Journal of Advances in Biology & Biotechnology
9(2): 1-5, 2016; Article no.JABB.27876
ISSN: 2394-1081

SCIENCEDOMAIN *international*
www.sciencedomain.org

Isolation and Screening of Fungi for Delignification of Paper Mill Effluent

Arifa Tahir[1], Mamoona Sarwar[1], Saima Aftab[1] and Shahid Raza[2*]

[1]Department of Environmental Science, Lahore College for Women University, Lahore, Pakistan.
[2]University of South Asia, Lahore, Pakistan.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2016/27876
Editor(s):
(1) Andrzej Kowalski, Department of Biochemistry and Genetics, Institute of Biology, Jan Kochanowski University, Kielce, Poland.
Reviewers:
(1) Jaime Solano, Universidad católica de Temuco, Chile.
(2) Nidhi Sahni, PCTE, Baddowal, Ludhiana, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/16086

Original Research Article

Received 23rd June 2016
Accepted 12th August 2016
Published 8th September 2016

ABSTRACT

Fifty fungi species were isolated from paper mill effluent i.e. *Penicillium* sp, *Trichoderma* sp, *Rhizopus* sp, *Mucor* sp, *Aspergillus* sp, and *Phanerochaete* sp. After screening of fungi, *Phanerochaete* sp, a white rot fungus was selected for delignification. The effect of different parameters on delignification was studied. It was found that pH (5), temperature (30℃), contact time (6 days) and moisture content (40%) were optimum for delignification by *Phanerochaete sp*. Different physicochemical parameters i.e., dissolved oxygen, turbidity, conductivity, chemical oxygen demand, biological oxygen demands were also determined.

Keywords: Phanerochaete; delignification; isolation; optimization.

1. INTRODUCTION

Pulp and paper mills are major energy and water exhaustive industries and it has been studied that 75% of water provided to these industries emerges in form of waste [1]. Treatment of this waste water is alarming for the environment because of its adverse effects. Physiochemical methods of treatment of industrial effluent are energy consuming as well as costly, so they are difficult to implement at industrial sites [2]. Researchers are therefore, using biological methods for the effluent treatment [3]. Biological delignification can be carried out with the help of

**Corresponding author: E-mail: dr.raza03@gmail.com;*

microorganisms as well as enzymes. There are several advantages of biological delignification including greater yield of product and low demand for energy [4]. It has been found that white rot fungi cause more efficient degradation of lignin as compared to other fungi through the supportive act of various lignolytic enzymes [5]. Several studies have been carried out to study pssulp delignification as well as bioremediation of effluent of paper mill using *Phanerochaete* [6]. *Phanerochaete* chrysosporium has also been involved in degradation of ligno-cellulosic materials as well as in soil remediation [7]. The objective of this study was optimization of different fermentation parameters for delignification using lignolytic fungi i.e., *Phanerochaete* sp.

2. MATERIALS AND METHODS

2.1 Sample Collection

Twenty wastewater samples were collected from Century Paper Industry near Phool Nagar, Lahore, Pakistan. These samples were taken in polypropylene bottles to avoid contamination and were bought to laboratory. Storage of samples was carried out at 4℃.

2.2 Analysis of Waste Water Samples

Samples were analyzed in terms of different physicochemical parameters. pH and temperature of sample was determined using portable pH and temperature meter (WTW Multi lab 540). Dissolved oxygen meter (WTW Multi lab 540) was used to calculate the amount of oxygen dissolved in wastewater samples. Turbidity was measured using a Hatch Model 2100N Ratio Turbid meter (Hatch Company, Loveland, CO). Electrical conductivity and Total Dissolved Solids were measured using WTW Ion Lab 02250017. COD of the wastewater samples were determined at 350 nm with a Hach DR/2000 spectrophotometer. BOD of the wastewater samples mg/L was carried out using standard methods [8]. Lignin concentration was determined using spectrophotometer [9].

2.3 Fungal Isolation and Screening

Malt Extract Agar (MEA) medium containing streptomycin (1.5 g/L) was used for isolation of fungi from waste water samples. The agar plates containing waste water samples were incubated at 30℃ for 72 hrs. Screening of fungus was done by growing fungal strain on media containing black liquor as lignin source at 30℃ for 10 days. The reduction in lignin content was determined using spectrophotometer.

