CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effect of ammonium sources on dye decolorization

Nitrogen is a macro nutrient and necessary for synthesis of proteins that play several roles in cell metabolism, especially, in this study, ligninolytic enzymes, which are an important factor in dye degradation. Therefore, nitrogen is the main parameter that should be considered. In general knowledge of microbial physiology, easiest available inorganic nitrogen source is ammonium followed by nitrate. Furthermore, in preliminary comparative study in effect of ammonium and nitrate salts on dye decolorization found that ammonium salts were able to induce rapid dye decolorization of the RC3 strain more than nitrate salts (data not show).

When considering on ammonium salts, dye decolorization rates are shown in Figure 4.1. Ammonium salt inducing the highest decolorization rate was ammonium oxalate, maximum decolorization (97-98% of decreased dye intensity when compared with pure water) could be obtained in 60 hours, however, in all other treatments could give a maximum decolorization within 84 hours including the control (no addition of any ammonium salts).

Oxalate was reported to involve in ligninolytic activities such as chelating unstable manganese ion and providing hydrogen peroxide (Jarosz-Wilkolazka and Gradd, 2003; Makela *et al*., 2002) and these enzymes were assumed to involve in dye decolorization (Duran and Esposito, 2000), then it was a best source giving highest decolorization rate.

Figure 4.2 show the pH change during decolorization, pH in the group of organic salts (ammonium oxalate, tartrate, and citrate) and control are slightly changed, while those of inorganic salts were markedly changed. This might be the result from high buffering capacity of organic salts, then pH of the culture broth altered from 6.0 to 3.5-4.5 while those of inorganic salts decreased to about 3.0. In control experiment, nitrogen limitation caused a low pH change that might be caused from low production of organic acid metabolites of the fungus.

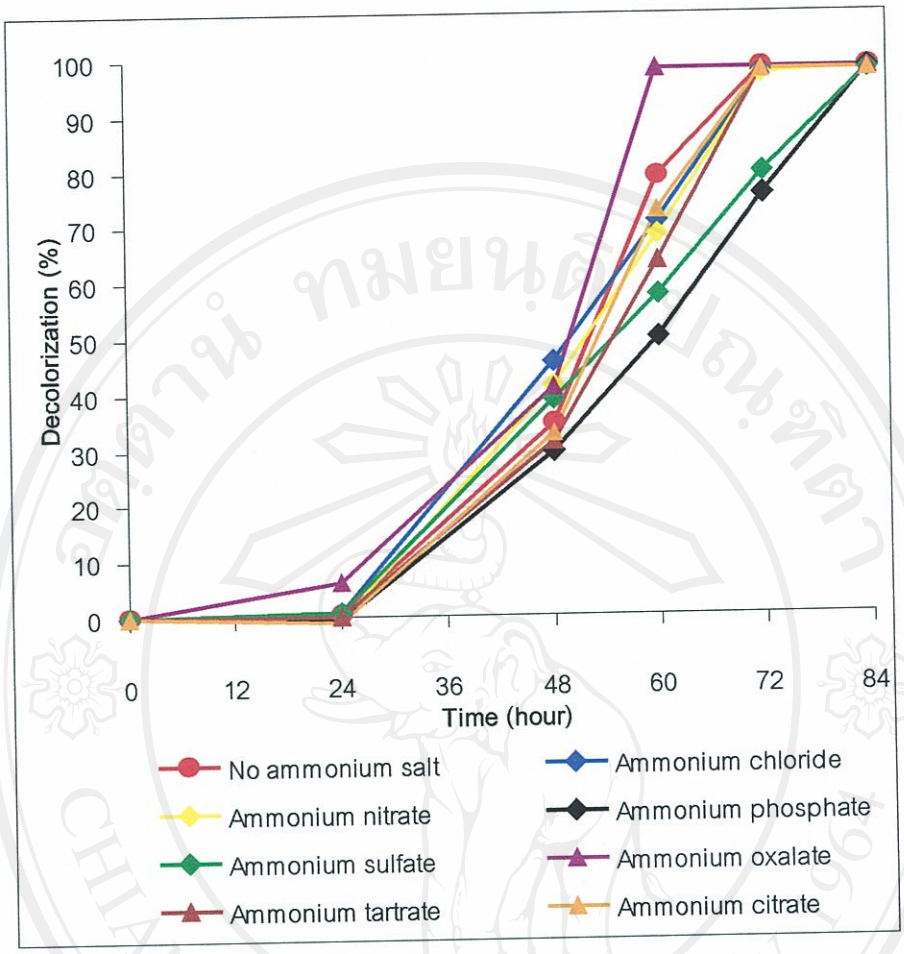

Figure 4.1 Decolorization of Orange II from various ammonium salts

Final biomass production (Figure 4.3), smallest biomass was obtained from the control but not significant different from the group of inorganic salts ($p > 0.05$). In case of control group, it possibly caused from the availability of other complex source of nitrogen as yeast extract. Addition of 0.04 g/l of ammonium ion was assumed to be a very low level and did not cause the difference between the treatment of inorganic salts addition and control unit. However, the group of organic salts gave a highest biomass yield, it was possible from effect of pH stability in the culture broth (Figure 4.2) and the additional carbon sources from organic anion; oxalate, tartrate, and citrate.

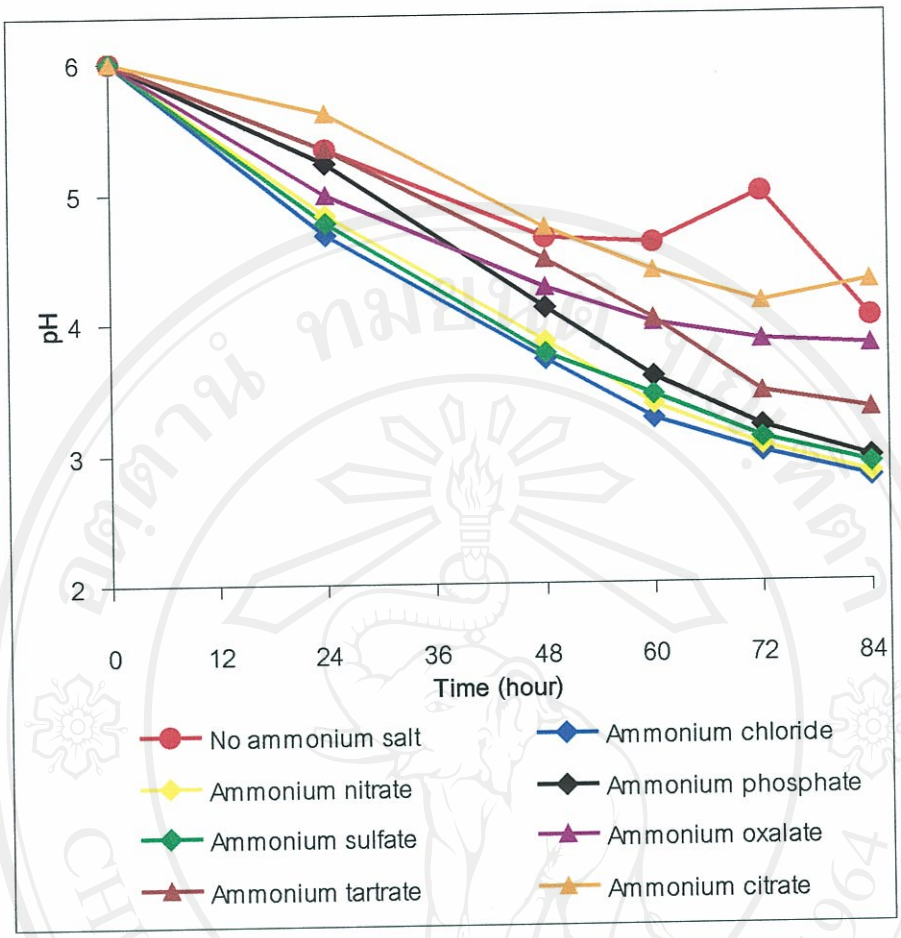

Figure 4.2 pH change during decolorization from various ammonium salts

4.2 Effect of glucose concentration on dye decolorization

Carbon sources are important to microbial growth especially fungi that can not fix carbon dioxide from atmosphere like plant, they need carbon sources from organic substances. In this study, dye consisting of hydrocarbon structure can be a carbon source for *C. versicolor* (Swamy and Ramsay, 1999). However, the complex structure of dye is difficult to degrade and addition of dye for carbon source is not enough. It is necessary to add other carbon sources in the culture broth to improve carbon availability and result the growing fungus (short lag phase) producing high activity of ligninolytic enzymes and, finally, the dye can be degraded easier. In this study, the easily available carbon source glucose was used.

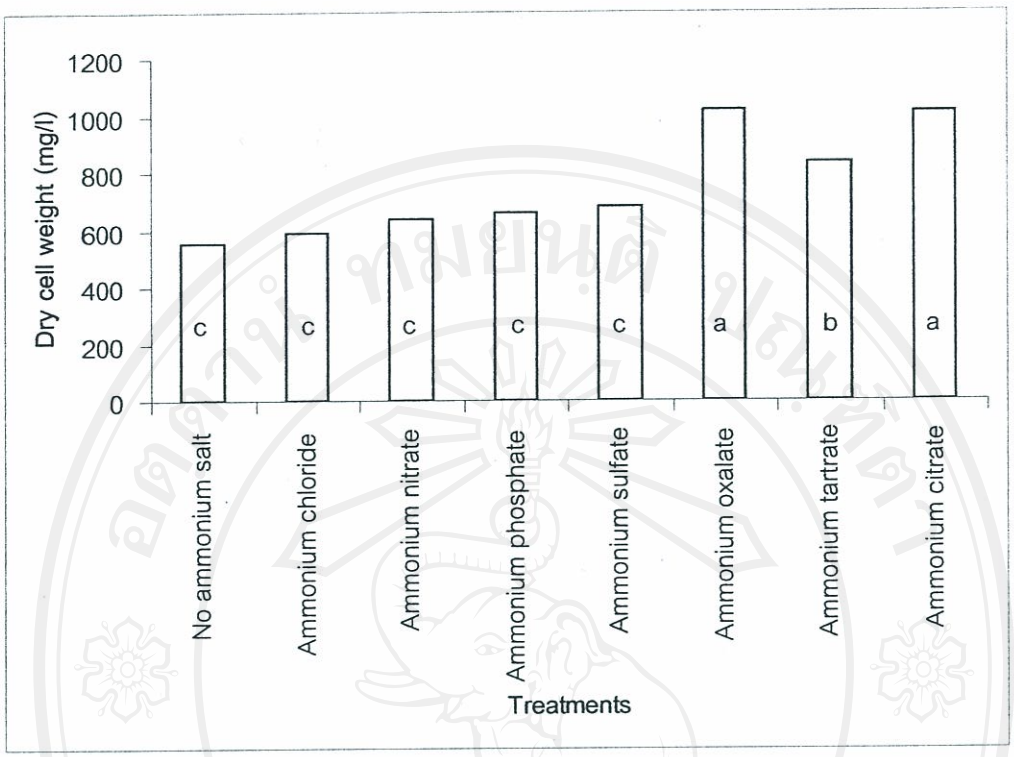

Figure 4.3 Final biomass from various ammonium salts

Different glucose level in the culture broth was investigated, the results showed that 1 g/l of glucose was a minimum level that could maintain a satisfying decolorization, maximum decolorization could be obtained in 72 and 60 hours under nitrogen limited and nitrogen rich condition, respectively (Figure 4.4a and b). In control experiment, no addition of glucose was resulted a poor decolorization rate in both condition of nitrogen availability.

