CHAPTER VIII

INVESTIGATION ON THE LIMITATION OF DECOLORIZATION EFFICIENCY

8.1 Investigation on the limitation of decolorization efficiency

It has been reported that melanoidins decolorization was dependent on bacterial growth conditions such as pH, nutrient levels, aeration and metabolites in liquid phase (Alkane et al., 2006; Ames et al., 1999; Gomaa et al., 2003; Kim and Shoda, 1999). Nutrients availability and metabolites accumulation might result in growth limitation and thereby decreased melanoidins decolorization. The aim of the experiments presented in this chapter 8 was, therefore, to conduct in-depth investigations in order to point out some of the key factors affecting the long-term performance of bacterial decolorization. Further, based on that the previous mentioned experiments, some strategies to enhance decolorization were also proposed.

To clarify the limitation of decolorization of the bacterial consortium MMP1, the used bacterial cells and the used medium were separately submitted to further study. The bacterial consortium was inoculated into synthetic melanoidins-containing wastewater medium and cultivated with shaking (200 rpm) at 30^{o}C for 48 h. Cells were harvested by centrifugation (10,000 rpm, 10 min, 4^{o}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^{o}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.

It was observed in Figure 8.1 that used cells suspension of the bacterial consortium MMP1 demonstrated much improved decolorization efficiency after cultivation with fresh medium. After cultivation for 72 h, the acclimatized bacterial consortium showed 15.4% decolorization of fresh synthetic melanoidins-containing wastewater medium, however, the fresh cells suspensions of bacterial consortium showed only 3.3% decolorization in used culture medium at the same incubation time (Figure 8.2)

Firstly, batch decolorization experiment confirmed the significant melanoidins decolorization capacity of the acclimatized bacterial cells in fresh medium. However, the decolorization efficiency of fresh bacterial consortium MMP1 in used medium was significantly higher than that one observed in the experiment of used cells in fresh

medium after the first 24 hours of incubation. As shown in Figure 8.2, it was observed that fresh cells suspension of the bacterial consortium MMP1 could decrease the color remaining in used culture medium after the first 24 hours of incubation. However, its decolorization efficiency dropped significantly after some hours of great decolorization. One of the possible causes of this phenomenon is might be due to the effect of toxicity of metabolites, which had been formed and accumulated during decolorization, thereby repressed the decolorization ability of fresh cells (Manjinder et al., 2005).

In general, the use of fresh cells suspension in used medium may not be an option for the continuous treatment of toxic compounds. Once the concentration of toxic compounds becomes too high or the process is operated for a long time, the amount of the original compounds and their metabolic products accumulated will reach saturation (Eccles, 1995). Beyond this point, the metabolism of living bacterial cells may be interrupted, resulting in death of the cells.

