DECOLOURIZATION OF WASTEWATER FROM PULP AND PAPER MILL EFFLUENT BY USING ISOLATED FUNGUS

Nang Aye Mya Mon¹, Weine Nway Nway Oo²
Department of Biotechnology
Mandalay Technological University
Mandalay, Myanmar

1ayemyatmon.nang@gmail.com
2Weinenno@gmail.com

Abstract— Three fungal strains were collected from decomposed woods in Kyaukse wood mill and five fungal strains from effluent sludge by continuous pulp and paper industry. Among the accumulated strains, WF4, WF5, WF8, SF1 and SF3 are able to grow up well on agar containing some pieces of powdered bamboo and sawdust indicating that they could utilize these powders as nutrient for their growth. When they were tested for their effectiveness on decolourization of the wastewater from the pulp and paper industry, it indicated that strain WF5 was the most efficient strain which has the ability to decolorize the wastewater by $(58\%\pm0.4)$ and degrade the lignin by (3104 ± 232) respectively.

Index Terms— pulp and paper, decolourization, lignin, fungal strains, agar

I. INTRODUCTION

Industrial effluents represent a significant environmental and economic problem. The pulp and paper industry typically generates large quantities of wastewater whose correct treatment prior to discharge into the environment is critical. Paper factory effluent is one of the major pollutants on the earth. The pulp and paper industry effluent is highly colored. [1]. One significant problem is the persistent dark brown color in the released effluent from wastewater treatment facilities of which the major contributors are lignin and its derivatives, such as chlorolignin, discharged from the pulp bleaching process.

The pulp and paper mill is a major industrial sector utilizing a huge amount of lignocellulosic materials and water during the manufacturing process. Recently, the impact of this industry on the environment has been closely examined. Even though the color of effluent from pulp and paper industry does not directly affect the environment, it extremely causes psychological trouble for people living in those areas. A number of researches demonstrated that the effluent from pulping process contains lignin compounds as major degraded compounds. These compounds are largely removed from the woody raw material in an alkali pulping stage. Some of these degradation products have exhibited toxicity and mutagenicity

and may be accumulated in the tissues of animals, fish and human beings [2].

Typically, wastewater treatment is able to improve the quality of wastewater discharged from any of the production processes; however, the color of the effluent is usually still intense according to lignin compounds. This is obvious when the effluent released to environments such as river or stream. Removal of lignin compounds from the effluent is therefore needed [3]. This could be achieved by using microorganisms that are able to completely degrade polymeric lignin. Fungi are the only known microorganisms found to degrade lignin and have been extensively studied [4] [5]. Based on the nature of degradation, wood decaying fungi are classified as soft rot fungi, brown rot and white- rot fungi. A number of studies have provided information on white-rot fungi that can secrete enzymes for degrading natural lignin as summarized by Kondo [6]. The prime objective of the present study is to evaluate effluent degradation potential of fungi isolated from decomposed woods and effluent sludge.

II. MATERIALS AND METHODS

A. Effluent

The effluent samples of pulp and paper industry were assembled from the Tharbaung pulp and paper industry in Ayeyarwady division, Myanmar. The samples were put into clean plastic containers, and then they had been taken to the laboratory and immediately kept in refrigerator at 4°C until used for further analysis. The collected effluent of pulp and paper industry was used in the present pulping stage for decolourization and delignification studies.

B. Isolation of fungal strains

Fungal strains were isolated from decaying wood by using direct method and from effluent sludge with continuous pulp and paper industry by employing standard serial dilution plating technique.

C. Preparatory examining for the capability of fungal strains in wastewater decolourization

Initial testing of the fungi could be carried out by growing the fungal strains on sobrouse dextrose broth (SDB) agar containing 0.5% and 1% bamboo and sawdust powder at 28°C for 5 days. The growth of isolated fungi was sized up as colony diameter. All the statements and datum were collected from the experiments performed in triplicate. The strains that were capable of decolorizing the wastewater from pulp and paper industries would be theoretically able to be alive and grow up well on this agar.

The pure fungal cultures were cultivated on SDB broth with shaking speed of 150 rpm at 28°C for 5 days. After that, 1000ul broth containing fungal mycelium was inoculated into 100ml of SDB provided with 1% bamboo powder (pH 8.5) with shaking speed of 150 rpm at 28°C for 5 days. The strains that grew well and removed the color of broth well were chosen and examined for their effectiveness for decolourization of wastewater.

D. Culturing of isolated fungi for decolourization of effluent and degradation of lignin

The cultures of the fungal species were aseptically inoculated into 150 ml conical flasks (reactors) containing 100ml of effluent from pulp and paper industry added with SDB 30g per effluent 1L. The fungal strains were grown at 28°C with shaking speed of 150 rpm for 5 days.

