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STANDARDIZATION OF FERMENTATION PARAMETERS FOR THE PRODUCTION OF FOOD BIO-COLOURS THROUGH SUBMERGED SHAKE FLASK FERMENTATION

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ABSTRACT
The food bio-colours are gaining importance and have become the focus of attention of many scientists all over the world due to toxicity problems caused by synthetic colours. The feasibility of the production of food bio-colours through submerged shake flask fermentation by using *M. purpureus* (MTCC 410) was investigated by optimizing the fermentation conditions. The higher yield of red, orange, yellow and total bio-colours were 57.42 OD Units/g dcm at 500 nm, 52.34 OD Units/g dcm at 475 nm, 9.38 OD Units/g dcm at 375 nm and 119.14 OD Units/g dcm respectively achieved through submerged shake flask fermentation at optimized process parameters including 200 rpm agitation speed, pH 6.0, incubation at 30ºC with 2% inoculum of 6 days old and an incubation period of 7 days by utilization of glucose (2% w/v) and peptone (1.5% w/v) as a carbon and nitrogen source respectively. The enhanced yield of bio-colours indicated that submerged shake flask fermentation process has good potentiality for the production of food bio-colours.

INTRODUCTION

With the advent of strict legislative regulations and growing awareness among the consumers about the food safety, bio-colours have become the choice in the foods as these are considered as safer than their synthetic counterparts. Bio-colours could be a dye, pigment or substance that can impart colour when added or applied to a food, drug, cosmetics etc. Bio-colours are of biological origin derived from plants, insects or microbes (Sharma, 2014). Micro-organisms have high growth rate and productivity for pigment (Babhita, 2009), which reduced the production time of bio-colours using a process with continuous operation (Hendry and Houghton, 1997). In addition, microbial production is flexible and can be easily controlled as compared to plant or animal sources. It is great advantageous to use microbes for the production of food bio-colours due to their intrinsic properties of high growth rate, no seasonal variation, high production rate and ease of manipulation (Joshi *et al.,* 2003).

The bio-colours have been produced from large number of bacterial, yeast and mold species. The microorganisms use as a bio-colour source should have some necessary features. Among the different microorganisms, *Rhodotorula* spp., *Achromobacter* spp., *Blakeslea* spp., *Micrococcus* spp., *Chromobacter* spp., *Sarcina* spp. and *Monascus* spp. are common bio-colours producing microbes (Joshi *et al.,* 2003). The application of *Monascus* bio-colours in food industry has been carried out traditionally in the oriental foods for hundreds of years (Babhita *et al.,* 2004; Teng and Feldheim, 2001). Bio-colours from this fungus widely used in food and pharmaceutical industries for therapeutic uses also (Kumar *et al.,* 2012).

At present, food bio-colours production at an industrial scale is not economical since the cost of production is still high. Therefore, the development of low cost comparatively viable process is needed for the production of food bio-colours. *Monascus* is probably a xerophilic fungus, which grows in a wide variety of natural substrates (Babhita *et al.,* 2004). *Monascus* fermentations have been performed mainly in solid cultures; however production yield is too low to compensate its economic availability and hence recent research efforts have focused on submerged shake flask fermentation to enhance the yield (Mukherjee and Singh, 2011). There are reports concerning mutation of strains, changes in nutrients and culture conditions. Submerged fermentation techniques have also been developed including fedbatch cultures, bioreactors and shake flask (Kim *et al.,* 2002). Moreover, studies on bio-colours synthesis by various strains of *Monascus purpureus* in submerged cultures revealed that the yield is affected by medium composition, temperature, pH, inoculums age, inoculums size, incubation time and agitation speed (Hamdi *et al.,* 1997). Particularly, composition of bio-colours varies significantly depending on the type of nutrients available such as carbon, nitrogen sources and the strain used. Therefore, optimization of various physical, chemical and nutritional parameters for maximum yield of bio-colours in

submerged shake flask fermentation was very important before isolation of food bio-colours.

Keeping in view the above, the present investigation was carried out to standardize the fermentation parameters for the production of food bio-colours through submerged shake flask fermentation by using *M. purpureus* (MTCC 410)

MATERIALS AND METHODS

Microorganism

The freeze dried culture of *M. purpureus* (MTCC 410) was obtained from Institute of Microbial Technology (IMTECH) Chandigarh, India. The stock culture was grown on PDA slants for 7 days at 30ºC and maintained at 4ºC in refrigerator by periodically sub-culturing after every 2 months.

