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Decolorization of synthetic melanoidins-containing wastewater by a bacterial consortium

Suhuttaya Jiranuntipon · Supat Chareonpornwattana · Somsak Damronglerd · Claire Albasi · Marie-Line Delia

Abstract The presence of melanoidins in molasses wastewater leads to water pollution both due to its dark brown color and its COD contents. In this study, a bacterial consortium isolated from waterfall sediment was tested for its decolorization. The identification of culturable bacteria by 16S rDNA based approach showed that the consortium composed of *Klebsiella oxytoca*, *Serratia mercescens*, *Citrobacter* sp. and unknown bacterium. In the context of academic study, prevention on the difficulties of providing effluent as well as its variations in compositions, several synthetic media prepared with respect to color and COD contents based on analysis of molasses wastewater, i.e., Viandox sauce (13.5% v/v), caramel (30% w/v), beet molasses wastewater (41.5% v/v) and sugarcane molasses wastewater (20% v/v) were used for decolorization using consortium with color removal 9.5, 1.13, 8.02 and 17.5%, respectively, within 2 days. However, Viandox sauce was retained for further study. The effect of initial pH and Viandox concentration on decolorization and growth of bacterial consortium were further determined. The highest decolorization of 18.3% was achieved at pH 4 after 2 day of incubation. Experiments on fresh or used medium and used or fresh bacterial cells, led to conclusion that the limitation of decolorization was due to nutritional deficiency. The effect of aeration on decolorization was also carried out in 2 L laboratory-scale suspended cell bioreactor. The maximum decolorization was 19.3% with aeration at KLa = 2.5836 h^{-1} (0.1 vvm).

Keywords Melanoidins · Molasses · Decolorization · Bacterial consortium · Wastewater

S. Jiranuntipon
Biotechnology Program, Faculty of Science,
Chulalongkorn University, Phyathai Road,
Patumwan, Bangkok 10330, Thailand

S. Chareonpornwattana (✉)
Department of Microbiology, Faculty of Science,
Chulalongkorn University, Phyathai Road,
Patumwan, Bangkok 10330, Thailand
e-mail: supat.c@chula.ac.th

S. Damronglerd
Department of Chemical Technology, Faculty of Science,
Chulalongkorn University, Phyathai Road,
Patumwan, Bangkok 10330, Thailand

C. Albasi · M.-L. Delia
Laboratoire de Génie Chimique, INP-ENSIACET,
5, rue Paulin Talabot Site Basso Combo,
BP 1301, Toulouse 31106, France

Introduction

Large quantities of wastewater in Thailand are generated from the alcohol distilleries which use sugar cane molasses as raw material. There are basically two types of wastewaters; one is high strength process wastewater or concentrated wastewater that originates from distillation process during alcohol production. The high strength wastewater is high in chemical oxygen demand (COD) and high concentration of organic matters (BOD). Moreover, it has low pH and large amount of dark brown color leading to serious impact to the environment. The other is low strength process wastewater or diluted wastewater that originates from the floor washing and equipment cleaning. It has low concentration of COD, BOD and color [31, 34, 37, 48].

The presence of dark brown color is known as melanoidins which are formed by Maillard amino carbonyl reaction [23]. The release of melanoidins into environment

from the effluent of alcohol distilleries has become a major concern in wastewater treatment since it can result in the reduction of photosynthetic activity and dissolved oxygen concentration in aquatic environment. Furthermore, it can also lead to eutrophication due to high organic loads. Finally, it affects aquatic plants and aquatic animals [39].

Accordingly, the method for removal of melanoidins before discharging into environment is necessary. Currently, the decolorization of melanoidins in wastewater is based mainly on physical and chemical methods such as ozonization, flocculation, chemical coagulation, precipitation, activated carbon adsorption and advanced oxidation of the wastewater [4, 17, 25, 28, 39]. However, the implement of physical and chemical methods eventually may generate significant amount of sludge, cause secondary pollution due to excessive chemical usage, and have some drawbacks such as high cost, formation of hazardous by-products and intensive energy consumption.

Several emerging technologies such as electrochemical destruction, photocatalysis and sorption are promising for molasses wastewater decolorization [21, 41]. However, these approaches often involve complicated procedures and are economically unfeasible. The conventional treatment process such as aerobic treatment and anaerobic treatment process can be accomplished as well but with only low removal of melanoidins and the COD of treated wastewater is still higher than the standard permission value of Department of Industrial Works, Ministry of Industry, Thailand [10].

Therefore, there are still demands to develop alternative means of melanoidins decolorization such as innovative biological methods capable of providing a more complete clean up of the pollutant in more economic fashion.

