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Decolorization of Distillery Effluent (Molasses Melanoidin) by Yeast Strain, *Candida Glabrata*

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KEYWORDS
xenobiotic, melanoidin, recalcitrant, spent wash, *Candida glabrata.*

ABSTRACT: Distillery effluent contains about 2% (w/W) of dark brown Xenobiotic compound, melanoidin. Melanoidin is major source of environmental pollution due to its recalcitrant nature, therefore may be treated before disposal. The aim of this study was to isolate potent melanoidin decolourizing yeast and characterize on physico-chemical and nutritional parameter in submerged system.

Among 14 yeast isolates from natural ecosystem *Candida glabrata* showing higher decolorization efficiency on distillery effluent was screened. After optimization, *Candida glabrata* Showed maximum decolorization 84% at 35˚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-5.5 within 30 hours.

This yeast strain can adopt in wide range of temperature as well as pH and required very less carbon (0.2%, w/v) and nitrogen sources (0.2%, w/v) in submerged fermentation system. Thus, this yeast can be utilized for melanoidin decolorization of spent wash at industrial scale.

INTRODUCTION:

Molasses based distillery is associated with the production of a high strength wastewater characterized by very high chemical oxygen demand (COD) and biological oxygen demand (BOD) content, high concentration of mineral salts and a dark brown in color. The main color compounds are melanoidins, which are recalcitrant, high molecular weight substances, acidic in nature and present nearly 2% (w/w) in distillery effluent. Several researchers have reported when melanoidin is discgarged from distilleries and fermentation industries makes complexation with various mutagenic, carcinogenic compounds and heavy metals due to its anionic nature (Chowdhary et al., 2018; Tripathi et al., 2021, Chandra et al., 2017).

There are much information regarding melanoidin structure, but in general melanoidins are considered to be non-linear polymers produced through non-enzymatic Maillard and polycondensation reactions resulting from the reaction of reducing sugars and amino compounds (Chandra et al. 2008).

Melanoidin is toxic as much as phenol, so discharge of molasses wastewater causes the problem, like reduction of sun light penetration, decreased photosynthetic activity and increased biological oxygen demand (B.O.D.) in aquatic ecosystem whereas on land, it causes reduction in soil alkalinity and inhibition of seed germination. Due to their antioxidant properties, melanoidins are toxic to many microorganisms involved in wastewater treatment (Plavsic M et al. 2006). Due to severe threats to the environment, various attempts have been made globally by the researchers using the chemical and biological method, for pretreatment of molasses-based melanoidin of distillery wastewater for its safe disposal in to the environment (Tripathi et al., 2021; Sharma et al., 2021).

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Degradation and decolourization of these wastewater by physicochemical methods such as chemical precipitation or coagulation(Chandra and singh, 1999; Liang et al. 2009b), flocculation treatment(Liang et al. 2009a), ozonation (Kim et al., 1985), activated carbon adsorption(Onyango et al. 2011), filtration(Satyawali and Balakrishnan 2008) and UV/H_2O_2 treatment have been reported, but these methods is quite expensive, requires large amount of reagents and also resulting in unstable decolorizing efficiency, fluctuation of the molasses based distillery effluent, generation of huge amount of sludge and formation of hazardous by-products (Sirianuntapiboon et al., 2004a,b). Biological methods have drawn attention of various workers world over due to their ecofriendly result, low operating costs and stable efficiency (Kaushik et al. 2010). Anaerobic biological treatment processes are commonly used. But, even after anaerobic digestion, the treated effluent may have a high organic loading and a dark color, requiring more treatment steps (Arimi et al. 2014).

Aerobic processes are usually applied as post anaerobic treatment methods. Different aerobic microorganisms such as bacteria, fungi, cynobacteria and yeasts present in nature have potential to decolorize spent wash. During aerobic treatment of spent wash, different biological mechanisms such as biodegradation by the presence of enzymes and bioadsorption have been reported.

Several yeast strains have been reported for their ability to remove dyes through biosorption and biodegradation mechanisms (Wu et al. 2011), such as Debaryomyces polymorphus and Candida tropicalis (Yang et al. 2008), C. zeylanoides(Martins et al. 1999), Kluyveromyces marxianus IMB3(Meehan 2000), Candida tropicalis(charumathi & Das 2012, Tan et al. 2013) have been reported.

