5. SUMMARY AND CONCLUSIONS

The research work on ‘Enhanced Production and Industrial Applications of Xylanase and Pectinase from Bacillus pumilus AJK’ was carried out with an aim to improve the xylano-pectinolytic titer with good pH and temperature stability for their effective application in various industries such as recycling of waste paper, processing of textile fibres and treatment of animal feed. Thus, to enhance the xylano-pectinolytic titer, mutagenesis and optimization of production conditions using statistical design methods was carried out. Characteristics of crude xylanase and pectinase and acidic and alkaline forms of xylanase enzyme were also studied. The immobilization of these enzymes on alginate beads will further reduce the cost, which provides a considerable potential in various industrial applications. A wide range of pH and temperature stability of these xylano-pectinolytic enzymes showed their efficacy for various industrial applications. Enhanced and cost-effective production of both the industrially important enzymes from a bacterial isolate concurrently in the same production medium using agro-wastes instead of commercial substrates will reduce the production cost and ultimately will help in making the process commercially viable.

The results have been summarized below.

1. **Mutagenesis**

- Both physical and chemical mutagenic agents were used to increase the potency of the parent strain, *Bacillus pumilus* AJK. First, the one time mutagenesis was carried out using different mutagens individually, and then sequential mutagenesis was carried out by selecting best mutants obtained after one time mutagenesis. In sequential mutagenesis, mutants were generated by sequential treatment with different potent mutagens one by one.

- The wild type strain produced $210 \pm 6$ IU/ml and $65 \pm 4$ IU/ml of xylanase and pectinase respectively.
• After UV treatment, best mutant (UV-13) gave maximum xylanase (285±11 IU/ml) and pectinase (102±6 IU/ml) activity, which showed an increase of 1.36 and 1.57 fold in xylanase and pectinase activity as compared to wild type strain.

• Among the various mutants obtained after MNNG treatment, the best mutant MNNG-8 gave 425±15 and 271±9 IU/ml of xylanase and pectinase, respectively showing an increase of 2.02 and 4.17 fold in xylanase and pectinase activity as compared to wild type strain.

• After treatment with DES, DES-12 mutant produced high level of both the enzymes (298±12 IU/ml for xylanase and 129±8 IU/ml for pectinase). This showed an increase of 1.42 and 1.98 fold in xylanase and pectinase activity as compared to wild type strain.

• Among the various mutants obtained after EMS treatment, EMS-10 showed the maximum activity of xylanase (384±14 IU/ml) and pectinase (302±11 IU/ml) enzymes respectively, which showed an increase of 1.83 and 4.65 fold in xylanase and pectinase activity as compared to wild type strain.

• Sequential mutagenesis of UV mutant was carried out using MNNG, DES and EMS mutagens and the best mutant, UMDE-4 showed maximum activity of 592±17 and 506±14 IU/ml for xylanase and pectinase respectively.

• Sequential mutagenesis of MNNG mutant was carried out using UV, DES and EMS mutagens and the best mutant, MUDE-6 showed maximum activity of 652±17 and 438±12 IU/ml for xylanase and pectinase respectively.

• Sequential mutagenesis of DES mutant was carried out using UV, MNNG and EMS mutagens and the best mutant, DUME-2 showed maximum activity of 750±18 and 480±14 IU/ml for xylanase and pectinase respectively.

• Sequential mutagenesis of EMS mutant was carried out using UV, MNNG and DES mutagens and the best mutant, EUMD-6 showed maximum activity of 762±23 and 423±15 IU/ml for xylanase and pectinase respectively.

