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EVALUATION OF *Rhizopus oryzae* POLYGALACTURONASE FOR BIO-SCOURING OF COTTON

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ABSTRACT

This study investigates the use of *Rhizopus oryzae* polygalacturonase (PGase) for cotton fabric pretreatment, aiming to reduce water pollution caused by traditional chemical methods. *R. oryzae* was cultivated in different media to determine optimal growth conditions for increased PGase production. *R. oryzae* PGase exhibited optimal activity at 40°C and pH 5. The concentrated enzyme was used for scouring cotton fabric. Ultrasonic treatment further reduced absorbency time indicating efficient removal of impurities from the fabric. This research showcases a sustainable approach to textile processing, blending enzymatic treatments with innovative physical techniques for enhanced efficiency and reduced environmental impact.

KEYWORDS: Enzyme Activity, Pectinase, Polygalacturonase, *Rhizopus oryzae*, Salt Glycerol Pectin Broth

The textile industry is at a decision-making stage to reconcile its manufacturing processes with environmental sustainability. Traditional textile processing methods heavily depend on hazardous chemical treatments, which presents significant challenges in environmental pollution, energy consumption and health hazards (Kadam A., 2015). In response to these concerns, industries are focusing on developing eco-friendly alternatives that minimise the ecological footprint of textile production.

One eco-friendly practice that garners a lot of interest is the enzyme-based pretreatment of textile materials. Enzymes gained importance in the textile industry because of their higher specificity, milder usage conditions, and eco-friendliness when the local governments imposed stricter limitations on releasing pollutants. Today, enzymes are an integral part of textile processing. Enzymes find various applications such as de-sizing, scouring, bleaching, mercerisation, peroxide neutralisation, bio-polishing, and fading of denim (Reddy and Sharma, 2011).

Enzymes are obtained from three primary sources: animal tissue, plants and microbes through fermentation. Fungi such as *Aspergillus spp.*, *Trichoderma spp.* and *Streptomyces spp.* and bacteria such as *Bacillus spp.* are used to produce many industrial enzymes. Different types of enzymes are used in the textile industry, such as amylase, pectinase, catalase and peroxidase (Mojsov K., 2011).

Among the enzymes explored for bio-scouring applications, polygalacturonase stands out for its ability to hydrolyse pectin, a key component of non-cellulosic impurities on natural fibres (Kunamneni A., 2002). *Rhizopus oryzae*, a filamentous fungus ubiquitous in soil and decaying organic matter, has attracted attention as a source for polygalacturonase production (Chowdhury *et al.*, 2017). In parallel, the integration of ultrasonic technology with enzymatic bio-scouring has emerged as a promising strategy to enhance the efficiency and efficacy of the scouring process (Ashokkumar M., 2007; Eren and Erişmiş, 2013; Yachmenev *et al.*, 2004). Ultrasonic waves, characterised by high-frequency mechanical vibrations, facilitate the penetration of enzymes into the fibre structure, thereby accelerating the removal of impurities (Easson *et al.*, 2018; Wang *et al.*, 2012; Wang L., 2010). The synergistic effects of ultrasound-assisted bio-scouring result in reduced processing times, improved scouring efficiency, and enhanced fibre properties (Bhushan and Anand, 2017).

The polygalacturonase enzyme obtained from *R. oryzae* has been studied for use in the food and bio-based industries but not in the textile industry. This study investigates the use of polygalacturonase enzyme synthesized from *R. oryzae* for bio-scouring of cotton knitted single jersey fabric. Enzyme production was characterized and optimized, and the effectiveness of the bio-scouring process was evaluated in comparison with the conventional high-temperature alkaline process. By combining the enzymatic action of polygalacturonase with the enhancing effects of ultrasonic technology, a green, bio-based process for the pre-treatment of cotton single jersey fabric has been developed.

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MATERIALS AND METHODS

Media and Growth Conditions

Rhizopus oryzae MTCC1987 was procured from Microbial Type Culture Collection, Chandigarh, India and routinely cultivated on potato dextrose broth (PDB) (Himedia) at 30°C in a rotatory shaker at 150 rpm. Agar was added to PDB when required at a concentration of 2%. Spores were enumerated using a Neubauer chamber under an Olympus OLS5100 3D laser microscope. Pectin was obtained from Loba Chemie Pvt. Ltd., India. Green Boost Scouring enzyme preparation and concentrated pectinase enzyme were obtained from Rossari Biotech Ltd., India. The wetting agent (Sarawet NF) was obtained from Sarex, while the sequestering agent (Verolan SE) was obtained from Rudolf Atul Chemicals Ltd. Single jersey kitted grey cotton fabric was obtained from a local vendor.

