CHAPTER V

RESULTS
(ADJUSTMENT OF SOME OPERATING CONDITIONS PARAMETERS)

5.1 Choice of the synthetic substrate

Decolorization of various brown color substances by bacterial consortium CONS8

Generally, wastewaters obtained from sugarcane molasses based distilleries have no consistency and uniformity, as the compositions in wastewater such as COD, BOD, chemical elements and color substances vary depending on time, day or season (Mogan, 2002). For this study, the variations in real sugarcane molasses wastewater would affect the results of all experiments. Moreover, sugarcane molasses themselves contain varied amounts of melanoidins, dark brown to black colored natural condensation products of sugars, and amino acids produced by Maillard reactions, depending upon the nature of its source. Apart from melanoidins, sugarcane molasses also contain other colorants such as phenolic compounds and caramel, whereas melanin is abundant in beet molasses (Godshall, 1999). Therefore, in order to prevent the lack of raw sugarcane molasses wastewater supply as well as variations in its compositions, various kinds of synthetic melanoidins-containing wastewater media were formulated.

The aim of this study was to determine the decolorization capabilities of the bacterial consortium CONS8 in various kinds of synthetic melanoidins-containing wastewater media and to look for the most suitable synthetic melanoidins-containing wastewater to be used for further decolorization experiments. Various synthetic melanoidins-containing wastewater media were prepared according to the method mentioned previously in Chapter 3. In brief, three kinds of melanoidins-containing substances were used after dilution with distilled water to concentrations corresponding to color and COD contents of raw sugarcane molasses wastewater. Then, the bacterial consortium CONS8 was grown at 30°C to investigate the decolorization of its model brown color substances. The characteristics of individual melanoidins color substances were indicated in Table 5.1 and the spectrophotometric determination of each melanoidins color substances is shown in Fig. 5.1.

Table 5.1 Characteristics of synthetic melanoidins-containing wastewater media

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

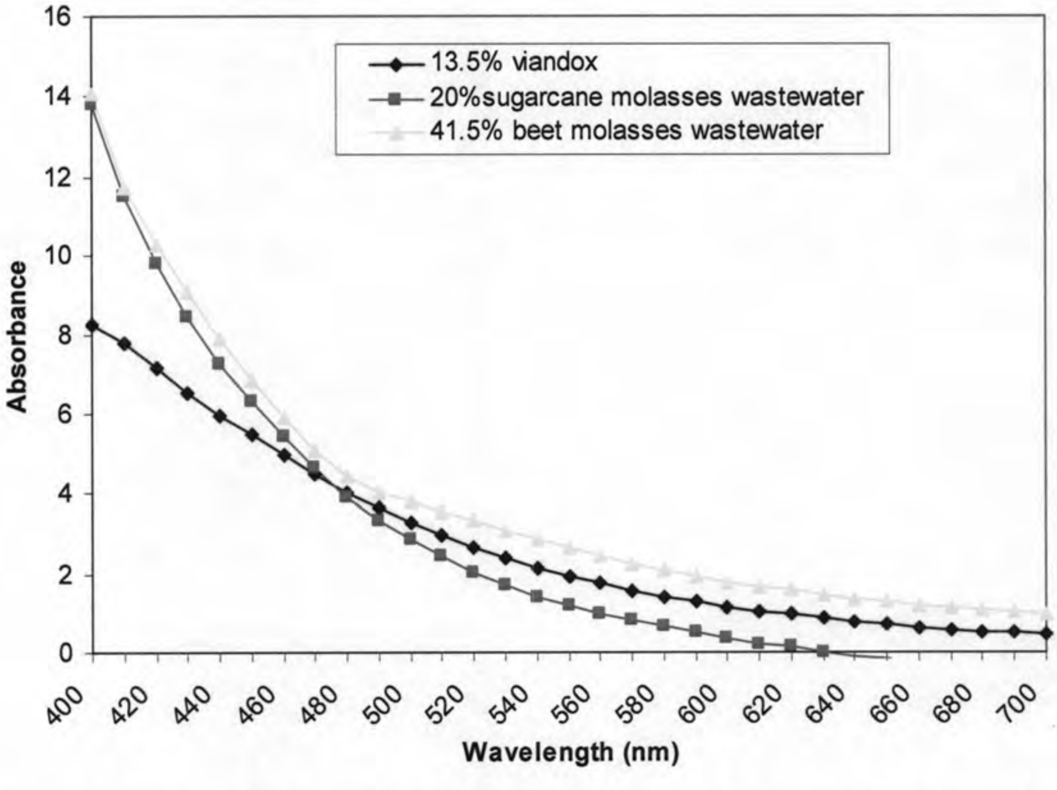

Figure 5.1 Typical visible spectrum of the individual synthetic melanoidins-containing wastewater medium before incubation of bacterial consortium CONS8.

