

# **UNIVERSITI PUTRA MALAYSIA**

# COMPARISON OF MUNICIPAL AND KITCHEN WASTE LEACHATES FOR THE PRODUCTION OF ORGANIC ACIDS AND POLYHYDROXYALKANOATES (PHA)

JAME'AH BINTI HAMED

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By

## JAME'AH BINTI HAMED

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

April 2003

### **DEDICATION**

## THE ONLY ONE ONLY WITH YOUR BLESSING GIVE ME HEALTH AND STRENGTH

## FATHER AND MOTHER WITH YOUR BLESS AND YOUR DOA YOUR LOVE AND SUPPORT

## BROTHERS AND SISTERS WITH YOUR HELP AND UNDERSTANDING

## TEACHERS FOR YOUR ADVICE AND GUIDANCE

## FRIENDS FOR YOUR HELP AND SUPPORT

## WITH ALL THIS I CAN HAPPILY GO ON WITH MY LIFE AND STUDY

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

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#### April 2003

#### Chairman: Professor Dr. Mohd. Ali Hassan

Faculty : Food Science and Biotechnology

The feasibility of using municipal solid waste leachate as carbon source for the production of polyhydroxyalkanoates (PHA) was investigated in this study. Leachates from MSW in Kuala Lumpur area were subjected to anaerobic treatment under specified condition for the production of organic acids. Two types of leachates were used, i.e. fresh leachate from the city council garbage trucks and combined leachate from several cells at the sanitary landfill. Initially, prior to the PHA production, the characteristics (pH, COD, BOD<sub>5</sub>, ammonium, total solid, suspended solid, organic acids and heavy metals) of the municipal solid waste leachate were analysed. The effect of pH and inoculum on organic acids production during anaerobic treatment was evaluated. Treatments were carried out at 30°C for 7-10 days under different conditions of pH, i.e. uncontrolled pH, adjusted to initial pH 7, controlled at pH 7 for 24 hours, and continuous control at pH 7 and pH 5.5.

The production of organic acids from fresh leachate was highest when the initial pH was adjusted to pH 7 with no further pH control. About 45 g/L total organic acids was produced, comprising of 28 g/L lactic acid, 8 g/L acetic acid and 9 g/L propionic acid. Based on the initial BOD<sub>5</sub>, the organic acid yield was about 80%. In contrast, with the combined landfill leachate the highest organic acids obtained was only 14 g/L when the pH was controlled at pH 5.5, with acetic acid as the main product. Temperature at  $37^{\circ}$ C gave the highest production on the second day. When the fresh leachate was autoclaved and seeded with 10% fermented kitchen garbage, the highest organic acids achieved were between 34-37 g/L. The highest selectivity of lactic acid (85%) was achieved during treatment of fresh leachate seeded with kitchen garbage without any pH adjustment. Overall, the results showed that the fresh leachate could be effectively converted to 45 g/L total organic acids by anaerobic treatment when the initial pH was adjusted to pH 7.

The supernatant collected from the treatment of highest organic acids production was used in the second stage for the production of polyhydroxyalkanoate (PHA). *Ralstonia eutropha* was used for the production of PHA using leachate as carbon source for the batch and fed-batch fermentation. In batch fermentation using shake flask, 3.4 g/L PHA was produced which gave 86% g PHA/g cell. In fed-batch fermentation the maximum PHA concentration was 6.9 g/L, corresponding to 85% (g/g) of cell dry weight. The PHA produced was plastic-like and resembles low density polyethylene. Thus the fresh leachate proved to be a suitable carbon source for organic acids and subsequently used in PHA production. Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

## PERBANDINGAN ANTARA CECAIR LELEHAN (LEACHATE) SISA PERBANDARAN DAN SISA DAPUR UNTUK PENGHASILAN ASID ORGANIK DAN POLIHIDROKSIALKANOATE (PHA)

Oleh

#### **JAME'AH BINTI HAMED**

April 2003

#### Pengerusi : Profesor Dr. Mohd. Ali Hassan

### Fakulti: Sains Makanan dan Bioteknologi

Dalam kajian ini cecair lelehan (leachate) dari sisa buangan pepejal perbandaran digunakan sebagai sumber karbon untuk penghasilan polihidroksialkanoate (PHA). Leachate dari sisa pepejal perbandaran di kawasan Kuala Lumpur secara subjektif dilakukan rawatan anaerobik dalam keadaan tertentu untuk penghasilan suatu produk yang bernilai (asid organik). Pada permulaannya ciri-ciri cecair lelehan dari sisa buangan pepejal dikenal pasti dari segi komposisinya. Ciri-cirinya termasuk pH, COD, BOD<sub>5</sub>, amonium, pepejal keseluruhan, pepejal terampai, asid organik dan logam berat. Peringkat seterusnya adalah untuk menilai kesan pH dan inokulum untuk penghasilan asid organik semasa rawatan anaerobik. Dua jenis cecair lelehan digunakan iaitu cecair lelehan yang baru dari lori sampah majlis perbandaran daerah dan cecair lelehan campuran daripada