Preparation of inoculum

The *M. purpureus* (MTCC 410) strain was grown on PDA slants for 7 days at 30ºC. Spores were harvested from slants by adding 8 ml of 0.85% sterile saline to each of the tube and scrapping of spores gently into saline solution under strict aseptic conditions.

Submerged shake flask fermentation

The chemical composition of synthetic medium for submerged shake flask fermentation of *M. purpureus* (MTCC 410) is as mentioned in Table 1 (Lin and Demain, 1991). The medium was autoclaved at 121ºC for 20 minutes and cooled to room temperature. The spore suspension was inoculated into the 250 ml Erlenmeyer flask containing 100 ml of sterile synthetic medium under aseptic conditions. This was incubated on a rotary shaker incubator for 12 days. At the end of the specified incubation period, the biomass was harvested and the bio-colours were extracted by solvent extraction to estimate the yield of bio-colours (Vanajakshi, 2006). The submerged shake flask fermentation process was performed as per the procedure depicted in Fig. 1.

Optimization of fermentation parameters

Different *Monascus* species have similar growth patterns and yield profiles, but their optimized conditions vary, depending upon the strain variability. Optimization of the fermentation process parameters is needed for improved production of food bio-colours to make the process cost effective.

Standardization of fermentation parameters

The optimum level of agitation speed was standardized by varying agitation speed (50, 100, 150, 200 and 250 rpm) for 100 ml of sterile synthetic medium. The incubation temperature was standardized by varying temperature ranges (20ºC, 25ºC, 30ºC, 35ºC and 40ºC).The medium was inoculated with 3 days old culture to 7 days old culture in 250 ml Earlenmayer flasks. The level of inoculum was standardized by varying levels of inoculum (1, 2, 3, 4 and 5%). The time of incubation was standardized by varying time periods (3, 5, 7, 9 and 12 days).The pH was standardized by varying initial pH of synthetic medium ranging from 3.0, 4.0, 5.0, 6.0 and 7.0 using 0.1N HCl.

Carbon sources

The best carbon source was selected by studying the effect of different carbon sources (maltose, sucrose, glucose, fructose and lactose). The various concentrations of best carbon source (1, 2, 3, 4 and 5%) were tried to obtain higher yield of bio-colours.

Nitrogen sources

The best nitrogen source was selected by studying the effect of different nitrogen sources (urea, ammonium sulphate, peptone, yeast extract and MSG). The various concentrations (0.5, 1, 1.5, 2 and 2.5%) of best nitrogen source were tried to obtain higher yield of bio-colours.

Extraction of food bio-colours

Extracellular bio-colours

At the end of fermentation, the fermented broth in each flask was filtered individually using Whatman No. 1 and the mycelia was washed two times with distilled water. The filtrate and the washings were made up to 100 ml (Carvalho *et al*., 2007; Velmurugan *et al*., 2011).

Intracellular bio-colours

The washed mycelia was suspended in 25 ml of alcohol, distilled water and hexane respectively incubated on a rotary shaker (150 rpm) at 30ºC for 30 minutes and filtered. Extraction was repeated 2-3 times. Finally, the filtered extracts were pooled together and made up to 50 ml (Carvalho *et al*., 2007; Velmurugan *et al*., 2011).

Estimation of bio-colours

Bio-colour concentration was determined by taking absorbance (OD) at specified wave-length of 500nm, 475nm and 375nm using UV-VIS spectrophotometer. The bio-colours yield (OD Units/g dcm) of individual bio-colour was calculated using the following formula.

$$\text{Bio colours yield} = \frac{OD_{(abs)} \times \text{Dilution factor} \times \text{Total vol. of bio colour}}{\text{Dry weight of biomass}}$$

The total red, orange and yellow bio-colours yield was obtained by summing up the extra and intracellular bio-colours yield at λ_{500}, λ_{475} and λ_{375} respectively (John and Stuart, 1991).

Determination of dry weight of biomass

The fungal biomass in the synthetic medium was filtered through pre-weighed Whatman filter paper and washed twice with deionized water followed by drying at 105°C for 3-5 hrs and weighed to estimate the dry weight of biomass (Velmurugan *et al.,* 2011).

Statistical analysis

The data obtained in the present investigation was statistically analysed by using completely randomized design as per method of Panse and Sukhatme (1989).

