UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF ORGANIC ACIDS FROM RESTAURANT WASTES AS SUBSTRATES FOR POLYHYDROXYBUTYRATE PRODUCTION BY RECOMBINANT *E. COLI*

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By

MAJD KHALID ESHTAYA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

June 2007

DEDICATION

ALLAH YOU ARE MY SUPREME LOVE

ALHAMDULILLAH ….THANK YOU FOR EVERYTHING

TO MY BELOVED COUNTRY PALESTINE

MY BELOVED PARENTS, SISTERS AND BROTHERS

THANKS FOR YOUR DOA', LOVE AND CARE

FRIENDS

THANKS FOR YOUR HELP AND SUPPORT

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman: Professor Mohd. Ali Hassan, PhD

Faculty: Engineering

In this study, restaurant waste was utilized as sole carbon source for the production of polyhydroxybutyrate (PHB) by recombinant *Escherichia coli*. PHB production was achieved via a two-stage process; organic acids production from fermented restaurant waste followed by PHB production utilizing the organic acids. In the first stage, the effect of initial pH, operating temperature and enzymatic pretreatment (hydrolysis using α-amylase and glucoamylase) were investigated to get highest organic acids production from restaurant wastes in anaerobic treatment. Anaerobic treatment was carried out in 500 mL Erlenmeyer flask using orbital shaking incubator. Three different temperatures were chosen; 30°C, 37°C and uncontrolled (room temperature). The initial pH of fermentation was also varied; adjusted at 7 and without adjustment. The organic acids produced were maximum (66 g/L) on the fifth day of fermentation that was carried out at 37°C, initial pH adjustment at 7 and enzymatic pretreatment with the yield of 0.723 g organic acids/g initial COD. The main organic acids produced was acetic acid (50%),

followed by lactic acid (33%), iso-butyric acid (9%) and n-butyric acids (8%). The results indicate that high concentration of organic acids were produced in anaerobic treatment of restaurant wastes, which could served as alternative and abundant substrate for the production of biodegradable plastics. The supernatant collected from the anaerobic treatment of highest organic acids production was concentrated by vacuum evaporation to be used for PHB production. The total organic acids obtained after evaporation process was about 100 g/L. In the second stage, the concentrated organic acids were successfully converted to PHB using batch and pH-stat fed-batch fermentation by recombinant *Escherichia coli* containing. In batch fermentation about 6.8 g/L of cell dry weight and 2.5 g/L of PHB were achieved after 18 hours of cultivation, resulting in PHB content of 36% and productivity of PHB 0.14 g L^{-1} h^{-1}. On the other hand, the maximum PHB concentration obtained was 9.2 g/L after 17 hours corresponding to 44% (g/g) of PHB content and 0.539 g L^{-1} h^{-1} productivity in pH-stat fed-batch fermentation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGHASILAN ASID ORGANIK DARIPADA SISA BUANGAN RESTORAN SEBAGAI SUBSTRAT UNTUK PENGHASILAN POLYHYDROXYBUTYRATE OLEH REKOMBINAN *E. COLI*

Oleh

MAJD ESHTAYA

Jun 2007

Pengerusi: Profesor Mohd Ali Hassan, PhD

Fakulti: Kejuruteraan

Di dalam kajian ini, bahan buangan restoran telah digunakan sebagai sumber karbon tunggal untuk penghasilan polyhydroxybutyrate (PHB) oleh rekombinan *Escherichia coli*. Penghasilan PHB dilakukan melalui dua peringkat; penghasilan asid organik daripada bahan buangan restoran yang dirawat seterusnya penghasilan PHB menggunakan asid organik tersebut. Dalam peringkat pertama, kesan pH permulaan fermentasi, suhu operasi and pra-rawatan berenzim (hidrolisis menggunakan α-amylase dan glucoamylase) telah dikaji untuk menggalakkan penghasilan asid organik daripada bahan buangan restoran dalam rawatan anaerobik. Rawatan anaerobik telah dijalankan dalam kelalang Erlenmeyer menggunakan inkubator penggoncang orbital. Tiga suhu yang berbeza telah dipilih; 30°C, 37°C dan tanpa kawalan (suhu bilik). pH permulaan proses fermentasi juga berbeza; pH 7 dan tanpa pembetulan pH. Maksimum asid organik yang dihasilkan ialah 66 g/L pada hari kelima fermentasi yang dijalankan pada suhu 37°C, pH permulaan 7, dan pra-rawatan berenzim memberikan angkali hasilan 0.723 g

