UNIVERSITI PUTRA MALAYSIA

UTILISATION OF FOOD WASTES FOR THE PRODUCTION OF ORGANIC ACIDS AND BIOPLASTICS

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By

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ALLAH
MY DESTINY IS HERE
DROP DOWN AND STRONG AGAIN
IT SO MEANINGFUL SPIRIT TO
UNDER YOUR BLESS
I'M SUCCESSFUL
ALHAMDULILLAH… THANK TO
FOR ALL THINGS
ITS

MY BELOVED PARENTS,
ABDUL WAHAB & ZAIMAH
THANK YOU FOR EVERYTHING
ANYTHING YOU DO
YOUR ENCOURAGE, YOUR GIVING, YOUR WISHING AND ALL
ALHAMDULILLAH…I LOVE YOU SO MUCH

ABANG, KAK, NYAH, YANG, AND YONG,
FOR THERE WISHING ME ALL THE BEST…
ALHAMDULILLAH…SO APPRICIATED

ADIK
AL-FATIHAH (28/7/2001)
DEEPLY MISSED BY FAMILY…
ALWAYS LOVED AND REMEMBERED….
MAY SHE REST IN PEACE
AMIN…

MY BELOVED,
HAMBALI
HAPPY AND SAD WTH ME
YOUR WISHING WITH ME
TO ENCOURAGE ME
TRUST AND LOYALTY
HAS MADE ME FEEL VERY LUCKY
ALHAMDULILLAH…INVALUABLE LOVE

FRIENDS
YOUR HELPESS AND YOUR ENCOURAGE
IT SO MEANINGFUL TO ME
I'M VERY APPRICIATE
THANK YOU
FOR ALL..

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

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October 2003

Chairman: Professor Dr. Mohd Ali Hassan

Faculty: Food Science and Biotechnology

In this study, food wastes were used as substrates for the production of polyhydraxyalkanoate (PHA) by *Ralstonia eutropha* (formerly known as *Alcaligenes eutrophus*). Restaurant waste obtained from a local restaurant and house was blended with an equal weight of water. PHA production was achieved via a two-stage process; organic acids production from food waste followed by PHA production from the organic acids. In the first stage, the study focused on the production of short-chain organic acids from complex organic fraction of food waste. The food waste was subjected to anaerobic treatment by the natural micro flora present in a 2-liter batch stirred tank bioreactor with mixing at 200 – 400 rpm. The effect of pH, temperature, substrate compositions of food waste on the production of volatile fatty acids (VFA) was evaluated. For the effect of different pH, the batch anaerobic medium was done [uncontrolled pH, controlled at pH 7 for different number of days (initial, one day, two days and whole duration)].

The effect of different temperatures (30°C, 37°C, 45°C) and different substrate compositions [kitchen waste (A), restaurant waste (B) and restaurant waste mixed with fruits waste (C)] have been done in the research. Restaurant waste mixed with fruit waste (substrate C) produced 58 g/L organic acids compared to 42 g/L from kitchen waste (substrate A) under same conditions at 37°C without control pH. Lactic acid was the most predominant acid, followed by acetic, propionic, butyric and formic acids. The study on the effect of temperature with uncontrolled pH shows that 37°C is the best temperature, producing 58 g/L organic acids, with 83% lactic acid. In the effect of pH, it was found that the highest amount of organic acids produced was 68 g/L, with 84% lactic acid when the initial pH was adjusted to 7.0 without further pH control.

Overall the best conditions for organic acids production is pH controlled at 7 in the initial treatment at 37°C using restaurant wastes containing fruit waste. Studies on microbial and enzyme activity was done on treated food waste to determine the factors contributing to the high organic acids especially lactic acid. From the microbes identified, *Lactobacillus* was predominantly involved in the fermentation to produce lactic acid. The enzyme lipase, protease, cellulase and α-amylase were detected throughout the treatments leading to the conversion of organic complex to sugar and subsequently to organic acids with α-amylase as the highest activity. In the recovery of organic acids from the fermented food waste, centrifugation was carried out to remove the solids and oils. The supernatant was collected and concentrated by evaporation for the second stage of bioplastic production.

The results show that by evaporation method, the organic acids were concentrated two-fold to about 100 g/L for use as substrates for fed-batch fermentation. PHA production from organic acids fermented from food waste, as a carbon source for fed-batch fermentation is 4.2 g/L with 88% of dry cell weight. From the experiment, C/N 10-20 because ammonium concentration in the original food waste was high. This encouraged cell growth in the medium rather than PHA production. In the PHA production, since C/N must be high (around 100-150), removal of ammonium was successfully achieved using Dowex 88wx resin for improved PHA production. The ammonia-deficient organic acids from food wastes were successfully converted to PHA by *Ralstonia eutropha* strain ATCC 17699, producing 8.9 g/L with 90%(g/g) PHA content.