Over the past decade, biological treatment has been investigated [32, 35, 46]. Microbial decolorization is an environment-friendly and cost competitive alternative to chemical decomposition process [32]. Several kinds of microorganism such as fungi (*Penicillium decumbens, Aspergillus* sp., *Aspergillus niger*, *Flavadon flavus*) [12, 19, 38, 42], white rot fungi (*Phanerochaete* sp., *Phanerochaete chrysosporium*, *Trametes versicolor*, *Coriolus* sp.,) [6, 16, 24], yeast (*Citeromyces* sp.) [47] and bacteria (*Bacillus* sp., *Pseudomonas* sp., acetogenic bacteria) [9, 13, 18, 47] have been reported regarding their abilities to remove melanoidins.

There are many reports showing that white rot fungi are very effective to remove melanoidins in molasses wastewater. But for the application in large scale treatment, it has been impeded of owing to lack of an appropriate reactor system capable of coping with relatively slow fungal degradation, loss of extracellular enzymes and mediator with discharged water [33, 49].

The decolorization of molasses wastewater by bacteria has also been investigated such as *Bacillus* sp., *Pseudomonas* sp., acetogenic bacteria [9, 13, 18, 47]. Nevertheless, the application of these strains to remove melanoidins from molasses wastewater was still inconvenient from the view point of stability and maintenance of removal activity due to culture condition, nutrient supplement and growth [24].

Pure bacterial or fungal cultures have been studied in order to develop bioprocess for melanoidins decolorization in molasses wastewater. However, the performance of fungal decolorization was limited by long growth cycle and moderate decolorization rate. In contrast, the bacterial decolorization is usually faster, but it may require a mixed community to decolorize melanoidins through combined metabolic mode of individual culture [1, 22]. The mixed culture of *Bacillus* spp. exhibited a two- to fourfold increase in melanoidins decolorization over that showed by any individual *Bacillus* isolate [22]. Also, Alkane et al. [1] reported that 69% decolorization of molasses spent wash was achieved using soil samples as inoculum instead of isolated microorganisms.

Hence, the bacterial consortium seems to be more competent for molasses wastewater treatment due to maintenance of microorganism and co-metabolism to enhance the efficiency of melanoidins decolorization.

In this study, the effect of operation parameters on melanoidins decolorization and the performance of the constructed bacterial consortium for treating the melanoidin-containing wastewater were investigated.

Materials and methods

Microorganisms

In this study, a bacterial consortium was collected from waterfall sediments in Maehongsorn province, Thailand. This consortium was screened and used for melanoidin decolorization in laboratory. It showed melanoidins decolorization when cultivated in the synthetic melanoidins-containing wastewater containing 20% (v/v) of sugarcane molasses wastewater from alcoholic distillery. This consortium exhibited the highest melanoidin decolorization of 20% within 48 h under aerobic condition and this consortium was selected for further study.

Preparation of synthetic melanoidins-containing wastewater medium

Four types of synthetic melanoidins-containing wastewater media were prepared using melanoidins-containing components including sugarcane molasses wastewater, beet molasses wastewater, Viandox sauce and caramel. Other components of the each synthetic melanoidins-containing

Table 1 Characteristics of synthetic melanoidins-containing wastewater

Color substances	Initial concentration % (v/v)	OD_{475}	COD (g/L)
Viandox	13.5	5.7081	22.8
Caramel	30	2.1355	243
Beet molasses wastewater	41.5	5.9014	30.75
Sugarcane molasses wastewater	20	5.7091	21.6

wastewater media were as follows: 0.01% $NaNO_3$, 0.2% K_2HPO_4, 0.1% KH_2PO_4, 0.01% $MgSO_4 \bullet 12H_2O$, 2% glucose and 0.1% yeast extract, and the initial pH was adjusted to 4. The characteristics of individual synthetic melanoidins-containing wastewater medium are indicated in Table 1. The sugar cane molasses wastewater was obtained from SangSom distillery, Nakhon-Pathom province, Thailand. Beet molasses wastewater, Viandox sauce and caramel were obtained from Laboratoire de Genie Chimique, Toulouse, France. Since the caramel had changed the physical property of synthetic melanoidins-containing wastewater medium as shown by high viscosity at the concentration up to 30%. Thus, this experiment could not synthesize melanoidins-containing wastewater medium using caramel with respect to color (OD_{475}) and COD contents based on analysis of sugarcane molasses wastewater.

