = pH 2; + = pH 3; - - - - = pH 4; ◆ = pH 4.5; ◇ = pH 5; ▲ = pH 5.5; Δ = pH 6; ■ = pH 6.5; □ = pH 7; ● = pH 7.5; ○ = pH 8; x = pH 9; _ = pH 10

Table 1. Effect of different initial culture pH on growth and red pigment production of *Monascus purpureus* FTC 5391

Initial pH	X_{max} (g/litre)	Red pigment concentration (UA_{500}^{max})	$Y_{x/s}$ (g cell/g glucose)	$Y_{p/s}$ (UA_{500} /g glucose)	Productivity P (g/litre.h)	P/X
2	2.85	2.01	ND	ND	ND	0.71
3	3.80	2.65	ND	ND	ND	0.70
4	3.86	2.66	ND	ND	ND	0.69
4.5	8.90	6.33	0.173	0.63	0.051	0.71
5	10.45	6.42	0.184	0.18	0.048	0.61
5.5	10.38	9.28	0.183	0.19	0.048	0.89
6	10.50	11.02	0.185	0.22	0.048	1.05
6.5	11.43	15.95	0.197	0.32	0.060	1.40
7	10.95	16.0	0.194	0.32	0.051	1.46
7.5	10.53	16.56	0.206	0.33	0.054	1.57
8	8.78	18.20	0.171	0.36	0.044	2.07
9	6.20	10.63	0.124	0.21	0.043	1.71
10	4.98	7.75	ND	ND	ND	1.56

ND = Not determined

red pigment production was observed, where the maximum concentration of the pigment obtained was ranged from 11.02 UA_{500} to 18.20 UA_{500} . On the other hand, at initial culture pH of 6.5, rapid red pigment production continued up to 168 h.

Substantially high cell concentration and red pigment production was obtained in fermentation with initial culture pH between 6 and 8. However, the highest red pigment production (18.20 UA_{500}) was obtained at initial culture pH 8. At high initial culture pHs (6–8), growth of *M. purpureus* FTC 5391 was enhanced and the ability of the cells to convert glucose to red pigment was also increased. Because of very low growth, red pigment was not produced in fermentation carried out at initial culture pH of 2–4. Chen and Johns (1993) reported that the highest red pigment production was obtained at pH 6.5 using glucose as a carbon source.

Glucose consumption increased with increasing culture pH and this was paralleled with increased in growth (Figure 1C). Cell growth began almost immediately and ended after 96 h due to glucose exhaustion, at which point glucose uptake also ceased. At initial culture pH between 2 and 4, growth

and glucose consumption were relatively poor, where about 10–25 g/litre glucose was still remained in the culture. The observed growth yield ($Y_{x/s}$), however, was higher than that at pH 6.5 (0.197 g cell/g glucose). On the other hand, the yield of pigment ($Y_{p/s}$) (0.32 UA_{500} /g glucose) was not significantly different for fermentation with initial pH between 6.5 and 8.

Effect of inoculum size in submerged fermentation

The time courses of red pigment fermentation by *M. purpureus* FTC 5391 using different inoculum sizes are shown in Figure 2 (A–C) and the performance of each fermentation is given in Table 2. Growth increased with increasing inoculum size up to 10% (v/v) and no lag phase was observed except in fermentation with inoculum size of 3% (v/v). Growth at inoculum size of 10% (v/v) reached a stationary phase after about 144 h with maximum cell concentration of 14.2 g/litre. This fermentation gave cell efficiency to produce red pigment (P/X) of 1.18, cell yield ($Y_{x/s}$) of 0.279 g cell/g glucose and pigment yield ($Y_{p/s}$) of 0.11 UA_{500} /g glucose. However, growth at inoculum size of between

