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ORIGINAL PAPER

Degradation and decolorization of monosodium glutamate wastewater with *Coriolus versicolor*

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Abstract Degradation and decolorization of monosodium glutamate wastewater (MSGW) with *Coriolus versicolor* were firstly carried out. The effects of various operation parameters namely wastewater concentrations, pH, culture time and incidence of sterilization on maximum percentage of degradation and decolorization of wastewater were investigated. Studies of mycelium and enzyme for *C. versicolor* degradation and decolorization were estimated in this study. Ten percentage of wastewater concentration and pH = 5.0 were found to be the most suitable ones among the other experiments. The highest degradation and decolorization efficiency of wastewater was obtained at the fifth day of cultivation, which was displayed with more than 70% chemical oxygen demand removal, 83% total sugar removal and 55% color removal, respectively. Sterile operation had no remarkable effect on the degradation and decolorization efficiency for *C. versicolor*. Mycelium and the extra cellular fungal enzyme were both necessary for the degradation and decolorization of MSGW. *C. versicolor* possesses great potential and economic advantages in MSGW treatment.

Keywords Degradation · Decolorization · *Coriolus versicolor* · Monosodium glutamate wastewater

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Introduction

Environmental problems are one of the most important concerns for society, especially the wastes produced by industrial activity. Monosodium glutamate, a flavor of food ingredient, is produced by the microbial fermentation processes using molasses or starch as carbon source and zeatin or ammonia as nitrogen source. Especially, effluents after being extracting glutamate contain both high concentration of organic matters such as organic acid, amino acid, nitrogenous compounds, etc. Also a large amount of dark brown pigments (Sirianuntapiboon et al. 1988) called melanoidin and formed by an amino acid and a carbohydrate through Maillard reaction (Wedzicha and Kaputo 1992) were remained in the monosodium glutamate wastewater. Approximately 10–15 ton monosodium glutamate wastewater (MSGW) is generated while producing 1 ton monosodium glutamate. If it were discharged directly into river, lake and sea, it would lead to serious environment pollution.

Physical and chemical methods such as adsorption, coagulation–flocculation, oxidation, filtration and electrochemical methods may be used for color removal and chemical oxygen demand (COD) reduction from the industrial wastewaters (Lin and Peng 1994, 1996; Calabro et al. 1991; Boon et al. 2000; Sangkil and Paul 2000). Even though these procedures prove to be efficient, the operational costs are relatively high and leads to other disadvantages like sludge formation, biomass accumulation, even may have secondary pollution problems. On the contrary, biodegradation and biological decolorization methods have some advantages over physical and chemical methods such as low-cost, and little or no secondary pollution.

Presently, biological treatments used for MSGW are aerobic and anaerobic biodegradation. For example, bio-treatment of MSGW by yeast was studied widely (Qingxiang Yang et al. 2005), but the yeast technology resulted only in COD removal and had little effects on melanoidin decolorization. In addition, the yeast technology required a special pure yeast strain, special fermentative tanks, a series of expensive sterilize operation.

Coriolus versicolor is the major white-rot strain used for delignification and decolorization purposes (Knapp et al. 1997; Heinfling et al. 1997). Many researchers are mostly focused on delignification and decolorization of various textile dyestuffs (Kapdan et al. 1999). Subsequently, *C. versicolor* had also been used to docolorize melanoidin and been applied in color-removal treatments of melanoidin-containing wastewater (Santos et al. 1993). However, there have no reports on degradation and decolorization of MSGW with *C. versicolor*.

The major objective of this study is to investigate the effects of various operation parameters such as wastewater concentrations, pH, culture time and incidence of sterilization on degradation and decolorization efficiency of wastewater by *C. versicolor*. And the studies of mycelium and extra cellular fungal enzyme elementary mechanism for *C. versicolor* degradation and decolorization of wastewater was also made clear.

Materials and methods

Fungal culture

The white-rot fungal culture, *C. versicolor* (AS 5.48), was obtained from the Department of Life Science, Henan Agricultural University as a pure culture. The culture was grown on potato-dextrose agar (PDA) plates for 5–6 days and were preserved at 4°C. The culture was re-cultivated periodically at the optimal growth temperature of 27°C.

