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MEDIA OPTIMIZATION FOR THE DECOLORIZATION OF DISTILLERY EFFLUENT BY *STREPTOCOCCOUS SP.*

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ABSTRACT

The wastewater released from distilleries and fermentation industries contain high BOD, COD alongwith melanoidin pigment. Melanoidin is a Xenobiotic dark brown colour compound, which is formed by the Maillard reaction between amino acid and carbonyl group of sugars. This recalcitrant compound causes soil and water pollution therefore must be treated before disposal. The aim of this study was to isolate and optimize a potential melanoidin decolorizing bacterium from natural resources for treatment of distillery effluent at industrial level.

In this study a strain of *Streptococus sp.* was isolated from distillery nearby natural ecosystem and optimized for decolorization of distillery effluent at various physico-chemical and nutritional

levels. This bacterium showed maximum decolorization, 87% at 40˚C using modified GYPE Medium i.e. 1% molasses medium (1%., Grade-C molasses, 0.2%., Yeast extract, 0.3%., Peptone, 0.05%., MgSO4, 0.05%., K2HPO4 with 3.5 OD effluent) pH-6.0 within 30 hours. This *Streptococcus sp*. can tolerate wide range of temperature as well as pH and survive in very less amount of carbon (0.2%) and nitrogen(0.2%) sources in submerged system. Hence, this bacterium has potential to degrade the melanoidin of distillery spent wash at industrial level for controlling environmental pollution.

Key words: Decolorization, *Streptococus* sp., Melanoidin, Maillard reaction, Xenobiotic, Decolorization

INTRODUCTION

Molasses based distilleries and Baker's yeast production industries produces dark brown color spent wash with a high Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), low pH and toxic substances such as phenols. The main problem in treating Distillery Spent Wash (DSW) is its color, which contains nearly 2% (w/w) of a dark brown recalcitrant pigment, melanoidin. Melanoidin is known as a natural browning polymer, produced by the "Maillard reaction" between amino and carbonyl groups of sugar and is closely related to humic substances in the natural environment.

several researchers have reported when melanoidin is discharged at higher concentration from distilleries and fermentation industries makes complexation with other environmental pollutants along with heavy metals due to its anionic properties (Chandra et al., 2017; Tripathi et al., 2021a, 2021b, 2021c, 2021d, 2021e).

Melanoidin is toxic as much as phenol, so when released in aquatic system, leads to reduction of sunlight penetration in rivers and lakes and thereby reducing the photosynthetic activity. On the other hand disposal on land causes reduction in soil alkalinity, manganese availability, and inhibits seed germination (Agrawal and Pandey 1994; Agarwal et al. 2010).

At present in India, there are more than 397 distilleries are operating, producing approx. 3.25×1010 liters of ethanol and generating 40.90×1015 liters of effluent annually (AIDA 2016). Therefore, distillery effluent requires a pretreatment before its disposal into the environment.

Different physico-chemical methods such as filtration (Satyawali and Balakrishnan 2008), flocculation (Liang et al. 2009a), adsorption (Onyango et al. 2011), chemical precipitation or coagulation (Chandra and Singh 1999; Liang et al. 2009b), UV/H2O2 treatment (Dwyer and Lant 2008) and ozone oxidation (Kim et al. 1985) has been reported for removal and decolourisation of distillery effluent, but these techniques are not feasible at industrial level due to high cost, requires large amount of reagents, generate large amount of sluge and formation of hazardous by-products (Pena et al., 2003; Mohana et al; 2007).

In contrast, the biological treatment methods are alternative to chemical decomposition process, have drawn attention of various workers world over due to their low cost, environmental friendly and publicly

acceptable (Kaushik et al. 2010). Under biological treatment process using fungi such as *Geotrichum candidum* (Kim and Shoda 1999) *Flavadon flavus* (Raghukumar and Rivonkar 2001), *Phanerochaete chrysosporium* (Thakkar et al. 2006), *Trametes sp.* (Gonzalez et al. 2000), *Coriolus hirsutus* (Miyata et al. 2000), *Pleurotus florida*, *Aspergillus flavus* (Pant and Adholeya 2009a), *Neurospora intermedia* (Kaushik and Thakur 2013), *Fusarium verticillioides* (Pant and Adholeya 2009b); yeast such as *Citeromyces sp*.(Sirianuntapiboon et al. 2004a), *Candida tropicalis* (Tiwari et al. 2012), *Candida glabrate* (Mahgoub et al. 2016); and certain bacteria such as Bacillus sp.(Kambe et al. 1999; Ponraj et al. 2011; Abhijit B. Shinde and Dhanraj B. Nakade 2021; Vineet kumar et al. 2022), Pseudomonas fluorescens(Dahiya et al. 2001; Agarry and Ajani, 2011; M.A. Boopathy and S.N.S. Senthikumar 2014), acetogenic bacteria(Sirianuntapiboon et al. 2004b), Pediococcus acidilactici B-25(Tiwati et al. 2013) have been reported for melanoidins degradation using different category of melanoidins. The fungus has been reported more effective for decolorization of distillery effluent due to prevalence of ligninolytic enzymes, which metabolizes melanoidins as a sole carbon and nitrogen sources (Miyata et al. 2000; González et al. 2008). However, at industrial scale these techniques have limitations due to poor growth cycle, huge spore formation, low pH range (3.0–5.0), and adverse submerged aquatic environment for growth of fungus (Arimi et al. 2014). Therefore, bacteria are promising alternative for higher decolourisation due to its faster growth rate in aquatic system, ability to grow on minimum nutrient supply, efficiency to perform in broader pH range(4-7) and high metabolizing capability of melanoidins by ligninolytic enzyme activity (Bharagava et al. 2009; Yadav et al. 2011).