2.4 Identification of Fungus

Identification of fungus was done on the basis of morphology i.e. hyphae, conidiophores, conidia etc. Morphological examination was performed using a light microscope equipped with a micrometer eyepiece with 400X magnification.

2.5 Optmization of Different Parameters for Delignification of Paper Mill Effluent by *Phaernerochaete* sp

2.5.1 Optimization of pH and temperature

pH concentration was optimized for lignin degradation by *Phanarochaete* at different ranges (2-6). Temperature was also optimized at different ranges (20-60℃).

2.5.2 Optimization of contact time and moisture content

Contact time (1-10 days) and moisture content (20-100%) was also optimized for delignification.

2.6 Lignin Degradation

The concentration of lignin in sample was determined using spectrophotometer. The absorbance was determined between 280-480 nm.

3. RESULTS

3.1 Physicochemical Characterization of Effluent Samples

The characteristics of effluent samples are given in Table 1. The concentration of lignin in these samples was found to be many times higher than levels permitted by the environmental legislation of NEQS and WHO.

3.2 Isolation and Screening of Fungi Capable of Delignification

Different fungi species were isolated from effluent using standard method by drop spore technique [10]. This technique was used to obtain samples on Malt Extract Agar media (pH 5-6) containing antibiotics (0.3 g/L penicillin and

1.3 g/L streptomycin). The agar plates were then incubated at 30℃ for 24 hours. Fungi isolated on Malt extract agar media are indicated in Table 2. The isolated fungi were further screened for delignification. Morphological examination was performed using a light microscope equipped with a micrometer eyepiece with 400X magnification. The strain capable of efficient delignification was identified as *Phanerochaete chrysosporium* which appeared white in color and it grows by producing thread like branching filaments known as hyphae as indicated in Fig. 1.

Fig. 1. *Phanerochaete chyrosporium* isolated on malt extract media

3.3 Optimization of Different Parameters for Delignification of Paper Mill Effluent

3.3.1 Optimization of pH

pH concentration was optimized at different ranges (2-6) as shown in Fig. 2. At pH 2 delignification efficiency was found to be 20%. A further increase in pH resulted in increase in delignification efficiency and at pH 5 maximum delignification upto 65% was obtained. Therefore, pH 5 was selected to be optimum for delignification by *Phanerochaete chyrsosprium.*

3.3.2 Optimization of temperature

Temperature was optimized at different ranges (20-60℃). At 15℃ delignification rate was found to be 30% and it increased with increasing temperature. At 30℃ maximum delignification of 75% was obtained as indicated in Fig. 3. A further increase in temperature leads to decrease in delignification rate and at 60℃ delignification efficiency was reduced to 12%.

3.3.3 Effect of contact time

Delignification by *Phanerochaete* sp was optimized at different time intervals (1-10 days). Delignification efficiency increased with time and maximum delignification efficiency of 90% was achieved after 6 days. However, further increase in time of incubation led to decrease in delignification efficiency as indicated in Fig. 4.

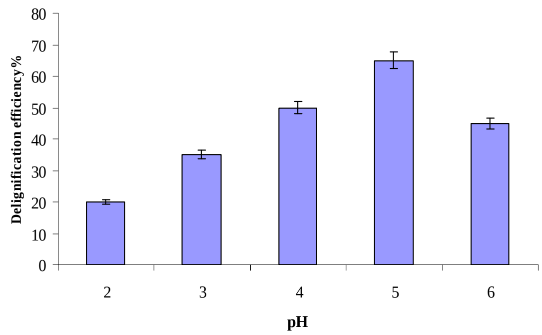

Fig. 2. Optimization of pH for delignification by *Phanerochaete chrysosporium.* Y error bars indicate standard deviation among three parallel replicates

