PRODUCTION OF LACTIC ACID FROM CO-FERMENTATION OF VEGETABLE WASTE AND FOOD WASTE IN OPEN FERMENTATION SYSTEM

by

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LIST OF ABBREVIATIONS

FS	Fish Scrap
FW	Food Waste
LA	Lactic Acid
MSW	Municipal Solid Waste
RW	Rice Waste
TCA	Trichloroacetic Acid
TS	Total Sugar
VW	Vegetable Waste

PENGHASILAN ASID LAKTIK DARIPADA KO-FERMENTASI SISA SAYURAN DENGAN SISA MAKANAN DI DALAM SISTEM FERMENTASI TERBUKA

ABSTRAK

Pembuangan sisa makanan di tapak pelupusan sampah telah dikaitkan dengan penyebab perlepasan gas-gas rumah hijau, bau busuk, cecair toksik dan tarikan kepada haiwan kotor serta penggunaan terlalu banyak tanah berpotensi. Di Malaysia, sisa ini berjumlah 4.404 tan daripada sisa pepejal perbandaran dalam tahun 2005 dan dianggarkan akan meningkat kepada 6.54 juta tan pada tahun 2020. Disebabkan oleh peningkatan jumlah sisa makanan serta kesan negatif yang terjadi hasil dari peningkatan ini, kaedah-kaedah menguruskan sisa organik ini dengan lebih baik telah pun diperkenalkan. Salah satu langkah ialah menggunakan sisa makanan untuk menghasilkan produk berfaedah seperti hidrogen dan metana serta asid laktik. Dalam penyelidikan ini, komponen kimia sisa makanan dibandingkan dengan komponen kimia sisa makanan dari negara Jepun. Kemudian, sisa makanan yang paling mampu menghasilkan lebih asid laktik ditentukan daripada tiga pecahan sisa makanan iaitu pecahan kanji (sisa nasi), pecahan selulosa (sisa sayur-sayuran), pecahan protein (sisa ikan) dan dibandingkan dengan sisa makanan. Keberkesanan sisa makanan dari segi penukaran bio turut dianalisiskan. Selain dari itu, masa, suhu dan pH awal yang terbaik untuk fermentasi sisa makanan turut ditentukan dalam penyelidikan ini. Tambahan pula, kaedah untuk meningkatkan hasil asid laktik dengan menambahkan pecahan sisa makanan yang paling berpotensi mengandungi asid laktik bakteria wujud semulajadi dengan sisa makanan turut dikaji. Semua sisa dan pecahan ini

dikisar berasingan sehingga halus dengan kadar nisbah 1:2 (v/v) dengan air suling ternyahion menjadi larutan sisa (tanpa sterilisasi), pH awal ditetapkan pada pH 7 dan diperap secara statik pada 25^{o}C. Pecahan makanan yang paling banyak menukarkan gula kepada asid laktik ialah pecahan selulosa iaitu sisa sayuran. Masa fermentasi yang paling sesuai adalah tiga hari dan suhu ialah 25^{o}C. Apabila pH awal ditetapkan pada pH 9, penghasilan asid laktik telah menurun di dalam sisa makanan tetapi meningkatkan penghasilan asid laktik di dalam sisa sayuran. Sebanyak 56.53-68.13 % penukaran bio telah tercapai apabila 10-70 peratus nisbah sisa sayur-sayuran telah ditambah kepada sisa makanan 1 dan 2. Keputusan menunjukkan bahawa penambahan sisa sayur-sayuran kepada sisa makanan bukan sahaja meningkatkan penghasilan asid laktik tetapi meningkatkan penggunaan gula di dalam larutan sisa.

PRODUCTION OF LACTIC ACID FROM CO-FERMENTATION OF VEGETABLE WASTE AND FOOD WASTE IN OPEN FERMENTATION SYSTEM

ABSTRACT

Food waste disposed in landfills has been associated as the cause of greenhouse gases, foul odour emission, toxic leacheate and vermin attraction as well as occupying of potential land area. In Malaysia, this waste amounts to 4.404 million tonnes in 2005 from total Municipal Solid Waste generated and is estimated to increase to 6.54 million tonnes in 2020. Due to the increasing number of waste generated and the negative outcomes, better ways of handling these organic wastes are currently being introduced. One of the steps is by using food wastes to produce beneficial products such as hydrogen and methane as well as lactic acid. In this study, food waste chemical composition was compared to Japanese food wastes and the food waste fractions that could produce highest yield were evaluated. The three food waste fractions namely starchy fraction (rice waste), cellulosic fraction (vegetable waste) and proteinaceous fraction (fish scrap) were compared against food waste. Food waste efficiency in terms of percent bioconversion was also analyzed. Furthermore, the duration of fermentation, temperature and initial pH for food waste fermentation were also confirmed in this research. Moreover, improvement of lactic acid production by adding certain concentrations of the most efficient waste fraction in food waste was also explored. All waste fractions and food waste were grinded separately in distilled deionized water 1:2 (v/v) to form food waste slurry (without sterilization), initial pH adjusted to pH 7 and fermented statically at 25^{o}C. The most

efficient food waste based on bioconversion of initial total sugar to lactic acid was vegetable waste. The most suitable fermentation time is 3 days and temperature is 25^{o}C. Setting initial pH 9 prior to fermentation suppressed lactic acid production in food waste but enhanced lactic acid production in vegetable waste. About 56.53-68.12 % bioconversion was achieved when 10-70 percent ratio of vegetable waste was added to food waste 1 and 2. The results indicated that addition of vegetable waste into food waste not only enhanced lactic acid production but also increased total sugar consumption in the slurry.

