

PRODUCTION OF CYCLODEXTRIN GLUCANOTRANSFERASE FROM
ALKALOPHILIC *Bacillus* sp. TS1-1 USING FED BATCH CULTURE

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*“Terima kasih Mami, kakak, abang, adik
dan semua yang terlibat secara langsung dan tidak langsung.
Ingatan yang berpanjangan dan Al-Fatihah untuk Allahyarham Daddy.”*

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ABSTRACT

The study of fed batch fermentation was carried out to enhance the production of CGTase from *Bacillus* sp. TS1-1. The microbes was grown in 2 %(w/v) soluble starch, 1 %(w/v) yeast extract, 0.1 %(w/v) K₂HPO₄, 0.02 %(w/v) MgSO₄.7H₂O and 10 %(w/v) Na₂CO₃ solution. Batch fermentation was carried out as control using 5 L fermentor with 4 L working volume. A maximum CGTase activity of 70.32 U/ml was observed during the stationary phase of growth with specific activity of 0.198 U/ μ g proteins. The fed batch study was carried out to obtain the best feeding mode, carbon and nitrogen sources. Constant feed rate fed batch result gave the highest increment in CGTase production by 25.3% as compared to the batch fermentation. Tapioca starch at concentration of 2 %(w/v) was selected as the best inducer for both CGTase and biomass production, where improvement of 35.6% and 25.7% was observed respectively, as compared to the batch fermentation. The addition of 0.5 %(w/v) nitrogen source in the feeding medium failed to improve the CGTase production, but on the other hand increased the biomass significantly. An increment of 69.3% in terms of biomass production as opposed to batch fermentation was obtained with yeast extract. The optimization of carbon and nitrogen concentration using tapioca starch and yeast extract was carried out using *Response Surface Methodology* (RSM). The optimum condition obtained were 3.3 %(w/v) of tapioca starch and 0.13 %(w/v) of yeast extract. The optimized medium improved the CGTase production up to 13.9% as compared to batch fermentation. The production of CGTase in repeated fed batch fermentation using 2 %(w/v) of tapioca starch was quite consistent even after the third addition of fresh medium with maximum activity fluctuating between 80 - 86 U/ml.

ABSTRAK

Kajian ke atas kaedah fermentasi suapan balik dijalankan untuk mengatasi had-had sekatan yang terdapat di dalam fermentasi berkelompok, seterusnya dapat meningkatkan lagi penghasilan siklodekstrin glukanotransferase (CGTase) daripada *Bacillus* sp. TS1-1. Kultur inokulum dibiakkan di dalam medium yang mengandungi 2 % (b/i) kanji terlarut, 1 % (b/i) ekstrak yis, 0.1 % (b/i) K_2HPO_4 , 0.02 % (b/i) $MgSO_4 \cdot 7H_2O$ dan 10 % (b/i) stok larutan Na_2CO_3 . Fermentasi berkelompok (kawalan) dijalankan menggunakan bioreaktor 5 L (isipadu kerja sebanyak 4 L), dan penghasilan CGTase yang maksimum (70.32 U/ml) diperolehi semasa pertumbuhan mula memasuki fasa pegun, dengan aktiviti spesifik sebanyak 0.198 U/ μ g. Proses penyaringan dijalankan untuk menentukan jenis suapan, sumber karbon dan sumber nitrogen. Suapan secara kadar tetap memberikan peningkatan maksimum aktiviti CGTase sebanyak 25.3% berbanding dengan penghasilan di dalam fermentasi berkelompok. Kanji ubi kayu berkepekatan 2 % (b/i) dipilih sebagai sumber karbon terbaik dengan peningkatan sebanyak 35.6% untuk CGTase dan 25.7% biomas berbanding dengan fermentasi kelompok. Penambahan 0.5 % (b/i) sumber nitrogen di dalam medium suapan menurunkan aktiviti CGTase, namun begitu pertumbuhan bakteria ini adalah sangat menggalakkan. Ekstrak yis memberikan peningkatan terbaik iaitu sebanyak 69.3%. Proses pengoptimuman kepekatan sumber karbon (kanji ubi kayu) dan nitrogen (ekstrak yis) dijalankan menggunakan kaedah gerakbalas permukaan (RSM). Penghasilan CGTase yang optimum adalah menggunakan 3.3 % (b/i) kanji ubi kayu dan ekstrak yis pada 0.13 % (b/i). Penghasilan CGTase sebanyak 80.12 U/ml diperolehi dengan peningkatan sebanyak 13.9% berbanding penghasilan di dalam fermentasi berkelompok (70.32 U/ml). Keputusan daripada percubaan aplikasi suapan balik ulangan menggunakan medium 2 % (b/i) kanji ubi kayu pula menunjukkan penghasilan CGTase yang agak konsisten walaupun selepas 3 kali kitaran dengan aktiviti maksimum diantara 80 - 86 U/ml.

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LIST OF SYMBOLS/ABBREVIATIONS

CCD	-	Central composite design
CD	-	Cyclodextrin
CGTase	-	Cyclodextrin glucanotransferase
D	-	Dilution rate
DO	-	Dissolved oxygen
F	-	Flowrate
g	-	Gram
H	-	Height
H ₀	-	Null hypothesis
H ₁	-	Alternative hypothesis
hr ⁻¹	-	Per hour
hr	-	Hour
L	-	Liter
M	-	Molar
mg	-	Miligram
min	-	Minutes
ml	-	Milliliter
mM	-	Milimolar
nm	-	Nanometer
S	-	Substrate concentration
S ₀	-	Initial substrate concentration
R ²	-	Regression coefficient
RSM	-	Response surface methodology
rpm	-	Round per minute
t	-	Time
T	-	Temperature

