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Bio-bleaching of ankara pulp with xylanase-producing bacterial consortium for sustainable handmade paper production

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ABSTRACT

The paper industry faces two critical challenges: the scarcity of raw materials and the environmental impact of chemical waste pollution. Addressing the first challenge involves harnessing alternative, sustainable raw materials, while the second challenge can be mitigated through the adoption of bio-bleaching processes, which significantly reduce chemical consumption while enhancing paper brightness and quality. This study proposes a solution to both challenges by using non-woody Calotropis procera (Ankara) and a xylanase-producing microbial consortium for sustainable handmade paper production, a combination not extensively explored in prior research. To evaluate this approach, the process was divided into three stages. In stage I, Ankara fibre was pulped through open hot digestion. In stage II, the pulp was subjected to bio-bleaching in two experimental setups: Set I (without sucrose) and Set II (with sucrose) for 5 days. In stage III, chemical bleaching was used to improve the final brightness of the treated pulps. A novel comparison was made between the bio-bleaching efficiency of an individual isolate g5 (BI) and a bacterial consortium (BC). This research highlighted that bio-bleaching with the consortium effectively removed lignin (140±60 mg/l) and colour (1830±50 PCU), especially in the presence of sucrose, compared to using a single xylanase isolate. Pulp residue/filtrate collected at each stage was estimated based on parameters such as colour and lignin content. After stage III (chemical bleaching), the release of colour and lignin in pulp filtrate was higher in BI compared to BC, indicating the consortium's effectiveness during bio-bleaching, which leaves fewer degradable lignin structures for the chemical bleaching stage. Papers crafted from consortium-treated pulp also exhibited higher brightness than those treated with the isolate. This study reveals the synergistic effect of microbial consortia, leading to more efficient lignin degradation and enhanced bio-bleaching capabilities, supporting the development of greener industrial processes. Ultimately, this study demonstrates a unique and eco-friendly approach to papermaking, combining C. procera and enzymatic bio-bleaching to reduce dependency on hazardous chemicals and support sustainable industry practices.

1. Introduction

The paper industry is facing a challenge of limited availability of wood based raw materials. Consequently, the focus has shifted towards non-woody raw materials, typically derived from agricultural residues and fast-growing plants. Commonly utilized sources include rye, bagasse, hemp, kenaf, reed, jute, and wheat and rice straw (Ashori, 2006). Despite the global trend towards digitalization, reducing paper usage remains a significant challenge. Therefore, researchers are continually seeking new non-wood sources to meet the growing demand for paper.

In line with this ongoing search, the first aim of the present study was to utilize Ankara fibre, derived from *Calotropis procera*, as a sustainable non-woody alternative for paper pulping. Abundant in certain arid regions of Rajasthan, Ankara fibre presents a unique opportunity to leverage local biodiversity for paper production. This study introduces the innovative use of a previously underutilized plant, demonstrating the potential for sustainable paper production.

Another significant challenge faced by the paper industry is the extensive use of chemicals during the pulping, bleaching, and final paper finishing stages of the production process. Microbial enzymes offer a cost-effective way to improve these processes. Xylanases, in particular,

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Table 1
Experimental design for treatment of pulp by xylanase producing bacteria cultures.

Sets		Dried pulp (25 g)	2% Bacterial culture		2% Sucrose (100 ml)	Sterile distilled water (100 ml)
			g5	Consortia		
Set-I (Without sucrose)	I-Control	+	-	-	-	+
	I-BI	+	+	-	-	+
	I-BC	+	-	+	-	+
Set-II (With sucrose)	II-Control	+	-	-	+	-
	II-BI	+	+	-	+	-
	II-BC	+	-	+	+	-

BI: Individual Bacteria; BC: Bacterial Consortium

Table 2
Comparison between xylanase activity of bacterial consortia.

Combinations	Xylanase activity (U/ml)			
Time (h)	g5	g1+g5	g1+g4+g5	g1+g4+g5+p2
24	2.4 ± 0.24^{a}	3.39 ± 0.59^{a}	3.7 ± 0.36^{a}	4.39 ± 0.36^{a}
48	3.47 ± 0.14^{b}	4.7 ± 0.24^{b}	4.62 ± 0.14^{b}	4.7 ± 0.24^{a}
72	4.39 ± 0.36^{c}	6.54 ± 0.4^{c}	6.54 ± 0.47^{c}	6.24 ± 0.27^{b}
96	8.47 ± 0.36^{d}	6.77 ± 0.4^{cd}	7.24 ± 0.24^{c}	7.54 ± 0.48^{d}
120	9.77 ± 0.61^{e}	7.62 ± 0.36^{d}	10.31 ± 0.71^{d}	8.62 ± 0.24^{d}
144	8.04 ± 0.03^{d}	6.04 ± 0.04^{c}	7.04 ± 0.04^{c}	6.54 ± 0.48^{b}

a – e represents homogenous subsets. Values not sharing a common lowercase superscript (a – e) differ significantly at $p \leq 0.05$ (Tukey's test).

are recognized as "pre-bleaching" or "bleach-boosting" agents due to their ability to facilitate pulp bleaching at a reduced cost by breaking down re-precipitated xylan on the fibres (Dukare et al., 2023). Xylanase treatment given before chemical bleaching is quite effective, as it cleaves the residual lignin to hemicellulose, thereby enhancing the entry of chemicals in pulp and improving the lignin extraction during subsequent stages of bleaching (Sharma et al., 2020; Azeri et al., 2010; Walia et al., 2015). This bleach-boosting effect of xylanases reduces the need for oxidizing chemicals by approximately 20% while still achieving the same level of brightness as traditional chemical bleaching (Çiçekler, 2022).

