Centre - Eau Terre Environnement

HIGH-CELL DENSITY FERMENTATION FOR PRODUCTION OF LACCASE ENZYME USING METHYLOTROPIC YEAST – *PICHIA PASTORIS*

Lalit Kumar[1,2], Ashwani Gautam[1], Rishabh Gangwar[1], Saroj Mishra[1],Vikram Sahai[1], R.D. Tyagi [2]

[1]**Indian Institute of Technology Delhi, Hauz Khas, New Delhi-110016, India**

[2] **Institut national de la recherche scientifique, Université du Québec, Québec, Canada G1K 9A9**

Corresponding author- lalit20.iitd@gmail.com

INTRODUCTION

➢ The Laccase enzyme: *p*-diphenol: oxygen oxidoreductase(EC 1.10.3.2)
- Multicopper enzyme belongs to blue oxidases
- Active site contains four copper ions (Cu^{2+}) per molecule
- **Broad substrate specificity**, **High oxidation capacity**, Uses oxygen as final electron acceptor.

➢ Source: Majority of laccase have been isolated from higher fungi – Ascomycetes, Deuteromycetes and Basidiomycetes; especially white-rot Basidiomycete.

➢ **Applications :**
- In **pulp & paper**, **textile** and **cosmetic** industries, for detoxification and decolouration of sewage
- In **organic synthesis**, for degradation of xenobiotics & **bioremediation** to create antimicrobial compositions
- In production of **wood-fiber plates**, **wood-blocks** & **cardboard** without using toxic linkers
- In **detergents** production
- In elaboration of **biosensors** and cathodes of **biofuel cells**.

➢ **Current production technologies & their limitations:**
- Most filamentous fungi produce several isoforms of laccase in high amounts.
- **However, an efficient production system at bioreactor level is still lacking**.

➢ **Our approach**:
- **Over-expression** of laccase in a suitable host
- Reducing the cost of laccase production by optimizing the fermentation strategy.

✓**Yeast** are suitable host for heterologous protein production because of **high capacity** for growth, **easy manipulation** of unicellular organism & a eukaryotic organization enables **post-translational modification**.

✓***Pichia*** is a well described & widely applied production system well known for high cell densities fermentation & hence **higher productivity** could be expected.

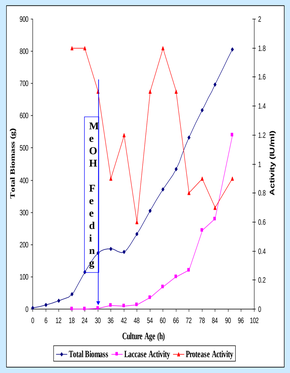


Fig 1:Process Chart

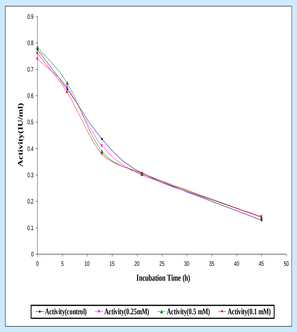
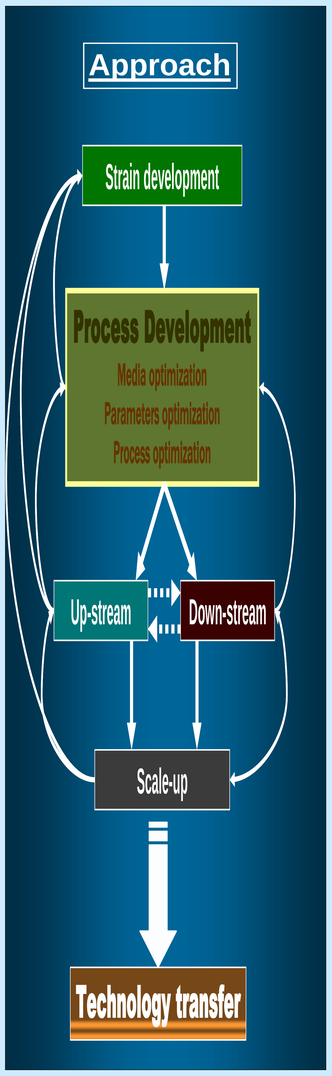
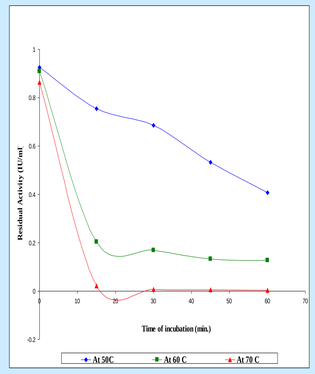