asid organik/COD awal. Asid organik utama yang dihasilkan ialah asetik (50%), diikuti oleh asid laktik (33%), asid iso-butirik (9%) dan asid n-butirik (8%). Keputusan tersebut menunjukkan asid organik pada kepekatan yang tinggi dapat dihasilkan dalam pra-rawatan bahan buangan restoran, dimana ia boleh membekalkan substrat yang murah dan banyak untuk penghasilan plastik boleh urai. Supernatan yang dikumpul daripada pra-rawatan yang paling tinggi penghasilan asid organik telah dipekatkan menggunakan pengewap kedap udara untuk digunakan seterusnya dalam peringkat penghasilan PHB. Jumlah asid organik yang dihasilkan selepas proses pengewapan adalah sekitar 100 g/L. Dalam peringkat kedua, asid organik yang dipekatkan telah berjaya ditukarkan kepada PHB menggunakan fermentasi sekelompok dan suapan sesekelompok oleh rekombinan *E.coli*. Dalam fermentasi sekelompok, 6.8 g/L berat sel kering dan 2.5 g/L PHB telah dihasilkan selepas inokulasi selama 18 jam menghasilkan kandungan PHB dalam sel sebanyak 36% dan produktiviti PHB ialah 0.14 g L^{-1} h^{-1}. Sebaliknya bagi fermentasi suapan sesekelompok pH-stat, kepekatan maksimum PHB yang didapati adalah 9.2 g/L selepas 72 jam fermentasi bersamaan dengan 44% g/g kandungan PHB dan 0.539 g L^{-1} h^{-1} produktiviti.

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I certify that an Examination Committee met on 15 June 2007 to conduct the final examination of Majd Khalid Eshtaya on her Master of Science thesis entitled "Production of Organic Acids from Restaurant Wastes as Substrate for Polyhydroxybutyrate (PHB) by Recombinant *Escherichia Coli*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as Follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of Supervisory Committee are as follows:

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Date: 9 August 2007

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

MAJD ESHTAYA

Date: July 2007

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LIST OF ABBREVIATIONS

CDM	Clean Development Mechanism
CDW	Cell dry weight
COD	Chemical oxygen demand
Da	Dalton
GC	Gas chromatography
HPLC	High performance liquid chromatography
HRT	Hydraulic retention time
MCL	Medium Chain Length
MSW	Municipal solid waste
NADH	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate (reduced form)
OD	Optical density
P(3HB-co-3HV)	Poly(3hydroxybutyrate-co-3hydroxyvalerate)
P(4HB)	Poly (4-hydroxybutyrate)
P(HBHHx)	Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PHO	Poly (3-hydroxyoctanoate)
PHV	Polyhydroxyvalerate
rpm	Rotation per minute

SCL	Short Chain Length
TKN	Total Kjeldahl Nitrogen
VFA	Volatile Fatty Acid

CHAPTER 1

INTRODUCTION

Over the past decades, plastics derived from petrochemicals, have become indispensable materials. Unfortunately, most of these materials are not biodegradable, thus they remain indefinitely in landfill sites and this causes serious environmental and waste management problems. On the other hand, when plastic waste are disposed of by incineration, numerous air pollution problems are generated from the incineration of the chemical additives in plastic formulations such as ash disposal, emission of dioxins, acid gases, carbon dioxide and other toxic gases (Wang et al., 2003).

Study by Khanna and Srivastava (2005) discussed about the problems concerning the global environment and solid waste management have created much interest in the development of biodegradable polymers as replacement to non-biodegradable petrochemicals plastics. Among the several biodegradable polymers under development, polyhydroxyalkanoates (PHAs) have attracted much attention because of their similar material properties to the conventional petrochemical derived plastics and their complete biodegradability in various environments. Polyhydroxyalkanoates (PHAs) are polyesters of hydroxyalkanoates that are synthesized and intracellularly accumulated as an energy and/or carbon storage material by numerous microorganisms. Polyhydroxybutyrate (PHB) was the first PHA to be discovered and is the most widely studied and best characterized PHA. It is accumulated as a membrane enclosed inclusion in many bacteria at up to 80% of the dry cell weight. It has mechanical properties very similar to conventional plastics like polypropylene.

One of the major drawbacks of employing PHAs in a wide range of applications is the high production cost, which makes it difficult to compete with petrochemical-based polymers. Therefore, much effort has been devoted to reducing the production cost of PHAs by the development of better bacterial strains and fermentation that is more efficient and recovery processes. Recombinant *Escherichia coli* strain, which has been thought to be a potential host for the production of PHAs, has been successfully employed for the production of PHB to a high concentration with high productivity. One of the important factors in determining the applicability of PHAs production on the industrial scale is the cost of raw materials (Ahn et al., 2001). In recent years, there has been a growing interest in the production of biodegradable plastics from renewable resources such as municipal solid waste, wastes from the food industry, household and waste from agriculture and forestry as a cheaper carbon substrate (Wang et al., 2003).