In the present investigation, an attempt was made to isolate such strains from natural ecosystem which has ability to grow at wide range of pH as well as temperature and higher percentage of melanoidin decolorization. Furthermore, the examination of their response towards modified GYPE medium and pysico-chemical parameters.

MATERIALS AND METHODS

Distillery Spent Wash (DSW): The molasses spent wash was collected aseptically from Masuadh sugarcane distillery India. The spentwash was centrifuged at 10,000 rpm for 15 min before use to remove the suspended solids and stored at 4°C (Pazouki et al., 2008). The stored distillery spentwash was filtered through (What man No: 1) filter paper and was diluted with distilled water.

Isolation, screening and identification of melanoidin decolorizing bacteria: Melanoidin decolorizing bacteria isolated from soil sample collected from Masaudh sugarcane distillery Faizabad, India, was grown on GPYE medium(Tiwari et. al. 2012) for 24-48 h incubation. Culture medium consisted of 0.5%, glucose; 0.2%, yeast extract; 0.3%, peptone; 0.05%, $MgSO_4$ and 0.05% $K2HPO_4$ with 3.5 O.D. effluent and the initial pH was adjusted to 6.0. In order to isolate molasses-decolorizing bacteria, 1g of soil was serially dilution up to 10^{-5}-10^{-6} and placed in Petri-plates along with the basal agar medium. The plates were subsequently incubated for 24-48 h at 34°C for bacteria. After 24-48 h of incubation decolorization effect was seen visually. The isolates showing higher decolorization of the melanoidin were selected for further studies, maintained on the same medium at 4°C in slants and sub-cultured after 15 days. The cultures were identified at genus level by Hi Media kit.

Inoculum preparation: Cell suspension was prepared by inoculating 1 mL of 24 h grown culture in 50 mL basal broth and then incubated at 34°C for 24 h to achieve active exponential phase of culture consisting 5×10^6 cfu/mL transfered into the flask and incubated in static condition. Quantitative decolorization value was determined on the basis of OD at 475 nm against the blank by UV-visible Spectrophotometer (Shimadzu UV-VIS modal 1601, Japan).

Decolourization assay of the spent wash: The melanoidin decolorizing bacterial isolates were inoculated in the basal broth medium and after incubation; broth was centrifuged at 10,000 rpm for 10 min. The supernatant of the centrifuged sample will read at absorbance maximum (Amax) of the melanoidin i.e., 475 nm using spectrophotometer (Ohmomo et al., 1988). The decolorization yield will be expressed as the decrease in the absorbance at 475 nm against initial absorbance at the same wavelength. Uninoculated medium will serve as control. The entire assay were performed in triplicate and compared with control. The decolourization efficiency of the different isolates will be expressed as per following equation: Decolourization (%) = I - F / I Where: I = Initial absorbance (Control) and F = Absorbance of decolourized medium broth

Optimization of modified GYPE medium for melanoidin decolorization: An experiment was conducted to optimize modified GYPE medium for efficient decolorization by bacterial strain. The media having different concentration of grade-c molasses instead of glucose were used to evaluate decolorization potential of the isolates.

Modified GYPE medium—different concentration of grade-c molasses(0.5%, 1%, 1.5% , 2% and 2.5%), 0.2 % yeast extract, 0.3 % peptone, 0.05 % MgSO4, 0.05 % K2HPO4 with 3.5 OD effluent.

Optimization of culture conditions for decolourization: Selection of physical parameters for melanoidin decolorization: The basal medium for melanoidin decolorization with different temperature viz. 25, 30, 35, 40, 45, 50°C and incubation period viz. 10, 20, 30, 40, 50 and 60 h were used for the melanoidin decolorization. The initial pH (6.0) was varied in the medium by adding either 1N HCl or 1N NaOH as required. The basal medium was then inoculated with 0.5% (v/v) inoculum of bacterial isolates having $5x10^6$ cfu/mL population respectively and incubated at different pH viz. 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 for optimization of melanoidin decolorization.