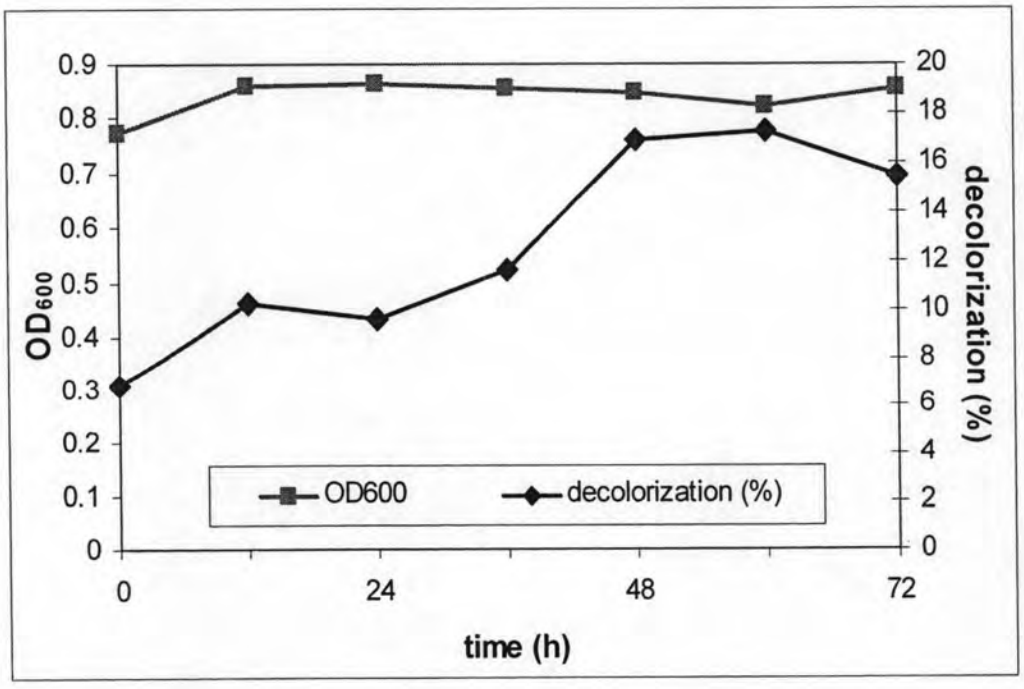

Figure 8.1 Limitation study of melanoidins decolorization by used bacterial cells in fresh synthetic melanoidins-containing wastewater medium.

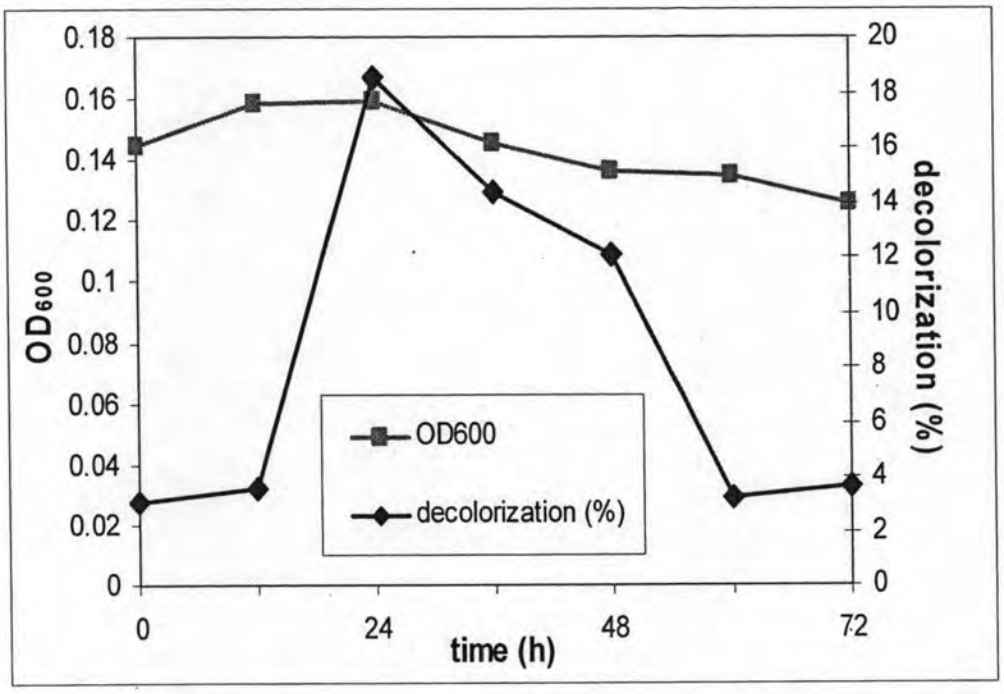

Figure 8.2 Limitation study of melanoidins decolorization by fresh bacterial cells in used synthetic melanoidins-containing wastewater medium

The result in Figure 8.2 indicated that the fresh cells suspension of bacterial consortium MMP1 could hardly removed the color in used synthetic melanoidins-containing wastewater medium during a given period. It was possible that the absence of nutrients markedly affected the decolorization of bacterial consortium MMP1. Adequate nutrients concentrations, including metal ions and vitamins, are required to support mixed bacterial cultures in wastewater treatment system in order to support viability of microbial community. In the cases where bacterial communities are dealing with a nutrient-limited wastewater, supplementation with nutrients can result in an enhanced degradation of pollutants (Singleton, 1994). Thus, in order to avoid decolorization efficiency drop, the effect of nutrient supplemented into used culture medium has been investigated in further study.