E. Decolourization of effluent using isolated fungi

Pulp and paper industry effluent was inspected in a spectrophotometer to determine the wavelength and the maximum absorbance was detected at 450nm and the rate of decolourization was monitored at the wavelength. For color determination, the effluent sample was centrifuged at 1000 rpm for 30 min to move away all the suspended matter. The percentage of the color reduction is the ratio of Absorbance of uninoclulated broth (A)-Absorbance of residual broth by (A) multiply by 100 [7].

F. Degradation of lignin

The value of lignin content was calculated by the method of Pearl and Benson [8]. In this method, the sample (50 ml) was mixed with 1ml CH₃COOH (10per cent) and 1ml (NaNo₂; 10per cent) after adjusting the pH 7.NH₄OH (2 ml) was added and absorbance (OD) was measured at 450nm.The absorbance lignin (ppm) is the ratio of Absorbance by 0.000247.

III. RESULTS AND DISCUSSION

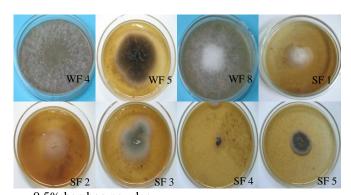
Three fungal strains (WF4, WF5 and WF8) were isolated on potato dextrose agar (PDA) plate by direct method and five fungal strains (SF1, SF2, SF3, SF4 and SF5) isolated on PDA plate by serial dilution method.

The eight fungal strains were hatched on SDB agar containing 0.5% and 1% bamboo powder (Figure 1) and

sawdust powder (Figure 2) at 28°C for 5 days to confirm their capability of growth in raw material from pulp and paper industry. (Table 1&2) illustrated that these five strains, WF4, WF5, WF8, SF1 and SF3 were able to grow well in this medium as observed by their growing mycelium whereas other strains could not grow on the same medium. So, these five fungal strains were selected to continue the following process.

After cultivation with shaking for 5 days, five fungal strains were growing very well and they reduced the color of medium broth .Among them, WF5, WF8 and SF3 removed above 50% the color of the medium (Figure 3). This brought about the result that when these three strains could be used for wastewater treatment.

The three selected strains were examined for their effectiveness on decolourization and delignification of wastewater. It was pointed that one out of three tested strains (WF5) (Figure 4) is the most efficient strain capable of lightening the color of wastewater by 58% (Figure 5) and removing the 5466ppm lignin of control into 3104ppm of WF5 (Figure 6).



0.5% bamboo powder

WF4

WF5

WF8

SF1

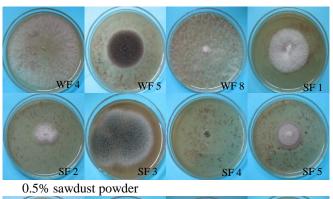
SF3

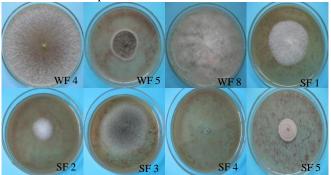
SF4

SF5

1% bamboo powder

Fig. 1. Capability of fungal strains growing on PDA agar containing bamboo powder





1% sawdust powder

Fig. 2. Capability of fungal strains growing on PDA agar containing sawdust powder

TABLE I. Growth Diameter of Isolated Fungi on PDA Supplemented with 0.5% and 1% Bamboo Powdered

Isol ates	Growth Diameter (cm) Mean±SD				
	0.5%		1%		
	5 Days after	8 Days after	5 Days after	8 Days after	
	Incubation	Incubation	Incubation	Incubation	
WF 4	9.0±0.0	9.0±0.0	9.0±0.0	9.0±0.0	
WF 5	4.6±1.1	9.0±0.0	3.1±1.3	9.0±0.0	
WF 8	9.0±0.0	9.0±0.0	9.0±0.0	9.0±0.0	
SF1	3.5±0.5	9.0±0.0	2.4±0.3	9.0±0.0	
SF2	3.3±0.3	9.0±0.0	2.2±0.3	9.0±0.0	
SF3	4.8±0.5	9.0±0.0	2.5±0.3	9.0±0.0	
SF4	0.7±0.3	0.7±0.3	1.0±0.3	1.0±0.3	
SF5	2.6±0.1	5.2±0.2	2.2±0.3	2.8±0.3	

TABLE II. . Growth Diameter of Isolated Fungi on PDA Supplemented with 0.5% and 1% Sawdust Powdered