Selection of nutritional parameters for melanoidin decolorization: Various carbon sources viz. glucose, fructose, maltose, sucrose, starch and lactose at 0.5% (w/v) were individually added in the basal medium and inoculated with 0.5% (v/v) of bacterial cultures separately with their respective optimized pH, temperature then incubated for decolorization. The best source of sugar will further optimized in different concentration viz. 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6% (w/v) for melanoidin decolorization. In another experiment, different organic and inorganic nitrogen sources viz. beef extract, yeast extract, peptone, ammonium sulphate and sodium nitrate were individually added into the basal medium at 0.5% (w/v). Active culture of individual bacteria was inoculated with 0.5% (v/v) inoculum having 5×10^6 cfu/mL. The best source of nitrogen will further optimized in different concentration viz. 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6% (w/v) for melanoidin decolorization.

Statistical analysis: All the experiments were carried out in triplicates and the results are presented as the mean of three independent observations. Standard deviation for each experimental result was calculated using Microsoft Excel.

RESULTS AND DISCUSSION

Isolation, screening and identification of melanoidin decolorizing bacterial isolates: A total of 30 bacterial isolates showing decolorization ability were isolated on the basal agar medium from the soil of distillery near by the Masudha distillery Faizabad, on qualitative basis. The isolates showing higher clear zone around the colony on molasses agar were selected, at pH 6.0 for 24-48h at 34°C. The clear zone diameter of more than 1 cm around the colony was considered as effective isolates for decolorization (data not shown). Secondary screening was made on quantitative basis using melanoidin broth medium containing molasses wastewater with distilled water to 3.5 OD consisted of 0.5%, glucose ; 0.2%, yeast extract; 0.3%, peptone; 0.05%, $MgSO_4$ and 0.05% K_2HPO_4(GYPE medium) with initial pH 6.0. Each isolates were inoculated in 50 mL of medium in 250 mL Erlenmeyer flask and kept for incubation at 34°C for 48 h for selection of melanoidin decolorizing bacteria individually. Among bacterial isolates, higher decolorization was shown by three selected bacterial isolates(BA-12, BA-14 and BA-5). The bacterial cultures were identified by Hi Media kit shown in Table 1 and identified as Streptococcus sp.(BA-12), Bacillus sp. (BA-14) and Bacillus sp. (BA-5).

DISCUSSION 1

Among the three bacterial strains Streptococcus sp. (BA-12) was found better than Bacillus sp.(BA-14) and Bacillus sp.(BA-5). Microorganisms have very diversified metabolic process and regulatory mechanisms for decolorization of distillery effluent.

Optimization of modified GYPE medium: The strain Streptococcus sp.(BA-12), Bacillus sp.(BA-14) and Bacillus sp.(BA-5) showed maximum melanoidin decolorization at 1% molasses medium i.e. modified GYPE Medium containing 1 %, grade-c molasses, 0.2 %, yeast extract 0.3 %, peptone 0.05 %, $MgSO_4$ 0.05 %, and K2HPO_4 with 3.5 OD(Table-2). Further increasing in concentration, decolorization not increased(Data not shown). These bacterial isolates were further studied for higher decolorization at different physico-chemical and nutritional parameters.

DISCUSSION 2

The availability of more carbon source and other factors in grade-c molasses of modified GYPE medium(i.e. 1% molasses medium) in comparison to GYPE medium could be the reason for better decolorization. Hence this medium was selected for optimization of physico-chemical and nutritional parameters to improve metabolic activity of enzyme production resulting into efficient melanoidin decolorization. (Mohana et al. 2007, Tiwari et al. 2012). The optimal physico-chemical and nutritional parameter for melanoidin decolorization depend on the variation of microbial strains and their genetic diversity as they have been isolated from a very wide range of climatic conditions.

Optimization of different physico-chemical and nutritional parameters for melanoidin decolorization:

Impact of different temperature on melanoidin decolorization: Effect of different temperature viz. 25-50°C was evaluated for melanoidin decolorization by three different bacterial strains at different physico-chemical and nutritional levels. Streptococcus sp.BA-12 showed best decolorization (83%) at 40°C and even up to 45°C, showing best decolorization ability as compared to Bacillus sp.BA-14 (74%) and Bacillus sp.BA-5 (53%) at 40°C. Further, increase in temperature, could not affect decolorization efficiency by the strains (Fig. 1).

DISCUSSION 3

It has been reported that temperature is an important factor for melanoidin decolorization(Kumar et al. 1997). According to Cetin and Donmez (2006), high temperature may cause loss in cell viability or deactivation of the enzymes responsible for decolorization resulted into suppressed decolorizing activity. In contrast, some workers have been reported maximum decolorization at lower temperature range of 25 to 40 °C (Chavan et al. 2006; Jiranuntipon et al. 2008). In this investigation, Streptococcus sp.(BA-12) could tolerate 35-45°C without affecting exponential growth phase which could mainly responsible for higher melanoidin decolorization. Therefore, the melanoidin decolorization efficiency of our strain Streptococcus sp. (BT-12) was undoubtedly better than other reports.

Impact of different incubation on melanoidin decolorization: Just after optimization of temperature for melanoidin decolorization in the liquid medium, incubation period was simultaneously optimized for decolorization. The results clearly indicated that Streptococcus sp.(BA-12) showed 83% decolorization in 30 h of incubation. Further increase in the incubation period did not increase the decolorization (Fig. 2). Bacillus sp. (BA-14) showed the 74% decolorization in 40 h of incubation while Bacillus sp. (BA-5) showed the highest decolorization (55%) in 40 h. Therefore, Streptococcus sp.(BA-12) showed higher decolorization in short time period.