CHAPTER 1

INTRODUCTION

This research is done to uncover the potential of different food waste fractions to produce lactic acid in unsterilized condition. Food waste is one of the organic fractions of the municipal solid waste (MSW) besides other fractions such as paper, plastics, glass, and textile and miscellaneous (Kathrivale *et al.*, 2003). Worldwide production of MSW is constantly increasing with increasing population, industrialization as well as changes in lifestyle (Singh *et al.*, 2011). The authors also stated most wastes are disposed in open dumps or landfills in developing countries. This disposal practice has caused many environmental issues such as diminishing of area for waste dumping and ground and surface water contamination, pest problems and odour problems (Singh *et al.*, 2011). Food waste has been regarded as the real cause for greenhouse gases, foul odour emission, toxic leacheate and vermin attraction in landfills (Kim *et al.*, 2010).

1.1 The Issue With Food Waste

Food waste is an organic waste rich in nutrients collected from homes, food eateries and restaurants. In Korea, the food waste generated amounts to 4.5 million tons per year (Yang *et al.*, 2006). About 30% of municipal wastes generated in Korea are food wastes (Lee *et al.*, 2007). In Beijing, China, the percentage of food waste generated has increased from 32.6% in 1989 to 63.39% in 2006 and has remained the major waste composition in municipal solid waste (Li *et al*., 2009). The increase of food wastes generated is because of the increase in population and economic development. In Malaysia, the food waste fraction from municipal solid waste in 2005 was 4.404 million tonnes and was estimated to increase to 6.54 million tonnes in 2020 (Kathrivale *et al*., 2003). Developed countries are already taking steps to reduce amount of food wastes generated. One of the steps is by using food wastes to produce beneficial products such as hydrogen and methane (Liu *et al*., 2006) as well as lactic acid (Yang *et al*., 2006; Ohkouchi & Inoue, 2007).

1.2 Lactic Acid As The Solution

Lactic acid or 2-hydroxypropionic acid is naturally produced from glucose metabolism during fermentation. At present, lactic acid is the third most important organic acid in the world behind acetic acid and citric acid. The lactic acid world demand is estimated around 130 000 to 150 000 tonnes per year (Mirasol, 1999). By the end of year 2011, lactic acid world demand is expected to rise up to 200 thousand tonnes (Ramesh, 2001). Lactic acid is an organic acid that is known to have many uses in food, chemical, pharmaceuticals, cosmetics and also in polymer industries (Wee *et al*., 2006). Production of lactic acid commercially is done either by chemical synthesis or natural synthesis (Wee *et al*., 2006; John *et al*., 2009). Alternatively, lactic acid also has been reported being produced from agricultural resources (Oh *et al*., 2005) and food waste by utilizing specific lactic acid-producing bacteria (Ohkouchi & Inoue, 2006). Lactic acid produced from food wastes are also synthesized into biodegradable plastics that is poly-lactic acid (PLA) (Sakai *et al*., 2004; Sakai & Ezaki, 2006). The factor that makes food wastes suitable for lactic acid production is the high content of carbohydrates mainly starch.

1.3 Methods of Lactic Acid Production

At the industrial level, lactic acid is either produced via chemical synthesis or carbohydrate fermentative technology (Wee *et al*., 2006). Through chemical synthesis, petrochemical resources were used as precursors in the production. However, due to the depletion of petroleum sources all over the world, this method is being replaced with renewable resources namely agricultural resources in carbohydrate fermentative technology. Besides the two known methods, numerous researches have been done to study other possible methods in fermentation technology. Earlier studies used specific lactic acid microorganisms and substrates that were sterilized prior to fermentation. Some methods used commercial enzymes to enhance degradation of agricultural resources (Oh *et al*., 2005). These methods become cumbersome especially when extra care is needed to maintain sterility during fermentation (Kim *et al*., 2009). In addition, cost of such maintenance, supplies and methods would cause a considerable amount of investment when applying to industrial scale. As more and more knowledge are accumulated, studies on lactic acid productions via fermentation shifted towards unsterilized condition in hope to overcome those issues (Sakai *et al*., 2000; Zhang *et al*., 2008).

1.4 Problem Statement

Many studies are being pursued to uncover potential renewable and cheaper resources to be used in lactic acid production. Waste from agricultural and food processing industry as well as MSW were being used as substrates in this production. However, the fermentation capability of different fractions of food wastes in relation to total sugar consumption and lactic acid production as well as optimal fermentation condition (fermentation duration, temperature and initial pH) for food waste fermentation were never truly discussed. A few studies used three days as fixed fermentation period (Ohkouchi & Inoue, 2007) while other used five days fermentation duration. Most researches conducted their experiments at temperature of 37^{o}C with the assumption that lactic acid bacteria indigenous of food wastes are active at that temperature (Sakai *et al*., 2000; Sakai & Ezaki, 2006). Previously, continuous and intermittently adjusting the pH of food waste during fermentation was discovered to considerably enhance lactic acid production (Sakai *et al*., 2000). However, another study showed that improvement of lactic acid production could be done by just adjusting the initial pH (Ohkouchci & Inoue, 2006).