Polygalacturonase Enzyme Production

Production of polygalacturonase was assessed by cultivating *R. oryzae* in different media. The media used were Potato Dextrose Pectin (PDP; PDB supplemented with 10 g/L pectin), Salt Pectin (SP; 3 g/L $(NH_4)_2HPO_4$, 2 g/L KH_2PO_4, 3 g/L K_2HPO_4, 0.1 g/L $MgSO_4$, 10 g/L pectin), Salt Xylose Pectin (SXP; SP supplemented with 10 g/L xylose), Salt Glycerol Pectin (SGP; SP supplemented with 10 g/L glycerol), and Yeast extract Pectin (YP; 2 g/L $(NH_4)_2HPO_4$, 2 g/L KH_2PO_4, 2 g/L K_2HPO_4 , 3 g/L yeast extract, 5 g/L pectin). 50 ml media were inoculated with approximately 3.5×10^6 spores, and the flasks were incubated at 30°C in a rotary shaker at 150 rpm for 5-6 days. Each day, a 1 ml sample was drawn and used to determine enzyme activity using dinitrosalicyclic acid (DNSA) reagent.

Enzyme Assay

PGase activity was determined using pectin as substrate and measuring the amount of galacturonic acid formed. DNSA reagent forms a coloured complex with galacturonic acid that can be measured using a spectrophotometer at 540 nm (Chowdhury *et al.*, 2017).

The reaction mixture consisted of 0.8 ml substrate (0.5% pectin solution) and 0.2 ml culture supernatant. The reaction was carried out for 30 min at 37°C. Following incubation, 1 ml DNSA reagent was added, followed by boiling at 100°C for 10 minutes. The coloured complex thus formed was diluted and measured.

Concentration of Enzyme

Ammonium sulphate was added to the culture supernatant to achieve 80% saturation and left undisturbed at 4°C overnight. The precipitate obtained by centrifuging at 3900 rpm for 20 min was dissolved in acetate buffer (pH 5.5) and dialysed using a 70 kDa membrane (Himedia). Sodium azide (0.1%) was added as preservative.

Enzyme Characterisation

Characterisation of polygalacturonase enzyme was performed to determine its optimum temperature and pH. For the determination of optimum temperature, enzyme assay was conducted at pH 6.5 and at six different temperatures: 30°C, 40°C, 50°C, 60°C, 70°C and 80°C. The optimum pH for enzyme activity was determined by conducting the enzyme assay at optimum temperatures at pH 4, 5, 6, 7 and 8.

Scouring of Cotton Knit

Bio-scouring was carried out using 3 g/l enzyme, 2 g/l wetting agent, 1 g/l sequestering agent and material to liquor ratio (MLR) of 1:20. Reaction was carried out for 30 min at 60°C. A bath sonicator (Labman Scientific Instruments, India) operating at a frequency of 40 KHz provided an ultrasonic power of 150 W. Additionally, the recipe used for conventional alkali scouring comprised 2 g/l wetting agent, 1 g/l sequestering agent, 3 g/l caustic soda and MLR of 1:20. Reaction was carried out for 60 min at 98°C.

Characterization of Fabric After Scouring

Absorbency Test

The AATCC 79 (2007) test method was used to measure absorbency. Briefly, a drop of water was released from a fixed height of 10 ± 1.0 mm (0.394 ± 0.04 in) onto the taut surface of the test specimen. The time taken for the water drop to get absorbed in the fabric was measured and recorded as the wetting time. Standard environmental conditions (21±1°C, RH 65% ± 2%) for sample testing were maintained. Five readings were taken for each sample, and the average was calculated.

Weight Loss

Fabric weight loss was calculated using the equation:

$$\text{Weight loss (\%)} = ((W1-W2)/ W1) \times 100$$

where W1 and W2 represent the fabric weights before and after the scouring process, respectively.

RESULTS AND DISCUSSION

Determination of Optimal Medium for PGase Production

R. oryzae was grown in five different liquid media, and PGase activity in the culture supernatant was

determined at different time periods (Table 1). The culture supernatant was also used to carry out bio-scouring of knitted grey single jersey fabric. The results indicate that scouring and PGase activity were not correlated. When *R. oryzae* was grown in SGP broth, PGase production was highest on the fourth day, while the most efficient scouring was found on the fifth day. This may be due to action of other, synergistically acting enzymes in addition to PGase present in the supernatant.