• After complete mutagenesis, the best mutant, DUME-2 gave maximum activity of 750±18 and 480±14 IU/ml for xylanase and pectinase, enzymes respectively which showed an increase of 3.57 and 7.38 fold in xylanase and pectinase activity as compared to parent strain respectively. This mutant produced larger substrate hydrolysis zones on agar plates containing xylan and pectin separately as compared to wild type strain.
2. **Xylanase and pectinase production under submerged fermentation**

- The mutant bacterial strain *Bacillus pumilus* AJK gave 750±18 IU/ml of xylanase and 480±14 IU/ml of pectinase, when grown in modified Horikoshi medium (unoptimized conditions).
- The different types of pectinases produced were exo-polymethylgalacturonase (252 IU/ml), endo-polymethylgalacturonase (2.1 IU/ml), exo-polygalacturonase (450 IU/ml), endo-polygalacturonase (3.7 IU/ml), polymethylgalacturonate lyase or pectin lyase (17.5 IU/ml), polygalacturonate lyase or pectate lyase (19.5 IU/ml) and pectin esterase (1.86 IU/ml).
- Xylanase showed two pH optima, one at 6.5 and other at 8.5, while pectinase showed maximum activity at pH 9.5. Two pH optima of xylanase showed that, at least two isozymic forms of xylanase may be present and these were named acidic and alkaline xylanase forms for convenience for further study.
- Both the enzymes showed maximum activity at 55°C. At a temperature of 50 and 60°C, the xylanase exhibited 94.09% and 87.13% activity, respectively while pectinase exhibited 93.02 and 98.63% activity, respectively.

3. **Optimization of production conditions for xylanase and pectinase by statistical design methods**

- Two stages statistical designs were used to optimize xylanase and pectinase production by *Bacillus pumilus* AJK under submerged fermentation. Initially PB (Plackett-Burman) design was used for the selection of significant factors affecting the enzyme production. Significant factors determined through PB were further evaluated through CCD (Central composite design).
- A total of 12 experiments were generated in Plackett-Burman design for xylanase and pectinase production simultaneously in the same fermentation medium.
- Ten physical and nutritional variables, i.e. Temperature, Incubation time, pH, Inoculum size, Peptone, Yeast extract, KNO₃, MgSO₄, Wheat bran and Citrus peel were selected to study their effect on xylanase and pectinase production.
- Each variable was studied at two extreme levels, i.e. higher and lower for their screening.
• Results of analysis of variance (ANOVA) of Plackett–Burman design for xylanase and pectinase showed that the model is significant.

• In case of xylanase production, P value (0.0246) less than 0.0500 indicates that the model terms are significant. In this case, B (Incubation time), C (pH), E (Peptone), H (MgSO₄) and J (Wheat bran) are significant model terms. In case of pectinase production, P value (0.0117) less than 0.0500 indicates that the model terms are significant. In this case B (Incubation time), C (pH), E (Peptone), J (Wheat bran) and K (citrus peel) are significant model terms.

• Significant factors determined through PB strategy were further evaluated through CCD–RSM for obtaining the enhanced xylanase and pectinase production under SmF. The minimum and maximum ranges of different variables were investigated.

• Each factor was studied at five different levels, that is, lowest (-2.82), low (-1), medium (0), high (+1), and highest (+2.82). A total of 86 experiments were generated in CCD.

• Results of analysis of variance (ANOVA) of central composite design for xylanase and pectinase showed that the model is significant. P value less than 0.0500 indicates that model terms are significant.

• Response surface plots showed the interaction between two or more variables.

• To validate and confirm the predictions obtained after CCD, 15 experiments were designed with different levels of significant factors according to predicted response generated by the model, taking the optimum production conditions after CCD. The model was successfully validated, as the values predicted by the model were in good agreement with the results obtained on validation for different levels of significant variables of incubation time, pH, peptone, MgSO₄, wheat bran and citrus peel.