DISCUSSION 4

During maximum growth, maximum enzyme production was achieved which are responsible for melanoidin decolorization by microorganism. Maximum growth also inhibits melanoidin decolorization due to production of some other enzymes or metabolites by the microorganism as a feedback inhibition mechanism during metabolism (Jadhav et al., 2011).

Filamentous fungi takes longer incubation period for melanoidin decolorization (Kim and Shoda, 1999). It was reported that Issatchenkia orientalis(yeast) and Citeromyces sp. WR43-6(yeast) required 7 days of incubation to show maximum decolourization [29, 30].

On contrary, Sirianuntapiboon (1999) reported 90.0 and 96.75 % decolorization by acetogenic bacterial strain no. 13A and no. BP 103, after 4 days of incubation. In another study, M. A. Boopathy and S.N.S. Senthikumar (2014) reported a maximum (74 %) decolorization by using Pseudomonas Fluorescence after

72 h of incubation. In the present investigation, bacterial strains showed maximum decolorization in a very short period i.e. 30-50 h of incubation when compared to fungi.

Impact of pH on color removal: Different pH viz. 4.0- 7.0 in the basal medium was evaluated for melanoidin decolorization by the bacteria at their optimal temperature and incubation periods. Streptococcus sp.(BA-12) showed higher 84% decolorization at pH 6.0. Bacillus sp. (BA-14) showed the 75% decolorization at 6.5 while Bacillus sp. (BA-5) showed the highest decolorization (54%) at pH 6.0 (Fig. 3) Further, increase and decrease in the medium pH reduced the decolorization.

DISCUSSION 5

In previous study, it has been found that enzymes formed by microorganisms during the decolourization were effective only in acidic conditions (Seyis, I. & Subasing, T. 2009). The increase in medium pH raises the color intensity of Sewage waste due to the polymerization of melanoidins which resulted into decrease in microbial decolorization.

In this investigation, maximum decolorization was recorded at pH 5.5-6.5 by the bacteria. Similar results were reported by others (Adikane et al., 2006; Pazouki et al., 2008; Ravikumar et al., 2011, Sirianuntapiboon et al. 2004a). Above and below of the optimum pH, melanoidin decolorization reduced due to inhibition of the enzyme production. All enzymes are proteinous in nature, therefore, some proteins denatured at higher or lower pH value.

Impact of different carbon sources on melanoidin decolorization: Various carbon sources viz. sucrose, glucose, maltose, fructose, starch and lactose at a concentration of 0.5% were individually tested in the basal medium at their optimal temperature, incubation period and pH to observe the effect on melanoidin decolorization by the bacteria.

Out of these carbon sources, glucose was found best for melanoidin decolorization by the bacteria followed by fructose. Higher decolorization (86%) was reported by Streptococcus sp. (BA-12), fructose favoured the decolorization. Bacillus sp. (BA-14) and Bacillus sp. (BA-5) showed 80 and 69% decolorization with glucose also. (Fig. 4).

DISCUSSION 6

According to previous study, various carbon sources are required for decolorization of distillery effluent(Santal et al.,2016). In this investigation, maximum melanoidin decolorization was observed in glucose as well as fructose as carbon source at the level of 0.5% and even 0.2% glucose showed same decolorization potential. Several reports suggested glucose as best carbon source however most of them used higher concentration to achieve optimum decolorization (Kumar et al. 1997 and Ohmomo et al. 1987). Spent wash contains a large amount of sugar but easily metabolizable carbon content of spent wash is almost negligible. The presence of easily available carbon sources in medium increased decolourization efficiency. The organism utilizes easily available carbon sources present in the medium during the initial growth phase and then it starts to degrade spent wash components for carbon source.

Impact of different concentration of glucose on melanoidin decolorization: In another set of the experiment, different concentrations of glucose (0.1- 0.6%) in the medium were tested for melanoidin decolorization at the same growth conditions at which carbon sources were evaluated. Streptococcus sp. (BA-12) showed 87% decolorization at 0.2% glucose concentration, while Bacillus sp. (BA-14) and Bacillus sp. (BA-5) were showed 81% and 71% decolorization at 0.6% concentration of glucose.

Streptococcus sp. (BA-12) was found to be the most effective decolorizer when compared with Bacillus sp. (BA-14) and Bacillus sp. (BA-5). Above and below of this concentration decolorization reduced (Fig. 5).

DISCUSSION 7

The decrease in melanoidin decolorization encountered with high sugar concentration in the medium is probably due to inhibition effect to the enzyme like lignolytic activity of laccase enzyme and oxidation activity of the peroxidase (Raghukumar and Rivonkar, 2001; Guimaraes et al., 2005; Pant et al., 2008; Jiranuntipon et al., 2008; Zhao et al., 2010; Ravikumar et al., 2011).

