

**STUDIES ON FUNGI MEDIATED SOLUTIONS FOR
FABRIC DESIZING AND DYE DEGRADATION IN
TEXTILE INDUSTRIES**

SHWETA KALIA



CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

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STUDIES ON FUNGI MEDIATED SOLUTIONS FOR FABRIC DESIZING AND DYE DEGRADATION IN TEXTILE INDUSTRIES

by

SHWETA KALIA

Centre for Rural Development and Technology

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CERTIFICATE

This is to certify that the thesis entitled '**Studies on fungi mediated solutions for fabric desizing and dye degradation in textile industries**' being submitted by **Ms. Shweta Kalia** to the Indian Institute of Technology Delhi for the award of '**Doctor of Philosophy**' is a record of bonafide research work carried out by her. She has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis. To the best of my knowledge the results contained in this thesis have not been submitted in part or full to any other university or institute for award of any degree or diploma.



Prof. Anushree Malik

Professor

Centre for Rural Development and Technology

Indian Institute of Technology Delhi

New Delhi-110016

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Shweta Kalia

ABSTRACT

The present study has attempted to provide a bio-based solution for the problems associated with textile industry. Different fungal mediated solutions were explored to provide an alternative to chemical desizing process and to treat the dye effluents generated from textile industry upto the Central Pollution Control Board (CPCB) discharge limits. To begin with, a visit to the textile industry was undertaken to understand the wet processing units of fabric manufacturing and identify the wastes generated from the industry. Various wastes such as solid wastes, starch effluent and dyes/dye effluents were procured and characterized. Different agro-wastes were also procured from nearby places and characterized as substrate for enzyme production through solid-state fermentation (SSF) for suitable application.

Further, screening of various fungal species for amylase production through SSF was done. *Trichoderma reesei* showed highest amylase production (19.22 U mL⁻¹ amylase activity on 7 d) using wheat bran as a substrate, which further enhanced to 25.48 U mL⁻¹ when supplemented with starch effluent generated from textile industry as an additional carbon source in SSF. Further, partial purification of crude amylase produced a yield of 58.39% with purification fold of 1.87. The optimum pH and temperature for amylase activity was 5.0 and 80 °C, respectively. The enzyme was thermally stable at 40 °C with 90% residual activity after 5 h and 70% residual activity at 50 °C after 3 h. The half-life and D-value of amylase was 6931.47 min and 23025.85 min, respectively at 40 °C. Using Michaelis-Menten kinetics analysis, the estimated K_m and V_{max} values for the partially purified amylase were found to be 4.7 mg mL⁻¹ and 18.65 U mL⁻¹, respectively. The cotton fabric was efficiently desized using amylase at 80 °C. The utilization of starch effluent as a growth supplement for fungal enzyme production seems to be an efficient way of resource recovery from the industrial wastewater stream. The in-house amylase production using effluents could eliminate the need for amylase procurement and its transportation cost.

Next, laccase production potential of *T. reesei* and *Pleurotus sajor caju* was studied. *P. sajor caju* showed 77.77 U mL⁻¹ laccase activity on 7 d using wheat bran and saw dust in 1:1 ratio as substrate in SSF. Partially purified laccase showed the optimum pH and temperature 3.0 and 30 °C, respectively and it was thermally stable at 30-40 °C retaining >85% residual activity after 6 h. The half-life and D-value of laccase was 6931.47 min and 23025.85 min, respectively at 30 °C. Using Michaelis-Menten kinetics analysis, the estimated K_m and V_{max} values for the partially purified amylase were found to be 0.07 mM and 43.47 U mL⁻¹, respectively. Partially purified laccase was further utilized for the treatment of textile dyes and effluents.

