Purification and characterization of some biotransformational polymers isolated from microbial species

Thesis Submitted For Ph.D. Degree in Biochemistry

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علما نافعا

ورزقا طيبا

وعملا متقبلا

بسم الله الرحمن الرحيم

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا
إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ

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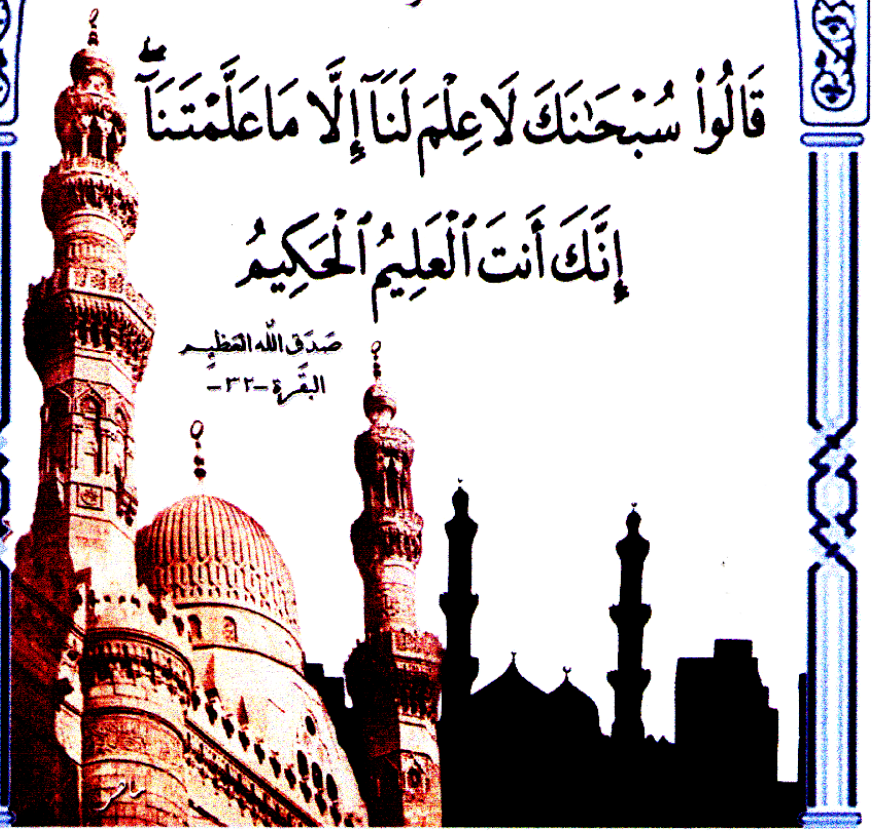

Declaration

This thesis has not been submitted to this or any other University.

Marwa Mohsen Mostafa

Abstract

In recent years the biotechnological production of balk biopolymer has focused on the synthesis of biodegradable polymers to replace their non-biodegradable counter parts derived from fossil resources. The microbial production of polyhydroxybutyrate (PHB) is a complex process in which the final quality and quantity of PHB depends on large number of process operating variables consequently the design and optimal operation of microbial process for efficient PHB production is an extremely interesting problem.

In trend toward suitable manufacturing bioplastic, that can be produced from renewable resources have attracted great attention. The present study investigates how key process operating variables affect biomass production rate and PHB accumulation in cells.

The obtained data reports that, bacterial strain which identified as *Bacillus cereus* capable of PHB production in medium containing glucose, tryptone, NaCl and yeast extract in submerged culture at 37 $^{\circ}$C, pH 6 for 48 hrs and the yield is approximately about 70% of cell dry weight. In addition, molasses is considered as the suitable and relatively cheap carbon feed-stocks have to be identified to maximize cost-effective production of PHB.

CONTENTS

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Marwa Mohsen Mostafa Sarhan

ABBREVIATIONS

ANP	:	Atrial natriuretic peptide
CDW	:	Cell dry weight
CGP	:	Cyanophycin granule peptide
cphA	:	Cyanophycin synthetase gene
FDA	:	Food and drug administration
FAs	:	Fatty acids
GDP	:	Guanosine diphosphate
GLC	:	Gas Liquid Chromatography
HA	:	hyaluronic acid
LB	:	Luria-Bertani medium
LDPE	:	Low Density Polyethylene
LPS	:	Lipopolysaccharide
MOL	:	Molasses
MSM	:	Minimal Salt Media
NADPH	:	Nicotinamide adenine dinucletide phosphate
NaOCl	:	Sodium hypochlorite
NMR	:	Nuclear Magnetic Resonance
P (3HB)	:	Poly (3-hydroxybutyrate)
PCL	:	Polycaprolactone
PDO	:	Polydioxanone
PGA	:	Poly-γ-glutamate
PHAs	:	Polyhydroxylalkanoates
PHB	:	Polyhydroxybutyrate
PHV	:	Polyhydroxyvalerates
Pl	:	ε-poly-l-lysine
SB	:	Sudan Black B
SDS	:	Sodium dodecyl sulfate
TLC	:	Thin layer chromatography
Tm	:	Melting temperature
UDP	:	Uridine diphosphate

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INTRODUCTION

Biotransformations are the basis of life. All natural organic and most inorganic molecules, the building blocks of organic and living matter, are subject to constant change and turnover. The turnover time is determined by the reactivity and the reaction partners of the respective compounds, and varies from millions of years to microseconds (***Nishino et al., 2010***).

Biotransformation differs from biosynthesis where complex products are assembled from simple substrates by whole cells, organs or organisms. They are also different from biodegradations in which complex substances are broken down to simple ones. It has great potential to generate novel products or to produce known products more efficiently (***Archana et al., 2001***).

Biotransformation capabilities of microorganisms and their enzymes for the production of a wide variety of fine chemicals are well known (***Ward and Singh, 2000***).

Nowadays management of plastic waste is a major worldwide concern. As a synthetic plastic is non-

biodegradable, it is creating a great havoc to environment. Plastic and synthetic polymers are mainly produced using petrochemical material and they contribute to environmental pollution .They are also dangerous to number of animal species (***Flora et al., 2010***).

Although plastic has many advantages its non-biodegradability is a major drawback, which forced us to think upon a material which can replace plastic. Earlier studies showed that Polyhydroxybutyrate (PHB) is biodegradable material, which has physical properties similar to the synthetic plastic. PHB has various applications in different areas like medicine, drug manufacture, agriculture and various industrial purposes. Some of the major applications of PHB are in drug delivery in developing medical sutures, bone marrow scaffold, tissue engineering devices and agriculture products (***Sarika et al., 2008***).

PHB is equivalent to a very basic polymer, polyhydroxyalkanoates (PHA). PHB is produced by microorganisms in the form of reserved food granules under stress condition like excess availability of carbon source but