Numerous studies have documented the use of microbial xylanase as a pre-bleaching agent in paper mills, utilizing both woody and non-woody raw materials. However, only a few studies (Saleem et al., 2009; Hui et al., 2013; Sharma et al., 2015; Sharma et al., 2020) have focused on its application in handmade papermaking. Additionally, it has been reported that the degradation of these lignocellulosic biomasses is more effective when using xylanases from bacterial consortia (Pandey et al., 2013) instead of individual cultures.

We hypothesize that Ankara fibre, when treated with a xylanase-producing microbial consortium, will enhance the bleaching efficiency, reduce the need for chemical bleaching agents, and improve the overall quality of handmade paper sheets.

Building on this insight, the second aim of the present study is to enhance the bleaching efficiency of Ankara pulp by employing a xylanase-producing microbial consortium prior to chemical treatment, thereby reducing chemical use and improving the quality of handmade paper sheets. This dual approach of using Ankara fibre and a microbial consortium for its biobleaching represents a novel advancement in sustainable papermaking.

2. Materials and method

2.1. Materials

Birchwood xylan was obtained from Sisco Research Laboratories Pvt. Ltd. (SRL), India. Commercially available Ankara fibre was procured from Kumarappa National Handmade Paper Institute (KNHPI), Jaipur, Rajasthan, India. All the reagents, media and chemicals used under study were of analytical grade (Hi-Media, Merck, Loba, SRL, and Sigma Aldrich).

2.2. Xylanase producing bacterial cultures

Four xylanase-producing bacterial cultures (g1, g4, g5 and p2) earlier isolated from compost soil were procured from Department of Microbiology and Biotechnology, IIS (Deemed to be University), Jaipur. All the cultures were inoculated in nutrient broth and incubated at 37 °C for 24 h. After incubation, the bacterial cultures were maintained on nutrient agar at 4 °C.

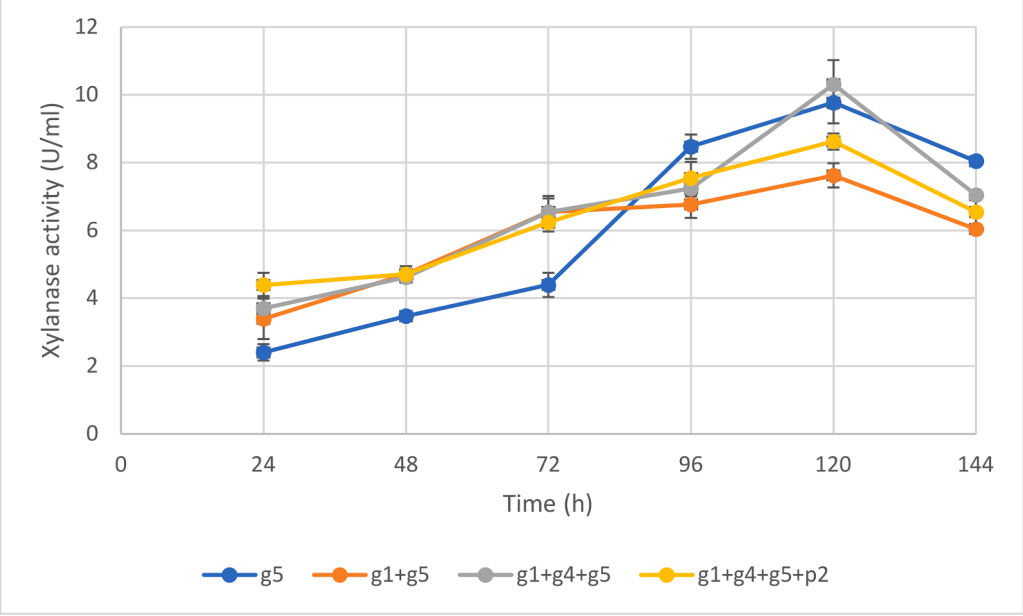


Fig. 1. Comparison of xylanase activity of different consortia.

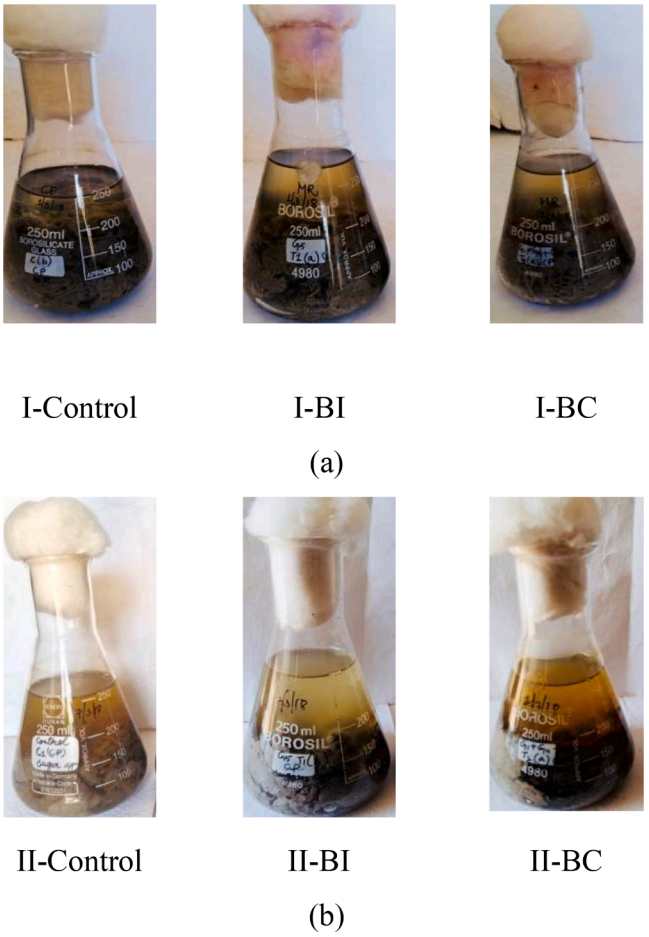


Fig. 2. Xylanase treatment of Ankara pulp (a) in absence of sucrose (Set I) and (b) in presence of sucrose (Set II).