Different fungal based methods were used for decolorization of Reactive blue 13, Reactive yellow 176, Reactive red 198 and Reactive black 5 dyes (100-500 mg L⁻¹) and effluents. *T. reesei* having amylase production potential showed 86-94% removal of all Reactive dyes (at 100 mg L⁻¹ concentration) in 48 h using minimal salt media for growth. The dye uptake capacity increased with increase in dye concentration from 100 mg L⁻¹ (21-31 mg g⁻¹) to 500 mg L⁻¹ (113-139 mg g⁻¹). The optimum concentration of glucose (5 g L⁻¹) and yeast extract (2.5 g L⁻¹) to achieve maximum dye decolorization was estimated followed by use of starch effluent as an alternative nutrient source for growth of *T. reesei*. Starch effluent spiked

with 3.5 g L⁻¹ glucose showed >85% decolorization of Reactive blue 13 (100-200 mg L⁻¹). Thus, starch effluent could bring down the amount of glucose and salts required for fungal growth and dye decolorization. On the other hand, *P. sajor caju* showed 75-85% decolorization of only Reactive blue 13 and Reactive black 5 in 8 d at 100 mg L⁻¹ concentration. The uptake capacity of *P. sajor caju* increased with increase in dye concentration from 100 mg L⁻¹ (29-31 mg g⁻¹) to 500 mg L⁻¹ (183-326.79 mg g⁻¹). As opposed to biosorption mediated phenomenon in *T. reesei*; the mechanism of decolorization in case of *P. sajor caju* was degradation mediated by laccase (39 U mL⁻¹). Both fungal species showed efficient decolorization of dyes, but could not decolorize textile effluents due to high toxicity, presence of alkalis, surfactants etc. that inhibit fungal growth. The spent fermented waste after laccase production by *P. sajor caju* was also utilized for decolorization of textile dyes and effluents. Spent fermented waste caused 63-87% dye (100 mg L⁻¹) removal in 60 min and 45-60% decolorization of textile effluents (10-50% v/v concentration) in 24 h. However, the secondary color of agro-wastes was released in the treated effluents. Hence, partially purified laccase (43 U mL⁻¹) produced from *P. sajor caju* was used for decolorization of textile dyes and effluents. It showed 51-89% dye (100 mg L⁻¹) removal in 100 min and 19-50% decolorization of textile effluents at 10-50% (v/v) concentration in 72 h at pH 3.0, 30 °C. Integrating the results from FTIR, HPLC and GC-MS of the dye and the degraded metabolites, enzymatic degradation pathway for Reactive blue 13 was proposed.

Due to incomplete decolorization of textile effluents, there emerged a need to combine the enzymatic process with a physical process. Electrocoagulation alone showed 86-93% decolorization of undiluted textile effluent under optimal conditions (pH 9.0 using zinc-coated iron electrode at current density 70 mA cm⁻²). However, it generated large amount of sludge (2.1-6.25 g L⁻¹) from both the textile effluents. To achieve complete decolorization and minimum sludge generation, Hybrid laccase treatment-electrocoagulation (LT-EC) process and Hybrid electrocoagulation-laccase treatment (EC-LT) process was used for both textile effluents. Hybrid LT-EC (25 mA cm⁻²) process showed 51-68% decolorization of 50% diluted textile effluents at pH 3.0 as compared to 39-43% decolorization achieved by only electrocoagulation. Hybrid EC (25 mA cm⁻²)-LT process showed 58-81% decolorization of undiluted textile effluent at ambient pH as compared to 19-46% decolorization using only laccase treatment. The sludge generation in hybrid processes reduced to 0.5-2.72 g L⁻¹. Further, to remove the residual color left after hybrid process and achieve the color (hazen) as per the CPCB discharge limits, hybrid processes were integrated with activated charcoal (AC) unit. The decolorization of textile effluent by Hybrid EC-LT process integrated with AC unit was 90.67% and Hybrid LT-EC process integrated with AC unit was 78.77%. Hybrid EC-LT integrated with AC unit was able to efficiently treat undiluted textile effluent to acceptable level (140 hazen). Overall, the results of this study demonstrated applicability of fungal mediated bio-base solutions for the textile industry.