• After validation of the model, the enzymes activity was increased up to $948.55 \pm 13.24$ IU/ml of xylanase and $802.28 \pm 12.25$ IU/ml of pectinase. So, the optimized fermentation conditions showed an increase of 1.26 and 1.67 fold in xylanase and pectinase activity as compared to unoptimized fermentation medium conditions.
4. Characterization of crude xylanase and pectinase

- The enzymes xylanase and pectinase produced by a mutant strain of *Bacillus pumilus* AJK were alkali tolerant, which is the requirement of various industries such as paper and pulp industry, deinking of paper industry, textile industry, etc.
- Both enzymes showed high stability over a broad range of pH.
- Xylanase was most stable at pH 8.0, retaining 78% activity up to 6 h. Enzyme retained 100% activity at pH 6.0-10.0 up to 2 h while at pH 7.0 and 8.0, it was 100% stable up to 3 h. The enzyme retained more than 94% residual activity at pH 6.0-10.0 up to 3 h of incubation while, at pH 10.0 the enzyme retained 63% activity even after 6 h.
- Pectinase was most stable at pH 9.0, retaining 100% activity up to 6 h. The enzyme showed a wide range of pH stability. It was 100% stable at pH 7.0-9.0 up to 5 h while, retained more than 70% residual activity at pH 5.0-11.0 up to 6 h of incubation. The pH stability studies revealed that, the pectinase is more stable under alkaline conditions.
- The xylanase showed 100% activity at 15°C to 37°C up to 6 h while, at 45°C the enzyme retained 76% residual activity up to 6 h. At 50°C, the xylanase showed more than 60% residual activity after 1 h while, at 55°C the enzyme retained 23% residual activity up to 1 h. The enzyme was unstable at 60°C retaining only 1.7% residual activity after 1 h of incubation.
- Pectinase was more thermostable as compared to xylanase. It was more than 80% stable at temperature 55°C after 1 h of incubation. The enzyme showed 100% activity at 15°C to 37°C up to 6 h while, at 45°C the enzyme retained 83% residual activity up to 6 h. At 50°C, the enzyme showed more than 40% residual activity up to 6 h while, at 55°C the enzyme retained 57% residual activity after 3 h of incubation. However, the enzyme showed 24% residual activity at 60°C after 3 h of incubation and retained only 9% residual activity after 6 h of incubation. The thermal stability profile of pectinase showed that, it is stable over a broad range of temperature, which makes the enzyme suitable for industrial use.
5. **Purification of Acidic and Alkaline forms of xylanase**

- Acidic and alkaline xylanases were purified to 11 fold with a total recovery of 47% using microfiltration, ultrafiltration technique (100 kDa and 3 kDa nominal molecular weight cutoff membranes), ammonium sulphate precipitation, ion exchange chromatography using Q-Sepharose and gel filtration chromatography through Sephadex G-100 column.
- Molecular weight of nearly 13 and 24.5 kDa were estimated for Xylanase I and II, respectively by gel filtration chromatography using standard gel filtration markers.
- The purified enzymes Xylanase I and II showed two and five bands on native PAGE. These bands were actually the isozymic forms of enzyme xylanase. This was confirmed by zymography.
- HPLC chromatogram of the purified enzyme also revealed the possibility of two and five isozymic forms in Xylanase I and II fractions.
- Purified Xylanase I and II lanes from native PAGE were cut and transferred on to the xylan containing agar plates for zymographic study. Their zymograms gave two and five clear, prominent activity bands for Xylanase I and II fractions respectively, thus confirming the presence of seven isozymic forms of xylanases.

6. **Characterization of purified forms of acidic and alkaline xylanase**

- The purified xylanase I showed pH optima at pH 6.5, while xylanase II showed pH optima at pH 8.5. Xylanase I showed 93% and 91% activity at pH 6.0 and 7.0 respectively. Xylanase II showed 93% and 91% activity at pH 8.0 and 9.0 respectively. Due to their pH optima, these enzymes were named as **acidic** and **alkaline** xylanase.
- Xylanase I showed maximum stability at pH 7.0 with 71% residual activity up to 12 h. It showed 100% stability at pH 6.0 and 7.0 up to 3 h. At pH 6.0, it was 67% stable up to 12 h. This enzyme showed more than 50% activity at pH range of 6.0 to 9.0 up to 12 h. Xylanase II showed maximum stability at pH 9.0 with 77% residual activity up to 12 h. It showed nearly, 100% stability at pH 8.0 to 10.0 up to 3 h. At pH 8.0 and 10.0, it was 74% and 64% stable up to 12 h. This enzyme
showed more than 55% activity at pH range of 7.0 to 10.0 up to 12 h while at pH 11.0, the enzyme showed 48% activity up to 12 h.