beberapa buah sel di kawasan kawalan kebersihan bahan buangan (sanitari landfil). Rawatan dilakukan pada suhu 30°C selama 7-10 hari di bawah keadaan pH yang berbeza iaitu tanpa kawalan pH, pH awal diubah kepada pH 7, dikawal pada pH 7 selama 24 jam, dan kawalan berterusan pada pH 7 dan pH 5.5. Penghasilan asid organik dari cecair lelehan baru adalah tertinggi apabila pH awal diubah kepada pH 7 tanpa kawalan pH selanjutnya. Lebih kurang 45 g/L asid organik dihasilkan bersamaan dengan 28 g/L asid laktik, 8 g/L asid asetik dan 9 g/L asid propionik. Berdasarkan dari nilai BOD<sub>5</sub> asal, asid organik yang terhasil adalah 80%. Berlainan pula dengan cecair lelehan campuran dari landfil bila mana penghasilan asid organik yang tertinggi hanya 14 g/L bila dikawal pada pH 5.5 berterusan dengan asid asetik sebagai produk utama. Pada suhu 37°C adalah suhu yang sesuai apabila penghasilan tertinggi pada hari kedua. Apabila cecair lelehan baru disterilkan dan ditambahkan dengan 10% sisa buangan dapur (inokulum), asid organik yang terhasil adalah diantara 34-37 g/L. Pemilihan asid laktik sehingga 85% adalah hasilan utama semasa rawatan terhadap cecair lelehan baru yang ditambahkan dengan inokulum apabila tiada perubahan pada pH dilakukan. Secara keseluruhan, keputusan menunjukkan bahawa cecair lelehan baru berupaya ditukarkan kepada asid organik sebanyak 45 g/L dengan cara rawatan anaerobik apabila pH awal diubah kepada pH 7.

Supernatan yang telah dikumpulkan dari rawatan anaerobik dengan penghasilan asid organik yang tertinggi digunakan dalam peringkat seterusnya iaitu untuk penghasilan polihidroksialkanoate (PHA). *Ralstonia eutropha* adalah mikrob yang digunakan untuk penghasilan PHA dengan menggunakan sumber karbon dari cecair lelehan untuk

fermentasi sesekelompok dan suapan sesekelompok. Dalam fermentasi sesekelompok menggunakan kelalang goncang, 3.4 g/L PHA bersamaan dengan 86% g PHA/g sel dihasilkan. Dalam fermentasi suapan sesekelompok, kepekatan maksimum PHA yang telah dihasilkan adalah 6.9 g/L bersamaan dengan 85% (g/g) dari berat kering sel. PHA yang dihasilkan adalah seperti plastik dan menyerupai polietelin berketumpatan rendah. Oleh itu sumber karbon daripada cecair lelehan yang baru dari lori sampah terbukti sesuai untuk penghasilan asid organik dan PHA.

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#### CHAPTER 1

#### **INTRODUCTION**

Landfilling is one of the alternatives for disposing the accumulated waste produced by human beings, especially municipal solid waste (MSW). Although the cost of other methods of treatment and disposal has generally decreased in recent years relative to the cost of direct landfill of untreated waste, landfilling is still by far the cheapest disposal method. MSW waste materials from household and commercial premises includes food garbage, sludge and garden waste. In Malaysia about 10 000 tons MSW is produced daily of which about 50% is organic matter (Loh *et al.*, 1999).

This dumped solid waste leached out liquid from interaction of degraded organic matter and chemicals giving rise to a polluted liquid known as leachate. The anaerobic process liquefies or dewaters the organic solid waste. The process proceeds by hydrolysis brought by extracellular enzymes or surface enzymes of organisms (Patrick, 1972). Leachate composition is governed by several factors such as climate, composition of the solid waste, site management, age of the landfill, etc. However little attention has been paid to the treatment of landfill leachate, which is now recognized as an environmental problem. Leachate from aged wastes maybe less strong, although concentration of ammonia may remain high for many years. Therefore treatment and containment of leachate are necessary to avoid or minimise contamination of adjacent ground or surface water resources. Leachate's  $BOD_5$  ranged from 20-30 g/L (Lema *et al.*, 1988) and due to the high BOD in leachate, high organic acid concentration could be obtained from leachate under appropriate treatment conditions.

Plastic materials have become an integral part of contemporary life, however most plastics are resistance to degradation upon disposal. Plastic materials account for about 20% by volume of MSW and reduce the capacity of precious landfill sites (Stein, 1992; Lee and Yu, 1997). These non-degradable plastics accumulate in the environments at the rate of 25 million tonnes per year (Lee, *et al.*, 1991). Other methods of plastic waste disposal is incineration but it is expensive and potentially dangerous. Hydrogen cyanide can be formed from acrylonitrile-based plastic and nylon, and hydrogen cloride is released from polyvinylchloride. These air pollutants can be also lead to water pollution (Brandl *et al.*, 1990). One of the solutions to reduce the plastic waste disposal is to develop degradable materials as a replacement to the non-degradable petrochemical-based plastic.