Fu and Viraraghavan (2001) reviewed that good carbon sources for white rot fungi in decolorization purpose were glucose, starch, and maltose. Five g/l of glucose was the most suitable concentration. Corresponding with this study, the best minimum glucose concentration resulting the highest decolorization rate was 5 g/l in nitrogen rich condition (see on 48 hours, Figure 4.4b)

Consideration on the final biomass (Figure 4.5), production of dry cell was limited about 500 mg/l under the nitrogen limited condition, while that was increased up to more than 1,700 mg/l in the nitrogen rich condition when glucose concentration was increased.

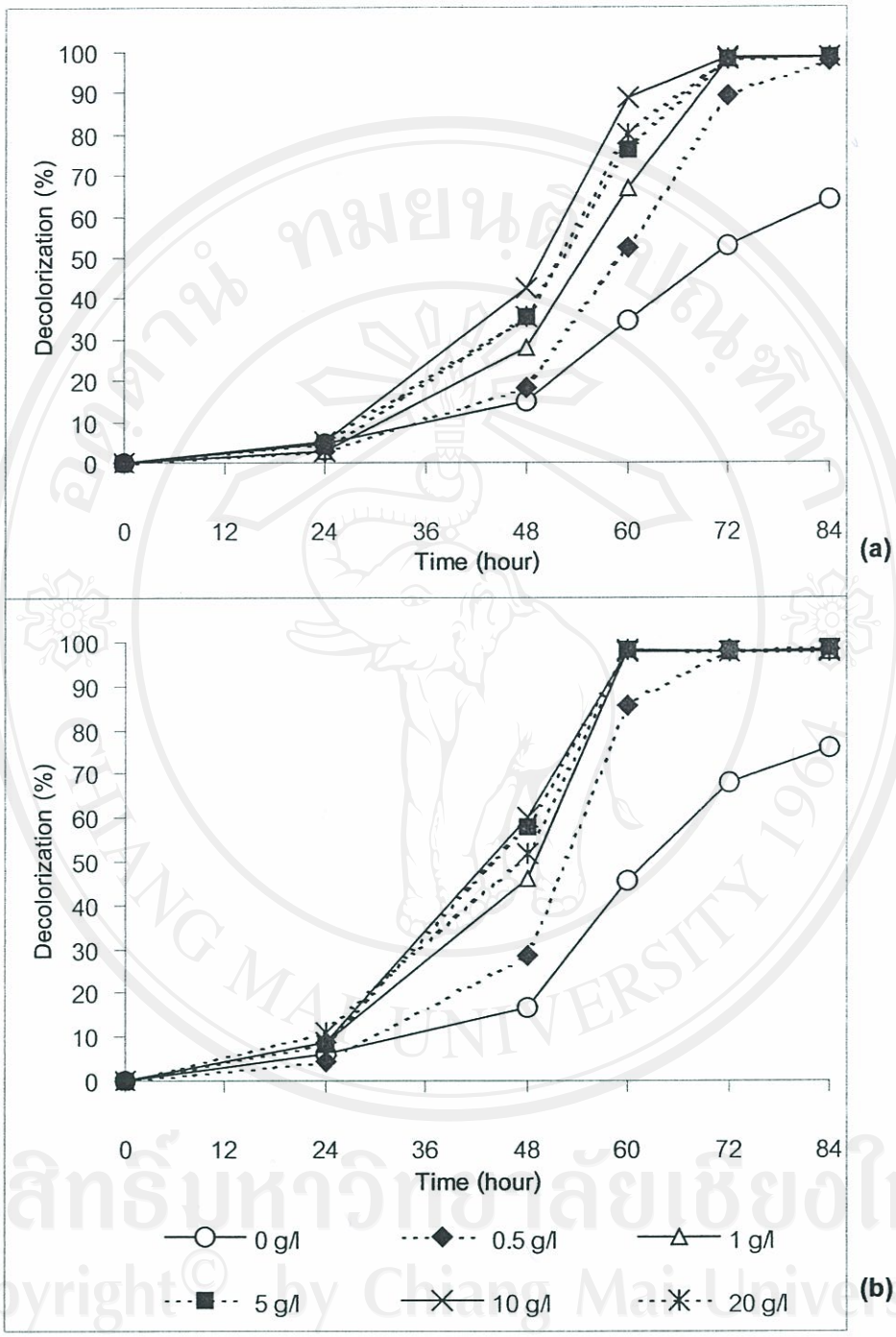

Figure 4.4 Decolorization of Orange II from various glucose concentration

(a) Nitrogen limited condition

(b) Nitrogen rich condition

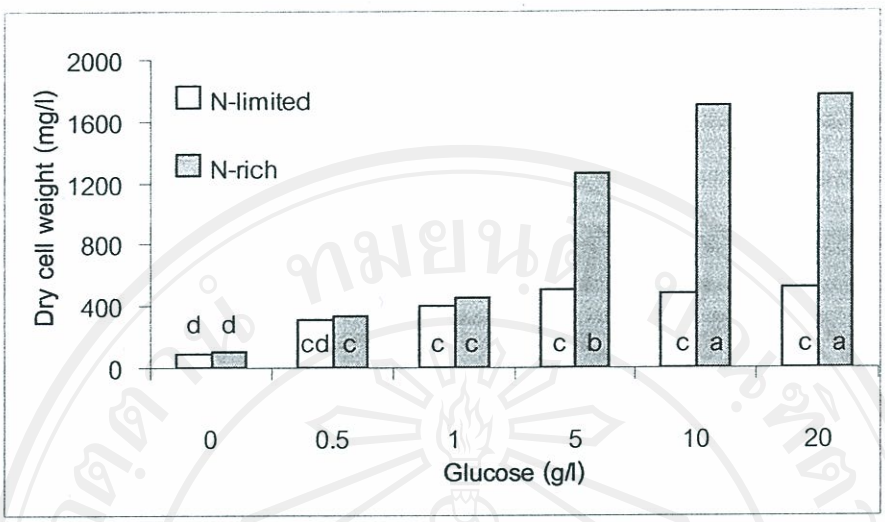

Figure 4.5 Final biomass from various glucose concentrations

Although the nitrogen rich condition with 5 g/l of glucose concentration was the best one in nitrogen rich condition and resulted the overall decolorization rate higher than the nitrogen limited condition, however low production of biomass is more suitable for using in continuous decolorization mode because high biomass production can easily clog the reactor (Lopez *et al.*, 2002). To avoid that problem 1 g/l of glucose in both nitrogen limited and nitrogen rich condition were selected and used in next experiment.

4.3 Effect of nitrogen concentration on dye decolorization

Microorganisms mostly require nitrogen, following to carbon. Nitrogen containing dyes can be utilized as nitrogen sources. Nitrogen will be liberated from dyes during ligninolytic processes and azo chromophore can be degraded by ligninolytic enzymes (Knapp *et al.*, 2001). Early research on ligninolysis by *Phanerochaete chrysosporium* showed that active ligninolysis was much more effective in the conditions of nitrogen limitation often associated with secondary metabolism (Knapp *et al.*, 2001). Accordingly, most early research on degradation of xenobiotics by white rot fungi used nitrogen limited condition. It is now clear that other fungi differ markedly from *P. chrysosporium.* As reason above, the suitable concentration of nitrogen for the RC3 strain have to be studied. In

addition, dyes are recalcitrant molecules, it is necessary to supply another easily available nitrogen source for fungal growth especially for decreasing of lag phase.

Effects of nitrogen concentration on dye decolorization are shown in Figure 4.6. Without addition of glucose (Figure 4.6a), all treatments showed a poor decolorization rate. Figure 4.6b showed the decolorization with addition of 1 g/l glucose, the fastest decolorization rate was obtained after 0.2 g/l ammonium oxalate was added (maximum at 60 hours). Additionally, 2 g/l ammonium oxalate resulted the slowest rate in 1 g/l glucose added condition (maximum at 84 hours). In the control, no addition of any ammonium salts, the maximum of decolorization occurred at 72 hours.

However, in the treatment of no ammonium salts supplementation, the maximum of decolorization was slower than that of 0.2 g/l ammonium oxalate just only 12 hours. When continuous decolorization mode was employed, there would be excess of active mycelia in the reactor. In addition no supplementation of nitrogen could be a factor of cost reducing of the treatment, then the condition of nitrogen limitation is attractive choice and it was selected to perform in next experiment.

For final biomass yield (Figure 4.7), in the condition of no glucose addition, the biomass yield was very low caused from lack of carbon source. While in the condition of 1 g/l glucose addition when increased nitrogen concentration the biomass could not increase because of glucose limitation at a level of 1 g/l.

4.4 Effect of initial pH on dye decolorization

The culture pH is an important factor for microbial growth as enzyme activities and nutrients availability are affected by pH. In general, fungi usually grow in acidic pH range about 5.0-5.5, however, in previous study, the RC3 strain produced acidic metabolites during the incubation period resulting the high acidity of culture broth that final pH could be detected lower than 3.0 in nutrient rich condition. The initial pH adjustment should be studied under the hypothesis that higher initial pH value may provide a longer period of growth, while too high initial pH value inhibit the fungal growth.

Effect of initial pH on dye decolorization is shown in Figure 4.8. At 60 hours, the fastest decolorization rate could be observed from initial pH 6.5, while maximum decolorization obtained from the initial pH 6.0 and 6.5 at 72 hours. The initial pH 6.5 was selected as the best for decolorization by this fungal strain.

