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Xylanase production by *Burkholderia* sp. DMAX strain under solid state fermentation using distillery spent wash

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Abstract

Xylanase production by a newly isolated strain of *Burkholderia* sp. was studied under solid state fermentation using anaerobically treated distillery spent wash. Response surface methodology (RSM) involving Box–Behnken design was employed for optimizing xylanase production. The interactions between distillery effluent concentration, initial pH, moisture ratio and inoculum size were investigated and modeled. Under optimized conditions, xylanase production was found to be in the range of 5200–5600 U/g. The partially purified enzyme recovered after ammonium sulphate fractionation showed maximum activity at 50 °C and pH 8.6. Kinetic parameters like K_m and V_{max} for xylan were found to be 12.75 mg/ml and 165 μmol/mg/min. In the presence of metal ions such as Ca^{2+}, Co^{2+}, Mn^{2+}, Ba^{2+}, Mg^{2+} and protein disulphide reducing agents such as β-mercaptoethanol and dithiotheritol (DTT) the activity of enzyme increased, where as strong inhibition of enzyme activity was observed in the presence of Cu^{2+}, Ag^{+}, Fe^{2+} and SDS. The crude enzyme hydrolysed lignocellulosic substrate, wheat bran as well as industrial pulp.

Keywords: Box–Behnken design; *Burkholderia* sp. DMAX; Distillery spent wash; Phylogenetic tree; Xylanase

1. Introduction

Distillery spent wash refers to the effluent generated from alcohol distilleries and pollution caused by it is one of the most critical environmental issue. On an average 8–15 l of effluent is generated for every liter of alcohol produced (Saha et al., 2005). There are 319 distilleries in India alone, producing 3.25 billion liters of alcohol and generating 40.4 billion liters of wastewaters annually (Pant and Adholeya, 2007).

The ever-increasing generation of distillery spent wash on the one hand and stringent legislative regulations of its disposal on the other has stimulated the need for developing new technologies to process this effluent efficiently and economically. A number of clean up technologies have been put into practice and novel bioremediation approaches for treatment of distillery spent wash are being worked out. Anaerobic treatment is a widely accepted practice and various high rate anaerobic reactor designs have been tried at pilot and full scale operations (Lata et al., 2002). However, anaerobically treated effluent still contains high amount of BOD (biochemical oxygen demand) and COD (chemical oxygen demand) and as such cannot be discharged directly (Nandy et al., 2002). At the same time, anaerobically treated distillery spent wash contains considerable nutrients in terms of potassium, sulfur, nitrogen and phosphorus. Moreover it also contains large amounts of micronutrients like Ca, Fe, Cu, Mn and Zn.

An emerging field in distillery waste management is exploiting its nutritive potential for production of various

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high value compounds. Several investigators have exploited the nutritive potential of distillery spent wash for production of various high value compounds such as biosurfactant (Dubey and Juwarkar, 2001), poly-β-hydroxybutyric acid (PHB) (Khardenavis et al., 2007), polyunsaturated fatty acids like docosahexaenoic acid and astaxanthin (Yamasaki et al., 2006) and single cell protein (Pathade, 2003). It has also been used for irrigation in agriculture (Ramana et al., 2002).

In the view of this, we attempted utilizing anaerobically treated distillery spent wash for production of an industrially important enzyme, xylanase, under solid state fermentation condition. Xylanases act on xylan, which is the major component of plant hemicellulose and is the second most abundant renewable hemicellulosic polysaccharide after cellulose. The key enzymes that constitute the xylanolytic system and which play the main role in depolymerization of xylan are endoxylanases (1,4-β-D-xylan xylanohydrolase, EC 3.2.1.8) and β-xylosidases (β-D-xyloside xylohydrolase, EC 3.2.1.37). Several accessory enzymes, such as α-arabinofuranosidase (α-L-arabinofuranoside arabinofuranohydrolase, EC 3.2.1.55), α-glucuronidase (α-D-glucuronidase, EC 3.2.1.131), acetylxylan esterase (EC 3.1.1.6), ferulic acid esterase and *p*-coumaric acid esterase are also necessary for hydrolysis of various substituted xylans (Saha and Bothast, 1999).

