IMPORTANCE OF CELLULASES IN BIOETHANOL FROM BIOMASS: A REVIEW

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INTRODUCTION

- The continous depletion of the fossils fuel reserves and consequent escalation in their price has stimulated an extensive evolution of alternative technologies and substrates to meet the global energy demand.
- Global crude oil production is predicted to decline from 25 billion barrels to approximately 5 billion barrels in 2050 due to the shortage of fossils fuels, the emission of green house gases and air pollution by incomplete combustion of fossils fuels has also resulted in an increased focus on production of biofuel from lignocellulosic biomass.

Useful Properties of Biofuel

- Most biofuel derived from biomass which is renewable ,low cost,easily available carbon source.
- Entailing little or no commitment on foreign exchange.
- Low green house gases emission.

Country wise production of Bioethanol

- Brazil is the largest producers of bioethanol (13.5 billion liters).
- U.S.A attained 6.4 billion liters.
- European union(France, U.K, Germany and Italy)having 2 billion liters.

Useful properties of bioethanol

- Ethanol has much higher latent heat of vaporization (855 MJ/Kg) than petrol (293KJ/Kg)
- Ethanol has a higher octane number (99) than petrol (80-100) as a result pre ignition does not occur when ethanol is used
- Ethanol is burnt more completely so that hydrocarbon emission is drastically lower as compared to petrol.
- Ethanol is much less likely to catch fire and explodes in case of fuel leakage.

Economic analysis of bioethanol production

- About 15 years ago the cost of ethanol production from cellulosic substrates was US$4.0 per gallon.
- But in recent years this cost has been lowered to US $ 1.8 per gallon by putting the effort of the results of recent scientific research .
- Further the projected cost as low as US $ 0.20 per liter in 2015 if enzymatic processing and biomass improvement together are met.

Role of cellulases

- Celluloses are one of the major structural components in all lignocellulosic wastes.The cellulose contained in those materials may be converted into ethanol by the application of cellulases.
- Cellulase enzyme system consist of at least three enzymes :
- Endoglucanase or CM Case or 1, 4 β D glucanglucanohydrolase, Cx (E.C. 3.2.1.4) - causing random scission of amorphous cellulose
- Exoglucanase or cellobiohydrolase or Avicelase or endo 1-4 β D glucan cellobiohydrolase E.C. 3.2.1.91) - causing exoattack on the non reducing end of cellulose
- β Glucosidase or cellobiase (E.C. 3.2.1.21) causing hydrolysis of cellobiose to glucose.
- Finally the glucose which is form by the reaction these enzymatic system can be utilised by the ethanol producing microorganisms for ethanol production either by two steps or by simultaneously in one step called simultaneous saccharification and fermentation.

Cost reduction strategies

- Ethanol production cost varies as a function of substrate price,Product yields and cellulase production cost.High cost of cellulase is one of the major hinderence to make the process commercialized. This can be reduced by adopting two strategies:
- One at the ethanol production level.
- And other at cellulase production level.

Advantages and limitations of SSF

- Attain a higher (upto 40%)yields of ethanol by removing end product inhibition.
- Eliminating the need of separate reactors for saccharification and fermentation .
- Shorter fermentation time.
- Reduced risk of contamination through external microflora.
- But the major drawback is that complete process occures at two different temperature optima and some ot the toxic substances arising from the pretreatment of lignocellulosics inhibits the action of fermenting microorganisms as well as the cellulase activity.

Improvement at ethanol production level

- By metabolic engineering approaches.
- By genetic engineering approaches.

Metabolic engineering approaches

In this approach engineered the metabolic pathways for conversion of renewable resources into ethanol. Various modes of central metabolism of 5 and 6 carbon sugars play an important role in deciding the ultimate fate of conversion of pyruvate ,the key 3 – carbon intermediate to ethanol.

Expanding the pentose utilizing capabilities of the hosts (*Saccharomyces* and *Zymomonas*) which are already efficient to converting hexose to ethanol. Direct carbon flow from the native fermentative product to ethanol in the hosts (*Escherrchia,Klebsialla,Erwinia*)which are already efficient in utilizing mixed sugars.

Genetic engineering approach

Genetic engineering is a very recent approach for getting the organisms with desired properties.

By this process *E.coli,Klebsialla oxytoca* and *Erwinia* sp to allow them directly ferment xylose to ethanol.

Key genes from the glucose fermenting bacterium *Zymomonas mobilis* have been incorporated into these organisms to allow ethanol production .

Yeast(*Saccharomyces and Pichia*)and bacteria *(E.coli,Klebsialla* and *Zymomonass mobilis*) have been genetically engineered to ferment glucose ,xylose and arabinose sugars .

By genetic engineering it is also possible to transfer cellulase genes from *Trichodermma* to *Saccharomyces cerevisiae* it is also possible for *Penicillium* ,*Neurospora* and *Aspergillus* to produce"Superstrains" via genetic enginnering capable of hydrolyzing cellulose and xylan along with fermentation of glucose and xylose into ethanol.

We have now develop a series of recombinant bacteria for the bioethanol production by introducing the *Zymomonas mobilis* genes encoding alcohol dehydrogenase and pyruvate decarboxylase to provide a functional ethanol pathway.

Utility of cellulases

The large market potential and the important role that cellulase play in the emerging bioenergy and biobased industry provide a great motivation to develop better cellulase preparations for plant cell wall cellulose hydrolysis.Enzyme cost is considered to be a major impediments in extensive commercialization of enzymatic cellulose hydrolysis.Cost of enzyme has decreased over the last 20 years but is still considered to be very high much research efforts have been focused on lowering the cost of enzyme .