2.3. Xylanase production from bacterial consortia

Three different bacterial consortia (g1+g5, g1+g4+g5, and g1+g4+g5+p2) based on the xylanase production and compatibility tests (data not shown here) were prepared in 20 ml of modified Czapek-mineral salt broth supplemented with 0.5% xylan as carbon source and incubated at 37 °C at 120 rpm for 6 days (Kumar et al., 2018). Xylanase production from these consortia were compared with highest xylanase producing bacterial isolate g5. The cultures were centrifuged at 10,000 rpm for 10 min and xylanase activity was determined in the cell-free extract each day (Bajaj and Singh, 2010). Bacterial consortium (BC) showing highest xylanase activity was selected for further studies.

2.4. Xylanase assay

Xylanase activity was quantified using the 3,5-dintrosalicylic acid (DNS) assay for reducing sugars (Miller, 1959). The assay mixture was prepared by mixing 0.5 ml of the crude enzyme extract with 0.5 ml of 1% (w/v) xylan substrate solution (1 g in 0.05 M potassium phosphate buffer, pH 7.0). The reaction mixture was incubated in water bath at 50°C for 30 min. DNS reagent (3 ml) was added and the reaction was terminated by boiling at 100°C for 10 min and absorbance was measured at 540 nm. Substrate with boiled crude enzyme was taken as blank. One unit (U) of xylanase was defined as the amount of enzyme that released 1 µmol of xylose equivalents from xylan per minute under the experimental conditions (Mandal et al., 2011).

2.5. Ankara fibre pulping (Stage I)

Open hot digestion of 500 g of uniformly chopped Ankara fibre (on oven dried basis) was carried out in 8% NaOH with bath ratio 1:10 for 3 h at boiling temperature (Chauhan et al., 2006). The mixture was stirred intermittently keeping the water level constant by addition of water to make up for the evaporation losses. The cooked pulp was washed with water after squeezing out all the black liquor. The washed pulp was squeezed and shredded for uniform distribution of the moisture and weighed sample aliquots of shredded pulp were kept in oven at 100 ±2°C. The dryness percentage and pulp yield were calculated using the given formula (Chauhan and Sharma, 2014):

$$Dryness\ \% = \left(\frac{WOD}{WAD}\right) * 100$$

$$Pulp\ Yield\ \% = \left(\frac{WOD}{WOF}\right) * 100$$

where, WOD, WAD, and WOF were weights of oven-dried pulp, air-dried pulp, and oven dried original fibre of Ankara, respectively.

The black liquor obtained during the pulp formation was characterized for total solids, pH, colour content, and lignin. Total solids (%) was calculated using the following formula (Chauhan et al., 2020):

$$Total\ solids\ (\%) = \left(\frac{WD}{WL}\right) * 100$$

where, WD and WL are weights of dried liquor and wet liquor, respectively.

The pH was determined at 30°C using the standard TAPPI Test method number T-625 CM-85. The lignin content was quantified according to TAPPI standard method T-222 om-21 by measuring absorbance by UV–vis spectrophotometer (Elico SL-164) at a wavelength of 280 nm. Colour was determined by measuring absorbance at 465 nm and converting it into the Platinum Cobalt Units (PCU) using the conversion factor (500 PCU=0.41 Absorbance) (Chauhan and Sharma, 2014).

$$Colour\ (PCU) = \frac{500}{0.41 * Absorbance * Dilution\ Factor}$$

2.6. Bio-bleaching with xylanase producing bacterial cultures (Stage II)

In a submerged fermentation experiment, 25 g of dried pulp was treated in a 250 ml flask using the experimental design (Table 1) in which two sets of treatments (Set I and Set II) were applied. Set I was without sucrose and set II with 2% sucrose solution. BI and BC represented xylanase producing g5 and bacterial consortium, respectively. The volume was adjusted with addition of 100 ml sterile distilled water. All the flasks were incubated at 37 °C at 150 rpm for 5 days in a shaker incubator. After five days, the pulp was harvested by filtering through the vacuum pump assembly and the filtrate was collected from each flask. The pulp was subjected to control washing using 250 ml of boiling water once followed by 250 ml tap water twice. The washed pulp pads were further used for bleaching experiments.

In order to find out the efficacy of the treatment, the pulp filtrate was analysed for the colour, lignin, and reducing sugar. Colour and lignin were analysed as per the methods given above. Reducing sugar was estimated using DNS method (Miller, 1959).

2.7. Chemical bleaching of pulp pads (Stage III)

All the treated pulp pads were chemically bleached using the modified method of Chapla et al. (2012). The bleaching was performed in three sequential steps i.e. peroxide bleaching, alkali extraction, and second peroxide bleaching to improve the final brightness of the pulps.