Research into microbial xylanase production is a welcome development as the enzyme has several important industrial applications such as bleaching of Kraft pulp, improving the digestibility of animal feed, clarification of juices, bioconversion of lignocellulosic waste into their constituent sugars, etc. (Shah et al., 2006). However, the problem faced by these industries is availability and cost of the enzyme. The cost of the enzyme is one of the main factors determining the economics of the process. Efforts on cost reduction have been directed towards increasing enzyme production by developing better microbial strains, efficient fermentation and recovery systems (Park et al., 2002; Xu et al., 2005). Solid state fermentation, an environment friendly and cost effective technology which involves the growth and metabolism of microorganisms on moist solids, generally low cost agro residues such as wheat straw, wheat bran, baggase, soya hulls, etc., in absence or near absence of any free flowing water and which also mimics the natural habitats of microbes has proven to be an efficient fermentation system in producing certain enzymes and metabolites including xylanases (Shah and Madamwar, 2005a; Khandeparkar and Bhosle, 2006).

In this study, we report the production of xylanase by a bacterial culture using distillery spent wash as the medium. Response surface methodology, using Box–Behnken design, was employed for optimizing the fermentation parameters. The properties of the partially purified xylanase preparation as well as its efficacy in saccharification of lignocellulosic waste have also been determined.

2. Methods

2.1. Collection and characterization of anaerobically treated distillery spent wash

Anaerobically treated distillery spent wash was collected from Kanoria Chemicals Ltd., Ankleshwar, Gujarat, India which treated distillery spent wash using an upflow anaerobic sludge blanket (UASB) reactor. The effluent samples were stored at 4 °C. The effluent was characterized and analyzed for pH, chemical oxygen demand (COD), biological oxygen demand (BOD), total solids (TS), total suspended solids (TSS), total organic carbon (TOC), total nitrogen (TN), phosphates, sulphates and metals based on the Standard Methods for Examination of Water and Wastewater (APHA, 1995).

2.2. Isolation and identification of xylanase producing culture

The xylanase producing bacterial cultures were isolated from soil samples collected from decaying agricultural waste. Screening of xylanase producing bacteria was carried out on xylan containing medium (in g/l: xylan, 2; peptone, 5; yeast extract, 1; NaCl, 5; K_2HPO_4, 1; $MgSO_4$, 0.2; $CaCl_2$, 0.1; and Na_2CO_3, 10). Sodium carbonate was sterilized separately and added to the rest of the medium after cooling (Gessesse, 1998).

The most promising bacterium was identified on the basis of morphological, biochemical as well as by 16S rDNA approach. Genomic DNA from the isolate was extracted as described by Ausubel et al., 1997. The genomic DNA was diluted appropriately to (~20–50 ng) and used as template in (30 μl) PCR reactions using universal eubacteria primers 16F27N (5′-CCA GAG TTT GAT CMT GGC TCA G-3′) and 16R1525 (5′-TTC TGC AGT CTA GAA GGA GGT GWT CCA GCC-3′) custom synthesized (MWG Biotech, Ebersberg, Germany). The amplified PCR product was purified as described previously (Mohana et al., 2007) and subjected to sequencing by automated DNA Analyzer 3730 using ABI PRISM® BigDye™ Cycle Sequencing kit (Applied Biosystems, Foster City, CA). The nearly complete sequence (>95%) of bacterial 16S rRNA gene (1476 bp) has been submitted to GenBank at NCBI. BLAST(n) program at NCBI server (Altschul et al., 1997) was used to identify and download the nearest neighbour sequences from the NCBI database. The phylogenetic tree was constructed by the neighbour joining algorithm using Kimura 2 parameter distance and more than 1000 replicates in Molecular Evolutionary Genetics Analysis 3.1 software (Kumar et al., 2001).

2.3. Xylanase production under solid state fermentation and enzyme extraction

Xylanase production was carried out in Erlenmeyer flask (250 ml) containing 5.0 g of wheat bran as the sub-