Food wastes contain high nutritive value and addition of nutrients such as protein and minerals are not necessary (Ohkouchi & Inoue, 2006). Furthermore, it was discovered previously that indigenous lactic acid bacteria were naturally found on surfaces of food materials, thus producing lactic acid from food waste are possible and simple modifications of methods could enhance its production (Daeschel & Fleming, 1984). Nevertheless, due to its variability of composition on day to day basis, food wastes could cause uncontrolled yield and production would vary tremendously.

Although food waste is a highly potential substrate for lactic acid production, the drawback for many researchers is the fact that food waste composition constantly differs at every discharge point and day-to-day basis. Thus, obtaining a constant yield is hardly possible and problematic especially if food waste was to be considered as substrate in pilot scale projects or even industrial production. Such problem could be solved if further knowledge on chemical composition was clarified and methods of predicting yield and ways to improve yield were discovered. Ohkouchi & Inoue (2007) have also revealed that estimating yield based on TS/N ratio is not sufficient since other components might also have an effect on yield. They have estimated yield by multivariate analysis of gathered data based on total carbohydrate content, fermentable sugars and lactic acid yield. However, this prediction model failed to estimate yield with food waste high in lignocellulosic content.

Even though production of lactic acid via unsterilized food waste was possible, little is known that the process can be much simplified without the use of enzymes, additional nutrients, and continuous adjustment of pH and addition of inoculum. A simple method can be applied by further understanding the behaviour of food waste fermentation. Thus, a study on how duration of fermentation, temperature and initial pH affect lactic acid production in food waste fermentation should be performed.

Most studies in lactic acid fermentation used food waste as it is. None of these studies characterize food waste into different fractions. Municipal solid waste was characterized into fractions of organic waste, inorganic waste, metals, plastics, glass and others (Kathrivale *et al.,* 2003) thus it would be appropriate to fractionise food waste as well. When fractions of the municipal waste are distinguished, treatments of the individual factions are easier compared to treating this waste as a whole entity. Similar concept should be applied to food waste. Food waste also contains several different fractions and studying these fractions would improve the final product yield.

Decades ago, brined vegetables or pickled vegetables are made by putting the vegetables in a jar containing salt water. The brining process occurs naturally by metabolic processes of the naturally occurring lactic acid bacteria on surfaces of vegetables (Daeschel & Fleming, 1984). With this understanding, raw vegetable waste would also contain abundance of lactic acid bacteria. This would affect production of lactic acid in food waste containing addition of vegetable waste. Thus, the effect of adding vegetable waste into food waste is also studied.

1.5 The Aim of This Study

1. To evaluate the lactic acid production of the different fractions of food waste namely starchy, proteinaceous and cellulosic components in open fermentation system.

2. To determine the best fermentation condition (cultivation period, temperature and initial pH) in terms of total sugar consumption and lactic acid accumulation.

3. To enhance lactic acid production via open fermentation by varying amount ratios of food waste and vegetable waste.

CHAPTER 2

LITERATURE REVIEW

In this chapter, subjects such as food waste, lactic acid, methods of production, related researches that are introduced in Chapter 1 are further expanded to cover definitions, history, problems, detailed methods and documented research pertinent to this study.

2.1 Municipal Solid Waste

Municipal solid waste (MSW) is waste generated from human settlements, small industries, commercial and municipal activities (Singh *et al*., 2011). Production of MSW is highly dependent on population growth and economy (Johari et al., 2012). The main issue with MSW is the massive disposal of waste into landfills that results in pollutions such as leacheate contamination, pest problems, land degradation and may also create health problems to residents living in close proximity to landfills dumps (Singh *et al*., 2011).

2.1.1 Municipal solid waste in the world

Recently, MSW production has caused many concerns as the amount generated are continuously increasing due to the increase in population and economy (Singh *et al*., 2011). Nevertheless, the latest data regarding the actual production of

MSW is complex to obtain due to difference in data collection activities of municipal town councils and also from country to country (Lisa & Anders, 2008). The most complete data at the moment is the 1991-1993 data obtained from Sakai *et al*. (1996) based on the International Ash Working Group's presentation at the "Seminar on Cycle and Stabilization Technologies of MSW Incineration Residues". The generation of MSW in some developed countries is shown in Figure 2.1.