RESULTS AND DISCUSSION

Effect of different fermentation parameters on yield of bio-colours

The results obtained during optimization of fermentation parameters by submerged shake flask fermentation with respect to its agitation speed, temperature, inoculum age, inoculum size, incubation time, pH also supplementation with carbon and nitrogen sources are categorized and discussed under suitable captions as follows:

Table 1: Composition of growth medium

Sr. No.	Chemical	g/L
1	Maltose	50
2	MSG	12.6
3	K_2HPO_4	2.40
4	KH_2PO_4	2.40
5	$MgSO_4$	1.00
6	KCl	0.50
7	$ZnSO_4.7H_2O$	0.011
8	$FeSO_4.7H_2O$	0.010
9	$MnSO_4.H_2O$	0.030

M. purpureus (MTCC 410) exhibited an increase in yield of bio-colours as the incubation conditions changed from static to shaken conditions at different agitation rates. It can be expected that an increase in shaking increases oxygen dispersal and yield of bio-colours. The total yield of bio-colours was increased up to 200 rpm (115.04 OD Units/g dcm) and reduced thereafter to the value of 105.40 OD Units/g dcm for agitation speed of 250 rpm. The expectation mentioned above were confirmed *i.e.* a higher stirred speed lead to a higher yield of red, orange and yellow bio-colours. The maximum yield of red, orange and yellow bio-colours were 58.20, 42.19

Table 2: Effect of agitation speed on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dcm)			
	Red	Orange	Yellow	Total
TA_1	54.17	39.06	7.19	100.42
TA_2	56.25	39.96	8.71	104.88
TA_3	57.29	40.00	11.88	109.38
TA_4	58.20	42.19	14.45	115.04
TA_5	55.42	39.17	10.83	105.40
SE ±	0.24	0.27	0.23	0.32
CD at 5%	0.73	0.79	0.70	0.96

(TA_1 = 50 rpm, TA_2 = 100 rpm, TA_3 = 150 rpm, TA_4 = 200 rpm and TA_5 = 250 rpm)

Table 3: Effect of temperature on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dcm)			
	Red	Orange	Yellow	Total
T_1	45.19	34.90	13.54	93.23
T_2	45.67	38.18	15.87	99.52
T_3	48.84	43.75	18.30	110.89
T_4	38.54	33.33	29.17	101.04
T_5	32.39	28.63	38.07	99.09
SE ±	0.14	0.19	0.17	0.22
CD at 5%	0.41	0.57	0.51	0.65

(T_1 = 20ºC, T_2 = 25ºC, T_3 = 30ºC, T_4 = 35ºC and T_5 = 40ºC)

Table 4: Effect of inoculum age on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dcm)			
	Red	Orange	Yellow	Total
TG_1	44.13	34.75	5.94	84.81
TG_2	44.89	37.27	8.86	91.02
TG_3	46.25	42.11	7.02	95.38
TG_4	52.94	48.09	15.59	116.62
TG_5	48.75	45.00	10.33	103.68
SE ±	0.16	0.29	0.24	0.30
CD at 5%	0.49	0.87	0.73	0.89

(TG_1 = 3 Days, TG_2 = 4 Days, TG_3 = 5 Days, TG_4 = 6 Days and TG_5 = 7 Days)

Table 5: Effect of inoculum size on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dcm)			
	Red	Orange	Yellow	Total
TS_1	54.17	39.06	7.19	100.42
TS_2	56.25	39.96	8.71	104.90
TS_3	57.29	40.00	12.08	109.38
TS_4	58.20	42.19	14.45	114.85
TS_5	55.42	39.17	10.83	105.40
SE ±	0.20	0.27	0.23	0.23
CD at 5%	0.60	0.80	0.69	0.67

(TS_1 = 1%, TS_2 = 2%, TS_3 = 3%, TS_4 = 4% and TS_5 = 5%)

Table 6: Effect of incubation time on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dcm)			
	Red	Orange	Yellow	Total
TT_1	37.75	34.00	4.75	76.50
TT_2	39.90	37.69	13.17	90.77
TT_3	43.50	41.75	16.25	101.50
TT_4	41.96	39.46	15.18	96.61
TT_5	41.46	37.19	11.56	90.21
SE ±	0.24	0.18	0.22	0.28
CD at 5%	0.71	0.53	0.65	0.84

(TT_1 = 3 Days, TT_2 = 5 Days, TT_3 = 7 Days, TT_4 = 9 Days and TT_5 = 12 Days)