The sugarcane molasses decolorization of bacterial consortium CONS8 with Viandox sauce, and beet molasses wastewater is given in Fig. 5.2. It shows that the decolorization of 17.5%, 9.5%, and 8.02% were achieved when using sugarcane molasses wastewater, Viandox sauce and beet molasses wastewater as a color substance of synthetic wastewater, after aerobic incubation for 48 h, respectively. The decolorization of the bacterial consortium CONS8 differed with the model color substances. The consortium could decolorize the model melanoidins pigments presented in sugarcane molasses wastewater, beet molasses wastewater and Viandox.

Figure 5.3 shows that bacterial growth was achieved when using real sugarcane molasses wastewater from Thailand, Viandox sauce, and beet molasses wastewater as brown colored substances in the synthetic melanoidins-containing wastewater.

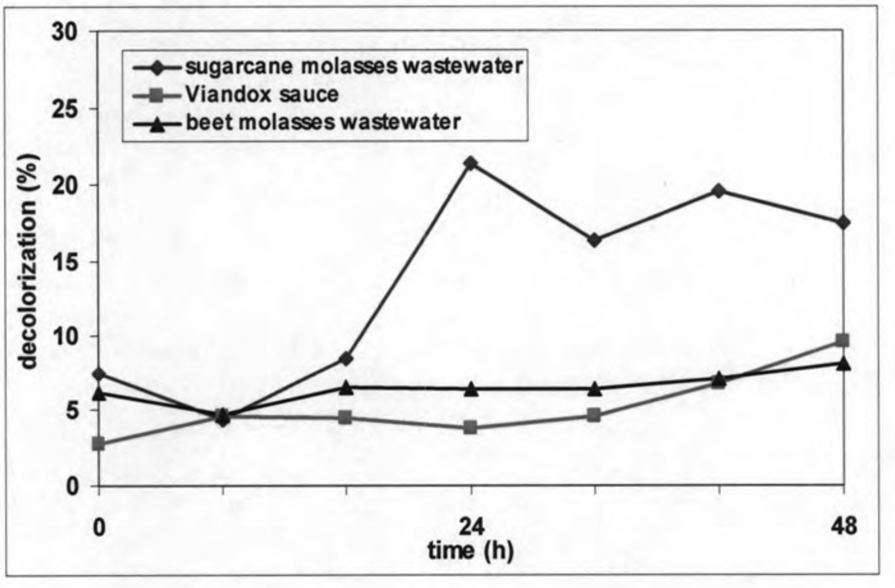

Figure 5.2 Decolorization of various synthetic melanoidins-containing wastewaters by the bacterial consortium CONS8. The data were obtained from three independent experiments.