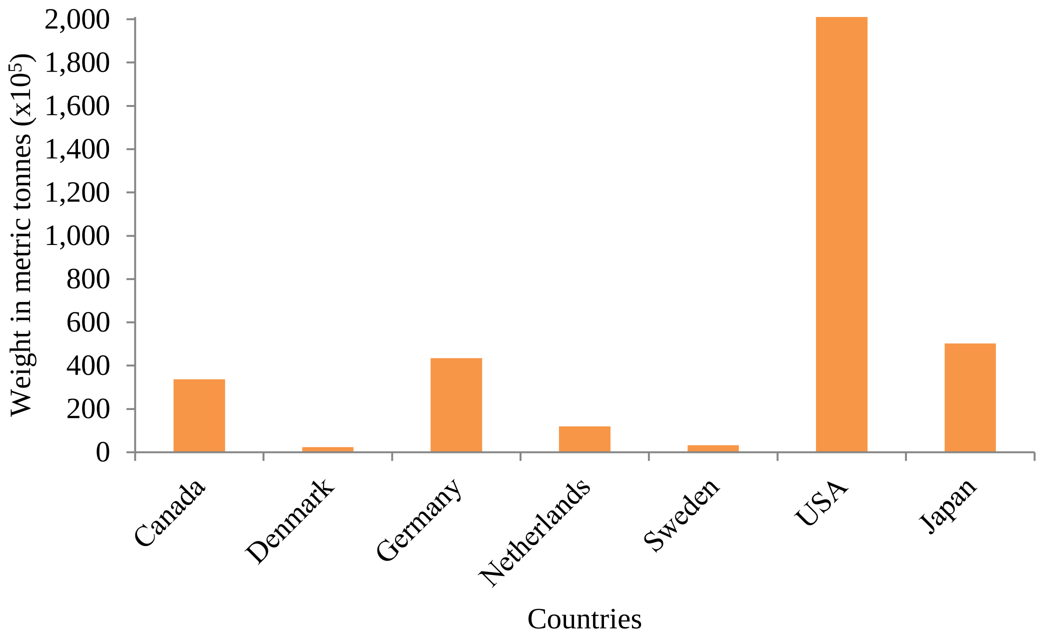

Figure 2.1 MSW generated based on data reported in 1991-1993 for seven developed countries (Sakai *et al*., 1996)

Global MSW generated in 1997 was about 0.49 billion tonnes (Johari *et al*., 2012). Figure 2.1, USA produced the most while Denmark produce the lowest garbage compared to other countries. USA produced the most MSW due to the high population density in the country (Sakai *et al*., 1996) whereas Denmark's MSW production was the lowest due to low overall population and recycling methods. Germany and Japan showed almost similar MSW production with Japan with slightly higher production than Germany. These developed countries managed their MSW by composting, recycling, incineration, landfill and sorting and recovery (Sakai *et al.*, 1996). The authors also reported approximately 33.2 % fraction of the MSW were organic waste and food waste.

2.1.2 Municipal solid waste in Malaysia

The Peninsular Malaysia generated about 6.2 million tonnes per year and range from 0.8-0.9 kg per household in general and about 1.62 kg per household in densely populated cities such as Kuala Lumpur (Kathrivale *et al.*, 2003). Figure 2.2 shows the composition of MSW in Kuala Lumpur.

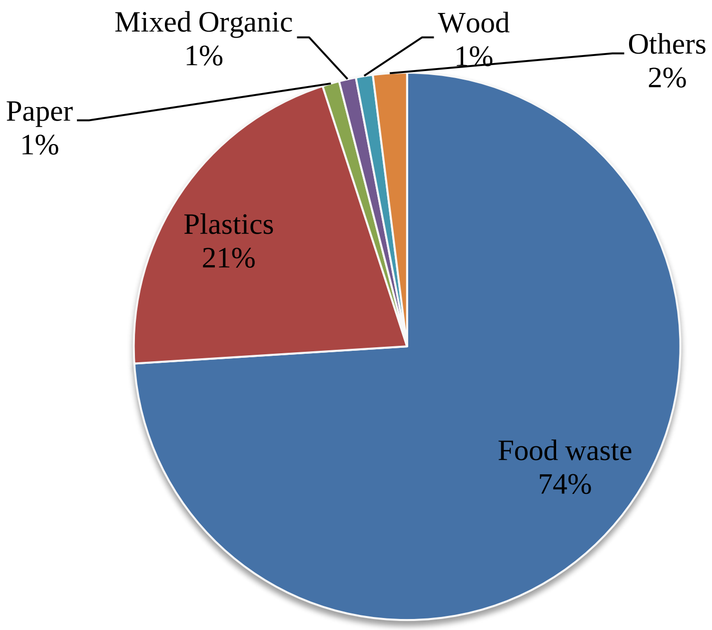

Figure 2.2 Fractions of Municipal Solid Waste of Kuala Lumpur (Budhiarta *et al.*, 2012).

From the figure, food waste is the largest fraction compared to other types of wastes. Other authors also confirmed that approximately 60% of MSW is food waste (Saeed *et al.*, 2009; Kathrivale *et al*., 2003). A study by Siti Rohana and Mohd Amir (2010) showed an average of 43.5 % of total household waste were food materials. The household wastes were collected from Gasing Indah Apartment, a low-cost flat in Petaling Jaya, Selangor.

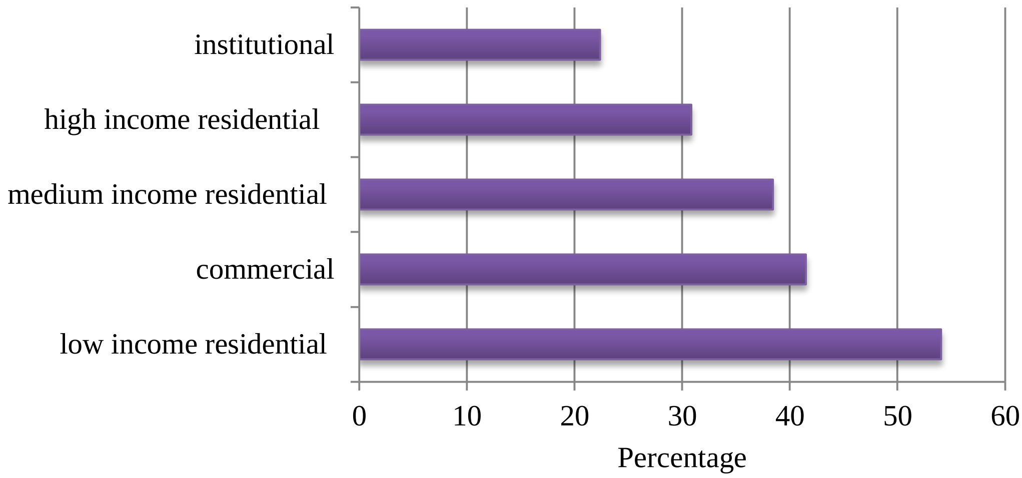

Figure 2.3 Food waste generated in Kuala Lumpur from different collected sources (Kathrivale *et al*., 2003).

