Some Properties of Coriolus sp.No.20 for Removal of Color Substances from Molasses Waste Water

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Coriolus No.20, belonging to the class Basidiomycetes, can remove color substances from molasses waste water (MWW). In the case of untreated MWW solution, the decrease was 82.5% in darkness under optimal conditions. This removal (= decolorization) was through the adsorption of melanoidin to the mycelium and the yield of adsorption was 9.67 and 12.12 mg per g of mycelium as dry basic in the case of autocaved mycelium and resting mycelium, respectively. The resting mycelium showed the highest adsorption yield (16.32 mg per g of mycelium) when the melanoidin solution was supplemented with 20% glucose solution.

In the fed-batch system, the strain showed a constant decolorization yield of 75% during four times replacements (32 days) in both untreated and treated MWW solutions.

In the continuous feed system for decolorization of untreated MWW solution, 10% of fresh untreated MWW solution was added every day after seven days of cultivation. The decolorization rate was constant (decolorization rate was 37.5%) during 12 days of operation

In the continuous decolorization of concentrated untreated MWW solution, the strain showed a constant decolorization yield (about 70%) during three times feeding of fresh concentrated untreated MWW solution. But 10 ml of 20% glucose solution had to be added after 13 days cultivation to keep the reducing sugar concentration in the culture broth at more than 1.0%.

Key words: *Coriolus* sp. Melanoidin, Decolorization, Molasses Waste Water

1. Introduction

Molasses contain about 50% of saccharides which cannot be removed commercially, and it has high commercial value due to its use as a carbon source in feed for domestic animals, biofertilizer and fermentation industries. However, the use of molasses as a raw material for fermentation industries such as alcohol fermentation, amino acid production, baker's yeast production, etc., has the disadvantage that a large amount of colored substances remain in the fermentation residue after recovery of the products, as the main colored substance, melanoidin, can not be effectively decomposed by biological treatment processes (1,2). Many chemical and physical treatment processes were introduced to remove the color substances from the waste water where BOD contents had already bean removed by usual biological treatment processes. However, these processes (3,4) still had disadvantages due to high cost, unstable removal efficiency and the production of solid waste. Consequently many researchers have tried to use biological processes for removal of the color substances from the waste water. Using Basidiomycetes, Deuteromycetes and bacteria (5,6,7,8). *Coriolus* sp. No.20 (7) class Basidiomycetes, was one of the strains which showed the ability to remove the colored substances from MWW. The ability to remove

the colored substances was expressed as the decolorization yield and the strain showed decolorization yield of about 80%. In this paper, we describe some poperties of *Coriolus* sp. No. 20 for decolorization in continuous process, fed-batch system and batch type process.

2. Materials and Methods

Molasses waste water (MWW) and melanoidin pigment (MP) solutions: A MWW sampled from the stillage of an alcohol factory and the effluent from a waste water treatment plant (UASB) were used in this study as untreated molasses waste water (U-MWW) and treated molasses waste water (T-MWW), respectively. These MWW were diluted with distilled water to a concentration corresponding to an absorbance reading of 3.5 at 475 nm, and adjusted to pH 6.0. A mixture of 1M glucose and 1M glutamate solution was adjusted to pH 9.0 with 1N NaOH solution and refluxed for 2 hrs. The brown black product was dialyzed against tap water for 2 days, and deionized water for 1 day. The non-dialyzable solution diluted with distilled water to a concentration corresponding to an absorbance reading of 3.5 at 475 nm was used as melanoidin pigment (MP) solution.

Microorganism, preparation of inoculation and medium: *Coriolus* sp. No. 20 which showed the highest decolorization ability in the previous study (8) was used in this experiment. The strain grown on the potato dextrose agar slant (10) for 7 days was inoculated into 50 ml of potato dextrose broth (10) in a 300 ml Erlenmeyer flask and then incubated at 30°C for 2 weeks. The mycelium grown on the surface of the liquid was harvested and a homogenized by a homogenizer (OMNI mixer Sorvall, Model 17106) with 20 ml of sterile distilled water at 17,000 rpm under aseptic conditions. The mycelial suspension thus prepared was used as the inoculum, which contained about 0.01 g of mycelium as dry weight per ml. The U-MWW and T-MWW mediums for the decolorization test were prepared from the U-MWW annd T-MWW solutions containing 2.5% glucose, 0.2% $NaNO_3$, 0.1% KH_2PO_4 and 0.5% $MgSO_4.7H_2O$

Decolorization of MWW solution under batch type system: Decolorization of U-MWW and T-MWW solutions with mycelium of *Coriolus* sp. No.20 was carried out in 500 ml shaking flasks under reciprocal shaking conditions (125 oscillations/min, 7 cm stroke) at 30°C. The 15 ml of mycelium suspension was inoculated into a 500 ml Erlenmeyer flask which contained 100 ml of U-MWW or T-MWW medium.

