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Nutrition regulation for the production of *Monascus* red and yellow pigment with submerged fermentation by *Monascus purpureus*

Clement Agboyibor, Wei-Bao Kong*, Ai-Mei Zhang, Shi-Quan Niu

College of Life Science, Northwest Normal University, Lanzhou, 730070, China

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ABSTRACT

The potential economic values and applications of natural yellow and red pigments in the food industry, cosmetic, and textile cannot be underestimated. So the improvement of a bioprocess for better production of *Monascus* pigments (MPs) using nutrition regulation under submerged fermentation was carried out. An organic acid together with fermentation conditions such as pH has proved to be effective for pigment enhancement as well as cell growth. The combined effect of 10 g/L peptone and 1.5 g/L pyruvic acid yielded yellow (99.81 ± 5.25 AU/ml) and red (97.22 ± 6.12 AU/ml) pigments which are about 5 folds compared to control. Further investigation indicated that the pigment production in the acidic medium is better than the nearly neutral medium. The highest yellow (574.76 ± 6.18 AU/ml) and red (570.98 ± 7.66 AU/ml) pigment production were attained at the initial pH of 5.0. The study again showed that the effect of fermentation time on pigments, pH curve, and biomass production did not reach the decline phase given an indication of adequate nutrient in the broth for probable growth and pigment production.

The present study gives the basic information on which the scaled-up production of valuable metabolites by filamentous fungi in submerged fermentation could be carried on.

1. Introduction

Natural pigments such as *Monascus* pigment MPs offers a better source of colorant in the textile and cosmetic industries, which is environment-friendly as a result of their enhanced biodegradability and better compatibility with the environment (Agboyibor et al., 2018; Basant and Jahan, 2016). MPs again has played an important role as the decolourable ink contains MPs used for ink-jet printing and these pigments from *Monascus* when exposed to irradiation of visible or ultraviolet light get discolored making it easy to recycle and reuse the papers in offices thereby conserving forest resources and reduced waste (Tsuyoshi et al., 2004). The natural coloring agents include plants, animals, and microorganisms. Among these sources, pigments from microorganisms have an advantage over other sources as it can be produced rapidly under controlled physicochemical conditions (Carvalho et al., 2007), they have a good quality for harvest, scale-up of production is easier and they are not subject to the vagaries of nature (Gomes and Takahashi, 2016). Nitrogen sources have been shown to regulate growth characteristics and metabolites production by the microorganism in terms of quantity and quality (Jung et al., 2003). Again, the growth of pigment-producing microorganism and secretion of secondary metabolites are influenced by the type of carbon sources used which generate energy to the cell. Glucose and its oligosaccharides have been reported to be the improved carbon sources for both growth and pigment production (Joshi et al., 2003).

Nonetheless, the production of high quantities of yellow and red fungal metabolites in submerged fermentation is one of the main challenges when using filamentous fungi such as *Monascus*. Therefore, this study was carried out for the improvement of a bioprocess for better production of yellow and red pigment by *Monascus purpureus* using different nutrients under submerged fermentation technology.

Great efforts have been made to improve the fermentation conditions and medium ingredients to enhance the production of *Monascus* pigments in submerged fermentation (Wang et al., 2013). According to Viggiano et al. (2017) and Hikino et al. (1973), when *Penicillium chrysogenum* was fed with an organic acid (pyruvic acid) enhanced the production of the yellow pigment, chrysogine. Chadni et al. (2017) also postulated that pigment production was reduced and cell growth inhibited as the dosage of an organic acid (malic and citric acid) increased. Addition of pyruvic acid to the fermentation broth has proven to be effective for the secretion of yellow and red *Monascus* pigment in this study. It seems to be feasible approaches to use pyruvic acid to augment the production of *Monascus* yellow and red pigments in submerged fermentation.

* Corresponding author.
E-mail addresses: agbeclement@yahoo.com (C. Agboyibor), kwbao@163.com (W.-B. Kong).

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1.1. Materials and methods

1.1.1. Microorganism

Monascus purpureus was obtained from the microbial culture collection of College of Life Science, Northwest Normal University Lanzhou, China and maintained on Potato Dextrose Agar (PDA) slants, sub-cultured for 5 days and preserved at 4 °C for further use.

1.1.2. Preparation of seed culture conditions

A suspension of spores was obtained by washing the Potato Dextrose Agar (PDA) slant cultures with sterile water, and around 10^8 spores were inoculated into 250 mL Erlenmeyer flasks containing 150 mL of seed culture medium. Seed medium (g/L) was prepared by using glucose 30, peptone 10, yeast extract, $NaNO_3$ 2, KH_2PO_4 1, $MgSO_4.7H_2O$ 1, KCl 0.5, and $FeSO_4.7H_2O$ 0.01 at a pH around 6.0. The seed culture was incubated at 30 °C for 4 days in a rotary shaker at 200 rpm.