Certain yeast such as Citeromyces sp. WR-43-6 (Sirianuntapiboon et. al., 2004), Issatchenkia orientalis (Tondee & Sirianutapiboon 2006; Tondee et al. 2008), C. tropicalis (Tiwari et al. 2012), Candida glabrata (Mahgoub et. al 2016) have been reported for action against different kind of melanoidin through biosorption and biodegradation mechanism.

However, isolated yeast strains usually show their low growth rate, especially in the presence of other competitors but present higher water activity (a_w) in comparison to other fungi. Therefore, yeast strains are suitable for submerged system. The aim of this work was the isolation of yeasts from natural ecosystem having higher melanoidin decolorizing ability with healthy growth rate, the examination of their response towards modified GYPE medium and the investigation of the effect of nutrients on their performance efficiency.

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 yeast: Melanoidin decolorizing yeast 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 yeast, 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 yeast. 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. One yeast culture was identified at species level by HIMedia kit (KB006 HiCandida ^{TM} Identification 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 population. Appropriate volume (0.5% v/v) of this cell suspension was used to inoculate the test flasks.

Decolourization assay of the spent wash: The melanoidin decolorizing yeast 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

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control. The entire assay was 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 yeast 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 % $MgSO_4$, 0.05 % K_2HPO_4 with 3.5 OD effluent.

Optimization of culture conditions for efficient melanoidin decolourization:

Selection of physical parameters for melanoidin decolorization: The modified GYPE medium was then inoculated with 0.5% (v/v) inoculum of yeast isolates having $5x10^6$ cfu/mL population respectively. The modified GYPE 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.

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 modified GYPE medium and inoculated with 0.5% (v/v) of yeast cultures separately with their respective optimized pH, temperature then incubated for decolorization. The best source of sugar will further be 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 modified GYPE medium at 0.5% (w/v). Active culture of individual yeast was inoculated with 0.5% (v/v) inoculum having 5×10^6 cfu/mL. The best source of nitrogen will further be optimized in different concentration viz. 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6% (w/v) for melanoidin decolorization.

Extracellular Lignin degrading enzyme assay for isolated yeast strain *Candida glabrata:*

To measure the ligninolytic activity during degradation, the yeast degraded supernatant was obtained by centrifugation at 6500g for 10 min at 4°C. The enzyme activity was expressed as international unit (IU), where 1 IU represents the amount of enzyme that forms 1µmol of product per minute under standard assay condition.

Lac enzyme assay:

Laccase activity was determined via the oxidation of o-methoxyphenol catechol monomethylether (guaiacol) as substrate. The reaction mixture contained 1 mL of 1 mM guaiacol in 0.1 M sodium phosphate buffer (pH 6.0) and 1 mL of crude enzyme solution was incubated at 30◦ C for 10 min. The oxidation of substrate was followed spectrophotometrically (A_{495}) by the increase in absorbance at 495 nm. (R. P. S. Dhaliwal et al., 1991).

MnP enzyme assay:

Manganese peroxidase (MnP) activity was determined using guaiacol as substrate. The reaction mixture contained 0.2 mL of 0.5 M Na-tartrate buffer (pH 5.0), 0.1 mL of 1 mM MnSO4, 0.1 mL of 1 mM H2O2, 0.25 mL of 1 mM guaiacol and 0.3 mL of crude enzymes. The oxidation of substrate at 30◦ C was followed spectrophotometrically at (A_{465}) (J. Putter, 1974)

LiP enzyme assay:

The lignin peroxidase (LiP) activity was measured via the oxidation of veratryl alcohol to veratryaldehyde at 310nm. One milliliters of enzymatic assay consisted of 0.4 mL of citrate-phosphate buffer (100 mM, pH 2.7), 0.1 mL of veratryl alcohol (20 mM), and 0.5 mL of fluid sample. Forty microliters of H_2O_2 (20 mM), which was freshly prepared daily and added to initiate the reaction (Tien M and Kirk T K 1988). The conversion to veratraldehyde is monitored in 1 mL quartz cuvette at(A_{310}) using UV-vis spectrophotometer.