Fed-batch system for decolorization of MWW solution: The fed-batch system for decolorization of U-MWW and T-MWW solutions with mycelium of *Coriolus* sp. No.20 was carried out in μ-carrier magnetic stirrer (Bellco, USA). Seventy five ml of mycelial suspension was inoculated into 500 ml of U-MWW or T-MWW medium prepared in 1,000 ml u-carrier spinner flask (Bellco, USA), and then incubated at 30°C at an impeller speed of 150 rpm. A half volume of culture broth was replaced with fresh medium with the same composition of the above medium, when a decolorization yield of about 75% and 70% had been attained.

Continuous feed system for decolorization of U-MWW solution: The continuous feed system for decolorization of U-MWW solution with mycelia of *Coriolus* sp. No.20 was carried out in μ-carrier magnetic stirrer (Bellco, USA). Forty five ml of mycelial suspension was inoculated in 300ml of U-MWW or T-MWW medium prepared in 1,000 ml u-carrier spinner flask (Bellco, USA), and then incubated at 30°C at an impeller speed of 150 rpm. After the color intensity of the culture broth dropped to 1 unit at 475 nm (about 7 days cultivation), 30 ml of fresh medium was added everyday.

Continuous system for decolorization of U-MWW solution: The continuous decolorization system for decolorizing of U-MWW solution with mycelia of *Coriolus* sp. No.20 was carried out in μ-carrier magnetic stirrer (Bellco, USA). Fourty five ml of mycelial suspension was inoculated in 300ml of U-MWW or T-MWW medium prepared in 1,000 ml μ-carrier spinner flask (Bellco, USA), and then inculated at 30°C at an impeller speed of 150 rpm. 30 ml of concentrated U-MWW solution (10 times

concentration of original U-MWW solution) was added when a decolorization yield of about 70% had been attained. Ten ml of 20% glucose solution was added to the culture broth when the reducing sugar of the culture broth dropped below 0.1%.

Melanoidin adsorption test: The melanoidin adsorption test of mycelium of *Coriolus* sp. No. 20 was carried out in an L-type tube. Two kinds of mycelium as resting mycelium and autoclaved mycelium were used in this experiment. The 10 ml of reaction mixture, containing 2% of mycelium in MP solution, was prepared in L-type tube and incubated in the Monad's shaker at 30°C for 20 hrs.

Estimation of the decolorization yield: Decolorization activity was determined as a decrease in optical density as absorbance at 475 nm(8). The decolorization yield was expressed as the degree of decrease in adsorbance at 475 nm against the initial absorbance at the same wavelength.

Assay: Residual sugar was determined by the Somogyi and Nelson's method (11,12). Mycelia washed 3 times with distilled water were used to determine the dry weight after drying at 105 °C overnight.

3. Results

Decolorization of MWW solutions by mycelium of *Coriolus* sp. No.20: The typical profiles of *Coriolus* sp. No.20 for decolorization of MWW solutions(U-MWW and T-MWW) in the batch type system under optimun conditions are shown in fig.1 and fig.2. The decolorization by this strain almost coincided with the increase of the amount of mycelium. The maximun decolorization yields were 82.5 and 77% in U-MWW and T-MWW solutions respectively, within 9 days of cultivation. The reducing sugar contents in the culture broths rapidly decreased during decreasing of color intensity in both U-MWW and T-MWW solutions. The maximum dry weight of the mycelium were 1.5 and 1.3 g/100 ml of culture broth within 6-day cultivation.

Comparison of the melanoidin adsorption ability between resting mycelium and autoclaved mycelium: The adsorption of melanoidin to resting mycelium and autoclaved mycelium of *Coriolus* sp. No.20 is shown in table 1. The adsorption degree of resting mycelium was 1.25 times higher than that of autoclaved mycelium. Under standard conditions, the maximum adsorption yield of autoclaved mycelium was 9.67 mg of pigments per g of mycelium (dry basic) while the maximum adsorption ability of resting mycelium was 12.12 mg of pigments per g of mycelium (dry basic). The melanoidin adsorption ability of resting mycelium could be increased by supplementing glucose (final concentration 2%) in the reaction mixture and reached 16.32 mg of pigments per g of mycelium.

Fed-batch system using resting mycelia: A typical time course for a fed-batch system for decolorization of U-MWW and T-MWW solutions is shown in fig.3 and fig.4. For the U-MWW solution, the decolorization yield was up to 75% and cell mass was about 1.2 g per 100 ml of culture broth within 7-day cultivation in the first batch. The decolorization yields in the second batch and third batch were 74% and 75%, respectively, within 5 days of cultivation. The maximum growth yield of the mycelium was about 1.2 g per 100 ml of culture broth in both second and third batches. For the T-MWW solution, the decolorization yield was up to 75% within 10 days cultivation in the first batch. In the second batch and third batch, the decolorization yield was up to 75% within 5 days of cultivation. The maximum growth yield was up to 1.2 g per 100 ml of culture broth within 10 days in the first batch and the concentation was constant in second and third batches.