8.2 Nutrient Supplements for Optimization of the Bacterial Decolorization

Adding nutrients to biological treatment processes is one possible approach to upgrading an existing facility in order to deal with increasing volumes and strengths of industrial wastewaters. Usually, growth required macronutrients consisting of carbon, oxygen, hydrogen, nitrogen, phosphorus and sulfur (Valo et al.,

1985; Singleton, 1994) and micronutrients including vitamins and trace elements (Lind, 1994; Lemmer et al., 1998). Burgess et al. (1999) have reported that the addition of micronutrient have beneficial effect to support microbial growth in activated sludge to treat wastewater as an unbalanced activated sludge community can lead to sludge handling problems.

Various studies on melanoidins decolorization by microorganisms have shown the similar results regarding to the effect of nutrient supplements. Melanoidins decolorization of 87% was reported after 12 days of incubation with *Geotrichum candidum* in the presence of 2% glucose and inorganic nutrients (Kim and Shoda, 1999). Removal of melanoidins from molasses waste of 84.16% using *Aspergillus niger* in the presence of glucose has also been reported (Gomaa et al., 2003). Although higher decolorization could be achieved using additional nutrient supplement but this might lead to addition of extra chemicals in the system (Gomaa et al., 2003).

The present study extends the earlier work investigating the limitation of decolorization of the synthetic melanoidins-containing wastewater medium by the constructed bacterial consortium MMP1. Previous studies, have shown that the limitation of decolorization might be due to the lack of nutrients required for microbial growth and metabolic activity. In this study, it has been hypothesized that addition of macro- and micronutrient may overcome these problems, thus increasing the decolorization of bacterial consortium MMP1.

To confirm whether the limitation of the synthetic melanoidins-containing wastewater decolorization was from the lack of macro- and micronutrients or toxicity of metabolites, the experiment was carried out by addition of either 0.5% (w/v) LB or B vitamins into used synthetic melanoidins-containing wastewater medium. Eight B vitamins were chosen as follows: 0.2 mg/l of ρ-aminobenzoic acid, 0.4 mg/l of pyridoxine-HCl, 0.2 mg/l of thiamine-HCl, 0.2 mg/l of riboflavin, 0.2 mg/l of nicotinic acid, 0.2 mg/l of vitamin B_{12}, 0.08 mg/l of biotin and 0.08 mg/l of folic acid. It was observed that addition of 0.5% (w/v) LB (Figures 8.3) or B vitamins (Figures 8.4) could effect growth and melanoidins decolorization efficiency of bacterial consortium MMP1.

It can be seen from Figure 8.3 that the addition of 0.5% (w/v) LB increased the growth of bacterial consortium in reused culture medium. However, the result shows that the addition of 0.5% (w/v) LB into reused synthetic melanoidins-containing wastewater medium can not improve the decolorization efficiency of the bacterial consortium MMP1.

The addition of eight B vitamins into reused synthetic wastewater had inhibitory effects on the bacterial consortium as reflected in the color removal and growth rates (Figure 8.4). It was possible that the vitamin B markedly affected the decolorization of bacterial consortium MMP1. Also, it might be due to the effect of toxicity of metabolites, which had been formed and accumulated during decolorization, thereby repressed the decolorization ability of fresh cells (Manjinder et al., 2005).