Fermentation affecting growth and red pigment production

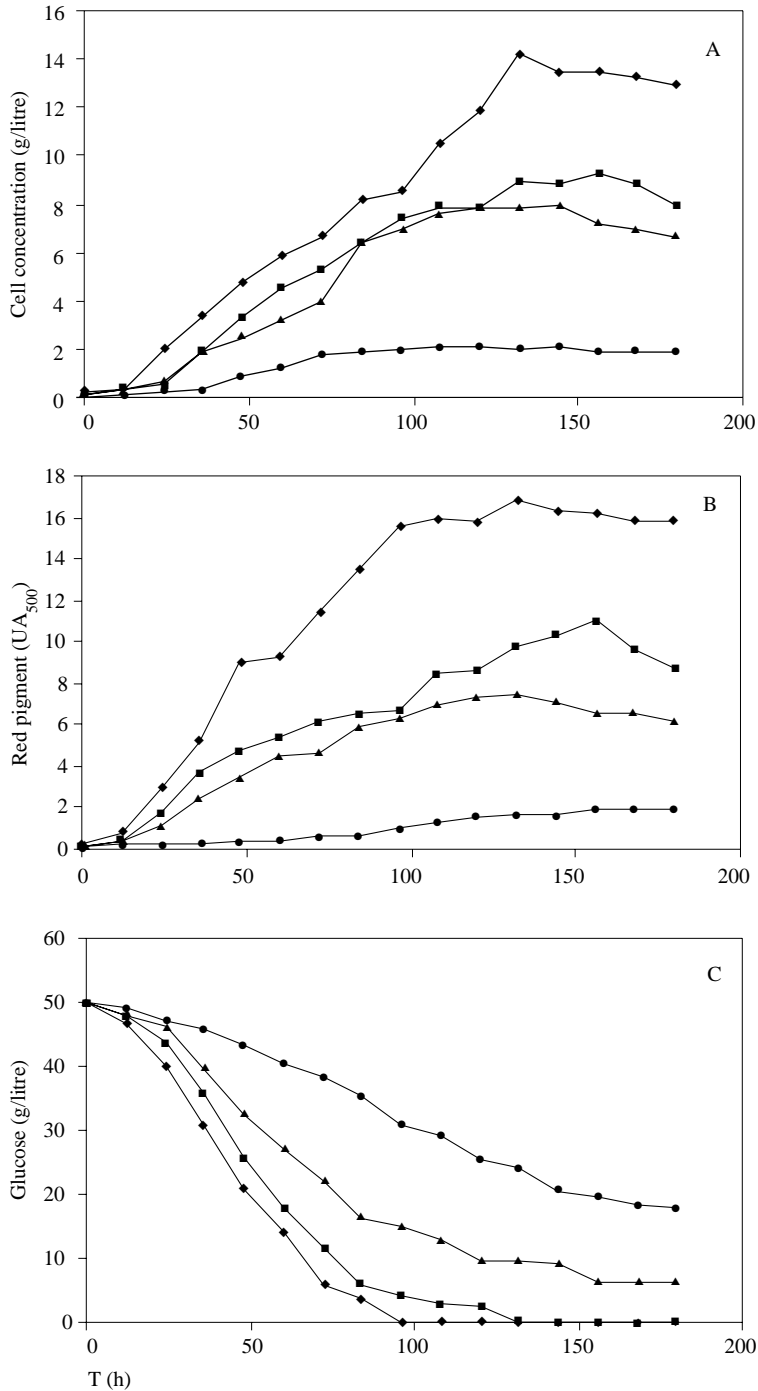


Figure 2. Effect of different inoculum sizes on growth of *Monascus purpureus* FTC 5391 and red pigment production: ● = 3% (v/v); ▲ = 5% (v/v); ■ = 7% (v/v); ◆ = 10% (v/v)

Table 2. Effect of different inoculum sizes on the performance of red pigment fermentation by *Monascus purpureus* FTC 5391

Inoculum size, % (v/v)	X_{max} (g/litre)	Red pigment concentration (UA_{500}) _{max}	$Y_{x/s}$ (g cell/g glucose)	$Y_{p/s}$ (UA_{500} /g glucose)	P/X
3	2.09	1.97	ND	ND	0.94
5	8.0	7.47	0.193	0.055	0.93
7	9.3	9.83	0.181	0.058	1.06
10	14.2	16.8	0.279	0.110	1.18

ND = Not determined

5% (v/v) and 7% (v/v) was not significantly different and growth reached a stationary phase after 144 h with maximum cell concentration of 8.0 g/litre and 9.3 g/litre respectively. A long lag phase and very slow growth was observed when inoculum size of 3% (v/v) was used.

Different inoculum sizes greatly influenced red pigment production by *M. purpureus* FTC 5391 (Figure 2B). At inoculum size of between 5% (v/v) to 10% (v/v), red pigment production stopped after 180 h and the maximum concentration obtained was between 7.47 UA_{500} to 14.2 UA_{500} . Maximum cell concentration obtained during the fermentation increased with increasing inoculum size. The highest red pigment production (14.2 UA_{500}) was obtained at inoculum size of 10% (v/v). Glucose consumption increased with increasing inoculum size and this was paralleled with growth (Figure 2C). From this experiment it was also observed that the risk of contamination increased with decreasing inoculum size.

As the inoculum size is increasing, there is a gradual build-up of cell concentration due to improved growth rate. Hence, it becomes imperative to look at the inoculum density together with the inoculum size. Manipulation of the quality of inoculum could be the alternative answer, in addition to the manipulation of culture medium and condition, to improve the red pigment yield.

Effect of nitrogen source in submerged fermentation

The time courses of red pigment fermentation by *M. purpureus* FTC 5391 using different types of nitrogen sources are shown in Figures 3 (A–C) and 4 (A–C) and the performance of each fermentation is given in Table 3. The various nitrogen sources where the total nitrogen content was fixed at 0.994 g/litre, either inorganic or organic compounds, were used to investigate their effects on the production of red pigment and growth of *M. purpureus* FTC 5391.