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

PENGGUNAAN SISA MAKANAN BAGI MENGHASILKAN ASID ORGANIK DAN BIOPLASTIK

Oleh

NORRIZAN ABDUL WAHAB

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Pengerusi: Professor Mohd Ali Hassan, Ph.D.

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Dalam kajian ini, sisa makanan digunakan sebagai substrat bagi penghasilan polyhydroxyalkanoate (PHA) oleh *Ralstonia eutropha* (juga dikenali sebagai *Alcaligenes eutrophus*). Sisa makanan yang diperolehi daripada restauran tempatan dan rumah kemudian ditimbang dengan jumlah berat air yang sama untuk dihancurkan (blender). Terdapat dua peringkat kajian bagi penghasilan PHA iaitu penghasilan asid organik dari sisa makanan dan diikuti dengan penghasilan PHA menggunakan asid organik yang diperolehi sebagai substrat. Dalam tahap pertama, kajian difokuskan kepada penghasilan rantaian pendek asid organik daripada pemecahan organik kompleks dari sisa makanan tersebut. Komposisi sisa makanan yang hampir sama bagi rawatan anaerobic dipengaruhi oleh kehadiran microflora semulajadi di dalam 2-liter bioreactor tangki berpengaduk yang dicampur pada kelajuan dari 200 hingga 400 rpm.

Kajian ke atas perbezaan pH [tanpa kawalan pH, pH dikawal pada pH 7 (awal rawatan, satu hari, dua hari dan sepanjang rawatan)], perbezaan suhu (30°C, 37°C, 45°C) dan komposisi substrat yang berbeza [sisa dapur (A), sisa restoran (B) dan sisa restauran yang mengandungi sisa buah (C)] dari sisa makanan dalam memberikan kesan ke atas penghasilan asid lemak bebas di nilai. Substrat C dari sisa restoran yang mengandungi sisa buah menghasilkan 58 g/L berbanding dengan substrate A dari sisa rumah yang hanya menghasilkan 42 g/L asid organik dalam keadaan yang sama iaitu pada suhu 37°C tanpa pengawalan pH. Umumnya, asid laktik merupakan asid yang paling banyak dihasilkan diikuti oleh asetik, propionik, butirik dan asid formik. Dalam kajian ke atas kesan suhu, suhu pada 30°C, 37°C dan 45°C digunakan tanpa kawalan pH. Didapati keputusan menunjukkan suhu 37°C merupakan suhu yang paling optimum menghasilkan 58 g/L asid organik dengan 83% asid laktik.

Bagi kesan pH, media di biarkan tanpa kawalan pH dan pengawalan pada pH 7 untuk bilangan hari yang berbeza. Didapati penghasilan asid organik yang tinggi iaitu 68 g/L, 84% asid laktik diperolehi, apabila pada awal rawatan, pH di kawal pada pH 7 dan seterusnya dibiarkan tanpa pengawalan pH. Dari keseluruhan keputusan, keadaan yang paling baik untuk penghasilan asid organik ialah dengan mengawal pH pada pH 7 hanya pada awal rawatan pada suhu 37°C dengan menggunakan substrat C. Kajian dalam microbiologi dan aktiviti enzim telah dibuat ke atas fermentasi sisa makanan bagi menentukan faktor yang menyumbang kepada kepekatan asid organik yang tinggi terutama asid laktik.

Didapati microb *Lactobacillus* memainkan peranan penting dalam fermentasi sisa makanan secara anaerobik menghasilkan produk laktik asid. Enzim lipase, protease, cellulase dan α-amylase telah dikesan sepanjang rawatan dalam proses pertukaran kompleks organik kepada gula dan seterusnya ditukarkan kepada asid organik dengan enzim α-amylase mempunyai tahap aktiviti yang tinggi. Bagi memperolehi asid organik daripada fermentasi, sisa makanan diempar bagi membuang pepejal dan minyak. Kemudian supernatan di kumpul dan dipekatkan dengan pemeluapan untuk digunakan bagi penghasilan bioplastik. Hasil dari pemeluapan, asid organik telah dipekatkan dua kali iaitu lebih kurang 100 g/L untuk digunakan sebagai substrat fermentasi suapan sesekelompok.