1.2. Experimental design of different trophic factors influence the MPs production by M. purpureus

1.2.1. Carbon sources and concentrations

The cultures in submerged fermentation of the carbon sources were carried out in 250 mL Erlenmeyer flasks containing 100 mL of the medium. The medium was prepared by using glucose 20 g/L, $NaNO_3$ 2 g/L, KH_2PO_4 1 g/L, $MgSO_4.7H_2O$ 1 g/L, and KCl 0.5 g/L. Various carbon sources; sucrose 20 g/L, lactose 20 g/L, maltose 20 g/L, glycerol 20 g/L and soluble starch 20 g/L were used to replace glucose and keeping the other nutrients and parameters constant. The initial pH maintains at 6.0 using NaOH or HCl. The contents of the flasks were mixed and autoclaved for 20 min at 115 °C. After cooling, each flask was inoculated with 5 mL of the seed culture and incubated in a rotary shaker of 200 rpm at 30 °C for 6 days.

The relationship among biomass, red and yellow pigment production by *Monascus purpureus*, were investigated using different glucose concentration with glucose concentration of (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 120, 140, 160 and 180 g/L) and the initial pH adjusted to 6.0 by NaOH or HCl. 100 mL culture medium containing 5 mL seed was cultivated in a 250 mL flask for this study. The procedure was repeated for glycerol with glycerol concentration of (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 g/L).

1.2.2. Nitrogen sources and concentrations

The cultures in submerged fermentation of the nitrogen source were carried out in 250 mL Erlenmeyer flasks containing 100 mL of medium. The medium was prepared by using $NaNO_3$ 2 g/L, glucose 20 g/L, KH_2PO_4 1 g/L, $MgSO_4.7H_2O$ 1 g/L, and KCl 0.5 g/L. Different nitrogen sources; KNO_3 2 g/L, $(NH_4)_2SO_4$ 2 g/L, NH_4Cl 2 g/L, urea 2 g/L and peptone 2 g/L were used to replace $NaNO_3$ and keeping the other nutrients and parameters constant. The initial pH of the fermentation culture medium was adjusted to 6.0 using NaOH or HCl. The contents of the flasks were mixed and autoclaved for 20 min at 115 °C. Each flask was inoculated with 5 mL seed culture after cooling and incubated in a rotary shaker of 200 rpm at 30 °C for 6 days.

The relationship among biomass, red and yellow pigment production in *Monascus purpureus*, was investigated using different KNO_3 concentrations with KNO_3 concentrations of (1, 2, 4, 6, 8, and 10 g/L). The initial pH adjusted to 6.0 using NaOH or HCl. 100 mL culture medium containing 5 mL seed was cultivated in a 250 mL flask for 6 days. The study was repeated for peptone with peptone concentrations of (1, 2, 4, 6, 8, 10, 12 and 14 g/L).

1.2.3. Inorganic salts and organic acids

The metabolic regulators with different concentrations used were $CaCl_2$, Pyruvic Acid and Malic Acid (0.50, 0.10, 1.50 and 2.00 g/L), Trisodium Citrate and Citric acid (0.50, 1.00, 2.00 and 4.00 g/L). Other nutrients and culture conditions and parameters were the same as previous work. 100 mL culture medium containing 5 mL seed, cultivated in a 250 mL flask. The pH was kept around 6.0 using HCl or NaOH.

1.3. Effect of pH

The relationship between biomass and pigment production in *Monascus purpureus* was investigated using different initial pH values (initial pH adjusted to 4, 4.5, 5, 5.5, 6, 6.5, and 7 using NaOH or HCl).

1.4. Determination of biomass and MPs contents

1.4.1. Determination of the biomass

The biomass was measured gravimetrically. For biomass determination, the mycelial collected were filtered via a pre-weighed filter paper under partial vacuum (200 mg Hg), washed three times with distilled water and dried at 80 °C overnight. The biomass concentration was expressed as mycelial dry weight per unit volume of culture medium (Mukherjee and Singh, 2010).

1.4.2. Quantification of pigments

The pigments quantification was done using UV–vis spectrophotometer. The concentration of the yellow and red pigments was assessed by measuring the absorbance of filtrates at 505 nm (red pigments) and 410 nm (yellow pigments) (Kim et al., 2002). The results were expressed as absorbance unit at the corresponding wavelength per ml (AU/ml) (Srianta et al., 2016; Tseng et al., 2000).

1.5. Data statistics and analysis

Each experiment was performed in triplicate and the results were expressed as the mean ± standard deviation (n = 3). All statistical analyses were performed using the software SPSS Statistics 19.0 and Microsoft Excel 2010. All the data obtained were analyzed by one way ANOVA, and tests of significant differences were determined by using Duncan's test at $p < 0.05$.