- Both purified forms of xylanase gave maximum activity at 55°C. Xylanase I showed more than 75% activity at temperature 50 and 60°C while Xylanase II showed more than 90% activity at these temperatures. However, Xylanase I showed more than 70% activity at temperature range of 45-60°C while Xylanase II showed more than 75% activity at temperature range of 45-65°C.

- The purified xylanases I and II retained 100% activity at 37°C up to 6 h of incubation. Both forms of xylanase I and II showed more than 70% residual activity at 45°C after 6 h of incubation, while at 50°C, these enzymes retained 18 and 29% residual activity, respectively after 6 h of incubation. At 55 and 60°C, both the enzymes were unstable showing very less residual activity.

- Both forms of xylanase showed 100% activity up to 6 months at 4°C after that, activity was not checked. At 25°C, xylanase I and xylanase II showed 76 and 80% activity up to 3 days while, at 37°C, these enzymes showed 56 and 61% activity respectively after 3 days of incubation.

- Among the various metal ions tested, Ba²⁺ increased the xylanase I activity whereas K⁺, Ca²⁺ and Co²⁺ brought slight fall in the xylanase I activity. All other metal ions such as Na⁺, Mg²⁺, Hg²⁺, Cu²⁺, Zn²⁺, Mn²⁺ and Fe³⁺ were found to be the potent inhibitors of xylanase I activity. In case of xylanase II, the activity was slightly increased in the presence of Ba²⁺ while, a slight fall in the activity was seen in the presence of K⁺, Ca²⁺, Cu²⁺ and Mn²⁺. All other ions such as Co²⁺, Na⁺, Mg²⁺, Hg²⁺, Zn²⁺ and Fe³⁺ were found to be the potent inhibitors of xylanase II activity. The activity of both the enzymes was strongly inhibited in the presence of Hg²⁺. Among the various additives tested, Tween-20, Tween-80, Triton X-100 and β-Mercaptoethanol enhanced the enzyme activity of both xylanase I and II, whereas urea significantly decreased the enzymes activity. A slight decrease in the enzyme activity of both xylanases was seen in the presence of EDTA.

- The Km and Vmax values of the purified xylanase I were found to be 6.67 mg/ml and 1538.46 IU/mg protein, respectively, while for the purified xylanase II, Km and Vmax were calculated to be 6.25mg/ml and 1666.67 IU/mg protein, respectively.
7. **Co-immobilization of xylanase and pectinase**