Table 7: Effect of pH on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dcm)			
	Red	Orange	Yellow	Total
TH_1	23.07	32.73	37.61	93.41
TH_2	24.48	32.81	39.58	96.68
TH_3	30.80	35.31	33.93	100.20
TH_4	40.54	33.98	31.25	105.46
TH_5	38.75	33.33	30.08	102.17
SE ±	0.23	0.18	0.23	0.42
CD at 5%	0.68	0.53	0.69	1.25

(TH_1 = 3, TH_2 = 4, TH_3 = 5, TH_4 = 6 and TH_5 = 7)

Table 8: Effect of carbon source on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dcm)			
	Red	Orange	Yellow	Total
TC_1	48.21	44.64	15.63	108.48
TC_2	44.89	42.05	12.95	99.89
TC_3	52.07	45.70	16.41	114.38
TC_4	42.61	39.20	12.50	94.18
TC_5	40.63	37.50	11.25	89.38
SE ±	0.13	0.12	0.12	0.20
CD at 5%	0.39	0.37	0.36	0.60

(TC_1 = Maltose, TC_2 = Sucrose, TC_3 = Glucose, TC_4 = Fructose and TC_5 = Lactose)

Table 9: Effect of carbon concentration on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dcm)			
	Red	Orange	Yellow	Total
TCC_1	48.44	42.71	14.58	105.65
TCC_2	50.83	47.50	15.83	114.17
TCC_3	52.46	51.09	17.58	121.33
TCC_4	51.34	46.43	16.07	113.84
TCC_5	48.56	46.63	13.46	108.65
SE ±	0.16	0.17	0.19	0.22
CD at 5%	0.47	0.51	0.57	0.65

(TCC_1 = 1%, TCC_2 = 2%, TCC_3 = 3%, TCC_4 = 4% and TCC_5 = 5%)

Table 10: Effect of nitrogen sources on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dcm)			
	Red	Orange	Yellow	Total
TN_1	50.42	42.08	12.50	105.00
TN_2	46.43	38.39	9.38	94.20
TN_3	55.88	47.06	15.81	118.55
TN_4	48.33	40.00	11.25	99.58
TN_5	52.34	44.96	13.67	110.94
SE ±	0.17	0.26	0.16	0.21
CD at 5%	0.50	0.77	0.48	0.63

(TN_1 = Urea, TN_2 = Ammonium sulphate, TN_3 = Peptone, TN_4 = Yeast extract and TN_5 = MSG)

Table 11: Effect of nitrogen concentration on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dcm)			
	Red	Orange	Yellow	Total
TNC_1	51.50	46.88	5.63	104.00
TNC_2	54.69	48.96	7.29	110.94
TNC_3	55.63	50.45	8.04	114.11
TNC_4	57.42	52.34	9.38	119.14
TNC_5	56.25	51.67	8.75	116.67
SE ±	0.23	0.15	0.19	0.40
CD at 5%	0.70	0.45	0.57	1.18

(TNC_1 = 0.5%, TNC_2 = 1%, TNC_3 = 1.5%, TNC_4 = 2% and TNC_5 = 2.5%)

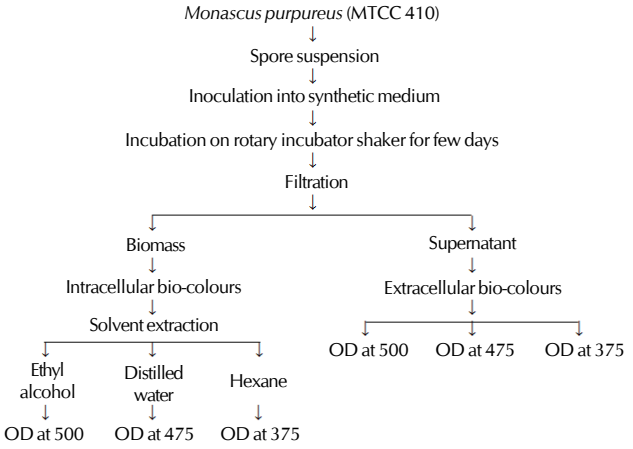

Figure 1: Production of food bio-colours through submerged shake flask fermentation

and 14.45 OD Units/g dcm respectively found at agitation speed of 200 rpm. In view of these results, it can be concluded that agitation speed significantly affect the morphology in submerged shake flask culture. The results of the present study are confirmed by the comparable results by Gunasekaran and Poorniammal (2008) reported that the pigment production was increased up to 200 rpm (920 mg/l) and reduced thereafter.