Table 1: PGase activity (a) and absorbency test (b) after scouring

PGase activity (IU/L)						
Medium	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
PDP	150	233	375	264	260	167
SP	122	223	340	248	182	123
SPX	138	186	218	313	211	135
SGP	180	252	388	435	253	179
YP	64	76	81	104	156	98

(a)

Water Drop test (sec)					
Medium	Day 2	Day 3	Day 4	Day 5	Day 6
PDP	39	35	29	18	38
SP	46	38	34	17	44
SPX	46	42	33	18	37
SGP	49	35	25	9	34
YP	198	70	45	46	34

(b)

Concentration and Characterisation of *R. oryzae* PGase

To concentrate the PGase present in the growth medium supernatant, ammonium sulphate precipitation was carried out. Table 2 presents the enzyme activity at each stage. The results indicate that the enzyme activity increased 2.25 times after concentration.

Table 2: Enzyme activity of crude and concentrated enzyme

Enzyme Activity (IU/L)		
Crude Enzyme	**After Precipitation**	**After Dialysis**
252	304	494

Effect of Temperature on Pectinase Activity

The effect of temperature on enzyme activity was investigated at pH 6.5 at different temperatures, and the results are presented in Table 3. The optimal activity was determined to be 40°C. Further investigations into the effect of pH were conducted at 40°C.

Table 3: Effect of temperature on PGase activity

Temperature (°C)	IU/L	% Activity
30	267	45
40	515	100
50	415	78
60	303	53
70	232	38
80	144	19

The results of the present study are in accordance with findings reported in the scientific literature. For instance, it was found that the maximal activity of the pectinase enzyme produced by *R. oryzae* by fermentation occurred at a temperature of 40°C (Chowdhury *et al.*, 2017). Another study reported that the maximum activity of the pectinase enzyme produced from *Aspergillus pulverulentus* was observed at 40°C (Wafaa *et al.*, 2020). Additionally, *R. stolonifer* produced acidic pectinase with optimum activity at a temperature of 50°C (Sari *et al.*, 2024).

Effect of pH on PGase Activity

The effect of pH on enzyme activity was investigated by conducting enzyme assays at the optimum temperature (40°C) at different pH levels (Table 4). The optimal activity was determined to be at pH 5.

Table 4: Effect of different pH on PGase activity

pH	IU/L	% Activity
4	420	76
5	533	100
6	509	96
7	409	74
8	332	58

The current findings are in agreement with results reported by Wafaa *et al.*, who found that the maximum activity of the pectinase enzyme produced by *Aspergillus pulverulentus* occurred at pH 5 (Wafaa *et al.*, 2020). Similarly, Ahmed *et al.* reported that the optimal pH for pectinase obtained from *Geotrichum candidum* was pH 5 Ahmed and Sohail, (2020) and Sari *et al.* (2024) found that pectinase produced by *R. stolonifer* displayed optimum activity at pH 5.

Scouring

Scouring was carried out using concentrated *R. oryzae* supernatant, commercial enzyme preparations and conventional alkali scouring. Despite the optimum conditions for *R. oryzae* PGase being in the range of 40°C and pH 5, the absorbency results obtained under these conditions were inferior to those obtained under scouring conditions of 60°C and neutral pH. This observation may be attributed to the presence of non-saponifiable oils or waxes in the cotton material, which need to be removed for the enzyme to effectively act on the cotton substrate. This removal typically occurs above 55°C, facilitated by the action of wetting agents on the material.

Ultrasonic-assisted scouring was performed using commercial enzyme and synthesized enzyme following the same recipe used for scouring without ultrasonic assistance. Tables 5 and 6 present the absorbency and weight loss results obtained after scouring. The results indicate that ultrasound conditions efficiently remove impurities present in the cotton single jersey knitted fabric.

Table 5: Absorbency times for different scouring conditions

Water Absorbency Time (sec)				
	Alkali Scouring	Commercial enzyme		*R. oryzae*
	Caustic	Pectinase	Green Boost	PGase
Without Ultrasonic	Instant	1-2 sec	1-2 Sec	6-7 Sec
With Ultrasonic	ND	Instant	Instant	3-4 Sec

Table 6: Weight loss percentage of fabric after different scouring

Weight Loss Percentage				
	Alkali Scouring	Commercial enzyme		*R. oryzae*
	Caustic	Pectinase	PGase	RO
Without Ultrasonic	5.70%	3.73%	3.74%	3.54%
With Ultrasonic	ND	4.10%	3.93%	3.70%

CONCLUSION

This study demonstrates the potential of polygalacturonase enzyme from *R. oryzae* for bio-scouring cotton knitted single jersey fabric, offering a sustainable alternative to traditional high-temperature alkaline processes. Bio-scouring was further enhanced by ultrasound, reducing absorbency times and improving impurity removal. The results highlight *R. oryzae* enzymatic bio-scouring as a viable, eco-friendly approach, significantly reducing environmental impact and promoting sustainability in textile processing.

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