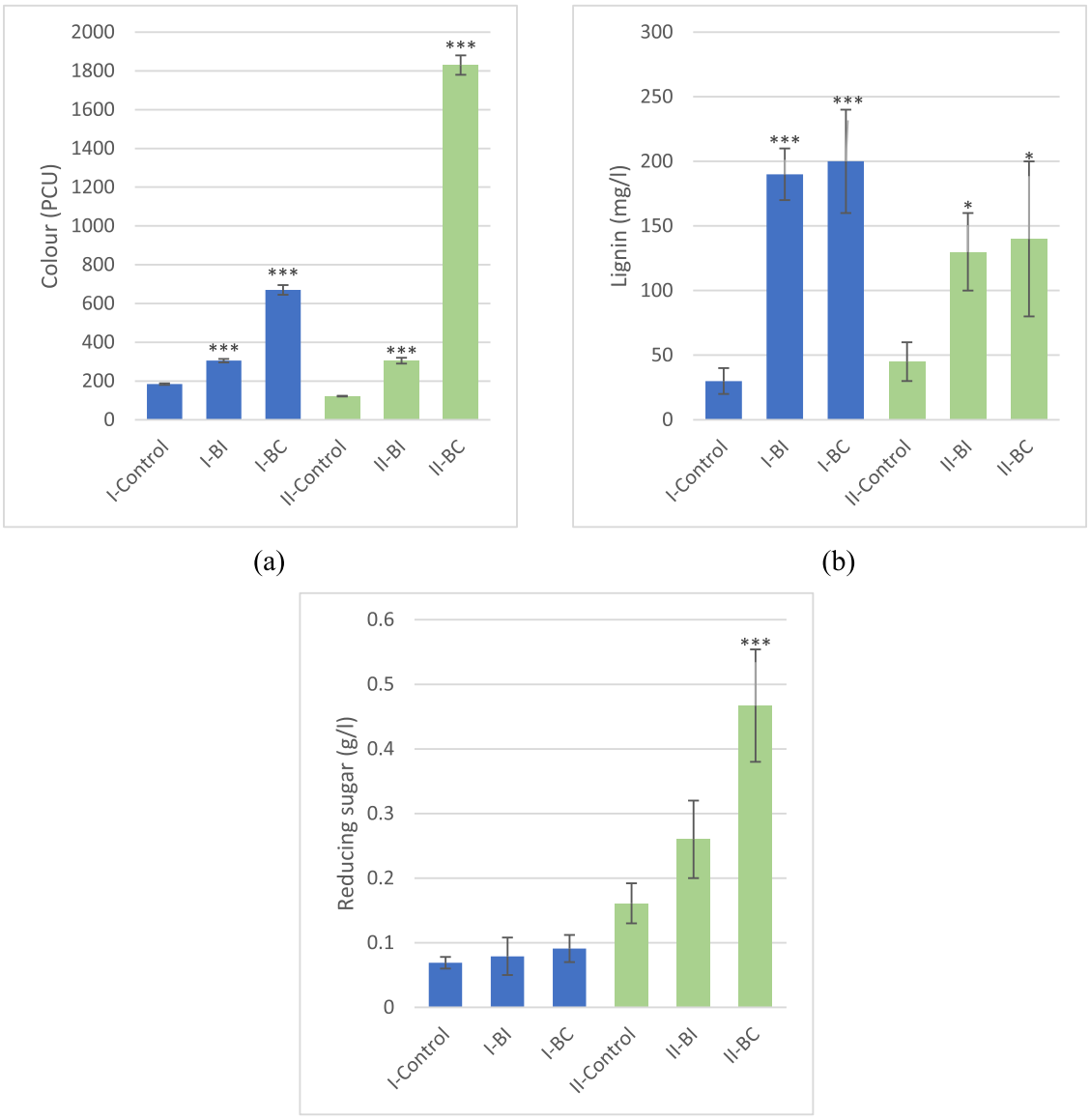


Fig. 3. Characterization of pulp filtrate of stage II (a) colour (b) lignin content, and (c) reducing sugar.

The pulp was maintained at 8% consistency using water at each bleaching step. The treatments were carried out in polyethene bags and temperature was maintained in water bath. The following conditions were applied at each bleaching step: First step: 2% NaOH and 2% H_2O_2 and 70 °C for 2 h; Second step: 2% NaOH and 60 °C for 2 h; Third step: 2% NaOH and 2% H_2O_2, 70 °C for 1 h. Pulp pads were prepared after control washing with water 2-3 times for pH neutralization. The final pulp filtrate was collected and colour and lignin were estimated as per the above protocol.

2.8. Preparation of handmade paper

Sheets were prepared from original pulp (Stage I), pulp after bio-bleaching (Stage II), and pulp after chemical bleaching (Stage III) by taking 5 g oven dried pulp to make two sheets of 2.5 g each using British Sheet Former (Hamzeh et al., 2013).