All inorganic nitrogen sources tested except ammonium persulphate [$(NH_4)_2S_2O_8$] and sodium nitrate ($NaNO_3$), were capable to support growth of *M. purpureus* FTC 5391. However, all inorganic nitrogen sources did not enhance red pigment production.

Very good growth (maximum cell concentration ranged from 10.99–15.68 g/litre) was observed in fermentation using organic nitrogen sources, except urea where growth of *M. purpureus* FTC 5391 was greatly inhibited. Similar to growth, red pigment production (ranged from 4.58–13.12 UA_{500}) was also enhanced when organic nitrogen sources were used, except urea. However, the pigment production (13.12 UA_{500}) was greatly enhanced when MSG was used as the sole nitrogen source.

Lin et al. (1992) and Lin and Demain (1995) reported that monosodium glutamate is the best nitrogen source for red pigment production by *M. purpureus* while

Fermentation affecting growth and red pigment production

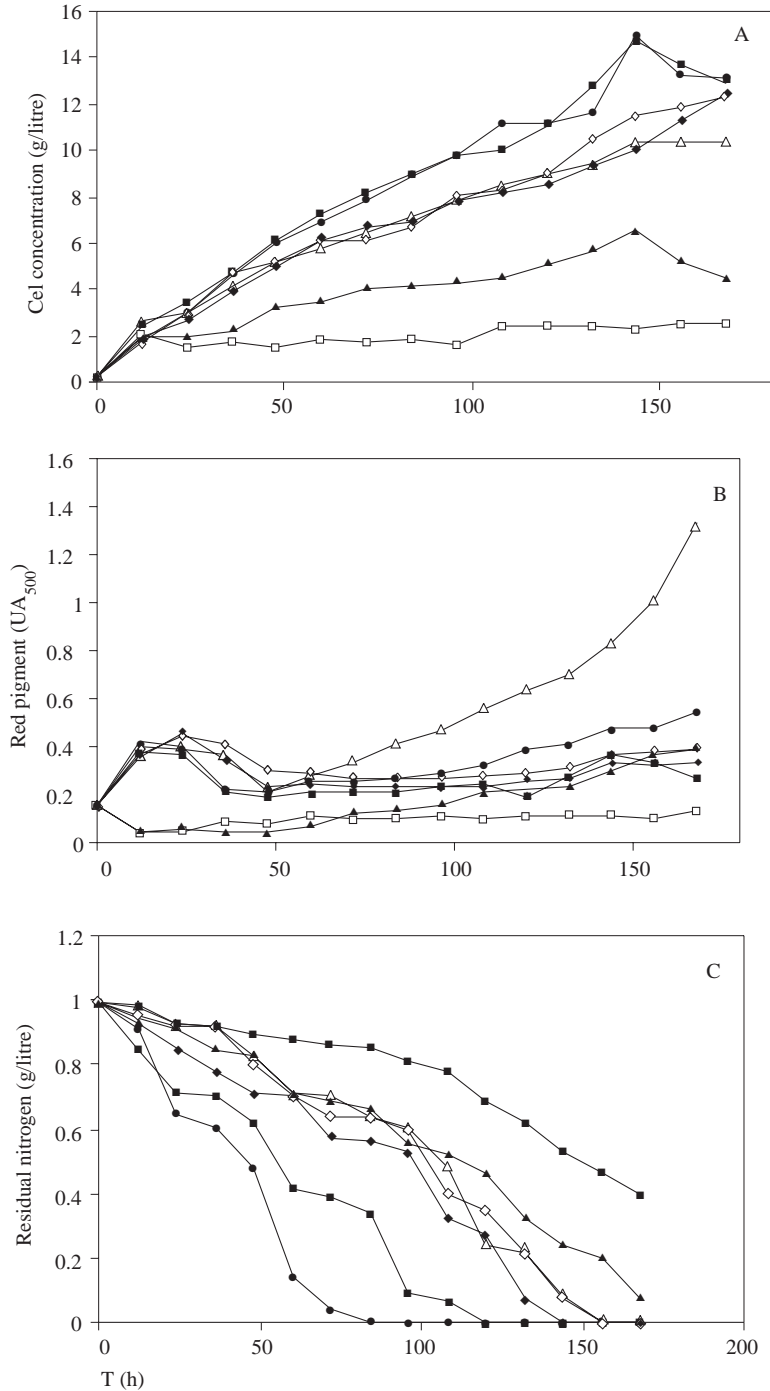


Figure 3. The influence of different types of inorganic nitrogen source on growth of *Monascus purpureus* FTC 5391 and red pigment production: ◆ = $(NH_4)_2HPO_4$; ◇ = $(NH_4)H_2P_4$; ▲ = $NaNO_3$; △ = NH_4NO_3 ; ■ = $(NH_4)_2SO_4$; □ = $(NH_4)_2S_2O_8$; ● = $(NH_4)Cl$

Table 3. Effect of different nitrogen sources on the performance of red pigment fermentation by *Monascus purpureus* FTC 5391