सार

वर्तमान अध्ययन में कपड़ा उद्योग से जुड़ी समस्याओं के लिए जैव-आधारित समाधान प्रदान करने का प्रयास किया गया है। रासायनिक डिसाइजिंग प्रक्रिया का विकल्प प्रदान करने के लिए और केंद्रीय प्रदूषण नियंत्रण बोर्ड (सीपीसीबी) की निर्वहन सीमा तक कपड़ा उद्योग से उत्पन्न टेक्सटाइल एफ्लुएंट का उपचार करने के लिए विभिन्न कवक मध्यस्थता समाधानों की खोज की गई। सबसे पहले, कपड़ा निर्माण की गीली प्रसंस्करण इकाइयों को समझने और उद्योग से उत्पन्न कचरे की पहचान करने के लिए कपड़ा उद्योग का दौरा किया गया था। विभिन्न अपशिष्ट जैसे ठोस अपशिष्ट, स्टार्च बहिःस्राव और डाई/डाई बहिःस्राव को प्राप्त किया गया और उनका गुणन किया गया। विभिन्न कृषि-अपशिष्ट भी आस-पास के स्थानों से खरीदे गए और उपयुक्त अनुप्रयोग के लिए ठोस-अवस्था किण्वन (एसएसएफ) के माध्यम से एंजाइम उत्पादन के लिए सबस्ट्रेट के रूप में चिह्नित किए गए।

इसके अलावा, एसएसएफ के माध्यम से एमाइलेज उत्पादन के लिए विभिन्न कवक प्रजातियों की जांच की गई। ट्राइकोडर्मा रीसी ने सबस्ट्रेट के रूप में गेहूं के चोकर का उपयोग करते हुए उच्चतम एमाइलेज उत्पादन (19.22 U mL^{-1} एमाइलेज गतिविधि 7वें दिन) दिखाया, जो एसएसएफ में एक अतिरिक्त कार्बन स्रोत के रूप में कपड़ा उद्योग से उत्पन्न स्टार्च प्रवाह के साथ पूरक होने पर 25.48 U mL^{-1} तक बढ़ गया। इसके अलावा, अपरिष्कृत एमाइलेज के आंशिक शुद्धिकरण ने 1.87 के शुद्धिकरण गुणा के साथ 58.39% की उपज का उत्पादन किया। एमाइलेज गतिविधि के लिए इष्टतम पीएच और तापमान क्रमशः 5.0 और 80°C था। एंजाइम 40°C पर 5 h के बाद 90% अवशिष्ट गतिविधि और 3 h के बाद 50°C पर 70% अवशिष्ट गतिविधि के साथ थर्मल रूप से स्थिर था। एमाइलेज की हाफ लाइफ और डी- मूल्य क्रमशः 6931.47 min और 23025.85 min था, जो 40°C पर था। माइकलिस-मेंटेन कैनेटीक्स विश्लेषण का उपयोग करते हुए, आंशिक रूप से शुद्ध एमाइलेज के लिए अनुमानित K_m और V_{max} मूल्य क्रमशः 4.7 mg mL^{-1} और 18.65 U mL^{-1} पाए गए। 80°C पर एमाइलेज का उपयोग करके सूती कपड़े को डिसाइज किया गया। फंगल एंजाइम उत्पादन के लिए वृद्धि के पूरक के रूप में स्टार्च प्रवाह का उपयोग औद्योगिक अपशिष्ट जल धारा से रीसोर्स रीकवरी का एक कुशल तरीका प्रतीत होता है। बहिःस्रावों का उपयोग करते हुए इन-हाउस एमाइलेज उत्पादन, एमाइलेज खरीद की आवश्यकता और इसकी परिवहन लागत को समाप्त कर सकता है।

इसके बाद, टी. रीसी और प्लुरोटस साजोर काजू की लैकेस उत्पादन क्षमता का अध्ययन किया गया। पी. सजोर काजू ने गेहूं के चोकर और लकड़ी के बुरादा (1:1) को सबस्ट्रेट के रूप में उपयोग करते हुए 7वें दिन 77.77 U mL^{-1} लैकेस गतिविधि दिखाई। आंशिक रूप से शुद्ध किए गए लैकेस (partially purified laccase) ने क्रमशः इष्टतम पीएच और तापमान 3.0 और 30°C दिखाया और यह 6 h के बाद $30-40^\circ\text{C}$ पर ऊष्मीय रूप ($>85\%$ अवशिष्ट गतिविधि) से स्थिर था। लैकेस का हाफ लाइफ और डी- मूल्य क्रमशः 6931.47 min और 23025.85 min था, जो 30°C पर था। माइकलिस-मेंटेन कैनेटीक्स विश्लेषण का उपयोग करते हुए, आंशिक रूप से शुद्ध एमाइलेज के लिए अनुमानित K_m और V_{max} मूल्य क्रमशः 0.07 mM और 43.47 U mL^{-1} पाए गए। आंशिक रूप से शुद्ध किए गए लैकेस का उपयोग कपड़ा रंगों और अपशिष्टों के उपचार के लिए किया गया था।