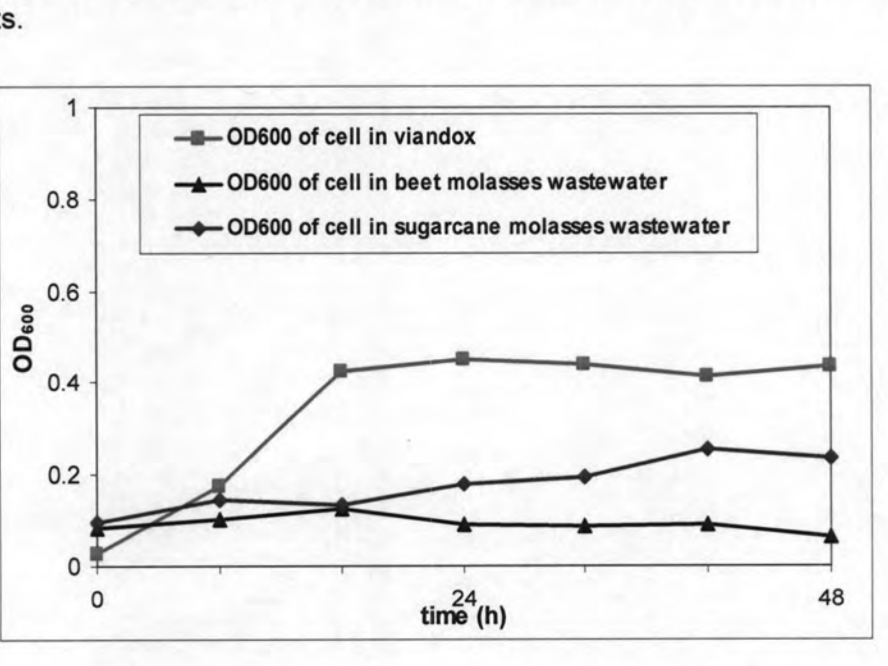

Figure 5.3 Growth of the bacterial consortium in various synthetic melanoidins-containing wastewaters. The data were obtained from three independent experiments.

It appeared that the Viandox sauce seemed to be the best source of brown colored substances in the model sugarcane molasses based distillery effluent. Then, the Viandox sauce will replace the real sugarcane molasses wastewater from Thailand since the variation of its compositions is rather lower than real sugarcane molasses wastewater. Therefore, Viandox sauce was used as the only colored substrate in the synthetic melanoidins-containing wastewater medium which will be used for all further experiments. However, the initial chosen concentration of Viandox at 13.5% (v/v) was not suitable as a colored substance in synthetic melanoidins-wastewater because this concentration could interrupt the bacterial growth (partial inhibition). Hence, the initial concentration of Viandox at 2% (v/v) was selected for further study.

In this study, another experiment was carried out to determine the variety of melanoidins decolorization of the bacterial consortium CONS8. Melanoidins models were also prepared in the laboratory using glucose and glycine as sugar and amino acid precursor for Maillard reaction (Dahiya et al., 2001). A mixture of 1 M glucose, 1 M glycine and 0.5 M sodium carbonate was autoclaved at 121°C for 15 min. The solution was adjusted to pH 7.0 with 1 N NaOH. The dark brown product, which assumed as the synthetic glucose/glycine model melanoidins, was used as a color substrate in the synthetic melanoidins-containing wastewater consisted of 0.01% $NaNO_3$, 0.2% K_2HPO_4, 0.1% KH_2PO_4, 0.01% $MgSO_4{\cdot}12H_2O$, 2% glucose and 0.1% yeast extract. The initial optical density (color) of medium at 475 nm was adjusted to the value of 3.5 by addition of synthetic melanoidins solution. The bacterial consortium CONS8 was investigated for its model melanoidins decolorization using the agar plate method and the shaking culture method as previously described in Chapter 3.

The results from both methods indicate that the bacterial consortium CONS8 did not show any decolorization against the glucose-glycine melanoidins model and the bacterial biomass content rather decreased along the incubation time like. This result might be due to the high inhibitory and antimicrobial activity of prepared melanoidins which could inhibit the growth and decolorization of bacterial consortium CONS8. This result is similar to those reported previously by many authors (Taylor et al., 2004; Rufian-Henares and Morales, 2006; Painter, 1998; Hiramoto et al., 1997).

It has been reported that melanoidins have negative biological effects such as mutagenic, carcinogenic and cytotoxic effects and give high inhibitory and antimicrobial activity (Painter, 1998; Taylor et al., 2001). Some harmful biological effects of melanoidins have been reported as followed: destruction of essential amino acids, inactivation of enzymes, inhibition of regulatory molecule binding, cross-linking of glycated extracellular matrix, abnormalities of nucleic acid function, altered macromolecular recognition, endocytosis and increased immunogenicity. Mutagenicity and DNA strand breaking activity of melanoidins from a glucose–glycine model was demonstrated by Hiramoto et al. (1997) who reported that the low molecular weight fractions acted as lipid sink (Larter and Douglas, 1980) and induced DNA damage, where the effect increased with the concentration added.

