



UNIVERSITI PUTRA MALAYSIA

**FEASIBILITY STUDY ON THE UTILISATION OF
RUBBER LATEX EFFLUENT FOR THE PRODUCTION OF
BACTERIAL BIOPOLYMERS**

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**FEASIBILITY STUDY ON THE UTILISATION OF
RUBBER LATEX EFFLUENT FOR THE PRODUCTION OF
BACTERIAL BIOPOLYMERS**

**By
TANG SOO NEE**

**Thesis Submitted in Partial Fulfilment of the Requirements for the
Degree of Master of Science in the Faculty of Engineering
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May 1999



Specially dedicated to,

*My beloved parents, brothers, sisters,
friends and Seong,*

For their invaluable love, sacrifices,

and wishing me all the best



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LIST OF ABBREVIATIONS

| | | |
|------------------|---|---|
| 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 |
| C ₀ | - | Concentration at inlet of column |
| CO ₂ | - | Carbon dioxide |
| COD | - | Chemical oxygen demand |
| DOE | - | Division of Environment |
| GC | - | Gas chromatography |
| HPLC | - | high pressure liquid chromatography |
| ICI | - | Imperial Chemical Industries |
| MCL | - | medium-chain-length |
| NMR | - | nuclear magnetic resonance |
| PHAs | - | polyhydroxyalkanoates |
| P(3HB) | - | poly(3-hydroxybutyrate) |
| PHBV | - | poly(hydroxybutyrate-co-hydroxyvalerate) |
| POME | - | palm oil mill effluent |
| SMR | - | Standard Malaysian Rubber |
| SS | - | suspended solids |
| μ_0 | - | linear superficial liquid velocity |
| t | - | time |
| VFA | - | volatile fatty acid |
| Z | - | column length |



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May 1999

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Faculty: Engineering

The natural rubber industry ranks second to the palm oil industry in Malaysia. It is estimated that about 100 million litres of effluent is discharged daily from rubber processing factories. Rubber latex effluent is the most polluting source, with a high biochemical oxygen demand (BOD). Utilisation of this effluent such as use of a coupled system not only can reduce the cost of treatment but also yield a fermentation feedstock for the production of bioplastic. This study initially was carried out to increase the production of organic acids by anaerobic treatment of rubber latex effluent. It was found that through anaerobic treatment the concentration of organic acids did not increase. This was caused by the bioconversion of the volatile fatty acids (VFA) to biogas. Consequently, separation of organic acids from rubber latex effluent by anion exchange resin were examined as a preliminary study of recovering acetic and propionic acids. However, the suspended solids (SS) content in the raw effluent was rather high which partially blocked the ion-exchange columns. Lime was used to



remove the SS in the rubber latex effluent. At pH 12, SS removal involved aggregation of the SS as flocs that can be easily sedimented. After the lime precipitation process, organic acids were found to adsorb strongly onto the anion exchange resin. Less adsorption of organic acids onto the resin was observed before the lime precipitation. This was probably due to more sites being occupied by colloidal particles on the resin thus inhibiting the adsorption of organic acids. Thus, removal of the SS was necessary. The breakthrough curve showed that the exchange capacity for treated effluent subjected to Dowex SBR anionic-exchange column after separation by Dowex 88 cationic-exchange column was improved. The initial concentration of organic acids in the raw effluent was 3.9 g/L. After ion exchange, the concentration of the organic acids increased to 27 g/L, which could be utilised for production of polyhydroxyalkanoates (PHA).

For PHA accumulation stage, concentrated rubber latex effluent obtained from ion exchange resins and synthetic acetic acid were used as the carbon source. Quantitative analyses from fed batch culture via HPLC showed that the accumulation of PHA in *Alcaligenes eutrophus* was maximum with a concentration of 1.182 g/L when cultivated on synthetic acetic acid, corresponding to a yield of 87% based on its cell dry weight. The dry cell weight increased from 0.71 to 1.67 g/L. On the other hand, using concentrated rubber latex effluent containing acetic and propionic acids resulted in reduced PHA content by dry weight (15%) but the dry cell weight increased from 0.49 to 1.04 g/L. The results clearly indicated that the cells grow in rubber latex effluent but no PHA was accumulated. This might be due to other toxic substances such as heavy metals contain in rubber latex effluent. Thus further work is



required before rubber latex effluent can be utilised as a substrate for PHA production industrially.