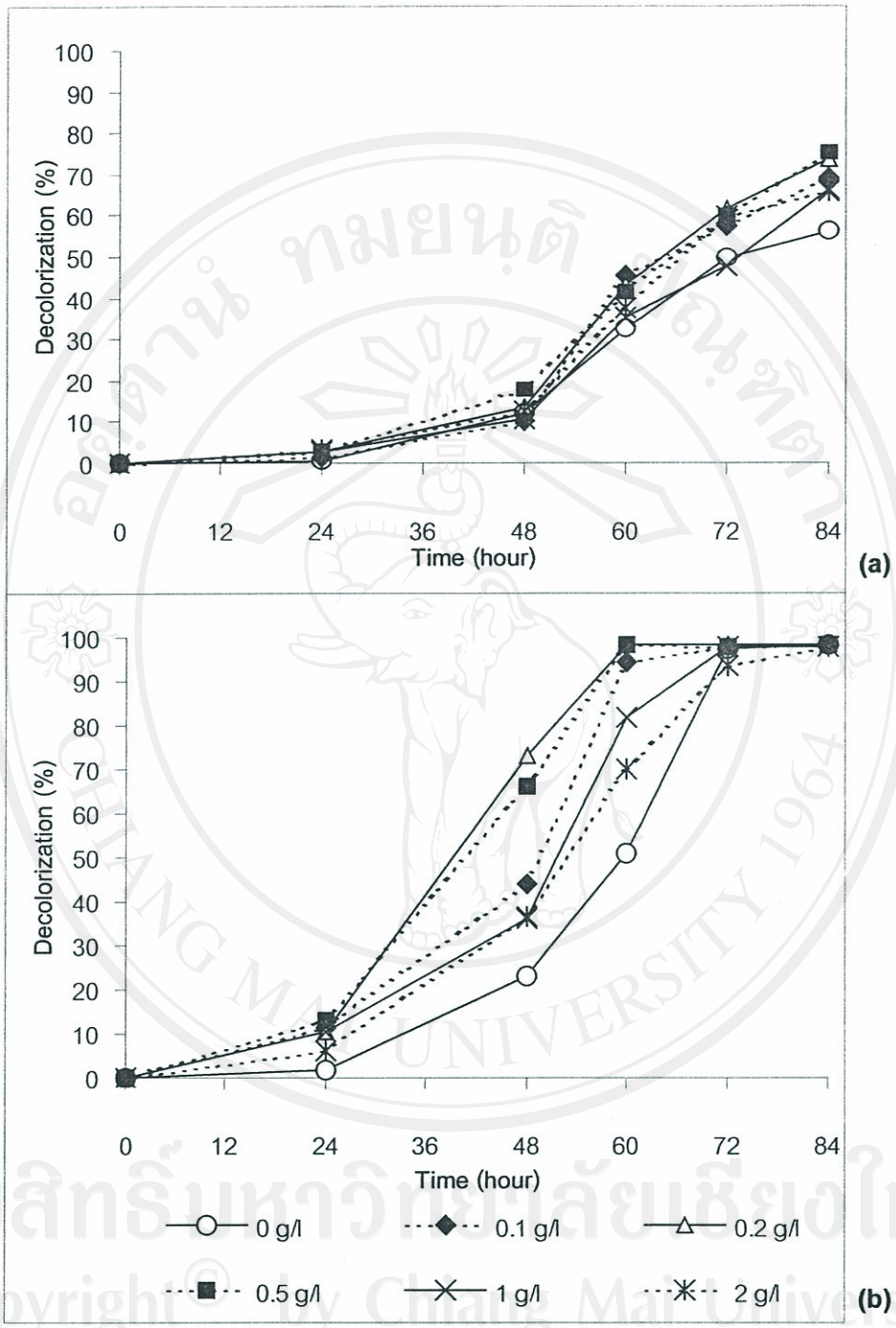

Figure 4.6 Decolorization of Orange II from various nitrogen concentration

(a) Without glucose

(b) With 1 g/l glucose

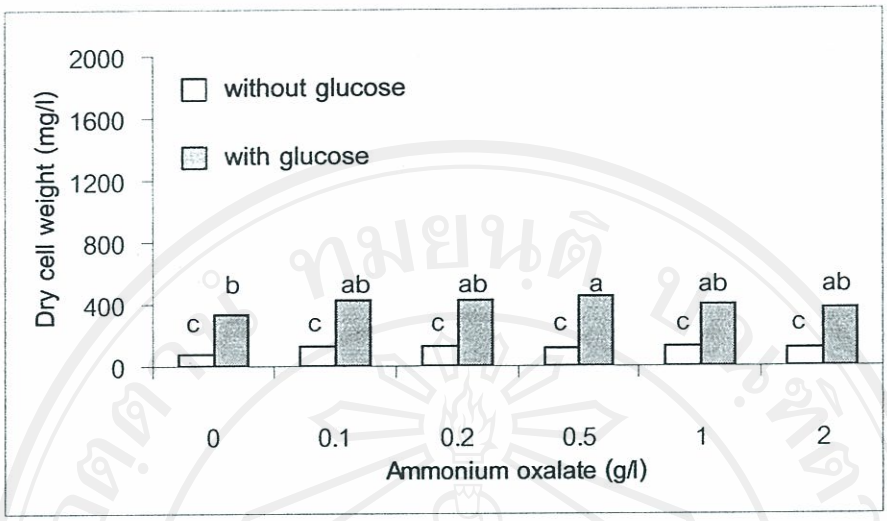

Figure 4.7 Final biomass from various nitrogen concentration

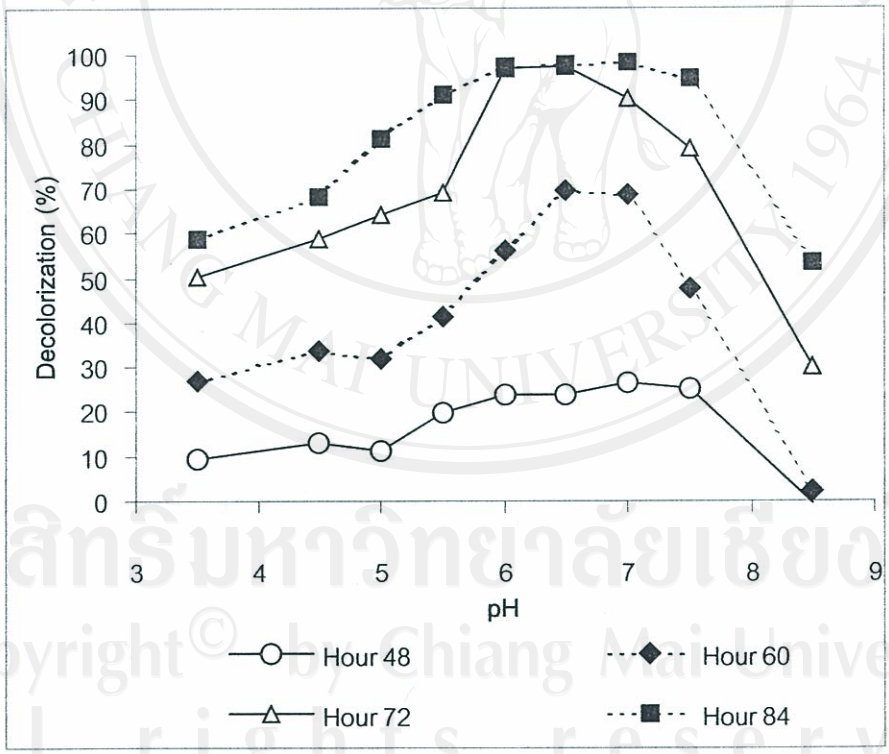

Figure 4.8 Decolorization of Orange II from various initial pH

Knapp *et al*. (2001) reported that decolorization of Orange II and cotton bleaching effluent has been shown to depend on initial pH. Optimal decolorization could obtain in non-excessive pH range (5.5-7.0). At high pH range (usually > 7), decolorization will not occur or it will only occur when pH is reduced to the optimal region and low initial pH can also disadvantageous.

The pH of all treatments dropped down to about 3.5-4.0 (Figure 4.9). When fungi grew in carbohydrate containing media generally caused acidification of the medium, the extent of which depends on the carbon source and the type and amount of buffering present. Acidity in the media derived from production of organic acids by the fungi, it is likely that fungi may produce their own buffering. However, later in the growth of the culture, utilization of accumulated acids may lead to a loss of buffering and further pH changes (Knapp *et al*., 2001).

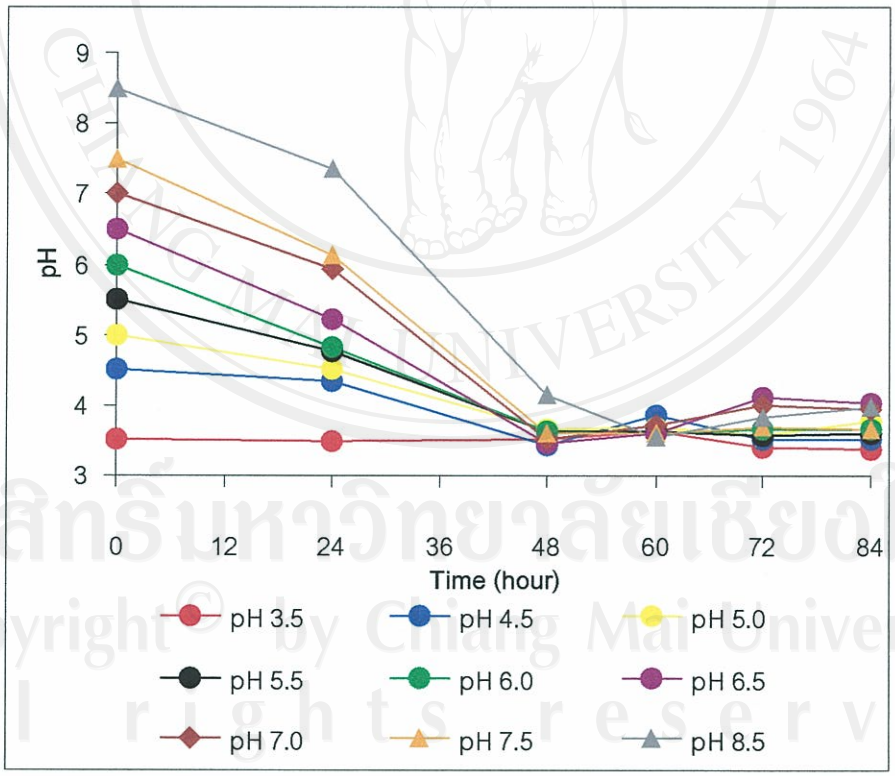

Figure 4.9 pH change during decolorization from various initial pH

Final biomass production is shown in Figure 4.10, the initial pH from 3.5-7.5 gave a same level of biomass yield indicating that the fungus can grow well in a wide range of pH while the initial pH 8.5 showed the slowest growth rate.

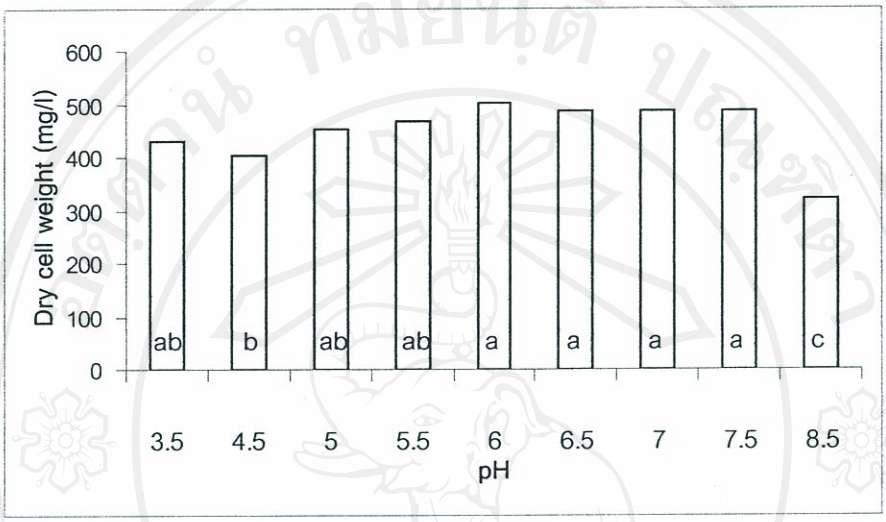

Figure 4.10 Final biomass from various initial pH

4.5 Effect of ambient temperature on dye decolorization

Different microbes show a different optimal temperature of growth and other activity. Most white rot fungi are mesophiles having optimal temperature at 27-30°C. In previous study, the RC3 strain well grew and decolorized the dye at 37°C, however, the effect of various ambient temperature on dye decolorization had to be studied.

The result of ambient temperature effect was demonstrated in Figure 4.11. The decolorization in various ambient temperatures was monitored at 48, 60 and 72 hours. It was found that the treatment of 30°C showed the highest decolorization rate. However, at 84 hours of cultivation, the decolorization of all treatment including 30, 35 and 37°C showed almost the same level. This indicated that ambient temperature in the range of 30-37°C could reach the same decolorization when incubation time was at least 84 hours. Moreover, at 40°C the decolorization activity still was found, however, there was no decolorization obtained at 45°C. This might be caused from the temperature was too high

over the metabolism limit of the fungus. Previously report was also concluded the optimal temperature of decolorization by *Coriolus* sp. was 35°C (Fu and Viraraghavan, 2001).