Decolorization of various synthetic melanoidins-containing wastewater medium by bacterial consortium

The inoculum was prepared by transferring of bacterial consortium into a flask containing 50 ml LB medium and incubated for 24 h with shaking (200 rpm) at 30 °C. The decolorization experiments were carried out by transferring of 10% inoculum into shake flasks containing 250 ml of four individual synthetic melanoidins-containing wastewater medium containing different colored substances. The consortium was incubated under shaking conditions (200 rpm) at pH 4, 30 °C. A separate set of uninoculated flasks was maintained in parallel as control. Experiments were performed in triplicate and samples were withdrawn with 8 h intervals for the determination of its growth and decolorization.

Identification of the bacterial consortium

Genomic DNAs of bacterium were amplified by PCR with the universal 16S rDNA primers 20F (5′-GAG TTT GAT CCT GGC TCA G-3′) and 802R (5′-TAC CAG GGT ATC TAA TCC-3′). The PCR products were used as template for DNA sequencing with UFUL primer (5′-GCC TAA CAC ATG CAA GTC GA-3′) [36] and Bigdye Termination v3.1 cycle sequencing kit. The sequences of 16S rDNA were compared with those available in the GenBank, EMBL and DJB databases using the gapped BLASTN 2.0.5 program through the National Center for Biotechnology Information server.

Optimal decolorization study

The bacterial consortium was transferred into 250 ml Erlenmeyer flasks containing 50 ml synthetic melanoidins-containing wastewater medium, using 2% (v/v) Viandox as a color substance, and then cultivated with shaking at 200 rpm, 30 °C for 48 h. Bacterial cells were harvested with 3 h intervals by centrifugation at 10,000 rpm, 4 °C for 10 min. Decolorization and bacterial growth were measured by optical density (OD) at 475 nm and 600 nm, respectively. In addition, the uninoculated control was also incubated under same conditions to determine the abiotic decolorization. To examine the effect of initial pH on the decolorization, synthetic melanoidins-containing wastewater medium was prepared at initial pH 4, 7 and 9.

Construction of bacterial consortia for optimal decolorization

In order to verify a bacterial composition for the effective color removal by mixed cultures, experiments were performed under various combinations of bacteria, namely *Klebsiella oxytoc*a (T1), *Serratia mercescens* (T2), *Citrobacter* sp. (T3) and unknown bacterium DQ817737 (T4). For construction of the active bacterial consortia, a loopful of each bacterium (T1, T2, T3 and T4) from LB plate was precultured in 50 ml LB at 30 °C with shaking at 200 rpm. After 24 h, the bacterial cells of each strain were harvested by centrifugation at 10,000 rpm at 4 °C for 10 min then washed with sterile normal saline solution. Washed bacterial cells at appropriate volume were subsequenctly inoculated into fresh synthetic melanoidins-containing wastewater media to obtain an initial OD_{600} of 0.2. Various bacterial consortia comprising of different bacterial compositions were constructed at the same initial cell density. These consortia were incubated with shaking (200 rpm) at 30 °C for 72 h. All assays were performed in triplicate and compared with respective uninoculated control. The factorial method was designed for this experiment [5, 7, 11].

Abiotic decolorization study

This study was carried out to verify whether the decolorization observed was due to biological or non-biological activity. The living and autoclaved cells of bacterial consortium with different cell concentrations at 5–50% (v/v) were added into 250 ml Erlenmeyer flask containing 50 ml of each synthetic melanoidins-containing wastewater medium. The flasks were

placed on rotary shaker (200 rpm) at room temperature for 48 h. Samples were withdrawn at respective time points and then centrifuged at 10,000 rpm for 10 min. The supernatants were read for the OD at 475 nm using spectrophotometer.

The study on limitation of decolorization

The bacterial consortium was inoculated into synthetic melanoidins-containing wastewater medium and cultivated with shaking (200 rpm) at 30 °C for 48 h. Cells were harvested by centrifugation (10,000 rpm, 10 min, 4 °C) and washed three times successively with sterile normal saline solution in order to eliminate the residual culture medium. Washed bacterial cells were resuspended in the fresh culture medium of the same volume and cultivated under condition as described above.

Meanwhile, the used culture medium was centrifuged again at 10,000 rpm for 10 min at 4 °C to completely remove the bacterial cells, then inoculated with fresh bacterial cells (10% w/v) and cultivated under the same condition as described above.

Effect of aeration on decolorization

The experiment was carried out in 2.5 L stirring bioreactor with working volume of 2 L. The agitation speed was set at 150 rpm and the temperature was maintained at 30 °C. The aeration varied at KLa = 0.3688 h^{-1} (0 vvm), KLa = 2.5836 h^{-1} (0.1 vvm), KLa = 4.6343 h^{-1} (0.2 vvm), and KLa = 8.9848 h^{-1} (0.4 vvm). Samples were withdrawn at 12 h intervals for the measurements of OD at 600 and 475 nm.