2. Results and discussion

2.1. Effects of various carbon sources and concentrations on the MPs production by M. purpureus

To speed cell growth and improve the production of yellow and red pigment, the addition of different carbon sources to the fermentation broth was employed. The degree of pigment intensification may be closely linked with the type of carbon and its interaction with the microbial cells. As a result, the effect of different carbon sources on biomass, yellow and red pigment production by *Monascus purpureus* were discovered (Table 1). Among the carbon sources tested, glycerol produced pigment faster than other carbon sources upon observation. According to (Table 1), *Monascus purpureus* inoculated on different carbon sources containing a medium grew faster on glucose than other carbon sources. However, glycerol produced more yellow (3.55 ± 0.10 AU/ml) and red (3.59 ± 0.05 AU/ml) pigment compared to other carbon sources and was significant at ($P < 0.05$). Glycerol which gave the highest yellow and red pigment did not enhance mycelia growth and this could be as a result that glycerol which is a complex carbon source was not directly and easily assimilated by *Monascus purpureus* and also function as an osmolyte (Kayingo et al., 2004).

Quite a few concentrations of glucose and glycerol were added to the fermentation broth at the beginning of fermentation and the effect on mycelial growth and production of yellow and red pigments were explored as shown in Fig. 1. Increasing the concentration of glycerol and glucose increased pigment production. At the lower concentration, glycerol produced more yellow and red pigments than glucose while at higher concentration glucose produced both higher biomass as well as

Table 1
The effects of different carbon sources on pigments and biomass production by *Monascus purpureus*.

Carbon sources	Pigment yield (AU/ml)		Biomass (g/L)
	Yellow pigment	Red pigment	
Sucrose	2.60 ± 0.19^{d}	3.05 ± 0.25^{d}	3.42 ± 0.001^{d}
Maltose	1.30 ± 0.21^{b}	1.42 ± 0.15^{b}	2.23 ± 0.001^{c}
Glycerol	3.55 ± 0.10^{f}	3.59 ± 0.05^{e}	1.33 ± 0.001^{b}
Lactose	0.92 ± 0.12^{a}	0.87 ± 0.11^{a}	1.11 ± 0.001^{a}
Glucose	3.27 ± 0.03^{e}	3.04 ± 0.05^{d}	4.22 ± 0.006^{f}
Soluble starch	1.90 ± 0.13^{c}	2.03 ± 0.13^{c}	3.57 ± 0.037^{e}

The fermentation processes for the carbon sources were conducted for 6 days at a pH of 6 in a rotary shaker at 200 rpm. Data are mean ± standard deviation (n = 3). Means in a column with different lowercase letters are significantly different (ANOVA, Dancan's test; $P < 0.05$).

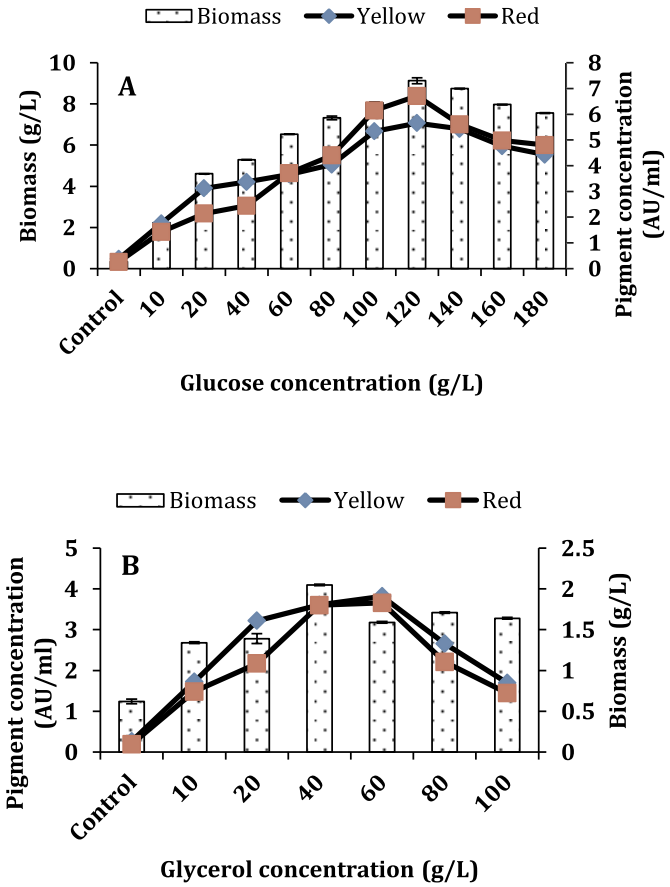

Fig. 1. Effect of different concentrations of glucose and glycerol on biomass, yellow and red pigment production. The work was incubated at 30 °C for 6 days with a pH of 6.0 in a rotary shaker at 200 rpm. The yellow and red pigment units were measured at optical density 410 and 505 respectively. Biomass and yellow and red pigments (mean ± standard deviation, n = 3). Fig. 1A. Glucose concentration (g/L), and Fig. 1B. Glycerol concentration (g/L). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

red and yellow pigments than glycerol Fig. 1. However, the optimum pigment production was achieved at glycerol concentration of 60 g/L Fig. 1B, and that of glucose was obtained at the glucose concentration of 120 g/L Fig. 1A.