Fig 3:Effect of Cu $^{2+}$ on the stability of the protein





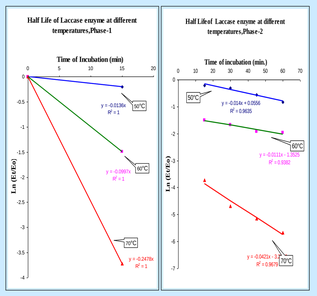


Fig 2 : Effect of different temperatures on Laccase stability (above); biphasic trend of temperature stability (below)

RESULTS

➢ Laccase activity was observed on induction with methanol.

➢ Maximum activities were observed on day 3 of the process on BMMGy medium (in shake flask)

▪Activity observed in the range of **100 IU/L** (with ABTS as substrate).

➢ Laccase activity was fairly stable up to 100 h when stored at 4 C

➢ Scale up was carried with a bench scale fermenter (5L) in a fed-batch mode
- Total production of **1200 IU/L** was obtained (fig 1).
- Final biomass concentration was **270 g/L** WCW (fig 1).
- Overall productivity of process was **300 IU/L.D**

👍 **Productivity with recombinant culture was 1.3 folds higher than the native fungus.**

➢ Temperature stability of laccase:
- Laccase showed biphasic trend of heat stability (fig 2).

Half life (min.)	At 50°C	At 60°C	At 70°C
Phase - 1	50.96	6.95	2.8
Phase -2	49.5	6.23	16.47

➢Effect of copper on laccase stability at room temperature

▪No significant effect of Cu^{2+} was observed on the stability of laccase with respect to control (Fig 3) when added in the supernatant.

FURTHER PLANS

Experimental work is planned to achieve:

✓ **complete characterization of recombinant protein.**

✓ **optimization of operation strategies to reduce the overall process time.**

✓ **an integrated approach for simultaneous fermentation & (semi) continuous product recovery.**

✓ **improved stability of recombinant product.**

✓ **minimal down-stream processing cost.**

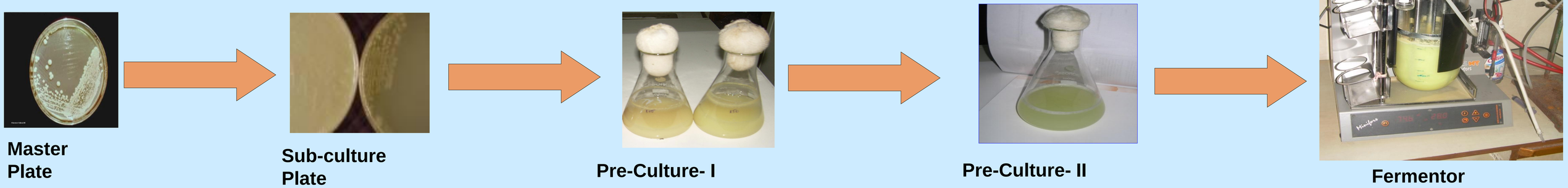
WORK DONE SO FAR

➢**Complete cDNA encoding laccase from *Cyathus buleri* was cloned, sequenced & expressed in *Pichia pastoris* under the influence of AOX1 promoter.**

➢ **Laccase secreted into the medium under the control of α-factor secretion signal of *Saccharomyces cerevisiae***

➢ **Cultivation of the developed *Pichia* strain has been done at shake flask level on different media - YPD and BMMGy.**

Fig 4.Schematic diagram of fermentation process with *P. pastoris*



Master Plate | **Sub-culture Plate** | **Pre-Culture- I** | **Pre-Culture- II** | **Fermentor**

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Canadian Association on Water Quality
Association canadienne sur la qualité de l'eau
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