The increase of food wastes generated in Kuala Lumpur is due to the rising food consumption from increase of population, the attitude of eating out during shopping as well as high living standards (Saeed *et al*., 2009). Kathrivale *et al.* (2003) showed that the highest food waste generated is by the low income residential that is 54.04% followed by commercial 41.48%, medium income residential 38.42%, high income residential 30.84% and institutional 22.36% based on the data collected from different sources in Kuala Lumpur (Figure 2.3). The high amount of wastes generated by the low income residents could probably be due to more self-cooking than the high income residents that eat out more.

2.2 Food Waste in Malaysia

Food waste in Malaysia are not segregated at source nor separated from other solid wastes at landfill sites before disposal. Solid wastes are not normally recycled by Malaysians either by composting or by other recycling methods. Recycling of wastes is normally done by factories that involve breaking down of the waste into raw materials in order to be used again to make new products. Most Malaysians also do not normally reuse solid wastes such as reusing plastic or glass beverage and food containers into useful household items. Separation of wastes such as papers, plastics, glass, rubber, ferrous and non-ferrous metals are usually done by garbage collection worker and scavengers at disposal sites (Hassan *et al*., 2000). Food wastes with other organic wastes such as garden waste and timber products are dumped directly into landfills without any treatment whatsoever. Nevertheless, a positive turn of events recently shows that Malaysian government is concerned about the ever rising solid waste issues in the country. The government will enforce Malaysians to separate solid waste at home according to category by giving free garbage bins by the year 2013. Datuk Nadzri Yahya, the Director General of the Department of Solid Waste Management, said that the Prime Minister, Datuk Seri Najib Razak demanded during the last cabinet meeting that Solid Waste Management Act and Public Cleansing 2007 to be executed as soon as early next year (Alias, 2010). For the execution of this act, two separate garbage bins will be provided for every household that is one for organic solid waste and one for recyclable waste material.

2.3 Food Waste Characteristics

Food waste is formally defined by Lechner and Schneider (2009) as food wasted along the value added chain of preparation, production, marketing and retail and finally consumption. This definition covers any parts, whole food during preparation or after consumption or other processes that are disposed of due to non-edibility, damaged, excessive production and also the wastes from food factories. Food wastes are generally produced by food processing factories, institutions, restaurants and residential areas. However, the biggest food wastes producers are from the residential areas (Kathrivale *et al*., 2003).

2.3.1 Classification of food waste

There were no specific classifications of food wastes. However, several authors have consistently used several terms to denote certain types of food waste. Some authors frequently used the term food waste to signify food scraps collected from a cafeteria (Ohkouchi & Inoue, 2006; Kim *et al*., 2009; Han & Shin, 2004). Food waste such as vegetable and fruit waste that contains lignin and cellulose content are called as lignocellulosic materials (Ohkouchi & Inoue, 2006). The term kitchen waste or kitchen refuse are repeatedly used by other authors to indicate waste collected from the kitchen (Sakai *et al*., 2000; Sakai *et al*., 2004; Sakai & Ezaki, 2006; Ohkouchi & Inoue, 2006; Zhang *et al*., 2008). Nevertheless, the term kitchen waste or the term food waste used in this study is similar as both waste samples were collected from the kitchen cafeterias or student canteen.

2.3.2 Composition of food waste

The composition of food waste was first and extensively studied by Sankai (1999) in Japan. His study was based on food waste collected in Japan and was used as reference to other research such as in the study of food waste composition (Sakai *et al*., 2000). The general composition of food waste is presented in Figure 2.4.

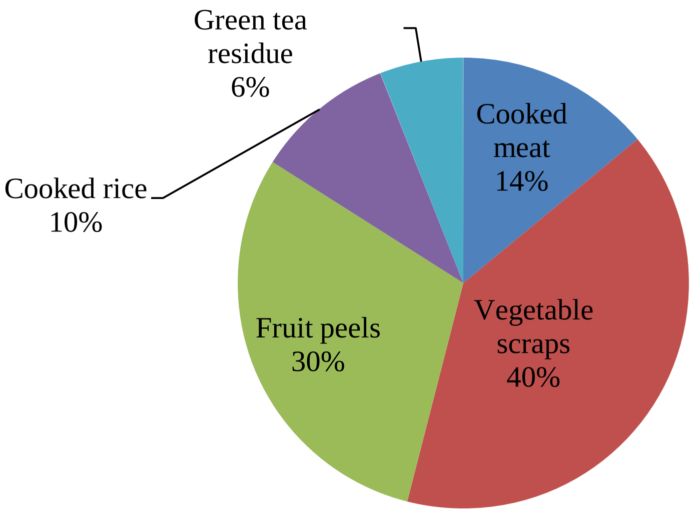

Figure 2.4 Composition of food waste adopted by Sakai et al. (2000) from the works of Sankai (1999)

From the pie chart, vegetable scraps and fruit peels represent the majority part of the Japanese food waste while only 24 % fraction represents wasted cooked food. The fractions that constitute the majority of material composition of food waste are cellulosic, starchy and proteinaceous fractions. This indicated that food waste is mostly composed of scraps from food preparation instead of cooked leftover or plate waste. The corresponding chemical composition from the above figure is presented in Figure 2.5.