In the case of glucose, increasing the glucose concentration leads to a significant increase in both mycelial growth and pigment production compared to control (Pandey, 2003), but the pigment production decreased after the concentration of 120 g/L Fig. 1A. As shown in Fig. 1A, the concentration of glucose had a significant effect on the production of yellow and red pigment. The production of yellow and red pigment increased with the rise in glucose concentration from 0 to 120.0 g/L. The highest yellow and red pigment production (5.66 ± 0.11 AU/ml) and (6.71 ± 0.11 AU/ml) respectively were obtained when 120.0 g/L of glucose was added to the fermentation broth which was 93.7% and 96.3% respectively higher than that of the control. Again at the higher concentration of glucose, the absorbance maxima shift from the yellow to red showing a tendency to produce more red pigments at a higher concentration. This may be due to the carbon-nitrogen ratio and the cultural conditions (Mukherjee and Singh, 2010). This is very important since the natural red pigment is used in East Asian countries as traditional food additives (Mukherjee and Singh, 2010; Pattanagul et al., 2007) and also as a food colorant (Feng et al., 2012).

However, in the result obtained from different glycerol concentration, there was no absorbance maxima shift of the yellow and red pigments at any level of the concentrations but rather shows that glycerol promotes yellow pigment production than red Fig. 1B. Again increasing glycerol concentration resulted in an increase in both mycelia growth and pigments production with yellow pigment being dominated, the highest yellow and red pigment production (3.82 ± 0.01 AU/ml) and (3.66 ± 0.9 AU/ml) respectively were attained when 60.0 g/L of glycerol was added to the fermentation broth which was 93.6% and 95.1% respectively higher compared to the control. The maximum biomass (2.05 ± 0.01 g/L) was produced at the glycerol concentration of 40 g/L Fig. 1B. This study is in line with Pandey (2003), as glucose remains the better carbon source compared to any other carbon source for both growth and pigment production by *Monascus purpureus*.

2.2. Effects of different nitrogen sources and concentrations on the MPs production by M. purpureus

To improve biomass as well as yellow and red pigment production by *Monascus purpureus* different nitrogen sources were added to the fermentation broth and the outcome shown (Table 2). Among the different nitrogen sources tested, KNO_3 offered both the maximum biomass and yellow and red pigment production followed by peptone. The result indicated that peptone and KNO_3 encouraged biomass production and there was no significant difference between biomass production by peptone and KNO_3. From the results, it was deduced that nitrogen sources also have a great influence on cell growth as well as yellow and red pigments production. The highest biomass (4.56 g/L) obtained was produced by KNO_3. The highest yellow and red pigments attained were (5.28 ± 0.04 AU/ml) and (5.08 ± 0.03 AU/ml) respectively (Table 2). Other studies stated that the best nitrogen sources used for good pigment production are NH_4Cl and peptone (Juzlová et al., 1996; Chen and Johns, 1993). NH_4Cl was recorded as the third-best nitrogen source and there was no significant difference in the production of the

Table 2
The effects of different nitrogen sources on pigments and biomass production by *Monascus purpureus*.

Nitrogen sources	Pigment yield (AU/ml)		Biomass (g/L)
	Yellow pigment	Red pigment	
$(NH_4)_2SO_4$	1.07 ± 0.03^{b}	0.94 ± 0.05^{b}	2.75 ± 0.004^{c}
KNO_3	5.28 ± 0.04^{e}	5.08 ± 0.03^{e}	4.56 ± 0.040^{d}
NH_4Cl	1.36 ± 0.10^{c}	1.69 ± 0.02^{d}	2.67 ± 0.002^{b}
$NaNO_3$	1.13 ± 0.09^{b}	1.2 ± 0.08^{c}	2.75 ± 0.072^{c}
Peptone	2.46 ± 0.12^{d}	1.730.11^{d}	4.53 ± 0.072^{d}
Urea	0.9 ± 0.09^{a}	0.64 ± 0.10^{a}	1.81 ± 0.002^{a}

The fermentation processes for the nitrogen sources were conducted for 6 days at a pH of 6 in a rotary shaker at 200 rpm. Data are mean ± standard deviation (n = 3). Means in a column with different lowercase letters are significantly different (ANOVA, Dancan's test; $P < 0.05$).