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Table 1. Optimal Lac, MnP and LiP enzyme activities produced by yeast strain *Candida glabrata:*

	Lac		MnP		LiP	
Isolate	**Enzyme activity (U/ml)**	**Period (h)**	**Enzyme activity (U/ml)**	**Period(h)**	**Enzyme activity (U/ml)**	**Period(h)**
Candida glabrata (YT-7)	Negative	……..	0.600	120	Negative	……..

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 yeast isolates: A total of 14 yeast 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%, MgSO4 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 yeast individually. Among yeast isolates, higher decolorization was shown by yeast isolates YT-7 identified by HIMedia kit (KB006 HiCandida ™ Identification Kit) as *Candida glabrata*.

Optimization of modified GYPE medium: This yeast strain *Candida glabrata* 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%, MgSO4 and 0.05%, K_2HPO_4 with 3.5 OD (data not shown). Further increasing in concentration, decolorization not increased. This yeast stain was further studied for higher decolorization at different physico-chemical and nutritional parameters.

The availability of more carbon source and other factors in 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 yeast strain YT-7 at different physico-chemical and nutritional levels. YT-7 showed best decolorization (77%) at 35°C and even almost upto 40°C. (Fig. 1).

The remarkable decolorization (77%) in the temperature range of 35–40°C reveals wide zone of temperature for the optimal activity of this yeast strain. Our strain showed better decolorization potential at higher temperature than Sirianuntapiboon et al. (2004) who reported a maximum of 68% spentwash decolorization at 30°C by Citeromyces sp. WR-43-6. In another study, Tondee and Sirianutapiboon. (2006) reported that Issatchenkia orientalis showing maximum 60% spentwash decolorization at 30 °C. Similerly, Samir Mahgoub et al. (2016) reported that Candida glabrata showing maximum 60% real melanoidin solution decolorization at 30 °C.

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

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deactivation of the enzymes responsible for decolorization resulted into suppressed decolorizing activity. In this investigation, Therefore, the melanoidin decolorization efficiency of our strain (YT-7) 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 YT-7 showed 78% decolorization in 30 h of incubation. Further increase in the incubation period did not increase the decolorization (Fig. 2).

On contrary Tondee and Sirianutapiboon. (2006) reported 60% decolorization by Issatchenkia orientalis, but after 7 days of incubation. In another study Sirianuntapiboon et al. (2004) had been reported a maximum 68% decolorization using Citeromyces sp. WR43-6 after 7 days of incubation. Similerly, Samir Mahgoub et al. (2016) reported that *Candida glabrata* showing maximum 60% real melanoidin solution decolorization within 2-5 days of incubation. 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). Therefore, decolorization efficiency of our strain YT-7 is certainly better than that reported by other researchers.

Impact of pH on color removal: Different pH viz. 4.0-7.0 in the modified GYPE medium was evaluated for melanoidin decolorization by the yeast at their optimal temperature and incubation periods. YT-7 showed higher 78% decolorization at pH 5.5 (Fig. 3). Further, increase and decrease in the medium pH reduced the decolorization.

Melanoidin decolorization from other yeast strain was also reported by several researchers having maximum decolorization activity in 5.0–6.0 optimum pH range (Tondee and Sirianutapiboon, 2006; Sirianuntapiboon et al. 2004). 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 and higher rate nutrient utilization which resulted into decrease in microbial decolorization (Adikane HV, 2006; Jiranuntipon S, 2008). Above and below of the optimum pH, melanoidin decolorization decreased due to loss of enzyme activity as well as 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 modified GYPE medium at their optimal temperature, incubation period and pH to observe the effect on melanoidin decolorization by the yeast.

Out of these carbon sources, glucose was found best for melanoidin decolorization by the yeast followed by fructose. Higher decolorization (80%) was reported by YT-7, fructose favoured the decolorization. (Fig. 4).

Melanoidin decolorization from other yeast are maximum in the presence of glucose have reported by others researchers also (Tondee and Sirianutapiboon, 2006; Sirianuntapiboon et al. 2004). It is, evident from our study that melanoidin decolorization by yeast strain YT-7 is remarkably stable in the presence of broad range of carbon sources employed in this study.

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 during the initial growth phase and then it starts to degrade spent wash components for carbon source (Kumar V, et al 1997).

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. Yeast strain YT-7 showed 79% decolorization at 0.2% glucose concentration. Above and below of this concentration decolorization reduced (Fig. 5).