At optimal concentration, glucose may generate more redox mediators which might act as electron donors for the reduction and cleavage of conjugated C=C, C=O and C≡N bonds of melanoidins (Chandra et al. 2018; Miyata et al. 2000; Kalawati et al. 2001; V. Kumar et. al 2022). Increase in glucose concentration may result in formation of excess gluconic acid (Chandra et al. 2018). Several workers have also reported similar observations during bacterial treatment of distillery spent wash (Chandra et al. 2018, Ghosh et al. 2002, Santal et al. 2011).

Ghosh et al. (2002) reported the use of 1 % glucose mandatory for growth and decolorization activity of P. putida. A facultative anaerobic culture L-2 reported by Kumar et al. (1997) showed 31% decolorization and 57 % COD reduction of spentwash in presence of 1.0 % glucose in 7 days. Mohana et al. (2007) also reported 67 % decolorization and 51 % COD reduction of distillery effluent by bacterial consortium in the presence of 0.5 % glucose in 72 h. The observations proved that our strain Streptococcus sp. (BT-12) is a better decolorizer than other reported microbe even in the presence of 0.2 % glucose within a short incubation period of 30 h.

Impact of different nitrogen sources on melanoidin decolorization: Inorganic and organic nitrogen viz. beef extract, malt extract, yeast extract, peptone, ammonium sulphate, ammonium nitrate, at the rate of 0.5% were used in the basal medium for melanoidin decolorization by the bacteria (Fig. 6). The melanoidin decolorization by the bacteria was almost similar in peptone amended medium, while other nitrogen sources did not increase in decolorization percentage. Streptococcus sp. (BA-12) was showed 87% decolorization with peptone while Bacillus sp. (BA-14) and Bacillus sp. (BA-5) were showed only 82 and 68% decolorization respectively.

DISCUSSION 8

Different nitrogen sources were optimized for melanoidin decolorization. Among different nitrogen sources (organic and inorganic), the highest melanoidin decolorization was reported with peptone at the level of 0.2%. Similarly various nitrogen sources were optimized by different workers for melanoidin decolorization, but peptone was the most effective for color removal (Ohmomo et al., 1988; Miyata et al., 2000; Ravikumar et al., 2011, Kumar and Chandra, 2018).

Impact **of different concentration of peptone on melanoidin decolorization:** Different concentrations of peptone (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 %) in the medium were also tested for melanoidin decolorization at the same growth condition at which nitrogen sources were evaluated. Streptococcus sp. (BA-12) showed better decolorization (87%) at 0.2% peptone concentration, while Bacillus sp. (BA-14) and Bacillus sp. (BA-5) showed 82 and 69% decolorization at 0.5% concentration of peptone. Further increasing in concentration, decolorization reduced (Fig. 7).

DISCUSSION 9

Further increase in peptone concentration inhibited decolorization process. This may be due to inhibition of bacterial growth by surplus addition of nitrogen source (Chadra et al. 2018, Tiwari et al. 2012). Similar effect was observed when low concentration of peptone was used as nitrogen source for decolorization of melanoidin pigment present in the spent wash.

Similarly Ravikumar et al. (2011) also reported that Cladosporium cladosporioides showed maximum decolorization at 1.0 gl^{-1} concentration of peptone. Similar effect was observed when low concentration (0.5 %) of peptone was used as nitrogen source for decolorizing melanoidin pigment present in spentwash using Phanerochaete chrysosporium (Dahiya et al. 2001). Hence, our bacterial culture utilized little amount of peptone for higher melanoidin decolorization compared to other researchers ever reported.

CONCLUSION

This is first report on melanoidin decolorization by using Streptococcus sp.(BA-12) in modified GYPE medium i.e. 1% molasses medium. The potential of this strain were showed maximum decolorization in the presence of minimum nutrient supply, short incubation period and wide range of pH as well as temperature. In the light of the above findings, promises its candidature for the development of cost effective and environmentally safe technology for sewage waste treatment at industrial level.

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CONFLICT OF INTEREST

There is no conflict of interest of any kind among the authors.

TABLE

Table 1: Decolorization potential of bacterial isolates and with their morphology and Gram reaction

Bacterial isolates	**Decolouriztion (%)**	**Morphology**	**Gram reaction**
BA-12	76	Cocci	Positive
BA-14	70	Rod	Negative
BA-5	50	Short rod	Positive

BA= Bacterial isolate

Table 2:Effect of 1.0% molasses media on melanoidin decolorization by of bacteria

Media	Decolorization (%)		
	BA-12	BA-14	BA-5
Medium: 1.0% molasses media	81	71	51