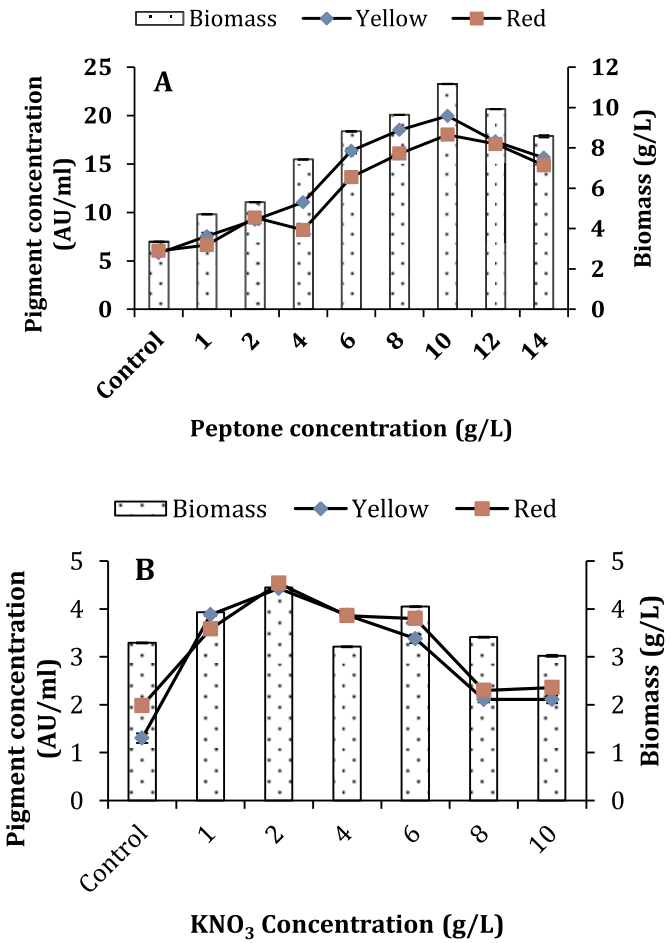

Fig. 2. The outcome of different concentration of peptone and KNO_3 on biomass, yellow and red pigments production. The experiment was incubated at 30 °C for 6 days with a pH of 6.0 in a rotary shaker at 200 rpm. The yellow and red pigment units were measured at optical density 410 and 505 respectively. Biomass and pigments (mean ± standard deviation, n = 3). Fig. 2A. Peptone concentration (g/L) and Fig. 2B. KNO_3 concentration (g/L). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

red pigments compared with peptone. The other nitrogen sources did not give any better pigment production.

The effect of different concentrations of KNO_3 and peptone on biomass and yellow and red pigments production in submerged fermentation of *Monascus purpureus* was studied and the results are shown in Fig. 2. Increasing peptone concentration stimulates cell growth and pigments production. Pigments production was significant ($P < 0.05$) at any peptone concentration level compared to the control as shown in Fig. 2A. KNO_3 obtained its maximum yellow and red pigments as well as biomass at the concentration 2 g/L. Further increase in KNO_3 concentration decreases pigment production Fig. 2B.

The biomass increased with increasing concentration of peptone from 0 to 10 g/L with the corresponding increase in yellow and red pigment. The optimum biomass (11.17 ± 0.01 g/L) and pigments produced were (19.97 ± 0.01AU/ml) for yellow and (18.03 ± 0.01AU/ml) for red at a peptone concentration of 10 g/L. Further increase in the peptone concentration resulted in a drop of biomass, yellow and red pigment which could be due to the harmful effect of high peptone concentrations on cell growth. Moreover, the results indicated that increasing the peptone concentration, perhaps resulted in a flow of pigments as cell membrane permeability may increase Fig. 2A.

However, with the KNO_3 concentration, the maximum pigment and biomass were found to be at the concentration of 2 g/L. The optimal yellow pigment, red pigment, and biomass production were found to be (4.43 ± 0.03AU/ml), (4.54 ± 0.02 AU/ml) and (4.45 ± 0.01 g/L) respectively. Furthermore, at the concentration of 4 g/L, both the biomass and yellow and red pigment decrease sharply probably triggered by the injurious effect of high concentrations of KNO_3. Again, further increased to 6 g/L of KNO_3 concentration resulted in a sharp rise of biomass and that could be owing to a change in cell membrane composition and enhance cell membrane absorbency. This effect did affect pigment production thereby resulted in a further decrease in both yellow and red pigment Fig. 2B.

2.3. Effects of different concentration of metabolic regulators on the MPs and biomass production by Monascus purpureus

A study of different metabolic regulators including pyruvic acid, malic acid, trisodium citrate, $CaCl_2$, and citric acid were conducted to promote biomass, yellow and red pigment production. The study was done by adding different concentrations of the metabolic regulators to the fermentation broth and the results shown in Fig. 3. Pyruvic acid and trisodium citrate enhanced pigment production while malic acid and citric acid inhibit pigment production. At the 0.5 g/L concentration of malic acid gave a significant increase in biomass production but did not affect pigment production as it showed no significant difference in both yellow and red pigment compared to control. Further increased in malic acid concentration decrease the yellow and red pigment significantly but stimulated biomass production compared to the control Fig. 3D. As shown in Fig. 3C, increasing the concentration of citric acid led to a significant decrease in both biomass and pigments production. Moreover, at the concentration, 4 g/L resulted in a very sharp increase in biomass production which could be as a result of variation in cell membrane structure and enhance cell membrane porosity. This study is in line with Chadni et al. (2017) which states that increasing the concentration malic and citric acid decreases pigment production. However, trisodium citrate enhanced pigment production and the maximum biomass (6.3 ± 0.04 g/L), yellow (7.9 ± 0.1 AU/ml) and red (7.01 ± 0.01 AU/ml) pigment production were obtained at the concentration of 0.5 g/L. This result is in agreement with that of (Bazaraa et al., 1998), in which the addition of citrate greatly increased the production of compactin in submerged fermentation of *P. cyclopium*. Further increase in trisodium citrate concentration resulted in a decrease in both biomass and yellow and red pigment but the yellow and red pigment showed a significant increase compared to the control. At the trisodium citrate concentration of 4 g/L, the yellow pigment showed no significant difference while red pigment decreases when they were compared to the control Fig. 3A.