Nitrogen source	Total nitrogen consumed (g/litre)	X_{max} (g/litre)	Red pigment concentration (UA_{500}^{max})	Yield, $Y_{X/N}$ (g cell/g nitrogen)	Yield, $Y_{P/S}$ (UA_{500} /g nitrogen)	Productivity, P (g/litre.h)	P/X
$(NH_4)_2HPO_4$	0.994	12.48	0.454	12.3	2.10	0.025	0.04
$(NH_4)H_2PO_4$	0.994	12.33	0.448	12.15	3.74	0.045	0.04
$NaNO_3$	0.920	6.55	0.385	8.37	0.23	0.001	0.06
NH_4NO_3	0.994	10.40	1.336	10.21	1.19	0.007	0.13
$(NH_4)_2SO_4$	0.994	14.73	0.383	14.56	0.23	0.001	0.03
$(NH_4)_2S_2O_8$	0.615	2.58	0.128	4.33	ND	ND	0.05
$(NH_4)Cl$	0.994	15.00	0.541	14.84	0.39	0.002	0.04
Peptone	0.917	12.38	4.510	13.30	4.73	0.028	0.36
Yeast extract	0.915	12.45	4.860	13.42	4.70	0.028	0.40
(MSG)	0.994	15.68	13.12	15.52	13.04	0.100	0.84
Urea	0.342	2.76	0.330	10.22	0.51	0.005	0.12
Tryptone	0.958	10.93	5.240	11.20	5.30	0.032	0.48

ND = Not determined

Fermentation with a fixed amount of nitrogen concentration (0.994 g/litre) and carbon concentration (18 g/litre)

Yongsmith et al. (1994) reported that MSG in combination with peptone remarkably enhances pigment production by *M. barkeri* KB10.

The profile of growth, red pigment production and nitrogen consumption during the fermentation by *M. purpureus* FTC 5391 using different organic nitrogen sources are shown in Figure 4 (A–C). The summary of the result of the effect of different types of nitrogen source is presented in Table 3. It can be seen that maximum cell concentration (X_{max}) was not significantly different while the maximum red pigment concentration (P_{max}), yield and productivity were the highest in fermentation using MSG. These results indicated that cell efficiency to produce red pigment where identified P/X (0.84) was obtained in fermentation using MSG while the pigment yield ($Y_{P/N}$) and cell yield ($Y_{X/N}$) were 13.04 UA_{500} /g nitrogen and 15.52 g cell/g nitrogen respectively. As a conclusion, organic nitrogen sources were more favourable for high cell growth and red pigment production as compared to inorganic nitrogen sources as for MSG was the best nitrogen source.

Effect of different concentrations of monosodium glutamate

The time courses of red pigment fermentation by *M. purpureus* FTC 5391 at different MSG concentrations are shown in Figure 5 (A–D) and the performance of each fermentation is given in Table 4. MSG concentration did not significantly affect cell concentration, though significant level of difference in red pigment production was observed. MSG concentrations between 7.5–30 g/litre (total nitrogen content 44.3–177.9 mM) did not significantly affect cell concentrations and reached stationary phase after about 132 h with maximum cell concentration of 10.8 g/litre with an exception of 5 g/litre MSG.

Correspondingly, the highest yield ($Y_{X/N}$) and pigment ($Y_{P/N}$) at 71.0 mM of total nitrogen content is 13.66 g cell/g nitrogen and 16.50 UA_{500} /g nitrogen, respectively. A long lag phase (72 h) and very slow growth was observed in culture without MSG (control), suggesting that growth of *M. purpureus* FTC 5391 was greatly inhibited in medium without nitrogen source (MSG) (Figure 5A). However, red pigment production was affected by the concentration of MSG as