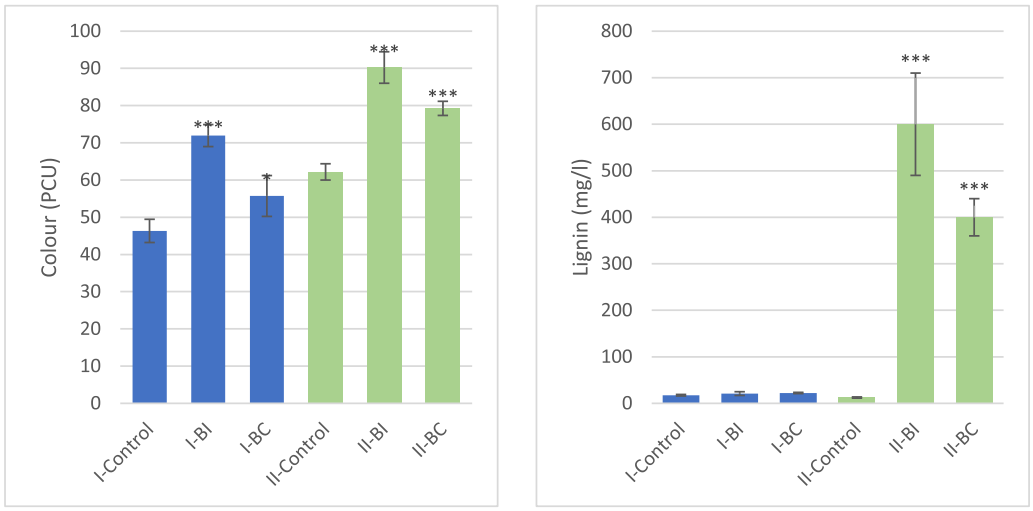
2.9. Statistical analysis

All experimental data were analysed using SPSS software (Version 22). Each experiment was performed in triplicate, and results were presented as mean ± standard deviation. Statistical analysis included an independent t-test and one-way ANOVA, followed by Tukey's post hoc test at a significance level of 0.05. A Multivariate Analysis of Variance (MANOVA) was conducted to examine the effects of bacterial treatment (BI/BC), sucrose addition (Set I/II), and stage (I/II/III) on lignin content and colour. Post-hoc tests with Bonferroni correction were applied to identify specific differences between groups.

3. Results and discussion

3.1. Xylanase production

Three bacterial consortia (g1+g5, g1+g4+g5, g1+g4+g5+p2) and



* = p ≤ 0.05 statistically significant, *** = p ≤ 0.001 highly significant

Fig. 4. Characterization of pulp filtrate after stage III (a) colour, and (b) lignin content.

g5 isolate were observed for xylanase production in modified Czapek-mineral salt broth with 0.5% xylan for 6 days. Xylanase production was observed to be maximum after 5 days of incubation for all three bacterial consortia as well as g5. Among all, highest production of 10.31 ± 0.71 U/ml was achieved with consortium g1+g4+g5 after 120 h of incubation (Table 2, Fig. 1). In a similar study on xylanase production from bacterial consortium, highest xylanase activity was achieved at day 6, with highest activity by pure strain A7 and lowest by AA3, and higher in the consortiums with M1 (A7 and AA3) (Zhang et al., 2016). In another study, Seo et al (2014) reported the xylanase activity of the *Bacillus licheniformis* using a minimal nutrient medium supplemented with copra meals. The xylanase activity in both the studies were lower as compared to the present study. On statistical comparison of the results of 120 days of all sets, xylanase production in set g1+g4+g5 was significantly higher as compared to g1+g5 (p=0.000) and g1+g4+g5+p2 (p=0.004). On the other hand, no significant difference (p=0.236) was observed in xylanase production between g1+g4+g5 and g5. Therefore, g1+g4+g5 (further mentioned as BC) was selected to explore the potential of their xylanase in bio-bleaching of paper pulp prepared from Ankara fibre.

3.2. Ankara fibre pulping (stage I)

Ankara fibre was prepared into pulp using open hot digestion in an alkaline solution at boiling temperature. The open hot digestion process effectively removes lignin and impurities, liberating and softening cellulose fibers (Chopra and Manikanika, 2022). The addition of alkali helps in dissolving carbohydrates, neutralizing organic acids, interacting with extractives, and most importantly, reacting with lignin, leading to a reduction in residual effective alkali (Jardim et al., 2022). Using this method, the pulp achieved a dryness percentage of 33.07 ± 0.51% and a yield of 77.84 ± 0.99%. On the contrary, Kumar and Mishra (2023) reported that ambient temperature with NaOH (8%) gave a slightly higher pulp yield from Ankara compared to boiling temperature at the same NaOH concentration. In another study, Musekiwa et al. (2020) reported pulp yield of 64.39 wt.% from groundnut shells. After the digestion of the fibres, the black liquor was collected and characterized for total solids, pH, colour, and lignin content. The total solids were estimated was 3.98 ± 0.15% and pH of the black liquor was 10. The colour of black liquor was found to be 53048.5 ± 1219.5 PCU and the lignin content was estimated as 16.75 ± 0.025 g/l of black liquor.