Analytical methods

Bacterial growth was determined by the OD at wavelength of 600 nm. The bacterial density (C^*) was calculated using Equation; $C^* = C_1 - C_0$; where C_1 is the OD value of culture broth; and C_0 is the OD value of supernatant obtained after centrifugation of culture broth. The color intensity in supernatant was determined by measuring OD at 475 nm. The COD content and total nitrogen, respectively, was determined by a spectrophotometric method using Hach COD reagent test kit and Hach Total nitrogen reagent test kit (HACH Company, USA).

Results and discussions

Formulation of synthetic melanoidins-containing wastewater for use as a wastewater model

Usually, wastewater obtained from distilleries has no consistency and uniformity, rather the composition in wastewater such as COD, BOD, chemical element and color substances vary in hourly, daily or seasonal fashion [30]. For this study, the variations in wastewater would affect the result of all experiments. Moreover, molasses itself contain various amounts of melanoidins depending upon the nature of its source. Apart from melanoidins, sugarcane molasses contains other colorants such as phenolic compounds and caramel, whereas melanin is abundant in beet molasses [14]. Therefore, in order to prevent the lack of raw sugarcane molasses wastewater supply as well as variations in its composition, various kinds of synthetic melanoidins-containing wastewater media were formulated. Four kinds of melanoidins-containing substances were used in this experiment after dilution with distilled water to a concentration corresponding to color and COD contents of raw sugarcane molasses wastewater. Figure 1 shows the decolorization efficiency of bacterial consortium which was conducted with Viandox sauce, caramel, beet molasses wastewater and sugarcane molasses wastewater.

The decolorization of 17.5, 9.5, 8.02 and 1.13% were achieved when using sugarcane molasses wastewater, Viandox sauce, beet molasses wastewater and caramel as a color substance of synthetic wastewater, respectively (Fig. 1). It was observed that the bacterial consortium could neither grow nor remove color substances in caramel. This was attributed to high processing temperatures, high acidity, high osmotic pressure and high specific gravity of caramel which were not supportive to microbial growth [3, 8]. Figure 1 shows that the decolorization of 9.5% was achieved using 13.5% (v/v) of Viandox sauce as a color substance of the synthetic melanoidins-containing wastewater.

For further investigation, the decolorization experiments were carried out in the synthetic melanoidins-containing

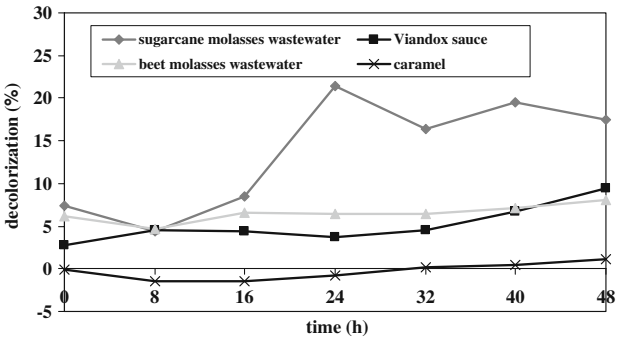


Fig. 1 Decolorization of various synthetic melanoidins-containing wastewaters by the bacterial consortium. The melanoidins-containing substances used as a color substance for each medium was Viandox (13.5%), caramel (30%), beet molasses wastewater (41.5%) and sugarcane molasses wastewater (20%). Negative value indicated the increase in color; the data were results obtained from three independent experiments

wastewater medium containing Viandox sauce as a color substance since the variation of its compositions was lower than sugarcane molasses wastewater. However, the initial concentration of Viandox at 2% (v/v) was selected for further study due to interruption of bacterial growth by Viandox at 13.5% (v/v) (data not shown).

Identification of bacterial isolates in the consortium

The 16S rRNA gene PCR amplified from the isolates using 20F and 802R primers was subjected to DNA sequencing with UFUL internal primers [36]. The sequences were then compared using NCBI BLASTN program. Pairwise alignments giving a closest match of 99% or more were chosen. The bacteria in consortium were identified as *Klebsiella oxytoca* (T1), *Serratia mercescens* (T2), *Citrobacter* sp. (T3) and unknown bacterium DQ817737 (T4).