As shown in Figure 8.3 and 8.4, the addition of 0.5% (w/v) LB and vitamin B provided the similar results and their decolorization did not increase significantly compared to the reused culture medium without nutrient supplementation. Therefore, the limitation of decolorization efficiency of the bacterial consortium MMP1 in this study was not directly due to a nutrient-limitation.

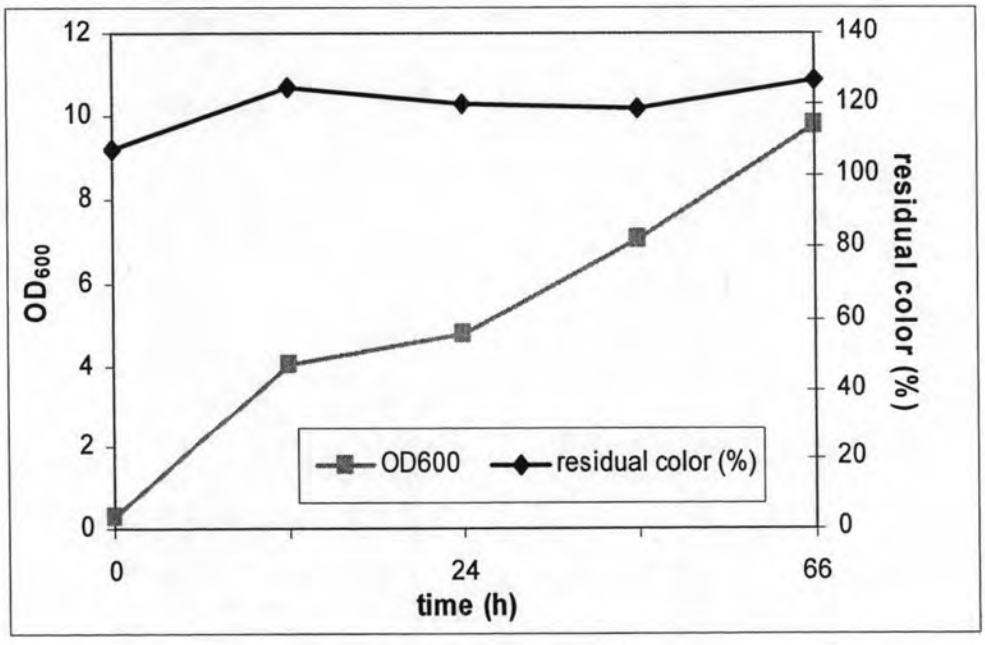

Figure 8.3 Melanoidins decolorization and growth of fresh bacterial cells in used synthetic melanoidins-containing wastewater medium supplemented with 0.5% (w/v) LB.