Fermentation affecting growth and red pigment production

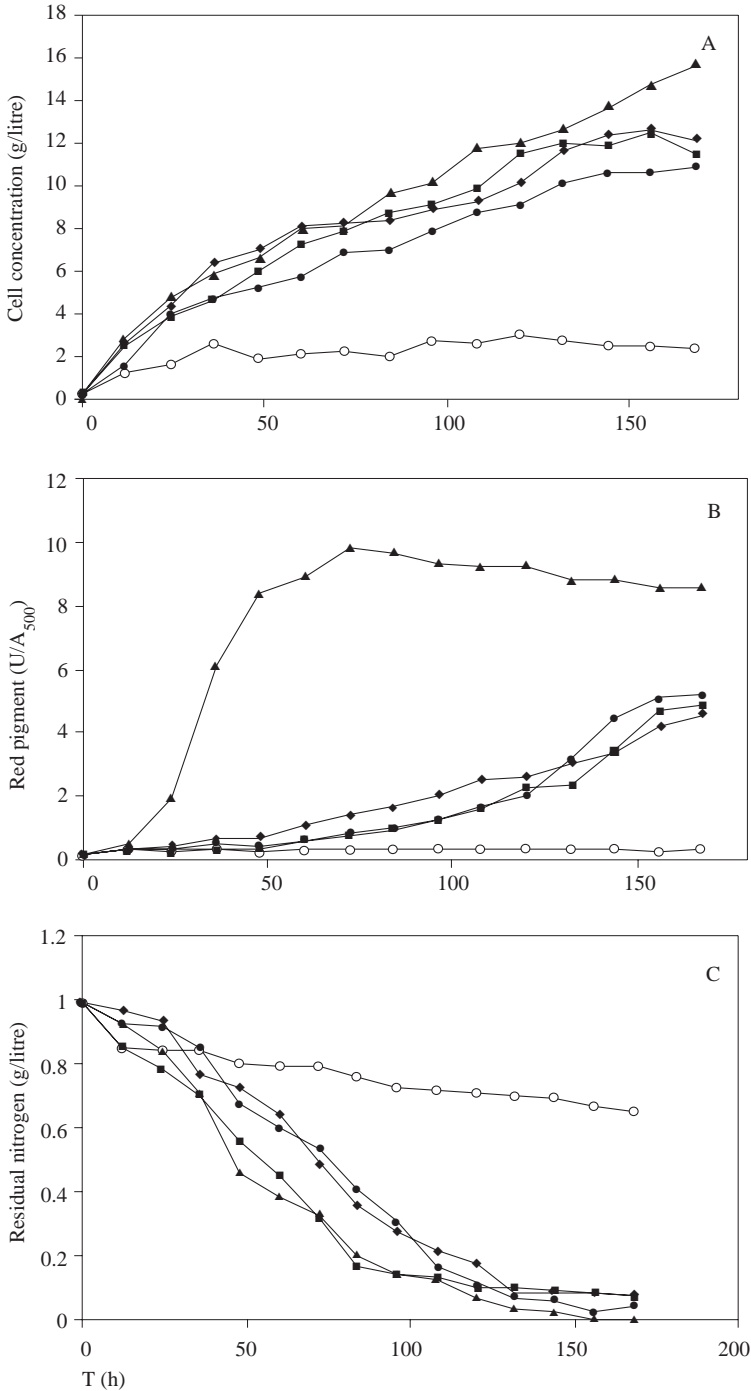


Figure 4. The influence of different types of organic nitrogen source on growth of *Monascus purpureus* FTC 5391 and red pigment production: ◆ = Peptone; ■ = Yeast extract; ▲ = Monosodium glutamate (MSG); ○ = Urea; ● = Tryptone

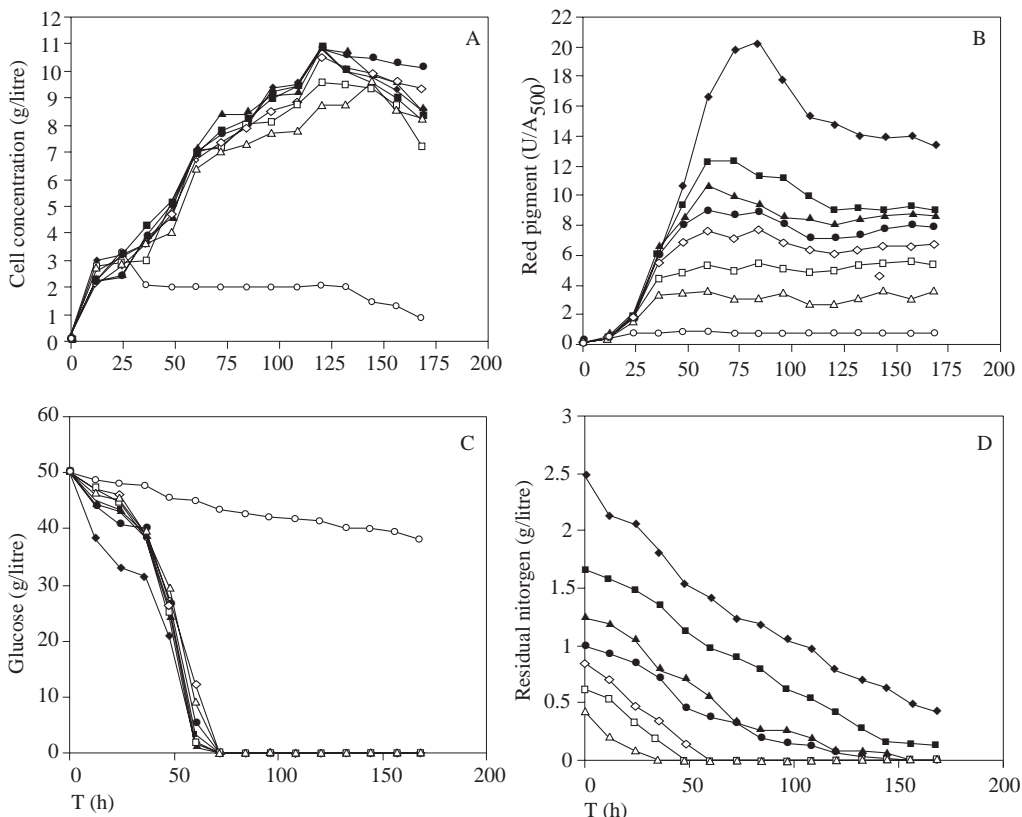


Figure 5. The influence of different concentrations of MSG on growth of *Monascus purpureus* FTC 5391 and red pigment production: ◆ = 3.0%; ■ = 2.0%; ▲ = 1.5%; ● = 1.2%; ◇ = 1.0%; □ = 0.75%; △ = 0.5%; ○ = 0% (Control)