Tondee and Sirianutapiboon. (2006) have also reported that Issatchenkia orientalis utilized 2.5% glucose for maximum decolorization (60%) and above this concentration of glucose there was decrease in the decolorization. Similarlly, Sirianuntapiboon et al. (2004) have reported that Citeromyces sp. WR-43-6 showed 68% decolorization in the presence of 2.0% glucose

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concentration. Ohmomo et al. (1987) have reported that glucose was the best carbon source, which utilized by Aspergillus fumigatus G-2-6 for maximum degradation of melanoidin and further increase in glucose concentration resulted in an increase in mycelial biomass but no further increase in decolorization yield. It is, therefore, evident from our study that melanoidin decolorization by yeast strain YT-7 is remarkably higher in the presence of 0.2% (w/v) glucose within 30 h of incubation when compared to other researchers.

The decrease in melanoidin decolorization encountered with high sugar concentration in the medium is probably due to inhibition effect to the lignin degrading enzymes 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 by sorbose oxidase enzyme in aerobic condition (Chandra et al. 2018).

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 modified GYPE medium for melanoidin decolorization by the yeast (Fig. 6). The melanoidin decolorization by the yeast was almost similar in peptone amended medium, while other nitrogen sources did not increase in decolorization percentage. YT-7 was showed 79% decolorization with peptone, it was practically high compared to the extent of decolorization reported by other workers (Mohana S, 2007). But in case of Issatchenkia orientalis and Citeromyces sp. WR-43-6, maximum decolorization was reported in the presence of 0.1% NH4Cl and 0.1% sodium nitrate (Tondee and Sirianutapiboon, 2006; Sirianuntapiboon et al. 2004). Kirk et al. (1978) have reported that enzymatic systems catalyze degradation of lignin and lignin-like materials during the secondary phase of the metabolic growth in the presence of peptone. Synthesis and secretion of ligninolytic are triggered by nutrient limitations such as carbon and nitrogen source.

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. Yeast strain YT-7 showed better decolorization (84%) at 0.2% peptone concentration, (Fig. 7). Further increase in peptone concentration inhibited decolorization process. This may be due to inhibition of yeast growth by excess of nitrogen source (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 yeast culture utilized little amount of peptone for higher melanoidin decolorization compared to other researchers ever reported.

Extracellular Lignin degrading enzyme assay for this isolated yeast strain *Candida glabrata* result were maximum activity of MnP enzyme analyzed at 120 hours while optimum melanoidin decolorization observed at 30 hours, showed negative test for other two enzyme i.e. Laccase and LiP. So, these lignolytic enzymes are not responsible for melanoidin decolorization. Therefore, decolorization takes place either through degradation by other enzymes or biosorption mechanism (Wu et al. 2011, Meehan 2000). Both mechanisms might be involved for decolorization of distillery effluent (Charumathi & Das 2012, Tusanee Tondee et al. 2007).

CONCLUSIONS:

This study demonstrated that isolated yeast strain *Candida glabrata* was capable of aerobically decolorizing 84% complex melanoidin compound in distillery effluent at wide range of temperature and pH, required little amount of carbon and nitrogen sources within a short incubation period of 30 h in submerged system. The decolorization could be attributed either to their degradation by other than extracellular lignolytic enzymes or to their adsorption on the yeast cell or both involve. Today, several industries in the world used a

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variety of consortia of various groups of microorganisms especially yeast supporting aeration for better decolorization. In the light of the above findings, it is concluded that isolated yeast strain could be a suitable candidature for the development of consortia, cost effective and eco-friendly technology used 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.

FIGURE:

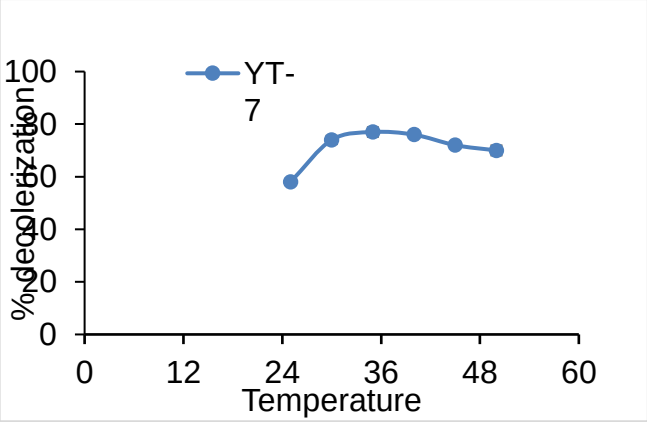

Fig. 1: Effect of different temperature on melanoidin decolorization. The inoculated flasks were incubated at different temperature (°C) for 24-48 h at static condition in medium. Error bars presented are mean values of ± standard deviation of triplicates