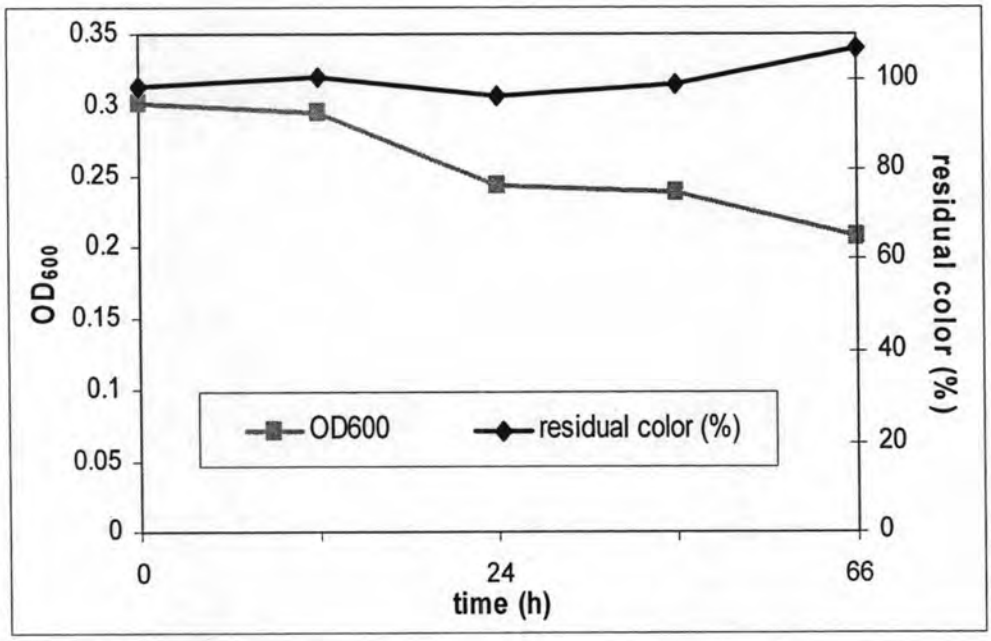

Figure 8.4 Melanoidins decolorization and growth of fresh bacterial cells in used synthetic melanoidins-containing wastewater medium supplemented with eight B vitamins.

8.3 Effects of trace elements on decolorization

One of the important differences between decolorization experiments which were carried out in different places, in Thailand and in France, may be the composition of trace elements in the tap water of each country. To prevent the effect of trace elements variation in synthetic melanoidins-containing wastewater medium and hence its suitability for bacterial decolorization, the decolorization experiments were carried out in 3 different synthetic melanoidins-containing wastewater media which were prepared by using different water sources as follow; double distilled water (DDW), tap water in Thailand and tap water in France, respectively.

The results in Figures 8.5 to 8.7 indicated that the bacterium consortium MMP1 could grow and showed decolorization in synthetic melanoidins-containing wastewater medium which was prepared with Tap water (in France) higher than the medium which was prepared with double distilled water (DDW). The decolorization obtained in the synthetic melanoidins-containing wastewater which were prepared by using double distilled water, tap water in Thailand, and tap water in France after incubation for 72 h were 16.4%, 14.5% and 27.5% (Figures 8.5, 8.6 and 8.7), respectively. The comparison of the decolorization of the bacterial consortium MMP1

in the synthetic melanoidins-containing wastewater medium which was prepared by tap water in France clearly revealed the presence of trace elements associated with bacterial decolorization.

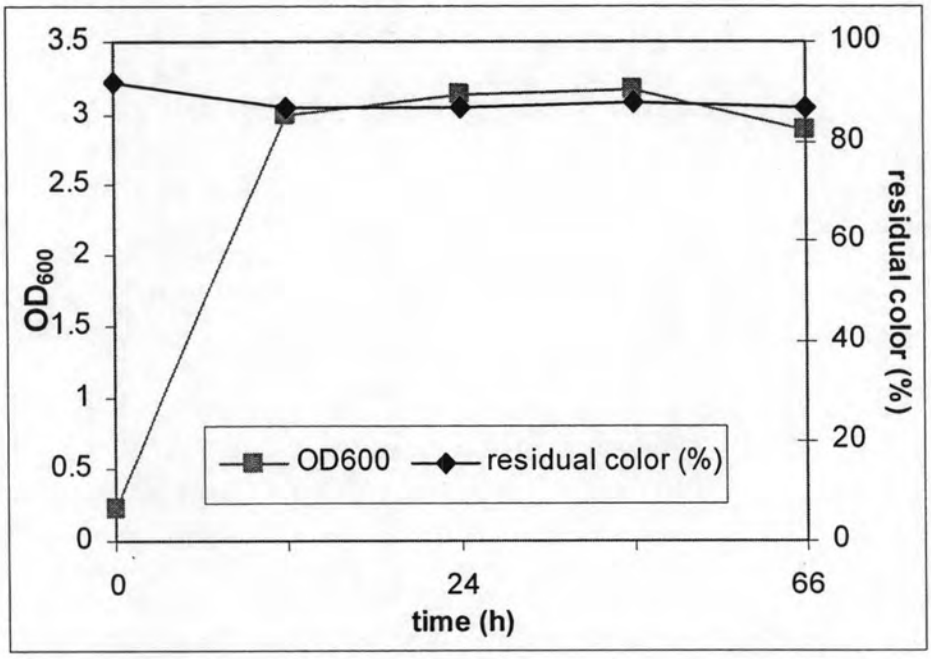

Figure 8.5 Growth and residual color of synthetic melanoidins-containing wastewater medium prepared with distilled water at different incubation times.