shown in Figure 5B. The highest red pigment concentration was observed at 30 g/litre (total nitrogen 177.9 mM) (20.35 UA₅₀₀) whilst the lowest was at 5 g/litre (total nitrogen 0.42 mM) (3.56 UA₅₀₀). Lower concentration of MSG resulted in lower yield of red pigment.

Although the C/N ratio usually affects the rates of biosynthesis of many metabolites, its influence on mycelial growth and pigment production in fungi has scarcely been demonstrated (Cho et al. 2002). The effect of the C/N ratio on pigment production was investigated using glucose-MSG. As shown in Table 4 and Figure 6, cell growth (10.88 g/litre) and pigment production (20.35 UA₅₀₀) were maximal at a C/N ratio of 8.4 mol/mol. It is noteworthy that increasing C/N ratio

resulted in a decrease in red pigment. This is comparable with the findings of Nam and Rhee (1991), in that the carotenoid content of pink pigment decreases as the C/N ratio increases.

Glucose was consumed and depleted at 72 h fermentation (Figure 5C). Of course, during the 50 h, simultaneous glucose consumption might occur. Glucose consumption was slow at first and became fast during 50–72 h. The pigment formation initiated at 72 h when the cell reached almost the maximum concentration and glucose was totally exhausted. The summary of the result of the effect of different MSG concentrations is presented in Table 4. It can be seen that maximum cell concentration (X_{max}) was not significantly different with increasing MSG concentration while the

Table 4. Effect of different concentrations of MSG on the performance of red pigment fermentation by *Monascus purpureus* FTC 5391

Concentration of monosodium glutamate (MSG)	Total nitrogen (g/litre)	C/N nitrogen consumed (g/litre)	Total (g/litre) (UA ₅₀₀) _{max}	X _{max} concentration nitrogen)	Red pigment (g cell/g nitrogen)	Y _{X/N} (UA ₅₀₀ /g)	Y _{P/N}	P (g/litre.h)	P/X
3.0%	2.490	8.4	2.07	10.88	20.35	0.212	0.41	0.090	1.87
2.0%	1.660	12.6	1.52	10.85	12.36	0.212	0.25	0.090	1.12
1.5%	1.245	16.8	1.25	10.85	11.72	0.214	0.23	0.070	1.08
1.2%	0.994	21.1	1.00	10.85	13.2	0.224	0.26	0.072	1.22
1.0%	0.830	25.3	0.83	10.55	7.78	0.206	0.16	0.086	0.74
0.75%	0.623	33.9	0.62	9.57	5.56	0.218	0.11	0.091	0.58
0.5%	0.415	50.0	0.42	8.74	3.56	0.202	0.07	0.060	0.41
0% (Control)	—	—	—	2.05	0.76	ND	ND	ND	0.37

ND = Not determined

Fermentation with a fixed amount of total carbon concentration (1,499.4 mM)

C/N ratio was calculated on millimolar basis

was also obtained in fermentation using 30 g/litre MSG. As a conclusion, the optimum red pigment production, yield and P/X were detected in fermentation with 12 g/litre MSG. This will save the cost of production by using less amount of raw materials but with maximum yield obtained.

Effect of trace elements

From this study, it was found that the phosphate concentration at 3.0 g/litre and above inhibited not only the growth but also red pigment production (data not shown). It was decided to decrease the concentrations of K₂HPO₄ and KH₂PO₄ in the medium to 2.5 g/litre of each. On the other hand, magnesium sulphate (MgSO₄.7H₂O), at concentrations higher than 2.0 g/litre, both growth and red pigment production were greatly reduced. In addition, growth and red pigment production by *M. purpureus* FTC 5391 increased almost linearly with increasing MgSO₄.7H₂O from 0.5 to 1.0 g/litre. For further studies, the concentration of MgSO₄.7H₂O was found to be 1.0 g/litre. Potassium chloride (KCl) has no significant effect on growth and red pigment production at concentrations higher than 0.5 g/litre. However, red pigment production was inhibited by KCl at lower concentration than 0.5 g/litre. Red pigment production was enhanced by the addition of zinc sulphate (ZnSO₄.7H₂O) and the optimal concentration of ZnSO₄.7H₂O for the red pigment production was 0.01 g/litre. Similar observation was also reported by Johnson and McHan (1975) and Bau and Wong (1979). With regard to manganous sulphate (MnSO₄.7H₂O) and ferrous sulphate (FeSO₄.7H₂O), the concentration of 0.03 g/litre and 0.01 g/litre respectively was found to be optimal for growth and red pigment production.