In this study, restaurant waste is used as the raw material for PHB production since food waste is a nutrition-rich resource and it can be used as a substrate in fermentation. Therefore, it is possible to consider coupling a restaurant waste treatment process with PHB production. This technique is beneficial in several ways. Firstly, it can reduce the quantity of restaurant waste, therefore reducing the costs of food waste treatment; secondly, large amount of organic acids (lactic, acetic, proionic and butyric acids) produced during anaerobic treatment of restaurant waste is used to produce PHB by recombinant *Escherichia coli* containing the *Ralstonia eutropha* PHB biosynthetic genes.

The objectives of this study are as follows:

1. The production of organic acids from restaurant waste in anaerobic treatment at various conditions.

2. To utilize the organic acids obtained from fermented restaurant waste as substrates for the production of polyhydroxybutyrate (PHB) by recombinant *Escherichia coli* containing in batch and fed-batch culture.

CHAPTER 2

LITERATURE REVIEW

2.1 Conventional Plastics

Within the last 50 years, petrochemical plastics have become one of our most applied materials. Plastics have wide range of strengths and shapes, lightness, low price, durability and resistance to degradation (Khanna and Srivastava, 2005) and the application of plastics in industry ranging from automobiles to medical (Zinn et al., 2001).

The problem of environmental pollution caused by dumping of plastic waste has assumed global proportions. Plastic waste, which accounts approximately 20–30% of the volume of municipal solid waste landfill sites are recalcitrant to microbial degradation (Ishigaki et al., 2004). Due to very fast development of society and the limitation of satisfactory landfill sites burial of plastics wastes in landfill is a time bomb (Suprakas and Bousmina, 2005). Solutions to plastic waste management include source reduction, incineration, recycling and bio- or photo-degradation (Khanna and Srivastava, 2005). Incineration plastics wastes are dangerous and expensive. During the combustion of plastic waste, a large amount of carbon dioxide produces and creates global warming and some times produces toxic gases like hydrogen chloride and hydrogen cyanide, which again contribute to global pollution. Furthermore, recycling has some major disadvantages, as it is difficult sorting the wide variety of plastics and there are changes in the plastic's material such that its further application range is limited (Reddy et al., 2003).

On these backgrounds, there is an urgent need for the development of friendly polymeric materials that would not involve the use of toxic or noxious component in their manufacture and could be degradable in the natural environmental conditions. For these reasons, throughout the world today, the development of biodegradable materials with controlled properties has been a subject of great research challenge to the community of materials scientists and engineers (Suprakas and Bousmina, 2005).

Three types of biodegradable plastics introduced are photodegradable, semi biodegradable and completely biodegradable. Photodegradable plastics have light sensitive groups incorporated directly into the backbone of the polymer as additives. However, landfills lack sunlight and thus they remain non-degraded. Semi-biodegradable plastics are the starch-linked plastics where starch is incorporated to hold together short fragments of polyethylene. Soil microorganisms degrade the starch easily, thus breaking down the polymer matrix while the polyethylene fragments remain non-degradable. The third type of biodegradable plastics is utilization by bacteria to form a biopolymer these include polyhydroxyalkanoates (PHA), polylactides (PLA), aliphatic polyesters, polysaccharides, copolymers and/or blends of the above (Reddy et al., 2003). Among the various biodegradable polymer materials, polyhydroxyalkanoates (PHAs) are attractive substitutes for conventional petrochemical plastics because of their similar material properties to conventional plastics and complete biodegradability after disposal

(Ojumu et al., 2004).

2.2 Polyhydroxyalkanoates (PHAs)

Polyhydroxyalkanoates (PHAs) are biological polyesters of various hydroxyalkanoates, which are accumulated as a carbon and/or energy storage material in various microorganisms usually under the condition of limiting nutritional elements such as nitrogen, sulphur, phosphate, oxygen, magnesium and potassium in the presence of excess carbon source. After the first discovery of poly (3-hydroxybutyrate) (PHB) from the bacterium *Bacillus megaterium* by the French scientist Lemoigne in 1926, many different PHAs possessing different numbers of main chain carbon atoms and different types of pendent groups have been found (Lee and Park, 2005). However only several PHA including PHB, copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate P(3HB-co-3HV), poly 4-hydroxybutyrate P(4HB), copolymers of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx) and poly 3-hydroxyoctanoate (PHO) are available in sufficient quantity for application or research purpose (Chen and Wu, 2005).

2.2.1 General Structure of PHA and Some Representative Members

More than 100 different monomer units have been identified as constituents of the storage PHA as shown in Figure 2.1. This enables producing different types of biodegradable polymers with an extensive range of properties. The monomer units are all in D (-) configuration due to the stereo-specificity of the biosynthetic enzymes which is important for their biodegradability and biocompatibility (Reddy et al., 2003).