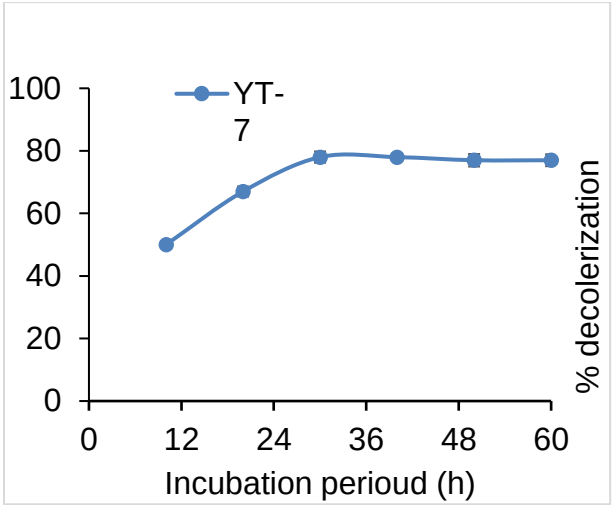

Fig. 2: Effect of different incubation periods on melanoidin decolorization. The inoculated flasks were incubated at different incubation period at 35°C under static condition in medium. Error bars presented are mean values of ± standard deviation of triplicates of three independent experiments

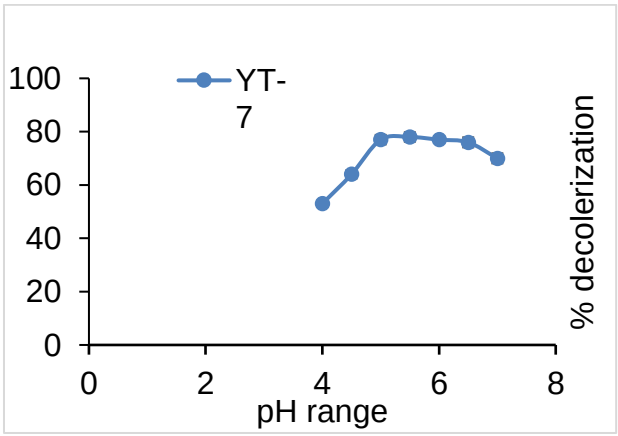

Fig. 3: Effect of different pH on melanoidin decolorization. The inoculated flasks were incubated at different pH at 35°C for 30 h under static condition in medium. Error bars presented are mean values of ± standard deviation of triplicates of three independent experiments.

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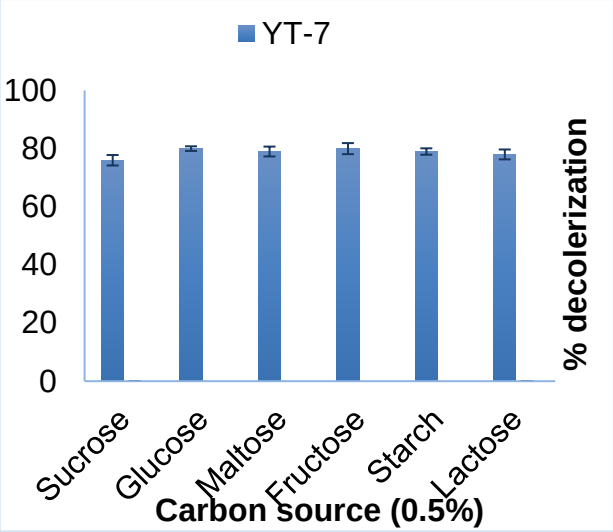

Fig. 4: Effect of different carbon sources on melanoidin decolorization. Test flasks contained different carbon sources in the medium at a level of 0.5 % (w/v). Inoculated flasks were incubated at 35°C for 30 h. Error bars presented are mean values of ± standard deviation of triplicates of three independent experiments