The main advantage of microorganisms having high optimal temperature does not lie in more rapid catalysis but in the fact that in large scale bioreactors removal of excess metalbolic heat will present less of a problem at a higher temperature, cooling costs will be less (Knapp *et al.*, 2001).

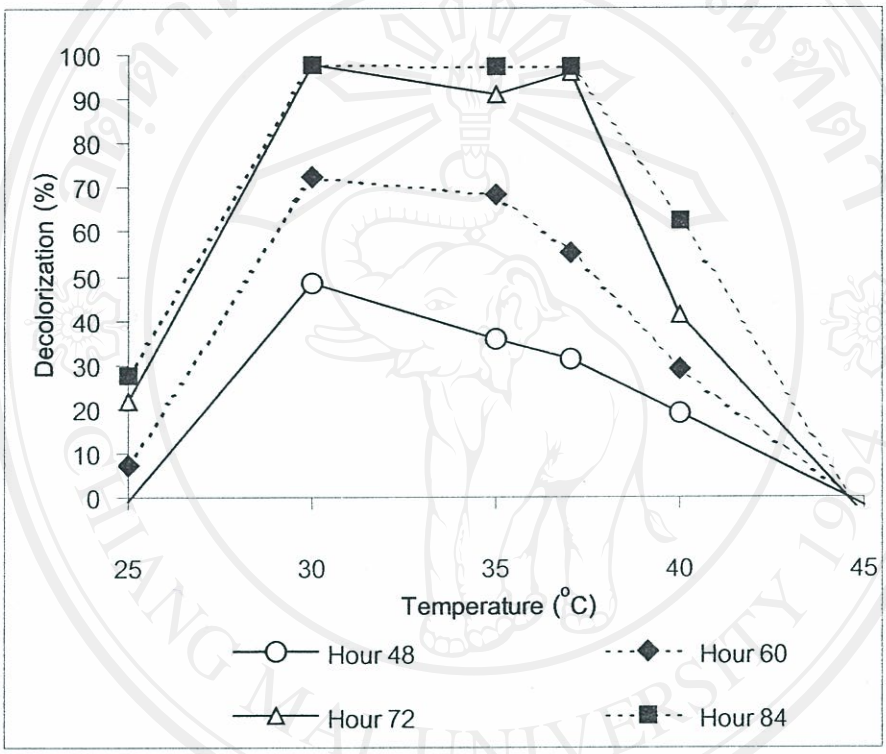

Figure 4.11 Decolorization of Orange II from various ambient temperature

About the biomass production, the temperature provided a highest final biomass yield was 37°C followed by 35°C (Figure 4.12). At 25, 30 and 40°C, the fungus showed a low level of biomass production and that was found in very low level at 45°C. This indicated that optimal temperature for growth of the RC3 strain was 37°C and it could be confirmed the thermotolerant property of the RC3 strain compared to other white rot fungi.

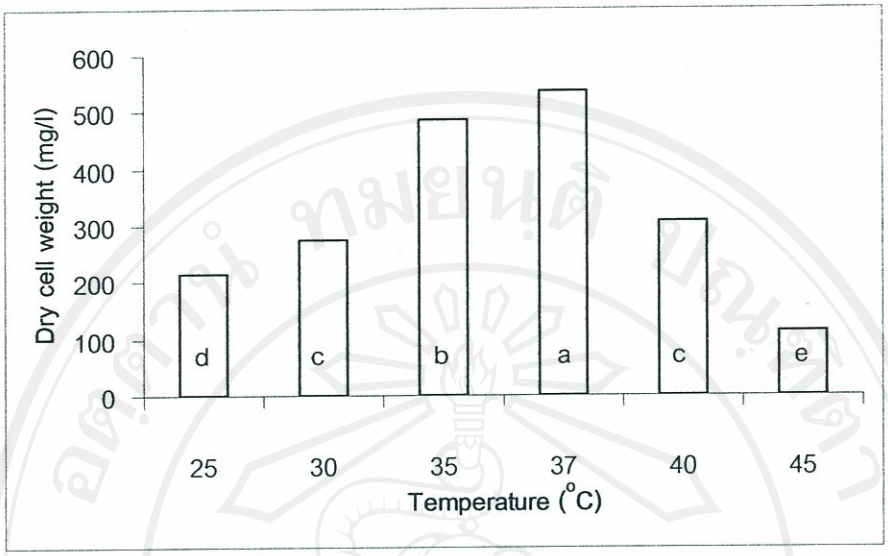

Figure 4.12 Final biomass from various ambient temperature

4.6 Monitoring of ligninolytic enzymes production during dye decolorization

As the literature review, ligninolytic enzymes are key points in decolorization mechanism and *Coriolus versicolor* in many reports were candidate sources for laccase production. Ligninolytic enzyme production during decolorization from this study found that the maximum decolorization obtained within between 72-84 hours (Figure 4.13a). A flask on the top of the triangle is control unit and the five ones below are experimental units. In control unit, no inoculation caused a non-decolorization. The average values from five replications are plotted in Figure 4.13b. The major ligninolytic enzyme produced by the RC3 strain was laccase and the increasing of enzyme activity corresponded with the increasing of decolorization percentage. The highest activity of laccase was found about 5-6 mU/ml at 84 hours. For other ligninolytic enzymes including manganese independent peroxidase and manganese peroxidase were found at very low level and lignin peroxidase could not be detected. pH of the culture broth dropped from 6.5 to about 4.0 at the end of the experiment.

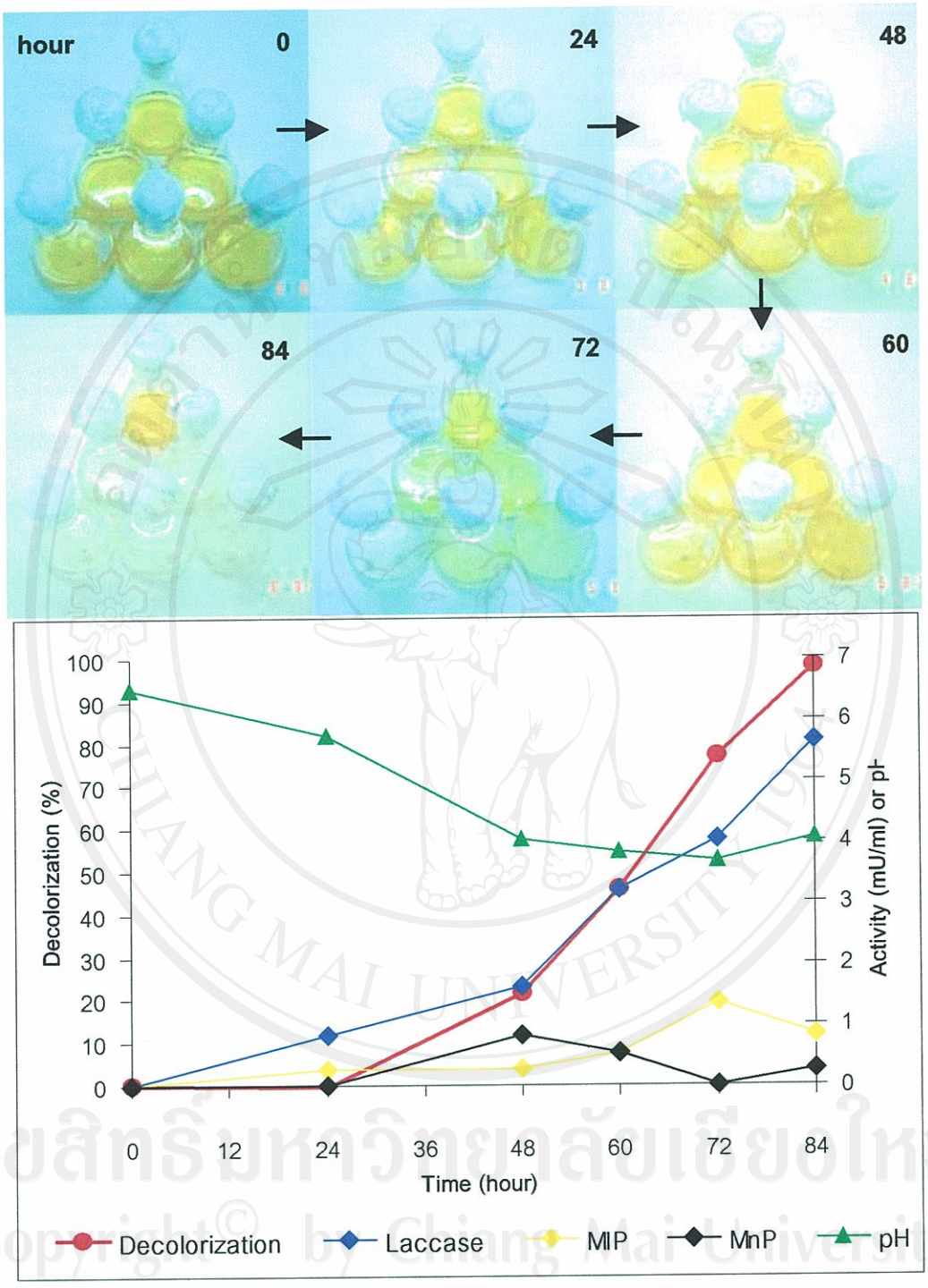

Figure 4.13 Ligninolytic enzymes production during Orange II decolorization

(a) Decolorization feature

(b) Ligninolytic enzymes production

4.7 Effect of polyurethane foam volume and incubation time on cell immobilization

As we know that most fungi favor to grow on solid state media, in this condition they can highly produce biomass and other products. However, Fungi can also grow in submerge culture. In this study, polyurethane foam (PUF) volume was varied for finding out the optimal ratio between PUF and medium broth volume that promote a highest cell density of *C. versicolor* RC3 on PUF. This optimal colonized PUF will be used in packed bed bioreactor in next experiments.

Figure 4.14a show cell density in PUF incubated in various volume of PUF and incubation time. The result showed that a small amount of PUF used promoted the high cell density because a large proportion between biomass and mass of PUF. However, the result showed an absolute amount of biomass yield in PUF per litter of the medium broth (Figure 4.14b). The treatment of 0.5 g PUF showed a lowest biomass yield, while other treatments showed a same level of higher biomass including the treatment of free cell (no PUF).

A best suitable immobilization condition was 1 g PUF per 50 ml of medium broth with 4 days of incubation. That obtaining cell density up to 200 g/kg PUF, which more higher than other two treatments (1.5 and 2.0 g PUF; Figure 4.14a) and the absolute biomass was about 4 g/l of culture broth that was not different from other three treatments below (Figure 4.14b). Proportion between PUF and culture broth used in other researchers were 1:45 w/v (Mielgo *et al.*, 2002 and Mielgo *et al.*, 2001), 1:55.5 w/v (Feijoo *et al.*, 1995) and 1:66.67 w/v (Kasinath *et al.*, 2003). However, those used different incubation condition and did not report about biomass yield.

In the treatment of 0.5 g PUF, after 3 days of incubation, the cell density in PUF and absolute biomass yield tend to be decreased when the incubation time was increased. This might be the result from the thick mycelial plate formed by mycelial growth over the top surface of PUF. Those mycelial plate would be easily washed out in washing process resulting a decreasing of retaining biomass (Figure 4.15; from left to right; 0.0, 0.5, 1.0, 1.5, and 2.0 g of PUF used, respectively and incubating for 4 days).