Moreover, biomass, yellow and red pigment production increase as the concentration of pyruvic acid increases from 0 to 1.5 g/L in the fermentation broth. The optimal biomass (5.95 ± 0.17 g/L), yellow (7.01 ± 0.17 AU/ml) and red (6.18 ± 0.05 AU/ml) pigment production were obtained when the pyruvic acid concentration was 1.5 g/L Fig. 3B. Our study agrees with (Viggiano et al., 2017) which states that pyruvic acid enhances pigment production.

$CaCl_2$ concentration gave the maximum biomass, yellow and red pigment production at the concentration of 1.5 g/L. The optimum biomass, yellow and red pigment obtained were (6.42 ± 0.02 g/L), (7.80 ± 0.15AU/ml) and (7.18 ± 0.15 AU/ml) respectively. Further increase in $CaCl_2$ decreases both biomass and yellow and red pigment production Fig. 3E.

However, 10 g/L peptone concentration in the fermentation broth was selected together with trisodium citrate, $CaCl_2$ and pyruvic acid for further studies.

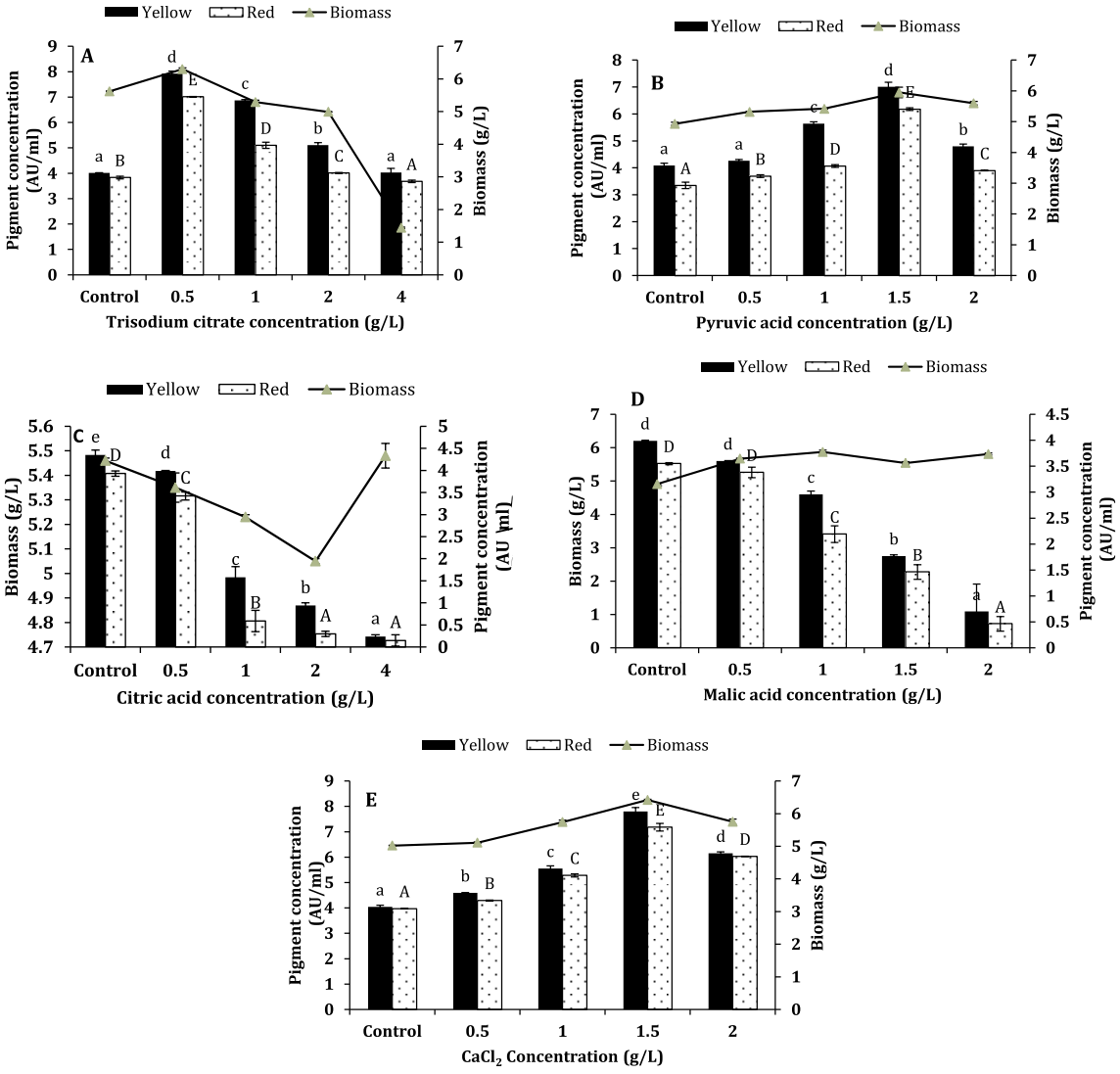

Fig. 3. Effect of different concentration of metabolic regulators on yellow and red pigment production. The study was incubated at 30 °C for 6 days with a pH of 6.0 in a rotary shaker at 200 rpm. The yellow and red pigments were measured at optical density 410 and 505 respectively. Biomass and yellow and red pigments (mean ± standard deviation, n = 3). Fig. 3A. Trisodium citrate concentration (g/L), Fig. 3B. Pyruvic acid concentration (g/L), Fig. 3C. Citric acid concentration (g/L) and Fig. 3D. Malic acid concentration (g/L) and Fig. 3E. $CaCl_2$ concentration (g/L). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.4. Enhancement MPs production by the addition of inorganic salts and organic acids to the fermentation broth containing 10 g/L peptone concentration

For pigment stimulation base on the above experiment, the broth containing 10 g/L of peptone concentration was considered. Concentration in g/L for trisodium citrate (1.5), $CaCl_2$ (1.5) and Pyruvic Acid (1.5) were added to the fermentation broth. It was expected that the addition of the selected metabolic regulators used will yield at most two folds of the pigment produced compare to the control with respect Fig. 3.