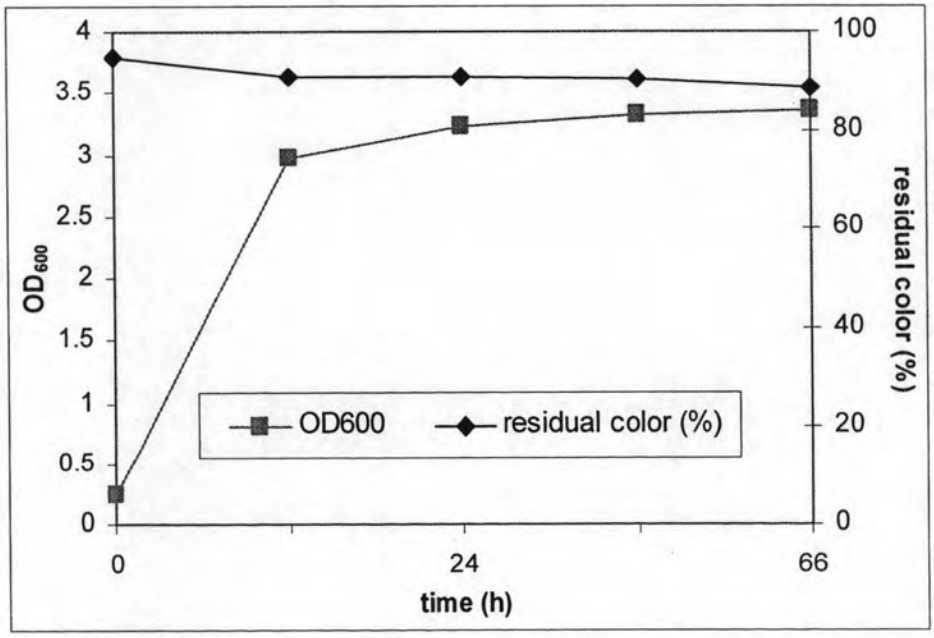

Figure 8.6 Growth and residual color of synthetic melanoidins-containing wastewater medium prepared with tap water in Thailand at different incubation times.