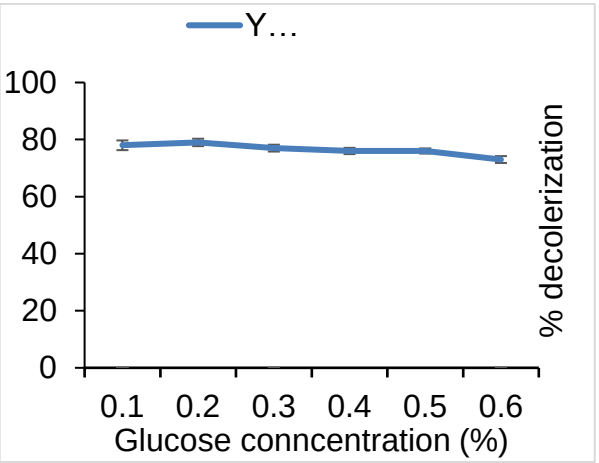

Fig. 5: Effect of different glucose concentration on melanoidin decolorization. Test flasks contained different concentration of glucose in the medium at a level of 0.6 % (w/v). Inoculated flasks were incubated at 35°C for 30 h. Error bars presented are mean values of ± standard deviation of triplicates of three independent experiments

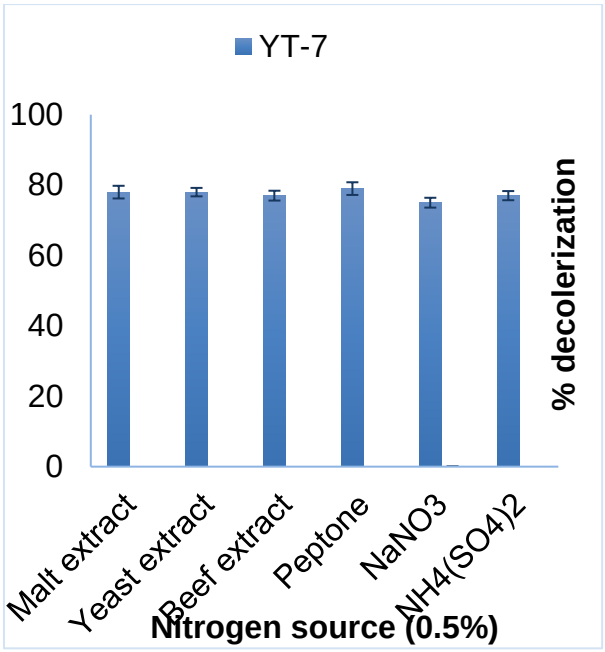

Fig. 6: Effect of different nitrogen sources on melanoidin decolorization. Test flasks contained different nitrogen sources in the medium at a level of 0.5 % (w/v). Inoculated flasks were incubated at 35°C for 30 h. Error bars presented are mean values of ± standard deviation of triplicates of three independent experiments

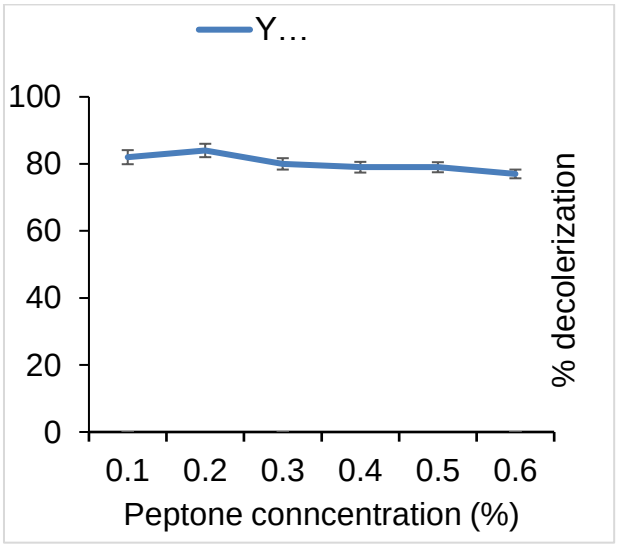

Fig. 7: Effect of different peptone concentration on melanoidin decolorization. Test flasks contained different concentration of peptone in the medium at a level of 0.6% (w/v). Inoculated flasks were incubated at 40°C for 30 h. Error bars presented are mean values of

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± standard deviation of triplicates of three independent experiments

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