According to a proposed biosynthesis pathway of secondary metabolites of *Monascus* in submerged fermentation (Agboyibor et al., 2018), $CaCl_2$, pyruvic acid, and trisodium citrate seem to participate in the bioprocess and had a stimulatory effect on its biosynthesis. When 1.5 g/L concentration of $CaCl_2$, Pyruvic acid, and trisodium citrate were added to the fermentation broth containing 10 g/L peptone concentration, shown a significant increase in yellow and red pigment as well as biomass production at $P < 0.05$ (Table 3). The optimum yellow and red pigment production which is almost 5 folds compared to the control was obtained when pyruvic acid was added to the fermentation broth, the yellow and red pigment yield were 99.81 ± 5.25 AU/ml and 97.22 ± 6.12 AU/ml respectively (Table 3). Moreover, the effect of fermentation time on pigment, pH curve, and biomass production was

Table 3
Effect of metabolic regulators on *Monascus* yellow and red pigment production.

Metabolic regulator	Yellow pigment (AU/ml)	Red pigment (AU/ml)	Biomass (g/ml)
Pyruvic acid	99.81 ± 5.25^{d}	97.22 ± 6.12^{d}	20.4 ± 0.8^{c}
Trisodium citrate	43.52 ± 4.11^{c}	42.69 ± 3.09^{c}	16.9 ± 0.6^{a}
$CaCl_2$	33.62 ± 2.03^{b}	31.97 ± 3.06^{b}	18.2 ± 0.7^{b}
Control	20.02 ± 0.51^{a}	19.31 ± 0.43^{a}	10.6 ± 0.4^{b}

Analysis of metabolic regulators as pigment enhancer when 1.5 g/L of each metabolic regulators were added to a fermentation broth containing 10 g/L peptone concentration at the beginning of the fermentation. The fermentation processes were conducted for 6 days. Data are mean ± standard deviation ($n = 3$). Means in a column with different lowercase letters are significantly different (ANOVA, Dancan's test; $P < 0.05$).

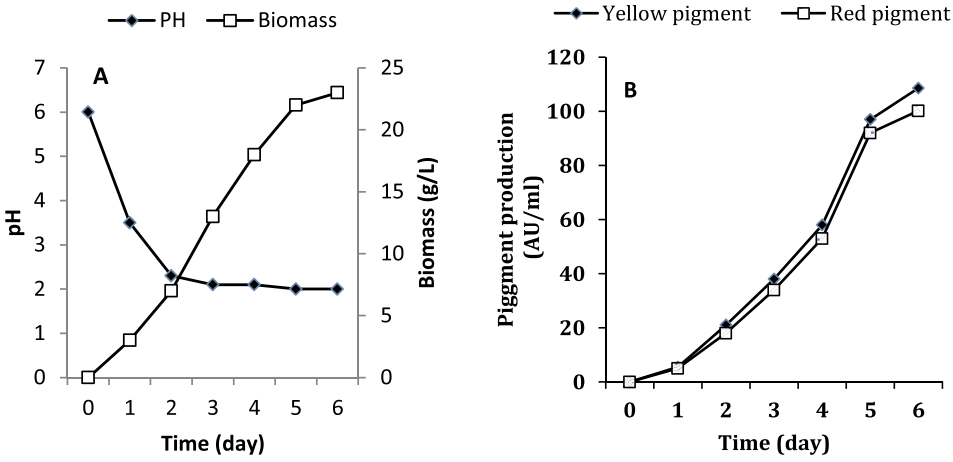

Fig. 4. The effect of fermentation time on yellow and red pigment, pH curve and biomass production when pyruvic acid was added to the fermentation broth containing 10 g/L peptone concentration. Growth and pigments yield (optical density of 410 and 505) of *Monascus purpureus* (mean ± standard deviation, n = 3). Fig. 4A. Effect of fermentation time on yellow and red pigment production and Fig. 4B. Effect of fermentation time on pH and cell growth.