Mycelium of *C. versicolor* in the form of pellets was used as inoculum. For preparation of pellets the fungus was first grown at pH 5.0 on PDA plates. Mycelia discs of about 1 cm diameter were cut from the zone of active growth and cultivated in 250 ml flasks containing 100 ml liquid PDA medium culture at 27°C for 5–6 days with 150 rpm in an oscillator. The mycelium grown in these conditions was then mixed for 20–30 s in order to obtain a homogeneous suspension of the mycelium. Pellets of approximately 1–3 mm diameter were obtained and used as inoculum in degradation and decolorization of wastewater.

Wastewater

Monosodium glutamate wastewater used in this study was obtained from a monosodium glutamate fermentation plant in Lotus Flower Company, Henan Province, China. It was a highly concentrated acid wastewater with pH value of 2–3. The main chemical properties of wastewater was shown in Table 1.

Experimental procedure

Experiments were performed by using 250 ml shake flasks in a temperature controlled incubator shaker. Flasks were prepared in triplicates and contained 100 ml wastewater. Ten milliliters of mycelium inoculum was transferred into wastewater. The cultures were incubated for 6 days at a

Table 1 The characteristics of MSGW used in this study

Component	Concentration (mg l^{-1})
COD	176,000 ± 5,000
BOD_5	86,500 ± 5,000
TOC	64,464.5 ± 300
NH_3–N^+ (amino nitrogen)	29,000 ± 100
SO_4^{2-}	102,000 ± 500
TP (total phosphorus)	1,500 ± 50
pH	2–3
Absorbance ($\lambda_{475\ nm}$)	0.25 (100-fold diluted)

shaking speed of 150 rpm. Samples were removed everyday and centrifuged at 4,000 rpm for 10 min. Analyses were carried out on clear supernatants.

Except the experiments with varying concentrations of wastewater, pH, the wastewater concentration in all experiments were 10% and pH = 5.0, respectively. Additionally, except the experiment of incidences sterilization and non-sterile of wastewater, all experiments were carried out under sterile condition. Control flasks contained only wastewater, but no fungi.

Wastewater concentrations and pH

Five different wastewater concentrations diluted with de-ionized water into 10, 20, 30, 40 and 50% were tested to investigate the effects of degradation and decolorization for *C. versicolor*. And seven different pH of wastewater adjusted to pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 with 5 N NaOH were also investigated.

Biodegradation and decolorization studies

The role of mycelium in degradation and decolorization was estimated by separating the mycelium and supernatant at the time at which the fungus showed maximum activity. The mycelium and supernatant were, respectively, obtained by centrifuging the culture of *C. versicolor* at 4,000 rpm for 20 min. To eliminate the enzyme in supernatant interference, the mycelium was washed three to four times with distilled water.

The experiment was dealt with two steps. During one step, the maximum activity of enzyme was obtained by measuring the enzyme activity of *C. versicolor* at different culture time. At the second step, the degradation and decolorization of wastewater with supernatant and mycelium was carried out in flasks, respectively.

Analytical methods

Degradation efficiency was determined by the COD and total sugar removal efficiency of wastewater. The COD was analyzed by CTL-12 type COD analyzer (Hua Tong Instrument Factory, China) and the total sugar was measured by the phenol-sulfate method (Dubis et al. 1956). The COD and total sugar removal efficiency were calculated by the difference between the initial wastewater COD (total sugar) and the final wastewater COD (total sugar) after *C. versicolor* cultivation.

Decolorization efficiency was determined in terms of the decrease in the absorbance at 475 nm against the initial absorbance of wastewater at the same wavelength (Sirianuntapiboon et al. 1988).

pH determination were made by a glass electrode with an aquatic pH meter 3S.

The laccase activity was analyzed by spectrophotometer (721-B, Shanghai, China) according to Wang et al. (1998), with 2,2′-azino-di (3-ethyl-benzo-thiazolin-sulphonate) as substrate. One unit was defined as the amount of enzyme that oxidize 1 μmol of substrate per minute and the activities are reported as U l^{-1}.

The dry mycelia weight was obtained by 105°C drying for 24 h.