Penghasilan PHA dari sisa makanan sebagai sumber karbon untuk suapan sesekelompok adalah 4.2 g/L dengan 88% berat sel kering. Didapati nilai C/N adalah rendah (10-20) disebabkan kepekatan amonia di dalam media pengeluaran semakin bertambah semasa fermentasi. Keadaan ini menggalakkan pertumbuhan sel dalam media. Bagi penghasilan PHA, C/N mesti tinggi (100-150), jadi pergurangan kepekatan amonia telah dibuat dengan menggunakan resin Dowex 88wx dalam proses meningkatkan penghasilan PHA. Asid organik dari sisa makanan yang mempunyai kepekatan amonia yang agak rendah telah berjaya ditukarkan kepada PHA oleh *Ralstonia eutropha* ATCC 17699 menghasilkan sebanyak 8.9 g/L dengan 98 % (g/g) kandungan PHA.

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Wassalam

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BOD_5	-	Biochemical oxygen demand (after 5 days incubation of sample at 20°C in the dark)
C	-	Concentration
C/N	-	Carbon to nitrogen ratio
CO_2	-	Carbon dioxide
COD	-	Chemical Oxygen demand
GC	-	Gas chromatography
HPLC	-	High pressure liquid chromatography
ICI	-	Imperial Chemical Industries
PHA_s	-	Polyhyroxyyalkanoates
P(3HB)	-	Poly (3-hydroxybutyrate)
PHBV	-	Poly (hydroxybutyrate-co-hydroxyvalerate
VFA	-	Volatile fatty acid
LAB	-	Lactic acid bacteria
TCA	-	Trychloroacetic acid
CHO	-	Carbohydrate

CHAPTER 1

INTRODUCTION

Food waste is on the increase with bigger population and affluence. Food waste is non-toxic, rich in organic matter and highly biodegradable. Most of these wastes end up in landfill sites, whereby the carbonaceous compounds are eventually decomposed to biogas. Apart from contributing to environmental pollution, such a system destroys the organic matter, which could be utilized as useful materials for producing value added products such as organic acids. Food wastes contain carbohydrates, proteins and lipids. These compounds are essential for the growth of microorganisms to synthesize desired products. Through biological treatment, food waste can be subjected for organic acids production, particularly for lactic acid production under controlled conditions. The organic acids can then be used as substrates by microbes to produce bioplastics.

It is known that many bacteria can produce biopolymers intracellularly under certain growth conditions, such as excess carbon and limiting nitrogen. These biopolymers have properties similar to normal plastics, with the advantage that they are biodegradable upon disposal in the soil within a few months. Through research, alternatives for energy-intensive chemical processes can be explored, thus reducing our national dependence on petroleum. Developing biodegradable plastic is one way to solve the waste disposal problem. Biodegradable plastics that do not pollute the global environment has become an extremely important product (Doi, 1990).

These biodegradable plastic materials must retain the desired material properties of conventional synthetic plastic, and should be completely degraded without leaving any undesirable residues when discarded. However, such bioplastics are not widely used currently due to their high costs. Currently, PHA in the form of poly (3HB-co-3HV) or PHB/V is being produced on a fairly large scale by Imperial Chemical Industries Limited (ICI) in Britain under the trade name Biopol using *Alcaligenes eutrophus* H16. Unfortunately, the high cost of PHA has limited their use on a wide scale. ICI's Biopol is sold for more than US$4.40/kg compared to polypropylene, which is only US$0.60/kg. This high cost is mainly due to the carbon source and the use of large quantities of organic solvents in the recovery process for extracting the intracellular polymers (Hassan, 1997).

The high PHA production cost can possibly be lowered by using unpurified, low cost organic wastes from agriculture and food processing plants (Cho *et al*., 1997). In this study, different compositions of food waste were chosen as substrates, which are rich in organic compounds necessary for bacterial growth. PHA or biodegradable thermoplastic polyhydroxyalkanoates can be produced from food waste in a two-stage process. During the first stage, the complex organic compounds are digested under anaerobic conditions by bacteria into volatile fatty acids (VFA) such as acetic, lactic, butyric and propionic acids. Then, in the second stage, the PHA-producing bacteria such as *Ralstonia eutropha (*previously known as *Alcaligenes eutrophus)* can utilize the VFA and polymerize the acids into PHA as carbon and energy reserves under nitrogen limited conditions.

Objectives

The objectives of this study are as follows:

- To obtain the best conditions such as substrate composition, temperature and pH in the utilization of food waste for the production of organic acids as materials for the production of PHA.

- To identify microbial and enzymatic activities affecting the production of organic acids during the fermentation of food waste.

- To produce polyhydroxyalkanoate (PHA) by *Ralstonia eutropha* using organic acids from anaerobically treated food waste.