Polyhydroxyalkanoates (PHA) are usually formed as intracellular inclusions under unbalanced growth conditions, when excess carbon sources are provided to the cells and if one nutrient essential for growth is depleted (Anderson and Dawes, 1990). PHA produced by microorganisms have attracted interest and attention worldwide as replacement of petrochemical plastics due to their biodegradability. The most studied organisms for PHA production are *Alcaligenes eutrophus* (now *Ralstonia eutropha*), *A. latus, Azotobacter sp.*, and *Methylobacterium sp.* Commercial interest has been focused on *A. eutrophus* strain because it is capable of accumulating very high PHB levels within the cells in a short time (Linko *et al.*, 1993a). *R. eutropha* produces PHB on a variety of substrates such as glucose, fructose and organic acids. *R. eutropha* accumulates high molecular mass PHAs and weight average molecular mass are frequently in excess of  $10^6$  (Ballard *et al.*, 1987; Doi, 1990).

A partial solution to the increase in plastic litter and the waste disposal problem is the development and the industrial production of degradable materials (Brandl *et al.*, 1990). Unfortunately, the high cost of PHA production limited their use in the market. The price of ICI Biopol product is US\$4.40/kg compared to petrochemical plastic being less than US\$1.00/kg (Ramsay *et al.*, 1993). One of the determining factors in the economics of PHA production on the industrial scale is raw material cost-especially carbon sources and downstream processing. Due to its high BOD value, leachate is a potential source to be used as a cheap carbon source for the production of PHA. Therefore, the strategy of using this wastewater to produce PHA represents converting into a high value product. The objectives of this study are:

- To characterize and determine the suitability of using municipal solid waste fresh leachate and percolated landfill leachate as carbon sources for production of organic acids and PHA.
- 2. To evaluate the effect of different pH regimes, temperature and inoculum in the utilisation of leachate from MSW for the production of organic acids.
- 3. To utilise the organic acids obtained from treated leachate as substrates for the production of PHA by *Ralstonia eutropha*.

#### **CHAPTER II**

#### LITERATURE REVIEW

#### Municipal Solid Waste (MSW)

The per capita generation rate was 0.81 kg per person per day. The increase in the income has resulted in a proportionate increase in consumption and consequently waste generation. In recent study of the World Bank, urban waste generation is predicted to increase substantially over the next coming years as GNP per capita increases. It is predicted that a total of 31.6 million tones per day waste generated in the next coming years (Einsiedel, 2000).

In developing countries such as Malaysia, it is also reported that 35% of the solid waste generated in some part of the region are disposed at unauthorized sites, and many areas do not have collection services (Chua and Garces, 1992). Some of the waste collected is also disposed off in open dump sites, which are not properly operated and maintained, thereby possibly a serious threat to public health.

Sources of organic wastes include sewage sludge, household kitchen, paper and garden waste, wood and food waste from process industries, livestock and crop waste from agriculture, and food and paper waste from commercial industries (James, 1993). The percentage of organic matter (food waste) was 36-70 % for the developing countries which were higher than those of the developed countries. These organic matter contains cellulose, lipids, proteins and other compounds that are readily biodegradable and generally no compounds are founds that are inhibitory to bacteria (Rintala and Ahring, 1994). The quantity of solid waste generation is generally correlated with the per capita income i.e. the higher the income the greater the amount of solid waste generated (Polprasert, 1996).

### Management of MSW

The predominant option for solid waste disposal worldwide is land disposal or landfill (Table. 2.1) followed by incineration and composting. In the developed countries, the trend is to divert the larger part of refuse away from landfill and integrated waste management planning including:

- Source reduction (including reuse of products and backyard composting of yard trimmings).
- 2. Recycling (including composting).
- 3. Waste combustion (preferably with recovery) and landfill.

Globally there is a need to conserve finite resources and to protect the environment. As a result the subject of waste disposal is often scrutinized to develop areas where resources may be recycled or used as energy. The aim of this study is to look over the organic waste situation and to discuss potential benefit that can be derived from the reprocessing of organic waste (leachate) products.

	Disposal method (%)		
Country	Land disposal	Incineration	Composting
Malaysia	85	5	10
Japan	25.9	74	0.1
Singapore	35	65	~
Thailand	85	5	10
Indonesia	85	5	10
Brunei Darussalam	100	-	-
Hong Kong	92	8	-
Philippines	90	-	10
Rep. Of Korea	100	_	-
USA	55.5	17.2	27.3

Table 2.1: Disposal methods for municipal solid waste in selected countries.

Source: (Einsidel, 2000; Village, 1998)

## Leachate

Leachate generally has a high concentration of contaminants, therefore some type of treatment is necessary prior to ultimate discharge of the wastewater (Pohland *et al.*, 1985). Leachate composition is governed by several factors such as climate, composition