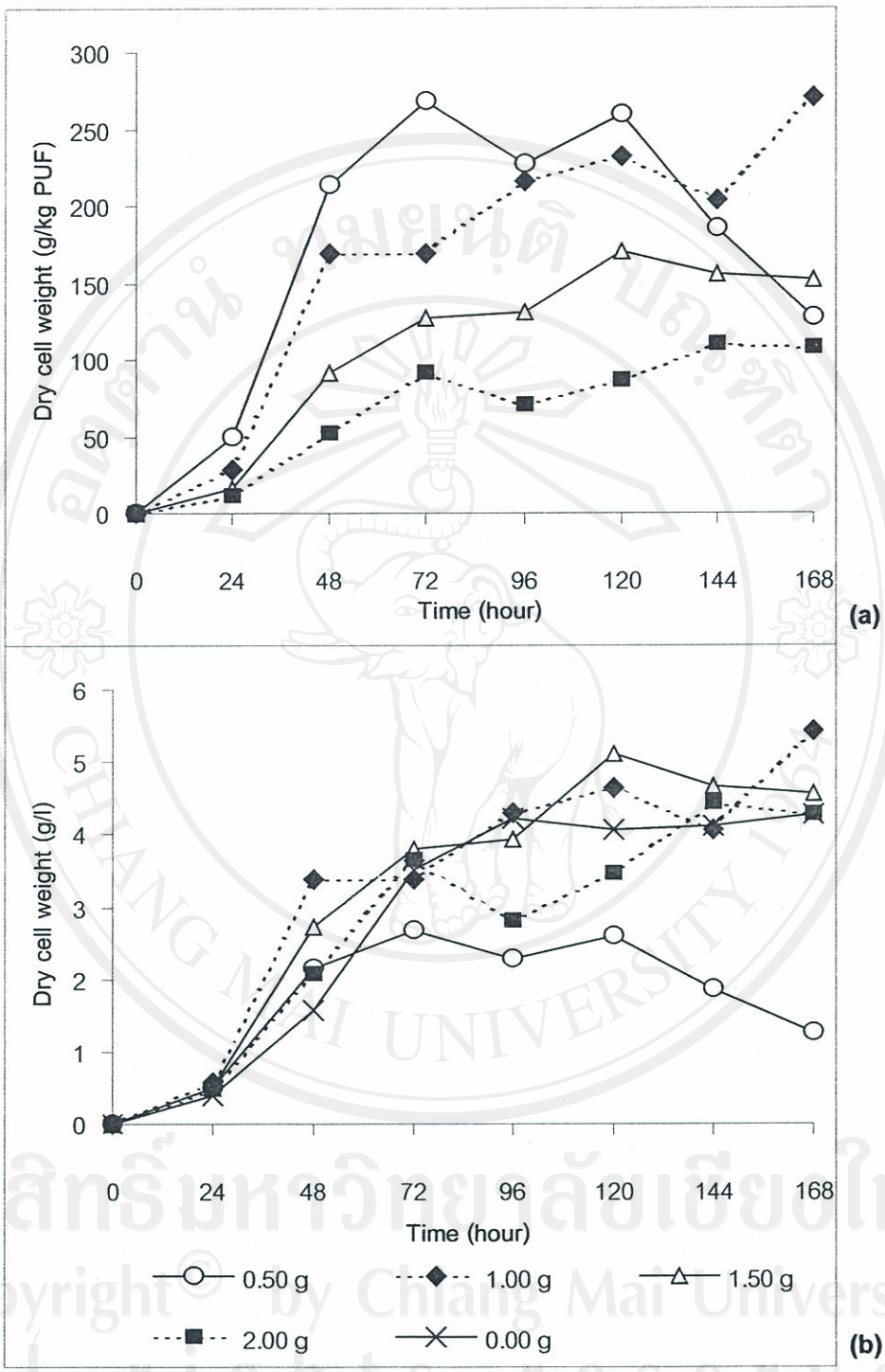

Figure 4.14 Immobilization of RC3 strain on various mass (g) of polyurethane foam

(a) Cell density on polyurethane foam at various incubation time

(b) Biomass yield in culture broth at various incubation time

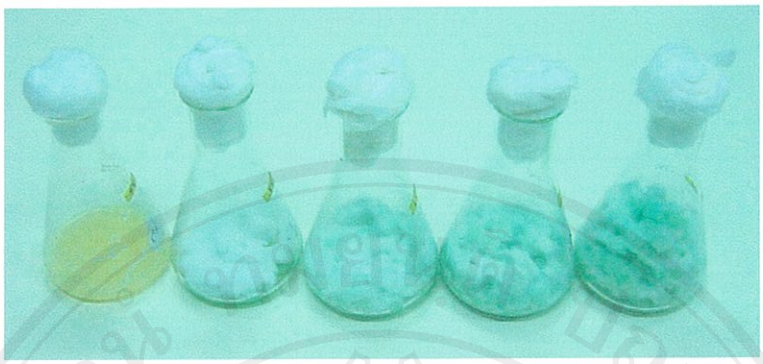

Figure 4.15 Colonization of RC3 strain on various polyurethane foam volume

4.8 Packed bed bioreactor design and configuration

A complete construction of packed bed bioreactor was followed a previous design and the operated setting was shown in Figure 4.16.

Figure 4.16 Packed bed bioreactor used in this study

4.9 Warming-up time of bioreactor

In the common sense of wastewater treatments, before releasing treated wastewater from any treatment plants, those should be in an acceptable condition. It is impossible that microorganisms in treatment plants can attack the wastes immediately after wastes influents were loaded into the plants. Adaptation to new environment is necessary for microorganisms. In this experiment, the PUF immobilized RC3 strain in packed bed bioreactor was filled by Orange II dye until reaching desired level. If the effluent were released immediately, the untreated dye would be passed through the reactor in large quantity. Therefore warming-up time of bioreactor before continuous feeding should be considered as one factor because the fungus and its ligninolytic enzymes need a period for adaptation to new substrate and environment as described above.

It was found that after the PUF immobilized RC3 strain in bioreactor was filled by the medium, more than 90% of decolorization could be obtained just 5 hours of incubation period and the pH of the broth was dropped from 5.3 to 3.9 (Figure 4.17). This result exhibited the high decolorization rate that might be caused from high density of immobilized fungal biomass in polyurethane foam.

4.10 Effect of hydraulic retention times on dye decolorization

Hydraulic retention time (HRT) indicate duration period of wastewater that will be retained in reactor system, it derive from proportion between working volume divided by hydraulic flow rate. Suitable HRT will provide a suitable reaction time between ligninolytic enzymes and dye molecules, which directly affect on efficiency of the reactor system.

The result from Figure 4.18 demonstrated continuous decolorization of Orange II with different HRT. It showed that at 5, 6 and 8 hours of HRT more than 90% decolorization could be obtained about 36, 72 and 120 hours of duration, respectively, and more than 80% decolorization could be obtained about 48, 96 and 156 hours of duration, respectively. At 12 hours of HRT, maximum decolorization could be achieved over a week indicating the corresponding of increased HRT with the increasing of decolorization duration.

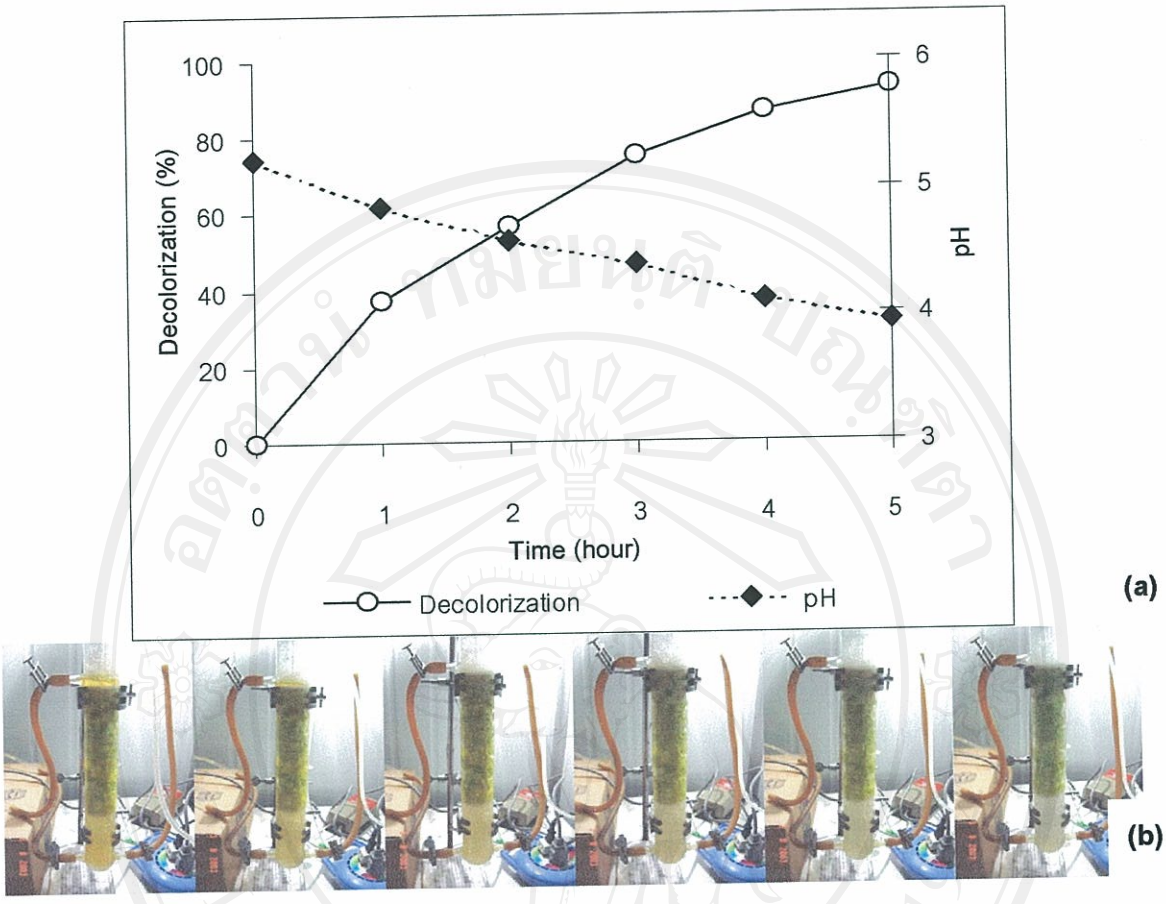

Figure 4.17 Decolorization in warming-up period

(a) Relation decolorization and pH change

(b) Decolorization feature from 0 to 5 hours, left to right

At low HRT or high medium flow rate directly affected to a stability of the system and ligninolytic enzymes are possible to be washed out easily. In the other hand, high HRT could maintain a long decolorization ability of the system, however, the reactor would be clogged by growth of the mycelia causing a decreasing of aerated space in the reactor. Although there were ligninolytic enzymes in the reactor, but oxygen involving in electron acceptor was insufficient resulting a poor decolorization subsequently. Average pH of broth during continuous decolorization was 3.5. Efficiency in each HRT is shown in Table 4.1, within a week, 8 hours of HRT gave the highest volume of treated broth.