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

**KAJIAN KEBERKESANAN KE ATAS PENGGUNAAN
SISA LATEKS GETAH UNTUK PENGHASILAN
BAKTERIA BIOPOLIMER**

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Industri getah asli di Malaysia merupakan industri kedua utama selepas industri minyak kelapa sawit. Adalah dijangka sisa air sebanyak 100 milion liter telah dilepaskan setiap hari daripada kilang pemprosesan getah. Sisa lateks getah merupakan salah satu sumber yang paling mencemar dengan kandungan keperluan oksigen biokimia (BOD) yang tinggi. Penggunaan sisa ini dengan sistem penduaan bukan sahaja dapat mengurangkan kos rawatan malah boleh memperolehi suapan stok fermentasi untuk penghasilan bioplastik. Kajian permulaan dijalankan untuk meningkatkan penghasilan asid-asid organik oleh rawatan anaerobik dari sisa lateks getah. Adalah didapati bahawa kepekatan asid-asid organik menerusi rawatan anaerobik tidak dapat ditingkatkan. Ini adalah disebabkan oleh bio penukaran asid lemak meruap (VFA) kepada biogas. Dengan demikian, pengasingan asid organik daripada sisa lateks getah oleh resin penukarganti anion telah diselidik sebagai kajian pendahuluan untuk mendapatkan semula asid-asid asetik dan propionik. Akan tetapi,

kandungan pepejal terampai (SS) di dalam sisa adalah agak tinggi di mana sebahagian akan menyekat kolum penukarganti ion. Kapur telah digunakan untuk menyingkirkan pepejal terampai di dalam sisa lateks getah. Pada pH 12, penyingkiran SS melibatkan penggumpalan pepejal terampai sebagai flok di mana mudah dimendakkan. Selepas proses pemendakan oleh kapur, asid-asid organik didapati menjerap pada resin penukarganti anion dengan kuatnya. Kurang penjerapan asid-asid organik pada resin diperhatikan sebelum pemendakan oleh kapur. Keadaan ini disebabkan oleh lebih ruang telah dipenuhi oleh SS yang menghalang penjerapan asid-asid organik pada resin. Lengkung penerobosan telah menunjukkan bahawa kapasiti penukargantian untuk sisa yang telah dirawat oleh kapur yang dilalui kepada kolum penukarganti anion Dowex SBR selepas pengasingan oleh kolum penukarganti kation telah ditingkatkan. Kepekatan awal asid-asid organik di dalam sisa mentah ialah 3.9 g/L. Setelah proses penukarganti ion, kepekatan asid-asid organik telah dinaikkan ke 27 g/L, di mana boleh digunakan untuk penghasilan polihidroksialkanoat (PHA).

Untuk tahap penghasilan PHA, sisa lateks getah yang telah dipisahkan dan sintetik asid asetik telah digunakan sebagai sumber karbon. Analisis kuantitatif dari penyuaapan fermentasi melalui HPLC menunjukkan bahawa pengumpulan PHA oleh *Alcaligenes eutrophus* adalah maksimum dengan kepekatan 1.182 g/L semasa pengkulturan di atas sintetik asid asetik, iaitu 87% daripada berat keringnya. Berat kering selnya meningkat dari 0.71 ke 1.67 g/L. Sebaliknya, sisa lateks getah yang mengandungi asid-asid asetik dan propionik telah didapati memperolehi kurang kandungan PHA daripada berat keringnya (15%) tetapi berat keringnya telah meningkat dari 0.49 ke 1.04 g/L. Keputusan ini jelas menunjukkan bahawa



pertumbuhan sel berlaku di sisa lateks getah tetapi tiada pengumpulan PHA. Ini mungkin disebabkan oleh kewujudan bahan-bahan toksik yang lain seperti logam-logam berat yang terdapat dalam sisa lateks getah. Oleh itu, kajian seterusnya dikehendaki disambungkan sebelum sisa lateks getah boleh digunakan sebagai satu substrat untuk penghasilan PHA di bidang industri.

CHAPTER I

INTRODUCTION

Rubber Industry and Rubber Effluent in Malaysia

The rubber industry being a major industry in Malaysia, is one of the principal agro-processing activities that generate the bulk of the wastes in the country. The annual Malaysian production of natural rubber in 1996 was around 1.08 million tonnes which was about 17.4% of the world production (Monthly Rubber Statistics Malaysia, 1997). Two main types of rubber processing include natural latex concentrate and Standard Malaysia Rubber (SMR). Skim latex and concentrated latex are obtained by centrifugation of field latex. Skim latex serum is derived from the coagulation of skim latex, a by-product of latex concentrate production. The serum containing a rich source of nitrogen, carbohydrates, proteins, lipids and trace metals, is the most polluting source in a latex concentrate factory. The main sources of wastewater from SMR manufacture include bulking, acid coagulation and milling. The effluents usually contain washing, cleaning and dilution from these operations. It is estimated that about 100 million litres of effluent is discharged daily from rubber processing factory (Sastry, 1995). Therefore, the treatment and disposal problems of rubber effluents have become important because of the concern for the living environment and the care of the earth.



Various feasible methods of effluent treatment and disposal were investigated including a conventional activated sludge plant; an oxidation ditch using extended aeration, an aerated lagoon, anaerobic digestion and land application. Current status of treatment involving an oxidation ditch or waste stabilisation pond (Ibrahim and John, 1986). In accordance with the Environmental Quality (Prescribed Premises) (Raw Natural Rubber) Regulation 1978, the DOE has adopted the methods of formulating suitable standards (Table 1.1) on the basis of available treatment technology and rendering the wastewater acceptable for discharge into natural waterways.