Results and discussion

Characteristics of monosodium glutamate wastewater

The characteristics of the concentrated MSGW are listed in Table 1. As can be seen, the wastewater was an acidic water with pH value of 2–3. It contained a large amount of COD and BOD_5 which were 176,000 and 86,500 mg l^{-1}, respectively, and the ratio of BOD_5 to COD is about 50.6%. There was about 64,464.5 mg l^{-1} of organic carbon in wastewater, and the concentrations of NH_3–N^+ (amino nitrogen) and sulfate were 29,000 and 102,000 mg l^{-1}, respectively. The absorbance of 100-fold diluted wastewater at 475 nm reached a value of 0.25.

All these above characteristics showed that the high content of organic and inorganic matters and pigments were contained of MSGW.

Effect of wastewater concentrations

Five different wastewater concentrations were tested to determine an optimal concentration for degradation and decolorization of *C. versicolor*. Figure 1 depicts degradation and decolorization curves obtained with different wastewater concentrations. Degradation and decolorization efficiencies decreased with the increase of wastewater concentrations after 6 days of incubation. When the wastewater concentration was 10%, degradation and decolorization efficiency were reached the highest values, which showed of 74.1% COD removal, of 83.9% total sugar removal and of 55.3% color removal, respectively. At the same time, the highest biomass yield was 7.8 g l^{-1}.

The wastewater concentrations had remarkable effects on degradation and decolorization efficiency of *C. versicolor*. As shown in Fig. 1, the COD removal, total sugar removal and color removal efficiency decreased with the increment of wastewater concentrations, and the dry mycelia weight also decreased. It was the reason that high wastewater concentration contained high content of organic or inorganic matters that caused the high osmotic pressure and led to inhibitory growth and low degradation and decolorization efficiency of *C. versicolor*.

Effect of pH

The adaptive growth pH for *C. versicolor* is from pH 5.0 to 6.0 However, such a low pH (pH = 3) of wastewater is not suitable for practical wastewater treatment operations. To find a more suitable pH for effective degradation and decolorization by the fungi, shake flask experiments were conducted at different initial pH values. Wastewater was adjusted to pH = 3.0–6.0 with 5 N NaOH. Acetic acid and phosphate buffers were used for pH = 3.0–4.0 and 5.0–6.0, respectively, to keep the pH of wastewater constant. Control flask contained wastewater and buffers, but no fungi.

Figure 2 showed that degradation and decolorization efficiency at pH = 5.0 were maximum, respectively, the maximum of COD removal, total sugar removal and color removal were 72.4, 83.4 and 55.8%, respectively, after 6 days. However, the efficiency decreased nearly 30% at pH 3, and at pH values between 5.5 and 6.0, the efficiency also decreased but not more than 10%. It was the main reason that the neutral pH was suitable for *C. versicolor* growth. Also, the difference of dry mycelia weight of *C. versicolor*

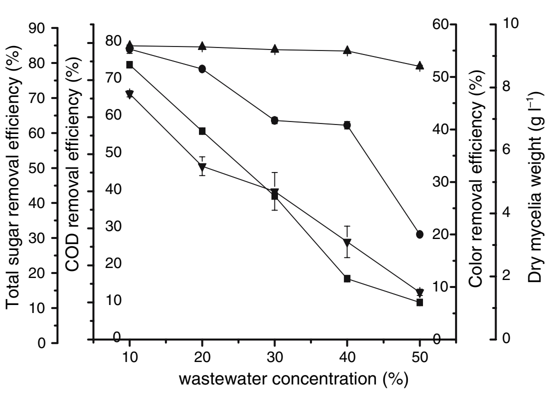

Fig. 1 Effect of wastewater concentrations on decolorization and degradation efficiency. (■) COD removal efficiency, (●) color removal efficiency, (▲) total sugar removal efficiency, (▼) dry mycelia weight. *Source*: Cuiying Jia et al