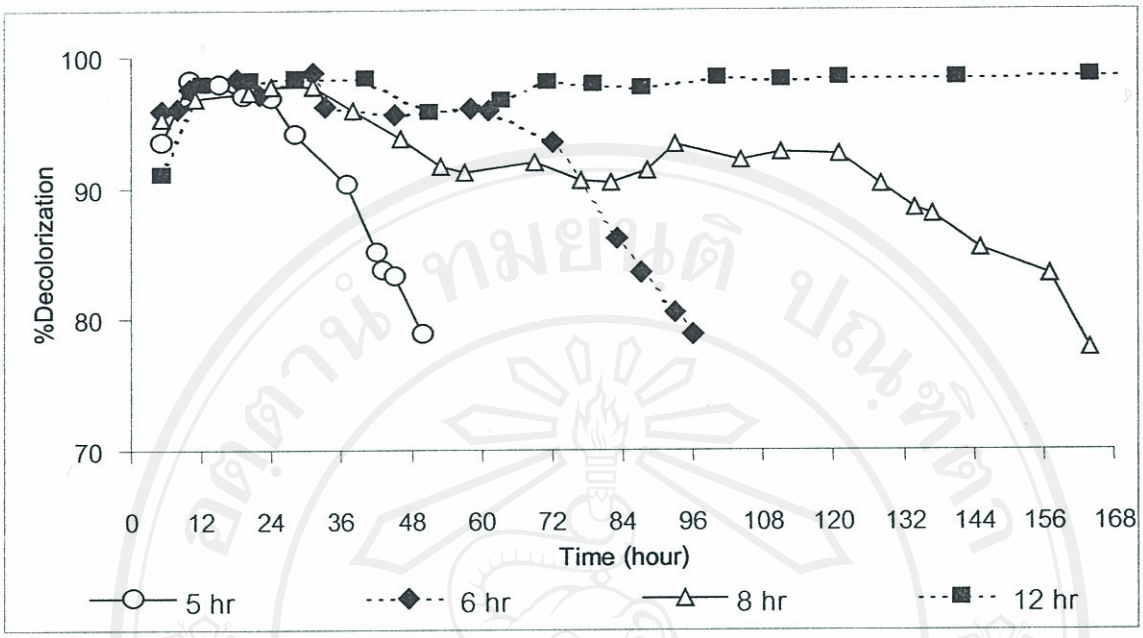

Figure 4.18 Continuous decolorization from various hydraulic retention time

Table 4.1 Comparison of treating efficiency in various HRT

HRT (hr)	Duration of >90% decolorization (hr)	Treated volume (ml)	Duration of >80% decolorization (hr)	Treated volume (ml)
5	36	1,440	48	1,920
6	72	2,376	96	3,168
8	120	3,000	156	3,900
12	>168	>2,772	>168	>2,772

4.11 Effect of polyurethane foam sizes on dye decolorization

Disadvantage of various packed bed bioreactors is clogging problem caused from growth of the packed cell. It is possible to avoid this problem, if bed density was improved. In this study, density of the packed bed was varied and the visualized reactor was compared as in Figure 4.19. When PUF size was increased, smaller weight of PUF

could be filled into the reactor. Applied air could pass through the reactor easier and longer duration in low bed density while the immobilized cell could grow in long period without clogging.

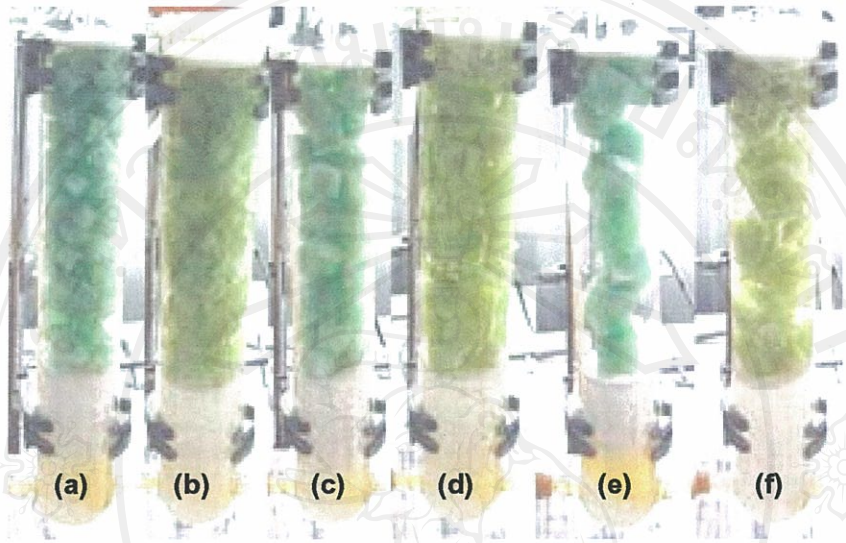

Figure 4.19 Comparison of bed density in packed bed bioreactor

(a) 1 cm^3 2.0 g PUF

(b) submerged 1 cm^3 2.0 g PUF

(c) 1.5 cm^3 1.75 g PUF

(d) submerged 1.5 cm^3 1.75 g PUF

(e) 2 cm^3 1.5 g PUF

(f) submerged 2 cm^3 1.5 g PUF

The results of decolorization and laccase activity are shown in Figure 4.20. Decolorization in a treatment of 1 cm^3 PUF showed a same pattern as the previous result (4.10), more than 90% and 80% of dye removal could be obtain within 156 and 192 hours, respectively. High cell density was found at 288 hours for 1.5 cm^3 PUF and affected to airflow in the reactor that obtained the fluctuation in decolorization. This could be suggested that ligninolytic enzyme strictly require oxygen in the reaction. The reactor was clogged by the mycelium completely at 360 hours, approximately, leading to the problem of air supplier, as air could not pass though glass bead zone of the reactor (see

also in Appendix E). For 2 cm^3 PUF, decolorization in first period (24-72 hours) was poor from high free space in the reactor. The large amount of dye could be loaded to the reactor, until 84 hours, the RC3 strain colonized in higher level provided a good stability of decolorization. At 300 hours, decolorization tended to be increased to 99% decolorization. This was explained as the causing from the increase of working area from mycelium colonization, that extended to glass bead zone including rubber tubes both influent and effluent vessels. However, the effluent port of the reactor was clogged by the mycelium at 360 hours and the culture broth could not pass through out the reactor (see also in Appendix E).

Laccase activity found in all three treatments showed a same pattern. At initial period of cultivation, the activities increased sharply with the peaks of 20.16, 25 and 16.13 mU/ml for 1, 1.5 and 2 cm^3 PUF, respectively. High fertility of the culture broth remaining in immobilized PUF from immobilization process and inducer as loaded dye are suggested to be the reason of high level of laccase activity from the RC3 strain. Laccase activity in immobilized PUF was measured and found only about 1-2 mU/ml in each batch of immobilization before put into the reactor. It was produced in higher level in the presence of some inducer, in this experiment Orange II can be acted as an inducer for ligninolytic enzymes instead of lignin. After 20 hours, the activities tended to be decreased rapidly, it was possible caused from the replacing of dye added minimal medium washed the old laccase out from the reactor. After 48 hours of incubation, the average enzyme activities were stable at 3.02, 4.65 and 6.19 mU/ml for 1, 1.5 and 2 cm^3 PUF, respectively. It could be concluded that the average laccase activity was increased when increased PUF size. pH of the effluents were dropped from about 4.0 to 3.2 during continuous decolorization (Figure 4.2).

Comparison to the provious reports about size of PUF, 1 cm^3 of PUF size was used by Kasinath *et al.* (2003) and Feijoo *et al.* (1995). The others used 1.2 cm^3 PUF (Fujita *et al.*, 2000) and 1.5 cm^3 PUF (Zouari *et al.*, 2002). There was no report about reason for use of any specified size of PUF. From this study, 1.5 cm^3 PUF show a best suitable when compare with other treatments as the comparative detail in Table 4.2. At 1.5 cm^3 PUF, the mycelium could be well retained in the reactor as 1 cm^3 PUF and there was no clogging problem at effluent port and tube as found with 2 cm^3 PUF. Initial decolorization at 24-72 hours showed a same good result as 1 cm^3 PUF and better than that of 2 cm^3 PUF. It could be run in longer than 1 cm^3 PUF.

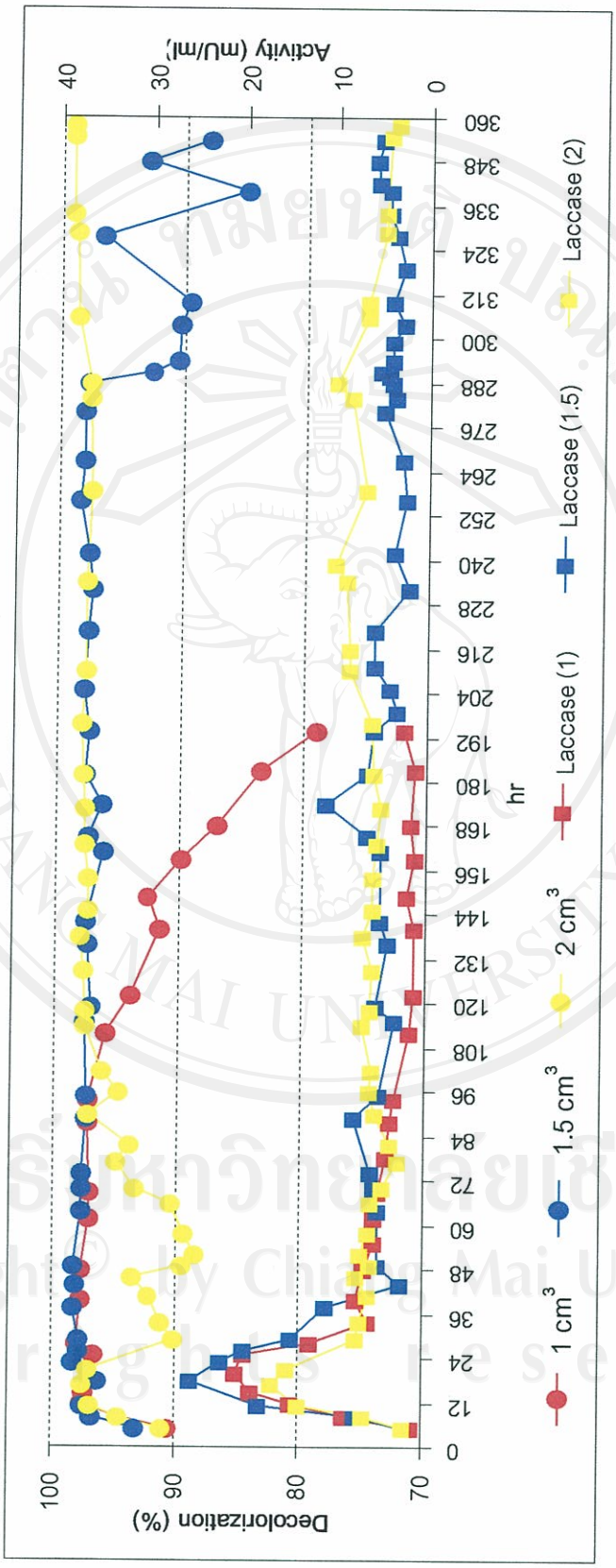

Figure 4.20 Decolorization and laccase production from various sizes of polyurethane foam