Some of bacterial strains have been reported as a molasses decolorizing bacteria. They were *Pseudomonas*, *Acenitobacter*, *Bacillus* and *Klebsiella* present in this bacterial consortium, all of which could decolorize colored components present in molasses wastewater [22, 29, 40]. Many researchers also reported the activity of *Serratia mercescens* on biodegradation of polycyclic aromatic hydrocarbons (PAHs) and lignin degrading activity [43].

Effect of initial pH on decolorization

In order to verify pH for the effective decolorization by bacterial consortium, experiments were performed at three different initial pH values (4, 7 and 9). The effect of initial pH on the decolorization and growth profiles of bacterial consortium is given in Fig. 2. The bacterial consortium could grow and decolorize synthetic melanoidins-containing wastewater containing 2% (v/v) Viandox at both pH 4 and 7 (Fig. 2a, b, respectively). However, this bacterial consortium gave the much lower decolorization at pH 9 (Fig. 2c). The highest decolorization of bacterial consortium was observed with an initial medium pH of 4 and the decolorization was decreased when the initial pH of medium was higher than 7 (data not shown). It appeared that the initial acidic pH has a critical effect on melanoidins decolorization. The similar pattern was also observed in another study where optimal decolorization of sugarcane molasses wastewater by soil inoculum was obtained at acidic pH [1]. Alkane et al. [1] reported that pH has a crucial role in melanoidins decolorization. An increase in pH of medium resulted in less microbial decolorization and the increase in color intensity. The increase in color may be due to the polymerization of melanoidins [1]. Hence, synthetic melanoidins-containing wastewater containing 2% (v/v) Viandox at initial pH 4 was selected for further studies since this condition gave the maximal decolorization of 18.3%

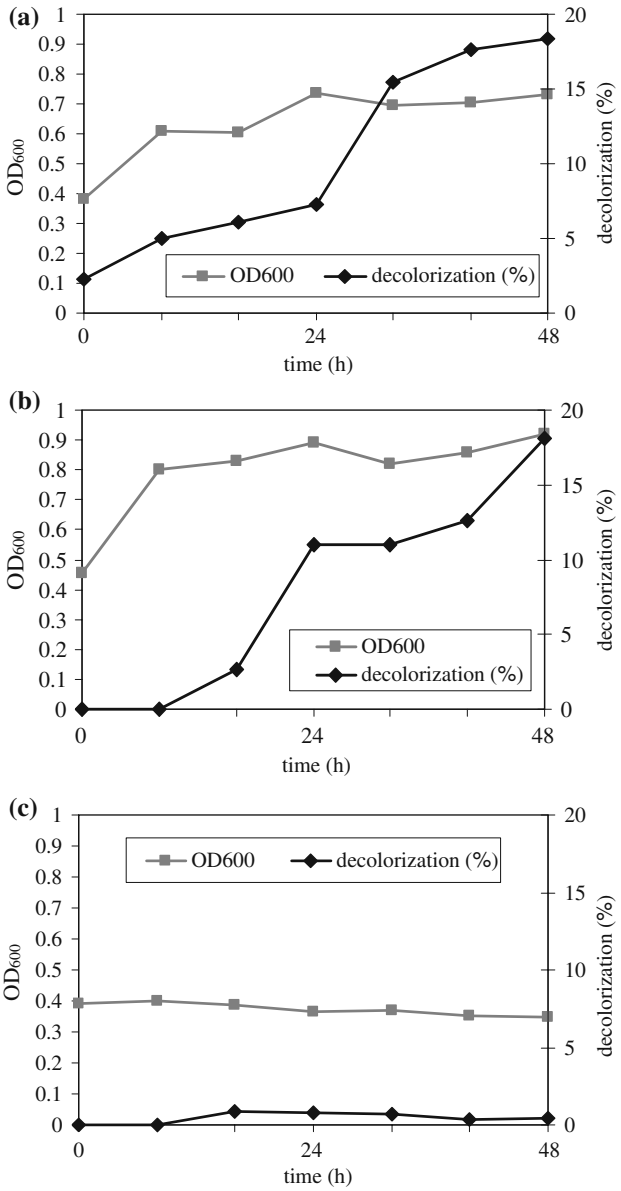


Fig. 2 Time course of growth and decolorization of bacterial consortium using synthetic melanoidins-containing wastewater medium containing 2% (v/v) Viandox at the initial pH 4 (**a**), 7 (**b**) and 9 (**c**)

under aerobic condition for 48 h (Fig. 2a). Moreover, pH 4 is closed to pH value of sugarcane molasses wastewater in Thailand.