BA = Bacterial isolate

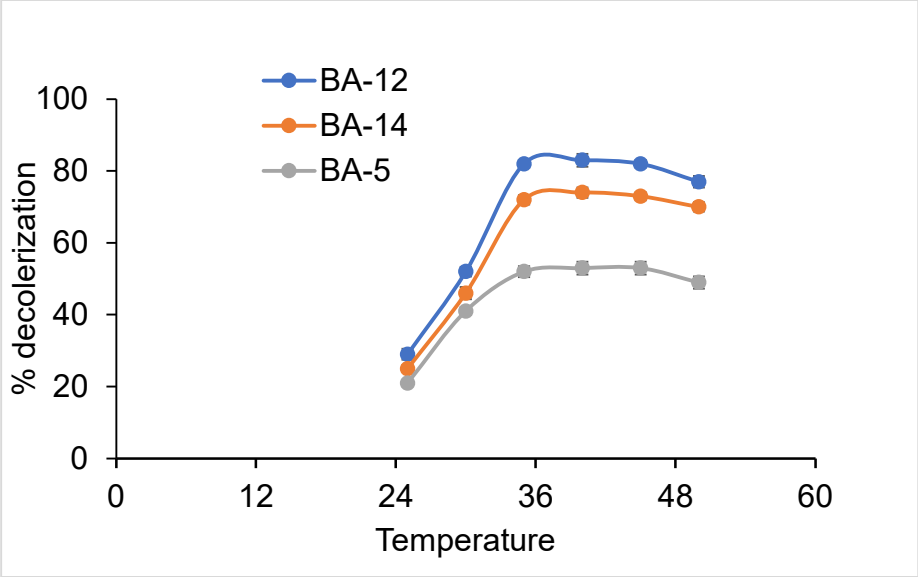

Fig. 1: Effect of different temperature on melanoidin decolorization. The inoculated flasks were incubated at different temperature (°C) for 24-48 h at static condition in medium. Error bars presented are mean values of ± standard deviation of triplicates

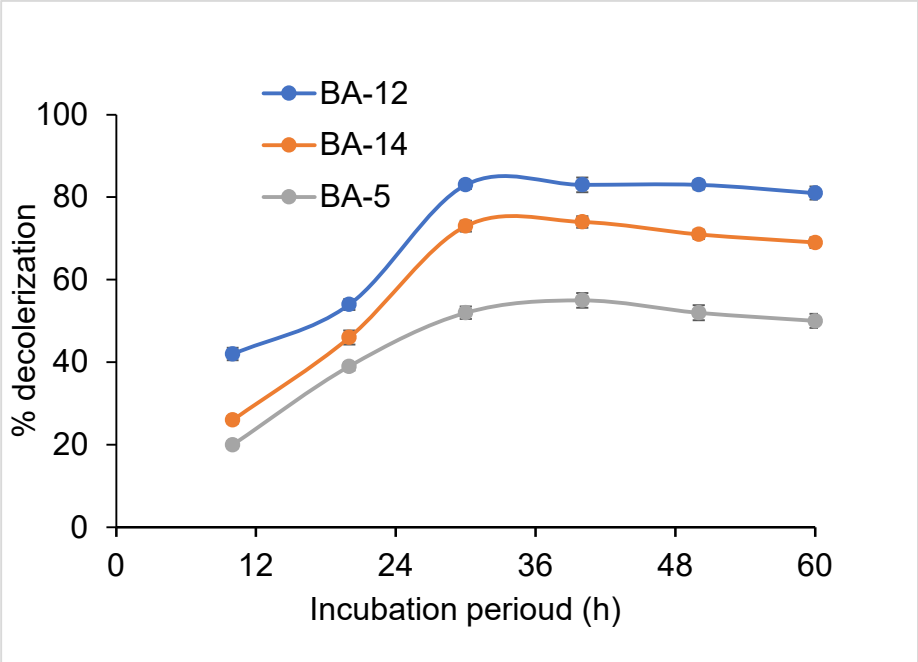

Fig. 2: Effect of different incubation periods on melanoidin decolorization. The inoculated flasks were incubated at different incubation period at 40°C under static condition in medium. Error bars presented are mean values of ± standard deviation of triplicates of three independent experiments