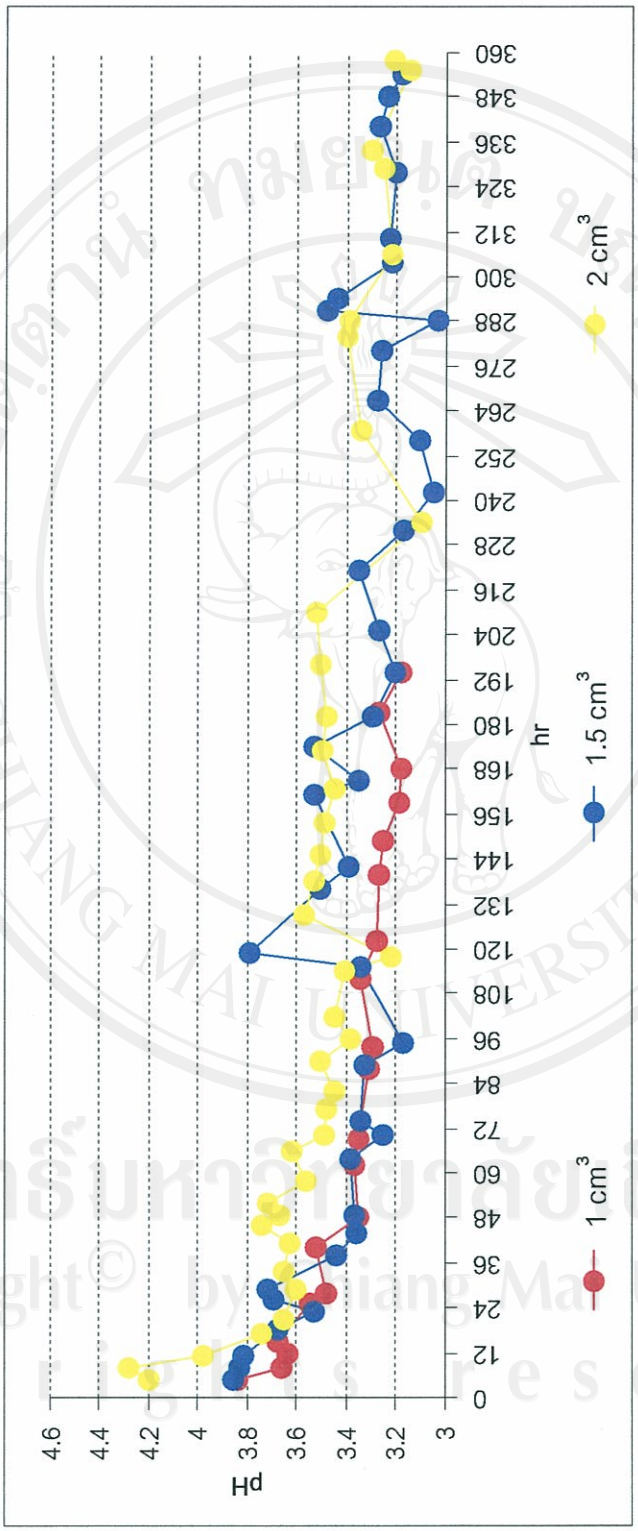

Figure 4.21 Change of effluent pH from various sizes of polyurethane foam

Table 4.2 Comparison of efficiency in three different size of PUF

Considerations	1 cm^3 PUF	1.5 cm^3 PUF	2 cm^3 PUF
Retention of mycelium in the reactor	Very good	Good	Poor There are mycelial's fragment released from the reactor through out the experiment, the effluent tube risked to clogging
Decolorization in first period	Very good	Very good	Poor The dye could be loaded to the reactor in large amount because low content of PUF and fungal cell in the reactor, this might be affected to decolorization efficiency until hour 84
Long duration decolorization	The reactor could clog easier then the other	Good	Good
Clogging position	PUF layer	Glass bead layer	Effluent port
Laccase activity (mU/ml), averaged from hour 48 to end	3.02	4.65	6.19

4.12 Effect of dye concentration on dye decolorization

It is necessary to find suitable dye concentration applied to the reactor. As 20 ppm Orange II was decided to used according to some other previous reports (Fu and Viraraghavan, 2001). In this study, the dye concentration was varied up to 50 and 100 ppm compared with previous study using the condition of 8 hour HRT and 1.5 cm^3 PUF. The result of decolorization was showed in Figure 4.22. Decolorization was markedly decreased when increased the dye concentration. The treatment of 50 ppm dye loading could be decolorized up to 98% decolorization in the first period (12-24 hours) corresponded with the maximum of laccase activity found in this period (Figure 4.23), and after that, decolorization was decreased. For 100 ppm dye loading, decolorization curve showed a peak at 20 hours, it was also the same peak of laccase activity (Figure 4.23), and after that, decolorization percentage was decreased rapidly. It was concluded that 50 and 100 ppm of dye loading were over a capacity level of the system. However, 8 hours of HRT was a very fast loading rate. Many researcher used 12-24 hours of HRT, and those reported that 50 and 100 ppm could be completely decolorized if the HRT of the system was increased (Knapp *et al.*, 2001; Mielgo *et al.*, 2001).

Laccase production showed a same pattern in all three treatments (Figure 4.23). The activity increased rapidly from hour 5 to hour 20 and decreased rapidly to stable consequently. This phenomenon was already discussed in 4.11.

When considering in absolute dye removal, it was found that the quantity of absolute dye removal was increased when increased dye concentration (Figure 4.24). In 20 ppm dye loading, It could be removed almost 20 ppm through out the experiment, while 50 ppm dye loading could be removed almost 50 ppm in first period of incubation and decreased corresponding with the increasing of incubation duration. Additionally, 100 ppm dye loading could be removed only 80 ppm of maximum in first period of incubation (at 20 hours) and the absolute dye removal decreased rapidly when increased incubation time. However, the effluent pH of all treatments were found to decrease from 3.9 and showed a same pattern like previous experiment (Figure 4.25).