The time of growth and red pigment fermentation by *M. purpureus* FTC 5391 using a medium containing optimal concentration of trace elements K₂HPO₄, 2.5 g/litre; KH₂PO₄, 2.5 g/litre; MgSO₄.7H₂O, 1.0 g/litre; KCl, 0.5 g/litre; ZnSO₄.7H₂O,

maximum red pigment concentration (P_{max}), yield and productivity were the highest in fermentation using 30 g/litre MSG. This result also indicated that cell efficiency to produce red pigment, calculated as (P/X)

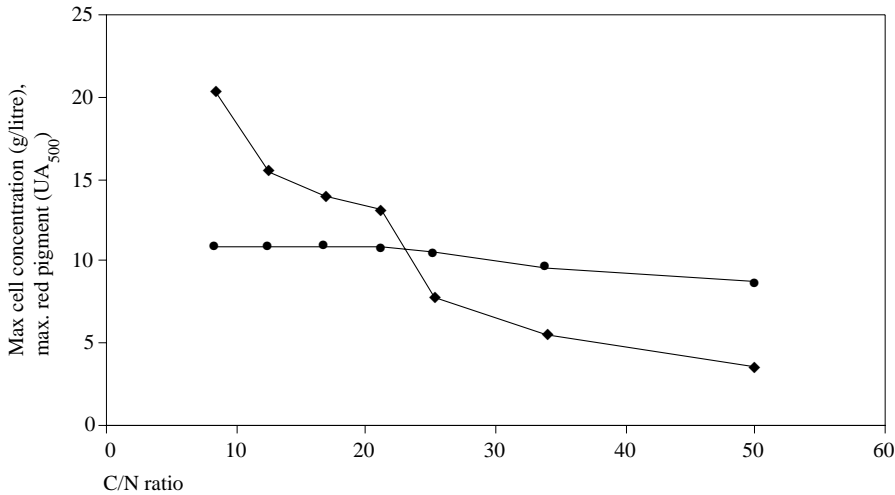


Figure 6. The relationship between C/N ratio and red pigment production in submerged fermentation by *Monascus purpureus* FTC 5391: ◆ = red pigment, ● = cell concentration

Table 5. Effect of trace elements on the performance of red pigment fermentation by *Monascus purpureus* FTC 5391

Parameter	X_{max} (g/litre)	Red pigment concentration (UA ₅₀₀) _{max}	Yield $Y_{x/s}$ (g cell/g) glucose	Yield $Y_{p/s}$ (UA ₅₀₀ /g glucose)	Productivity P (g/litre.h)	P/X
Medium supplied with trace elements	15.68	15.95	0.224	0.32	0.072	1.02
Medium without trace elements	8.24	8.87	0.216	0.18	0.095	1.08

0.01 g/litre; FeSO₄·7H₂O, 0.01 g/litre and MnSO₄·H₂O, 0.03g/litre, are shown in Figure 7. The performance of each fermentation is given in Table 5. Both growth and red pigment production by *M. purpureus* FTC 5391 were enhanced by the addition of trace elements. The maximum cell concentration (X_{max}) in fermentation with trace elements was 15.68 g/litre, while in fermentation without trace element the X_{max} was only about 8.24 g/litre. It is also interesting to note that growth in fermentation without trace elements decreased after about 70 h, indicating the cell was dying when trace elements were not present. Red pigment production in fermentation with trace elements (15.95 UA₅₀₀) was about two times higher as compared to fermentation without trace

elements (8.87 UA₅₀₀). Rate of glucose consumption was higher in fermentation with trace elements as compared to control, suggesting that trace elements may play important roles in the metabolites of glucose by the cells.

Conclusion

The optimized medium for red pigment fermentation by *M. purpureus* FTC 5391 was proposed in this study. The composition of the optimum fermentation is as follows, glucose as a carbon source at a concentration of 50 g/litre, MSG as the nitrogen source at concentration of 12 g/litre, trace elements K₂HPO₄ (2.5 g/litre), KH₂PO₄ (2.5 g/litre), MgSO₄·7H₂O (1.0 g/litre), KCl, (0.5 g/litre), ZnSO₄·7H₂O (0.01 g/litre), FeSO₄·7H₂O (0.01 g/litre) and