Construction of bacterial consortium for optimal decolorization

To verify whether molasses wastewater decolorization is more effective by active mixed culture than a single bacterial isolate, the experiment was carried out by constructing feasible bacterial consortia. Totally, 16 different

experiments were carried out according to the factorial method for four bacteria (T1-T4) in comparison with control. Figure 3 illustrated that the bacterial consortia were able to decolorize synthetic melanoidins-containing wastewater at a significantly higher level as compared to those achieved by individual isolates. The results showed that unknown bacterium DQ817737 (T4) accounted for the majority of decolorization of synthetic melanoidins-containing wastewater. Although strain T2 was not an effective decolorizer, its presence might still play an important role in affecting optimal color removals of bacterial consortia. In this study, the bacterial consortium namely MMP1, comprising of T1, T2 and T4 was subjected to further study since it gave the highest decolorization of 17.52%. However, decolorization did not occur in sterile cell-free medium, suggesting the absence of abiotic decolorization. The higher decolorization of MMP1 may be due to the enhanced effect of coordinated metabolic interactions on melanoidins decolorization [27, 44, 45].

Decolorization at optimal condition

The consortium was cultured in the synthetic melanoidins-containing wastewater under the optimum condition with 2% (v/v) Viandox as color substance at an initial pH 4. Figure 4 showed the typical culture profiles of the constructed bacterial consortium MMP1. Its highest decolorization approximately 22% was observed after cultivation for 72 h, while total nitrogen decreased from 390 mg/L at the beginning of this experiment to 290 mg/L after incubation for 72 h. The pH of cultured medium was not significantly changed along the experiment period (Fig. 3).

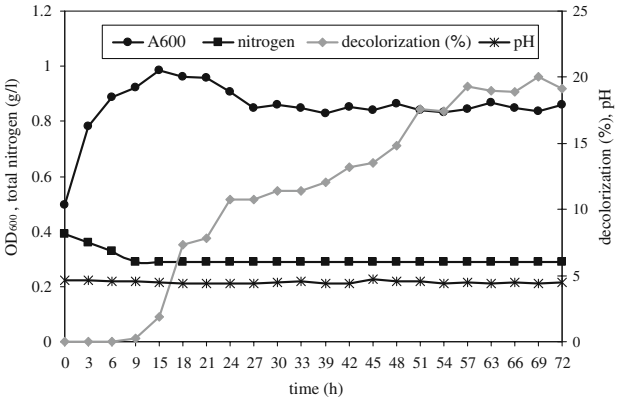


Fig. 4 Growth and decolorization of bacterial consortium MMP1 at optimal condition using 2% (v/v) Viandox as a color substance at initial pH of 4. The data were from three independent experiments

Decolorization by living and autoclaved cells

Abiotic decolorization study was carried out to verify whether the decolorization was obtained from biological activity or non-biological activity. It showed that the experiment with the different initial cell concentrations (5–50% v/v) of autoclaved cell of consortium MMP1, exhibited no melanoidins decolorization after incubation for 48 h (Fig. 5). In contrast, the melanoidins decolorization was occurred by living cell of MMP1 (Fig. 5). Additionally, to confirm that the melanoidins decolorization occurred by biological activity but not adsorption mechanism, NaOH extraction method was adopted [47]. The cell pellets of both living cell and autoclaved cell were resuspended with equal volume of NaOH 0.1 M to extract color substances adsorbed to cell surface. The extracts were