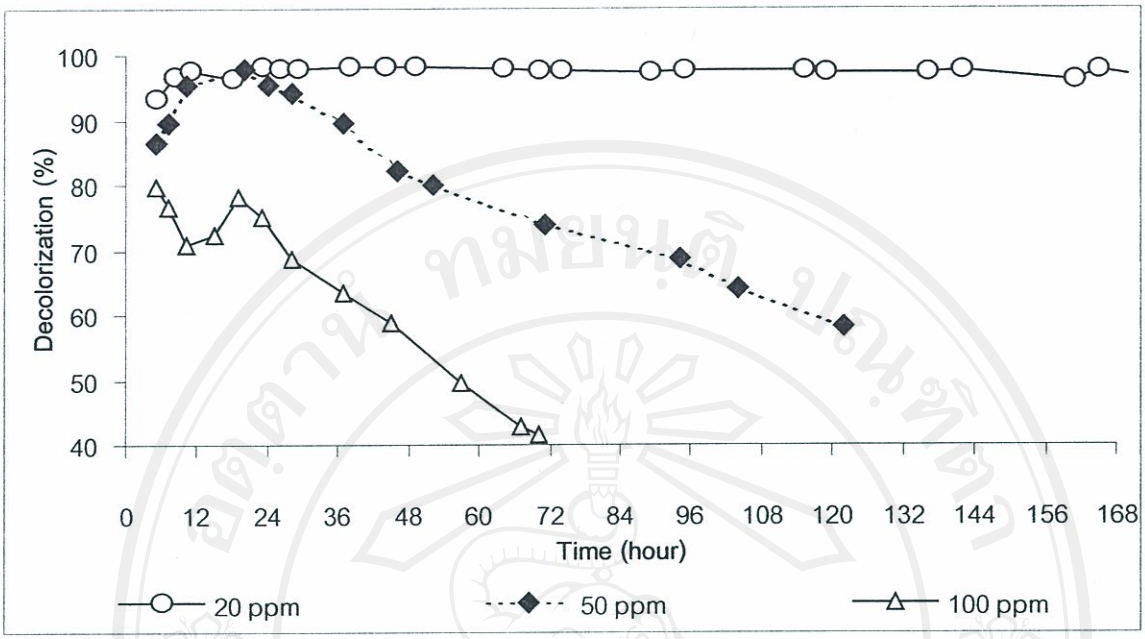

Figure 4.22 Continuous decolorization from various dye concentration

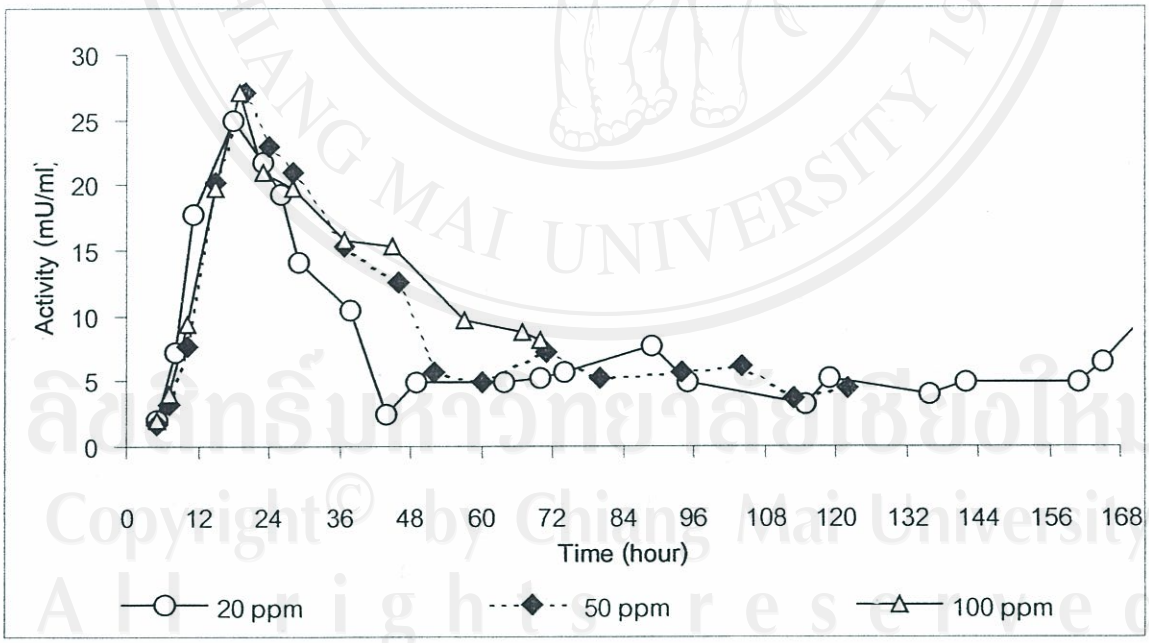

Figure 4.23 Laccase production from various dye concentration

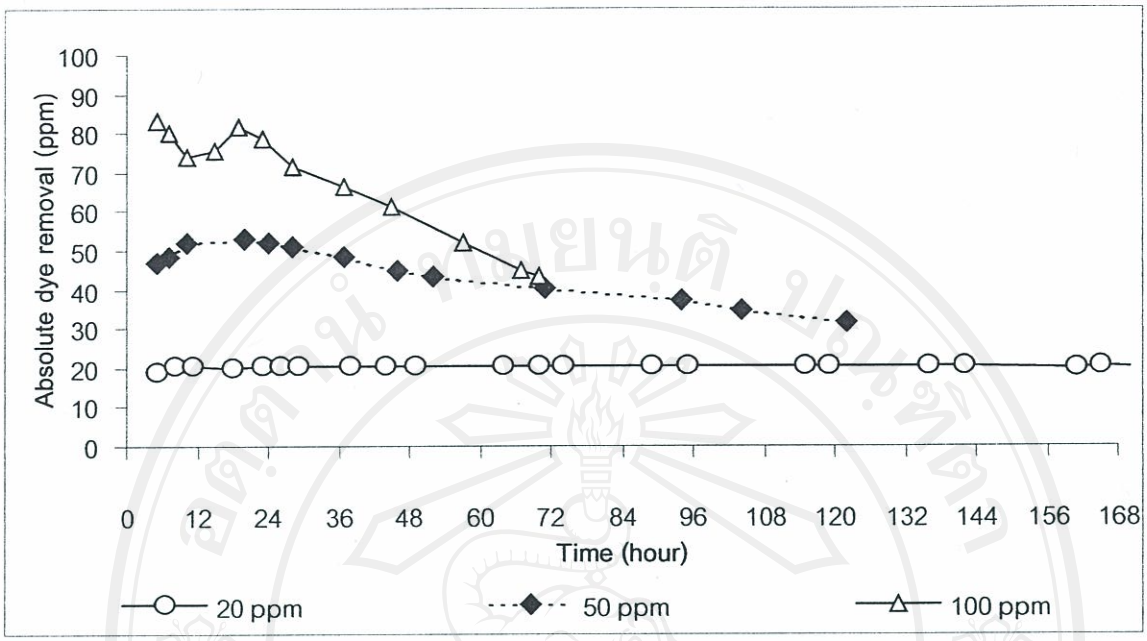

Figure 4.24 Absolute dye removal from various dye concentration

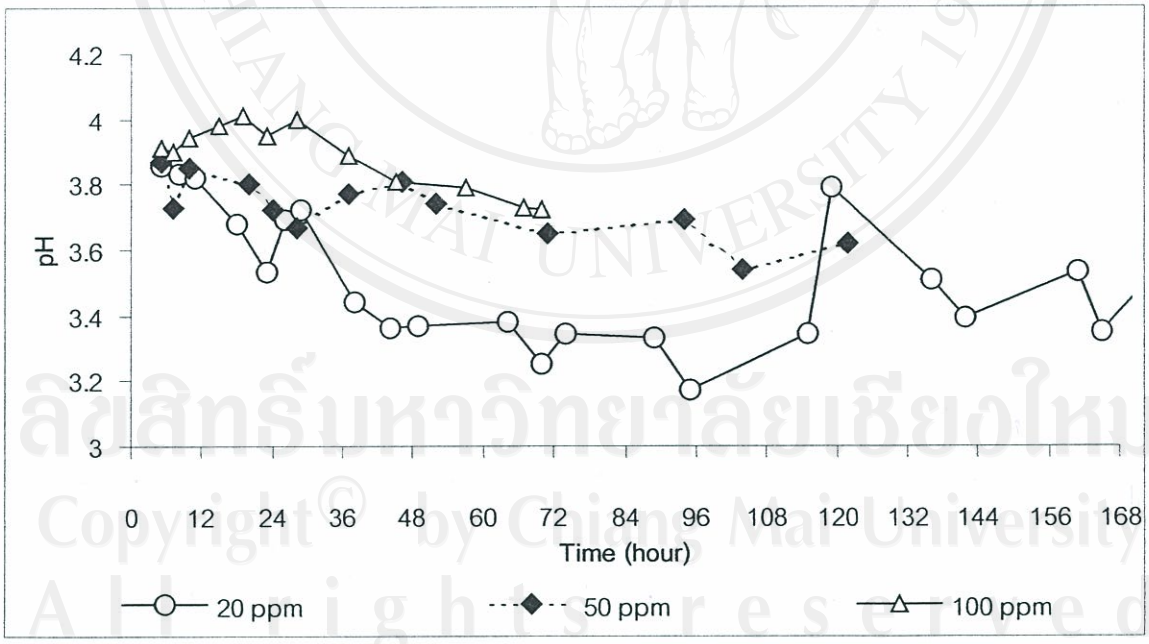

Figure 4.25 Change of effluent pH from feeding of various dye concentration

4.13 Decolorization of Batik wastewater

The results were found that the RC3 strain could efficiently decolorized the Batik wastewater as showed in Figure 4.26 and 4.27. Maximum decolorization (83.1%) was obtained from medium supplemented 100% wastewater (100M) cultivated for 4 days (Figure 4.26), while medium supplemented 50% wastewater (50M) was maximum at 73.56% decolorization. In glucose supplemented at 100% and 50% wastewater (100G and 50G, respectively), up to only 60% decolorization at 5 days incubation was obtained. In non-nutrient supplemented 100% and 50% wastewater (100I and 50I, respectively), decolorization was just likely to be detected after 3 days for 50I and decolorization was not found in 100I. This can be explained that addition of nutrient may be important or helpful for decolorization of Batik wastewater in high concentration. For control experiment (100C and 50C) as no inoculation, decolorization was not found (Figure 4.27).

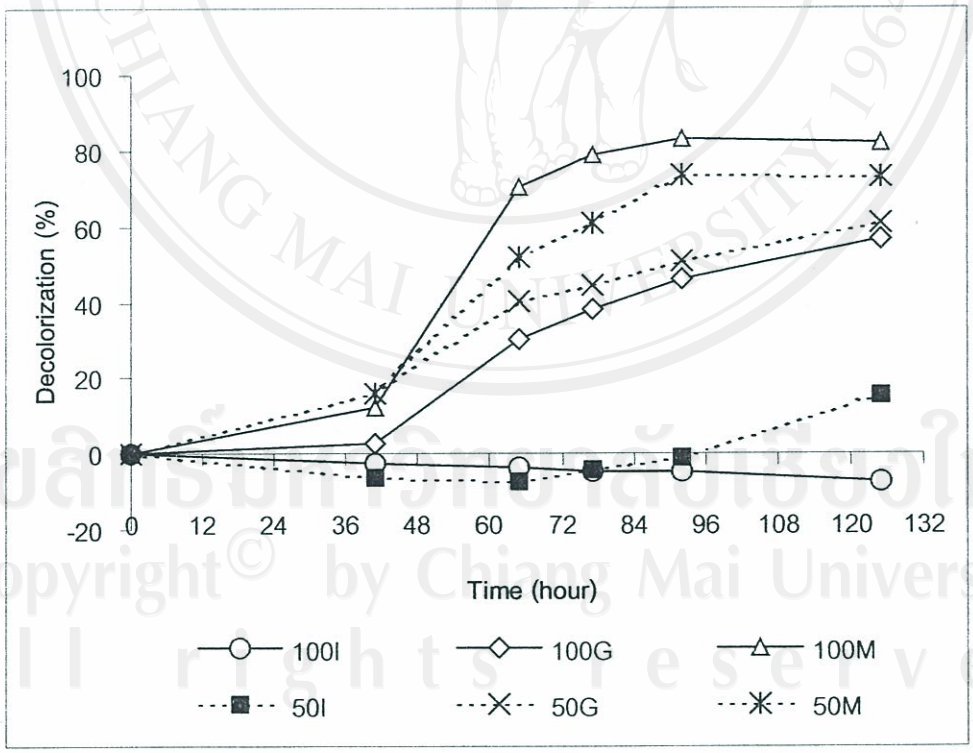

Figure 4.26 Decolorization curve of Batik wastewater

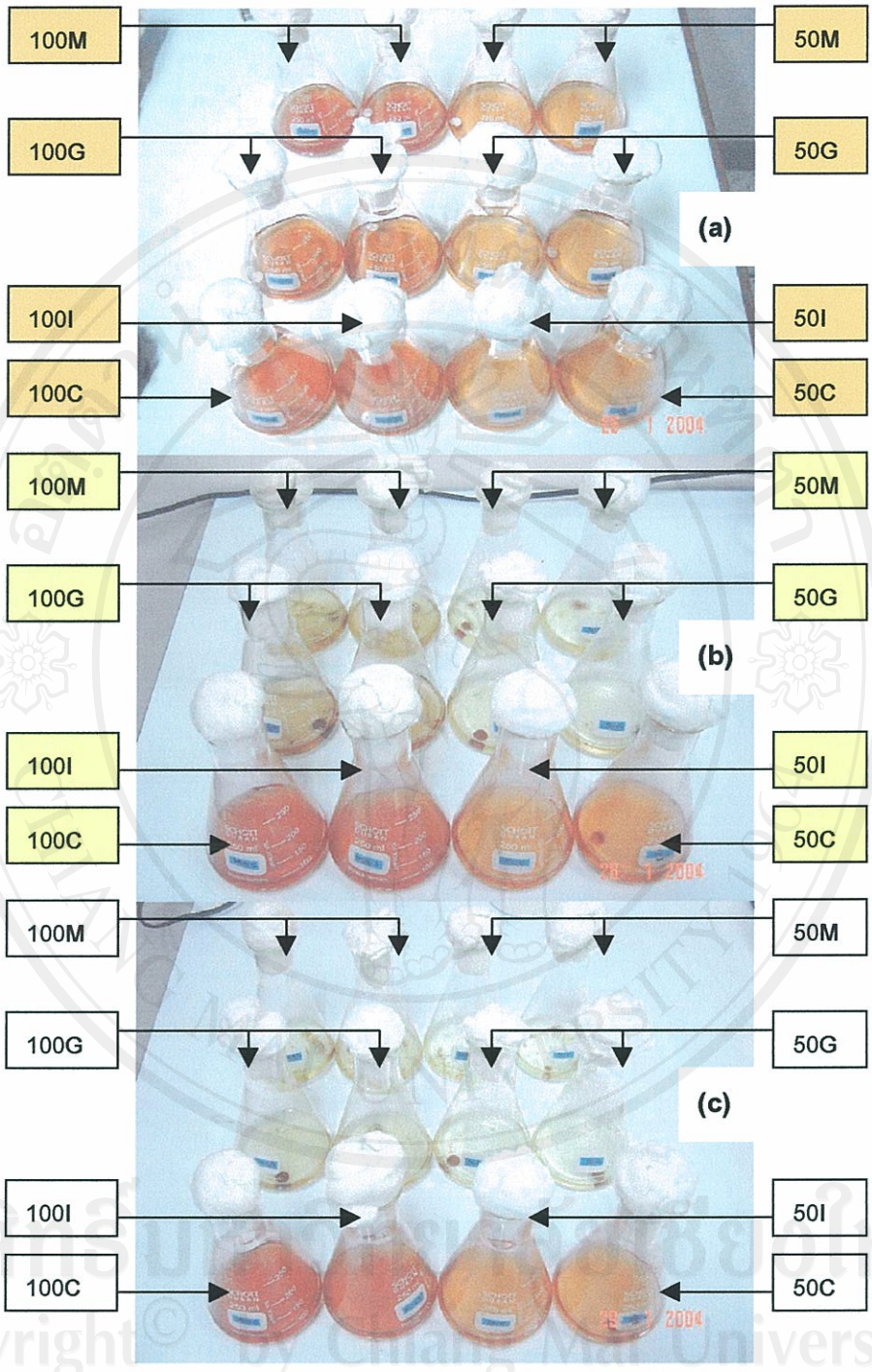

Figure 4.27 Decolorization feature of Batik wastewater

(a) Before decolorization (o day)

(b) 3 days decolorization

(c) 4 days decolorization

However, real dye contaminated wastewater is contained with several uncertainty factors such as dye types, dye concentration, pH, detergent substances, availability and so on. Those factors are difficult to control when textile wastewater was selected and used in any decolorization experiments. Experimental planing and wastewater characterization should be investigated before started the experiment.