ABSTRACT

In the current study, cellulase free thermo-alkali-stable xylanase was produced through a cost effective method; followed by its immobilization and application in paper pulp bio-bleaching. A potent thermophilic xylanolytic bacteria; identified as *Geobacillus thermodenitrificans* X1 (GTX1) was isolated from Tattapani hot spring soil. The process of xylanase production from GTX1 was optimized by two methods: "one-factor-at-a-time method" (OFAT) and "response surface methodology" (RSM). Highest xylanase titer of 18.5 U/mL was observed after optimization by OFAT, whereas 1.3-fold elevation in the titer (24 ± 2 U/mL) was observed after RSM. For minimizing the production cost, commercial xylan was substituted by lignocellulosic residues. The xylanase titer achieved by utilizing lignocellulosic residues in the present study is highest in comparison to that, reported from other *Geobacillus thermodenitrificans* isolates. The crude GTX1 xylanase was further immobilized to form xylanase cross-linked enzyme aggregates (Xy-CLEAs). Various parameters influencing the preparation of Xy-CLEAs were optimized. GTX1 Xy-CLEAs rendered spherical shape with 202 nm diameter. After immobilization variation in the temperature and pH optima of xylanase was observed. Also, Xy-CLEAs rendered better stability at high temperature (70ºC) and alkaline pH (10) in comparison to free xylanase. The promising commercial potential of Xy-CLEAs was reflected through its reusability for six consecutive cycles with 53.5% retained xylanase activity and storage stability with an efficiency of 86% activity after being stored for 8 weeks at 4 ºC. Immobilization through preparation of Xy-CLEAs was observed to be advantageous in terms of enhanced functional and operational stability. The only loophole in the process was decreased xylanase activity after immobilization. Further, for exploring the potential of GTX1 xylanase as a bio-bleaching agent in the paper industry, crude xylanase was used. Bio-bleaching application of GTX1 xylanase was conducted in R&D laboratory of Kauntum Papers Ltd. situated in District Hoshiarpur, Punjab, India. Xylanase was used at a dosage of 50U/mL/g of agropulp (wheat straw-bagasse) for bio-bleaching. Application of GTX1 xylanase improved the paper properties such as freeness, breaking length and tear factor. The brightness of the paper produced was >80 ISO and there was 20% reduction in the chlorine consumption during the pulp bleaching process after xylanase pretreatment. Thus, GTX1 xylanase proved to be efficient bio-bleaching agent that could

potentially cut down the consumption of harsh chemical bleaching agent, without compromising with the paper quality and environment safety.

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