Fermentation affecting growth and red pigment production

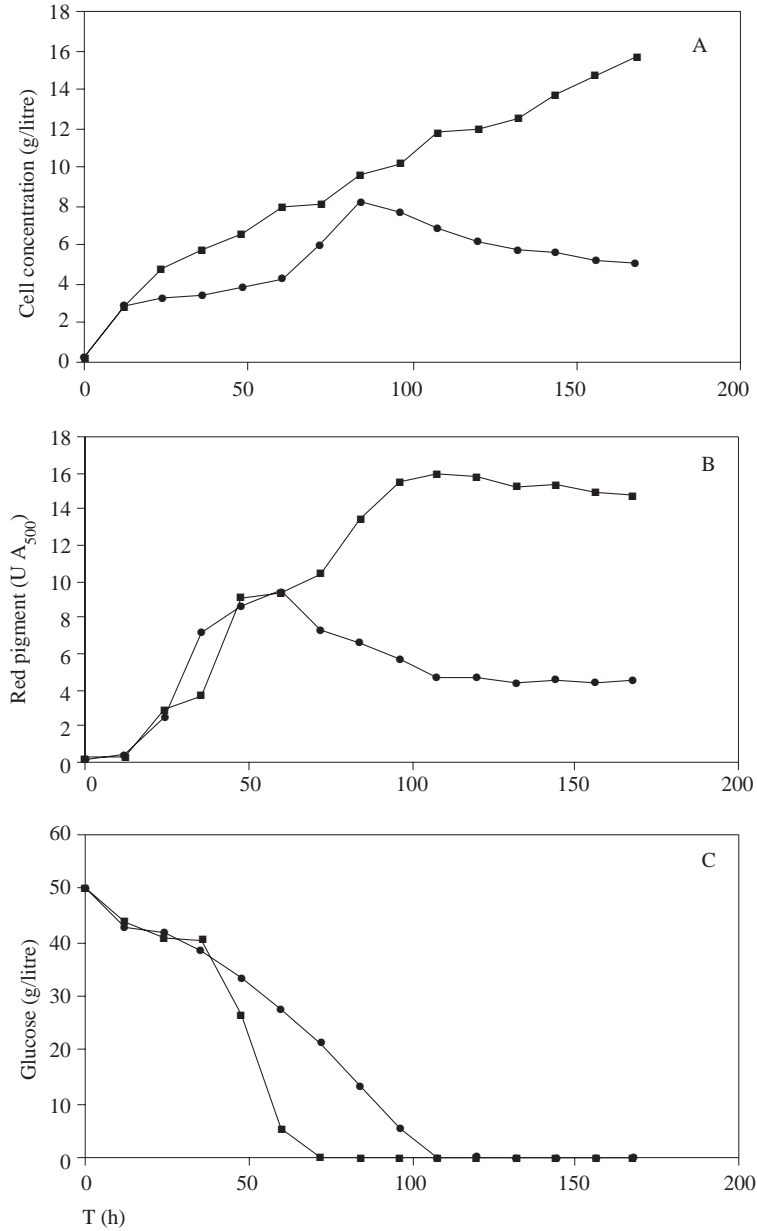


Figure 7. Effect of trace elements on growth of *Monascus purpureus* FTC 5391 and red pigment production. A) cell concentration, B) red pigment concentration, C) glucose consumption: ■ = contains trace elements; ● = without trace elements

MnSO₄.H₂O (0.03g/litre) was obtained at initial culture pH of 6.5 and inoculum size of 10% (v/v). By using this optimized medium, the maximum concentration of red pigment obtained in batch culture using shake flask was 15.95 UA₅₀₀ which gave yield ($Y_{p/s}$) and productivity (P) of 0.32 UA₅₀₀/g glucose and 0.072 g/litre.h respectively.

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Abstrak

Kajian pengoptimuman keadaan fermentasi untuk pertumbuhan sel dan penghasilan pigmen merah oleh *Monascus purpureus* FTC 5391 telah dijalankan di dalam kelalang bergoncang pada suhu 37 °C. Kesesuaian pH pemulaan bagi kultur untuk penghasilan pigmen merah ialah pH 5.5–9, tetapi daripada kajian ini didapati pH pemulaan yang optimum ialah 6.5. Saiz inokulum yang optimum ialah 10% dan penurunan saiz inokulum akan memberi kesan terhadap pertumbuhan miselium dan penghasilan pigmen merah. Daripada kajian penggunaan sumber nitrogen yang berbeza ($(\text{NH}_4)_2\text{HPO}_4$, $(\text{NH}_4)_2\text{H}_2\text{P}_4$, NaNO_3 , NH_4NO_3 , $(\text{NH}_4)_2\text{SO}_4$, $(\text{NH}_4)_2\text{S}_2\text{O}_8$, $(\text{NH}_4)\text{Cl}$, peptone, ekstrak yis, monosodium glutamate, urea dan triptone, didapati monosodium glutamate merupakan sumber nitrogen terbaik bagi pertumbuhan dan penghasilan pigmen merah oleh *M. purpureus* FTC 5391. Hasil kajian pengoptimuman medium menunjukkan medium yang menggunakan 50 g/liter glukosa dan 12 g/liter monosodium glutamate dengan penambahan unsur-unsur surih K_2HPO_4 (2.5 g/liter), KH_2PO_4 (2.5 g/liter), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1.0 g/liter), KCl , (0.5 g/liter), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.01 g/liter), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.01 g/liter) dan $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (0.03g/liter) memberikan keadaan yang optimum untuk pertumbuhan sel dan penghasilan pigmen merah.