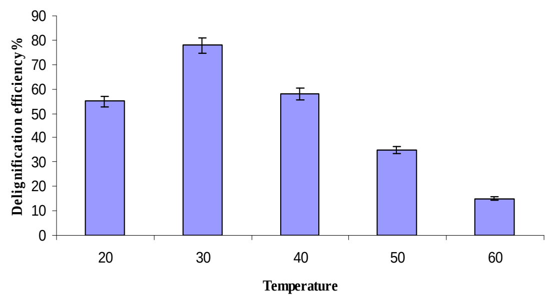

Fig. 3. Optimization of temperature for delignification by *Phanerochaete chrysosporium* at pH 5 and temperature 60℃. Y error bars indicate standard deviation among three parallel replicates

Table 1. Physicochemical analysis of effluent samples collected from century paper mill

Sr. no.	Parameters	Values	NEQ'S
1	pH	7.33	6 – 10
2	Temperature	60℃	40℃
3	Dissolved oxygen	12 ppm	8.7 ppm
4	Turbidity	109	-
5	Conductivity	3.34	-
6	Chemical oxygen demand	170 mg/L	150 mg/L
7	Biological oxygen demand	70 mg/L	< 30 mg/L
8	Lignin	50 gm/kg	< 20 gm/kg

Table 2. Different fungal species isolated from effluent samples and their morphological characteristics

Fungal culture isolated	Total no of isolates	Macroscopic characteristics
Penicillium sp	6	Slow growing grey smooth mycelial mat
Trichoderma sp	5	Green and yellow, fast growing mycelia
Rhizopus sp	4	White, fast growing cottony mass of mycelia
Mucor sp	5	Dark green, slow growing, smooth mycelial mass
Aspergillus sp	8	Moderate growing strain with dark black spores
Phanerochaete sp	10	White, fast growing mycelia with filamentous thread like structure

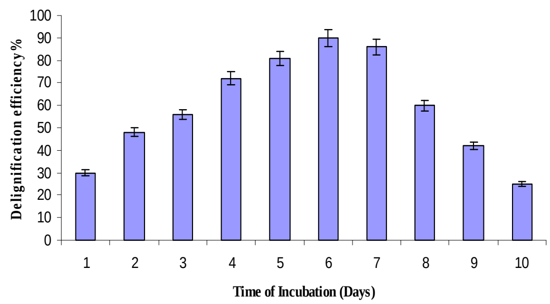

Fig. 4. Optimization of contact time for delignification by *Phanerochaete chrysosporium* at pH 5 and temperature 60℃. Y error bars indicate standard deviation among three parallel replicates

3.3.4 Effect of moisture conditions

Moisture content was optimized at different ranges (20-100%). Maximum delignification rate (92%) was obtained at moisture content of 40%. Further increase in moisture content led to decrease in moisture content as indicated in Fig. 5.

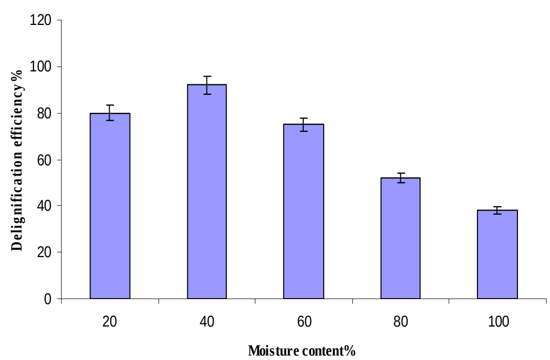

Fig. 5. Optimization of moisture content for delignification by *Phanerochaete chrysosporium* at pH 5 and temperature 60℃. Y error bars indicate standard deviation among three parallel replicates

4. DISCUSSION

The research work was conducted to examine and remove the lignin contents in waste water from Century Paper Mill using fungal sp. Physicochemical analysis revealed that effluent samples were acidic and have high lignin content of 50 g/kg. *Phanerochaete chrysosporium* was found to be potential bio degraders of lignin. Culture conditions affecting lignin degradation by *Phanerochaete chrysosporium* were examined. Delignification efficiency was greatly influenced by pH. The value of pH 5 was found to be optimum for lignin degradation. Similar results were reported [11] who suggested pH 5 to be optimum for growth of *Phanerochaete chrysosporium*. [11] also suggested that pH in range of 4-8 are most appropriate for effluent treatment. Therefore, acidic pH was favorable for growth of *Phaeneochaete*. Variation in temperature also influences delignification efficiency. Maximum lignin removal was obtained at temperature of 30℃. Any further increase in temperature reduced delignification efficiency. This might be due to fact that high temperature is unfavorable for fungal growth. Contact time also play a fundamental role in delignification process. Lignin content in effluent was reduced to 90% after 6 days of incubation. However after 6 days a decrease in delignification efficiency was observed. This might be due to fact that production of secondary metabolites resulted in decrease in delignification [12]. The moisture content was a key factor for fungal growth. Too low moisture contents limit fungal delignification without providing sufficient water to fungal growth. Higher moisture contents cause clogging inter-particle spaces, limited oxygen circulation, often inhibited aerobic solid state cultivation and increased susceptibility to bacterial contamination [13].