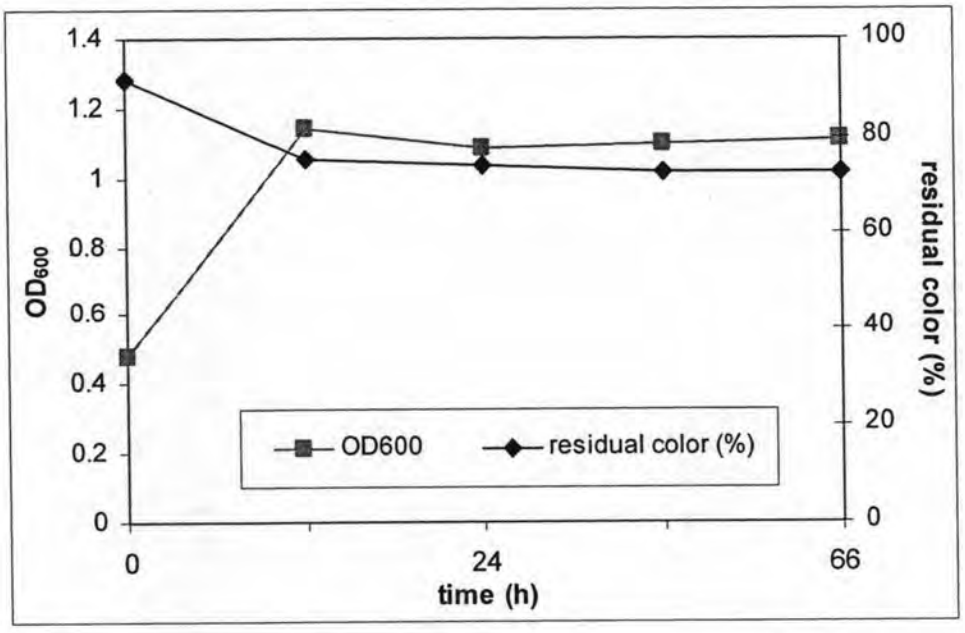

Figure 8.7 Growth and residual color of synthetic melanoidins-containing wastewater medium prepared with tap water in France at different incubation times.

Because of the higher decolorization, tap water in France had also drawn the attention of author to further experiments. Moreover, the different compositions of trace elements in tap water in Thailand and France might have variable effects on decolorization of bacterial consortium MMP1. Mahler and Cordes (1966) have reported that trace elements are taken up as components of enzymes and maintenance of enzyme structure. In the other hand, in some cases, excess trace elements have toxic effects (Madoni et al., 1996)

Hence, in order to elucidate the probable effect of trace elements on decolorization activity, the experiments were carried out. At the first step, the trace elements analysis of tap water in France was determined by ICP-Spectroscopy and the summaries of the roles of some trace elements indicated in Table 8.1. From ICP-spectroscopy results, the key trace elements of tap water in France were identified as follow; calcium, sulfur, sodium, magnesium, potassium at the concentration of 44.3, 31.0, 9.3, 4.3, and 1.3 mg/L, respectively. Then, the synthetic melanoidins-containing wastewater medium was prepared by using water which had key trace elements similar to tap water in France. To mimic the key trace elements available in tap water in France, the double distilled water were added with Na_2SO_4, $(NH_4)_2SO_4$, $CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$ and KCl at the concentration of 28.85, 75.08, 162.27 and 44.12, 2.42 mg/L, respectively. The distilled water supplemented with trace elements was used as a water source for preparation of the synthetic melanoidins-containing wastewater medium hereinafter.

The growth and decolorization efficiency of bacterial consortium MMP1 in synthetic melanoidins-containing wastewater medium supplemented with trace elements was showed in Figure 8.8. The result indicated that the bacterial consortium showed lower growth and decolorization in synthetic melanoidins-containing wastewater medium prepared with double distilled water containing trace elements (Figure 8.8) compared to culture medium which was prepared by double distilled water (Figure 8.5). Trace amount of decolorization activity at 12.4% was also observed in the synthetic melanoidins-containing wastewater medium prepared with DDW containing trace elements after incubation for 72 h.

It appeared that the trace element supplementation did not improve decolorization efficiency of the bacterial consortium MMP1. Therefore, further decolorization experiments were carried out by using the synthetic melanoidins-containing wastewater medium prepared with distilled water without any supplementary trace element.

Table 8.1 Trace element requirements and the concentrations present in tap water in France