5.2 Effect of initial pH on decolorization of synthetic melanoidins-containing wastewater

Environmental factors like pH, colored substances, aeration and nutrients play vital role in bacterial decolorization of molasses based distilleries as the metabolism and activity of enzymes are greatly influenced by these environmental factors. In order to characterize the pH influence for the effective decolorization by bacterial consortium CONS8, 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 were given in Figure 5.3. The bacterial consortium could grow and decolorize synthetic melanoidins-containing wastewater made of 2% (v/v) Viandox at both pH 4 and pH 7 (Figure. 5.4a and 5.4b, respectively). However, this bacterial consortium led to the much lower decolorization at pH 9 (Fig. 5.4c). The highest decolorization of bacterial consortium was observed with an initial medium pH of 4 whereas; the decolorization was decreased when the initial pH of medium was higher than 7 or highly alkaline.

It has been demonstrated previously that the initial acidic pH has a critical effect on melanoidins decolorization. The similar effect was also observed in another study where optimal decolorization of sugarcane molasses wastewater by soil inoculum was obtained at acidic pH (Alkane et al., 2006). Alkane et al. (2006) 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 (Alkane et al., 2006). The decrease in color removal efficiency in highly alkaline pH might be due to the fact that the melanoidins responsible for color were more soluble in the alkaline pH, whereas, the melanoidins might be precipitated and removed easily in the acidic pH.

Therefore, initial acidic pH seems to favor the decolorization of this bacterial consortium. Indeed, pH 4 is closed to pH value of sugarcane molasses wastewater in Thailand. 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% under aerobic condition for 48 h (Fig. 5.4a). In addition, the progressive decolorization of synthetic melanoidins-containing wastewater medium containing 2% (v/v) Viandox as a colored substrate at the initial pH 4 by bacterial consortium CONS8 can be observed in the visible spectral sequence presented in Fig. 5.5. The absorbance from 400 to 700 nm decreased along the incubation time.

(a)

(b)

(c)