5. CONCLUSION

It was concluded from the current investigation that the fungi *Phanerochaete* sp. displayed tremendous removal potential for lignin content from industrial effluent. The effects of different parameters were studied. The results of this research work clearly showed that the optimization of various parameters maximize the lignin removal efficiency of the fungi. *Phanerochaete chrysosporium* could be used for biodegradation of waste.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumar V, Dhall P, Naithani S, Kumar A, Kumar R. Biological approach for the treatment of pulp and paper industry effluent in sequence batch reactor. Journal of Bioremediation and Biodegradation. 2014;5:218.
2. Singh P, Mishra LC, Iyengar L. Biodegradation of 4-aminobenezesulphonate by a newly isolated bacterial strain PNS-1. World Journal of Microbiology and Biotechnology. 2004;20:845-849.
3. Nagarathnamma R, Bajpai P, Bajpai PK. Studies on decolourization, degradation and detoxification of chlorinated lignin compounds in kraft bleaching effluents by ceriporiopsis subvermispora. Process Biochemistry. 1999;34:939-948.
4. Lee J. Biological conversion of lignocellulosic biomass to ethanol. Journal of Biotechnology. 1997;56:1-24.
5. Karp SG, Faraco V, Amore A, Letti LAJ, Soccol VT, Soccol CR. Statistical optimization of laccase production and delignification of sugarcane bagasse by *Pleurotus ostreatus* in solid state fermentation. Biomed Res Int. 2015;Article ID 181204.
6. Saritha M, Arora A, Nain L. Biological pretreatment of lignocellulosic substrates for enhanced delignification and enzymatic digestibility. Indian Journal of Microbiology. 2012;52(2):122-130.
7. Reddy HKY, Srijana M, Reddy MD, Reddy G. Coculture fermentation of banana agro-waste to ethanol by cellulolytic thermophilic *Clostridium thermocellum* CT2. African Journal of Biotechnology. 2010;9:1926–1934.
8. Lokeshwari N, Srinikethan G, Joshi SG, Shasikala I, Srikanth B, Ahmed B, Sushma L. Isolation and screening of fungi for aerobic delignification and reduction of AOX of pulp and paper mill effluent. International Journal of Biological Sciences and Engineering. 2013;7(12).
9. Ademoroti CMO. Standard methods for water and effluent analyis. Foludex Press Ltd, Ibadan. 1996;3:29-118.
10. Kheiralla ZH, Badr El-Din SM, Abdel Malik SM, Abdel Aziz DH. Optimization of cultural conditions for lignin peroxidase production by *Phanerochaete chrysosporium* and *Pleurotus ostreatus*. Academia Journal of Biotechnology. 2013; 1(6):87-95.
11. Lara MA, Rodríguez OJ, Holmquist O, Gonzalez AM, Bullona J, Penaloza N, Araujo E. Blackliqour lignin degradation by *Trametes elegans*. Int Biodeter Biodegr. 2003;52:167-17.
12. Freitas AC, Ferreira F, Costa AM, Pereira R, Antunes SC, Gonçalves F, Rocha-Santos TAP, Diniz MS, Castro L, Peres I, Duarte AC. Biological treatment of the effluent from a bleached kraft pulp mill using basidiomycete and zygomycete fungi. Sci Total Environ. 2009;407:3282-3289.
13. Shi J, Chinn MS, Sharma-Shivappa RR. Microbial pretreatment of cotton stalks by solid state cultivation of *Phanerochaete chrysosporium*. Bioresource Technology. 2008;99:6556–6564.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/16086