Recently the US department of energy has added more resourse to the cellulase enzyme research contracts to the world leading industrial enzyme producers:Novozymes (www.novozymes.com)and Genencor international (www.genencor.com) with the goal to reducing cellulase costs for commodity biomass conversion.

Improvement at cellulase production level

The following strategies can be applied for reducing the cellulase production cost

- By strain improvement methods.
- By isolating novel and overproducing strains.
- By using mixed cultures or co cultures of organisms.
- By using novel,cheap,renewable and easily available cellulosic wastes.
- By using fed batch system.
- Applying recent trends in solid state fermentation.

Strain improvement methods

- By Mutagenesis.
- By Protoplast Fusion Technology.
- By Genetic Engineering and Recombinant DNA Technology.

Mutagenesis

This is one of the important process using in strain improvement.

- A mutant EMS-UV 8 of *P.janthellum* capable of showing enhanced zone walseth cellulose hydrolysis was isolated using EMS treatment followed by UV irradiation of the spores. All these mutants produced two times higher FPAse and CMCase activities than the parent strain .
- Successive UV and nitrsoguanidine treatments on *Aspergilus terreus* ATCC 52340 resulted in isolation of strain UNGI-40 having 3.5,4.6 and 3.3 fold increase in filter paper ,β-glucosidase and carboxymethyl cellulase activity respectively compared to *Aspergillus terreus* ATCC 52430 parental strain.

Protoplast Fusion Technology

Protoplast fusion technique is a powerful tools In the strain improvement.

- This process is helpful in the production of a complete set of cellulase by the protoplast fusion of *T.reesei* and *A.niger* (one produced more amount of endo and exoglucanase and other produced more β- glucosidase .
- Isolated protoplast from *Trichodermma reesei* strain PTr2 showed high CMCase activity with 80% of fusants and more than two fold increment in enzyme activities with two fusants SFTr2 and SFTr3 as compared to the parental strain PTr2.

Genetic engineering and RDT

Recombinant DNA technology and genetic engineering offers the possibility of fusing different lignocellulolytic genes or section of genes from different organism to give rise to novel chimeric gene products with improved properties.

The low amount of β-glucosidase results in a shortage of capacity to hydrolyze the cellobiose to glucose resulting in a feed back inhibition of enzyme production. This can be overcome by engineering β-glucosidase genes into the organism so that it is overproduced.

Isolation of novel and overproducing strains.

Isolation and screening of new microbial sources of highly efficient hydrolytic enzymes for cellulosic ethanol production.

Mixed cultures or cocultures

In order to enhance cellulose activity ,mixed cultures are generally used.Application of mixed cultures is an alternative tool to overcome feedback inhibition and catabolite repression.

The mixed culture of *T.reesei* and *A.phoenicus* could produce cellulase containing a high level of β-glucosidase from dairy manure. β-glucosidase activity and filter paper activity from the mixed culture system could reach 0.64 IU/ml and 1.54 FPU/ml respectively

Novel,cheap and renewable carbon source

Due to the high production cost of pure cellulose such as Solka flok and Avicel the various lignocellulosic raw material is proved to be an best alternative for cellulase production such as wheat straw.Rice straw,Corn straw etc.

Xia et al reported the cellulase production by solid state fermentation using corncob residue, a lignocellulosic waste from the xylose industry, as the substrate of *Trichoderma reesei ZU*.

Fed batch system

Fed batch fermentation involves a slow additions of highly concentrated nutrients into the bioreactor . this operation can maintain low nutrient levels to minimize catabolite repression or to extand the stationary phase by nutrient addition to obtain additional product.

Fed batch with feed back control in case of substrate inhibition is widely used in industrial fermentation for increasing the cellulase productivity.

Recent trend in solid state fermentation

Solid state fermentation is the more productive way for production of enzyme but most of the solid state fermentation suffer with a critical problem related to heat transfer.

This major problem can be removed by using periodical dynamic change of air(Including air pressure pulsation and internal circulation of air) instead of agitation and rotation in SSF.

Enzyme engineering

Enzyme engineering opens up numerous possibilities for designer enzymes with various engineered properties.

The active carboxylic residues in *T.reesei* endoglucanase identified by site direct mutagenesis opening up future research prospects for modifying those to improved activity.

A number of designer enzymes also called glucosynthases including cellulase and hemicellulase have been engineered by replacing the nucleophilic residue thus resulting in higher yields of different oligosaccharides.

Rationale design methods

This is the one of the strategy applied for improving the individual cellulase component .

Rational design is the earliest approach to protein engineering was introduced after the development of recombinant DNA technology and site directed mutagenesis.

Rationale design appears to be a logical method for researchers to examine the possible amino acid site near to the active site or the binding pocket in a 3-dimensional structure.

Engineering cellulase mixtures

NREL researches determines that a 90:9:1 mixture of a cellobiohydrolase from *T.reesei*(CBHI) a thermotolerant endoglucanase from *Aspergillus cellulolyticus* (EI) and a β-glucosidase was capable of nearing the performance observed for a leading commercial *T.reesei* preparation at comparable protein loading.

Proteonomics

For more complex biomass feedstocks,understanding the roles and relationship of component enzymes from the *Trichodermma reesei* cellulase system acting on complex substrates is the key to developed of efficient artificial cellulose systems for the conversion of lignocellulosic biomass to sugars.

Endoglucanases,Cellobiohydrolase,xylanase,β-glucosidase,β-mannnaase at NREL these enzyme are being fingerprinted from *T.reesei* broth by 2-D gel electrophoresis and confined by direct peptide sequence analysi

THANK YOU