- Three methods were used for immobilization using sodium alginate *i.e.* Entapment, Covalent and Ionic immobilization.
- After entrapment, maximum immobilization and activity yield of xylanase and pectinase was obtained at alginate concentration of 3.0%, protein concentration of 22.5 µg/ml alginate and CaCl\(_2\) concentration of 0.2M. After optimization of all parameters, the maximum immobilization yield for xylanase and pectinase was 63.53 and 57.50%, respectively, while the activity yield was 51.73 and 44.92% for xylanase and pectinase, respectively.
- In covalent immobilization, maximum immobilization and activity yield of xylanase and pectinase was obtained, when beads were activated with 2.0% glutaraldehyde. A contact time of 45 min between beads and glutaraldehyde was sufficient for activation of beads. Binding of the xylanopepticolytic enzymes with the beads was maximum at pH 8.5, of 10 mM buffer concentration. A protein concentration of 7.5 µg/g beads was found to be optimum. Maximum immobilization and activity yield was obtained at beads to buffer ratio of 1:2. Shaking of beads at 80 rpm resulted in the maximum immobilization and activity yield of xylanase and pectinase. Among a temperature range of 4 to 37 °C, the maximum immobilization and activity yield of xylanopepticolytic enzymes was obtained at 25 °C. The substrate concentration of 7.5 mg of xylan and 10 mg of pectin per gram beads was found to be optimum for maximum immobilization of xylanase and pectinase, respectively. A contact time of 4 h was sufficient for the maximum binding of enzymes with the beads. Glutaraldehyde at a concentration of 2% was optimized for cross linking of immobilized xylanopepticolytic enzymes. A crosslinking time of 30 min was sufficient for maximum crosslinking of immobilized xylanopepticolytic enzymes. Additional incubation at 4°C further increased the immobilization yield. Optimization of different process variables for covalent immobilization increased the immobilization yield up to 76.80 and 68.75, while the activity yield at this temperature was 67.70 and 56.36%, respectively for xylanase and pectinase.
- In ionic immobilization, binding of the xylanopepticolytic enzymes with the beads was maximum at pH 9.0 of 10 mM buffer concentration. The protein
concentration of 7.5 µg/g beads was found to be maximum binding capacity of beads. Maximum immobilization and activity yield was obtained at beads to buffer ratio of 1:2. Shaking of beads at 70 rpm resulted in the maximum immobilization and activity yield of xylanase and pectinase. Among a temperature range of 4 to 37 °C, the maximum immobilization and activity yield of xylanopecticolytic enzymes was obtained at 25 °C. The substrate concentration of 10 mg of each substrate, xylan and pectin per gram beads was found to be optimum for maximum immobilization of xylanase and pectinase, respectively. A contact time of 2 h was sufficient for the maximum binding of enzymes to the beads. Glutaraldehyde at a concentration of 2.5% was found to be optimum for cross linking of immobilized xylanopecticolytic enzymes. A crosslinking time of 30 min was sufficient for maximum crosslinking of immobilized xylanopecticolytic enzymes. Additional incubation at 4°C further increased the immobilization and activity yield. After ionic immobilization, the immobilization yield was reached up to 67.60 and 70.25, while the activity yield was 54.73 and 58.36%, respectively for xylanase and pectinase.

8. Characterization of immobilized xylanase and pectinase

- Free xylanase enzyme showed two pH optima, one at 6.5 and other at 8.5, which remained same after immobilization by entrapment method while, the immobilized xylanase enzymes after covalent and ionic immobilization showed maximum activity at pH 8.5. Free pectinase and immobilized pectinase after immobilization by entrapment method showed maximum activity at pH 9.5 while, the immobilized enzymes after covalent and ionic immobilization showed maximum activity at pH 9.0.
- Although, the free and immobilized xylanase enzymes were stable in a broad range of pH, but showed the maximum stability at pH 8.0. At pH 8.0, the free xylanase enzyme was 63% stable up to 12 h, while this stability was increased up to 74% after entrapment, 91% after covalent immobilization and 88% after ionic immobilization.
- Temperature optima of free xylanase was at 55°C, which remained same for immobilized enzyme after entrapment, while, after covalent and ionic
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immobilization it was shifted to 60°C. Optimum temperature for free pectinase was 55°C while, after immobilization it was shifted to 60°C for all the immobilized enzyme after entrapment, covalent and ionic immobilization.

- Free and immobilized xylanase was 100% stable at 37°C up to 6 h. At 45°C, free xylanase was 76% stable up to 6 h, while this value was increased up to 81, 89 and 87% after entrapment, covalent and ionic immobilization. Free enzyme was 23% stable at 50°C up to 6 h, while, the enzyme showed 60, 84 and 78% residual activities after entrapment, covalent and ionic immobilization. Free xylanase was unstable at 55 and 60°C, showing only 0.8 and 0.13% residual activities after 6 h of incubation, while, the residual activities were increased up to 24, 38 and 29% at 55°C and 19, 28 and 23% at 60°C after 6 h of incubation after entrapment, covalent and ionic immobilization.