3.3. Bio-bleaching with xylanase producing bacterial cultures (stage II)

While some amount of lignin is removed, residual lignin typically remains, which may require additional bleaching or treatment steps to achieve the desired level of purity and brightness in the final pulp. For this reason, biobleaching followed by chemical bleaching was performed (Campioni et al., 2019). For biobleaching, pulp was treated in two sets (with and without sucrose) using g5 and BC (Fig. 2). After 5 days of incubation, the pulp filtrate was collected and the remaining was processed for washing. Colour, lignin and reducing sugars were analysed to characterise the pulp filtrate. In set I, the maximum colour of 670±25 PCU was obtained in consortium (I-BC) which was significantly higher (p =0.000) than I-BI (305±9 PCU) and control (184±4 PCU). The amount of lignin released was 6-times higher in I-BI and I-BC as compared to control. Though no significant difference was observed in the reducing sugar in all three sets. The addition of sucrose in set II, increased the amount of colour released in II-BC to 1830±50 PCU which was approximately 3-times higher as compared to I-BC. The amount of lignin released in II-BC was 140±60 mg/l which was higher than II-BI and control. Similarly, the amount of sugar released in II-BC (0.47 ±0.09 g/l) was significantly higher to II-BI (p =0.00) and control (p =0.001) (Fig. 3). Bio-bleaching efficiency measured in terms of release of colour, lignin, and reducing sugars was more in consortium as compared to individual isolate. The types of enzymes produced by the consortium versus the single isolate can differ significantly. The enzyme production can target specific lignin bonds more effectively when produced in a consortium. Consistent with these findings, Parab and Khandeparker (2021) also reported that treatment of hardwood and chemical bagasse pulp with *Bacillus* sp. NIORKP76 xylanolytic enzyme consortia produced better biobleaching results than treatment with single xylanase Though they documented the use of purified or crude bacterial xylanase for biobleaching, Kaushal et al. (2022) concluded that directly applying *Bacillus pumilus* to soda pulp in solid substrate fermentation was more efficient as compared to using enzyme. This method is straightforward, time-efficient, and suitable for industrial bio-bleaching operations. It also eliminates the need for enzyme extraction and low-temperature maintenance, making it more cost-effective.

Comparison of I-BC and II-BC showed significant difference in the amount of colour (p =0.000) and sugar (p =0.002) released whereas the difference observed in lignin was not significant. This suggests that the

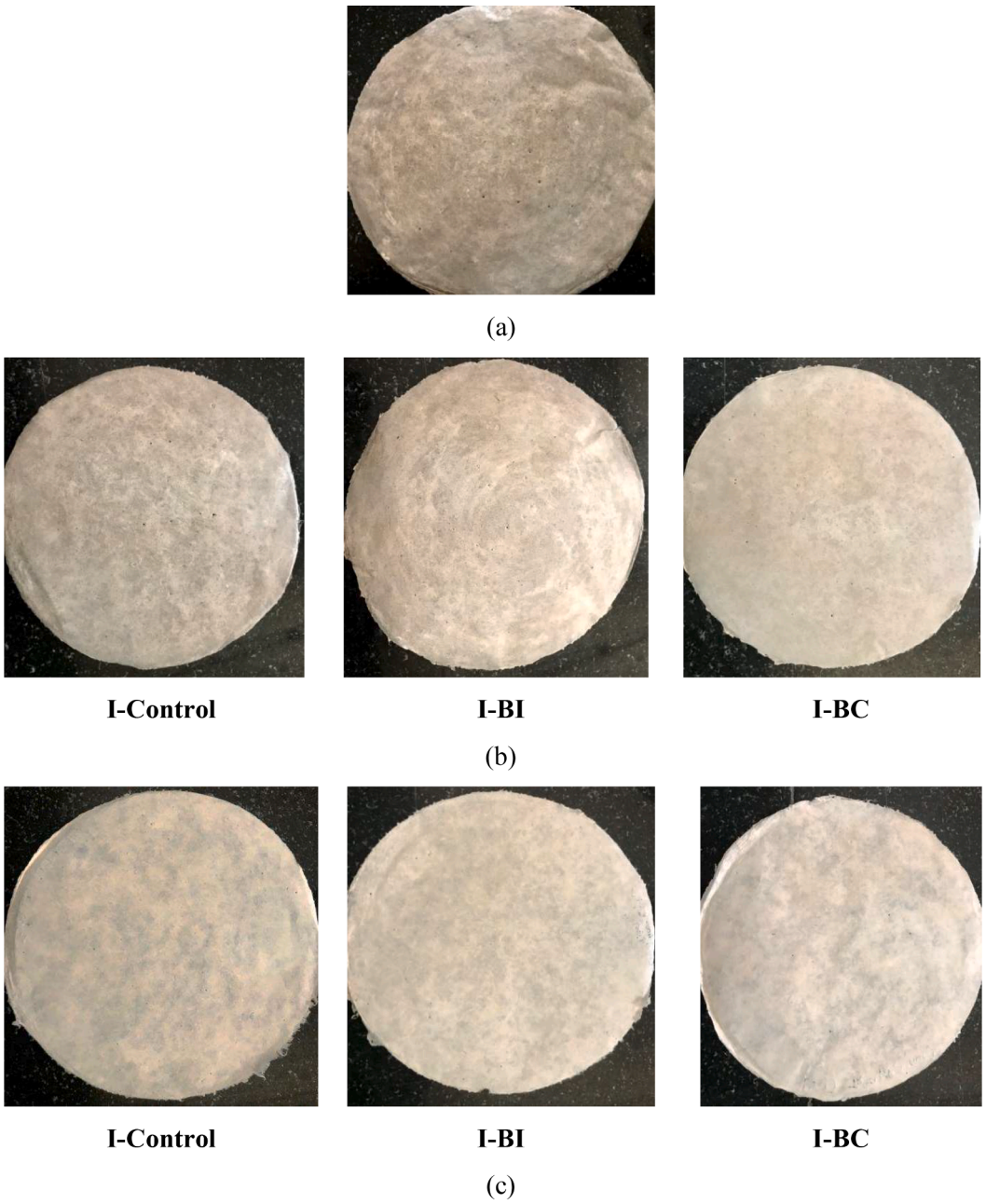


Fig. 5. Sheets of Ankara pulp prepared in Set I (without sucrose) after (a) Stage I, (b) Stage II, and (c) Stage III.

effect of selected consortium can be improved by supplementing sucrose in pulp leading to higher amount of release of colour and reducing sugar. It has already been reported in the literature that sucrose addition enhances microbial growth that leads to better activity of the consortium (Canon et al., 2020). This indicates that the efficiency of bio-bleaching process can be improved by using bacterial consortium and supplementing pulp with sucrose.