रिएक्टिव ब्लू 13, रिएक्टिव येलो 176, रिएक्टिव रेड 198 और रिएक्टिव ब्लैक 5 डाई ($100-500 \text{ mg L}^{-1}$) और टेक्सटाइल एफ्लुएंट के रंग हटाने के लिए विभिन्न कवक आधारित विधियों का उपयोग किया गया। एमाइलेज उत्पादन क्षमता वाले टी. रीसी ने विकास के लिए न्यूनतम नमक (salt) मीडिया का उपयोग करके 48 h में सभी प्रतिक्रियाशील रंगों का 100 mg L^{-1} एकाग्रता पर 86-94% डीकोलेराइजेशन। डाई अपटेक क्षमता 100 mg L^{-1} ($21-31 \text{ mg g}^{-1}$) से 500 mg L^{-1} ($113-139 \text{ mg g}^{-1}$) तक डाई एकाग्रता में वृद्धि के साथ बढ़ी। ग्लूकोज (5 g L^{-1}) और यीस्ट एक्सट्रैक्ट (2.5 g L^{-1}) की अधिकतम सांद्रता को अधिकतम डाई डीकोलेराइजेशन प्राप्त करने के लिए अनुमान लगाया गया था, इसके बाद टी रीसी के विकास के लिए वैकल्पिक पोषक स्रोत के रूप में स्टार्च प्रवाह का उपयोग किया गया था। 3.5 g L^{-1} ग्लूकोज के साथ स्टार्च प्रवाह ने रिएक्टिव ब्लू 13 ($100-200 \text{ mg L}^{-1}$) का $>85\%$ डीकोलेराइजेशन दिखाया गया। इस प्रकार, स्टार्च बहिःस्राव कवक के विकास और डाई डीकोलेराइजेशन के लिए आवश्यक ग्लूकोज और लवण की मात्रा को कम कर सकता है। दूसरी ओर, पी. सजोर काजू ने 100 mg L^{-1} एकाग्रता पर रिएक्टिव ब्लू 13 और रिएक्टिव काले 5 ने 8 दिन में 75-85% डीकोलेराइजेशन दिखाया। पी. सजोर काजू की अपटेक क्षमता 100 mg L^{-1} ($29-31 \text{ mg g}^{-1}$) से 500 mg L^{-1} ($183-326.79 \text{ mg g}^{-1}$) तक

g⁻¹) तक डाई एकाग्रता में वृद्धि के साथ बढ़ी। टी. रीसी में बायोसॉरशन की मध्यस्थता वाली घटना के विपरीत; पी. सजोर काजू के मामले में डीकोलेराइज़ेशन की क्रियाविधि लैकेस (39 U mL⁻¹) द्वारा मध्यस्थता में गिरावट थी। दोनों कवक प्रजातियों ने रंगों का कुशल विवर्णीकरण दिखाया, लेकिन उच्च विषाक्तता, क्षार, सर्फेक्टेंट आदि की उपस्थिति के कारण टेक्सटाइल एफ्लुएंट का डीकोलेराइज़ेशन नहीं कर सका, जो कवक के विकास को रोकते हैं। पी. सजोर काजू द्वारा लैकेस उत्पादन के बाद किण्वित कचरे (spent fermented waste) का उपयोग कपड़ा रंगों और टेक्सटाइल एफ्लुएंट के डीकोलेराइज़ेशन के लिए भी किया गया था। किण्वित कचरे (spent fermented waste) ने 60 min में 63-87% डाई (100 mg L⁻¹) डीकोलेराइज़ेशन किया और टेक्सटाइल एफ्लुएंट (10-50% v/v) का 24 h में 45-60% डीकोलेराइज़ेशन किया। हालांकि, उपचारित टेक्सटाइल एफ्लुएंट में कृषि-अपशिष्ट का द्वितीयक रंग (secondary color) पाया गया था। इसलिए, पी. सजोर काजू से उत्पादित आंशिक रूप से शुद्ध लैकेस (43 U mL⁻¹) का उपयोग कपड़ा रंगों और टेक्सटाइल एफ्लुएंट के डीकोलेराइज़ेशन के लिए किया गया था। इसने पीएच 3.0, 30 °C पर 51-89% डाई (100 mg L⁻¹) डीकोलेराइज़ेशन 100 min और टेक्सटाइल एफ्लुएंट (10-50% v/v) का 19-50% डीकोलेराइज़ेशन 72 h पर किया। एफटीआईआर, एचपीएलसी और डाई के जीसी-एमएस और अवक्रमित मेटाबोलाइट्स के परिणामों को एकीकृत करते हुए, रिएक्टिव ब्लू 13 के लिए एंजाइमेटिक डिग्रेडेशन मार्ग प्रस्तावित किया गया था।