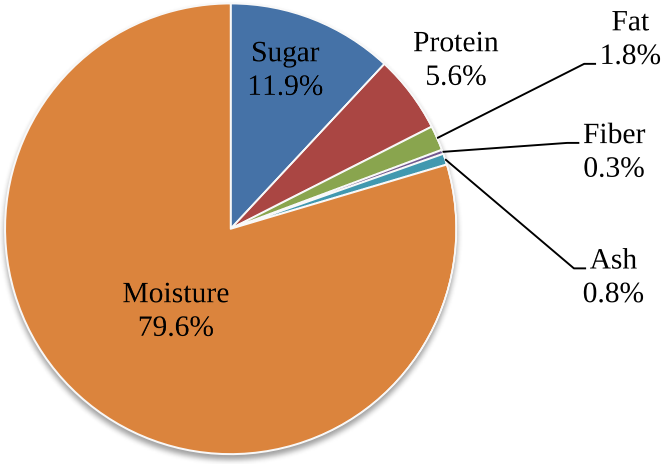

Figure 2.5 Chemical composition of food waste based on the composition in Figure 2.4 (Sankai, 1999).

Moisture is the largest constituent of food waste. This could be due to the high content of lignocellulosic materials from vegetables and fruit peels that normally contain high moisture (Ohkouchi & Inoue, 2007). Sugar and protein content is within the amount sufficient for lactic acid production (Ohkouchi & Inoue, 2007; Sakai *et al*., 2000). Fats, fiber and ash comprised less than 3 % of the total chemical composition.

2.3.3 Chemical composition of food waste

In general, food waste is chemically composed of carbohydrates, protein, fiber, fats, moisture and ash. However, the different amount of these chemicals present in one food waste makes it different from other food waste.

Carbohydrates mostly starch is important for lactic acid production and food waste contains an average 11.22 % starch based on percent wet weight (Ohkouchi & Inoue, 2006). The authors showed that starch content in food waste is as reliable as soluble starch. Other studies on starch utilization in food waste for lactic acid production also showed excellent results (Wang *et al*., 2008). This is due to the amylolytic capabilities of lactic acid bacteria that could degrade starch into simple sugars such as glucose (Reddy *et al*., 2008). Nonetheless, the most important component in food waste is total sugar as this is the most preferred substrate by all lactic acid bacteria. Ohkouchi and Inoue (2007) observed that when food waste contain initial total sugar of lesser than 60 g/L, 78 % bioconversion on average can be achieved while higher initial total sugar would inhibit lactic acid production.

Besides carbohydrates, protein is also a very important nutrient required by lactic acid bacteria for growth and survival. Ohkouchi and Inoue (2007) reported protein amount of 3.75-3.99 % wet weight in food waste is already sufficient nitrogen source for lactic acid production utilizing specific strain of lactic acid bacteria namely *L.manihotivorans* LMG18011. The authors found that when kitchen wastes containing protein amount of 3.88 % (w/w) was fermented, lactic acid produced was 97.5 g/L from food waste. This indicated clearly that amount of protein present in the current food waste is sufficient for lactic acid production.

Other chemical components that are normally found in food waste are fiber (cellulose, hemicelluloses and lignin) and lipid (fats or oils). The amount of fiber and lipids in food waste are approximately 3 % and 5 % respectively (Ohkouchi & Inoue, 2007). Sources of fiber are normally from vegetables and fruit peels as well as tea leaves and coffee grounds. However, the same authors discovered that cellulose or carboxy methyl cellulose in food waste is not utilized by *Lactobacillus manihotivorans* LMG 18011. Tea leaves and coffee grounds contain considerably higher lignin content (5.13-8.48 % w/w) than in kitchen waste (0.16-0.65% w/w). Thus, lactic acid yield obtained from these materials are lower than from kitchen waste that contains high starch content

Since lipid content in food waste is considerably low, there were very few reported results on lipid metabolism by lactic acid bacteria. However, Ye *et al*. (2008) discovered that *Lactobacillus plantarum* BP04 that are dominant in fermented food waste had proteolytic and lipolytic capabilities to hydrolyze crude protein and short chain fatty acids. This shows that lipid will also be metabolized into lactic acid.

2.3.4 Indigenous microorganism of food waste

All types of food putrefy after a certain time due to the actions of microorganisms naturally exists on the surfaces of the food. Food waste has been shown to contain various indigenous microorganism namely lactic acid bacteria (Sakai *et al*., 2000; Kim *et al*., 2009; Daeschel & Fleming, 1984). Sakai *et al*., (2000) isolated 29 bacterial colonies from food waste (after 72 hours fermentation) that are all Gram-positive, non-motile, non-sporeforming, catalase negative rods and accumulated lactate as a main organic acid. This indicated the characteristics of lactic acid producing bacteria and most probably from the genus *Lactobacillus*. The authors also discovered that 14 of the strains were *Lactobacillus plantarum* and was confirmed by analyzing partial sequence of amplified 16S rDNA. Another 11 of the strains were identified as *L.brevis* while the remaining strains are *L.paracasei* subsp. *paracasei*, *L.delbueckii* subsp. *lactis* and *L.fermentum*. Kim *et al.* (2009) also discovered that the dominant bacteria in food waste are lactic acid bacteria. The lactic acid bacterial strains detected and confirmed by analyzing 16S rDNA which were amplified by polymerase chain reaction (PCR) are *Lactobacillus sakei, Lactococcus lactis*, and *Lactobacillus casei*.