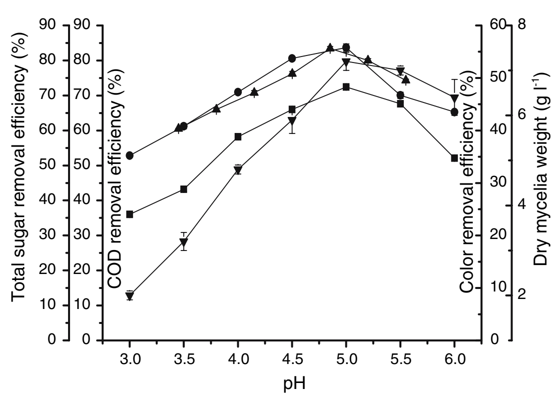

Fig. 2 Effect of pH of wastewater on decolorization and degradation efficiency. (■) COD removal efficiency, (●) color removal efficiency, (▲) total sugar removal efficiency, (▼) dry mycelia weight. *Source*: Cuiying Jia et al

at different initial pH was also approved this point.

The fungus was capable of degradating and decolorizing wastewater with lower efficiency at pH of 3.0 and 4. The highest of degradation and decolorization efficiency was obtained at pH 5.0. No significant color change was observed in the control flasks at different pH values.

Effect of sterilization

The optimal pH for *C. versicolor* growth is near to neutral and sterilization operation should be considered at this pH. So the incidences of sterilization and non-sterilization were investigated for *C. versicolor* degradation and decolorization of MSGW.

The results were shown in Fig. 3. As shown in Fig. 3a, whether MSGW was sterilized or not, all parameters of removal efficiency such as COD, color and total sugar removal efficiency displayed no significant difference, the highest color removal of 56.1%, COD removal of 78.7% and total sugar removal of 90.6% were obtained at the fifth day, respectively. Under non-sterile condition, the rate of COD removal and total sugar removal was 2,198.8 and 213.9 mg l^{-1} day^{-1}, respectively, and under sterilization condition, the rate of COD and total sugar removal of 2,353.6 and 303.6 mg l^{-1} day^{-1} was achieved, respectively. All these results were shown that sterilization accelerated the rate of COD and total sugar degradation of *C. versicolor* but did not affect the COD and total sugar removal efficiency.

As shown in Fig. 3b, under sterilized condition, growth of the cells were inhibited by the additional melanoidin, which was generated in sterilization, and about 50% slower than that of the cells under non-sterilized condition at the first