3.4. Chemical bleaching of pulp pads (stage III)

After chemical bleaching, the pulp filtrate was collected before the washing of the pulp and the characterization of the extract was done by the estimation of colour and lignin (Fig. 4).

In set I, there was a significant difference between Control, BI and BC (p =0.001) in release of colour in pulp filtrate with maximum observed in the case of g5 (71.95±2.96 PCU) as compared to BC (55.72±5.49 PCU). Whereas no significant difference was observed in concentration of lignin released in pulp filtrate (p =0.143) in both the cases.

In set II, release of both colour and lignin was significantly different in Control, BI and BC (p =0.000). The release of colour was higher in the case of g5 (90.24±4.23 PCU) as compared to the BC (79.26±1.9 PCU) (p =0.004) whereas the concentration of lignin was significantly higher in individual isolate as compared to the consortium (p =0.011).

The results so obtained showed higher amount of colour and lignin released from g5 as compared to bacterial consortium that indicates differential degradation capacities of both in stage II. During the stage II (bio-bleaching), members of the consortium acted synergistically that might have allowed them to break down lignin and other organic substrates more completely or efficiently than single strain (Lin, 2022). This could have resulted in the consortium-treated pulp having less residual lignin and colorants entering stage III. The effectiveness of consortium during bio-bleaching leaves fewer degradable lignin structures for the chemical bleaching stage. Whereas, in case of g5 treatment, the residual amount of lignin in the pulp was more which then reacted during the

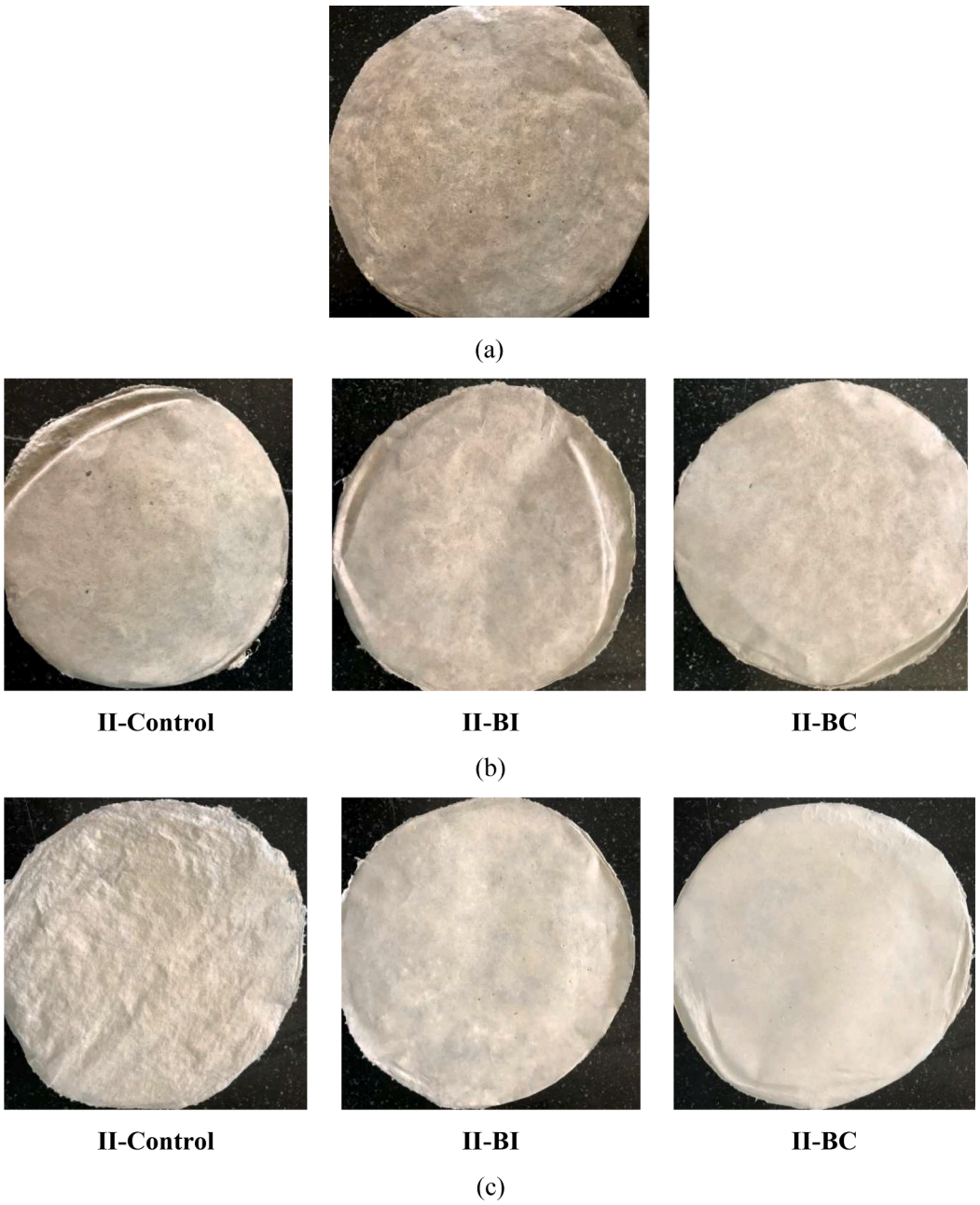


Fig. 6. Sheets of Ankara pulp prepared in Set II (with sucrose) after (a) Stage I, (b) Stage II, and (c) Stage III.

chemical bleaching stage, leading to greater release of colour and lignin.