Continuous feed system for decolorization of U-MWW: A typical time course of a continuous feed system for decolorization of U-MWW solution is shown in fig 5. The U-MWW solution having the initial color intensity as absorbance about 3.5 at 475 nm was cultivated with mycelia of *Coriolus* sp. No. 20 for 7 days and the color intensity of the culture broth was reduced to absorbance about 1.0 at 475 nm. 30

ml of fresh medium was added everyday, the color intensity of the culture broth was reduced constantly as absorbance from 1.3 to 1.0 each day during 5-day continuous feeding process.

Continuous feed system for decolorization of concentrated U-MWW: A typical time course for continuous decolorization of concentrated U-MWW solution is shown in fig 6. The U-MWW medium having initial color intensity as absorbance about 3.5 at 475 nm was cultivated with mycelia of *Coriolus* sp. No. 20 for 7 days and the decolorization activity was up to 70%. After adding the 30 ml of concentrated U-MWW solution (10 times of U-MWW solution), the decolorization yield was up to 70% within 7 days of cultivation in the second batch. For the third batch, more than 70% of added concentrated U-MWW solution (30ml) was decolorized within 7 days.During the continuous decolorization process, the reducing sugar in the culture broth was controlled to be not below 0.1%. However, after 13 days of cultivation, the reducing sugar in the culture broth dropped below 0.1%, and the decolorization yield also dropped. When 10 ml of 20% glucose solution was added, the decolorization activity recovered as shown in fig.6.

4. Discussion

Coriolus sp. No.20 showed good ability for dedcolorization of both U-MWW and T-MWW solutions as shown in fig.1 and fig.2. But the decolorization yield in T-MWW solution was 6.7% lower than in U-MWW solution. This may be explained by some toxic substances produced during the concentration of MWW repressed the decolorization activity of this strain or this strain could not effectively attack the colored substances in the T-MWW solution due to a change in the chemical structure during the waste water treatment process (13,14).

The decolorization of melanoidin by *Coriolus* sp. No. 20 occurred by adsorption mechanism and decomposition by intracellular enzyme (3,13,14). As shown in table 1, the resting mycelium showed adsorption degree 1.25 times higher than that of autoclaved mycelium and the adsorption yield of the resting mycelium was increased to 1.69 times higher than that of autoclaved mycelium when the MP solution contained 2% glucose. The resting mycelium showed both the adsorption ability and decomposition ability by intracellular enzymes (3,13,14) at the same time while the autoclaved mycelium could show only adsorption ability. And the decolorization mechanism might be serial mechanism (14). Namely, the melanoidin is adsorbed onto the mycelium first, then incorporated into the cell and then decomposed by intracellular enzyme.

As to applications, the fed-batch system and continuous feed system for decolorization of MWW solutions were carried out. For the fed-batch system, the decolorization of U-MWW and T-MWW solutions with reuse of resting mycelia was carried out in μ- carrier magnetic stirrer three times (32 days). The decolorization ability remained almost constant and the decolorization yields were about 75% and 70% in U-MWW and T-MWW solutions, respectively, as shown in fig.3 and fig.4. The continuous feed system showed a constant decolorization rate of about 37.5%in each day during 12-day operation as shown in fig.5.

For the continuous decolorization of concentrated U-MWW solution, the decolorization yield was constant during 3 times feeding. However, the reducing sugar of culture broth had to be adjusted by up to 0.1% by adding 10 ml of 20% glucose solution.

From the results above, we believe that the application of this *Coriolus* sp. No.20 might be most suitable due to the high decolorization yield. This strain could be used for treating both U-MWW and T-MWW with the decolorization ability more than 75%. It means that this strain could be easily applied to the conventional treatment processes which is usually used in alcohol factories. And the application form of the processes should be chosen from either the continuous feed system, semi-continuous feed system or fed-batch system according to the situation of each treatment plant due to the age of the treatment plant, quality and quantity of waste water, period of operation of the factory, quality of effluent, and so on.

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Table 1 : Melanoidin adsorption ability of autoclaved and resting mycelium of *Coriolus* sp. No.20.

The mycelium of *Coriolus* sp. No.20 which was cultivated in MWW medium was separated into 2 parts. One was used as resting mycelium after being washed with cold deionized water three times. The other was used as autoclaved mycelium after being washed with deionized water three times and sterilized at 121°C for 15 min. The melanoidin adsorption activity of both kinds of mycelium was assayed as described in the text.

Mycelium (2%)	Adsorption yield (mg melanoidin/g mycelium)	
	non-glucose supplemented melanoidin suspention	glucose supplemented melanoidin solution
Autoclaved mycelium	9.67	9.68
Resting mycelium	12.12	16.32