Table 4
Effect of pH on yellow and red pigment production when 1.5 g/L pyruvic acid was added to the fermentation broth containing 10 g/L peptone.

pH	Yellow pigment (AU/ml)	Yellow pigment (AU/ml)	Biomass (g/L)
4.0	395.82 ± 5.97^{e}	385.30 ± 4.24^{e}	22.5 ± 0.5^{d}
4.5	438.72 ± 8.28^{f}	432.92 ± 3.55^{f}	25.2 ± 0.3^{e}
5.0	574.76 ± 6.18^{g}	570.98 ± 7.66^{g}	27.7 ± 0.1^{f}
5.5	354.66 ± 5.50^{d}	343.65 ± 9.50^{d}	24.8 ± 0.2^{e}
6.0	123.82 ± 5.44^{c}	116.85 ± 6.09^{c}	19.4 ± 0.2^{c}
6.5	105.72 ± 4.34^{b}	102.93 ± 2.02^{b}	17.9 ± 0.1^{b}
7.0	87.90 ± 0.13^{a}	86.64 ± 0.58^{a}	17.4 ± 0.1^{a}

Conditions: Each shaking-flask culture containing 1.5 g/L pyruvic acid and 10 g/L peptone and other parameter been same as previous work was incubated at 30 °C for 6 days in a rotary shaker at 200 rpm, the yellow and red pigment units were measured at Optical density 410 and 505 respectively. Biomass and yellow pigments (mean ± standard deviation, n = 3) having different lowercase letters, respectively, have a significant difference (ANOVA Dancan's test; $p < 0.05$).

also examined as shown in Fig. 4. The production of *Monascus* yellow and red pigment conducted with an initial pH of 6.0 and at 200 rpm in 6 days. It was expected that the fermentation process will have four growth phases including the lag phase, the logarithmic phase, the stationary phase, and the decline phase from the growth curve of *Monascus purpureus*. However, only 3 growth phases were observed; (0–1) the lag phase, (1–4) the logarithmic phase, and (4–6) the stationary phase Fig. 4A. A decline phase was not observed indicating adequate nutrient and oxygen supply that could aid in a probable growth and pigment production. Again, in the first 4 days, the yellow and red pigment accretion in this period was relatively slow, even though the biomass of *Monascus purpureus* grew faster. Subsequently, rapid accretion of yellow and red pigments was conveyed with the mycelial growth reaching the stationary phase. The pigment concentration reached the maximum level of 108.6 AU/ml for yellow and 100.2 AU/ml for red on the 6 days Fig. 4B. The pH curve in the medium also showed the relationships between biomass productions, nutrient consumption and pH changes in the medium. The change and difference of pH in medium might be due to the nutrient assimilation and organic acids generation during fermentation of *Monascus purpureus*.

The effects of different initial pH values when pyruvic acid was added 10 g/L peptone concentration on the biomass and yellow and red pigment production were also examined (Table 4). The biomass and yellow and red pigment production increased as the pH increased from 4.0 to 5.0, above which substantial inhibition ($P < 0.05$) was discovered. This study is in agreement with (Lv et al., 2017). The finest initial pH for mycelial growth was 5.0, with the biomass 27.6 g/L, while the maximum yellow and red pigment concentration were (574.76 ± 6.18 AU/ml) and (570.98 ± 7.66 AU/ml) respectively at ($p < 0.05$) acquired at the same pH value. The developments of yellow and red pigment yield along with the different initial pH were the same as the biomass yield. The results showed that *Monascus* mycelial tend to grow better at an acidic pH than a near-neutral pH and the higher biomass accord with the higher yield of yellow and red pigment production. The pH as well as the pyruvic acid disturb the fungal cell wall structure and modify the conformation of proteins protruding from the plasma membrane, which exercise effect on the lipid organization and function of cellular membranes (Liu et al., 2015). Therefore, the effect of pH and pyruvic acid on mycelial growth development could be closely linked to its effect on the exterior features of the fungal cells.

3. Conclusion

The results of our work indicated the viability and applicability of pyruvic acid, an organic acid, for submerged fermentation production of yellow and red pigments from *Monascus purpureus*. Addition of pyruvic acid significantly enhanced the production of yellow and red pigment in submerged fermentation of *Monascus purpureus.* The mycelial growth of *Monascus purpureus* was significantly influenced by the culture conditions such as the initial pH which further exerted a great impact on the production of yellow and red pigments. The current finding gives us the basic information on which the scaled-up production of valuable metabolites by filamentous fungi such as *Monascus* in submerged fermentation could be carried on.

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