However, the natural habitats of the genus *Lactobacillus* are in the human oral cavity, intestinal tract and vagina as well as on plants, plants materials and cereal products such as rice and wheat (Stiles & Holzapfel, 1997). Fermented vegetable is an example of utilizing indigenous microorganism to undergo the fermentation process (Daeschel & Fleming, 1984). The authors have previously categorized indigenous microbial growth during natural fermentation of vegetables into four stages; initiation, primary fermentation, secondary fermentation and finally post fermentation. During initiation and primary fermentation, the growth of lactic acid bacteria becomes dominant thus dropping the pH further to 4.0 that also acts as an inhibitor for other pathogenic bacteria.

Lactic acid bacteria are anaerobic, heterotrophic and proliferate on food waste due to the abundance of nutrients as these bacteria lack many biosynthetic capabilities (Reddy *et al*., 2008). Conditions or environments that lack sufficient nutrients are unable to support abundant growth of lactic acid bacteria (Daeschel & Fleming, 1984; Reddy *et al*., 2008). Amylolytic lactic acid bacteria (ALAB) such as *Lactobacillus plantarum* and *Lactobacillus manihotivorans* produce α-amylase and normally used in food fermentation such as sour rye bread and salt bread (Reddy *et al*., 2008). The authors also stated that lactic acid bacteria are mesophilic bacteria that could grow at temperatures from 5^{o}C to 45^{o}C and tolerant to acidic conditions although optimal pH would be at pH 5.5-6.5.

2.4 Food Waste Usage

Food waste contains high nutritive value and has been used to produce several products such as hydrogen (Shin & Yuon, 2005; Kim *et al*., 2009; Kim *et al*., 2011), methane (Han & Shin, 2004), lactic acid (discussed in detail in Unit 2.6.2) and ethanol (Tang *et al*., 2008). Besides these products, food waste has also been converted to oil similar to petroleum (Minowa *et al*., 1995) and to poly-L-lactate plastic (Sakai *et al*., 2004).

The following Figure 2.6 shows several usage of food waste in three categories which are gases, organic acids and fuel.

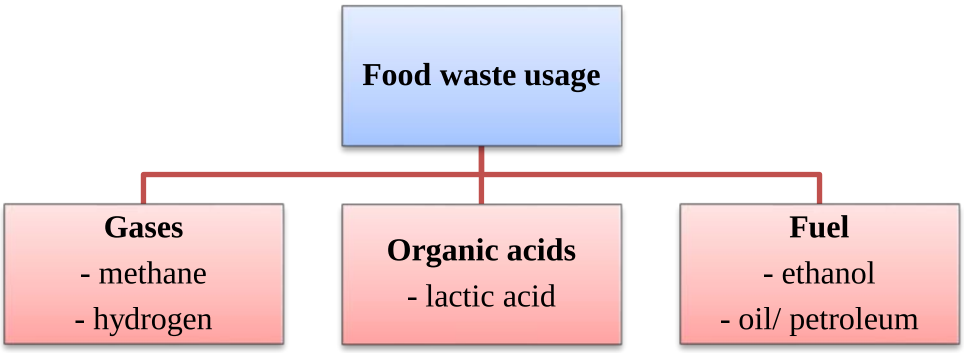

Figure 2.6 Usage of food waste to produce beneficial products.

2.4.1 Production of gases

There are many researches using food waste especially in the production of hydrogen and methane that performed these fermentations in open condition. Most studies are conducted by harnessing the naturally occurring fermentation by manipulating the environmental factors such as temperature, pH, and incubation time. In addition, the studies mainly innovates a new system that generally consists of fermenters or bioreactors, sensors (pH and temperature) and gas collector (Shin & Youn, 2005; Han & Shin, 2004; Kim *et al*., 2009). The study by Shin and Yuon (2005) and Han and Shin (2004), utilizes seed sludge microbes from anaerobic digestion tank in a sewage treatment plant. The seed sludge is then treated and mixed with food waste in the fermenter. Whereas, Kim *et al.* (2009) used heat treatment on food waste to select hydrogen-producing microbes, Clostridium species. All experiments proved efficient in producing hydrogen but only the work of Kim *et al*. (2009) shows the most practicality as it is simpler and does not require start-up period and less cumbersome whereby sterility and maintenance of inoculums was not required.

2.4.2 Production of other products

Food waste has been used to produce oil by thermochemical liquefaction (Minowa *et al*., 1995). In this study, food waste was constructed using cabbage, boiled rice, boiled and dried sardine, butter and shell of short-necked clam. The food waste was then heated under pressure at different temperatures for a few hours with a catalyst. Highest oil obtained was 27.6 % on an organic basis in the condition of 4 % catalyst added, temperature of 340^{o}C, pressure of 18 MPa and holding time of 0.5 hours.