The results (Table 3) indicated that maximum total yield of bio-colours was obtained at 30ºC. The highest total yield of bio-colours was 110.89 OD Units/g dcm noted at 30ºC while the lowest total yield was 93.23 OD Units/g dcm recorded at 20ºC of incubation temperature, production decreased drastically at higher temperatures due to the mesophilic nature of *Monascus* spp. The results are comparable with previous literature which showed that incubation of 30ºC favoured best growth and bio-colours yield (Lee *et al.*, 2001). The higher yield of red bio-colour (48.84 OD Units/g dcm) was observed at 30ºC and thereafter the yield get decreased. The highest yield of yellow bio-colour *i.e.* 38.07 OD Units/g dcm was noticed at 40ºC of incubation temperature. Carvalho *et al.* (2005) reported a shift in absorbance maxima of bio-colours extract at different incubation temperatures. The present findings are in consistent with the results reported by (Chatterjee *et al.*, 2009; Dikshit and Tallapragada, 2011; Mukherjee and Singh, 2011; Reeba panesar, 2014) who observed good bio-colours production in the temperature range of 30-35ºC. Kamala Das *et al.* (2013) observed the maximum production of phytase by at 28ºC incubation temperature.

The results indicated that the medium inoculated with 6 days old culture gave the better yield of bio-colours *i.e.* red bio-colour (52.94 OD Units/g dcm), followed by orange bio-colour (48.09 OD Units/g dcm) and yellow bio-colour (15.59 OD Units/g dcm) as shown in the Table 4. The 6 days old inoculum also gave a highest total yield of bio-colour *i.e.*116.62 OD Units/g dcm, while the culture of 3 days old gave a lowest total yield of 84.31 OD Units/g dcm. The data is clearly supported by the comparable results given by Cho *et al.* (2002) and Gunasekaran and Poorniammal (2008). Amongst several fungal physiological properties, the inoculum age usually plays an important role in fungal development (Glazebrook *et al.*, 1992; Bae *et al.*, 2000).

The amount of inoculum is of prime importance in determining the morphology and the general pattern of fungal fermentation. It is clearly revealed from the Table 5 that higher total yield of bio-colour was (114.85 Units/g dcm) obtained with 4% of inoculum level. The medium inoculated with 4% of spores suspension maximized the yield of red bio-colour (58.20 OD Units/g dcm), followed by orange bio-colour (42.19 OD Units/g dcm) and yellow bio-colour (14.45 OD Units/g dcm). The results are in agreement with previous studies (Pandey *et al.*, 2003; Babitha *et al.*, 2006; Chakradhar *et al.*, 2009). The lower levels of inoculum resulted in insufficient biomass and lower yield of bio-colours, whereas too much inoculum produced excessive biomass and depleted the nutrients required for bio-colours formation (Babitha *et al.*, 2006).

As depicted in Table 6, remarkably higher yield of red, orange and yellow bio-colours were observed on 7th day of fermentation. The comparatively higher yield of red, orange and yellow bio-colours were 43.50 OD Units/g dcm, 41.75 OD Units/g dcm and 16.25 OD Units/g dcm respectively recorded after the completion of 7 days of incubation period. Significant variation in yield of bio-colours was observed during different fermentation periods. The yield of bio-colours obtained with respect to varied incubation time during submerged shake flask fermentation were more or less in conformity with the results observed by Mukherjee and Singh (2011) and Reeba panesar (2014). The variations in incubation time to achieve maximum yield of bio-colours may be due to strain specificity. It has been reported in previous literature that the maximum yield of bio-colours was observed on 7th day of fermentation using *M. purpureus* (MTCC 410) by Dikshit and Tallapragada, (2011).

The initial pH value of the medium markedly influenced red

and yellow bio-colours formation. The results indicated that pH 3.0, 4.0 and 5.0, the maximum absorbance shifted to 375 nm and 475 nm respectively. The red bio-colour (*i.e.* 40.54 OD Units/g dcm) yield was maximal at pH 6 and yellow bio-colour (*i.e.* 39.58 OD Units/g dcm) was maximal at pH 4 (Table 7). The higher total yield (105.46 OD Units/g dcm) of bio-colours was observed at pH 6, while lesser total yield of bio-colours (93.41 OD Units/g dcm) was noticed at pH 3. It has been previously reported that *Monascus* spp. were able to grow at wide range of pH values (2.5-8.0) with maximum in the range of 4.0-7.0 (Carvalho *et al.,* 2005). pH influenced the physiology of fungi, conidial development and pigment synthesis. The low pH (3.0-4.0) favoured yellow bio-colour production while high pH (6.0-7.0) dominated the production of red bio-colour in chemically defined media using *Monascus purpureus* (NFCCI 1756). However, the orange fraction maintained a steady state irrespective of pH condition (Mukherjee and Singh, 2011). The overall findings of this study were closely related to findings reported by Cho *et al*. (2002) who observed the optimal initial pH 6.0 for both mycelial growth and pigment production. Raja Husain *et al*. (2015) and Kamala Das *et al*. (2013) also reported that the optimum pH of the enzyme activity of amylase and phytase was found to be 6.