These treatment systems are either costly or technically unsatisfactory. Electricity costs in the oxidation ditch process for rubber effluent are quite exorbitant (Ibrahim, 1992). It would of course make more economic sense if the wastes can be exploited as substrates to generate high value added and readily saleable components. Utilisation of the rubber latex effluent to yield valuable substances can reduce the cost of effluent treatment or even generate additional revenue for rubber factories.

Table 1.1: Standard for SMR and Latex Concentrate Factory Effluent

| Parameter | SMR | Latex concentrate |
|------------------------|------------|-------------------|
| pH | 6.0 – 9.0 | 6.0 – 9.0 |
| BOD ₃ | 100(50*) | 100(50*) |
| COD | 250 | 400 |
| Ammoniacal N | 40 | 300 |
| Total N | 60 | 300 |
| Total Suspended Solids | 150 (100*) | 150 (100*) |
| Total Solids | - | - |

(All parameters except pH are expressed as mg/L)

This additional limit is the arithmetic mean value determined on the basis of a minimum of four samples taken at least once a week for four weeks consecutively.

The Problem with Plastics

In recent years, the rapidly expanding production and use of plastic materials have been increasing drastically in Malaysia, particularly at waste disposal sites. This has manifested the need to practise a different solid management alternative as opposed to the traditional method. Plastic materials have become an integral part of contemporary life because of many desirable properties including durability and resistance to degradation. These nondegradable plastics accumulate in the environment at a rate of 25 million tonnes per year (Lee *et al.*, 1991). A waste composition analysis for the city of Chicago (1990) showed that 9.4% by weight of its municipal solid waste was plastic and these plastics generally occupy 20% by volume of landfill discards. Since the main bulk of waste is made up of plastics, there is a great deal of interest in recycling plastics and in producing materials that can be safely and easily disposed of in the environment.

Plastic products have gained universal use not only in food, clothing and shelter, but also in the transportation, construction, medical and leisure industries as well of our daily life. Whereas previously conventional plastics were developed as durable substitute products, manufactured from fossil fuels such as oil, contribute heavily to the global problem of waste disposal. It can take at least 50 years for them to break down and there is a limit on how often they can be recycled. Conventional plastics are giving rise to the most environmentally harmful wastes. In response to increasing public concern about the harmful effects of petrochemical-derived plastic materials in the environment, many countries are conducting various solid waste

management programs, including plastic waste reduction by developing biodegradable plastic materials. These biodegradable plastic materials must retain the desired material properties of conventional synthetic plastics, and should be completely degraded without leaving any undesirable residues when discarded. Therefore, this futuristic type of plastic that is anticipated to replace existing types of plastics and resolve our current environment disruption issues.

Recently, science and industry have focused on the development and application of biodegradable plastic materials (Brandl *et al.*, 1995). Biodegradable plastics are defined by the American Society for Testing and Materials (1992) as degradable plastics in which the degradation results from the action of naturally occurring microorganisms such as bacteria, fungi, and algae with a lapse of time and ultimately decomposed into water and carbon dioxide.

According to a survey, two thirds of the American population agree with the assertion that plastics pose the greatest threat to the environment because of their nonbiodegradability (Brandl *et al.*, 1995). It has been estimated that one million marine animals are killed every year either by choking on floating plastic items or by becoming entangled in nondegradable plastic debris (Doi, 1990). In industrialised countries, waste reduction has been the focus of many debates. In certain waste incineration plants the amount of plastics in the waste stream is almost 20% because of the separation of the waste before incineration (Brandl *et al.*, 1995). It is assumed that the utilisation of degradable plastics would help to reduce the percentage of plastics in the waste. In

PERPUSTAKAAN

addition, the use of these materials is based on renewable resources and contributes to a material cycling analogous to the natural biogeochemical cycles in nature.

Polyhydroxyalkanoates (PHA)

PHA are intracellular carbon and energy reserve materials accumulated by a variety of bacteria under conditions of nutrient imbalance, especially nitrogen, phosphorus and oxygen, in the presence of excess carbon source (Anderson and Dawes, 1990; Dawes and Senior, 1973; Doi, 1990; Lee, 1996a). PHAs show thermoplastic or elastic properties depending on the polymer composition, and has become of industrial interest to evaluate PHA polyesters as natural, biodegradable, and biocompatible polyester (Cox, 1994; Holmes, 1985; Kang *et al.*, 1996). Poly(3-hydroxybutyrate) (PHB) is the best known representative of the PHA family. Some bacteria are also capable of forming copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (Anderson and Dawes, 1990). The polymer PHB has a number of interesting characteristics and can be used for a wide range of possible application similar to many conventional synthetic plastics now in use such as surgical sutures, long term carriers for drugs, or moulded plastics and films (Anderson and Dawes, 1990; Brandl *et al.*, 1990; Doi, 1990).

It is estimated that the global market for biodegradable polymers will reach 1.4 million tonnes per year by the year 2000 and is expected to grow (Lee, 1996a). However, one of the problems facing the development of biodegradable polymers as