टेक्सटाइल एफ्लुएंट के अधूरे डीकोलेराइज़ेशन होने के कारण, एंजाइमी प्रक्रिया को एक भौतिक प्रक्रिया के साथ संयोजित करने की आवश्यकता उत्पन्न हुई। अकेले इलेक्ट्रोकोएग्यूलेशन ने इष्टतम स्थितियों (वर्तमान घनत्व 70 mA cm⁻² पर जस्ता-लेपित लोहे (zinc-coated iron) इलेक्ट्रोड का उपयोग करके पीएच 9.0) के तहत अविरलित टेक्सटाइल एफ्लुएंट का 86-93% विवर्णीकरण दिखाया। हालांकि, इसने दोनों टेक्सटाइल एफ्लुएंट से बड़ी मात्रा में कीचड़ (sludge) (2.1-6.25 mg L⁻¹) उत्पन्न किया। पूरी तरह से रंग हटाने और न्यूनतम कीचड़ (sludge) उत्पादन को प्राप्त करने के लिए, हाइब्रिड लैकेस ट्रीटमेंट-इलेक्ट्रोकोएग्यूलेशन (एलटी-ईसी) प्रक्रिया और हाइब्रिड इलेक्ट्रोकोएग्यूलेशन-लैकेस ट्रीटमेंट (ईसी-एलटी) प्रक्रिया का उपयोग दोनों टेक्सटाइल एफ्लुएंट के लिए किया गया था। हाइब्रिड एलटी-ईसी (25 mA cm⁻²) प्रक्रिया ने पीएच 3.0 पर 50% पतला (diluted) टेक्सटाइल एफ्लुएंट का 51-68% डीकोलेराइज़ेशन दिखाया, जबकि केवल इलेक्ट्रोकोएग्यूलेशन ने तुलना में 39-43% डीकोलेराइज़ेशन दिखाया। हाइब्रिड ईसी (25 mA cm⁻²)-एलटी प्रक्रिया ने केवल लैकेस उपचार का उपयोग करते हुए 19-46% डीकोलेराइज़ेशन की तुलना में परिवेशी पीएच पर बिना पतला (diluted) टेक्सटाइल एफ्लुएंट का 58-81% डीकोलेराइज़ेशन दिखाया। संकर प्रक्रियाओं में कीचड़ (sludge) का उत्पादन घटकर 0.5-2.72 g L⁻¹ हो गया। इसके अलावा, हाइब्रिड प्रक्रिया के बाद बचे अवशिष्ट रंग को हटाने और सीपीसीबी डिस्चार्ज सीमा के अनुसार रंग (हेज़ेन) प्राप्त करने के लिए, हाइब्रिड प्रक्रियाओं को सक्रिय चारकोल (एसी) इकाई के साथ एकीकृत किया गया था। एसी यूनिट के साथ एकीकृत हाइब्रिड ईसी-एलटी प्रक्रिया द्वारा टेक्सटाइल एफ्लुएंट का डीकोलेराइज़ेशन 90.67% था और एसी इकाई के साथ एकीकृत हाइब्रिड एलटी-ईसी प्रक्रिया 78.77% थी। एसी यूनिट के साथ एकीकृत हाइब्रिड ईसी-एलटी, अविरलित टेक्सटाइल एफ्लुएंट को स्वीकार्य स्तर (140 हेज़ेन) तक प्रभावी ढंग से ट्रीट करने में सक्षम था। कुल मिलाकर, इस अध्ययन के परिणामों ने कपड़ा उद्योग के लिए कवक की मध्यस्थता वाले जैव-आधार समाधानों की प्रयोज्यता का प्रदर्शन किया।

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