It is clear from the results that glucose proved to be better than other carbon sources in terms of bio-colours yield (Table 8). The use of glucose 3% (w/v) as a carbon source is pronounced with a maximum red bio-colour yield of 52.07 OD Units/g dcm, orange bio-colour yield of 45.70 OD Units/g dcm and yellow bio-colour yield of 16.41 OD Units/g dcm after 7 days of fermentation. The researchers have reported that glucose was a superior substrate for maximum bio-colours yield by *Monascus* spp. (Juzlova *et al*., 1994; Yoshimura *et al.,* 1975; Border and Koehler, 1980; Lin and Demain, 1991). The type and concentration of the carbon sources are known to directly affect the growth of *Monascus* spp. (Lee *et al.*, 2001).

The yield of bio-colours was examined at different glucose concentrations (1-5% w/v), higher yield of bio-colours was obtained at a glucose concentration of 3% (w/v) (Table 9). The use of glucose (3% w/v) increased yield *i.e.* 52.46, 51.09 and 17.58 OD Units/g dcm for red, orange and yellow bio-colours respectively. However, above this concentration bio-colours yield were drastically reduced due to crabtree effects. The crabtree effect involves a shift in metabolism from aerobic to partly anaerobic even though plenty of oxygen may be available (Chen and Johns, 1994; Carvalho *et al.*, 2007), which generally occur in yeast batch fermentation with high sugar concentrations and inhibit respiratory enzyme and increase ethanol production. Chen *et al*. (1971) supported these findings by stating that glucose at 2 g/L was optimum for red pigment production and it was reduced above this concentration, perhaps due to respirofermentative metabolism. Mukherjee and Singh (2011) also found the glucose concentration of 18 g/L to be optimum for bio-colours production.

Among all the other nitrogen sources tested, peptone (1% w/v) as a nitrogen source gave the highest yield of bio-colours (Table 10). It was also observed that total yield of bio-colours obtained from peptone (118.55 OD Units/g dcm) was higher as compare with the MSG (110.94 OD Units/g dcm). When the medium was supplemented with 1% (w/v) of peptone the remarkable increase in red, orange and yellow bio-colours were 55.88, 47.06 and 15.81 OD Units/g dcm respectively noted. The literature on the use of above tested nitrogen sources for the yield of microbial bio-colours is very scarce, however, it has been reported that organic nitrogen sources gave better yield than inorganic nitrogen sources by *Monascus* spp. (Pastrana *et al.,* 1995; Dufosse *et al.,* 2005). It has been also observed that the yield of bio-colours varies with nitrogen supplementation by *Monascus* spp. (Jung *et al*., 2003).

The effect of peptone concentration on yield of bio-colours is shown in Table 11. Optimum peptone concentration was determined to be 1.5% (w/v) by shake flask culture for maximum yield of bio-colours. The maximum yields of red, orange and yellow bio-colours were 57.42, 52.34 and 9.38 OD Units/g dcm respectively with 1.5% (w/v) peptone as a nitrogen source. Increased peptone concentrations increased biomass, but concentrations of over 1.5% (w/v) of peptone decreased yield of bio-colours. The observations of present investigation are in agreement with the data reported by Cho *et al*. (2002) and Dikshit and Tallapragada (2013). The optimum concentration of nitrogen sources influences growth, sporulation and the yield of bio-colours produced by *M. purpureus* (MTCC 410). The consumption of nitrogen sources produces different pH profiles in controlled fermentation and this also affect the pattern of growth and bio-colours yield (Wong *et al*., 1981; Martinkova and Patakova, 1999).

The outstanding yield of red, orange, yellow and total bio-colours were 57.42, 52.34, 9.38 and 119.14 OD Units/g dcm respectively achieved with submerged shake flask fermentation at optimized process parameters including 200 rpm agitation speed, pH 6.0, incubation at 30ºC, inoculation with 2% spore suspension of 6-days old culture and an incubation period of 7 days by utilization of glucose (3% w/v) and peptone (1.5% w/v) as a carbon and nitrogen source respectively.

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