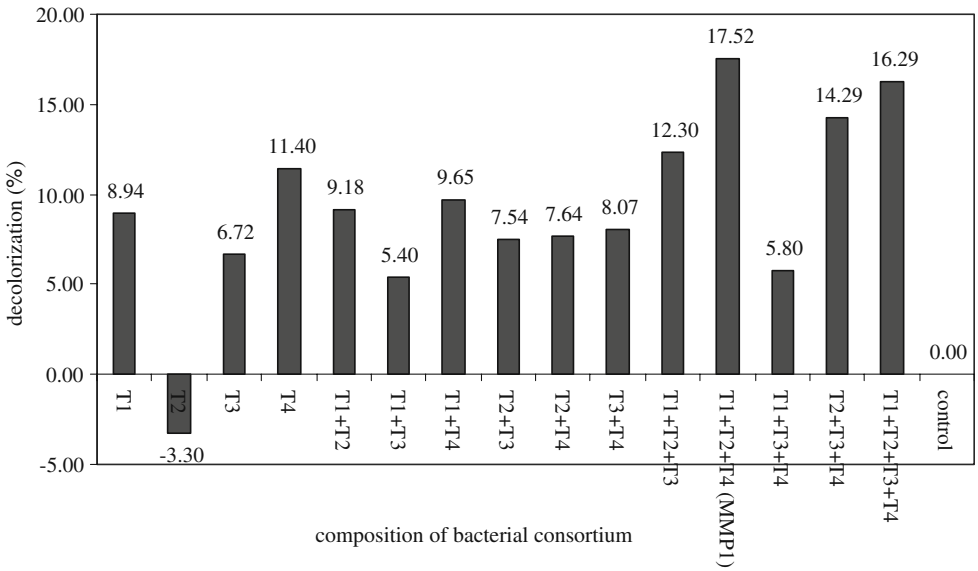


Fig. 3 The composition of bacterial consortium for decolorization of the synthetic melanoidins-containing wastewater medium containing 2% (v/v) Viandox; negative value indicated the increase in color; the data were results of three independent experiments

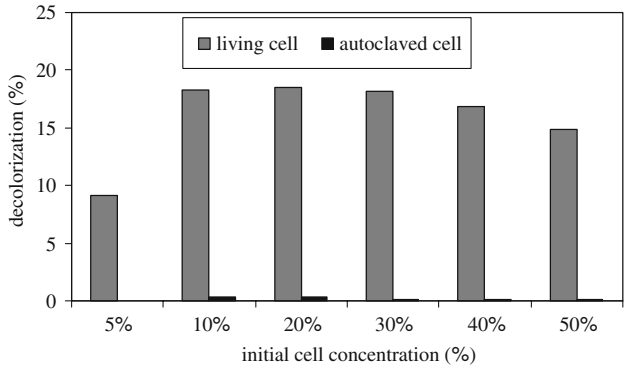


Fig. 5 Decolorization of living cell and autoclaved cell of bacterial consortium MMP1. The data were from three independent experiments

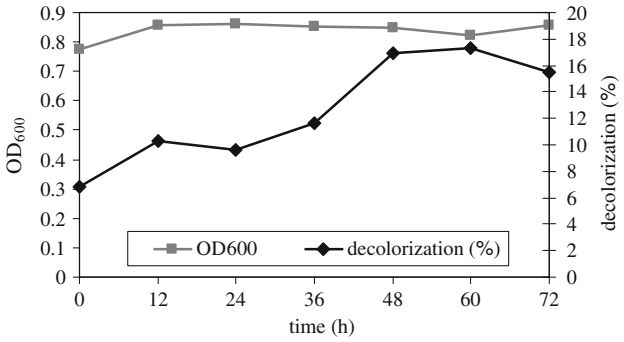


Fig. 6 Limitation study of melanoidins decolorization by used bacterial cells in fresh synthetic melanoidins-containing wastewater medium

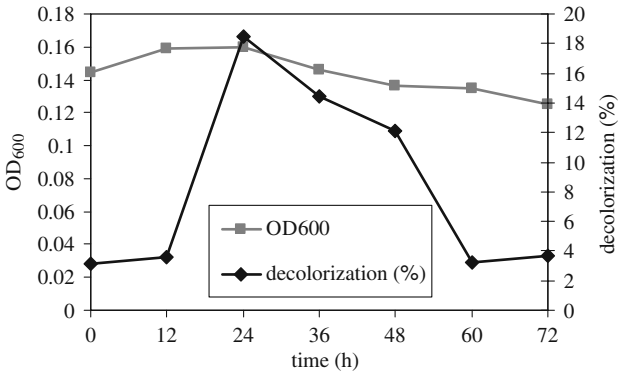


Fig. 7 Limitation study of melanoidins decolorization by fresh bacterial cells in used synthetic melanoidins-containing wastewater medium

centrifuged and OD was measured at 475 nm. It showed that the final fractions of NaOH-extractable color substance were negligible. These results clearly indicated that the decolorization of Viandox by consortium MMP1 was due to biological mechanisms.

The study on limitation of decolorization

Since melanoidins decolorization was dependent on bacterial growth conditions such as pH, nutrient levels, aeration and metabolites in liquid phase. Nutrient availability and metabolite accumulation might result in growth limitation and thereby consequently decreased in melanoidins decolorization. To clarify the limitation of decolorization of the bacterial consortium MMP1, the used bacterial cells and the used medium were separately subjected to further study. Used cells were inoculated into fresh medium and, meanwhile, used medium was inoculated with fresh bacterial cells. After cultivation, it was found that the used cells could decolorize of freshly prepared medium (Fig. 6). Figure 7 shows that the fresh bacterial cells could decolorize the melanoidins remaining in used culture medium during the first 24 h of incubation and its decolorization was then decreased afterward. As shown in Fig. 7, it was observed that the color of Viandox sauce could be hardly removed by fresh bacterial cells in used medium. This might be due to the effect of toxicity of melabolites, which had been formed and accumulated during decolorization, thereby repressed the decolorization ability of fresh cells [26]. Also, it was possible that the absence of nutrients markedly affected the decolorization of bacterial consortium MMP1.