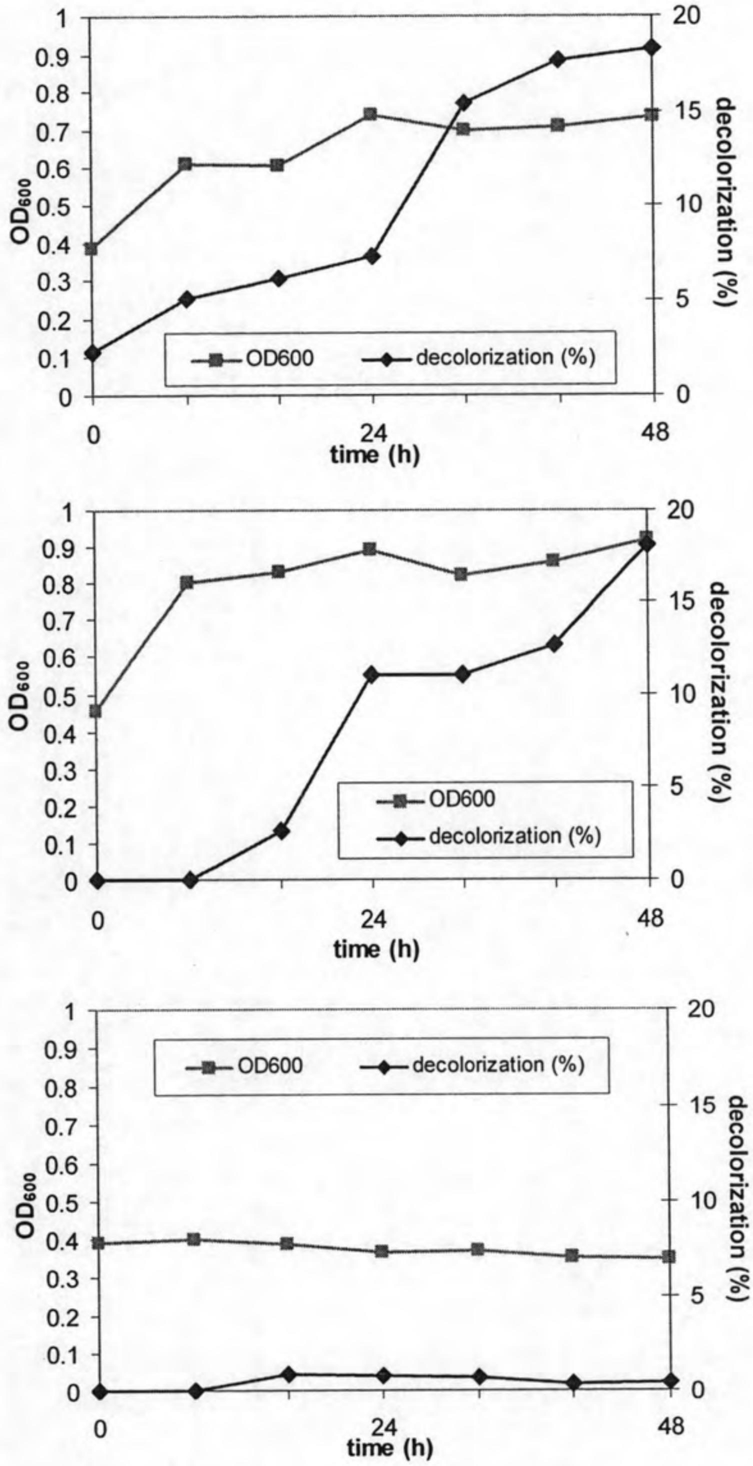

Figure 5.4 Time course of growth and decolorization of bacterial consortium using synthetic melanoidins-containing wastewater medium containing 2% (v/v) Viandox as a colored substrate at the initial pH 4 (a); pH 7 (b) and pH 9 (c).

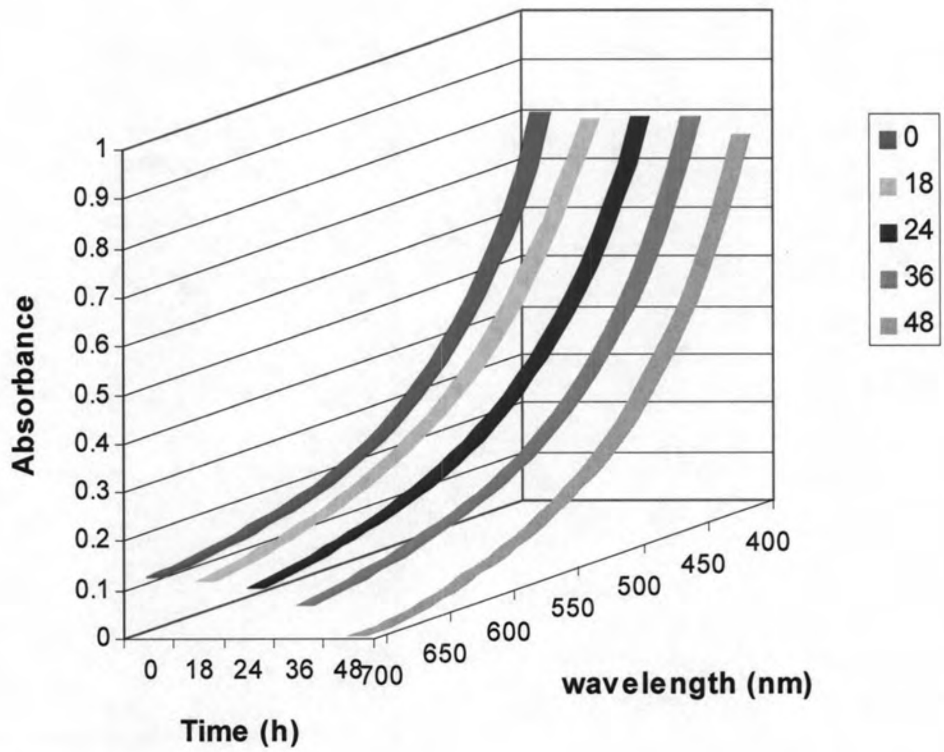

Figure 5.5 Typical visible spectra of supernatant from aerobic decolorization of synthetic melanoidins-containing wastewater medium containing 2% (v/v) Viandox as a colored substrate at the initial pH 4 by bacterial consortium CONS8 under the optimum condition at various incubation times.