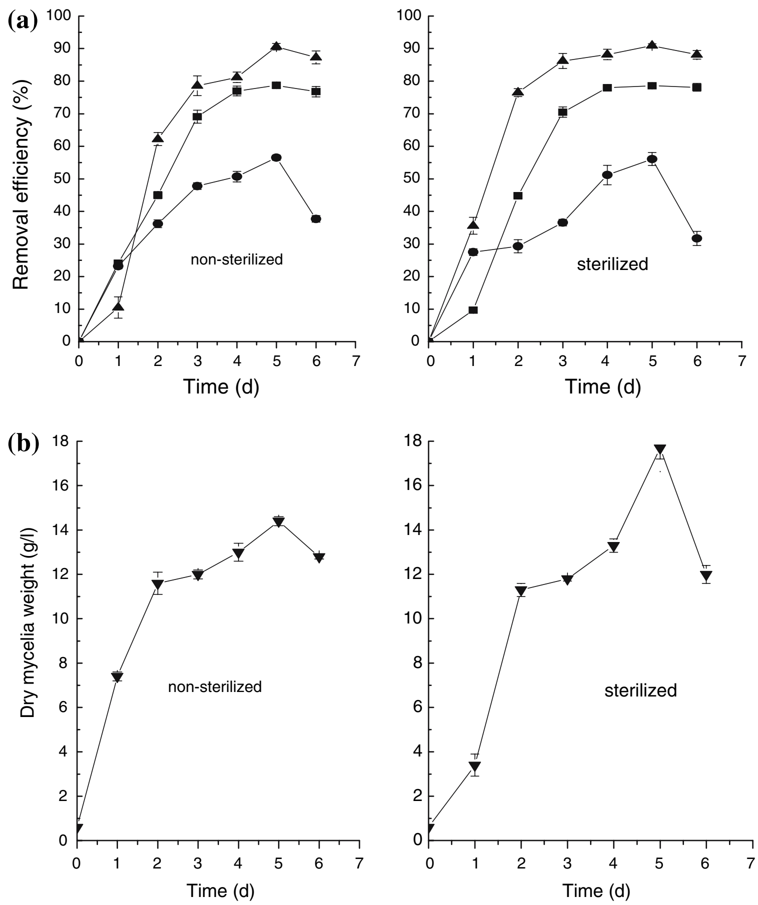

Fig. 3 Effect of sterilization on degradation and decolorization efficiency. (**a**) COD, color and total sugar removal efficiency. (■) COD removal efficiency, (●) color removal efficiency, (▲) total sugar removal efficiency. (**b**) Dry mycelia weight of *C. versicolor*. (▼) dry mycelia weight. *Source*: Cuiying Jia et al

day. Although the growth rate recovered at the second day and reached the highest mycelia dry weight of 17.1 g l^{-1} at the fifth day, which was 3 g l^{-1} higher than that of non-sterile wastewater treatment, the treatment efficiency benefited a little from the higher cell density.

The above results indicated that *C. versicolor* possessed a great capability for MSGW decolorization and biodegradation. Sterilization had no influence on biodegradation efficiency and inhibited *C. versicolor* growth in the prophase of cultivation. The omitting of sterilization avoids a high energy consuming process and regeneration of melanoidin, and the treatment costs were reduced remarkably.

Degradation and decolorization studies of wastewater

Table 2 showed the enzyme activity of *C. versicolor* at different culture time. The maximum activity was obtained at 5–6 days of cultivation, and also the highest dry mycelia weight was achieved. Therefore, to obtain the maximum activity, the culture time of *C. versicolor* should continue 5–6 days.

Table 3 showed the degradation and decolorization of wastewater with supernatant and mycelia. For supernatant, 58% COD removal and 61.3% color removal were, respectively, obtained; for mycelia, the COD and color removal efficiency of wastewater were 61.9 and 57.8%, respectively. Compared to the results of degradation and decolorization with enzyme and mycelium, the degradation and decolorization of wastewater with enzyme was equivalent to with mycelium, however, it was actually considerable that the mycelium grow and enzyme was produced again during the degradation and decolorization of wastewater, that was to say, 61.9% of COD removal and 57.8% of color removal with mycelia shown in Table 3 should contain the part of enzyme catalysis. So, the degradation and decolorization of wastewater were mostly realized by enzyme catalysis.

Table 2 The results of enzyme activity analysis

Culture time (days)	Laccase activity (U l^{-1})	Dry mycelia weight (g l^{-1})
5	256.2 ± 2.5	7.8 ± 1.2
6	254.5 ± 2.5	7.6 ± 1.2
7	192.3 ± 2.5	6.5 ± 1.2
8	185.4 ± 2.5	6.0 ± 1.2

Conclusions

The white-rot fungus, *C. versicolor*, showed the high ability of degradation and decolorization during the MSGW treatment. Effect of various environmental conditions on degradation and decolorization of MSGW by *C. versicolor* were investigated. The highest COD, total sugar and color removal for MSGW in present work were found to be more than 70, 80 and 55%, respectively, at the following optimum conditions: wastewater concentration 10%, pH 5.0, 5 days cultivation. Similarly, experiments were carried out under sterilization and non-sterile conditions, respectively. It was found that sterilization operation had no remarkable effect on degradation and decolorization efficiency of wastewater, therefore the omitting of sterilization avoids a high energy consuming process and regeneration of melanoidin, and the treatment costs were reduced remarkably. These results suggest that *C. versicolor* possesses great potential and economic advantages in MSGW treatment.

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Table 3 The results of degradation and decolorization efficiency of enzyme and mycelia

	Initial COD (mg l^{-1})	Final COD (mg l^{-1})	COD removal (%)	Initial absorbance	Final absorbance	Color removal (%)
Enzyme	26,453 ± 50	11,110 ± 50	58 ± 0.015	0.62 ± 0.02	0.24 ± 0.02	61.3 ± 0.02
Mycelia	26,453 ± 50	10,052.3 ± 50	61.9 ± 0.002	0.64 ± 0.02	0.27 ± 0.02	57.8 ± 0.02

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