Various studies on melanoidins decolorization by microorganisms have shown the similar results regarding to the effect of nutrient supplements. A melanoidins decolorization of 87% was reported after 12 days of incubation with *Geotrichum candidum* in the presence of 2% glucose and inorganic nutrients [20]. Removal of melanoidins from molasses waste of 84.16% using *Aspergillus niger* in the presence of glucose has also been reported [15]. Although higher decolorization could be achieved using additional nutrient supplement but this might lead to the addition of extra chemicals in the system [15].

Effect of aeration on decolorization

The effect of aeration on decolorization of synthetic melanoidins-containing wastewater medium by bacterial consortium was carried out in 2 L laboratory-scale suspended cell bioreactor. Figure 8 shows that the consortium could decolorize synthetic melanoidins-containing wastewater medium at aeration rate of KLa = 0.3688 h^{-1} (0 vvm), KLa = 2.5836 h^{-1} (0.1 vvm), KLa = 4.6343 h^{-1} (0.2 vvm), and KLa = 8.9848 h^{-1} (0.4 vvm) up to 18.92, 19.32, 16.94 and 8.31% within 48 h, respectively. Further increase in the aeration rate did not improve the decolorization. At the end of these experiments, the optimum aeration rate for melanoidins decolorization was found to be KLa = 2.5836 h^{-1} (0.1 vvm).

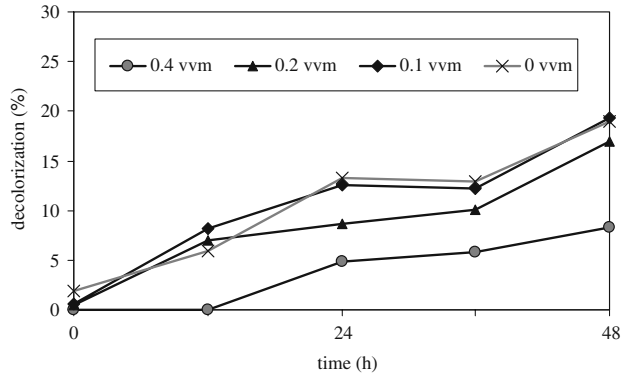


Fig. 8 The effect of aeration rate on decolorization of synthetic melanoidins-containing wastewater containing 2% (v/v) Viandox as a color substance

In general, several microorganisms that have been shown to degrade melanoidins are not best suited for treating melanoidins-containing effluent from distilleries. This is because they are depleted in oxygen, which is necessary for oxidative degradation of melanoidins [2]. However, the results presented in this study showed that color removal under low aeration conditions relatively higher than the highly aerobic condition. Hence, the decolorization mechanisms of melanoidins-containing wastewater by bacterial consortium MMP1 in this study might be due to metabolism of bacterial cell under facultative and anaerobic conditions such as fermentation and anaerobic respiration [2, 48].

Conclusions

Bacterial consortium comprising of *Klebsiella oxytoca* (T1), *Serratia mercescens* (T2) and unknown bacterium DQ817737 (T4), namely MMP1, was developed for this study. This bacterium consortium exhibited increased decolorization compared to that shown by any single isolate. This may be due to the enhanced effect of coordinated metabolic interactions on melanoidins decolorization. Also, the bacterial consortium MMP1 could be utilized for the decolorization of various kinds of melanoidins present in various industrial effluents including sugarcane and beet molasses wastewaters.

The consortium showed high growth and melanoidins decolorization at the initial pH of 4 under low aeration condition. Thus, the consortium MMP1 might be suitably applied to the acid formation phase of conventional aerobic or anaerobic treatment systems of alcoholic distillery wastewater.

The used bacterial cells inoculated in fresh medium showed that the addition of nutrients affecting the decolorization of the consortium MMP1. This suggested that the decolorization of melanoidins ran parallel with the decomposition of nutrients. Therefore, nutrients could affect the growth and melanoidins decolorization of consortium MMP1.

The comparison of decolorization of consortium MMP1 with abiotic control has proved that the color removal for synthetic melanoidins-containing wastewater medium containing 2% (v/v) Viandox was due to biotic activity of bacteria but not adsorption of color substances on cell surface.

For process application, our bacterial consortium has the potential to serve as an inoculum for decolorization of melanoidin-containing wastewaters since its highest decolorization took place under the condition similar to the real distillery wastewaters.

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