This shows that though the chemical bleaching treatment was the same for both the samples, the starting conditions (post-bio-bleaching) were different due to the varying effectiveness of the bacterial treatments. It indicates that chemical bleaching efficiency can be heavily influenced by the initial conditions of the pulp and results suggested that integrating bio-bleaching in the process has improved the bleaching efficiency (Wei et al., 2021).

The MANOVA tests revealed significant effects for stage (p =0.001), bacteria ($p < 0.001$), and sucrose ($p < 0.001$) on the combined dependent variables of lignin content and colour. Additionally, significant interactions were found between stage and bacteria (p =0.001), stage and sucrose ($p < 0.001$), and bacteria and sucrose ($p < 0.001$). The three-way interaction between independent variables was also significant ($p < 0.001$). Tests of between-subjects effects showed significant ($p < 0.001$) results for lignin content with respect to stage, bacteria, and sucrose, including significant interactions. For colour, only the intercept was significant ($p = 0.000$), with no significant main effects or interactions. Post-hoc comparisons using the Bonferroni method indicated significant differences in lignin content between all stages, bacteria types, and sucrose conditions. For colour, significant differences were observed between Stage I and both Stage II and III, but not between Stage II and III. No significant differences were found between bacteria treatments for colour. These findings suggest that optimizing the combination of stage, bacterial treatment, and sucrose addition can significantly enhance lignin and colour extractions during the pulping and bleaching processes. The significant multivariate effects and interactions observed indicate that these factors interact in complex ways to affect the overall effectiveness of the treatments.

3.5. Hand-sheets preparation from Ankara pulp

The hand-sheets were prepared after every stage (I, II and III) and visually compared on the basis of brightness and finish. The sheets

prepared after stage I were rough and less bright. The smoothness and brightness improved with subsequent bio-bleaching and chemical bleaching (Fig. 5 and 6). The brightness of the pulp treated with BC was higher than that of the pulp treated with BI, corresponding to the higher amount of colour and lignin extracted after bleaching. This suggests a synergistic action of xylanase produced by the constituent members of the consortium. The addition of sucrose further enhanced the brightness and improved the texture of the paper produced.

The xylanase from *Bacillus coagulans* demonstrated a beneficial effect on the brightness of three different pulps, particularly notable in wheat and rice straw pulps at elevated pH levels. For jute pulp, the greatest increase of 4 points, was achieved at a pH of 7, which was significantly higher than the points at pH 8.5. Despite the enzyme's optimal pH being 7, the highest brightness increases of 5.1 points occurred in rice straw pulp at a pH of 8.5. Notably, improvements in the brightness of rice straw and jute pulps were evident even after the enzyme treatment phase (prior to bleaching process). In contrast, brightness gain in wheat straw pulp was observed only after the bleaching process (Chauhan et al., 2006).

Recently, Bakry et al. (2024) also reported whiteness enhancement of wastepaper using the thermotolerant xylanase enzyme produced by *Bacillus haynesii* K6. Angural et al. (2020) documented combinatorial bio-bleaching of ligninolytic and hemicellulolytic enzyme for papermaking. Chauhan et al. (2015) reported bio-bleaching of the handmade paper pulps of paper mulberry using white rot fungal cultures. Kumar (2021) reviewed bio-bleaching as an eco-friendly approach to reduce chemical consumption and pollutant generation in pulp and paper industry. In biobleaching, xylanolytic treatment of pulp reduced hypochlorite consumption to 50% during the chlorine treatment (Parab and Khandeparker, 2021). Therefore, this makes the bio-bleaching process both economical and environmentally sustainable.

4. Conclusion

This study successfully demonstrates the potential of Calotropis procera (Ankara fibre) as a sustainable and viable raw material for handmade paper production. Utilizing this non-woody plant not only provides an alternative to traditional wood-based raw materials but also leverages local biodiversity, particularly in arid regions like Rajasthan. This research underscores the importance of exploring new, eco-friendly sources for papermaking, supporting the industry's shift towards more sustainable practices.

Additionally, the use of xylanase-producing bacterial consortia enhances the bio-bleaching process of C. procera pulp. By comparing the efficacy of an individual bacterial isolate and a consortium, significant improvements in lignin reduction and colour lightening in the treated pulp were observed. These findings highlight the advantage of utilizing microbial consortia over single isolates. Moreover, directly applying bacteria to pulp is more efficient compared to using enzymes alone, eliminating the need for enzyme extraction and low-temperature maintenance, making it more time-efficient and cost-effective. The study also demonstrates that bio-bleaching can effectively reduce chemical use in pulp and paper production, supporting the development of greener industrial processes in alignment with global efforts to minimize environmental impact and promote sustainability.

Overall, this research contributes to ongoing efforts in exploring eco-friendly alternatives and emphasizes the critical role of microbial consortia in industrial applications, affirming the potential of C. procera in supporting eco-friendly industry practices.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT4.0 in order to improve language and readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

CRediT authorship contribution statement

Meenakshi Rajput: Investigation, Writing – original draft. **Disha Pamecha:** Data curation, Writing – review & editing. **Preeti Kumari:** Investigation. **Payal Chaturvedi:** Data curation, Formal analysis, Supervision, Validation, Writing – original draft, Writing – review & editing. **Charu Sharma:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Rahul Mishra:** Funding acquisition, Project administration. **Sunita Chauhan:** Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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