



BCIL *seeks partners to license...*

Technology for the Production of Cellulase-free Xylanase Enzyme

Biotech Consortium India Limited (BCIL) is seeking companies interested in commercializing a technology used for production of Thermostable Cellulase-free Xylanase enzyme. This technology has been developed at the Department of Microbiology, Guru Nanak Dev University (GNDU), Amritsar, India, keeping in view the potential application and current global status on the use of xylanase enzymes in paper and pulp industry.

BCIL was incorporated as public limited company in 1990 under the Indian Companies Act 1956. It is promoted by the Department of Biotechnology, Government of India and is financed by several all India financial institutions, venture capital funds and the corporate sector. BCIL has been actively involved in technology transfer, project consultancy, fund syndication, information dissemination, and manpower training & placement related to biotechnology over the last decade and half. BCIL has transferred more than 15 technologies in the last 5 years using its expertise in facilitating licensing agreements that allows a healthy and productive cooperation between the inventor and the licensee.

Introduction

The past few decades of the twentieth century have witnessed spectacular advances and betterment of living standards. In the field of chemical technology, where manufacture of a variety of products on large scale has resulted in serious effluent and hazardous waste disposal problems, the need for safer and 'environmental friendly' technologies has become imminent. Therefore enzyme alternatives to polluting chemical technologies, is a glimmer of hope to save the environment and yet achieve the goals of chemical technology in several frontier developments in this area. One such area is the Pulp and Paper Industry where the quantities of raw materials processed are huge, as well as the use of naturally hazardous chemicals are also large.

Xylanase is the name given to a class of enzymes which degrade the linear polysaccharide beta-1, 4-xylan into xylose, thus breaking down hemi-cellulose, which is a major component of the cell wall of plants. Microorganisms are rich sources of xylanase enzymes, which are produced by diverse genera and species of bacteria, actinomycetes and fungi. While several *Bacillus* species and filamentous fungi secrete high levels of extracellular xylanase, its secretion often accompanies cellulolytic enzymes. To use xylanase enzymes for pulp treatment, it is preferable not to have any accompanying cellulolytic activity, since the cellulase may adversely affect the quality of the paper pulp. Hence, the present technology imparts a step to address this problem by providing Cellulase-free Xylanase Enzyme.



Technology for the Production of Cellulase-free Xylanase Enzyme

Technology

- ❖ **Product:** Cellulase-free Xylanase Enzyme.
- ❖ **Source:** Strain D₂W₃ which has been developed by screening and selection amongst the wild type strains of thermophilic fungus isolated from the composting soils of Amritsar (India).
- ❖ **Process:** The following are the two methods to achieve maximum enzyme activity of Xylanase enzyme;
 - **Submerged culture production:** 4% of 48h old culture of developed thermophilic fungus strain D₂W₃ was inoculated in the shake flask culture containing complex medium (corn cobs, yeast extract and mineral salt solution) and incubated for 144h at 50°C under shaking conditions (200rpm). The maximum quantity of Xylanase activity (i.e. 2900 units/ml) was produced.
 - **Solid State Fermentation (SSF):** The production medium with sorghum straw (substrate) as carbon source and a cheap synthetic medium containing (NH₄)₂SO₄ as nitrogen source has been developed. A maximum level of xylanase activity (48000units/g substrate) was achieved at 50°C after 144 h of incubation under solid- state fermentation.
- ❖ **Optimization for achievement of Maximum Xylanase Activity:**
 - 🧪 **Method:** Optimization was carried out by response surface methodology using Box-Behnken design of experiments.
 - 🧪 **Validation:** Optimized levels were validated using repeated shake flask culture experiments.
 - 🧪 **Assay:** The culture filtrates were assayed for xylanase activity using Birchwood xylan.
- ❖ **Scale at which the technology has been developed:** The technology has been developed at the bench scale.

Salient Features:

- **Maximum Enzyme Activity:** Under solid-state fermentation maximum level of xylanase activity (48000units/g substrate) was achieved.
- **Thermostable:** The crude enzyme showed maximum activity in range of 70°C - 80°C and showed high thermostability where 60% activity was recovered after the enzyme was incubated at 55°C for 48h.
- **Optimum pH:** The crude enzyme was optimally active at pH 7.0 (100 %) and stable at the alkaline conditions (i.e. 66% enzyme activity at pH 8.6).
- Scaling-up at flask level did not resulting any change in the enzyme activity.