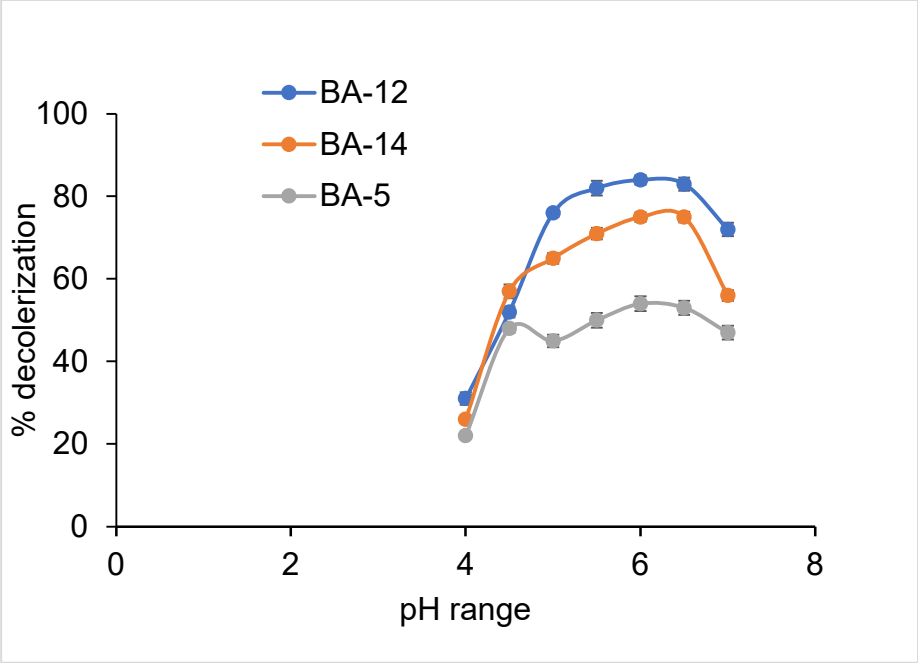

Fig. 3: Effect of different pH on melanoidin decolorization. The inoculated flasks were incubated at different pH at 40°C for 30 h under static condition in medium. Error bars presented are mean values of ± standard deviation of triplicates of three independent experiments.

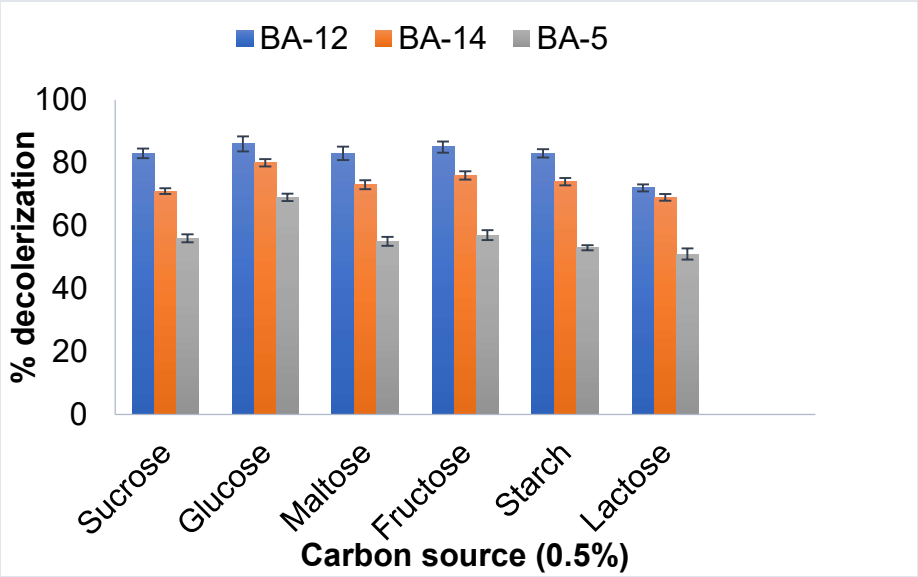

Fig. 4: Effect of different carbon sources on melanoidin decolorization. Test flasks contained different carbon sources in the medium at a level of 0.5 % (w/v). Inoculated flasks were incubated at 40°C for 30 h. Error bars presented are mean values of ± standard deviation of triplicates of three independent experiments

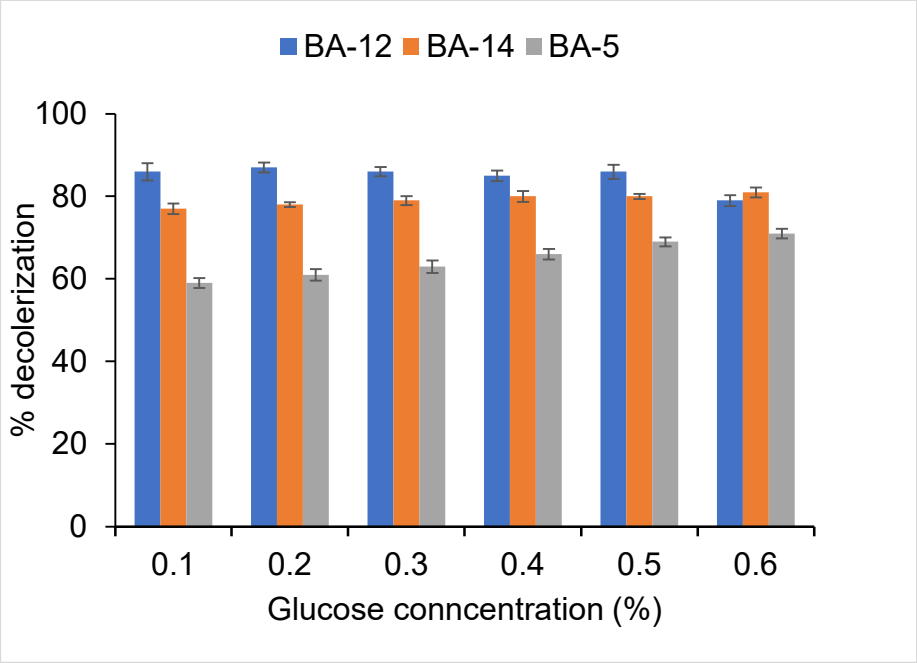

Fig. 5: Effect of different glucose concentration on melanoidin decolorization. Test flasks contained different concentration of glucose in the medium at a level of 0.6 % (w/v). Inoculated flasks were incubated at 40°C for 30 h. Error bars presented are mean values of ± standard deviation of triplicates of three independent experiments