Trace elements	Reported requirements (mg/L)	Role of trace metal	Concentration detected (mg/L)
Ca	0.4-1.4	Cell transport systems and osmotic balance in all bacteria. Bridging anionic ECP and aiding flocculation. Increase growth rates. Requirements and effects vary.	44.301
K	0.8->3.0	Cell transport systems and osmotic balance in bacteria.	1.268
Fe	0.1-0.4	Growth factor in bacteria, fungi and algae. Adsorbed in proportion to the concentration available. Electron transport in cytochromes. Synthesis of catalase, peroxidase and aconitase.	0.004
Mg	0.4-5.0	Enzyme activator for a number of kinases and phosphotransferase in heterotrophic bacteria.	4.350
Mn	0.01-0.5	Activates bacterial enzymes. Often interchangeable with magnesium in kinase reactions. Lower affinity for binding sites than other metals but still can inhibit metabolism at 1 mg/L	0.002
Cu	0.01-0.5	Bacterial enzyme activator required in trace quantities. Can inhibit metabolism. Chelates other substances, reducing their toxicity.	0.059
Zn	0.1-0.5	Bacterial metallic enzyme activator of carbonic anhydrase and carboxypeptidase A. Dissociable on active site of enzymes. Stimulates cell growth. Can exacerbate toxic effects of other metals and inhibit metabolism.	0.491
Mo	0.2-0.5	Molybdenum is a common limiting nutrient	0.043
Co	0.1-5.0	Bacterial metallic enzyme activator. Dissociable on active site of enzymes. Activates carboxypeptidase for synthesis of vitamin B12 but otherwise toxic. Can inhibit metabolism.	0.001

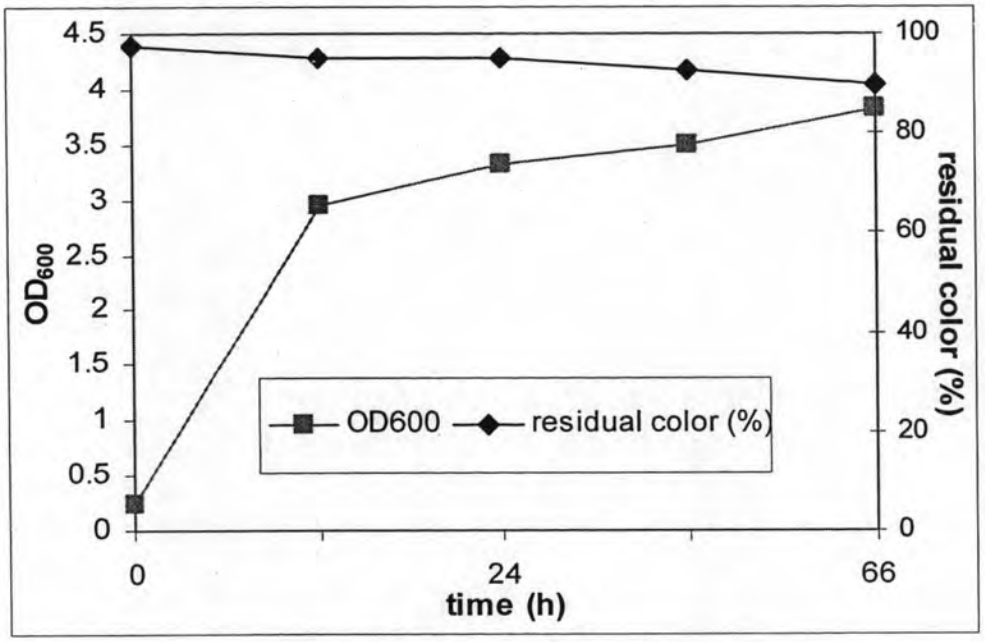

Figure 8.8 Growth and residual color of synthetic melanoidins-containing wastewater medium prepared by using DDW supplemented with trace elements at different incubation times.

In this chapter, the limitation of decolorization efficiency of bacterial consortium MMP1 was investigated. These results indicated that the nutrient supplement did not show significant effect on melanoidins decolorization of bacterial consortium MMP1, the addition of 0.5% (w/v) LB had supplementary effect so that the growth of bacterial consortium had a much higher than used culture medium without nutrient supplementation.

The effect of trace elements on decolorization of bacterial consortium MMP1 in various synthetic melanoidins-containing wastewater media has been investigated, and such results would be required when selecting water sources for the preparation of culture medium at different places in the world. The data indicated that the impact of understanding the nutrients required for melanoidin decolorization could be useful to optimize bacterial efficiency for the proposed treatment of melanoidins-containing wastewaters.