- Free pectinase and immobilized pectinase after entrapment, covalent and ionic immobilization also showed 100% residual activity at 37°C up to 6 h. At 45°C, free pectinase showed 83% residual activity up to 6 h, while, this value was increased up to 89, 97 and 94% after entrapment, covalent and ionic immobilization. Free enzyme was 41% stable at 50°C up to 6 h while, the enzyme showed 68, 86 and 76% residual activities after entrapment, covalent and ionic immobilization. Free pectinase showed less stability at 55 and 60°C, showing only 18 and 9% residual activities after 6 h of incubation, while, the residual activities were increased up to 26, 41 and 37% at 55°C and 18, 29 and 21% at 60°C after 6 h of incubation after entrapment, covalent and ionic immobilization.

- Operational stability was checked at 55°C. A gradual decrease in the xylanase activity was seen after each cycle. Similar, results were obtained for pectinase. This was due to less stability of enzymes at 55°C.

- At 25°C, free xylanase enzyme showed 71% residual activity after 3 days of incubation, while, it showed 91% residual activity after entrapment and 100% residual activity after covalent and ionic immobilization. At 37°C, free xylanase was 52% stable up to 3 days of incubation while it was 65, 86 and 77% stable after entrapment, covalent and ionic immobilization. All the enzymes (free and immobilized) were 100% stable at 4°C even after 60 days.
Pectinase was more stable as compared to xylanase. At 25°C, free enzyme showed 24% residual activity after 30 days of incubation while, it showed 33, 57 and 48% residual activity after entrapment, covalent and ionic immobilization. At 37°C, free pectinase was 11% stable up to 30 days of incubation, whereas it was 18, 31 and 23% stable after entrapment, covalent and ionic immobilization. All the enzymes (free and immobilized) showed 100% stability at 4°C even after 60 days.

Km values of free and immobilized xylanase after entrapment, covalent and ionic immobilization were 13.33, 16.67, 11.76 and 11.11 mg/ml, respectively. Km value of free pectinase was 2.63 mg/ml, while, after entrapment, covalent and ionic immobilization, the Km values were found to be 3.33, 2.17 and 2.33 mg/ml for immobilized pectinase, respectively.

Vmax values for free xylanase and immobilized by entrapment, covalent and ionic binding were 1111.11, 909.09, 1408.45 and 1333.33 IU/mg protein, respectively. Free pectinase and immobilized by entrapment, covalent and ionic binding showed Vmax values of 662.25, 571.43, 740.74 and 724.64 IU/mg protein, respectively.

9. Application of xylano-pectinolytic enzymes in deinking of different types of waste papers

For effective deinking of waste paper, different process variables were optimized using “one variable at a time approach” in order to obtain the best effective biodeinking conditions.

Xylano-pectinolytic enzyme released maximum reducing sugars at 10% pulp consistency for deinking of school waste paper, mixed office waste paper and news paper waste. An enzyme dose of 15 IU of xylanase and 3.0 IU of pectinase per gram of pulp for school waste paper, 10 IU of xylanase and 8.0 IU of pectinase per gram of pulp for mixed office waste paper and 12.5 IU of xylanase and 10 IU of pectinase per gram of pulp for news paper waste, were found to be sufficient for ink removal. These xylano-pectinolytic enzymes released maximum ink at pH 8.5 from the pulp of all waste paper types. A treatment period of 150 minutes was found to be optimum for deinking of school waste paper and news
paper waste, whereas a treatment period of 120 minutes was required for deinking of mixed office waste paper. Performance of enzymes was maximum at 50°C. Maximum values of different optical properties were achieved, when non-ionic surfactant (Tween-80) was used at a concentration of 1% (volume on oven dried pulp basis) for school waste paper and mixed office waste paper, while Tween-80 concentration of 1.25% was required for maximum ink removal from news paper waste.

- After optimizing all necessary conditions, maximum values of brightness and whiteness were achieved, which were higher than control pulp sample by 4.12 and 3.30 units for school waste paper, 4.88 and 4.16 units for mixed office waste paper and 3.12 and 2.78 units for news paper waste, respectively. A decrease was also seen in the yellowness of the pulp by 1.36 units for school waste paper, 1.06 units for mixed office waste paper and 0.84 units for news paper waste. Handsheets deinked with these xylano-pectinolytic enzymes showed less residual ink as compared to control, showing deinking efficiency of 25.03% for school waste paper, 22.45% for mixed office waste paper and 19.55% for news paper waste.