Isol	Growth Diameter (cm)
ates	Mean±SD

		0.5%		1%	
		5 Days	8 Days	5 Days	8 Days
		after Incubation	after Incubation	after Incubation	after Incubation
4	WF	9.0±0.0	9.0±0.0	9.0±0.0	9.0±0.0
5	WF	4.7±0.2	9.0±0.	4.1±1.2	9.0±0.0
8	WF	9.0±0.0	9.0±0.0	9.0±0.0	9.0±0.0
	SF1	4.8±0.3	9.0±0.0	4.3±0.3	9.0±0.0
	SF2	3.1±0.1	9.0±0.0	1.9±0.2	9.0±0.0
	SF3	6.2±0.4	9.0±0.0	5.5±0.5	9.0±0.0
	SF4	0.7±0.3	0.7±0.3	0.4±0.1	0.4±0.1
	SF5	3.3±0.2	3.3±0.2	2.1±0.1	2.1±0.1

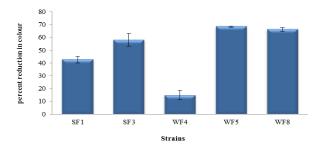


Fig. 3. Preliminary screening of fungal strains capable of wastewater decolourization

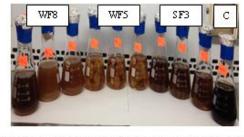


Fig. 4. Color removal of pulp and paper mill effluent using isolated fungi (C=control)

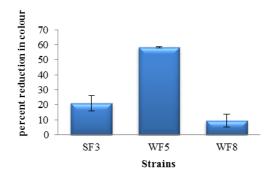


Fig. 4. Effectiveness of fungal strains on decolourization of treated effluent

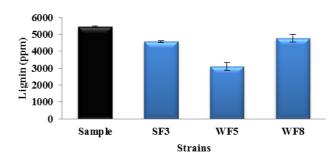


Fig. 5. Effectiveness of fungal strains on reduction of lignin of the treated effluent

IV. CONCLUSION

From the above examining, it can be concluded that fungal strains having decolorizing activity can be isolated from pulp and paper mill effluent sludge and wood decay. Preliminary screening showed that three strains (SF3, WF5 and WF5) were able to grow on SDB agar containing bamboo and sawdust powder by capable of wastewater decolourization 58, 68 and 66%, respectively. Among than, we have seen that WF5 causes the color of wastewater and reduction of lignin better than other fungus. It is reasonable to assume that WF5 should be used for wastewater treatment of pulp and paper mill effluent for further experimental studies.

V. ACKNOWLEDGMENT

The author would like to express her gratitude to Dr. Myo Myint, Associated Professor and Head, Department of Biotechnology and to her teachers from Department of Biotechnology at Mandalay Technological University. Finally, I especially acknowledge my great special thanks to my family.

REFERENCES

- [1] Bajpai P, Bajpai PK (1994) Biological colour removal of pulp and paper mill wastewaters. J Biotechnol 33:211-220
- [2] Bajpai, P., P.K Bajpai and R. Kondo. 2000. Biotechnology for Environmental Protection in the Pulp and Paper Industry. Springer-Verlag Berlin Heidelberg, New York. 65-85 p.
- [3] Kirk, T.K. and D. Cullen. 1998. Enzymology and Molecular Genetics of Wood Degradation by White-Rot Fungi. pp. 273-302. In Environmentally Friendly Technologies for the Pulp and Paper Industry. A.Y. Raymond and A. Masood. John Wiley & Sons, Inc.
- [4] Hatakka, A. (2001): Biodegradation of Lignin. In: Biopolymers, Vol. 1, Lignin, Humic Substances and Coal, M. Hofrichter and A. Steinbuchel, eds. Wiley-VCH, Weinheim, Germany, 129-180.
- [5] Evans, C.S., and Hedger, J.N. (2001): Degradation of plant cell wall polymers. In: Fungi in Bioremediation, G.M. Gadd, ed. Cambridge University Press, Cambridge. 1-27.
- [6] Kondo, R. 1998. Waste Treatment of Draft Effluent by White-Rot Fungi, pp. 515-539. In A.Y Raymond and A. Masood, (eds.). Environmentally Friendly Technologies for the Pulp and Paper Industry. John Wiley & Sons, Inc.
- [7] Thanga, V. S.G., P. Marimuthu and M. Maheswari, 1997. Degradation of azodyes by anaerobic consortia. In: 6th Natl. Symp. Environ. Tamil Nadu Agril. Univ., Coimbatore, pp: 91-98
- [8] Pearl I A & Benson H K, The determination of lignin in sulphide pulping liquor, Pap Trade J, 111 (1940) 35. Science, 1989.