U	-	Unit (enzyme activity)
V	-	Volume
V_0	-	Initial volume
v/v	-	Volume per volume
W	-	Width
w/v	-	Weight per volume
X_{\max}	-	Maximum biomass concentration
μm	-	Micrometer
μg	-	Microgram
μ	-	Specific growth
\AA	-	Angstrom
$^{\circ}\text{C}$	-	Degree Celsius
%	-	Percentage

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CHAPTER 1

INTRODUCTION

1.1 Introduction and Problem Statement

Microbial extra cellular enzymes are responsible for much of the polymeric organic matter cycling. Diversity of substrates, environments, and organisms has led to the evolution of a prolific variety of enzymes. Amongst the amylases, α -amylase was long thought to be the only bacterial starch-degrading enzyme. At present, it had been discovered, that there were endo and exo-acting amylases with and without α -(1,6) debranching activity with every imaginable optimum temperature and pH. This diversity had fuelled the enzyme industry for the past few decades and still provides sufficient novel enzyme to justify further screening (Priest, 1992). One of the important extra cellular enzymes in biotechnology is called cyclodextrin glucanotransferase (CGTase), which is capable of synthesizing a unique product called cyclodextrin (CD).

CGTase is known to have been produced by various genera of bacteria such as *Bacillus*, *Klebsiella*, *Psuedomonas*, *Brevibacterium*, *Thermoanaerobacterium*, *Corynebacterium*, *Micrococcus* and many more. All known CGTases produced a mixture of α -, β - and γ -cyclodextrins and their proportion being characteristic of the individual enzymes. CGTase producers can be found in various places such as soil, waste, plantation, hot springs and even deep-sea mud (Georganta *et al.*, 1993). Some known CGTase producers are *Bacillus macerans* (Kim *et al.*, 2000), *Bacillus circulans* (Bovetto *et al.*, 1992), *Bacillus firmus* (Goel and Nene, 1995), *Bacillus*

stearothermophilus (Kabaivanova *et al.*, 1992), *Bacillus coagulans* (Akimaru *et al.*, 1991) and *Klebsiella pneumonia* (Gawande and Patkar., 2001).

The production of CGTase is normally carried out as batch and fed-batch processes or variations of these procedures. Several authors have reported that CGTase production was highly dependent on strain, medium composition and culture conditions. Very little information has been obtained regarding CGTase production using fed batch culture (Chen *et al.*, 1994; Park *et al.*, 1997 and Gawande *et al.*, 2003). Most papers focused on CGTase production in fed batch fermentation using the recombinant organism. Journals reviewing the wild strain or novel-producing enzymes are very scarce. Fed batch culture is used to remove the repressive effects of rapidly utilized carbon sources, reduce the viscosity of the medium and the effect of toxic medium constituents or simply to extend the product formation stage of the process for as long as possible. The fed batch method has also been used to improve the phycocyanin production (Zhang and Chen, 1997), protease from *Bacillus sphaericus* (Singh *et al.*, 2004), recombinant β -1,3-glucanase (Shene *et al.*, 1999) biomass of *Spirulina platensis* (Alberto *et al.*, 2003), polysaccharide and ganoderic acid from *Ganoderma lucidum* (Tang and Zhong., 2002), polysialic acid (Xiaobei *et al.*, 2002) and xylitol from from *Bacillus licheniformis* (Yoon *et al.*, 2000).

The main purpose of this work is to explore the potential of fed-batch culture as a fermentation system for CGTase production that is produced by an alkalophilic bacteria, *Bacillus* sp TS1-1. The effect of various parameters, e.g. types of culture, nutrients and concentration of substrates on the kinetic aspects of fermentation, were also studied.

1.2 Objectives of Study

The objective of this research is to enhance the production of CGTase from *Bacillus* sp. TS1-1 in batch, fed batch and repeated fed batch fermentation. Besides that, the effects of various carbon and nitrogen sources and C/N ratio in the feed stream on the CGTase production were also studied.

1.3 Scopes of Study

The scope of the research consists of four parts:

- i) To evaluate the feeding controlled strategy for the fed batch fermentation in order to find the best feeding mode that produce the highest CGTase yield.
- ii) To investigate the best carbon and nitrogen sources that give the highest increment for CGTase production.
- iii) To optimize the of carbon and nitrogen concentration in the feed medium to give the maximum CGTase production using Central Composite Design.
- iv) To carry out a comparative study of CGTase production in batch, fed batch and repeated fed batch fermentation.

can be extended in the future in order to diversify the use of the species economically. Several recommendations on the studies are listed below:

1. β -cyclodextrin which has been found to be dominantly produced by CGTase from *Bacillus* sp.TS1-1 using only the crude enzyme has given new dimensions to explore and study on the bioconversion of cyclodextrins.
2. Cloning and expression of CGTase gene from *Bacillus* sp.TS1-1 can also improve the CGTase production. The study should help to understand the characters of the recombinant and mechanisms involved through modeling of the CGTase structure. Besides, the modification of the genetic structure of the bacteria is expected to give a high yield of CGTase. Furthermore, the mutation of the specific gene also helps to improve the product of interest and this can be studied through various methods.
3. The production of CGTase by novel *Bacillus* sp. TSI-1 in fermenter has been applied using batch and fed batch systems. Therefore, other types of fermentation principles such as continuous fermentation and immobilized cell system can be applied in order to increase the production of CGTase.
4. The characters of CGTase can be further studied in the section of purification processes. The isolation of one particular protein from other contaminating proteins may stimulate the studies of its structure and other properties. Once a suitable cellular source of the protein has been identified, the protein is liberated into the solution and separated from the contaminating material by sequential use of a series of different fractionation techniques or separations. Full characterization of the purified CGTase will be expected to give the increment of the CGTase production.

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