Food waste has also been processed into ethanol. Tang *et al*. (2008) used kitchen waste that was kept fresh for a week by spraying with lactic acid bacteria, saccharified it with glucoamylase enzyme and fermented the enzyme-treated liquid with flocculating yeast *Saccharomyces cervisiae* KF-7 to produce ethanol. The enzyme-treated residue was further placed in thermophilic anaerobic continuous stirred tank reactor to produce methane gas. The authors have managed to treat food waste by producing energy products that are ethanol and methane.

Another use for food waste is by converting food waste to plastic (Sakai *et al*., 2004). The authors first fermented the food waste in sterile condition and inoculating with *Propionibacterium freundenreichii*. The crude fermented media was then filtered and purified to produce L-lactic acid. After that, L-lactic acid was polymerized to LL-lactide that further underwent ring-opening polymerization to produce poly-L-lactic acid polymer.

2.5 Lactic Acid and Its Chemical Structure

Lactic acid is a hydroxycarboxylic acid which is a 3-carbon organic acid molecule with one carboxyl and a hydroxyl in its structure. Another familiar name of lactic acid is lactate in ionized form and 2-hydroxypropionic acid. Lactic acid bearing the chemical formula of CH_3-CHOH-COOH or $C_3H_6O_3$, can be found as two different optical isomers (stereoisomers) that are in a form of L(+) and D(-)-lactic acid (Figure 2.7).

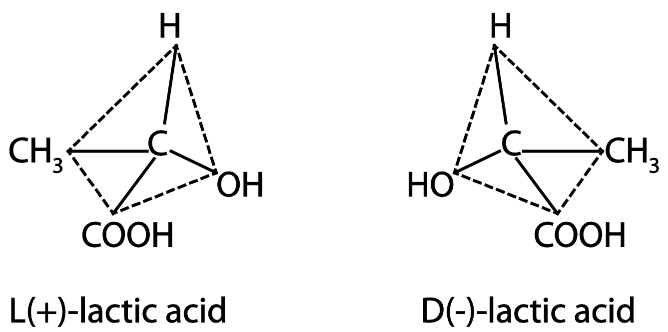

Figure 2.7: Molecular structure of lactic acid isomers (Neidhardt *et al*., 1990)

Naturally, lactic acid is produced in living cells of microbes and animals undergoing anaerobic respiration that is respiration without oxygen. Almost all bacterial cells produce lactic acid as one of the common metabolite. Nevertheless, the most efficient lactic acid producers are homofermentative lactic acid bacteria that only produce lactic acid from glucose (Reddy *et al*., 2008). Food waste also has been reported to contain the most lactic acid bacteria that naturally exist in it (Sakai *et al*., 2000, Kim *et al*., 2009).

2.5.1 Biochemical pathway from glucose to lactic acid

One molecule of glucose theoretically makes two molecules of lactic acid by homolactic acid bacteria (Ohkouchi & Inoue, 2007). Other lactic acid producing bacteria however, produce not only lactic acid but also ethanol, diacetyl, formate, acetic acid and carbon dioxide (Neidhardt *et al*., 1990; John *et al*., 2009). The pathway from glucose to lactic acid is schematically shown in Figure 2.8.

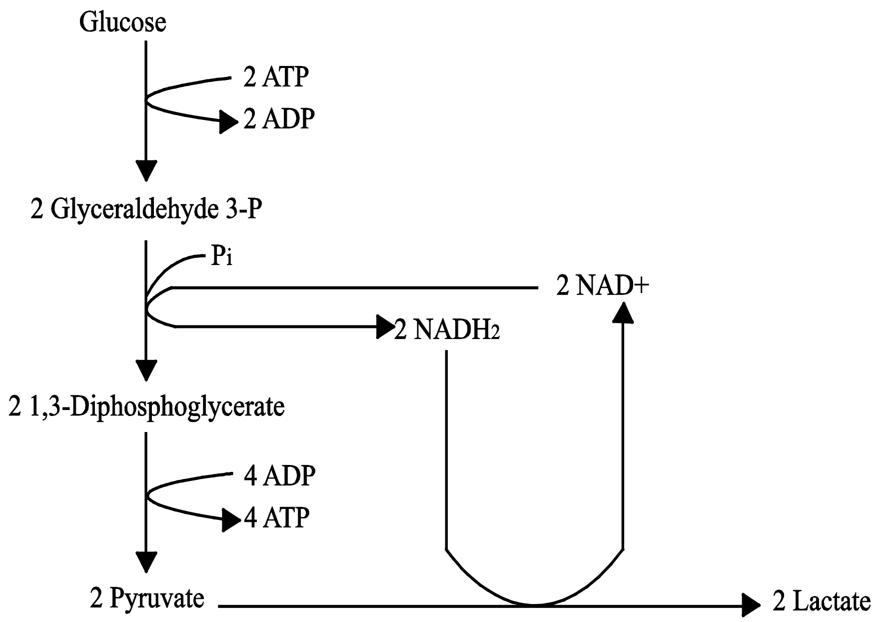

Figure 2.8 Homolactic fermentation (Neidhardt *et al*., 1990)

Glucose is first converted to pyruvate through a series of reactions of cleaving the molecule into two 3-carbon smaller molecules and rearrangements of molecular structure and makes two net ATP (adenosine triphosphate). This process is called