- When enzyme treated pulp was subjected to chemical treatment of different composition (10%-80% reduction in NaOH, Na₂SiO₃ and H₂O₂), a reduction in the requirement of NaOH, Na₂SiO₃ and H₂O₂, was seen. Using this biodeinking plus chemical deinking methodology, a reduction of 50, 60 and 40% was seen in the deinking chemicals for school waste paper, mixed office waste paper and news paper waste, respectively.

- There was a gain of 10.71, 11.65 and 9.64% in viscosity, 7.49, 6.33 and 5.82% in breaking length, 10.52, 9.38 and 8.57% in burst factor, 6.25, 7.5 and 6.45% in tear factor after treatment with xylano-pectinolytic enzymes plus less consumption of chemicals for school waste paper, mixed office waste paper and news paper waste, respectively.

- This combined method also decreased the BOD and COD values of effluents as compared to conventional chemical deinking by 20.51% and 22.64% for school waste paper, 17.90% and 19.75% for mixed office waste paper and 18.89% and 17.68% for news paper waste respectively.
10. Application of xylano-pectinolytic enzymes in processing of textile fibres/fabrics

- All the necessary conditions were optimized for effective removal of non-cellulosic impurities from the fibres/fabrics to make them softer for textile utilities.
- Enzymes showed maximum activity at pH 8.5 and 50 mM buffer molarity. Effect of bioscouring was more at MLR (material to liquid ratio) of 1:20 for jute fabrics and ramie fibres while a MLR of 1:15 and 1:25 was required for bioscouring of cotton fabrics and sisal fibres. Xylanase and pectinase dose of 2.5 and 2.0 IU for jute fabrics, 5.0 and 4.0 IU for cotton fabrics, 7.5 and 6.0 IU for ramie fibres and 10.0 and 8.0 IU for sisal fibres was found to be optimum for maximum removal of non-cellulosic impurities. Maximum removal of these impurities was achieved after a treatment period of 60 min for jute fabrics, cotton fabrics and sisal fibres while, a treatment period of 90 min was required for ramie fibres at a temperature of 50 °C. Agitation speed of 60 rpm was required for treatment of jute and cotton fabrics, whereas bioscouring was highest at an agitation speed of 55 rpm for ramie and sisal fibres. EDTA (chelating agent) at a concentration of 0.5, 1.0, 1.5 and 2.0 mM was optimized for effective removal of impurities from jute fabrics, cotton fabrics, ramie and sisal fibres, respectively. Different wetting agents were used in order to improve the water absorbancy of the fibres/fabrics. Tween-80 (Non-ionic surfactant) at a concentration of 1% for jute and cotton fabrics, 1.25 and 0.50% for ramie and sisal fibres was optimized to increase the efficiency for these enzymes on removal of impurities.
- After enzymatic treatment, the whiteness and brightness of bioscour ed fabrics were increased by 7.30 and 19.45% for jute fabrics, 4.31 and 6.70% for cotton fabrics, 7.10 and 9.72% for ramie fibres and 6.83 and 11.52% for sisal fibres, respectively as compared to control. A decrease was also seen in the yellowness of the fibres/fabrics. After bioscouring, a decrease of 4.22, 12.24, 14.45 and 7.11% in yellowness was noticed for jute fabrics, cotton fabrics, and ramie and sisal fibres, respectively.
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- Alkaline scoured fibres/fabrics showed more weight loss as compared to bioscourred fibres/fabrics, which may be due to damage of the fibres/fabrics by the chemicals used in alkaline scouring.

- Wetting time of all bioscourred fibres/fabrics was found to be less than one second, while after alkaline scouring, the wetting time of the scoured fibres/fabrics was 1.5, 2.7, <1 and 2.2 seconds for jute fabrics, cotton fabrics, ramie and sisal fibres, respectively, which proved that bioscourred fibres/fabrics were more hydrophilic in nature.