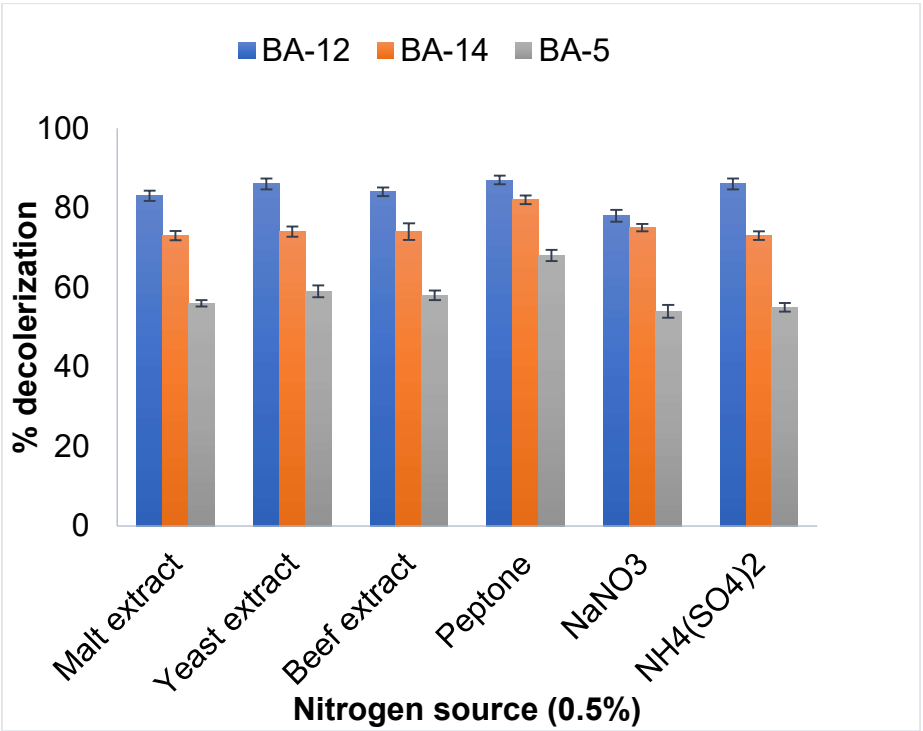

Fig. 6: Effect of different nitrogen sources on melanoidin decolorization. Test flasks contained different nitrogen sources in the medium at a level of 0.5 % (w/v). Inoculated flasks were incubated at 40°C for 30 h. Error bars presented are mean values of ± standard deviation of triplicates of three independent experiments

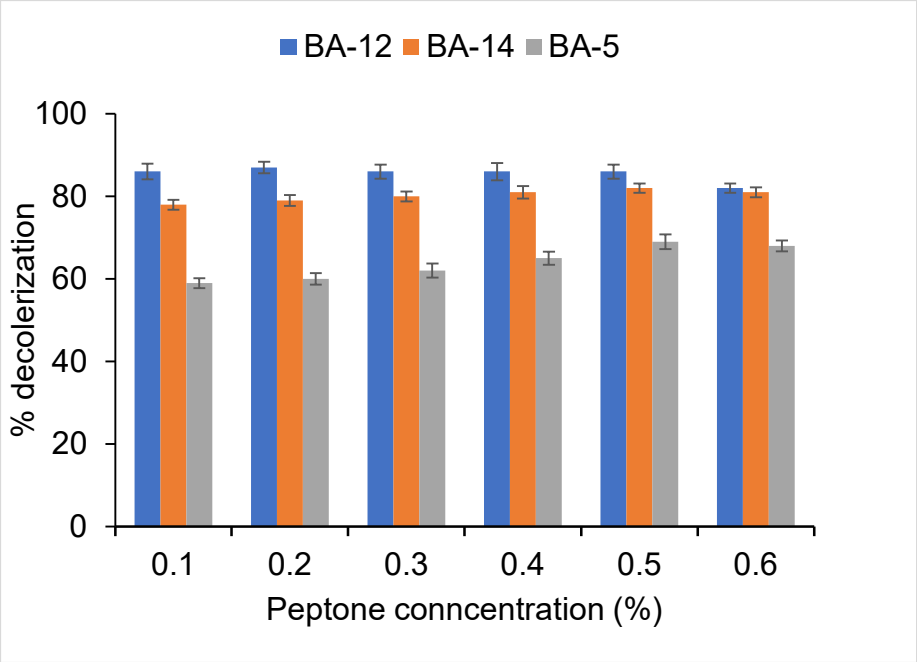

Fig. 7: Effect of different peptone concentration on melanoidin decolorization. Test flasks contained different concentration of peptone in the medium at a level of 0.6% (w/v). Inoculated flasks were incubated at 40°C for 30 h. Error bars presented are mean values of ± standard deviation of triplicates of three independent experiments

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