- The characteristic peaks in the UV region showed the release of lignin during the treatment of fibres/fabrics.

- The results showed, the use of xylanase and pectinase completely replace the alkaline scouring method. Thus, enzymatic scouring is ecofriendly and energy saving, since it replaced the use of toxic chemicals used in alkaline scouring.

- After bleaching, an increase of 14.56 and 27.63% for jute fabrics, 2.18 and 2.33% for cotton fabrics, 1.03 and 2.53% for ramie fibres and 11.58 and 23.75% for sisal fibres, was obtained in whiteness and brightness of bioscourred plus bleached fibres/fabrics as compared to scoured plus bleached fibres/fabrics. Yellowness was also decreased by 30.05% for jute fabrics, 4.61% for cotton fabrics, 7.79% for ramie fibres and 27.99% for sisal fibres. Similarly, the tensile strength of bioscourred plus bleached fabrics was also improved by 29.06% for jute fabrics, 11.74% for cotton fabrics and 16.26% for sisal fabrics as compared to scoured plus bleached fibres/fabrics.

- The bioscouring efficiency of xylano-pectinolytic enzymes was also supported by the microscopic images of raw, bioscourred and alkaline scoured fibres using light microscope at 1000X.

11. Application of immobilized xylano-pectinolytic enzymes in deinking of school waste paper

- Enzyme released maximum reducing sugars and blue ink from the school waste paper pulp at 2.5% pulp consistency. These xylano-pectinolytic enzymes released maximum reducing sugars and ink at pH 8.5 from the pulp. An enzyme dose of 12.5 IU of xylanase and 7.5 IU of pectinase per gram of pulp was found to be
sufficient for ink removal. Enzymes were more efficient in removing ink, when the reaction mixture was agitated at 90 rpm. Performance of enzymes was maximum at 50°C. A treatment period of 150 minutes was found to be optimum for maximum deinking. Maximum values of different optical properties were achieved, when non-ionic surfactant (Tween-80) was used at a concentration of 1.25%.

- Enzymes released maximum reducing sugars from the pulp as compared to control, by a value of 5.14 mg/g pulp. Maximum values of brightness and whiteness were achieved, which were higher than control pulp sample by 3.91 and 3.16 units, respectively. Handsheets deinked with these xylano-pectinolytic enzymes showed less residual ink as compared to control, showing deinking efficiency of 23.10%.

- The reusability of the immobilized enzymes was analyzed by measuring the reducing sugars and blue colored ink released during deinking after each cycle and the residual activity was calculated by taking these values 100% at the first cycle. Immobilized xylano-pectinolytic enzymes showed nearly 90 and 80% efficiency after second and third cycle, while, the efficiency was reduced to nearly 60 and 40% after the fourth and fifth cycle.

12. Application of immobilized xylano-pectinolytic enzymes in treatment of poultry feed

- Enzyme released maximum reducing sugars at material to liquid ratio of 1:5. These xylano-pectinolytic enzymes released maximum reducing sugars at pH 8.0. Performance of these enzymes was highest at 50°C. An enzyme dose of 2.5 IU of xylanase and 2.0 IU of pectinase per gram feed was found to be sufficient for maximum release of reducing sugars from the feed. Enzymes released maximum sugars, when the reaction mixture was agitated at 55 rpm. A treatment period of 120 minutes was found to be optimum for maximum release of reducing sugars. After optimizing all necessary conditions, enzymes released maximum reducing sugars from the feed as compared to control, by a value of 9.24 mg/g feed.

- The reusability of the immobilized enzymes was analyzed by measuring the reducing sugars released after each cycle and the residual activity was calculated
by taking the reducing sugars released at the first cycle as 100%. Immobilized xylano-pectinolytic enzymes showed nearly 90 and 75% efficiency after second and third cycle, while, the efficiency was reduced to nearly 65 and 40% after the fourth and fifth cycle.